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An experimental study on the migration of the African armyworm moth, Spodoptera exempta (Walker) (Lepidoptera : Noctuidae).

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An Experimental Study on the Migration of the African armyworm moth, Spodoptera exempta (Walker) (Lepidoptera:Noctuidae).

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by

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SUMMARY.

A laboratory investigation into the factors controlling migration in the African armyworm moth, Spodoptera exempta (Walker) was carried out using a tethered-flight technique.

The methods used to culture S.exempta in the laboratory are described and evaluated. Larvae could not be satisfactorily reared on artificial diet, and a successful culture was only established when larvae were reared on freshly-cut maize leaves.

A series of experiments were carried out to determine the effects of environmental conditions acting on the larvae on the flight performance of the resulting moths. Depriving larvae of food, or feeding them on leaves from water-stressed maize plants, did not have any significant effects on the flight performance of female moths. However, when larvae were reared at high densities, a significant increase in the proportion of moths defined as "migrant" on an arbitrary criterion was observed. There was no significant difference between the wing-loadings of "migrant" and "non-migrant" moths.

Observations made during the above experiments suggested that a signifcant genetic component contributed to the determination of flight capacity. Selection experiments and the calculation of heritability estimates confirmed these expectations, and suggested that flight capacity was a polygenic character.

Flight capacity of "migrant" moths generally declined with age. Feeding and mating did not have any significant effects on the flight capacity of four-day old moths. No direct association could be found between flight capacity and rate of ovarian development.

An hypothesis for the control of migration in S.exempta in East Africa is described. Differential selection is seen as operating between the wet and the dry seasons, with "flight" genotypes selected for in the wet season when the potential habitat range of S.exempta expands, and "non-flight" genotypes being selected for during the dry season when habitats are isolated and topographically restricted.

GENERAL INTRODUCTION.

The African armyworm, Spodoptera exempta (Walker) is a major pest in east, central, and southern Africa. The larvae occur in outbreaks or plagues and attack almost exclusively Graminaceous crops, feeding on the foliage. These include the most important staple food crops in this part of Africa, which include maize, sorghum millet, and finger millet, as well as more recent introductions such as wheat, barley, rice, and oats. Pasture and range grazing is also severely attacked. The armyworm also feeds on plants in other families, notably the Cyperaceae, and isolated reports of larvae feeding on species from other families including the Iridaceae, Leguminosae, Malvaceae, and Solanaceae were cited by Brown and Dewhurst (1975). Although some of these families contain species of economic importance, these are rarely seriously attacked by S.exempta. A full list of known host-plants was compiled by Brown (1962), and this has recently been updated (Brown and Dewhurst,1975). The latest additions to the list of host-plants are given by Baker (1978) who reported larvae feeding on Tritonia crocosmiflora (Lemoine) Nich. (Iridaceae) in Papua New Guinea , and Yarro et al. (1981), who found a small infestation of larvae on the dwarf coconut Cocos nucifera L. (Palmae) at Mombasa in Kenya.

Despite being known as the African armyworm, the distribution of S.exempta extends beyond Africa, and it has been reported in a number of continental and island countries throughout the Near and Far East, and in Australia (Brown and Dewhurst,1975). However given the lack of published data, S.exempta does not appear to be a major pest in these regions.

Within Africa, the distribution of S.exempta varies greatly with

season, although it may remain unimportant or unreported for much of the year in some areas (Rose,1975,1979; Rainey and Betts,1979). Its potential range covers most of the central and eastern side of the continent from South Africa to Ethiopia and Somalia, and may extend as far as the Yemen in some years (Brown and Dewhurst,1975). It is also found in West Africa, where serious outbreaks can occur (Betts,1976), and it is likely to become an increasingly important pest in this region as grassland replaces the cleared natural forests. However it is in East Africa that outbreaks are particularly severe (Swaine,1963), and therefore the work in this thesis is primarily related to the current armyworm situation in the East African region, particularly Tanzania and Kenya. Despite this, the conclusions reached in this work may be equally applicable to other areas where S.exempta occurs.

The armyworm outbreak season in East Africa coincides with the rains, and extends from December to May (Brown,1965). The first outbreaks often (although not always) occur in central Tanzania, particularly in the Kilosa/Morogoro area (Odiyo,1981,1982), and these are followed by a series of outbreaks at approximately generation intervals which occur progressively further north (Brown,1965, Brown et al.,1969). There is a corresponding southward movement into Zimbabwe, Botswana, and South Africa from the Malawi/northern Mozambique area (Blair and Catling,1974). Even in seasons in which no major outbreaks are reported, numbers of armyworm moths caught in light-traps fluctuate more than those of related species (Brown,1965), implying that local populations levels are not stable but subject to considerable variation, probably as a result of extensive immigration and emigration.

Spodoptera exempta has a typical Lepidopteran endopterygote life-cycle. Adult females lay eggs in large batches (typically 200-400 per

batch), usually on a suitable host-plant. On hatching, the larvae commence feeding, after wind-assisted dispersal on silk threads (Whellan,1960). Only larvae landing on young grass or cereals survive (Hattingh,1941). They may pass through five or six instars (Hattingh,1941; Matthee,1946-see below), then leave the host-plant and burrow into the soil to pupate. On emergence, the adults tunnel to the surface, and climb up suitable vegetation to expand their wings. Mating normally occurs within 2-4 days of emergence, and oviposition commences immediately thereafter. Under optimum conditions, the duration of the life-cycle is c. 1 month. A detailed account of the life-cycle is given by Whellan (1954).

The larvae of S.exempta show a distinct density related phase variation first described by Faure (1943). Because of the superficial similarity between this phenomenon and the well documented phase variation in locusts (reviewed by Kennedy,1956), Faure used the terms "gregaria" and "solitaria" to describe the two forms of larvae. However, the armyworm shows no true larval aggregation behaviour, and no overt phase variation in the adults has yet been reported. In recognition of this, Whellan (1954) coined the terms "active" (gregaria) and "passive" (solitaria) to describe the larvae, based on behavioural differences between the two phases. This terminology is used throughout this work.

Active phase larvae, the true armyworms, are characteristically found in outbreaks where larval densities can reach several hundred per m^2 (Khasimuddin,1981a; Odiyo,1981). They are typically a velvet black colour with black head-capsules (although they tend to become paler when nearing a moult, Faure,1943) and are extremely vigorous and irritable (Whellan,1954). They pass through six instars (Hattingh,1941;

Matthee,1946) and their development is highly synchronised (Khasimuddin,1981a; Brown et al.,1969). The black colouration develops fully at the moult from the third to the fourth instar (Faure,1943), at which point the larvae tend to move to the upper parts of the host-plant (Whellan,1954). In the later instars their rate of feeding is drastically increased, and it is at this stage that major crop damage occurs (Brown and Odiyo,1968). If all the available food in a particular location is eaten, their propensity towards movement enables them to travel to a new plant or area. Movement of this type involving vast numbers of larvae at high densities is termed "marching", providing the derivation of the name "armyworm". However movement is rarely unidirectional (Whellan,1954). Although damage to crops is often serious, foliar regeneration can occur in younger plants if damage is not too severe (Brown and Mohamed,1972).

Passive phase larvae are predominantly green in colour throughout their development, and often have yellow head-capsules (Faure,1943). Most pass through five instars, although a small percentage were found to pass through six instars (Matthee,1946). They are found where larval densities are low, although they need not necessarily be completely solitary (Whellan,1954). Rose (1975) reported passive phase larvae at densities as high as $10/m^2$, and they may even be found within relatively high density outbreaks. However, they are generally sluggish and tend to remain at the bases of grasses. This behaviour, coupled with their cryptic colouration, renders them inconspicuous even at relatively high densities (Rose,1975), and they are often difficult to distinguish from other Noctuid larvae (Whellan,1954).

Larvae intermediate in colour between the active and passive phases are also found (termed "transiens" by Faure,1943). They are relatively

uncommon, but emphasise the fact that the two main phases represent the opposite extremes of a continuously graded spectrum of variation, and are not discrete morphs.

Biochemical and physiological differences between the two larval phases have also been reported. Matthee (1945) found that final instar active phase larvae had a higher lactic acid and fat content than final instar passive phase larvae, and Khasimuddin (1981b) reported that passive phase larvae took longer to develop (17 days from hatch to pupation at 25°C as opposed to 14 days for active phase larvae). He also found that passive phase larvae had more protein fractions and higher titres of juvenile hormone in the final instar. These differences were thought to reflect the differing modes of life which the two larval types exhibit (Khasimuddin,1981b-see below).

In assessing the general biology of the armyworm, a detailed understanding of which is an essential pre-requisite to a successful regional control strategy, the adaptive significance of phase variation in this species is of central importance. Rose (1975) suggested that the majority of larvae in natural habitats are passive phase. Khasimuddin (1981b) elaborated on this by suggesting that the two phases were physiologically distinct, and that the natural form of larva for this species is the passive phase. Thus whereas the active phase is highly efficient at exploiting abundant but possibly ephemeral food resources characteristic of the outbreak (rainy) season, the passive phase ensures the long term survival of the species throughout the year, especially during the off (dry) season, when food availability and environmental conditions place the emphasis on survival (Khasimuddin,1981b). Evidence for successive low density generations in favourable areas during the

outbreak season has been found by Khasimuddin and Lubega (1979), who monitored three consecutive low density generations in the Lambwe Valley in Kenya, and Rose (1975) studied three successive generations of the armyworm which occurred at relatively low densities in a localised area in Zimbabwe. Recently, pheromone traps maintained in damp highland areas in Kenya have caught moths in every month of the year, suggesting that low density populations do persist throughout the off season in areas previously thought to be clear of armyworm at this time (Page,1982). Field cage experiments have also demonstrated the ability of the armyworm to survive throughout the year in coastal regions in Kenya, but not at other inland sites (Persson,1981). The evidence thus suggests that areas where low density populations do survive during the off season are likely to be topographically restricted, and to vary in their precise location from year to year.

There is some evidence to suggest that under certain conditions S.exempta may undergo either a pupal (Khasimuddin,1977) or a larval (Fonseca-Ferrão and Santos,1965) diapause to enable it to survive the off season. However diapause has yet to be demonstrated under controlled conditions, and it appears to be rare under field conditions. Its importance in the biology of the armyworm is yet to be clarified.

In terms of the practicalities of control in the field, an understanding of how and where outbreaks are likely to occur is also of major importance. S.exempta moths are known to be capable of long-distance migratory flight, and this has important implications for the possible mechanisms leading to the occurrence of outbreaks. Evidence for migration is derived from several sources. Circumstantial field evidence was collected by Brown and Swaine (1966), who, on the basis of fluctuating numbers of moths caught in light-traps, the low rate of

fertilization of trapped females, and the low ratio of males to females in trap samples when compared to the non-migratory Spodoptera triturata (Walker), concluded that S.exempta is a stongly migratory species. Direct field observations by Rose and Dewhurst (1979) suggested that many moths emigrated from emergence sites within 24 hours of emergence, and these observations are supported by radar studies on the flight behaviour of S.exempta moths at outbreak sites in Kenya (Riley et al.,1981). Laboratory evidence for migration has also been found. Aidley (1974) demonstrated that moths were capable of sustained flight for several hours on a flight mill, and these observations have recently been confirmed by Gatehouse and Hackett (1980), who showed that moths readily flew for several hours on a tethered-flight system incorporating spontaneous take-off and landing facilities. Studies on the heterogeneity between six isoenzymes in moths collected from widely different locations in Africa also suggested that wide gene mixing and therefore extensive migration occurs in S.exempta (den Boer,1978). Furthermore, outbreaks can often be attributed to an influx of migrant moths into a particular area brought about by the transportation and eventual deposition of migrant moths by convergent wind-systems (Betts,1976; Blair,1972; Haggis,1971; Blair and Catling,1974; Rose and Law,1976; Blair et al.,1980; Brown et al.,1969; Tucker et al.,1982). Mass egg-laying then follows, and young larvae hatch to feed on the flush of new vegetation promoted by the rain associated with cyclonic weather systems (Brown et al.,1969). Douthwaite (1978) found no association between peak catches of armyworm moths (in a light-trap at Muguga, Kenya) and wind direction or wind shifts (cf. Haggis,1971), and suggested that low wind speeds were generally responsible for high trap

catches. Mark/recapture experiments are currently being undertaken in Kenya to ascertain the direction of dispersal of moths from known infestations, and if this can be linked to the prevailing winds at the time. As yet, no firm conclusions have been reached (Page,1982).

The armyworm forecasting system which covers the East African region (reviewed by Odiyo,1979) also largely bases its predictions on the contention that moths are concentrated by convergent wind-systems. Synoptic weather charts and rainfall records are used to identify possible convergence zones, and if these coincide in space and time with reports of large numbers of moths in the regional light and pheromone trap network, then large scale breeding is forecast as being likely downwind (Odiyo,1979; Brown et al.,1969). The fact that this system generally works satisfactorily (Betts,1976; Odiyo,1979) adds credence to the idea of wind convergence concentrating migrant moths.

The origin of moths causing outbreaks, and in particular the first outbreaks of the season, is a matter of some controversy. There are two hypotheses. One suggests that the overall armyworm population can be regarded as a number of relatively high density, highly mobile regional populations which fluctuate in size. These move within weather systems in a similar manner to locusts (Betts,1976,1982; Rainey,1976,1982; Rainey and Betts,1979). Many of these populations are unreported for much of the time when their movements take them beyond the network of light and pheromone traps in East Africa, or beyond areas of human habitation. Occasional catches of a few moths in the trap network could be indicative of the edge of such populations (Rainey and Betts,1979). First outbreaks are envisaged as occuring when moths are brought in on westerly airstreams, possibly from Angola, Zambia, Zaire, or even West Africa (Rainey,1982). Control measures should be directed against as

many outbreaks as possible, even those not directly threatening crops, thus reducing the size of the populations contributing to further outbreaks in a potentially cumulative manner (Rainey and Betts,1979).

The second hypothesis stresses the role of low density populations in initiating the first outbreaks of the season (Rose,1979), and in providing additional sources of moths for concentration during the outbreak season. These are envisaged as supplementing the moths derived from outbreaks which are considered to be the major sources of moths for further outbreaks. Moths are not seen as moving in discrete swarms; instead the tendency is for moths to become widely dispersed (Rose,1982). Populations are thought to exist on two levels; either they are at low densities where passive phase larvae survive predators through cryptic colouration and behaviour, or they are at high densities (outbreaks) where they are conspicuous but survive predation and disease through weight of numbers. The severity of any particular outbreak season depends on the biotic and abiotic mortality factors affecting the survival and population growth of low density populations at the onset of the rains (Rose,1982). Virus infections may be particularly important in controlling population levels under certain conditions (Persson,1981). High population growth leads to large early outbreaks, and vice versa. Later in the season, the population becomes more dispersed as the potential range of the insect expands, and widespread low density populations become more important in outbreak initiation (Rose,1982). Hidden low density populations are thought to occur in many areas during the rainy season, and there is evidence that these persist in favourable areas during the off (dry) season (see above). Nyierenda (1982) has also recently produced evidence of continuous year round

survival of the armyworm in Malawi, which could act as an important source of moths for those outbreaks which occur in Zimbabwe and other countries to the south. The conclusion of this hypothesis is that if the first outbreaks(s) of the season can be effectively controlled, the number and severity of further outbreaks could be reduced. This approach is the current aim of the regional control strategy (Rose,1982).

It is clear from the foregoing discussion that the colonization of new habitats (and hence outbreak initiation) by the armyworm is dependent on large scale adult migration, both in terms of the numbers of moths involved and the potential distances covered. Migration therefore has a central role in the life-history strategy of the armyworm and contributes greatly to its pest status. The consequences of this have been stressed by Joyce (1976), who stated that "the importance of a study of insect flight to pest control is that the first essential requirement for crop protection, namely defining the temporal and spatial extent of the threatening pest population, cannot be reached without this knowledge." This is particularly applicable to migrant pests such as S.exempta. However, although the physiological capability of S.exempta moths to fly for long periods has been demonstrated (Aidley,1974), little is known of the intrinsic and extrinsic factors promoting or suppressing migratory flight. Without such knowledge, an appreciation of the numbers and quality of migrants arising from any particular population, which has far-reaching effects on the ways in which the armyworm is dispersed over its range, is impossible.

The aim of this study is the elucidation of the mechanisms controllimg migration in this species.

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CHAPTER ONE.

Culturing Spodoptera exempta

in the laboratory.

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1. INTRODUCTION.

The first requirement for any laboratory study of insects is a steady supply of insect material. This can be achieved either by collecting insects from the field if they are abundant in the locality, or by setting up a laboratory culture. A culture may be the only option if the insect under study is highly seasonal, or not indigenous to the particular location where the work is being carried out. A culture has the additional advantage of enabling the conditions under which the insects are being reared to be strictly controlled, thus reducing to a minimum any variation due to environmental effects. This is particularly important if behaviour is being studied.

Descriptions of insect culturing techniques in the literature are numerous, and a detailed review is not attempted here. However, with particular reference to Lepidoptera, the types of methods employed can conveniently be classified into three categories based on the type of larval diet used. These are listed below, and are then briefly reviewed.

1. Artificial diets.

2. Semi-synthetic diets.

3. Natural host-plants.

Artificial diets are chemically defined media, most commonly wheat-germ and agar based. They are widely used, and numerous examples can be found in the literature (Bot,1966; McMorran,1965; Vanderzant,1967; Burton and Perkins,1972; Moore and Navon,1964; Hoffman et al.,1966; Lyon et al.,1972; Vanderzant, Richardson, and Fort,1962). The popularity of artificial diet is due to the fact that once larvae have been placed on the diet, they require little further attention until pupation. This is labour saving, reduces mortality due to injury caused during transfer to

fresh food (Wongsiri and Randolph,1962), and if individual and/or disposable containers are used, the risk of disease or secondary fungal contamination spreading through the culture can be minimized (Ignoffo and Boening,1970). The process of diet preparation and dispensing lends itself to mechanization, and hence it is often used in mass-rearing programmes (Perkins,1979) where cost is a major limiting factor.

Problems of host specificity are also overcome by the use of artificial diet, enabling different species to be reared on the same medium (Ignoffo and Boening,1970). However, the exact nutritional requirements of individual species may vary (Vanderzant,1967), and identifying the shortcomings of a particular diet for a given species is time-consuming (Burton and Perkins,1972). Work carried out on the basic dietary requirements of several species emphasises this point (Vanderzant et al.,1957; Vanderzant, Pool, and Richardson,1962). However, the wealth of published literature covering this subject may alleviate this problem in some circumstances.

Semi-synthetic diets are essentially artificial diets containing some fresh or dry whole-plant material (Ignoffo,1963; David and Gardiner,1966; Beckman et al.,1953; David et al.,1975). The plant material used need not necessarily originate from a preferred host-plant. Shorey (1963) successfully used a diet based on dried Lima beans to rear the Cabbage looper Trichoplusia ni Hb., and a similar diet was used by Shorey and Hale (1965) to rear nine species of Noctuid moths. However, Harper (1970) found that this latter method resulted in a rapid decline of egg viability. A combination of diet and natural food allowed a vigorous culture to be maintained. Use of host-plant material may be a short cut to avoid the need to identify exact nutritional requirements for a specific species (cf. artificial diet), or to provide necessary

phagostimulants for larvae which do not readily feed on the basic diet (David and Gardiner,1965). Natural host-plant material is a potential source of pathogens, although this problem may be reduced if the material is dried before incorporation into the diet. Seasonal availability and time-consuming preparation of material (e.g. drying and grinding leaves) are also limiting factors (David and Gardiner,1965). Such problems mitigate against the widespread use of semi-synthetic diets in mass-rearing programmes, although in most other respects their advantages and disadvantages are similar to those for artificial diet.

Natural host-plants have been successfully used to rear a number of Lepidopteran species (Long,1953; Macaulay,1973; Rivnay and Meisner,1965; David et al.,1975), although detailed assessments of methods and mortality are relatively uncommon in the literature. The number of insects which can be reared using this method is relatively small, the main constraints being the availability of host-plants (David and Gardiner,1965), and the high handling time involved in transferring larvae to fresh food, sometimes necessary every 1-2 days (Brown and Swaine,1965). However, larvae may develop faster on natural hosts compared to those on artificial diet, possibly due to the optimum nutritional status of the host plant (David and Gardiner,1965). Boller (1972) suggested that the use of host-specific token stimuli (implicit in natural host-plants) was important in maintaining wild-type behavioural traits in cultured insects if this was desired. For example, Lepidopteran larvae reared initially on artificial diet lost their host specificity to varying degrees when subsequently tested against non-host plants (Ma,1976; Schoonhoven,1967). The use of natural food plants may be impossible if endemic insect pathogens are carried into the culture

on food material despite stringent sterilization procedures (Brown and Swaine,1965).

Handling techniques for pupae, adults, and eggs are varied. Some inert material such as sawdust (Moore and Navon,1964), vermiculite (Bot,1967), or sterilized soil (David et al.,1975) can be provided if larvae normally pupate in the soil. Pupation may also satisfactorily occur in or on artificial diet (Shorey and Hale,1965). Adults can be kept in jars or cages, and provided with food and suitable substrates or host-plants for oviposition. Optimum environmental conditions for mating and oviposition may vary for different species. Fecundity may be affected by both larval (Rivnay and Meisner,1965; Zaher and Long,1959) and pupal (Miller,1982) rearing conditions.

Attempts to culture Spodoptera exempta in the laboratory have been made using all the methods outlined above, with varying degrees of success. Early workers on the armyworm used natural host-plants such as barley (Hattingh,1941) and maize (Faure,1943; Matthee,1945). Unspecified diseases were reported as being "somewhat troublesome" by Faure (1943), while Hattingh (1941) reported a "wilt disease" associated with damp rearing conditions. Brown and Swaine (1965) also attempted to culture S.exempta using maize, but their culture suffered heavily from epidemics of a nuclear polyhedral virus (NPV), thought to originate from contaminated leaves, and subsequently transmitted between larvae via contaminated frass. There is also evidence that trans-ovarial transmission of the virus may occur (Swaine,1966). The NPV affecting S.exempta has been studied in detail by Odindo (1977-see Discussion). An artificial diet suitable for rearing S.exempta was described by Bot (1967), who reported a low disease incidence using this method, although precise details of mortality were not given. David et al. (1975)

successfully reared S.exempta on a semi-synthetic diet and on growing maize, but egg viability declined rapidly to less than 25% by the ninth generation. This was thought to be due to the accumulation of deleterious genotypes caused by inbreeding (David and Ellaby,1975).

In general, the two main problems associated with insect culturing are disease, and maintenance of viability in terms of a genetically diverse stock (Mackauer,1976). Due consideration should therefore be given to these when choosing a culturing technique. Ideally, the culture method employed should be dependent on the type of work for which the insect material is required (Boller,1972). However, problems specific to particular species (e.g. disease in S.exempta) may necessitate a compromise between the ideal method and what is practical given the problems involved and the resources available.

The aim of setting up a culture of Spodoptera exempta at UCNW Bangor was to provide a year round supply of moths for experimental work. Ideally, these were to be derived from active phase larvae, whose rearing conditions had been monitored and controlled. This chapter describes the methods used to achieve this. A detailed analysis of durations of stages, number of instars, and factors causing mortality was considered to be outside the scope of this work, and was not attempted.

2. MATERIALS AND METHODS.

2.1. Artificial diet.

A wheat-germ and agar based artificial diet (Table 1.1), modified from that described by Hoffman et al. (1966), and similar to that used by Bot (1967) was the first method of larval rearing attempted.

2.1.1. Origin of insect material.

A stock of c. 100 larvae and pupae was obtained in December 1979 from the cultures then maintained at the Insect Virology Unit, Wytham Wood, Oxford. This culture was thought to be virus-free, and had been reared on the artificial diet described in Table 1.1 for several years. No new stock had been recently introduced in to this culture because of the need to avoid the introduction of pathogens, particularly virus.

2.1.2. Environmental conditions.

Temperature and humidity were monitored using thermohygrographs. Eggs and larvae were maintained in a constant temperature room held at 25±2°C. Humidity was not rigidly controlled, but a water-bath in front of a fan maintained a level of 40-60% R.H. Pupae and adults were kept in a separate room maintained at 26±1°C and 75±4% R.H. The photoperiod in both adult and larval rooms was 13h light:11h dark.

2.1.3. Rearing procedure.

Adults were maintained in wire-framed fine netting or muslin cages (either 20x20x20cm or 30x30x30cm). A sleeve allowed access to the cage. Moths were provided with a cotton wool pad soaked in a 20% w/v sucrose solution in a petri-dish or plastic lid placed on the floor of the cage. This method of feeding adults was similar to that used in the cultures at Oxford. The sucrose solution was renewed (usually every two days) when the pad had dried out or the sucrose solution had started to

TABLE 1.1.

Ingredients and method of preparation of artificial diet.

Ingredients	g	Procedure
Agar	15.0	1. MIX.
Casein	26.5	
Wheatgerm	57.5	
Salts mixture*	7.5	
Dried Brewers Yeast	11.5	
Sugar	23.5	
Cholesterol	0.75	
Methyl p hydroxy benzoate (Nipagin)	0.75	
Sorbic acid	1.2	
	ml	
Linoleic acid	1.5	2. Add to dry ingredients.
Water	580.0	3. Autoclave for 20 mins, allow to cool.
	g	
Choline Chloride	0.75	4. Add when diet has cooled to 60-70°C.
Vitamin and antibiotic mixture*	4.5	
Benlate	0.3	

5. Pour diet into containers while still warm.

*See Appendix 1 for details.

ferment. Initially distilled water was also provided, but as a labour saving measure this was later discontinued with no apparent adverse effects on the adults. The number of moths per cage was not strictly controlled, but did not exceed 7 males and 7 females.

The cages were inspected daily for eggs. These were normally laid on a piece of thin paper towelling attached to one wall of the cage, or occasionally on the cage netting or wire frame. They were usually laid in batches consisting of 1-3 superimposed layers of eggs, and the early batches were normally covered with black hairs from the abdomen of the female. The eggs were pale yellow when first laid, later becoming brown and then black when close to hatching. These descriptions conform to those given by Hattingh (1941). Eggs were surface sterilized on the day following collection (see Appendix 1), and were then placed in a clean petri-dish in the larval culture room.

On hatching, larvae were left until they had eaten the egg-shells, and moved off in search of food, usually 2-4h after hatching. They were then transferred to the artificial diet using a fine paint brush. Two types of container were used, both made of plastic.

(a) Large diet pots. c. 295 ml containing c. 100 ml of diet.

(b) Small diet pots. c. 10 ml containing c. 5 ml of diet.

Suitable plastic lids lined with filter-paper were used. The plastic lids were punctured to allow adequate ventilation. The density regimes of larvae maintained in the two types of pot are shown in Table 1.2.

Diet was freshly prepared for each generation. As it was often found necessary to replace diet during larval development (section 3.1) enough was made up to cater for this replacement. Spare pots containing diet were stored in a refrigerator.

Larvae were allowed to pupate either in or on the diet. Pupae were

TABLE 1.2.

Densities of larvae reared on artificial diet in small (10ml) and large (295ml) diet pots.

Instar	No./10ml pot	No./295ml pot
1-3	1-3	60
4	1-3	40
5-pupation	1-3	20

Notes.

1. The exact number of larvae/10ml pot was dependent on the availability of diet and larvae.

2. The excess larvae arising from the reduction in larval density in the large pots at 4th instar were transferred to small pots if enough of these were available. At 5th instar, larvae were divided into two large pots containing 20 larvae each.

collected within two days of the last larva pupating, and surface sterilized (see Appendix 1). They were then sexed by the method described by David et al. (1975), and placed in clean filter-paper lined petri-dishes. If fungal growth on the diet was severe, pre-pupae were transferred to filter-paper lined petri-dishes. On the afternoon prior to emergence, pupae were transferred to a petri-dish in a cage. Pupae within 6-8h of eclosion can be easily identified, as at this time they change in colour from a chestnut brown to a deep chocolate brown. On eclosion, the adults climbed up the cage netting to expand their wings.

2.1.4. Sterilization of diet pots.

After use, any remaining diet was removed, and pots and lids were washed in hot water containing an industrial detergent (Decon 90) in a sink used only for this purpose. They were then soaked overnight in a bucket containing 0.1% sodium hypochlorite solution, and dried using paper towelling in a room isolated from the larval and adult culture rooms.

2.2. Natural host-plants.

Due to the difficulties encountered in rearing larvae successfully on artificial diet (section 3.1), it was decided to rear larvae on natural food-plants. In initial attempts, wheat (Triticum sp.) seedlings were used for one generation in June 1980. These were not successful. However in September 1980, after another failure with artificial diet, a successful culture using maize (Zea mays L.) grown specially in a greenhouse was established.

2.2.1. Origin of insect material.

The initial stock of insects was obtained from the armyworm cultures maintained at the International Centre for Insect Physiology and Ecology

(I.C.I.P.E.), Nairobi, Kenya. Further stocks were received from I.C.I.P.E. at irregular intervals, as well as from the armyworm culture at the Kenya Agricultural Research Institute (K.A.R.I.), Muguga, Kenya. One batch of pupae was also received from the Centre for Overseas Pest Research (C.O.P.R.), London, U.K. The C.O.P.R. insects also originated from the K.A.R.I. cultures. All new stock was kept separate from the main culture for one generation to allow the incidence of diseases in incoming insect material to be monitored. If mortality in this initial generation was comparable with that in the main culture, the new stock was interbred with the main stock in the following generation.

2.2.2. Enviromental conditions.

Temperature and relative humidity in the larval culture room were as described in section 2.1.2. Adult and pupal conditions were also similar except for the addition of 120W of tungsten lighting above the bench on which the adults were maintained. These lights were connected to an automatic dimmer, which provided an artificial dawn and dusk of c. 45m duration. The heat from the lighting produced a diurnal temperature variation of 3-5^{o}C in the rearing room.

2.2.3. Rearing procedure.

Adults were maintained in 450ml glass jars (1lb Kilner jars). A lid was provided by covering the top of the jar with a piece of filter-paper held in place by a screw-ring. Filter-paper was also used to line the floor of the jars, and a further piece of crumpled filter-paper was provided loose in the jar to act as a hiding place during the day, and to provide an additional substrate for oviposition. The maximum number of moths per jar was 3 males and 3 females; these were chosen for their diversity of genetic background as far as was possible. Moths were

provided with a cotton wool pad soaked in a 20% v/v honey solution in a small plastic lid placed on the floor of the jar. This was changed every two days. Adults were maintained until all the females had died, or arbitrarily until the fourth day of oviposition.

Jars were inspected daily for eggs, which were normally laid on the filter-paper surfaces in the jar. Eggs required for breeding were collected from as many adult jars as possible and surface sterilized (Appendix 1) on the day following collection. If eggs were not required they were destroyed. The time-consuming process of egg sterilization was eventually abandoned, even for new stock, with no apparent adverse effects on mortality in the culture. Eggs were allowed to hatch either in petri-dishes or plastic vials.

Larvae were fed on leaves from 4-6 week old maize plants. After hatching, c. 60 1st instar larvae were placed in a 450ml glass jar (1lb Kilner jar) containing a small thin new leaf from the base of a plant. The cut end of the leaf was placed in water in a 20ml glass vial stoppered with cotton wool. Jars had filter-paper lids and their floors were also lined with filter-paper. They were inspected daily. If the leaf was still apparently fresh and the area consumed was small, the water in the vial was replenished and the leaf was left for another day. However, if it appeared that the leaf would not provide sufficient fresh food to support the larvae for a further 24h, a fresh piece of leaf in a new vial was put into the jar, and the old one removed. The larvae were maintained in this way until they reached 4th instar. At this point, numbers were reduced to c. 25 larvae per jar. The rate of food consumption by the larvae now increased rapidly, and floor filter-papers, water vials, and food were replaced daily. The daily change was carried out by tipping the larvae, frass, and old leaf material on to a

piece of paper towelling in a plastic tray. Larvae were handled using a paint brush and a filter-paper scoop. Any larvae apparently lagging behind in development (more than one instar behind the majority of larvae in the jar) or showing any symptoms of disease (section 3.2.2) were removed. Because of the need to insert a large amount of leaf into the jar in order to provide these later instar larvae with sufficient food for 24h, the mid-ribs were stripped out of the larger leaves, the cut ends still being placed in a water vial. During the early generations of the culture, late sixth instar larvae which had ceased feeding and pre-pupae were removed from the jars and placed in clean jars containing c. 5cm of vermiculite, into which the larvae burrowed to pupate. Pre-pupae are easily recognised by their cessation of feeding followed by extreme activity. They then appear moist, possibly due to secretions from the epidermal glands (Clark,1980), and start to contract. A small piece of leaf was placed in the jar in case larvae still required some food. This procedure was later simplified by allowing the larvae to pupate in the accumulated frass in the bottom of the larval jars. This proved to be satisfactory, and prevented disturbing or possibly damaging larvae and pre-pupae during transfer to the pupation jars.

Pupae were collected 2 days after pupation. Although surface sterilization was initially carried out, this was again found to be unnecessary and time consuming, and was discontinued after several generations. Pupae were however still washed in distilled water prior to sexing, and were then kept in the adult room until emergence.

Adults were allowed to emerge either in cages (section 2.1.3) or in 55ml plastic vials containing a piece of filter-paper for the moths to

climb up on emergence.

2.2.4. Sterilization procedures.

All glassware was heat-sterilized for at least 3h and usually overnight in an oven maintained at 170°C. All used food and soiled filter-papers were placed in a bucket containing 0.1% sodium hypochlorite solution, or put in the oven to be heat-sterilized. All dead or diseased insects, including adults, were also heat-sterilized. In the early generations, leaves were washed in 0.1% sodium hypochlorite solution prior to being fed to larvae. However, this procedure was also later abandoned as unnecessary, and thereafter leaves were only washed if they were severely infested with aphids and consequently sticky and dirty.

3. RESULTS.

3.1. Artificial diet.

Stage-specific mortality and overall mortality which occurred in 7 out of the 9 generations reared on artificial diet are shown in Fig.1.1. Although overall mortality remained within tolerable limits for the first three generations (40-50%), a sharp increase in overall mortality from 40-80% was recorded in the fourth generation, and a peak of 85% was reached in the fifth generation. Over the next two generations, overall mortality declined to 75%. It then increased again, reaching 100% by the ninth generation. Reference to Fig.1.1 clearly shows that larval mortality accounted for the greater part of the overall mortality in all generations, the combined effect of pre-pupal and pupal mortality rarely accounting for >20% of the overall mortality. The high larval mortality was due mainly to a suspected viral or bacterial infection which eventually became uncontrollable. Infected larvae were characterised by reduced feeding rate, slow development, sluggishness, and generally stunted appearance. Fungal contamination of the diet was also a major problem, despite the inclusion of fungal inhibitors (Benlate and Nipagin-Table 1.1). The most common fungus formed black spores, and could rapidly colonize the diet. Although the fungus did not appear to be directly pathogenic to the larvae, a heavy growth drastically increased the humidity in the larval pots, apparently rendering the larvae more susceptible to viral or bacterial infection.

Some larval mortality was also due to 1st instar larvae drowning in the surface film of water on the diet if this was not dried off prior to introducing the larvae. There was limited cannibalism in later instars, but only if larval densities were high, or if fungal infestation of the

FIGURE 1.1.

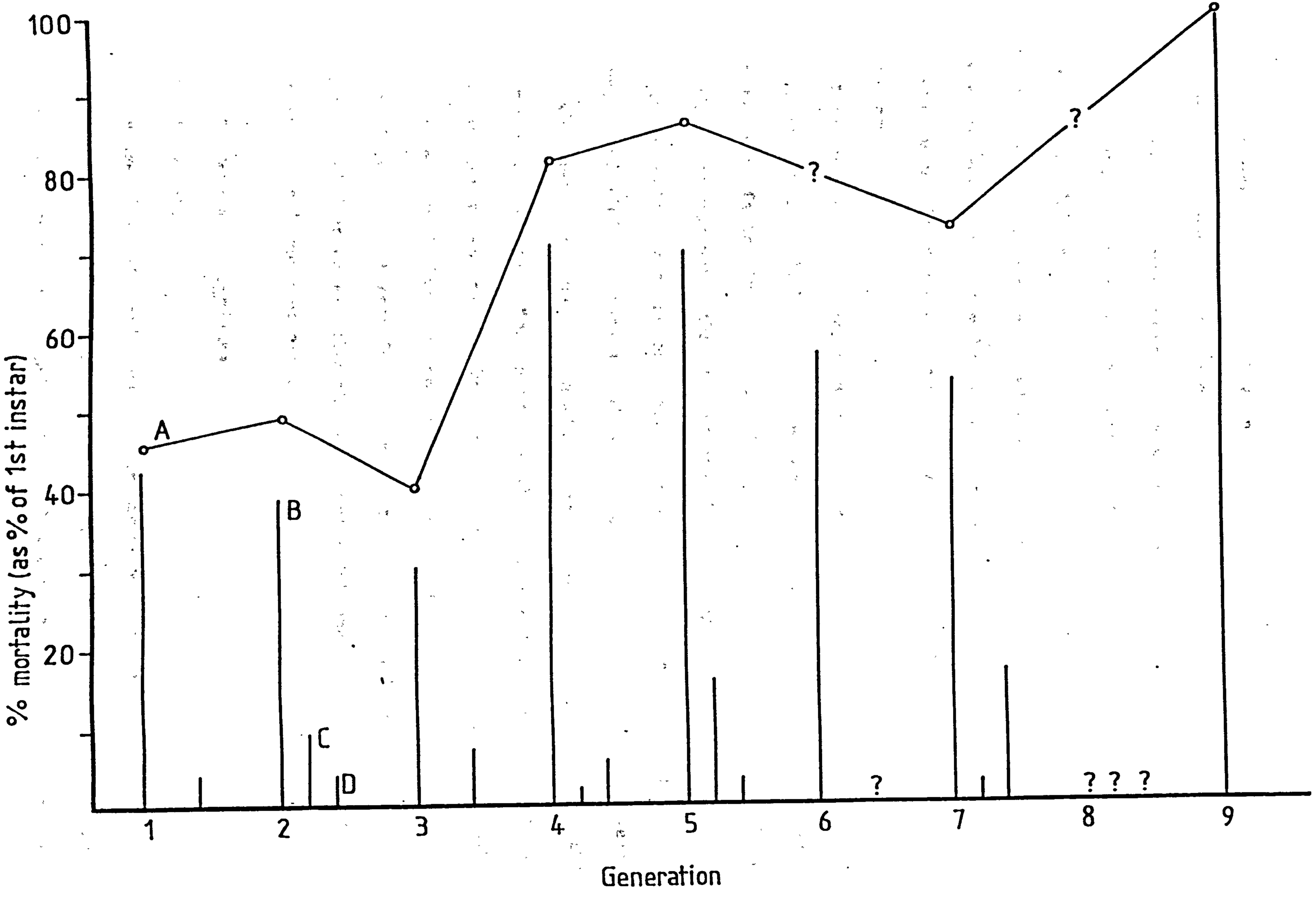
Stage-specific and overall mortalities of insects reared in the artificial diet culture.

A. Overall mortality.

B. Larval mortality.

C. Pre-pupal mortality.

D. Pupal mortality.


100
80
60
40
20
% mortality (as % of 1st instar)
A
B
C
D
?
?
?
?
?
?
1
2
3
4
5
6
7
8
9
Generation

diet rendered it inaccessible and/or unpalatable due to the spread of fungal hyphae over the surface.

Pre-pupal and pupal mortality may have been due to a low-level virus infection, which can become fatal at this stage (Vaughn,1974). Some pupae were deformed, occasionally appearing pinched between the head and thorax with an associated small area of incomplete pupal cuticle. More commonly pupae simply had a distorted thorax or abdomen. All these pupae usually died or produced adults which failed to emerge successfully.

Data on the percentage emergence of normal adults from full-term pupae was not collected, but this was generally high. Adults survived and mated satisfactorily. No detailed data was collected on egg viability, although it was noted that percentage hatch of eggs was variable.

Approximate durations of stages for each generation, and sample mean pupal and adult weights are shown in Tables 1.3 and 1.4 respectively. Total generation time (egg to adult) varied between 27 and 32 days, with a mean of 28.5 days. Mean pupal weight in the small sample taken was 142.8mg for males, and 139.2mg for females. Mean adult weight was 94.0mg for males, and 95.8mg for females. Male and female mean weights were not significantly different for either adults or pupae. All larvae reared on artificial diet were active phase regardless of whether they were reared in small or large diet pots.

3.2. Natural host-plants.

3.2.1. Wheat.

The use of wheat seedlings to rear larvae was only attempted once, and was a complete failure. Approximately 150 larvae in 3 batches of *c.* 50 larvae each were introduced on to freshly cut wheat seedlings held in a

TABLE 1.3.

Stage-specific durations and overall generation times (in days) for insects reared in the artificial diet and maize cultures.

Artificial diet.

Generation no.	Egg	Larval	Pupal	Total
1	2	14	9	27
2	2	15	10	27
3	2	19	9	32
4	2	17	10	29
5	2	16	9	27
6	?	14	8	?
7	2	17	8	29
$\bar{x}$ (n=7)	2	16	9	28.5

Maize.

$\bar{x}^*$(n=23)	2±0	13.4±0.47	6.7±0.44	22.0±0.50

*±95% confidence limits (corrected by a value of t equal to p=0.05).

water vial in standard 450ml rearing jars. However, no larvae developed beyond second instar, and those surviving longest were stunted and sluggish. Possible reasons for this failure are discussed later (section 4.2).

3.2.2. Maize.

Stage-specific and overall mortalities in the maize culture covering a period of 19 consecutive generations (Nov.1980-Feb.1982) are shown in Fig.1.2. Overall mortality was high in the initial generations (60-70%), largely due to high larval mortality. This level of mortality may be artificially high due to the ruthless "weeding out" of larvae showing any symptoms of disease during this period. However, from the 4th generation, larval mortality fell rapidly, resulting in an overall mortality of 26% by the 8th generation. Thereafter, larval mortality was maintained below c. 25%. The combined effect of pre-pupal and pupal mortality rarely exceeded 25%, and thus from the 8th generation overall mortality was maintained at 25-55%. The 13th generation was an exception, where overall mortality increased to 62%, largely as a result of abnormally high pupal mortality. The density at which larvae were reared was increased during this generation, thus possibly increasing the numbers of larvae contracting a sub-lethal virus infection, sufficient to kill the insects during the pupal stage (Vaughn,1974). However, as there was no evidence of even a small increase in larval mortality, which would have been expected if disease was involved, it is possible that pupal mortality may have been due to the fact that pupae were being washed within two days of emergence at this time. In the following two generations, pupae were washed earlier (within two days of pupation) and pupal mortality fell, resulting in a concomitant fall in overall mortality to 38% in the 15th generation.

FIGURE 1.2.

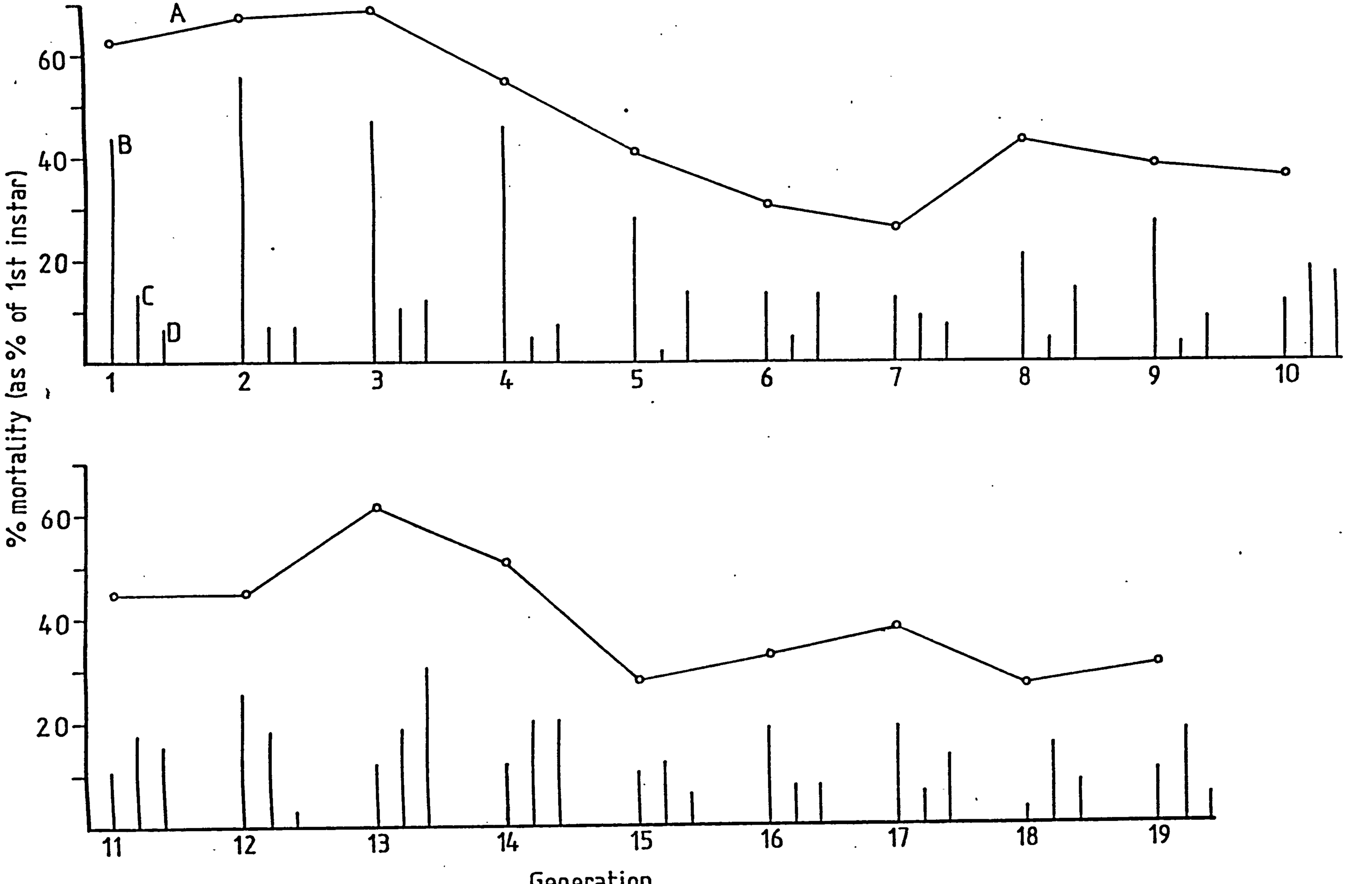
Stage-specific and overall mortalities of insects reared in the maize culture over a period of 19 consecutive generations.

A. Overall mortality.

B. Larval mortality.

C. Pre-pupal mortality.

D. Pupal mortality.


A
B
C
D
60
40
20
% mortality (as % of 1st instar)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Generation

The symptoms of viral infection in the early generations were not normally apparent until larvae reached the 4th instar. Stunted and sluggish larvae then became conspicuous. Later instar larvae often had assymetric white swollen areas beneath the posterior dorsal cuticle, and/or similar white blotches on the ventral surface. Infected larvae either failed to complete development, or produced deformed pupae characterised by the pinching between the head and thorax described in section 3.1. These pupae subsequently shrivelled up, presumably through dehydration, and died. In later generations the larval and pupal symptoms described above became rare, and diseased larvae were more commonly characterised by slow development leading to stunted growth. Such larvae often became tinged with brown, stopped feeding, and eventually died. Dead larvae were flaccid, and contained a thick brown fluid. However, the cuticle was not easily ruptured. Infected pupae went black and the pupal case became fragile, releasing a brown fluid when ruptured.

Adults survived and mated satisfactorily. The pre-oviposition period varied between two and four days. Egg viability was again found to be variable (as in the artificial diet culture) but there were no overt indications of any long-term decline in either adult fecundity or egg fertility.

Mean minimum stage-specific durations (the time taken by the fastest developing individuals in each stage) and mean overall generation times (egg to adult) calculated from 23 consecutive generations are shown in Table 1.3. Eggs always hatched in c. 2 days. Larval period varied between 12 and 16 days, with a mean of 13.4 days, and pupal period varied between 6 and 9 days, with a mean of 6.7 days. Mean overall

TABLE 1.4.

Pupal and adult mean weights (in mg) insects reared in the artificial diet and maize cultures (±95% confidence limits).

	Pupal wt.		Adult wt.	
	Male	Female	Male	Female
Artifcial diet.	142.8±26.05 (n=9)*	139.3±28.83 (n=9)	94.0±26.70 (n=8)	95.8±28.25 (n=7)
Maize culture.	117.7±4.32 (n=55)	131.3±9.12 (n=12)+	71.6±3.51 (n=45)	80.1±6.32 (n=12)+

* Where n<30, 95% confidence limits have been corrected by a value of t equal to p=0.05 with n-1 degrees of freedom.

+Calculated from the 12 mean values plotted in Fig.1.3. Total no. of insects=374 pupae, 321 adults.

generation time was 22.0 days, varying between 20 and 24 days. Synchrony of larval development was good, with the majority of larvae reaching pupation within 2 days of each other. All larvae reared in this culture were active phase.

Data demonstrating the variation in mean weight of female pupae and adults from 12 out of 16 consecutive generations is given in Fig.1.3. These insects were reared under identical larval conditions (larval density and amount of food strictly regulated). Mean pupal weight declined from 136.6mg to 104.1mg over the first 4 generations. It then increased to 151.0mg by the 9th generation, before again declining to 122.3mg by the 15th generation. An increase to 149.0mg was recorded in the 16th generation. The variation in mean adult weight closely reflected that shown by mean pupal weight. Overall mean female pupal and adult weights for these 12 generations are given in Table 1.4.

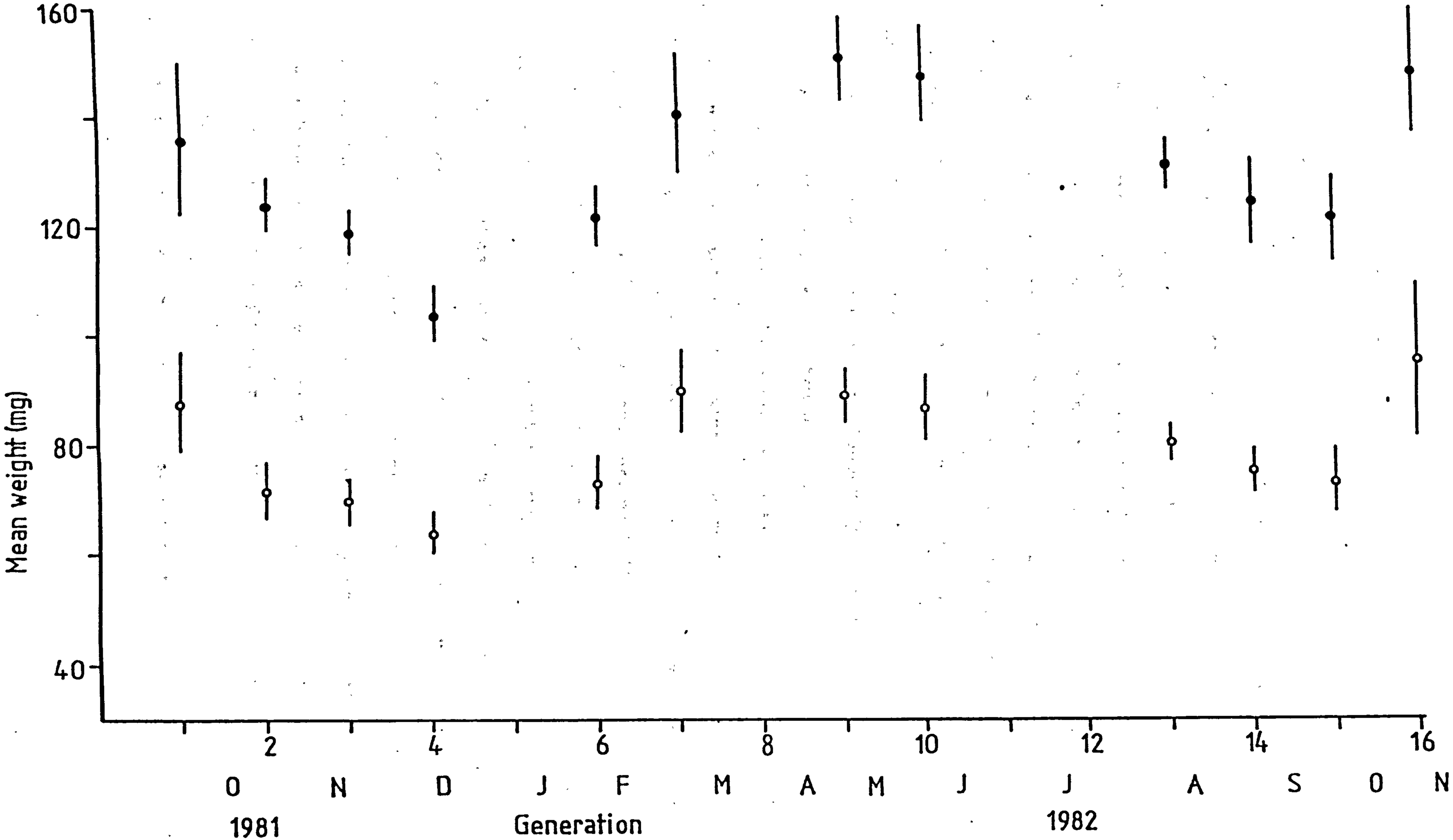
Mean weights of male pupae and adults from a representative sample of insects reared under the same larval conditions as the females in Fig.1.3 are given in Table 1.4. Mean pupal weight was 117.7mg, and mean adult weight was 71.6mg.

FIGURE 1.3.

The variation in mean female pupal and adult weight (±95% c.l.) of insects reared in the maize culture over a period covering 16 consecutive generations (data for 12 generations only).

• Mean pupal weight.

o Mean adult weight.


160
120
80
40
Mean weight (mg)
2
4
6
8
10
12
14
16
O
N
D
J
F
M
A
M
J
J
A
S
O
N
1981
Generation
1982

4. DISCUSSION.

4.1. Artificial diet.

The failure of the artificial diet culture after nine generations (Fig.1.1) can be attributed largely to the problems of secondary fungal contamination of the diet, and more especially to the marked build up of the suspected virus infection, which was undoubtedly responsible for the greater part of larval mortality in the final generations.

The failure of the fungal inhibitors, Nipagin and Benlate, to suppress the growth of fungus adequately may have been due to fungal resistance to these chemicals. Reports of major secondary fungal contamination of insect artificial diets are not common in the literature. Ignoffo (1963) found that larvae of the cabbage looper Trichoplusia ni would not develop on diets seriously contaminated with yeasts, although he found that these were satifactorily controlled by Aureomycin, which is present in the vitamin and antibiotic mixture (Appendix 1) of the diet used in this study. Other fungal contaminants can be controlled by incorporating formaldehyde in the diet (Burton and Perkins,1972). These latter authors reported that 5-20% of their individual diet pots used to rear Heliothis zea (Boddie) and Spodoptera frugiperda (J.E.Smith) contracted some fungal contamination, and concluded that an increase in formaldehyde concentration in the diet may have reduced this problem. David et al. (1975) apparently had no problems with fungal growth when rearing Spodoptera exempta on a semi-sythetic diet containing formaldehyde, and it is therefore possible that the use of formaldehyde would have helped to reduce fungal contamination of the diet used in this study.

The manner in which the larval disease spread through the culture over

several generations is consistent with it being a relatively low-level virus infection. These spread slowly through a culture, causing abnormal growth and lower fecundity, resulting in a generally weakened stock (Vaughn,1974). The majority of larvae were reared at high density and, as frass was not removed from the pots, the diet surface quickly became contaminated, resulting in a rapid transmission of the infection between larvae, even if only a few individuals were affected initially. Other workers have reported few or no problems with disease when S.exempta was reared on artificial (Bot,1967) or semi-synthetic (David et al.,1975) diets, even though the latter authors reared larvae at high initial densities. The use of antibiotics to control bacterial infections was found to be unnecessary when Spodoptera frugiperda was reared on artificial diet (Burton and Perkins,1972). The symptoms and possible identity of the larval diseases encountered in this study are discussed in more detail below (section 4.3).

The low larval mortality caused by cannibalism might have been eliminated by improved suppression of fungal growth which would almost certainly have led to better diet palatability. Moore and Navon (1964) reported that cannibalism in Spodoptera littoralis (Boisd.) (cited as Prodenia litura F.) could be minimized by reducing the density at which larvae were reared in the final instars. Such a procedure was also used in this study (Table 1.2). Mortality due to first instar larvae drowning in moist diet was also reported by Bot (1967), who overcame it by laying strips of filter-paper on the diet surface in his rearing tubes.

The problems of fungal contamination of the diet and larval disease were compounded by inadequate sterilization of diet containers. Although strong alkalis (such as sodium hypochlorite) are known to dissolve virus polyhedra (Thomas,1974), fungal spores were almost certainly not

destroyed by washing the diet pots in alkaline solutions, as fungal growth often started around the edge of fresh diet where it came into contact with the side of the pot. The fact that the suspected virus infection also appeared to persist from generation to generation suggests that not all virus particles were destroyed when the pots were washed. Increasing the concentration of the alkaline solution may have reduced this problem. Under the sterilization regime used, however, the number of contaminated pots increased, as when larvae were transferred from infected diet to uncontaminated pots, some fungal spores and diseased larvae were inevitably introduced with them. Virus particles may also have been transmitted trans-ovarially (Swaine,1966), although if this was by surface contamination it should have been precluded by the process of egg sterilization. It is also possible that transmission of virus particles occurred via contaminated dust particles or moth scales.

Strictly in terms of its suitability for development, there is no doubt that the artificial diet was nutritionally adequate, and that larvae would readily feed on it. Generation times (Table 1.3) were similar to those given by David et al. (1975) for S.exempta reared on Bot's (1967) diet under comparable environmental conditions. Variations in larval period (Table 1.3) may have been caused by differing levels of fungal growth on the diet in different generations, or by qualitative differences in the diet, particularly moisture content. Beckman et al. (1953) reported drying medium as slowing larval development of the pink bollworm Pectinophora gossypiella (Saund.). There is no apparent direct link between larval period and the level of larval mortality for any given generation, even though diseased larvae generally developed more

slowly. However, the development times shown in Table 1.3 are minimum times, and are therefore likely to reflect the development rate of the healthiest individuals.

There are no published data on weights of S.exempta pupae and adults reared on artificial diet. However the weights recorded in this study (Table 1.4) are comparable with those of insects reared on maize (Table 1.4 and Fig.1.3).

4.2. Natural host-plants.

The poor larval performance on wheat could be attributed to host-plant preference. However, Brown (1970) states that if no choice of host is available, larvae will readily feed on any species of host-plant. Since wheat has been widely reported as a common host-plant for S.exempta (Brown,1962,1970; Brown and Dewhurst,1975), it seems unlikely that it could have been unacceptable or nutritionally inadequate for larval development. The high and rapid larval mortality recorded on wheat in this study was probably due to an acute attack of larval disease, possibly virus. Even if larvae had survived, it is doubtful if sufficient wheat could have been grown to satisfy the required demand for insect material.

The maize culture fulfilled the basic requirement for a long term supply of insect material, and with the help of irregular injections of new stock, it is continuing to do so after more than 30 generations. Apart from the initial generations, larval mortality was low (Fig.1.2) and this was undoubtedly the reason why the culture was successful. Maintenance of low larval mortality was probably due to a combination of effective sterilization of larval and adult jars, and the weeding out of diseased individuals before any infection had a chance to spread. The

fact that the population was split amongst a number of containers also helped to reduce the likelihood of any infection spreading, as it could be quickly isolated. Good quality food and relatively low larval densities also helped to reduce stress likely to induce a flare-up of disease as a result of lowered larval tolerance. Disease levels within the culture were thus held at a low enough level to obviate the need for stringent sterilization procedures, hence the abandoning of egg and pupal surface sterilization. Such laxity would not be possible in areas where virus is endemic; Brown and Swaine (1965) found it impossible to maintain a successful culture of S.exempta on maize in Kenya because of severe problems with virus infections brought in on food plants. Persson (1981) states that washing leaves in 0.1% sodium hypochlorite solution helps to reduce this problem. David et al. (1975) did not report disease as being a problem when S.exempta was reared on maize in the U.K. Average stage-specific durations and overall generation times (Table 1.3) were similar to those recorded by Faure (1943), but several days shorter than those given by Hattingh (1941) and David et al. (1975), even though the latter authors used similar environmental conditions. These discrepancies are probably due to small variations in rearing temperature or differences in larval food quantity or quality.

The high percentage emergence of normal adults is indicative of the nutritional suitability of maize for development. Pre-oviposition periods were consistent with published reports for both wild and laboratory reared insects (Hattingh,1941; Whellan,1954; David et al.,1975), although they were never as short as one day (Hattingh,1941; Rose,1975). The observation that not all females with access to males laid fertile eggs may be due to the fact that not all females will mate (Khasimuddin,1978), although mated females will also lay infertile eggs

(David and Ellaby,1975).

The variation in female pupal and adult weights (Fig.1.3) must be due largely to variations in maize quality, as all other factors likely to contribute to weight variation (e.g. food quantity and larval density) were constant. Fluctuations in maize quality may have arisen from seasonal variations in the chemical composition of the leaves; there is some suggestion that mean female adult and pupal weights declined during the autumn (October-December 1981), and increased again during the spring (February-May 1982; Fig.1.3). However, variations in the nutrient content of the compost in which the plants were grown may also have accounted for some of this variation. For instance, it is known that the compost was deficient in nitrogen at the time when the lowest weights were recorded (November-December 1981), and plant growth was poor at this time (see also Chapter 2, section 4.3.1). An aphid transmitted plant virus which stunted plant growth also became troublesome in the later generations, and may have had some secondary effects on the nutritional quality of the leaves.

4.3. Disease.

Disease was the major constraint on the establishment of a successful culture of S.exempta, and it is therefore important to consider the pathogens involved in some detail. Diseases specific to pupae and adults were not encountered, although some pupal mortality may have occurred as a result of an infection carried over from the larval stage.

The precise identification of insect pathogens is often difficult without specialised knowledge and techniques, particularly as the external symptoms of the two most common groups of pathogens, viruses and bacteria, overlap to some extent (Faust,1974; Vaughn,1974). However,

a general diagnosis is usually sufficient to allow appropriate control measures to be taken.

S.exempta larvae suffering from a known infection of a nuclear polyhedral virus (NPV) become lethargic, cease feeding, and void quantities of fluid. Later the skin becomes fragile and ruptures easily to release a milky fluid containing polyhedra. Dead larvae are characteristically found hanging from one or two pairs of prolegs (Odindo,1977; Brown and Swaine,1965). The integument may also become paler, and larvae show a tendency to move towards the light (Odindo,1977). These symptoms are not entirely consistent with those shown by larvae thought to be suffering from a virus infection in this study. Very few larvae either from the artificial diet or maize cultures became fragile, although most became flaccid, and only a few cadavers were observed hanging from their prolegs. However, the intensity of symptoms may vary with the level of infection (Vaughn,1974), and as many obviously sick larvae were removed from the culture and destroyed before the infection reached a late stage, it is possible that some of the later symptoms were never observed. The characteristic swellings (posibly fat body hypertrophy) shown by larvae in the early generations of the maize culture (see above, section 3.2.2.) were consistent with the symptoms described by Vaughn (1974) for a granulosis virus infection. Freshly killed specimens of larvae showing these symptoms were sent to the Insect Pathology Unit at the Glasshouse Crops Research Institute (G.C.R.I.), Littlehampton, Sussex, and their investigations confirmed the presence of a low-level granulosis virus infection. However, the level of infection was not considered high enough to be pathogenic to the larvae, and this observation would be consistent with

the suggestion (section 3.2.2) that some pupal mortality was caused by a low grade virus infection carried over from the larval stage.

The symptoms of some diseased larvae reared on artificial diet, and particularly those of larvae and pupae dying in the later generations of the maize culture, are indicative of a bacterial infection. The general external symptoms of a bacterial infection, namely decreased mobility, loss of appetite, and oral/rectal discharges, are superficially similar to those for a virus infection. However, after death, the body (particularly of larvae) may darken to brown or black, and become soft and shapeless. Internal tissues become viscous and foul-smelling (Faust,1974). These symptoms are similar to those described above (section 3.2.2) for some diseased larvae and pupae in the maize culture. Mortality due to such diseases was particularly prevalent when conditions in the larval jars were excessively damp, often resulting in heavy late larval or pre-pupal mortality. David et al. (1975) reported a high larval and pre-pupal mortality due to bacterial disease when larvae were reared at high humidities on artificial diet, and Hattingh's (1941) "wilt disease" associated with damp rearing conditions was probably a bacterial infection. Some larval mortality on artificial diet in this study may have been due to a bacterial infection as a result of high humidities within larval pots caused by wet diet or fungal infestation. Specimens of larvae and pupae showing these symptoms were again sent to G.C.R.I., but the presence of the most common bacterial insect pathogen, Bacillus thuringiensis, could not be detected. However, it was suggested that this may have been due to the age of the specimens, and the high level of secondary bacterial contamination. Given the close association between the published symptoms (see above) and those shown by some insects in this study, it seems unlikely that no pathogenic bacterial

infection was present in the culture.

It is possible that a mixed infection of virus and bacteria occured at times. Certain non-sporeforming bacteria (e.g. Pseudomonas sp.) are often found in the guts of insects and are generally non-pathogenic. When the insect is stressed by factors such as starvation or infection by another pathogen, the bacteria may become pathogenic (Faust,1974). Some of the confusion over the exact nature of larval diseases in this study may be as a result of such multiple infections. The investigations at G.C.R.I. also revealed the presence of an unidentified Protozoan infection in some diseased insects, but it is not clear if this was pathogenic.

4.4. The quality of cultured insects.

A laboratory colony of insects intended to represent a species in laboratory research , as was the case in this study, must retain as many properties of the wild population as possible or misleading conclusions may result from experiments used to predict events in wild populations (Stock and Robertson,1982). The quality of insects produced in the laboratory should therefore be routinely monitored, and appropriate criteria need to be chosen when assessing the type of "quality" required (reviewed by Chambers,1977). Huettel (1976) suggested that "quality" could be defined in terms of a trait or a particular set of traits, expressed as the difference between a laboratory trait and the same trait in wild insects. Traits suitable for monitoring should be easily measured, and include dispersal ability, survival, mating site location, courtship and mating, and oviposition (Huettel,1976). Ancillary data collected throughout this study on mortality, durations of stages, and pupal and adult weights (e.g. Fig.1.3) performed a monitoring function

to a certain extent, although detailed data on deviations from wild-type patterns of behaviour or characteristics such as percentage mating and pre-oviposition period, similar to those reported for Heliothis virescens (F.) by Raulston (1975), were not collected. Ideally, data on the performance of cultured insects should be compared with similar observations made on wild populations (Huettel,1976), and observations made by other workers on both laboratory and field populations of S.exempta (Hattingh,1941; Khasimuddin,1978; Rose,1975; David et al.,1975) generally compared favourably with those made during this study (section 4.2), although detailed comparisons were not always possible.

Maintenance of wild-type traits in cultured insects largely depends on the maintenance of genetic variation, as the factors contributing most to genetic decay within a culture are inbreeding, genetic drift, selection, and the founder effect (Mackauer,1976). Some non-genetic behavioural conditioning may also occur (Chambers,1977). Cultured insects are inevitably forced through a genetic bottleneck which tends to alter or reduce genetic variation (Boller,1972). This may in turn lead to the accumulation of deleterious genotypes which can have potentially serious effects, such as the decline in the viability of S.exempta eggs reported by David and Ellaby (1975). Encouragingly, no such long term decline was noted in the present study. However, genetic changes cannot always be directly linked to parallel changes in physiology, behaviour, or other phenotypic traits (Tsakas and Zouros,1980).

The work undertaken in this study was largely concerned with adult flight activity, and this is often selected against in cultured insects

(Boller,1972). The founding population is also important in determining the traits of a culture (Mackauer,1976). A very low level of flight activity was recorded in moths derived from the artificial diet culture, probably because the original stock had already been in culture for several years. However, the irregular injections of new stock into the maize culture helped to maintain genetic variability, and flight activity was generally satisfactory, although at the time of writing it is possibly declining. Guthrie and Carter (1972) showed that larval survival of the European corn borer Ostrinia nubilalis (Hb.) reared on a meridic diet could be vastly improved by injecting wild stock in to the culture, and it is possible that a similar boost to overall vigour is now required for the present culture of S.exempta.

4.5. Conclusions and recommendations.

4.5.1. Artificial diet.

The use of artificial diet as the basis for a culture proved to be problematical, and the full benefits of its use such as reduced labour, year-round availability, and standard nutrition of all insects were not realised. However, the success of other workers using this method (Bot,1967; David et al.,1975) clearly shows that under the right conditions a successful culture can be maintained.

With hindsight, there is no doubt that better precautions could have been taken to reduce the scale of the problems encountered, particularly as these stemmed primarily from problems of disease and secondary contamination rather than any inadequacies in nutrition or diet palatability. Ideally, disposable containers should have been used, although this would have been more expensive. Failing this, improvements could have been made in sterilization procedures, such as the use of

heat-sterilizable diet containers. However the necessity of rearing larvae in crowds, and its attendant problems of a "flare out" of disease would still have been present. To combat this, larval densities could be reduced, and a system devised whereby frass would not accumulate on the diet (e.g. by keeping the diet pots upside down or on their sides), thus possibly reducing surface contamination of the diet and hence the spread of any infection. Chemical control of fungi could be improved by experimenting with different inhibitors which, although time-consuming, would be beneficial in the long run.

4.5.2. Maize culture.

The maize culture produced good results, but was extremely labour intensive and consequently very time-consuming. The current methods employed have been streamlined to maintain the culture at its present level with the minimum expenditure of time. Despite this, daily attention is still required, and only a limited number of insects can be reared given the resources available. The major consraint is the availability of larval food, as the supply of maize plants tends to vary seasonally, and the plants themselves are susceptible to pest and disease attack. This problem could be alleviated by ensuring that sufficient plants were grown, preferably in more than one place, to act as a contigency reserve. The time spent rearing stock insects could be reduced by infesting growing plants in the laboratory, thus necessitating a change of food only when the plants became defoliated. However this method is not sufficiently controlled for the rearing of experimental insects.

Diseases proved easier to control in this culture, and the viability of the insects appears to have remained good. However, variations in the quality of the maize may well affect the quality of the insects in terms

of weight (Fig 1.3), and the extent to which this affects experimental results is not clear.

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CHAPTER TWO.

The effects of environmental conditions acting on the larvae on the flight performance of S.exempta moths.

TABLE OF CONTENTS.

1. INTRODUCTION.

Insect migration is now accepted to be a distinct physiological, behavioural, and ecological syndrome (Dingle,1972; Kennedy,1961), and it is therefore intimately related to other aspects of life-history such as growth, maturation, timing of reproduction, and total reproductive effort (Dingle,1974). Thus migration has generally come to be regarded as an evolved adaptation rather than a response to current adversity (Johnson,1960; Kennedy,1975), although Southwood (1981) has recently pointed out that these two views of migration are in fact the opposite extremes of the same spectrum of migratory stategies. However, the idea of involuntary dispersal (whereby an insect "accidentally" migrates by, for example, being caught and carried by the wind) is now practically dead (Kennedy,1975).

Insect migration can be defined as "the act of moving from one spatial unit to another" (Baker,1978). However, it is a reflection of the phenomenon that this definition is so broad as to be meaningless (Southwood,1981). More specifically, migration must entail the departure of the insect from its current habitat (Johnson,1969), where habitat is defined as "the area that provides the resource requirements for a discrete phase of the animal's life" (Southwood,1981). In behavioural terms, a migrant can be defined as having three distinct characteristics; persistent locomotion, straightened out locomotion, and suppression of vegetative responses such as feeding and mating (Kennedy,1975). Migration is also usually post teneral and pre- (or occasionally inter-) reproductive, the period available for migration being limited by the length of the pre-oviposition period. The complex association between migration and reproduction which occurs in many

insects has been termed the "oogenesis-flight syndrome" (Johnson,1963,1969), and is discussed in more detail in Chapter 4.

The prime evolutionary advantage of migratory movement lies in its enabling a species to keep pace with changes in the location of its habitat (Southwood,1962), and indeed migrants are usually considered to be denizens of ephemeral habitats. There is an enormous variation in migratory strategies, but they are all linked by the common thread of adaptation to shifting environments (Dingle,1980). Thus whereas diapause is an escape in time from adverse conditions, migration is an escape in space, although it was stressed by Dingle (1978) that the two phenomena are often intimately linked. He cited the milkweed bug Oncopeltus fasciatus (Dallas) as an example. Temperate populations of O.fasciatus exhibit a short-day-induced reproductive diapause, and this delay in reproduction permits extensive migratory flight to occur which is enhanced by diapause. Solbreck (1978) suggested that insect migrants could also be viewed as "bet-hedgers", spreading egg production in space and time and thus guarding against total reproductive failure.

A successful migration strategy must include an accurate method of assessing the suitability of the current habitat for future generations, and the role of environmental cues in triggering migration has been studied in detail for many species (see below). The characterization of habitats is often complicated (Southwood,1977), and in some species there may be an hierarchical organisation of the ways environmental information is used to control phenotypic switches between direct development, migration, and possibly diapause (Solbreck,1978). He showed that for the seed bug Neacoryphus bicrucis (Say), photoperiod was at the top of the hierarchy. Shortening day-length always induced diapause, and bugs were insensitive to information about mates or food resources.

Under constant photoperiods, females responded to a lack of food or mates by migrating, and only fed and mated females reproduced without migrating.

The type of environmental cues that Spodoptera exempta might use to trigger migration should be considered in the light of its habitat and work on other insects. In temperate regions, shortening photoperiods marking the onset of winter have been found to stimulate migration. This has been demonstrated for the migratory skipper butterfly Parnara guttata guttata (Bremer and Grey) in Japan (Ono and Nakasuji,1980), and Elsey (1974) showed that in the predatory stilt bug Jalysus spinosus (Say), diapause and the associated migration was influenced by photoperiod regardless of food conditions, although summer migrations were affected by food availability. In other insects, the effect of photoperiod is related to the availability of food. Short photoperiod and poor food stimulates migration by extending the pre-oviposition period in Oncopeltus fasciatus (Caldwell,1974). Temperature also varies considerably with season in temperate latitudes, and may influence the proportion of migrants in a given population. Exposure to temperatures above 15^{o}C induced flight muscle development in Cenocorixa spp. (Scudder and Meredith,1972). In Oncopeltus fasciatus, relatively high temperatures reduce the time available for migration by shortening the pre-oviposition period (Caldwell,1974; Dingle,1968), although other environmental factors are also involved in the control of migration in this species (see above). However, S.exempta is found largely in the tropics, where large seasonal variations in daylength and temperature are not found; such factors are therefore unlikely to have any major significance in the migratory strategy of the African armyworm.

Seasonal variations do nonetheless occur in the tropics, mainly as a result of contrasting periods of high and low rainfall, and as host-plants become available for exploitation they often attract migrant insects reproductively programmed to take advantage of temporarily abundant resources (Dingle,1982). The changing patterns of vegetation growth and distribution with season result in large variations in food availability and quality; consequently these are likely to be important cues in controlling migration in tropical insects such as S.exempta. Quality and availability of food have been shown to affect the incidence of migratory flight in several species of insect, notably Hemiptera. These include Oncopeltus fasciatus (Dingle,1968), Cicadulina spp. (Rose,1972), Dysdercus spp. (Dingle and Arora,1973), and the Rutherglen bug Nysius vinitor Bergroth (Kehat and Wyndham,1973a,b). In Lepidoptera, there is some evidence of the quality of larval food influencing adult flight behaviour in the spruce budworm moth Choristoneura fumiferana (Clem.) (Blais,1953-see Discussion).

A further environmental cue found to be of importance in migrant insects is crowding, which is to some extent related to food availability. An increase in the proportion of migrant individuals in response to high larval or adult densities has been shown for several species, including aphids and locusts (reviewed by Johnson,1969), the mosquito Aedes taeniorhynchus (Wied.) (Nayar and Sauerman,1969), and the brown planthopper Nilaparvarta lugens (Stål.) (Kisimoto,1956). High larval densities have also been shown to result in an increase in the number of the "active" (flight) form of the cowpea weevil Callosobruchus maculatus (F.), although the high temperature associated with larval crowding has been found to be the major determinant in the production of the flight form rather than larval interaction (Sano,1967; Utida,1972).

Conversely, crowding was found to inhibit flight in fed females of the cotton stainer Dysdercus superstitiosus (F.) (Gatehouse and Hall,1976).

The study of insect flight in the laboratory necessitates the use of specialised techniques which must reflect as accurately as possible the aspect of flight being studied, be it aerodynamic, behavioural, or physiological. Many controlled flight techniques have been developed, all of which have certain limitations. The most satisfactory in terms of allowing the insect practically free flight was the air-treadmill used by Kennedy and Booth (1963) to study the flight of Aphis fabae Scop. It consists of a small vertical wind-tunnel with a light at the top, and a means of controlling the strength of the air-flow, which is directed downwards. Phototactic and photokinetic responses make the insect fly up towards the light, and the insect's position can be held in the wind-tunnel by adjusting the rate of air-flow to equal the insect's rate of climb. This technique was modified by Laughlin (1974) for use with larger insects such as the Rutherglen bug Nysius vinitor (Kehat and Wyndham,1973a,b). However, it is not suitable for large powerfully flying insects, or for the continuous monitoring of very long flights or activity patterns over several days. Neither is it suitable for nocturnal insects which do not show appropriate photo-responses.

Activity patterns and flight durations of larger insects can be recorded using actographs. The insect is placed in a small cage with some means (either mechanical or electrical) of detecting movement. Actographs have been used to study flight behaviour specifically (Baker,1970; Edwards,1960; Macaulay,1972a,b) and generalised locomotor activity, including flight (Hsiao,1978; Leppla et al.,1979). Although the insects are not tethered using this method, they are severely

constrained by the size of the cage, and cannot establish prolonged undisturbed flight. Many actographs do not distinguish between flight and walking, thus making the interpretation of results difficult.

The flight potential of many insects in terms of range and speed has been studied using flight mills (roundabouts), where the insect is suspended on a counterweighted arm which rotates about a bearing (Aidley,1974; Cooter,1982; Green,1962; Hocking,1953; Koerwitz and Pruess,1964; Kishaba et al.,1967). A means of counting the number of revolutions is usually incorporated in the system. Several insects can also be flown simultaneously on the same mill (Krogh and Weis-Fogh,1952; Kennedy et al.,1948). This technique provides the insect with the optomotor input (albeit assymetric as a result of its constant angular velocity) of the substrate passing beneath it, and the relative wind of flight which is necessary to maintain flight in some insects, such as locusts (Fraenkel,1932). However, problems such as assymetric wing-beating (Hocking,1953), and the outward flexing of the abdomen as a result of attempts to "steer off" the mill (Green,1962) are indicative of the abnormal sensory input which the insect is receiving whilst flying on the mill. Tethered-flight techniques in general allow the insect little control over its aerodynamic position, although occasionally some provision is made for a certain amount of adjustment in the crucial pitch, roll, and yaw planes (Chambers and O'Connell,1969).

Another widely used method of assessing the flight potential of individual insects is static tethered-flight. Although this is a relatively crude technique, it is simple and gives sufficiently representative results to allow comparisons to be made between insects subjected to different experimental treatments; although the actual

flight durations recorded are usually arbitrary. The insect is suspended on a stick or piece of wire, and wing-flapping is initiated by a standard procedure starting with the removal of tarsal contact. The technique described by Dingle (1965) for use with the Oncopeltus fasciatus has been widely used for the assessment of flight capacity in bugs, which generally do not require any optomotor input or relative wind (Eguagie,1975; Dingle and Arora,1973; Gatehouse and Hall,1976; Rankin and Riddiford,1977,1978). Flight duration is taken as the time spent wing-flapping, and suitable arbitrary criteria are chosen to define migratory flight (Dingle,1965). A variation of this technique was used by Hwang and How (1966) to study flight activity in the Oriental armyworm Mythimna (cited as Leucania) separata (Wlk.). Moths were suspended from an arm which vibrated against a smoked kymograph drum while the moth was flying. This technique was also used by Macaulay (1974) to study flight in Plusia gamma L.

A major disadvantage with most tethered-flight techniques is that the insects cannot take-off and land spontaneously. As a result, flight is often terminated by exhaustion, which is rare under field conditions where insects will normally land with sufficient reserves remaining for trivial movements associated with vegetative behaviour, such as mating, location of a host, and oviposition. Thus unless specific criteria are set to define migratory flight, data on absolute flight duration will have little behavioural and ecological significance. For instance, some insects may fly considerably longer in the laboratory if they have no means of landing than they would in the field. This was noted for Aphis fabae by Cockbain (1961), and for the European pine shoot moth Rhyacionia buoliana (Schiff.) by (Green,1962). Tethered-flight systems

incorporating spontaneous take-off and landing facilities give a more accurate assessment of flight capability in behavioural terms, and allow activity to be monitored over several days if necessary. A rare example is the "flight swing" described by Hackett (1980).

A further problem with active or delicate insects is that they have to be immobilized either by chilling (Berry et al.,1978; Rowley et al.,1968) or anaesthesia (Hackett,1980; Koerwitz and Pruess,1964) to avoid damaging them during tethering. This treatment may affect not only the subsequent flight of the insects (Green,1962), but also other physiological processes such as oviposition (White et al.,1970). Allowing sufficient time for recovery may be difficult when dealing with insects which may migrate within hours of emergence.

To date, there is no published data on environmental cues thought to control migration in S.exempta. Gatehouse (unpublished report*), using a novel tethered-flight technique described by Gatehouse and Hackett (1980), found that moths derived from active phase larvae flew significantly longer than those derived from passive phase larvae. This raised the possibility that it is the larvae which are sensitive to changing environmental conditions, particularly with respect to food quantity and quality. This chapter describes an investigation into the effects of larval food deprivation, host-plant quality, and larval crowding on the flight capacity of the resulting moths, using a modified version of the tethered-flight technique described by Gatehouse and Hackett (1980).

* Gatehouse,A.G. (1979). Report of a visit to Kenya to carry out research on flight activity of the African armyworm moth (Spodoptera exempta) in support of the COPR/DLCOEA East Africa Armyworm Project, 29th March-31st May 1979.

2. MATERIALS AND METHODS.

2.1. Flight technique.

The tethered-flight technique used throughout this study was the flight balance system described by Gatehouse and Hackett (1980) for flying S.exempta moths. This technique permits continuous automatic recording of spontaneous flights by individual moths over any period up to several days. For the purposes of this study, the design of the actual balance was altered to make it lighter and more compact, and the counterweighting system was simplified. The provision of a relative air-flow over the flying moths (present in the original technique) was also dispensed with as moths were found to fly satisfactorily in still air. The definitive apparatus is described below; procedures for mounting and attaching moths to the equipment are given in section 2.1.3.

2.1.1. The definitive flight technique.

Fig.2.1 is a diagrammatic representation of the flight apparatus used for all definitive flight experiments. The moth is attached to the arm of the balance (1) which is made from hypodermic needle tubing. When at rest, the moth sits on a black paper drum (16) which rotates freely and is mounted on a simple gimbal at the end of an aluminium arm (10). When the moth takes off, the lift it generates tilts the beam of the balance around the pivot (3) and the thrust pulls the balance forward around another pivot at (4). The combination of these forces pulls the foil flag (6) out of the infra-red photo-emitter detector (7). This has the effect of switching a relay to operate an event-recorder pen, and also activates the solenoid (13) which is attached to a pulley (9). This rotates in an anti-clockwise direction to pull away the paper drum. The movement of the balance is damped by a small vane in liquid paraffin at

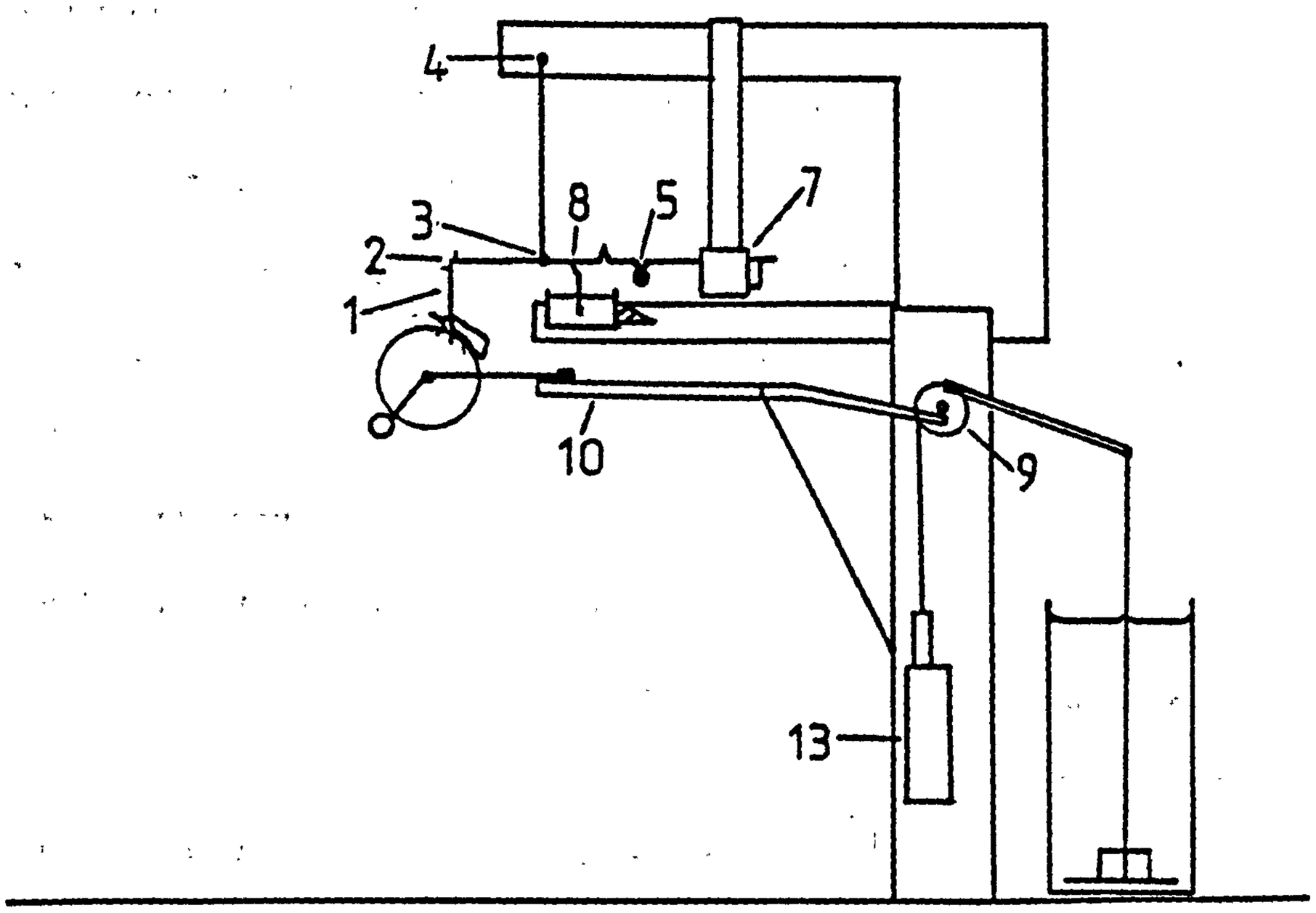
FIGURE 2.1.

A diagrammatic representation of the definitive tethered-flight apparatus showing (a) a moth at rest and (b) a moth in flight.

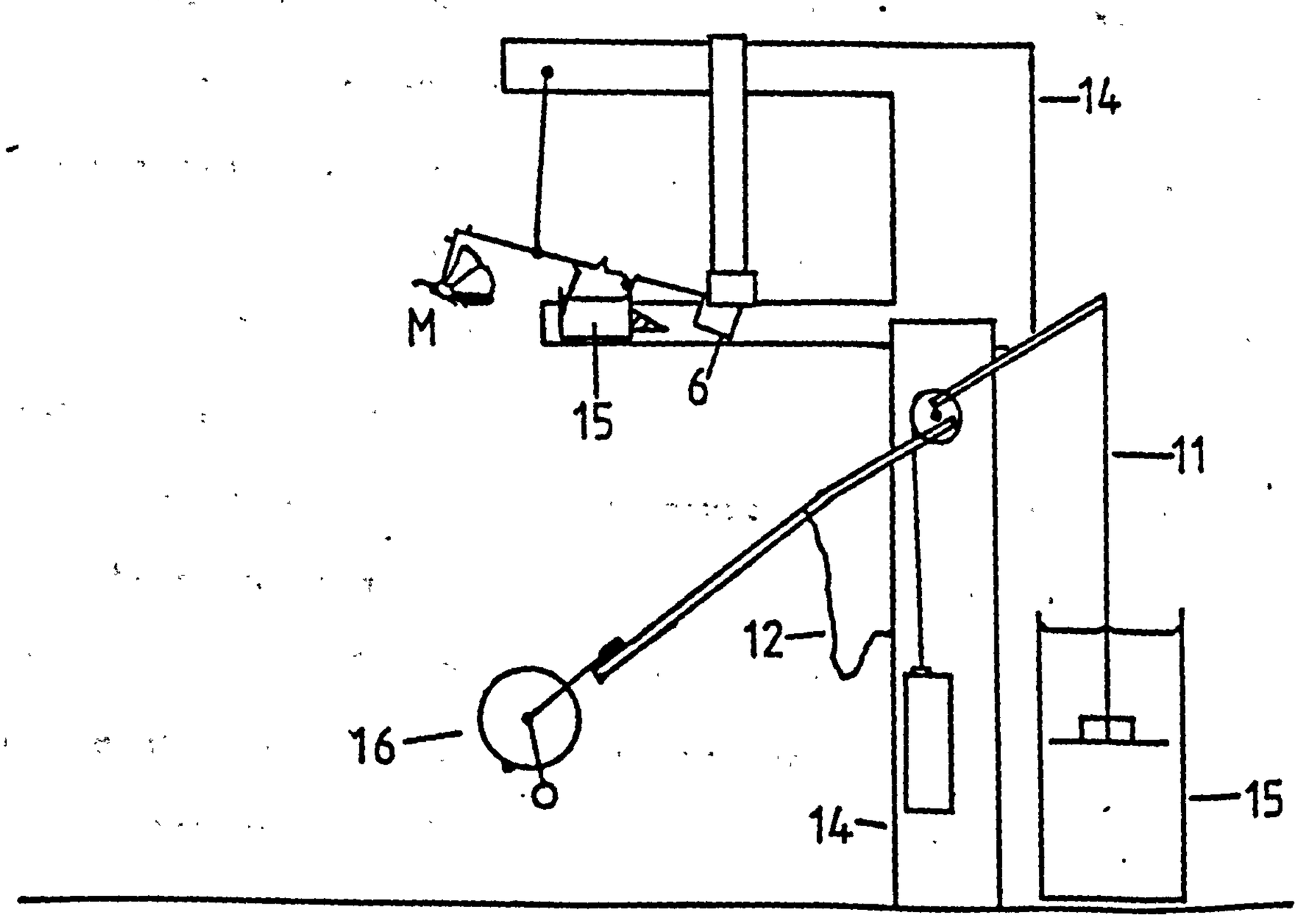
1. Flight balance.
2. Bearings allowing roll and yaw.
3. Balance pivot.
4. Balance support pivot.
5. Counterweight.
6. Aluminium foil flag.
7. IR photo-emitter detector.
8. Balance damper.
9. Landing wheel pulley.
10. Landing wheel arm.
11. Counterweighted arm.
12. Cotton restrainer.
13. Solenoid.
14. Plastic supports.
15. Dash pot containing liquid paraffin.
16. Black paper landing drum.

M. Moth.

(a)



(b)



(8). A small plasticine counterweight is attached at (5). Bearings at (2) allow the moth to roll and yaw through c. 60°.

When the moth ceases to generate enough lift and thrust to keep the flag out of the IR photo-emitter detector, the flag cuts the IR beam which de-activates the event-recorder pen and the solenoid. The paper drum is then returned by the counterweighted arm (11) which is also liquid paraffin damped, and the moth lands back on the wheel. The speed at which the wheel is returned can be varied by adjusting the counterweight, and its limit of upward travel is governed by a length of cotton (12). A total of 14 channels were available on the control boxes, of which 12 were used. Balances were spaced at c. 60cm intervals facing a plain white wall. The bench below the balances was cut away, with the result that moths flew over a drop of c. 1.2m.

2.1.2. Initial counterweighting experiments.

Moths flying on the balance do not generate enough lift to support their own weight. This is mainly due to the fact that a moth's ability to adjust its aerodynamic position on the balance, especially in the pitch plane which influences the angle of attack, is constrained by the tether. The lack of relative wind also probably reduces the lift which a moth generates. The balances therefore need to be counterweighted in order to ensure the correct operation of the apparatus. A standard procedure for this was adopted by Gatehouse and Hackett (1980). However, as moths lost weight at a rate which varied with environmental conditions and especially with the amount of flight, it was necessary to adjust the counterweight twice daily, and after every 5-6 hours of prolonged flight. To avoid the necessity of this time-consuming readjustment, some initial experiments were carried out to quantify the amount of weight moths lose with prolonged flight and with age after

emergence, with the aim of developing a simplified counterweighting system which would obviate the need for any readjustment once the moths had been attached to the balances.

(i) Insect material. The majority of moths were derived from active phase larvae reared in the early generations of the maize culture. Some moths emerging from pupae received from the armyworm cultures at I.C.I.P.E. were also used.

(ii) Environmental conditions. Experiments were carried out in the adult culture room at 25±2°C and 55% R.H., under a 13h light:11h dark photoperiod. In these experiments, the lights were switched off at 1530h G.M.T, and switched on at 0230h G.M.T.

(iii) Weight loss with flight duration. One and two-day-old starved moths were used, all of which were fitted with a mounting bracket (section 2.1.2). Both males and females were flown, and all experiments were carried out during the daylight period of the light-cycle. A simple static tethered flight system was used to record flight duration. Moths were attached to the bottom end of a vertically mounted 10cm section of stiff wire, the top of which was attached to an aluminium tubing cross-beam. Three tethers were fixed to the beam at 20cm intervals. The apparatus was positioned in front of a video camera connected to a time-lapse video recorder fitted with a timer. Flight was initiated by removing tarsal contact, and if necessary by blowing gently over the moth. The duration of flight of each moth was ascertained by playing back the video tape and recording the time each moth spent "flying" (i.e. wing-flapping). Only a few moths flew continuously; most flew for periods of 3-20m at a time, ceased beating their wings for 1-3m, and then commenced flying again. During these periods, the wings were not

closed but held in a low dihedral as if gliding. Some moths did not fly at all, and these were discounted from the analysis. Moths were weighed immediately prior to attachment, and then at 30m intervals after the start of the experiment, regardless of how long they had actually flown. The flight test for any particular moth was terminated when the individual in question had not flown for 15m, or 5h after the start of the experiment.

(iv) Weight loss with age. Newly emerged male and female moths were weighed (after ensuring that the meconium had been voided) and then placed in individual 9ml glass test-tubes which allowed virtually no movement. The tube was closed by a small section of nappy liner held in place by an elastic band. This allowed condensation to escape. Moths were weighed on the following day at 1330h and 1930h (light-cycle times), and at the same times on the subsequent day. The experiment ended after the 1330 weighing on Day 3, the longest that moths were envisaged as being attached to the balances. All moths were starved throughout the experiment.

(iv) Results. Fig.2.2 shows the results of the "weight loss with flight duration" experiment. After 5h of flight, females had lost 12.3$\pm$6.38% of their initial weight, and males had lost 18.9$\pm$2.07%. The rate of weight loss in the first hour of flight was more rapid than in subsequent hours, possibly due to faeces being voided during this period. Although significant, the differences between males and females were not considered sufficiently large to warrant separate treatment in the development of a new counterweighting system.

Fig.2.3 shows the results of the "weight loss with age" experiment (males and females pooled). By Day 2, moth weights were reduced to 78.1$\pm$3.60% of their initial weight, and this had dropped further to

FIGURE 2.2.

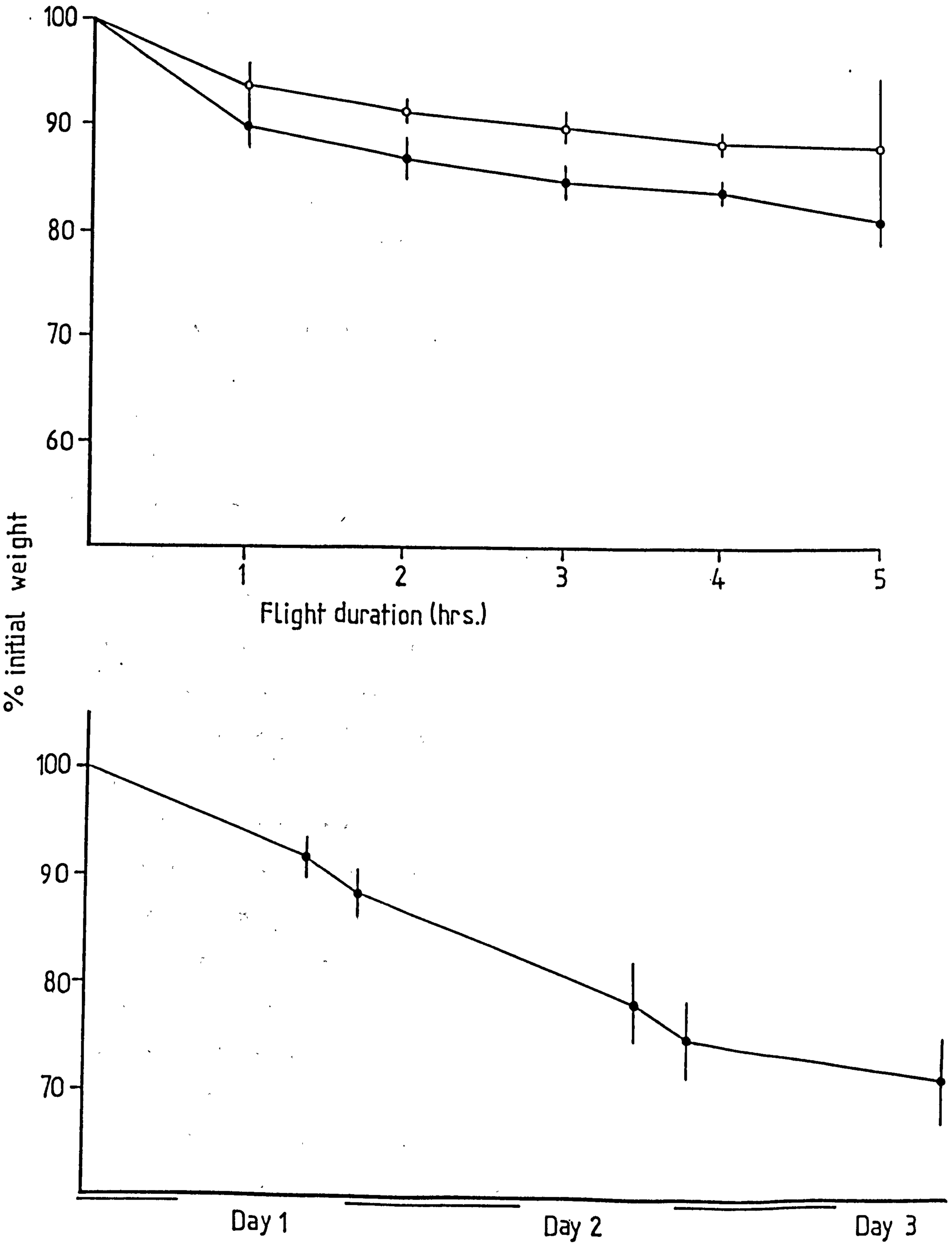
Weight loss with flight duration of moths flying on a static tether at 25±2°C and 55% R.H. (±95% confidence limits).

o Females (total n=21).

• Males (total n=20).

FIGURE 2.3.

Weight loss of starved moths with age when maintained in 9ml test-tubes at 25±2°C and 55% R.H. (±95% confidence limits). Males and females pooled (n=21).


100
90
80
70
60
% initial weight
1
2
3
4
5
Flight duration (hrs.)
100
90
80
70
Day 1
Day 2
Day 3

71.1±4.05% by Day 3. Weight loss was approximately linear throughout the experiment.

The majority of moths in definitive flight experiments were originally envisaged as being attached to the balances at emergence, and removed on Day 2. It was decided that moths should be able to fly for at least 9h and still be heavy enough to land. Extrapolating from the curve in Fig.2.2 suggested that a 9h flight would result in a c. 20% weight loss. Weight loss due to age up to Day 2 would also be c. 20%, of which c. 5% would occur during a 9h flight. This gave a maximum potential overall weight loss of c. 35% for the proposed experimental period. Allowing for variations in weight loss between individual moths, it was decided to give each moth a counterweight equivalent to 60% of its initial weight on attachment to the balance (i.e. a maximum weight loss of 40% would be possible), and not to attempt any readjustment thereafter. Although, in practice, the weight loss of individual moths on the balances was found to be variable, this system worked well, and most moths flying for >9h were able to land again, with only a small proportion being left suspended.

2.1.3. Mounting and attachment of moths to the balances.

(i) Mounting procedure. Mounting brackets were made from a c. 3mm piece of 0.40mm i.d. polythene catheter tubing, into one end of which was fitted an aluminium foil flange with a surface area of 0.8-1mm^2. To avoid the need to anaesthetize moths, brackets were attached to the pharate adult before eclosion (Gatehouse and Hackett,1980). A small rectangular piece was cut out of the pupal cuticle above the thorax, and the normally still damp scales underneath were removed. The bracket was then attached to the mesothorax using Evostick impact adhesive. Mounting

was normally carried out 6-8h prior to eclosion. The pupal cuticle at this time becomes brittle and fragile, and is easily torn by light pressure of a scalpel blade. If a drop of fluid formed at the site of the first incision, then pupae were not close enough to eclosion to ensure proper emergence. Moths mounted too early usually died, although occasionally moths successfully emerged over 24h after being fitted with a mounting bracket.

(ii) Attachment procedure. Moths were weighed immediately prior to attachment to the balances (section 2.2.4), and a small plasticine counterweight (equivalent to 60% of its initial weight, section 2.1.2) was weighed out for each moth. The counterweight was attached to the downward pointing prong of the balance (5, Fig.2.1). Once the counterweight was in position, the moths were attached to the balance by seizing the catheter tubing of the mounting bracket with a pair of forceps, and pushing the open end of the tubing over the arm of the balance to give a tight-fit connection. Each moth was disturbed into flight to ensure that the apparatus was operating correctly, and was then settled back on to the paper drum. The flight test period then commenced, and no further adjustment to the counterweight was necessary. At the end of each test period, the moths were removed and weighed. The mounting bracket was either removed and re-used, or left in place if the moth was to be flight-tested again later.

2.2. Experimental.

2.2.1. Insect material.

All moths were reared in the maize culture between April 1981 and February 1982. The insect stock was derived from pupae received from K.A.R.I., Muguga, Kenya in September 1980, and a further batch of pupae

was obtained from C.O.P.R., London, U.K. in July, 1981. This stock was also derived from the K.A.R.I. armyworm cultures.

2.2.2. Environmental conditions.

Larvae were reared under a 13h light:11h dark photoperiod at 25±2°C. Humidity was uncontrolled, but was normally in the region of 40-60% R.H. Pupae were transferred to the adult culture room 2 days after pupation (Chapter 1, section 2.2.3). Adult flight experiments were carried out in a separate room which had a cycling diurnal temperature regime varying between 25 and 30°C. The temperatue variation was caused by supplementary tungsten lighting connected to an automatic dimmer, which provided an artificial dawn and dusk of c. 45m duration. This resulted in an approximate 13h light:11h dark photoperiod, with the main lighting being switched off at 1800h G.M.T, and switched on at 0600h G.M.T. The artificial "dusk" commenced at 1745h G.M.T, and the "dawn" at 0515h G.M.T. Relative humidity varied to a certain extent with temperature, but was maintained at 60-70 % R.H.

2.2.3. Larval treatments.

All larvae were reared under normal culture conditions (Chapter 1, section 2.2.3) until they reached fourth instar to ensure proper establishment and development in the early instars. Larval treatments were initiated at fourth instar. Concurrent controls were run throughout. The larval treatments were as follows:

(i) Food deprivation. Larvae were subjected to three levels of food deprivation by removal of maize for 4, 6, and 8h daily from fourth instar to pupation. Food deprivation was carried out during the light period, and was usually initiated at the same time each day. Larvae were maintained at a density of 15-20 per rearing jar, and a control was provided by larvae fed ad lib.

(ii) Larval density. Larvae were reared at 10, 20, and 40 larvae per rearing jar from the fourth instar (these treatments were denoted T10, T20, and T40 respectively). Each jar was provided with the same measured quantity of maize leaf every 24h; thus the higher the density, the less food per larva was available. This feeding regime is described in Table 2.1. To ensure that larval densities did not drop below the required number, back up jars were initially run as reservoirs to make up any losses in the main jar. However, as this latter method was time-consuming and wasted maize leaves, the following procedure was later adopted. When larvae reached fourth instar, the number per rearing jar was reduced to just over the final rearing density (e.g. 22 larvae for the T20 treatment). Larval mortality over the next two days usually reduced the numbers of larvae in the jar to the density required. If this did not occur, the excess larvae were removed on the third day of the treatment. Further mortality did not usually occur, as larvae were now at 5th instar and the vast majority successfully moulted to 6th instar.

(iii) Feeding on water-stressed maize. In these experiments, larvae were fed on leaves from maize plants subjected to limited water-stress. When plants are deprived of water, biochemical changes occur in the leaves which could possibly be used by the larvae as indicators of the deteriorating quality of the environment (see Discussion for details). In all the experiments, larval densities and the amount of food were regulated as for the T20 treatment described in Table 2.1. All insects were reared in the same jar up to fourth instar, and then randomly split into control and test groups. The treatments are described below.

Treatment A: larvae fed on leaves from maize plants water-stressed

TABLE 2.1.

Larval feeding regime for density and water-stressed maize experiments.

Day	Amount of leaf (cm^2)	Instar	T20 treatment (no. of larvae)
1	90	4	22
2	210	4-5	22
3	550	5	20
4	550	5-6	20
5	550	6	20
6	Pupating	6-Pre.P.	20
7	Pupating	Pre.P.	20

Notes.

1. The amount of leaf was estimated from a table of areas calculated for maize leaves of different sizes. A leaf was assumed to consist of 4 right-angle triangles, and the area of a leaf was the sum of the area of the triangles.

2. The actual area of leaf fed to larvae was obtained by trial and error, and was the amount of leaf which would provide just sufficient food for 20 larvae for 24h.

3. When larvae started pupating, the amount of maize was reduced to:

$$\frac{550 \times F \times 0.75}{D}$$

where F= no. of larvae still feeding, and D=larval density.

(deprived of water) for 3-4 days. By this time, the leaves were becoming pale and fragile as a result of the loss of turgour. The cut ends of these dried leaves were held in a water-vial in the rearing jar to prevent excessive drying.

Treatment B: as for Treatment A except that the maize leaves were not held in a water-vial in the rearing jar, and hence dried out rapidly.

Treatment C: larvae fed leaves from maize plants deprived of water for 5-6 days; the leaves were not provided with water in the rearing jars.

Treatment D: control-larvae fed on leaves from maize plants watered daily; the leaves were provided with water in the rearing jars.

2.2.4. Adult treatments.

(i) Flight testing. Initially, moths were attached to the balances on the night of emergence (designated Night 0). However, it was found that few moths showed migratory activity on Night 0 (Appendix 2), and this is consistent with laboratory (Gatehouse and Hackett,1980) and field (Rose and Dewhurst,1979) observations which suggest that the first migratory flight does not usually occur until Night 1 (the first night after the night of emergence). Consequently, the majority of moths were attached to the balances between 1000h and 1200h on Day 1. Flight activity was then recorded during the remainder of Day 1 and throughout Night 1. All moths were removed from the balances between 0900h and 1000h on the following day (Day 2). Because of the pressure of space on the balances, only females were flown in sufficient numbers to warrant analysis. Moths flight-tested in all experiments described in this chapter were starved and unmated.

(ii) Weights and morphometric data. Male and female pupae were weighed on the afternoon prior to emergence, and adult females were weighed just prior to attachment to the balances (see above). Data was

collected on moths from all larval treatments. Morphometric measurements (body length, fore-wing length and width, hind-wing length and width) were made on all moths flown in the larval density experiment, and on a few flight-tested moths derived from larvae fed on water-stressed maize. Measurements were made using a vernier caliper rule accurate to 0.1mm.

2.2.5. Analysis of flight results.

The frequency distribution of all individual flights of >6m duration (Fig.2.4) shows that the majority of flights (80-90%) were <30m duration, with only c. 20% of flights in all treatment groups being >30m, and even fewer (c. 5%) being >120m. On the basis of this data, a standard criterion was formulated to define migratory flight. This was that any moth giving a total flight duration of >120m in individual flights of >30m during the test period should be classified as possessing the capacity for migratory flight. Any moth not fulfilling this criterion was classified as a non-migrant. In fact, individual flights of >120m duration (and up to >12h) were common (c. 95% of individuals) in moths categorized as migratory on this criterion. This was considered to be a more satisfactory method of assessing the migratory capacity of individual moths than using the longest single flight as the basis for analysis. There were two reasons for this. Firstly, moths migrating in the field have to reduce the amount of lift they generate over a significant period of time to be able to descend and land, as they may fly at a height of up to several hundred metres (Riley et al.,1981). Any reduction in the lift produced by moths on the balances would result in an immediate landing opportunity as the paper drum (Fig.2.1) would be returned. Thus the flight apparatus may have a tendency to terminate flight early (Gatehouse and Hackett,1980).

FIGURE 2.4.

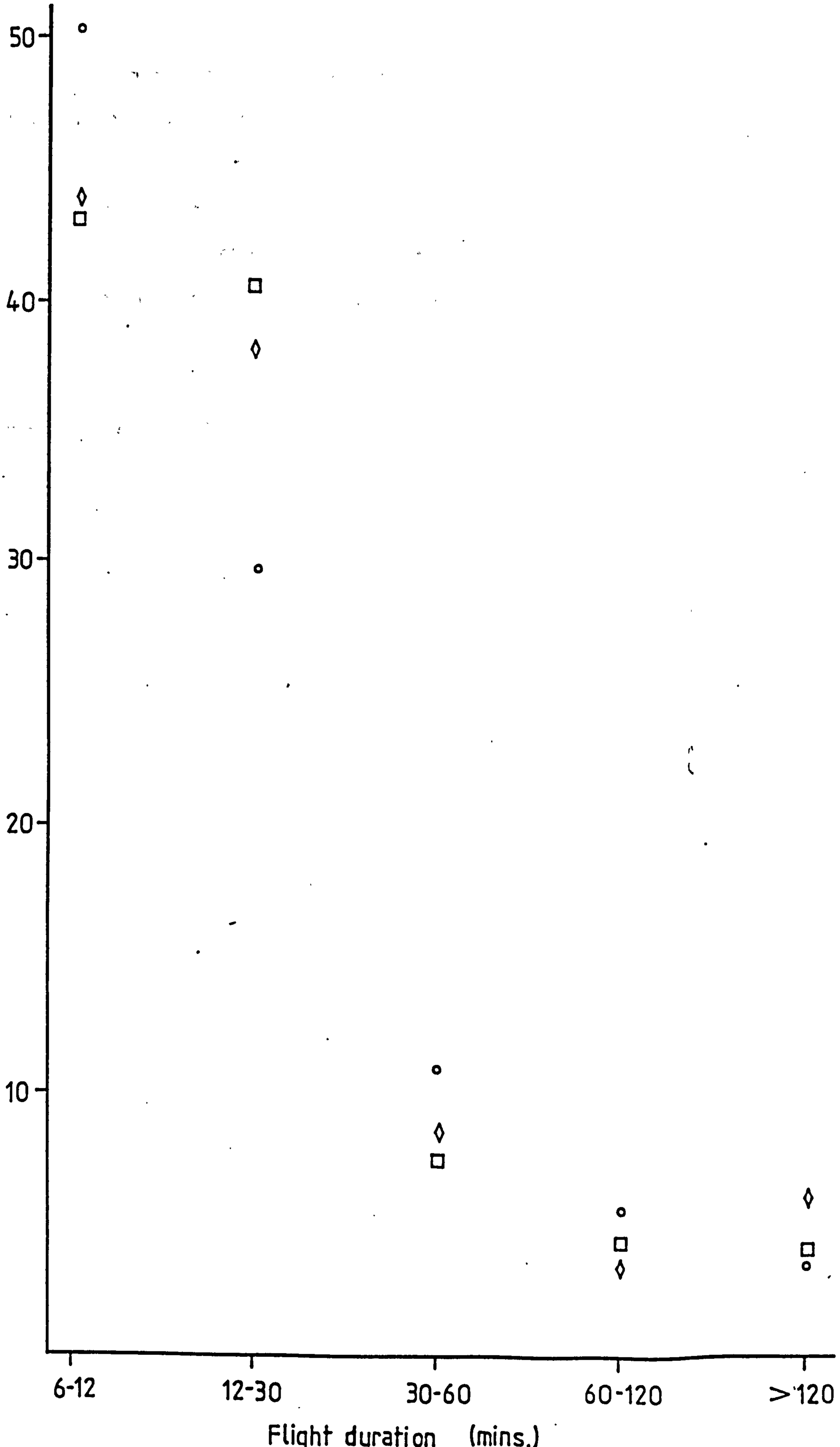
The flight duration frequency of all flights >6m given by female moths on Night 1.

□ Food deprivation group; n=108, 378 flights.

o Larval density group; n=84, 442 flights.

◊ Water-stressed maize group; n=171, 923 flights.

See section 2.2.3 for details of treatment groups.


50
40
30
20
10
% of all flights
6-12
12-30
30-60
60-120
>120
Flight duration (mins.)

Secondly, many moths made several flights of >30m during the test period, and the ability to fly for >30m could be taken as indicative of the ability to disperse, as in the field a downwind flight of 30m duration could result in significant displacement.

Significant differences between the proportions of migrant moths (as defined above) in control and test groups were tested using the chi-square statistic in a 2x2 contingency table incorporating Yates' Correction (Bailey,1959). Where expected values were <5, Fisher's exact test was used (Bailey,1959).

3. RESULTS.

3.1. The effects of larval rearing conditions on flight.

3.1.1. Time of take-off.

The distributions of the numbers of female moths taking off on flights of >1m duration during Night 1 in both control and test groups for selected larval treatments are shown in Figs 2.5-2.7. Although there was some variation in the distributions between treatments, the underlying pattern was generally the same. A marked peak of take-off at "dusk" (1800-1900h) by c. 90% of moths was followed by a rapid decline in activity. The level of activity then gradually increased to a broad peak at 2400-0200h (60-80% of moths), declined slightly, then increased to a "dawn" peak of 60-90% of moths. Generally, the level of activity was higher in the latter half of the night, and the "dawn" peak was occasionally absent or ill-defined, e.g. in the control group in Fig.2.6. This pattern is essentially similar to that described by Gatehouse and Hackett (1980) for laboratory moths tested under the natural light cycle in Kenya. However, the recovery of numbers of moths taking off following the well defined "dusk" peak was c. 1-2h later in the present study. The dawn peak reported by Gatehouse and Hackett (1980) was also much more well defined. The apparent discrepancy of 1h between the "dawn" peaks of take-off in Figs 2.5 and 2.6 (0500h) and Fig.2.7 (0600h) is an artifact of the analysis, caused by the fact the main lighting in the earlier experiments (Figs 2.5 and 2.6) was being switched on just prior to 0600h, whereas in Fig.2.7, it was switched on just after 0600h. Since the percentage of moths taking off in each hour period was recorded, an apparent 1h difference resulted in the time of peak take-off, when the actual difference was only c. 5m.

FIGURE 2.5.

The distribution of the number of female moths taking off in each hour period throughout Night 1. Data from the larval food deprivation experiments.

---o--- Control (n=23).

—x— 8h larval food deprivation treatment (n=21).

See section 2.2.3 for details of larval treatments.

FIGURE 2.6.

The distribution of the number of female moths taking off in each hour period throughout Night 1. Data from the water-stressed maize experiments.

---o--- Control (n=18).

—x— Treatment A (n=31).

See section 2.2.3 for details of larval treatments.

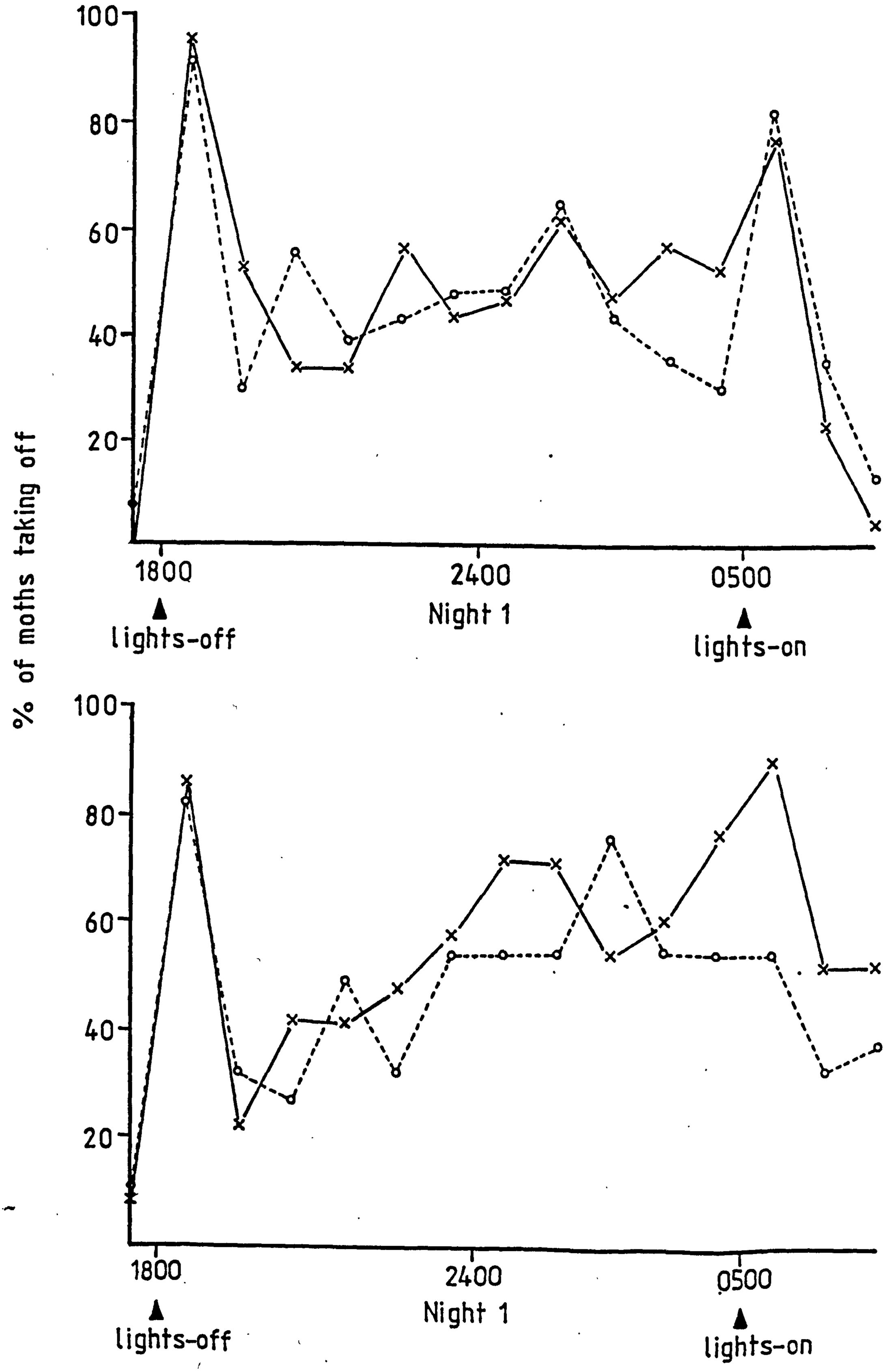

100
80
60
40
20
% of moths taking off
1800
2400
0500
lights-off
Night 1
lights-on
100
80
60
40
20
1800
2400
0500
lights-off
Night 1
lights-on

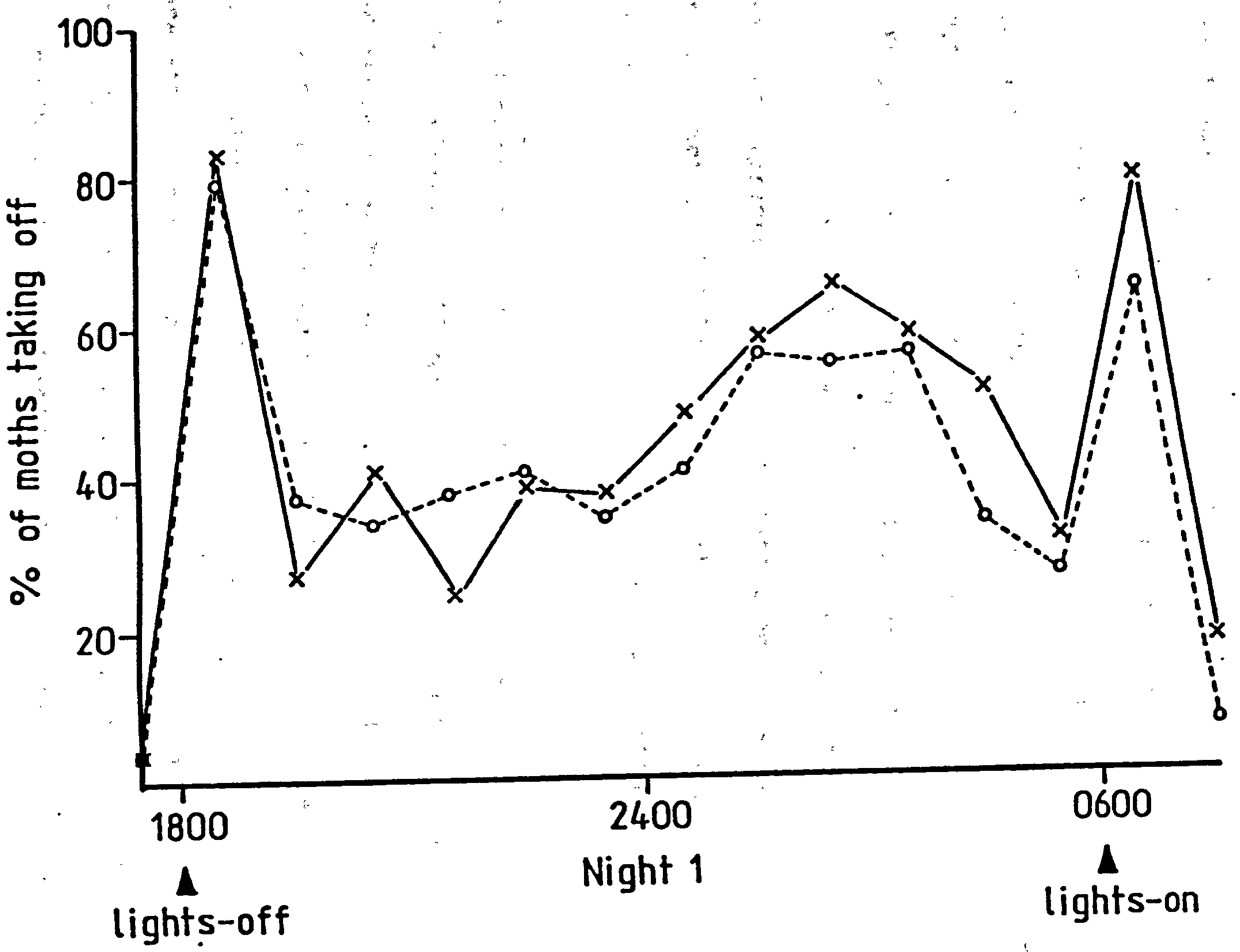
FIGURE 2.7.

The distribution of the number of female moths taking off in each hour period throughout Night 1. Data from the water-stressed maize experiments.

---o--- Control (n=29).

——x—— Treatment C (n=29).

See section 2.2.3 for details of larval treatments.


% of moths taking off
100
80
60
40
20
1800
2400
0600
Night 1
lights-off
lights-on

3.1.2. Larval conditions and flight.

(i) Food deprivation. The results from the 4h and 8h larval food deprivation experiments are shown in Figs 2.8 and 2.9 respectively. Although moths from the 6h larval food deprivation experiment were also flight-tested, it was later found that a significant genetic component was involved in flight capacity (Chapter 3). Examination of the culture records showed that the genetic match between moths in control and test groups was good in the 4h and 8h food deprivation experiments, but not sufficiently close to warrant analysis in the 6h food deprivation experiment. No significant difference in the proportion of migrant moths (section 2.2.5) was found between control and test groups for either the 4h or 8h food deprivation experiments (4h vs control, $p=0.68$; 8h vs control, $p=0.25$). In both treatments, the proportion of moths classifiable as migrants in control and test groups was only 10-20%, and the majority of moths (50-80%) gave no single flight >30m duration. Very few moths had total flight durations of 30-60m or 60-120m (<10% in both cases).

(ii) Larval density. The results of this experiment are shown in Fig.2.10. No significant difference in the proportion of migrant moths was found between the T10 and T20 groups ($p=0.155$). The proportions of migrants in these two groups were 17.25% and 25% respectively. The proportion of migrants in the T40 group (the highest density) was 55%. This represented a significant increase over the proportion of migrant moths in both the T10 and T20 groups (T10 vs T40, $p<0.01$; T20 vs T40, $p<0.05$). The genetic match between the moths in the three treatments, although not precise, was good enough to allow direct comparisons to be made. The majority of moths in the T10 and T20 groups (50-60%) gave no single flight of >30m duration. However, as a result of the high

FIGURE 2.8.

The effect of depriving larvae of food for 4h a day from fourth instar to pupation on the flight capacity of the resulting female moths.

4h larval food deprivation (n=20).

Control (n=15).

FIGURE 2.9.

The effect of depriving larvae of food for 8h a day from fourth instar to pupation on the flight capacity of the resulting female moths.

8h larval food deprivation (n=23).

Control (n=23).

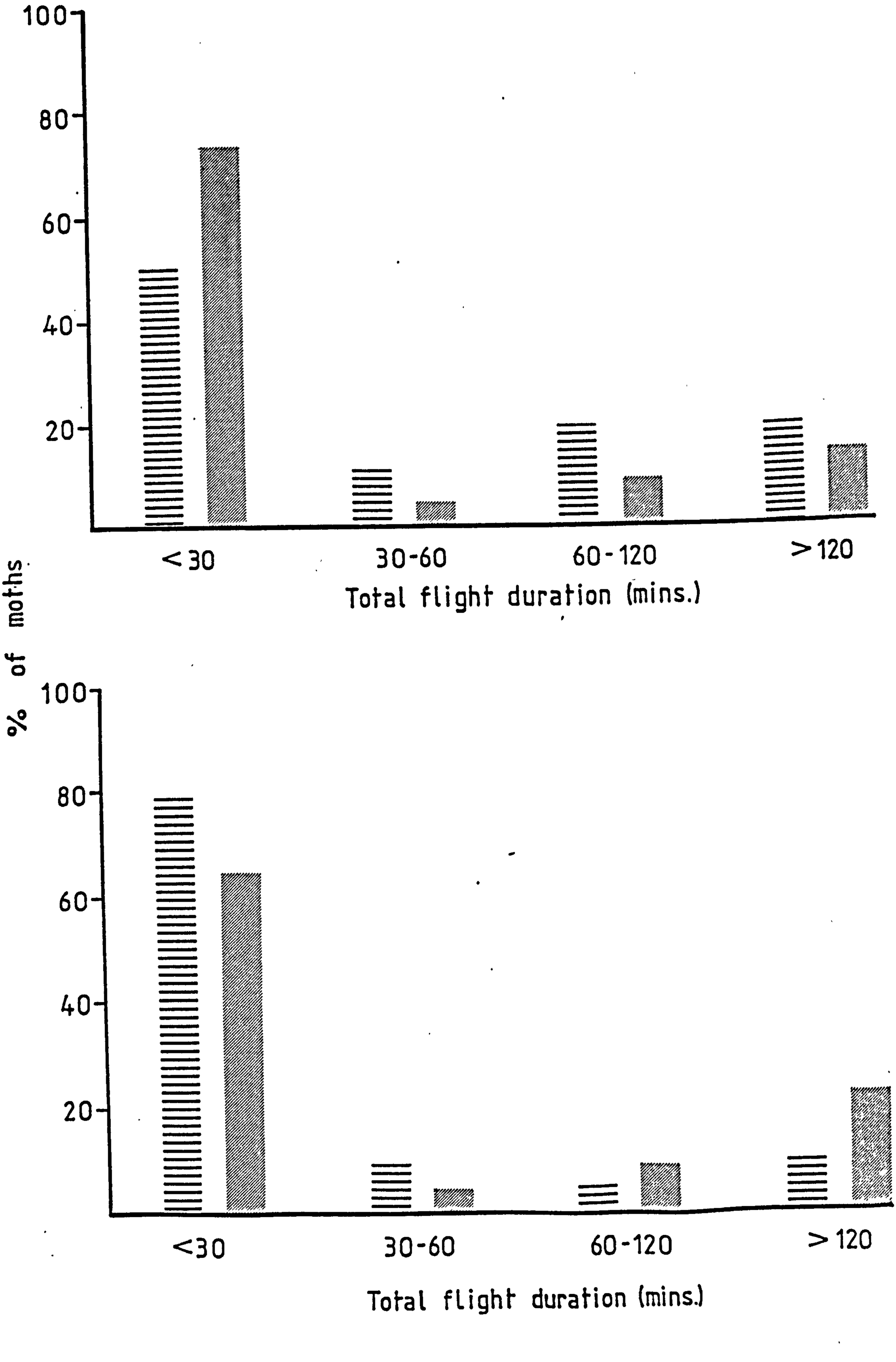

100
80
60
40
20
% of moths
<30
30-60
60-120
>120
Total flight duration (mins.)
100
80
60
40
20
<30
30-60
60-120
>120
Total flight duration (mins.)

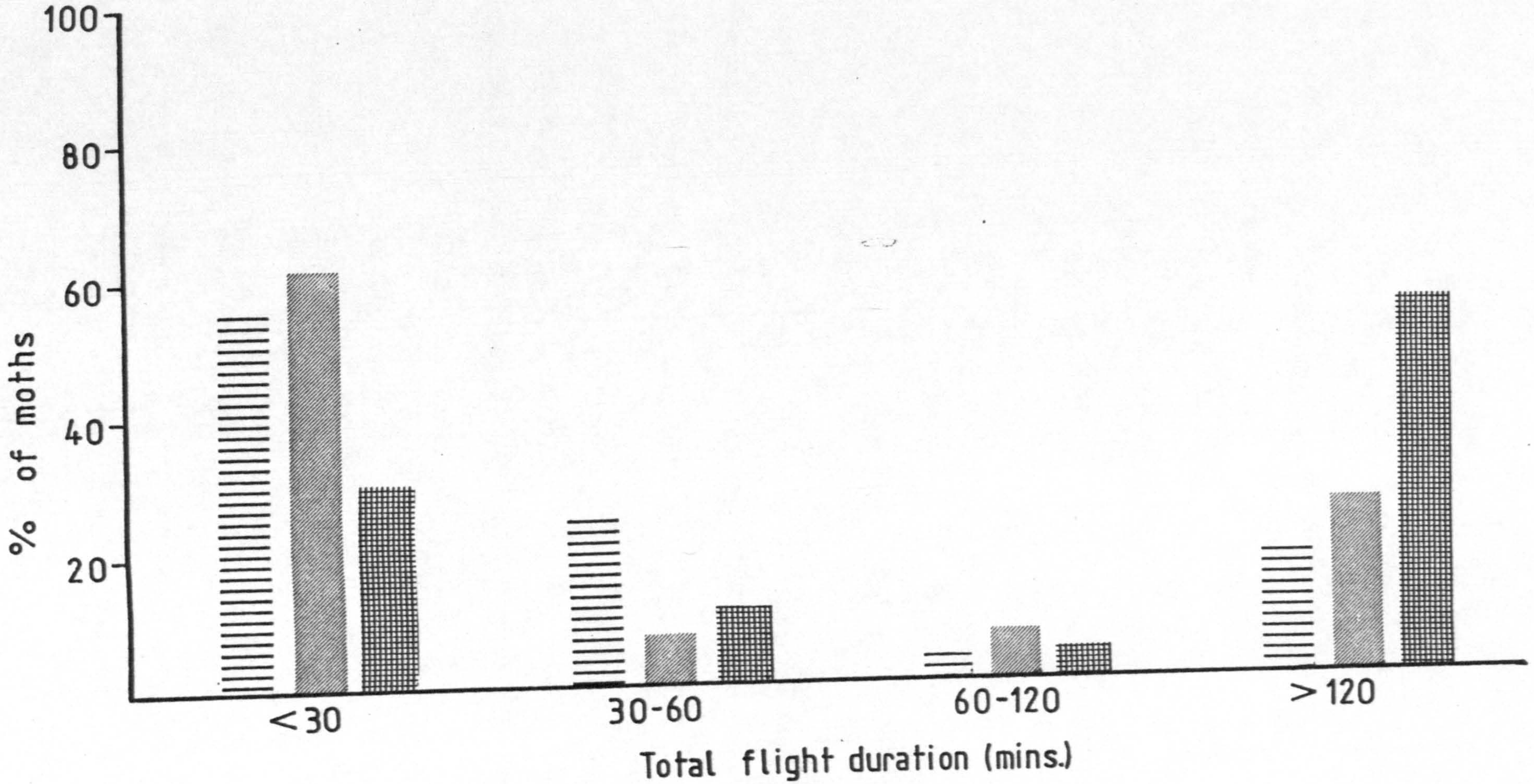
FIGURE 2.10.

The effect of rearing active phase larvae at densities of 10, 20, and 40 per 450ml rearing jar on the flight capacity of the resulting female moths.

10 larvae/jar (n=29).

20 larvae/jar (n=28).

40 larvae/jar (n=27).


100
80
60
40
20
% of moths
<30
30-60
60-120
>120
Total flight duration (mins.)

percentage of migrant moths in the T40 group, the percentage of T40 moths giving no flight of >30m duration was reduced to 30%. The percentage of moths giving total flight durations of 30-60m and 60-120m was again generally <10% in all treatments, the exception being the T10 group in the 30-60m flight duration category, where 24% of moths gave total flight of 30-60m duration.

(iii) Feeding on water-stressed maize. The results from this series of experiments are shown in Figs 2.11-2.13. No significant differences between the proportions of migrants in control and test groups were found in any of the 3 experiments (Treatment A vs control, p=1.00; Treatment B vs control, p>0.05; Treatment C vs control, p>0.05). However, the percentage of migrants in both control and test groups was relatively high (>20% in all cases), and particularly in Treatment B (Fig.2.12), where the percentage of migrants in control and test groups was 56.25% aand 40.6% respectively. The percentage of moths giving no single flight of >30m duration consequently dropped to 30-40% in Treatment B compared to 40-60% of moths in the other two experiments. The percentage of moths giving total flight durations of 30-60m and 60-120m was again low (<15%) in all treatments. Due to the fact that both control and test insects were derived from the same larval jar (section 2.2.3), the genetic match between moths in control and test groups in all these experiments was exact.

3.2. Weights and morphometric results

3.2.1. Adult and pupal weights.

(i) Larval food deprivation. Male and female mean pupal weights decreased significantly with larval food deprivation (Fig.2.14). Mean pupal weight for control insects was significantly higher than those

FIGURE 2.11.

The effect of feeding larvae on leaves from maize plants deprived of water for 3-4 days (Treatment A, section 2.2.3 iii) on the flight capacity of the resulting female moths.

Treatment A (n=31).

Control (n=18).

FIGURE 2.12.

The effect of feeding larvae on leaves from maize plants deprived of water for 3-4 days and kept dry in the rearing jars (Treatment B, section 2.2.3 iii) on the flight capacity of the resulting female moths.

Treatment B (n=32).

Control (n=32).

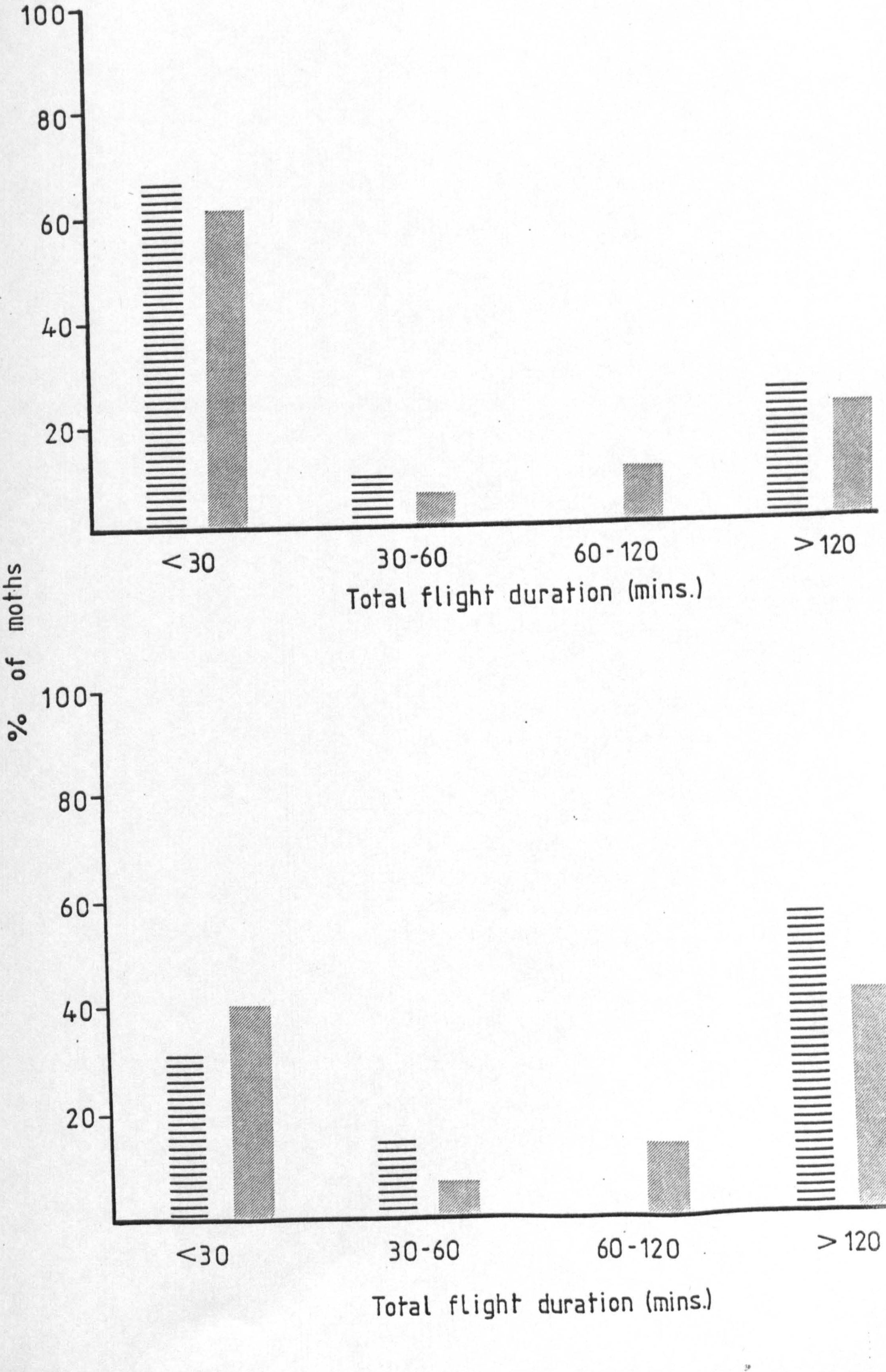
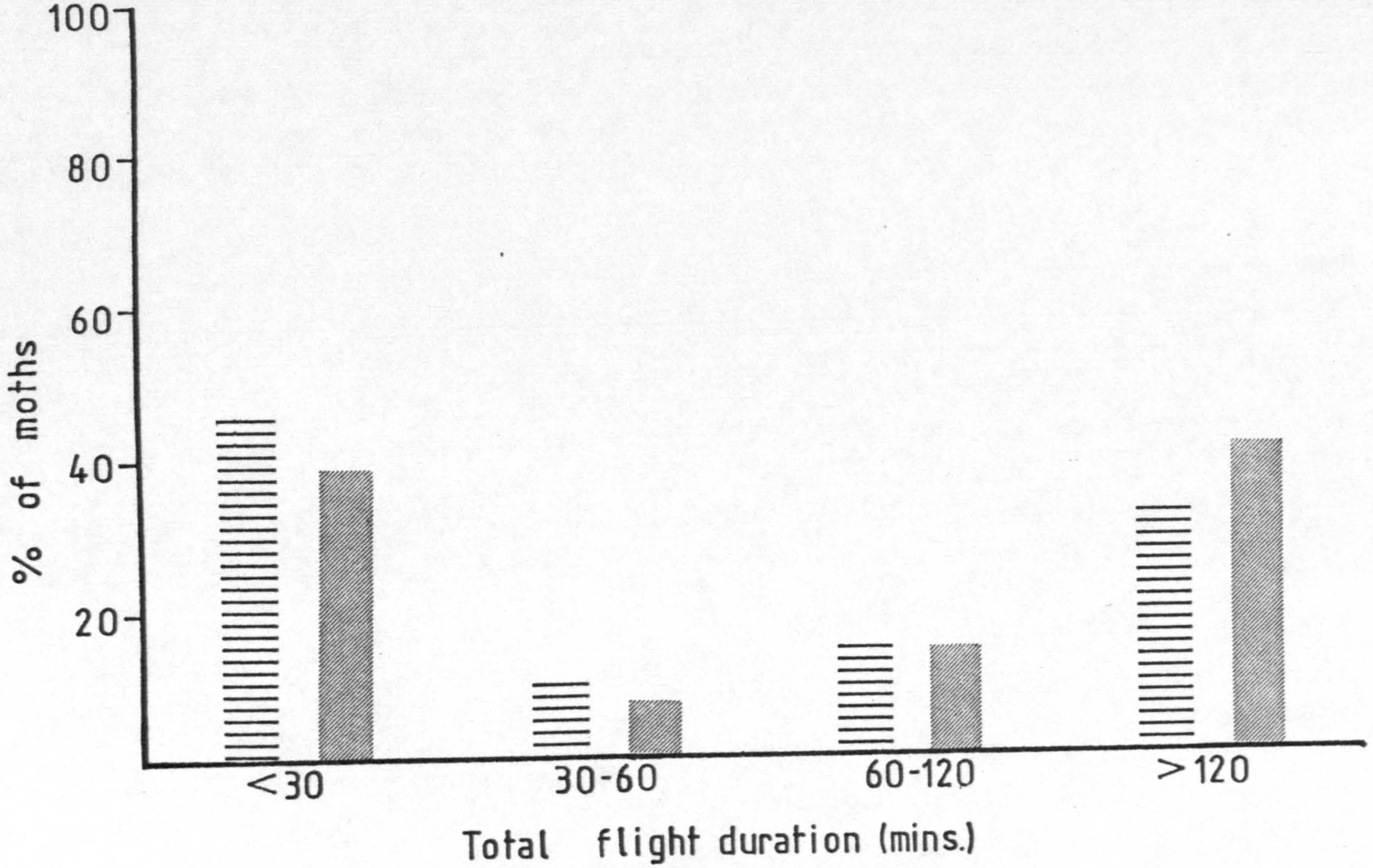

100
80
60
40
20
< 30
30-60
60-120
> 120
Total flight duration (mins.)
% of moths
100
80
60
40
20
< 30
30-60
60-120
> 120
Total flight duration (mins.)

FIGURE 2.13.

The effect of feeding larvae on leaves from maize plants deprived of water for 5-6 days and kept dry in the rearing jars (Treatment C, section 2.2.3 iii) on the flight capacity of the resulting female moths.

Treatment C (n=29).

Control (n=29).


100
80
60
40
20
% of moths
<30
30-60
60-120
>120
Total flight duration (mins.)

subjected to 4, 6, and 8h of larval food deprivation a day (males: control vs 4h $t=3.73$ $p<0.001$; control vs 6h $t=7.7$ $p<0.001$; control vs 8h $d=6.74$ $p<0,001$; females: control vs 4h $d=7.66$ $p<0.001$; control vs 6h $d=12.6$ $p<0.001$; control vs 8h $d=14.07$ $p<0.001$). There was also a significant decrease in mean weight between the 4h and 6h groups (males: $t=3.09$ $p<0.01$; females: $d=5.94$ $p<0.001$). Mean pupal weight in the 6h group for both males and females was slightly lower than that for the 8h group, but the differences were not significant.

Mean adult female weight (Fig.2.14) for control moths (mean 115.5mg) was also significantly higher than those of moths from the 4h (mean 97.3mg), 6h (mean 83.5mg), and 8h (mean 80.9mg) food deprivation groups (control vs 4h $t=4.15$ $p<0.001$; control vs 6h $t=8.1$ $p<0.001$; control vs 8h $t=9.4$ $p<0.001$). There was also a significant difference between the 4h and 6h groups ($t=3.1$ $p<0.01$), but not between the 6h and 8h groups.

(ii) Larval density. Mean adult and pupal weights were significantly reduced with increasing larval density (Fig.2.15), but the reduction was less marked than that recorded in the larval food deprivation experiment. Male pupal weight in the T10 group (mean 117.7mg) was variable, and therefore there was no significant difference between this and the T20 group (mean 121.3mg). However, there was a signifcant reduction in mean weight between the T20 and T40 (mean 108.4mg) groups ($d=2.63$ $p<0.01$). Female pupal weight followed a similar pattern; there was no significant difference between the T10 (mean 129.3mg) and T20 (mean 126.4mg) groups, but there was a significant weight reduction between the T20 and T40 (mean 105.9mg) groups ($d=4.67$ $p<0.001$). Mean male pupal weights tended to be lower than those for females, although the females were marginally lighter in the T40 group.

FIGURE 2.14.

The effect of depriving larvae of food for 4, 6, and 8h a day from fourth instar to pupation on the mean weights (±95% confidence limits) of male and female pupae and female adults (pupae weighed on the afternoon prior to emergence, adults weighed at emergence).

• Mean female pupal weight (0h, n=70; 4h, n=47; 6h, n=23; 8h, n=43).

× Mean male pupal weight (0h, n=60; 4h, n=22; 6h, n=23; 8h, n=32).

o Mean female adult weight (0h, n=37; 4h, n=15; 6h, n=20; 8h, n=43).

FIGURE 2.15.

The effect of rearing larvae at densities of 10, 20, and 40 per 450ml rearing jar from fourth instar to pupation on the mean weight (±95% confidence limits) of male and female pupae and female adults (pupae weighed on the afternoon prior to emergence, adults weighed on Day 1).

• Mean female pupal weight (10/jar n=39; 20/jar n=59; 40/jar n=40).

× Mean male pupal weight (10/jar n=21; 20/jar n=33; 40/jar n=32).

o Mean female adult weight (10/jar n=32; 20/jar n=42; 40/jar n=32).

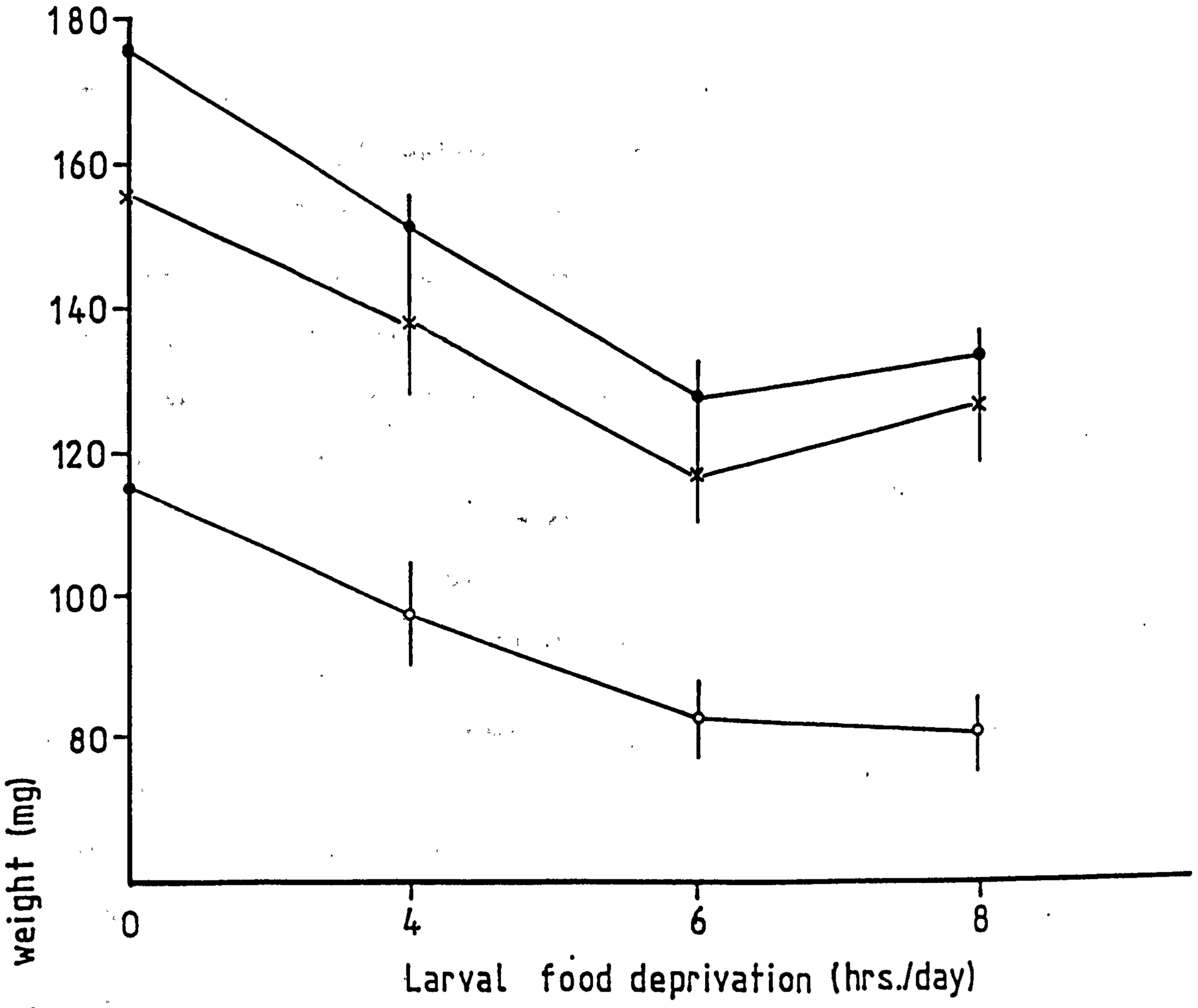

180
160
140
120
100
80
Mean weight (mg)
0
4
6
8
Larval food deprivation (hrs./day)
160
140
120
100
80
60
Mean weight (mg)
10
20
40
Larval density

TABLE 2.2.

Mean pupal and adult weights of insects from the water-stressed maize experiments (±95% confidence limits*). All weights in mg.

Treatment A: larvae fed on maize leaves from plants deprived of water for 3-4 days; the leaves were kept in water in the rearing jars.

Treatment B: as for Treatment A except leaves were not kept in water in the rearing jars.

Treatment C: Larvae fed on maize leaves from plants deprived of water for 5-6 days; the leaves were not kept in water in the rearing jars.

Control: larvae fed maize leaves from plants watered daily.

	Female		Male
Treatment	Adult	Pupal	Pupal
Control	74.0±4.17 (n=20)	119.2±6.31 (n=20)	112.5±6.51 (n=19)
Treatment A	72.2±4.46 (n=34)	125.5±6.24 (n=35)	110.0±9.61 (n=18)
Control	64.0±4.05 (n=37)	104.1±5.06 (n=40)	103.3±7.83 (n=24)
Treatment B	56.9±2.81 (n=33)	99.9±3.93 (n=37)	89.5±5.99 (n=24)
Control	76.1±4.55 (n=35)	127.4±6.61 (n=39)	114.0±5.95 (n=33)
Treatment C	77.5±4.54 (n=32)	128.1±6.58 (n=34)	115.6±5.38 (n=36)

* Where $n \leq 30$, 95% confidence limits have been corrected by a value of t equal to p=0.05 with n-1 degrees of freedom.

Adult female mean weight was also reduced with increasing larval density (Fig.2.15). There was no significant difference between the T10 (mean 81.3mg) and T20 (mean 73.3mg) groups although the T20 mean weight was lower. However there was a significant weight reduction between the T20 and T40 (mean 58.6mg) groups ($d=4.56$ $p<0.001$).

(iii) Water-stressed maize experiment. Mean male and female pupal weights and mean adult female weights from this experiment are shown in Table 2.2. Feeding larvae on water-stressed maize had little effect on mean pupal and adult weights, even in the most extreme treatment (Treatment C), where larvae were fed maize leaves from plants deprived of water for 5-6 days. In practically all cases, the differences in mean weights between control and test insects were not significant, and indeed some of the mean weights of control and test insects were very similar (e.g. Treatment C adult weight and its control). The only exceptions were in Treatment B (larvae fed maize leaves from plants deprived of water for 3-4 days), where mean adult weight was significantly reduced from the control weight of 64mg to 56.9mg ($d=2.88$ $p<0.01$). Mean male pupal weight was also significantly reduced ($t=4.10$ $p<0.001$). However, all the weights in Treatment B were markedly lower than those in Treatments A and C. Possible reasons for this are discussed below (section 4.3.1).

3.2.2. Morphometric results.

(i) Body length and wing dimensions. The mean values for all morphometric measurements taken on moths from the larval density experiment are shown in Table 2.3. There was a sigificant decrease in body length between the T10 (mean 13.6mm) and T20 (mean 13.1mm) groups ($d=2.72$ $p<0.01$), but no significant decrease between the T20 and T40 (mean 12.84mm) groups. Mean values for wing dimensions and wing area all

TABLE 2.3.

Mean morphometric measurements of moths from the larval density experiment (±95% confidence limits*). All lengths are in mm.

		Fore-wing		Hind-wing		
Treatment	Body length	Length	Width	Length	Width	Half wing area (mm^2)
T10 (n=30)	13.6±0.30	14.1±0.32	6.6±0.19	10.9±0.26	9.1±0.37	96.3±4.56
T20 (n=32)	13.1±0.22	14.0±0.30	6.8±0.24	10.6±0.21	9.1±0.36	95.6±4.10
T40 (n=26)	12.8±0.36	13.7±0.34	6.7±0.26	10.6±0.26	8.9±0.41	93.1±4.81

See section 2.2.3 for details of treatments.

*Where n≤30, 95% confidence limits have been corrected by a value of t equal to p=0.05 with n-1 d.f.

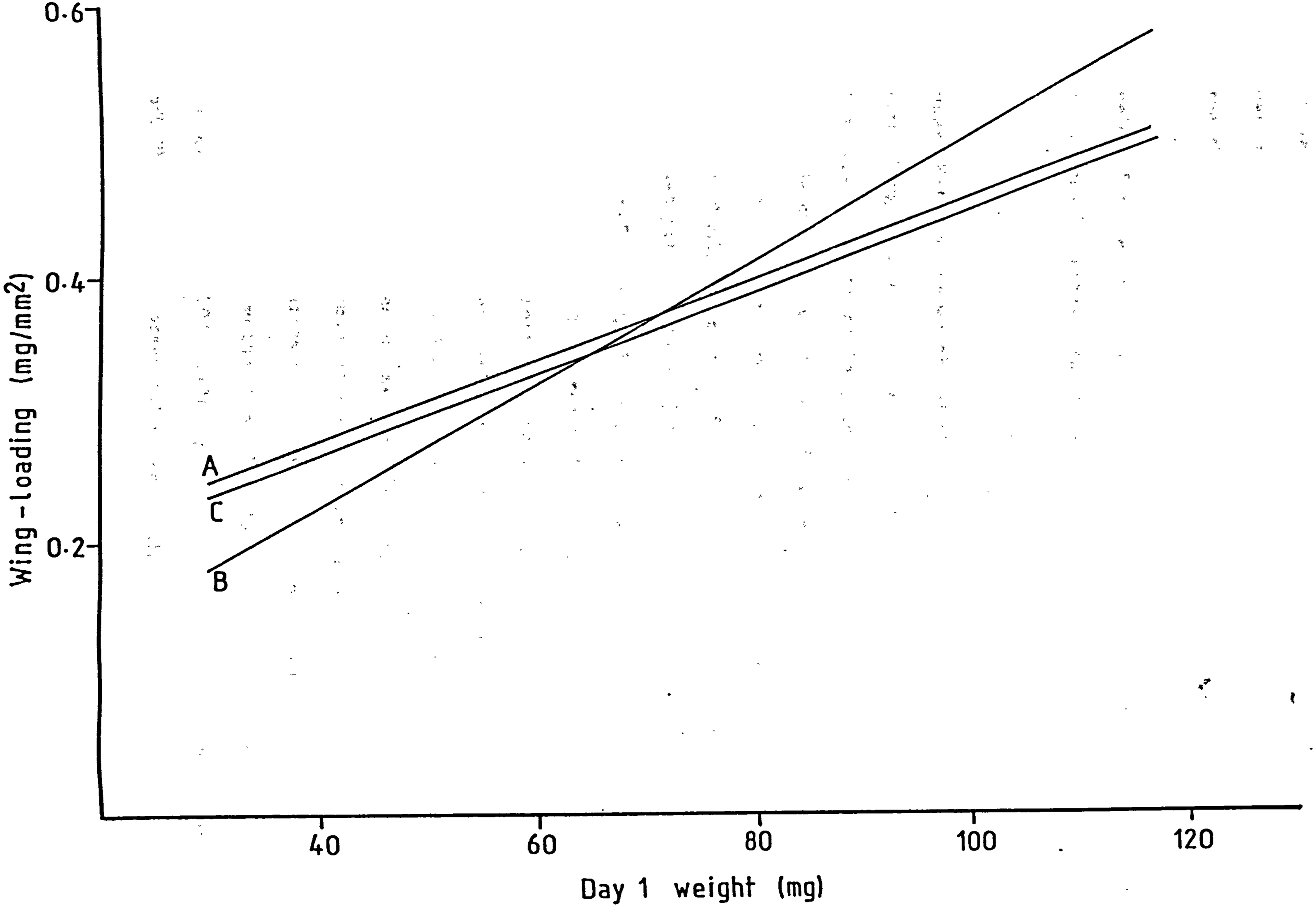
FIGURE 2.16.

The regressions of wing-loading on weight for moths derived from larvae reared at 10, 20 and 40 larvae per 450ml rearing jar.

A: 10 larvae/jar: y=0.161+0.003x (n=30).

B: 20 larvae/jar: y=0.051+0.0046x (n=32).

C: 40 larvae/jar: y=0.153+0.003x (n=26).


0·6
0·4
0·2
Wing-loading (mg/mm2)
A
C
B
40
60
80
100
120
Day 1 weight (mg)

showed a tendency to decrease with increasing density; however there were no significant differences in mean values for any dimension between the three density groups.

(ii) Wing-loading: Fig.2.16 shows the regression of wing-loading on weight for the moths from the three larval density groups. Wing-loading is defined in aeronautical terms as:

$$\frac{W}{A}$$

where W=the gross weight of the aeroplane or glider, and A=gross wing area. Wing area was calculated for the fore and hind-wings by assuming that they were triangles, and calculating the area for each on this basis. As only the right-hand wings were measured, total wing area was obtained by doubling the area of the right-hand wings. The regressions shown in Fig.2.16 were significant for all three larval density groups (T10: $b=0.003$, $t=5.76$ $p<0.001$; T20: $b=0.0046$, $t=7.43$ $p<0.001$; T40: $b=0.003$, $t=5.76$ $p<0.001$), but there were no significant differences between any of the three regression lines.

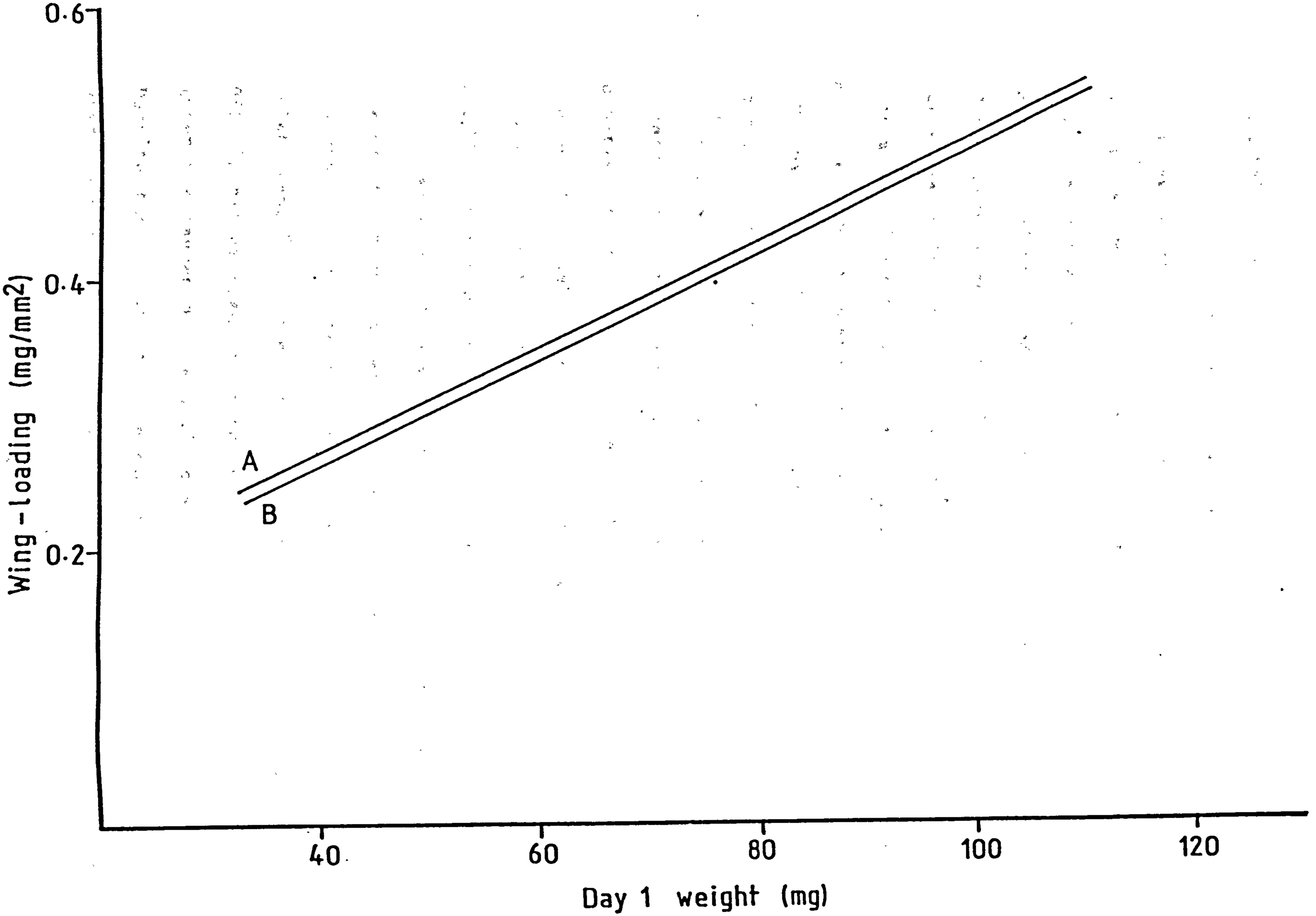
In a further effort to identify morphometric differences between different groups of moths, all data on moths from the larval density experiment were pooled with additional data on a few moths derived from the water-stressed maize experiments. Moths were then split into migrants and non-migrants on the criteria described above (section 2.2.5), and the regression of wing-loading on weight was calculated for the two groups of moths (Fig.2.17). Both regressions were significant (migrants, $b=0.004\pm0.0004$ $p<0.001$; non-migrants, $b=0.004\pm0.0003$ $p<0.001$), but were practically identical.

FIGURE 2.17.

The regressions of wing-loading on weight for female moths classified as migrants or non-migrants on Night 1 (data on moths from the larval density and water-stressed maize experiments).

A: non-migrants: y=0.11+0.004x (n=79).

B: migrants: y=0.10+0.004x (n=37).


0·6
0·4
0·2
Wing-loading (mg/mm2)
A
B
40
60
80
100
120
Day 1 weight (mg)

4. DISCUSSION.

4.1. Validity of the flight technique.

The tethered-flight apparatus generally operated satisfactorily, giving few mechanical or electrical problems despite being in continuous use for over two years. No obvious discrepancies were discernible in the flight records which could be attributed to variations between the individual balances, and the behaviour of moths on the balances (such as flight posture and daylight behaviour) was generally similar to that described by Gatehouse and Hackett (1980). The new counterweighting system also eliminated disturbance caused by counterweight readjustment once the moths had been attached to the balances.

The pattern of flight activity, as reflected by the timing of take-off by females during Night 1 (Figs 2.5-2.7), approximated to that recorded in previous laboratory experiments (Gatehouse and Hackett,1980), and in direct (Rose and Dewhurst,1979) and radar (Riley et al.,1981) observations of flight by S.exempta moths in the field in Kenya. However, there was no evidence of a pre-dawn peak of activity similar to that reported for field moths (Rose and Dewhurst,1979; Riley et al.,1981). The dawn peak of activity reported by Gatehouse and Hackett (1980) was apparently associated with sunrise; however, examination of the event-recorder tapes suggested that the "dawn" peak of activity observed in this study (when present, e.g. Fig.2.5) occured as a "disturbance" response to the main fluorescent lighting coming on (at 0600h), rather than in response to the gradual increase in light intensity caused by the artificial "dawn" which started at 0515h. Despite the slight inconsistencies between field and laboratory observations, the agreement between them was close enough to suggest

that the tethered-flight technique did accurately reflect the behaviour patterns of free-flying moths. Further, there was no evidence of a change in behaviour patterns with time, as the distributions of times of take-off shown in Figs 2.5-2.7 were recorded at intervals of c. 5 months over an eleven month period. Nocturnal patterns of activity similar to those observed for Spodoptera exempta in this study were also reported for the closely related Spodoptera exigua (Hübner) and S.frugiperda by Leppla et al. (1979).

The use of total accumulated flight (in terms of flights of >30m duration) as a criterion to define the flight capacity of individual moths (section 2.2.5) also appeared to be satisfactory. A marked discontinuity in the frequency distribution of flight duration usually occurred between the percentage of moths giving no single flight >30m and those classified as migrants, with relatively few moths falling into the 30-60m and 60-120m flight duration categories (e.g. Fig.2.12). However, it should be stressed that the experiments carried out in this study were comparative in nature. As such, flight durations recorded in the laboratory were thought to be comparable to those which would be given by the same moths under field conditions, but they are unlikely to be exactly similar, due at least in part to the probable tendency of the tethered flight apparatus to terminate flight early (Gatehouse and Hackett,1980-see section 2.2.5).

4.2. The effect of larval conditions on flight.

In general, it was found that the proportion of moths classifiable as migrants in the various experiments varied considerably, from as low as 9% in the 8h food deprivation treatment (Fig.2.9) to 56% in the control group of Treatment B in the water-stressed maize experiment (Fig.2.12).

These discrepancies were accounted for by the periodic occurrence of groups of individuals giving prolonged flight in both control and test groups. A similar inconsistent level of migratory activity in standard groups of insects was also observed by Elsey (1974), in studies of flight in the stilt bug Jalysus spinosus. Despite the variations in the proportions of migrant moths, the general trend was towards a skew distribution of flight capacity, with the majority of individuals making only short flights, and only a few being capable of flights of several hours duration (see also Fig.2.4). This pattern is characteristic of many migrant insects (Johnson,1976).

No evidence of a significant effect on the proportion of migrant female S.exempta moths was obtained when insects were subjected to restricted availability of larval food, or when larvae were fed on drying out leaves from water-stressed maize plants. Short-term water deprivation in plants is known to lead to physiological changes in the leaves, such as the cessation of leaf elongation (Acevedo et al.,1971), and a decrease in transpiration rate (Janes,1968). Biochemical changes also occur, such as an increase in the rate of hydrolysis of starch to sugar and proteins to amino acids (reviewed by Levitt,1980). The level of plant nitrogen can also increase in some tissues and decrease in others in response to reductions in moisture content (reviewed by Mattson,1980). An increase in the concentration of certain solutes in the leaves as a result of water deprivation (e.g. glucose and fructose, Jones et al.,1980), or even simply the depressed leaf water content, could have been used by larvae as a indicator of poor larval conditions and hence acted as a cue for migration. However, the results from the water-stressed maize experiments gave no evidence for the existence of any such mechanism.

Lack of larval food has been found to act as a cue for migration in other tropical insects (see Introduction); Derr (1980) found that a combination of increasing food deprivation and moisture stress caused a greater proportion of nymphs of the bug Dysdercus bimaculatus Stål to retain their wing-muscles and migrate. Dingle (1968) reported an increase in short-range non-migratory flight when adult Oncopeltus fasciatus were deprived of food. However, there was no evidence that larval food deprivation in S.exempta had a similar effect on adult moths.

The significant increase in the proportion of migratory moths which followed an increase in the density under which active phase larvae were reared from the fourth instar extends the data obtained by Gatehouse (unpublished report, see Introduction), which demonstrated a significantly higher incidence of prolonged flight in female moths from active phase as opposed to those from passive phase laboratory-reared larvae. Although the genetic match between moths in the three larval density treatments was not exact, comparisons made between sub-samples of moths with comparable genetic backgrounds from the different treatments suggested that the differences were real. Corroborative evidence for this assertion is provided by Hirata (1956) cited in Johnson (1969), who reported an increase in adult locomotor activity associated with increased larval density in the closely related cabbage armyworm moth Mamestra (cited as Barathra) brassicae (L.). However, Gatehouse (unpub. data) in experiments with field-collected moths in Kenya, found that the proportion of moths from high density infestations giving prolonged flights was small, although such individuals were present in most samples. Denno and Grissell (1979) found that the

proportion of macropters produced in response to nymphal crowding in the planthopper Prokelisia marginata (Van Duzee) also differed between populations. The situation pertaining to larval density and migration in S.exempta is thus likely to be complicated by the involvement of other mechanisms. Further, moths in this study were only flight tested on Night 1, and significant flight on other nights may also occur (Chapter 4).

Only females were flight-tested in the experiments described in this chapter. Johnson (1963,1969) stressed that males and females should be considered separately in studies on insect migration, largely because the relationship between gonad development and migratory flight seems to differ in the two sexes from species to species. However, tethered flight studies (Aidley,1974) and indirect field evidence (Brown and Swaine,1966) suggest that S.exempta males do migrate. Experiments described in Chapter 3 showed that males can give prolonged flights, and it would be logical if their migratory behaviour was controlled by the same mechanisms as those in the females. Evidence for this hypothesis is provided by Gatehouse (unpublished report-see Introduction), who demonstrated an increase in the proportion of migratory males as a result of larval crowding in a similar manner to that reported for females.

4.3. Weights and morphometrics.

4.3.1. Adult and pupal weights.

The reduction in pupal and adult weights as a result of high larval densities was expected, and was consistent with work on other Lepidoptera. Pupal and adult weight reductions as as result of larval crowding (as opposed to food deprivation) have been reported for many

species. These include Trichoplusia ni (Henneberry and Kishaba,1966), Pseudaletia separata (Haw.) (cited as Leucania separata Haw.) (Iwao,1959), Lymantria (cited as Porthetria) dispar (L.) (Leonard,1968), Plusia gamma (Long and Zaher,1958), and Spodoptera littoralis (cited as Prodenia litura F.) (Zaher and Moussa,1961). However, little data is available on the effect of larval food deprivation per se on weight. Nayar (1967), working on the mosquito Aedes taeniorhyncus, reported that lack of food combined with high larval density resulted in smaller adults, but he did not consider either factor in isolation. The larval food deprivation experiments in this study were carried out at relatively low larval densities, but the consequent weight losses were more marked than those observed in the larval density experiments. This was possibly a reflection of the relative severity of the treatments, although there must have been an element of food deprivation involved in the larval density experiments. High larval density and food deprivation need not always occur together, although in practice it may be difficult to separate their relative effects.

The lack of any major weight loss in pupae and adults as a result of feeding larvae on water-stressed maize was surprising in view of the fact that some of the maize fed to larvae was extremely dry. These results contrast with those of Scriber (1977), who found that when larvae of Hyalophora cercropia L. were fed moisture-deficient host-plants, they produced lighter pupae when compared to those fed on leaves fully supplemented with water. Iwao (1962) suggested that crowded larvae of Pseudaletia separata were more tolerant of starvation and unpalatable food than solitary larvae, and the same may be true of S.exempta. Khasimuddin (1980) found that the Juvenile Hormone analogue Farnesol, which is thought to increase in concentration in water-

stressed graminaceous plants, significantly increased the larval period when fed to larvae in an artificial diet. However, larvae fed water-stressed maize in this study developed as fast as control insects, and this suggests that larvae are indeed tolerant of poor host-plant quality, at least in terms of water content. These results again contrast with those of Scriber and Feeny (1979), who studied the growth of several species of herbivorous caterpillars, including Spodoptera eridania (Cramer), in relation to the growth form of their food-plants. They found that larvae grew faster and more efficiently on herbacious plants (which generally have a high water content), than on the foliage of shrubs and trees which have a relatively low water content. Nitrogen content, toughness, and fibre content were also thought to contribute to the differences in growth rates.

The anomalous drop in weight in the Treatment B group of insects in the water-stressed maize experiment (Table 2.2) was probably an artifact. At this time larvae were (unwittingly) being fed leaves from maize plants grown in nitrogen deficient compost (see also Chapter 1, section 4.2.), and this appears to have had a significant effect on both control and test insects. Yarro (1980) found that the size of S.exempta moths varied not only with species of larval host-plant, but also with the same species of host growing under differing environmental conditions; host-plant nutritive qualities are therefore undoubtedly important in determining size in S.exempta. Observations made on the light brown apple moth Epiphyas postvittana (Walker) by Danthanarayana (1975), suggested that size variation in adults is similarly influenced by larval food plant and the time of year of feeding, presumably through seasonal variations in nutritive quality of the host. Variations of this

type are known to occur, such as the seasonal increase in the tannin content of oak leaves, which, probably due to the fact that proteins are rendered less available (Bernays, 1983), results in a reduction in the level of infestation of the winter moth Operophtera brumata (L.) (Feeny,1968; Feeny and Bostock,1968).

Although the majority of larval treatments had little effect on the proportion of migrants in a given sample, important secondary effects on the adults, particularly the females, could nonetheless result from them. A reduction in pupal or adult weight is known to reduce fecundity in some Lepidoptera (Klomp,1966; Leonard,1968), presumably due to lower reserve levels, and the same may be true for S.exempta (Dr A.Gunn, pers.comm.). Danthanarayana et al. (1982) found that when larvae of E.postvittana were reared at even relatively low densities (2-5 larvae per leaf), fecundity and egg viability were reduced. Conversely, Henneberry and Kishaba (1966) found that egg viability in Trichoplusia ni remained unaffected by larval crowding, and Zaher and Long (1959) found that larval crowding in Plusia gamma resulted in increased fecundity and a shorter pre-oviposition period. These latter authors also found that mean egg weight was higher in "solitary" P.gamma compared to those reared at higher densities; egg size has been shown to have important effects on the wind-assisted dispersal of first instar larvae of Lymantria dispar (Capinera and Barbosa,1976), where larvae from larger eggs were found to disperse more than those from small eggs. First instar S.exempta larvae also undergo a wind-assisted dispersal phase (Whellan,1960), but the effect of variations in egg size on this is not known. In common with other Lepidoptera (Leonard;1970; Wellington,1964), marked changes in population quantity (in terms of absolute numbers) and quality (in terms of the size of individuals,

activity, rate of development etc.) are known to occur in S.exempta (Rose,1975). Physiological effects on moths of the type outlined above, caused as a result of varying larval conditions, are probably important in such variations, but are as yet largely undefined for S.exempta.

4.3.2. Morphometric data.

The aim of identifying specific groups of moths, especially those likely to be migrants, on the basis of morphometric measurements was not achieved. The significant reduction in size (in terms of body length and weight) which occurred as a result of larval crowding has also been reported for other Lepidopteran species (Hodjat,1970; Long and Zaher,1958; Zaher and Moussa,1961; Danthanarayana et al.,1982). However, in common with work by Long (1959) on Plusia gamma and Pieris brassicae L., the significant decrease in emergence weight with increasing larval density was not accompanied by a decrease in wing area in the same proportion. Thus in theory, smaller and lighter moths should on average have a proportionately greater wing area in relation to their weight and, consequently, lower wing-loadings. The significant regressions of wing-loading on weight (Figs 2.16 and 2.17) shows this to be true for S.exempta. Blais (1953) found that when larvae of the spruce budworm Choristoneura fumiferana occurred at high densities on host-trees, only old needles were left for the next generation, and consequently smaller moths with the ability to fly prior to oviposition were produced. Danthanarayana (1976), working on Epiphyas postvittana, also found that smaller moths, produced when climatic and larval host conditions indicated adverse breeding conditions, had lower wing-loadings. From these data he concluded that small moths were better adapted to dispersal. Superficially, the same argument could be applied to

S.exempta; high larval density reduces mean adult weight and also results in a higher proportion of migrants in the population. However, Fig.2.16 clearly demonstrates that for any given weight, moths reared at high larval densities did not have consistently lower wing-loadings than those reared at lower densities, and the regression of wing-loading on weight for migrant and non-migrant moths (Fig.2.17) confirmed that migratory moths did not have significantly lower wing-loadings. Wing-loading in S.exempta is therefore purely a function of weight and is not directly related to flight capacity.

Measurements made on field collected moths have indicated significant size differences between those collected from different generations in the same area (Rose,1975) and from widely separate locations (Aidley and Lubega,1979). Such gross morphometric differences between populations have been used to help identify influxes of migrant moths into an existing population (Page,1982). It has also been suggested that larger or smaller moths may be more likely to migrate (Rose,1975; Aidley and Lubega,1979), the rationale behind these suggestions being that it may be possible to identify the relative numbers of migrant and non-migrant individuals in a field collected sample of moths on the basis of size. This would be a powerful tool in any study on the population dynamics of S.exempta. However, in the light of the results discussed above, this possibility would seem to be remote. Size variations between different populations are probably largely accounted for by differences in local larval conditions (even within outbreaks), and these are likely to be complex, involving biotic and abiotic factors such as the level of larval crowding, food availability, disease level, climatic factors, the type of host-plants available and their nutritive quality. Attempting to correlate size variations in moths with one or two assumed variables

without knowledge of the conditions under which the larvae developed is therefore probably impossible.

4.4. Conclusions.

The largely negative results obtained from the experiments on the effect of larval conditions on flight suggest that factors such as larval food deprivation and feeding on poor quality though preferred hosts are unreliable as indicators of future environmental trends. However, the fact that high larval density appears to act as a cue for migration indicates that an environmental component is involved in the control of migration in this species. The possible significance of the use of larval density for this purpose is discussed later (see General Conclusion). The precise nature of the way larval density controls migration would certainly benefit from further investigation; work to ascertain a possible threshold density above which the phenotypic "switch" to migration is thrown would be of particular value.

Adult size variation as a result of differing larval densities has been shown to be irrelevant in terms of migration, but the possible physiological and behavioural side-effects on the adults of factors resulting in size variation and their consequent impact on the biology of the armyworm require further investigation (see also Chapter 4). Possible differences between moths derived from active or passive phase larvae require particular attention.

Despite the confirmation that high larval densities increase the proportion of migrants in a given sample, several important factors suggested that other mechanisms were also involved in the control of migration. These were 1) the widely varying proportions of migrant moths in both control and test groups regardless of larval treatment; 2) the

results obtained by Gatehouse (unpub.data) in Kenya which suggested that not all high density populations produced a high percentage of migrant moths; 3) the failure to find any association between environmentally-determined size and migratory capability. These observations suggested that further as yet unidentified environmental factors are involved, or that migration is under genetic control. On the available evidence, the latter possibility seemed more likely, and further work was undertaken to investigate this possible aspect of the control of migration in S.exempta.

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CHAPTER THREE.

A study of the genetic basis for flight capacity in S.exempta.

TABLE OF CONTENTS.

1. INTRODUCTION.

The existence of dispersal polymorphisms among insects, whether environmentally or genetically determined, is well established. In the past however, studies on the behavioural and physiological mechanisms controlling insect migration have largely concentrated on the role of environment in cueing the phenotypic switches between migratory and non-migratory patterns of behaviour (see Chapter 2), and it has only recently been stressed that the role of genetic differences in controlling such factors as wing length and variations in flight behaviour within any particular species is little understood, and is therefore in need of much more detailed study (Dingle,1980,1982; Harrison,1980).

Dispersal polymorphisms in insects can be categorized into three groups. The most obvious of these is polymorphism for wing length, which in some insects (e.g. aphids) has been taken to the extreme of producing winged (alate) or wingless (apterous) morphs. Many other species, principally from the Coleoptera and Hemiptera, show two or more morphs representing classes of insects with different wing lengths. The mechanisms controlling the production of morphs have been studied (mainly with respect to environmental factors) for several species, including the cowpea weevil Callosobruchus maculatus (Utida,1972; Nwanze and Horber,1975), the brown planthopper Nilaparvata lugens (Kisimoto,1956), and the cricket Gryllodes sigillatus (Walk.) (McFarlane,1964). Other morphological differences associated with flight capacity may also occcur. Rose (1972), in a study of three species of Cicadulina, identified short-bodied forms associated with fliers, and long-bodied forms associated with non-fliers.

Another common, though less obvious, dispersal polymorphism involves variation in wing-muscle development. Reduction in wing-muscles is common, and often occurs as a result of arrested development. Jackson (1933) found that unfavourable environmental conditions during development were probably responsible for the production of flightless Sitona hispidula F. weevils found to have undeveloped wing-muscles, and Scudder and Meredith (1972) showed that the Corixid bugs Cenocorixa bifida (Hung) and C.expleta (Uhler) arrested flight muscle development in response to low autumn temperatures. It should also be noted that wing-muscle histolysis also commonly occurs immediately prior to the onset of reproduction; this has been demonstrated for several species, including the Carabid beetle Agonum retractum Leconte (Carter,1976), and for three species of Dysdercus (Dingle and Arora,1973). However, this type of physiological change is not a dispersal polymorphism.

A third major group of dispersal polymorphisms are those based on variations in flight behaviour; these usually occur only in species in which all individuals are capable of flight. Variations in flight behaviour are difficult to observe in the field, but tethered flight studies have revealed that within species, insects can often be classified as "fliers" or "non-fliers". Examples in the literature are numerous, and include the black-fly Simulium ornatum Meigen (Cooter,1982), the milkweed beetle Tetraopes tetraopthalmus (Forster) (Davis,1980), the milkweed bug Oncopeltus fasciatus (Dingle,1965), the weevil Hylobius abietis L. (Solbreck,1980), the European pine shoot moth Rhyacionia buoliana (Green and Pointing,1962), the Reduvid bug Triatoma infestans (Klug) (Ward and Baker,1982), and the brown planthopper Nilaparvata lugens (Baker et al.,1980). Occasionally an intermediate category of "brief flyers", observed in Cicadulina spp. (Rose,1972) and

Aphis fabae (Shaw,1970), can also be distinguished.

Despite the apparently clear delineation between the above types of polymorphisms there is, in fact, often some overlap. Gregarious and solitary locusts exhibit both morphological and behavioural differences (Kennedy,1956), and Shaw (1970) found that alate Aphis fabae could be categorized as "fliers", "brief fliers", and "non-fliers". The whole subject of insect dispersal polymorphisms and their adaptive significance is comprehensively reviewed by Harrison (1980).

The extent to which genetic (as opposed to environmental) factors operate in the maintenance of dispersal polymorphisms has not received wide attention. Most of the evidence so far accumulated on the subject is derived from the study of easily identifiable morphometric characters such as wing length (Carter,1976; Caswell,1960; Harrison,1979; McFarlane,1964; Rose,1972). Such studies have usually suggested that wing length is under the complex control of genes at several loci (often modulated by environmental factors), although Jackson (1928) found good evidence to suggest that the brachypterous (short-winged) condition in the weevil Sitona hispidula is under the control of a single dominant Mendelian gene. Vepsäläinen (1978) developed a model to explain seasonal variations in wing length in Gerris spp., which included both environmental control and a genetic switch at one locus; however the evidence for this is unconvincing (Harrison,1980).

Work on the genetics of variation in flight behaviour in species which exhibit no associated wing polymorphism has been limited by the fact that flight capacity (usually measured in terms of flight duration) is a continuously varying character, and therefore insects showing variation in flight behaviour do not readily fall into discrete, easily

identifiable groups. However, the available evidence suggests that the effect of genetic variation on flight behaviour may be high. Dingle (1968) found that selecting for long flight in Oncopeltus fasciatus raised the percentage of bugs making long flights from 20-30% to 60% in one generation, and concluded that the behavioural polymorphism of migrants and non-migrants is largely genetic. Rankin (1978) was able to delay the age of maximum flight activity in O.fasciatus by several days by selecting for late flying bugs. Estimates of heritability (h^2), which measure the ratio of additive genetic variance to total phenotypic variance and thus indicate the potential effectiveness of natural selection (Falconer,1981), have also occasionally been made on flight duration. Caldwell and Hegmann (1969) found that the heritability value for flight duration in the bug Lygaeus kalmii Stål was consistent with those found for other behavioural traits, and concluded that the substantial amount of variation in flight performance was mostly attributable to genetic differences between individuals.

To date, there is no published evidence to suggest that migration in Spodoptera exempta is under genetic control. Aidley and Lubega (1979) suggested that variation in size between different populations could be due to genetic differences, but pointed out that den Boer (1978), in a study on the allele frequencies of six isoenzymes from samples of moths from widely dispersed locations in Africa, had found no evidence of genetic heterogeneity between populations and concluded that wide gene mixing occurred, presumably as a result of extensive migration. However, the results obtained from the experiments on the effect of larval experience on flight, and observations associated with them (Chapter 2), provided sufficient grounds for suspecting a possible genetic involvement in the control of migration.

This chapter describes an investigation into the possible role of genetic differences in producing variation in flight behaviour in S.exempta.

2. MATERIALS AND METHODS.

2.1. Insect material.

All moths were reared in the maize culture between December 1981 and January 1983. The majority were derived from the main stock culture (denoted CK), but a "wild" strain (denoted KF) of insects derived from pupae collected in the field at Kakamega in Kenya on the 26th March 1982 were also used for one series of selection experiments (see below, section 2.4.2).

2.2. Environmental conditions.

These were as described in Chapter 2, section 2.2.2.

2.3. Flight testing.

All moths in the experiments described below were flight tested using the flight balances described in Chapter 2. They were attached to the balances between 1000h and 1200h on Day 1 (i.e. the day following the night of emergence), and removed between 0900h and 1000h the following day (Day 2). All moths were starved and unmated.

2.4. Experimental.

2.4.1. Re-examination of data from the larval experience experiments.

The initial approach taken was to re-examine the data accumulated during the series of experiments carried out to assess the effect of environmental conditions acting on the larvae on flight (Chapter 2). The aim of this was to try and establish a link between known groups of migrant moths (migrant moths being defined by the standard criterion described in Chapter 2, section 2.2.5) by tracing their ancestry back through the culture records over a period covering approximately three generations, with the aim of determining whether one group of migrants

were the progeny of another. Detailed culture records were kept throughout most of this study, and it was therefore possible to follow individual "lines" of moths within the culture with some accuracy. A "group of migrants" was defined as occurring when >50% of moths tested over a three day period gave total flight durations >120m on Night 1.

2.4.2. Selection experiments.

The aim of these experiments was to ascertain if populations of moths responded to directional selection for both increased and decreased flight potential (in terms of the percentage of migrants in the population) on Night 1. The two lines were denoted "Migrant" and "Non-migrant" respectively, where "Migrant" moths were defined as all those giving total flight >120m in flights of >30m duration on Night 1, and "Non-migrant" moths as all those failing to meet this criterion.

The initial hypothesis was that flight capacity was under the control of a single gene, although in the light of subsequent results this view was modified. Thus the initial basis for selection was that all moths classifiable as migrants, whether derived from the "Migrant" or "Non-migrant" lines, were suitable for breeding in the "Migrant" line. Conversely, all non-migrant moths, whatever their origin, were considered suitable for breeding in the "Non-Migrant" line. In practice, however, the tendency was to select moths from the extremes of the migrant and non-migrant categories of flight duration, with the result that either very long-flying moths or those giving virtually no flight were selected for further breeding. However, the practice of cross-breeding between lines was abandoned in later generations (see section 4.1). Both males and females were flown in all experiments. The origins of the various breeding lines are described below.

(i) CK lines: the "Migrant" line (denoted CKA) was initiated in December 1981 when a particularly large group of migrant moths appeared in the water-stressed maize experiments (see Chapter 2). Twelve female moths were selected from this group and were mated with twelve males (not flight-tested) which had the same genetic background. Three males and three females were maintained in each of four rearing jars. The progeny from these moths were then bred through unselected for one generation. The F_2 moths (males and females) were flight-tested and migrant individuals selected for further breeding.

The "Non-migrant" line (denoted CKN) was initiated by flying a sample of moths from the stock (denoted CK) culture at the same time as the F_2 moths from the "Migrant" line were being flight-tested, and selecting non-migrant moths for further breeding. Some non-migrant moths from the "Migrant" line were also used for breeding in the initial "Non-migrant" generations.

(ii) KF lines: selection was not initiated in this line until the fifth generation after the arrival of the original breeding moths from Kenya. In the fifth generation, a large sample of moths (25 males and 15 females) was flown; migrant and non-migrant moths were selected and divided into separate breeding lines. The overall level of flight activity in the initial generation was low, and therefore obtaining sufficient stock to start a "Migrant" line was difficult.

In order to minimise any possible variation due to environmental effects, larvae in all lines were reared under the standard T20 larval regime described in Table 2.1. The exceptions were the third generation of the CKN ("Non-migrant") line, and the fifth generation of the CKA ("Migrant") line, which were reared concurrently under the T10 larval regime (Chapter 2, section 2.2.3). The reason for this is discussed

below (section 4.1). Breeding was carried out on a single pair basis, and eggs from as many pairs as possible were used to initiate the subsequent generation. Adults were provided with a 20% v/v honey solution once they had been placed in the breeding jars. Progeny from more than one set of parents were not mixed during the larval or pupal stages, enabling the flight performance of all the offspring from any particular breeding pair to be followed.

2.4.3. Heritability estimates.

The proportion of the phenotypic expression of flight capacity which could be attributed to additive genetic variance (a polygenic basis for flight capacity is assumed) was investigated by calculating heritability estimates for flight duration. Heritability (h^2) is defined as the ratio of additive genetic variance (V_a) to total phenotypic variance (V_p) (Faloner,1981). Thus:

$$h^2=V_a/V_p.$$

Total phenotypic variance can be split into 2 components, genetic variance (V_g) and environmental variance (V_e). Thus:

$$V_p=V_g+V_e.$$

V_g can also be further subdivided into dominant genetic variance (V_d), additive genetic variance (V_a), and epistatic genetic variance (V_i). Thus, overall, V_p can be partitioned as:

$$V_p=V_d+V_a+V_i+V_e.$$

In effect, heritability estimates measure the degree of resemblance between parents and offspring, and are obtained by calculating the regression of mean offspring value on mid-parent value of the trait being investigated. The heritability value is equal to the slope of the line. If only one parent or one sex of offspring is used in the

calculation, the value of the slope obtained must be doubled in order to obtain the correct value of the heritability. A high heritability indicates a high degree of genetic heterogeneity in the population, whereas a low heritability indicates that the phenotypic variation in the character under study is not due to genetic variation between individuals. Heritability estimates also predict the level to which the character in question will respond to directional selection (Falconer,1981).

For the purposes of this study, three approaches were taken to estimate the heritability of flight duration in Spodoptera exempta.

(i) Overall heritability: the regression of mean offspring flight duration on mid-parent flight duration (males and females pooled).

(ii) Heritability due to paternal influences: the regressions of sons on fathers and daughters on fathers (in terms of mean flight duration of offspring, and actual flight duration of fathers).

(iii) Heritability due to maternal influences: the regressions of sons on mothers and daughters on mothers (in terms of mean flight duration of offspring, and actual flight duration of mothers).

To increase the precision of the estimates, only data on moths derived from the CK "Migrant" and "Non-migrant" lines reared under the T20 larval regime (Table 2.1) were used (i.e. all insects were reared under identical environmental conditions), and preference was given to data on moths from generations already subjected to some selection (Falconer,1981). Mean offspring and mid-parent values were calculated on the basis of total accumulated flight consisting of flights of >30m duration. Moths giving no flights >30m were given a standard flight duration value of 30m.

2.4.4. Statistical considerations in the calculation of heritability.

The phenotypic variances of male and female offspring (in terms of flight duration) were found to be comparable (F=1.34; 94 males, 133 females) and therefore the pooling of the sexes in the calculation of overall heritability was valid (Falconer,1981 p.153).

Ideally, mean offspring values used to calculate the regressions should be corrected for family size, which in this study varied between 2 and 22. This can be achieved by calculating the regression using every value of y (offspring) for each value of x (mid-parent) (Sokal and Rohlf,1969 p.430), or by applying a correction factor involving the calculation of the intraclass correlation (t) (Falconer,1981 p.167). However, both these methods involve an analysis of variance of the raw offspring data. Detailed observation of this data (see also Fig.3.10, section 3.3) showed that the distribution of flight duration within families was either highly skewed towards long or short flight, or approximately U-shaped (several long and short flights, but few of intermediate duration). Thus an analysis of variance (which assumes normally distributed data) cannot be carried out. No suitable transformation could be found to render the data approximately normal, and therefore for the purposes of this study, uncorrected offspring means were used in the calculation of the regressions, and it was assumed that these mean values were normally distributed. The use of median offspring value instead of the mean was considered, on the grounds that this gave a more accurate representation of the data, and tended to smooth out anomalous mean values due to small families. However, it was decided that the use of median values to calculate regression coefficients was untenable statistically, and would not allow comparisons to be made between regressions.

3. RESULTS.

3.1. The genetic association between groups of migrant moths.

Detailed examination of the culture records covering the period between October and December 1981 strongly suggested that a genetic association was occurring between groups of migrant moths. Fig.3.1 is a schematic representation of the occurrence of such groups of migrants, and the tendency for these to occur at generation intervals regardless of larval treatment is clearly demonstrated. Since all other environmental conditions remained constant during this period, the clear inference was that one group of migrants tended to produce progeny which themselves had a high proportion of migrant individuals. The particularly large group which occurred between the 30th of November and the 14th of December was the result of an overlap of two migrant groups, and it was this group of moths which provided the original insect material for the "Migrant" (CKA) line selection experiments.

3.2. Selection experiments.

The indications that flight capacity on Night 1 may have a genetic basis were confirmed by the results of the selection experiments.

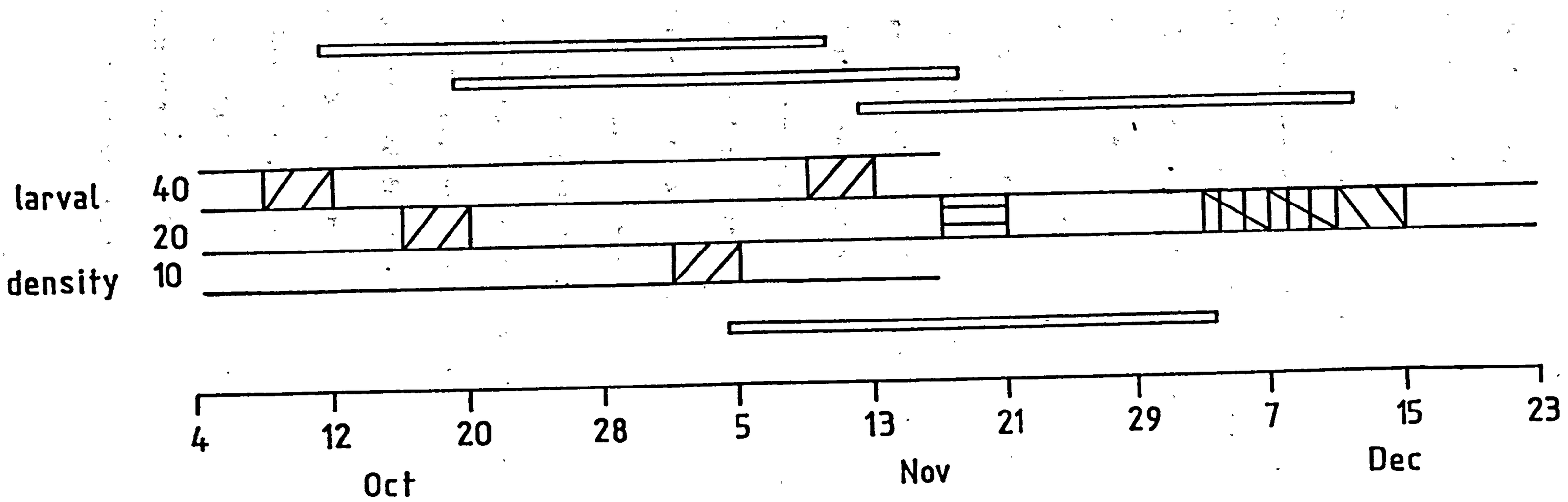
3.2.1. CK lines.

The results of this experiment are shown in Figs 3.2 and 3.3; these show the effects of selection for migrant and non-migrant individuals on females and males respectively.

(i) Females. Selection for migrant moths in the parental and F_2 generations of the experiment resulted in an increase in the proportion of migrants from 38% in the original stock culture to 75% by the 3rd generation (Fig.3.2). The percentage of migrants in the stock culture at this time (36%) was significantly lower than that in the "Migrant" line

FIGURE 3.1.

The incidence of high levels of flight activity (>50% of moths tested over 3 day periods giving total flight >120m on Night 1) in female moths. Data from a series of experiments designed to examine the effect of different environmental conditions acting on the larvae on flight capacity. Different hatching indicates different larval treatments; narrow bars indicate one generation intervals.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(chi-square=4.63, $p<0.05$). Further selection over the 4th and 5th generations in the "Migrant" line produced 68% and 80% of migrants respectively. The latter percentage was also significantly higher than that observed in the unselected line (47.5%) at the same time (chi-square=4.53, $p<0.05$). Selection was relaxed between the 5th and 6th generations, and the proportion of migrants fell to 53%, a figure comparable to that for moths from the unselected line in the 5th generation (47.5%). Migrant moths were again selected in the 6th generation, but not in the 7th. Despite this, the proportion of migrants in the 8th generation was found to be 80%. Further selection over the following 4 generations maintained this high level of migrant moths at 70-80%. Selection was again relaxed over the 14th generation, and the proportion of migrants fell to 42% by the 15th generation.

The selection for non-migrant moths was unsuccessful in the initial generations of the "Non-migrant" line, and the proportion of migrants in this line remained similar to that in the unselected line for the first 2 generations. There was no significant difference between the "Migrant" and "Non-migrant" lines at this time. In the 3rd generation, the proportion of migrants in the "Non-migrant" line rose sharply to 78%, which was very close to the value recorded in the concurrent "Migrant" generation (possible reasons for this are discussed in section 4.1). Selection was then relaxed for three generations, and the proportion of migrants fell to 42% by the 6th generation. This was significantly lower than the proportion of migrants in the concurrent "Migrant" generation (chi-square=5.66, $p<0.05$). Selection in the 6th generation reduced the proportion of migrants to 22.7% in one generation, again significantly lower than the level of activity in the concurrent "Migrant" generation

FIGURE 3.2.

The results of selecting moths for long flight (total flight >120m on Night 1) and short flight (total flight <120m on Night 1) on the proportion of migrant female moths in the population when flight-tested on Night 1.

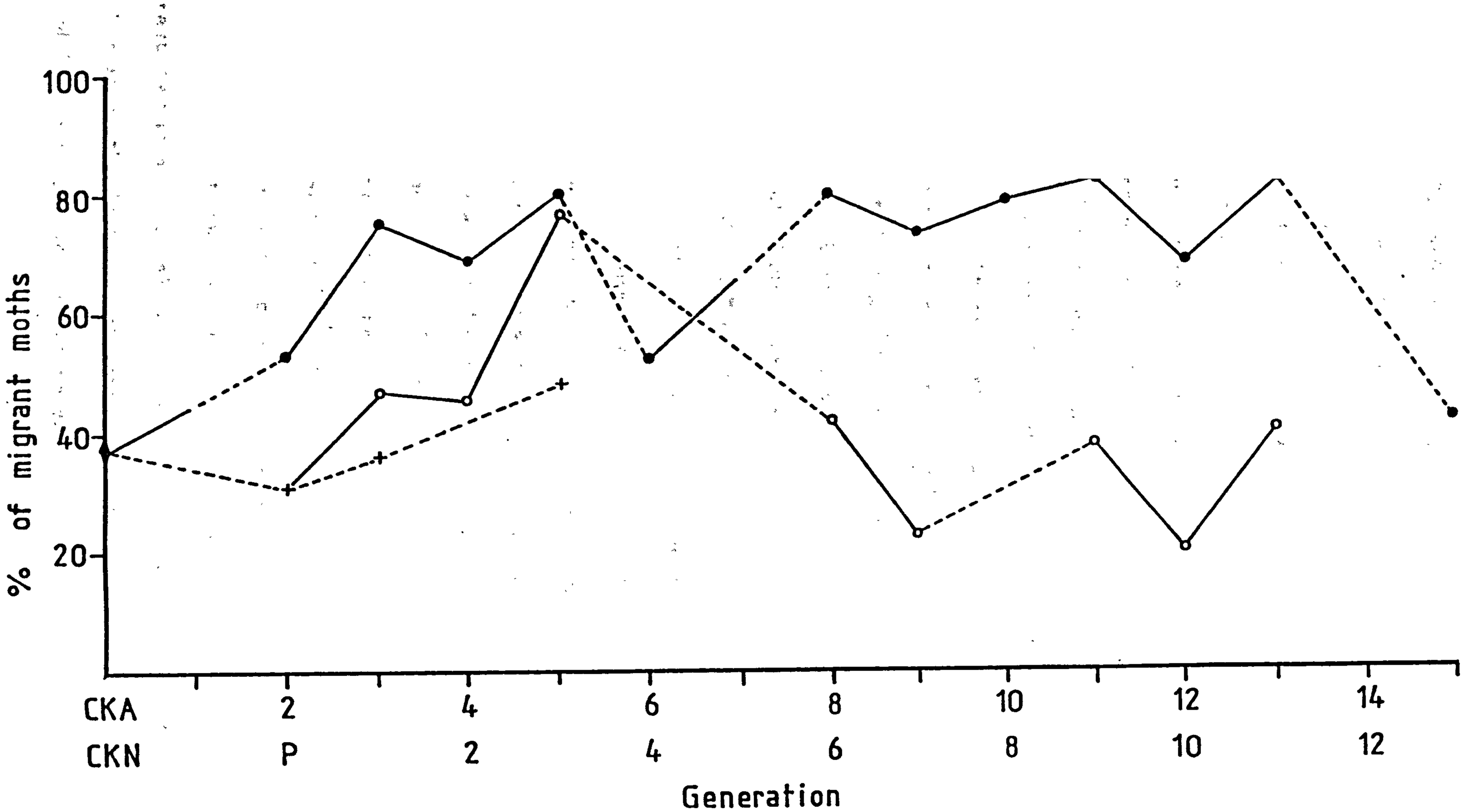
• CKA "Migrant" line (long flight selection).

o CKN "Non-migrant" line (short flight selection).

+ Unselected line.

P Parental generation.

Dotted lines indicate that moths were not flight-tested in the preceeding generation, and therefore no selection occurred.


100
80
60
40
20
% of migrant moths
CKA
CKN
2
P
4
2
6
4
8
6
10
8
12
10
14
12
Generation

(chi-square=8.7, $p<0.01$). Selection was then relaxed in the 8th generation, and the proportion of migrants rose to 38.4% in the 9th generation; this was still significantly lower than the percentage of migrants in the "Migrant" line (Fisher's exact test, $p=0.018$). Further selection resulted in levels of activity of 20% and 43% for the 10th and 11th generations respectively, both significantly lower than the appropriate percentage in the "Migrant" line (Gen.10: chi-square=5.59, $p<0.05$; Gen.11: Fisher's exact test, $p=0.011$).

(ii) Males. The effect of selection on males was broadly similar to that on females, although there were some differences (Fig.3.3). Selection for both migrants and non-migrants had little effect on the proportions of male migrants in both the "Migrant" and "Non-migrant" lines in the initial generations. Indeed, the proportion of migrants in the "Non-migrant" line rose steadily over the first 3 generations, reaching a peak of 73.7% in the 3rd generation, in a similar manner to the females in the "Migrant" line. The percentage of male migrants in the "Migrant" line remained little changed in the first 2 generations (30-40%), then rose to 56% in the 3rd generation. There was no significant difference between the "Migrant" and "Non-migrant" lines in these early generations. The relaxation in selection between the 5th and 6th generations in the "Migrant" line resulted in a drop in the proportion of male migrants to 35%, in a similar manner to that observed for the females. Selection for migrants in the 6th generation but not in the 7th resulted in an increase in the proportion of migrants to 60% by the 8th generation. Continued selection over the next three generations resulted in an initial fall in the percentage of migrants to 43% but thereafter the level of activity rose rapidly to 80% and 69% in the 11th and 12th generations respectively.

FIGURE 3.3.

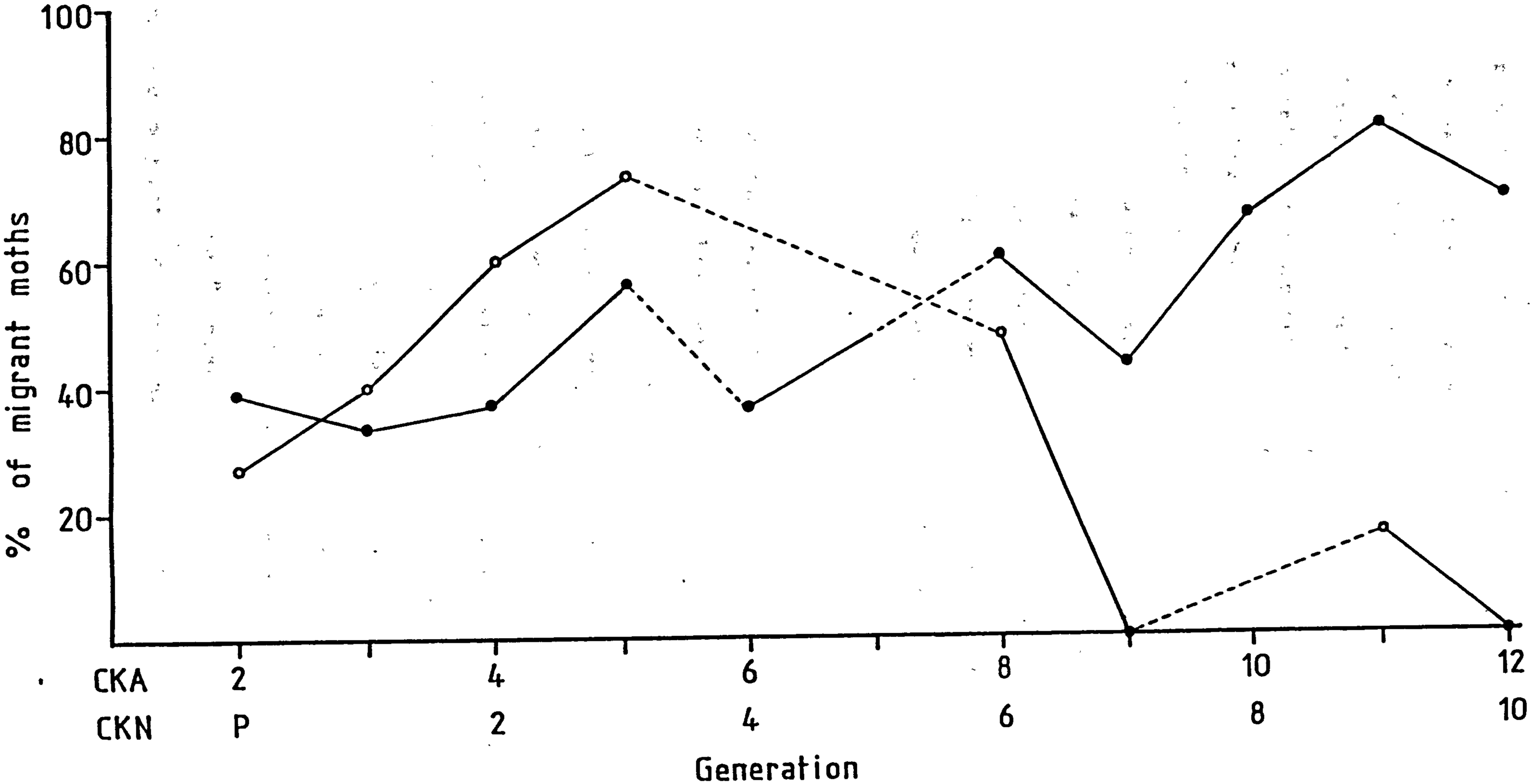
The results of selecting moths for long flight (total flight >120m on Night 1) and short flight (total flight <120m on Night 1) on the proportion of male migrants in the population when flight-tested on Night 1.

● CKA "Migrant" line (long flight selection).

○ CKN "Non-migrant" line (short flight selection).

P Parental generation.

Dotted lines indicate that no moths were flight-tested in the preceeding generation, and therefore no selection occurred.


100
80
60
40
20
% of migrant moths
CKA 2 4 6 8 10 12
CKN P 2 4 6 8 10
Generation

No selection for three generations in the "Non-migrant" line resulted in a fall in the level of activity to 48% by the 6th generation. This was not significantly different from the level of activity in the "Migrant" line. However, further selection in the 6th generation immediately reduced the proportion of migrants to 0% in one generation, significantly lower than the concurrent level of activity in the "Migrant" line (Fisher's exact test, $p=0.016$). Selection was then relaxed in the 8th generation, and the percentage of migrants rose to 17% in the 9th generation, still significantly lower than that in the "Migrant" line (chi-square=8.49, $p<0.01$). Further selection again reduced the proportion of migrants to 0% in one generation, which was again significantly lower than the "Migrant" line (Fisher's exact test, $p=0.0023$).

3.2.2. KF lines.

The results of this experiment are given in Fig.3.4. Selection was only carried out over three generations, and only females were flown in sufficient numbers to warrant detailed analysis. The initial level of activity was low (27%), and therefore selection for migrants was not particularly good. However, by the 3rd generation, the percentage of migrants had risen to 73%, although only 8 female moths were flight-tested in this generation. In contrast, the level of activity in the "Non-migrant" line fell to 0% in 2 generations, recovering slightly to 7% in the 3rd generation. The only significant difference between the "Migrant" and "Non-migrant" lines in this experiment was in the final generation, where the "Migrant" line had a significantly higher proportion of migrants (Fisher's exact test, $p=0.0086$).

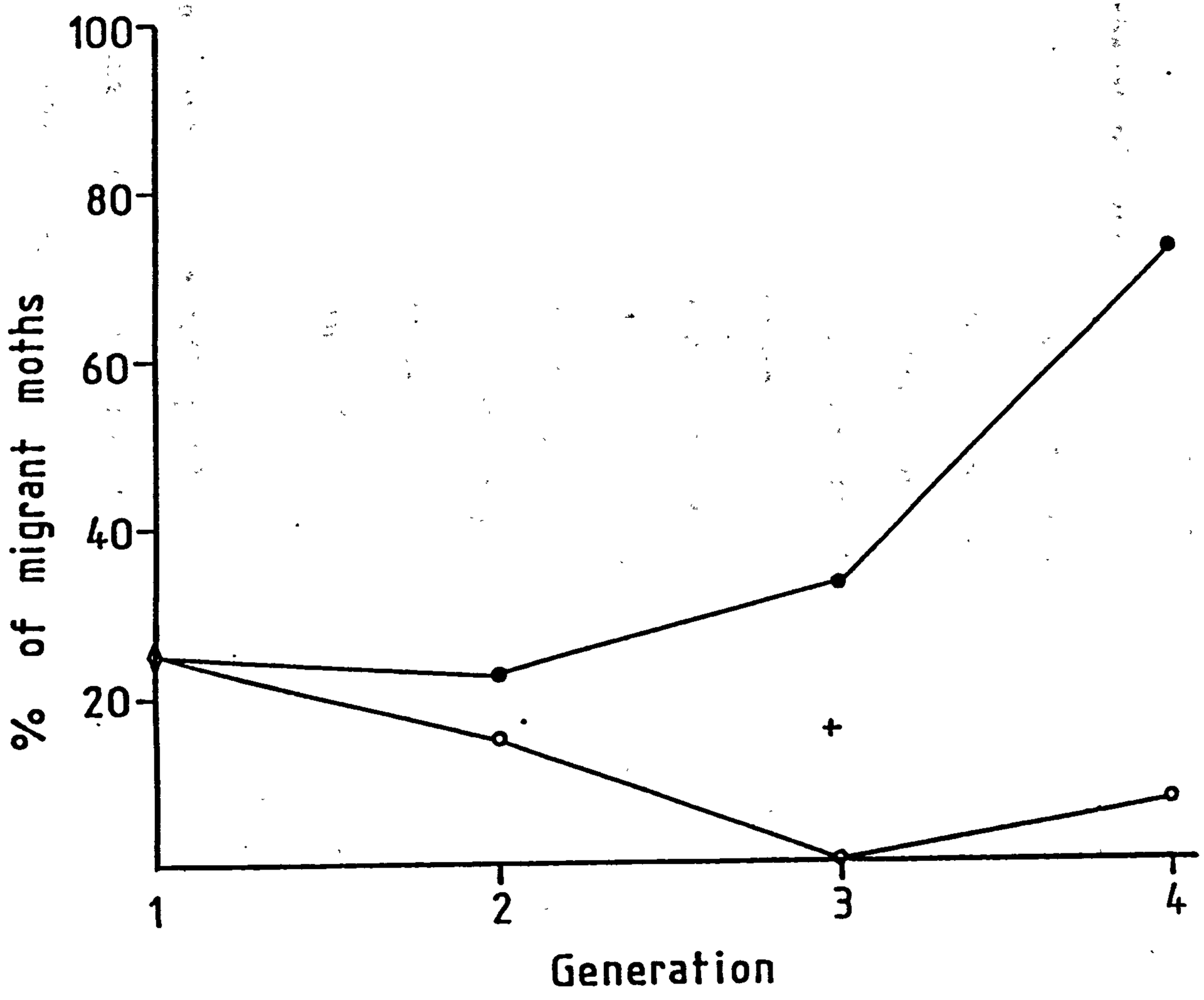
FIGURE 3.4.

The results of selecting moths for long flight (total flight >120m on Night 1) and short flight (total flight <120m on Night 1) in the Kakamega (KF) line on the proportion of female migrants in the population when flight-tested on Night 1.

• "Migrant" line (long flight selection).

o "Non-migrant" line (short flight selection).

+ Unselected stock.


100
80
60
40
20
% of migrant moths
1
2
3
4
Generation

3.3. Heritability estimates.

3.3.1. Overall heritability.

The overall regression of mean offspring flight duration on mid-parent flight duration is shown in Fig.3.5. There is a clear tendency for long flying parents to produce long flying offspring and vice versa. The regression is significant ($t=5.58$, $p<0.001$), and the slope of the line (b) is 0.40, showing that in this particular population of moths, 40% of total phenotypic variance in terms of flight duration can be attributed to additive genetic variance (Table 3.1).

3.3.2. Paternal and maternal effects.

The regressions of daughters on fathers and daughters on mothers are shown in Figs 3.6 and 3.7, and the heritability values are given in Table 3.1. Both regressions were significant (daughters on fathers, $t=3.44$, $p<0.002$; daughters on mothers, $t=2.40$, $p<0.05$). Heritability due to paternal effects (71.2%) was found to be significantly higher than that due to maternal effects (50.8%; comparison of regression coefficients $t=7.92$, $p<0.001$). Similarly, the regressions of sons on fathers ($t=4.23$, $p<0.001$) and sons on mothers ($t=2.34$, $p<0.05$) were both significant (Figs 3.8 and 3.9), and again heritability due to paternal effects (see Table 3.1) was significantly higher than that due to maternal effects ($t=12.87$, $p<0.001$).

3.3.3. Flight duration frequencies.

The flight duration frequencies of a representative sample of individual families of moths used in the calculation of heritability estimates are shown in Fig.3.10. It is apparent that these frequency distributions vary considerably, with some families containing both migrant and non-migrant individuals resulting in an approximately U-shaped distribution, while other families consist entirely of either

FIGURE 3.5.

Overall heritability of flight duration (males and females pooled): the regression of mean offspring flight duration on mid-parent flight duration; y=131.441+0.400x (n=28).

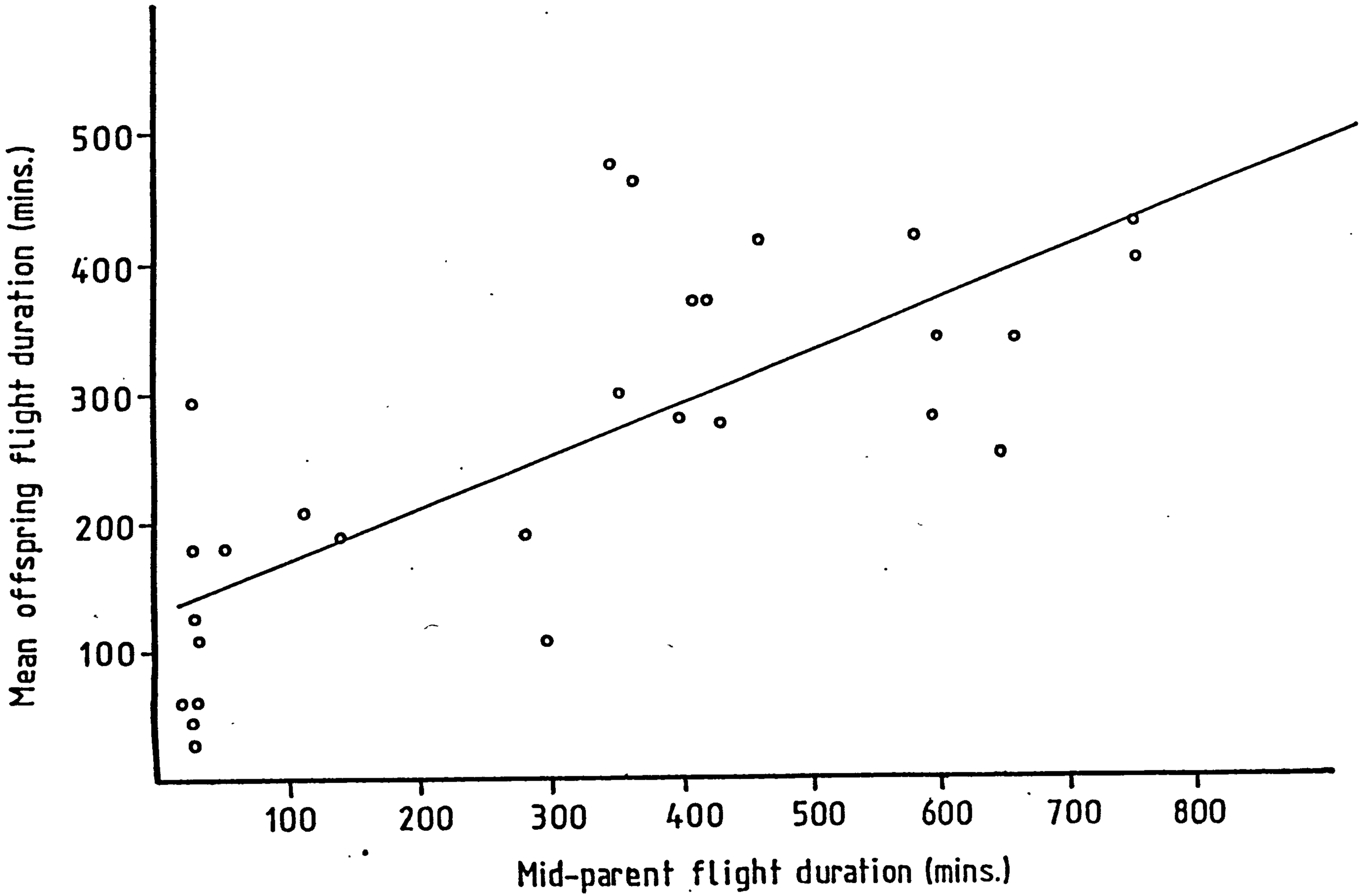

Mean offspring flight duration (mins.)
500
400
300
200
100
100
200
300
400
500
600
700
800
Mid-parent flight duration (mins.)

FIGURE 3.6.

The heritability of flight duration: the regression of mean female offspring flight duration on male parent flight duration; y=189.70+0.356x (n=30).

FIGURE 3.7.

The heritability of flight duration: the regression of mean female offspring flight duration on female parent flight duration; y=207.56+0.254x (n=30).

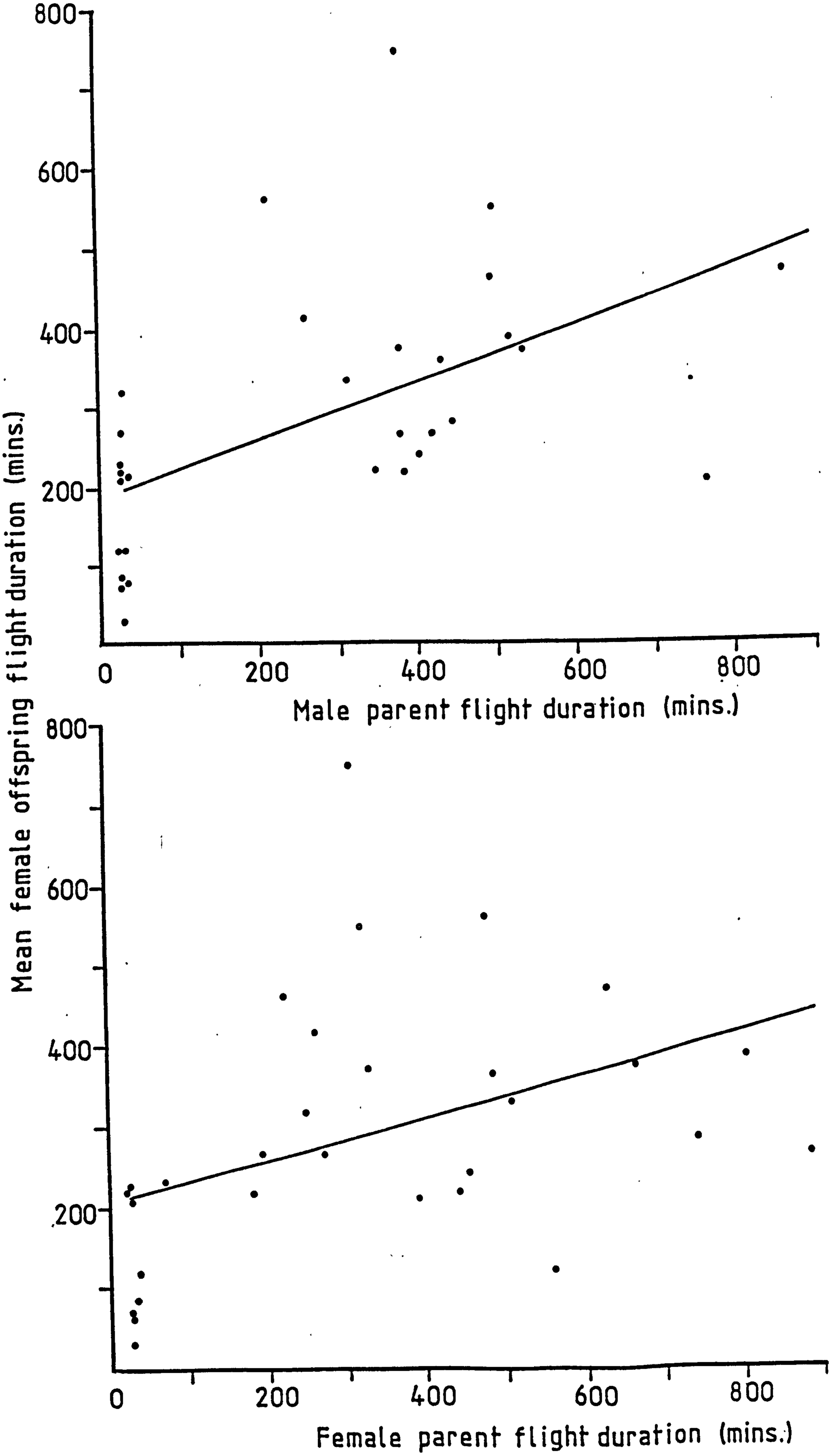

800
600
400
200
0
200
400
600
800
Male parent flight duration (mins.)
Mean female offspring flight duration (mins.)
800
600
400
200
0
200
400
600
800
Female parent flight duration (mins.)

FIGURE 3.8.

The heritability of flight duration: the regression of mean male offspring flight duration on male parent flight duration; y=123.0+0.442x (n=24).

FIGURE 3.9.

The heritability of flight duration: the regression of mean male offspring flight duration on female parent flight duration; y=156.13+0.271x (n=24).

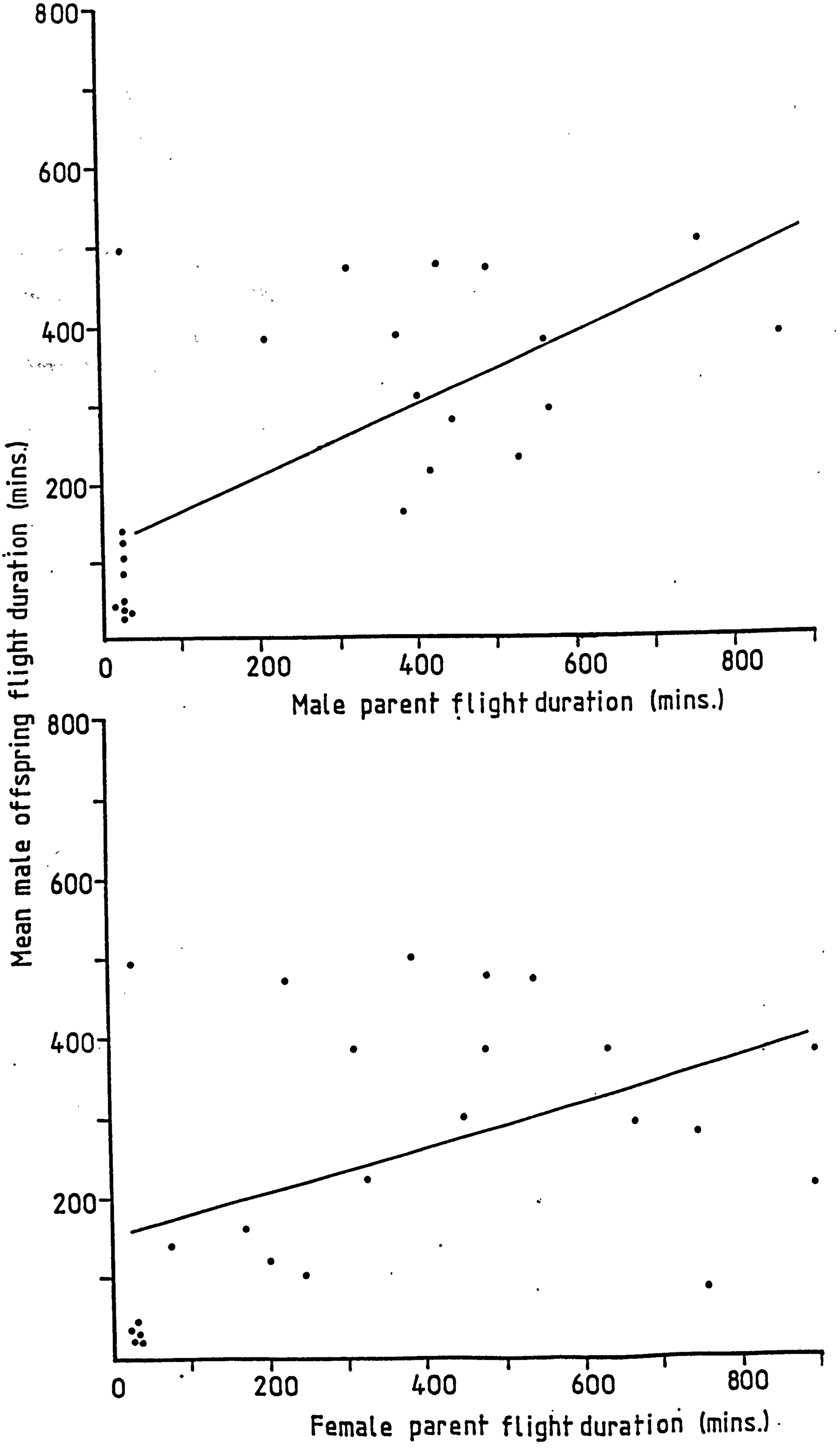

800
600
400
200
0
200
400
600
800
Mean male offspring flight duration (mins.)
Male parent flight duration (mins.)
Female parent flight duration (mins.)

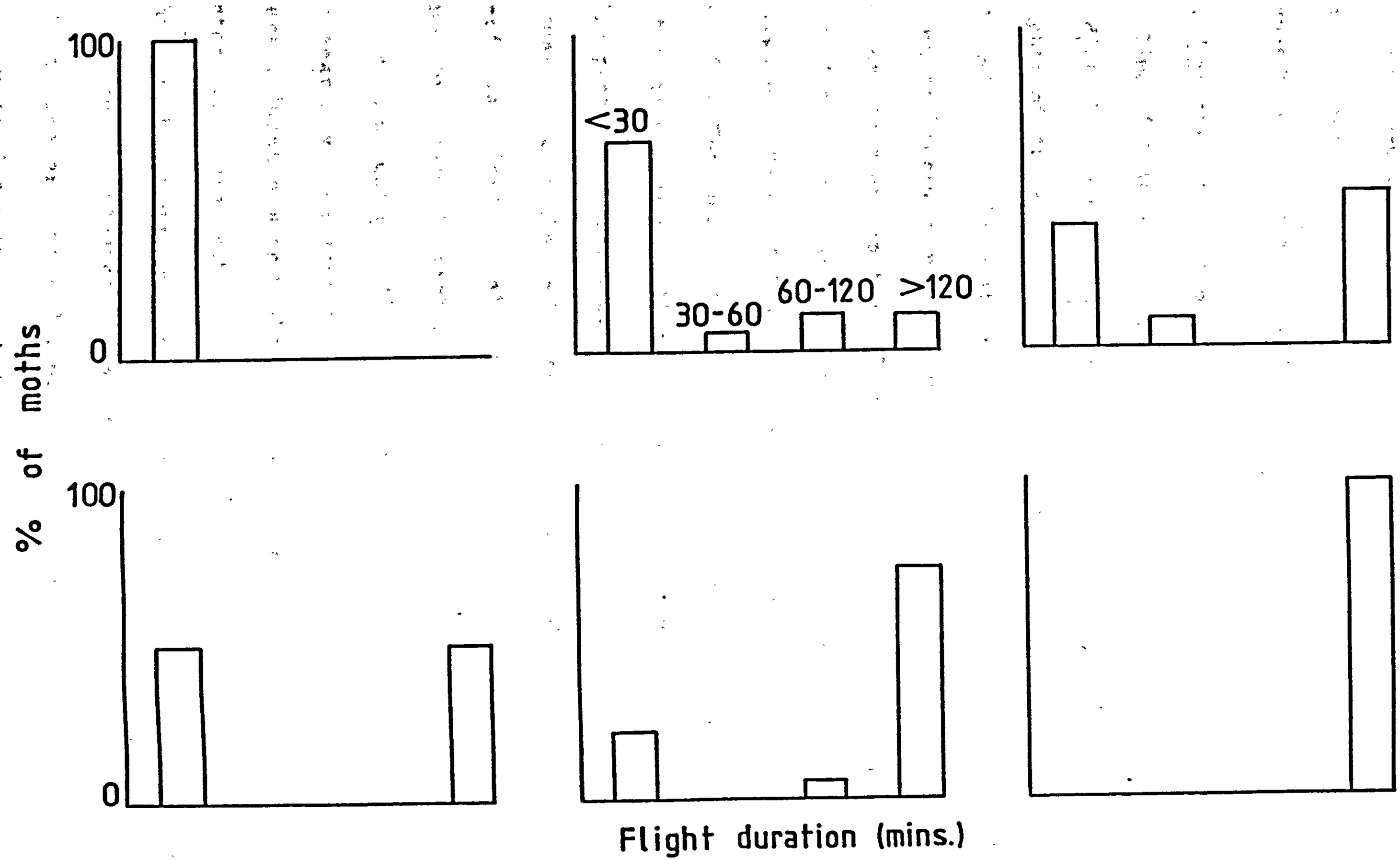
TABLE 3.1.

Percentage heritabilities of flight duration on Night 1.

	Parents		
Offspring	Male	Female	Both
Male	88.4	54.2	-
Female	71.2	50.8	-
Both	-	-	40.0

FIGURE 3.10.

The flight duration frequencies of a representative sample of individual families of moths used in the calculation of heritability estimates. Each distribution represents one family.


100
0
% of moths
<30
30-60
60-120
>120
100
0
Flight duration (mins.)

migrant or non-migrant individuals. This pattern suggests that in many families a segregation of alleles is taking place, with some resulting genotypes producing migrant moths, and others non-migrant individuals.

3.4. Characteristics of migrant moths.

3.4.1. Time of take-off on longer flights.

The distribution of the times of take-off of female moths on Night 1 on flights of different durations is shown in Fig.3.11. There was a peak of take-off in the hour after "dusk" for all categories of flights of >1h, which became more pronounced with increasing flight duration. Thereafter, take-off in the 1-2h and 2-6h categories remained at a low level (5-15% of all flights) throughout the night, although this tailed off between 0400h and 0600h. However, no moths took off on flights >6h after 0200h. This may be a reflection of the fact that moths tended to stop flying at dawn, although this was not invariably the case. A similar pattern of activity was shown by the males (Fig.3.12), although the "dusk" peak in the 2-6h category was relatively less well defined; take-off on flights of all durations was maintained at 5-15% of all flights throughout the night, again tailing off towards "dawn".

3.4.2. Adult weight and flight duration.

The mean Day 1 weights of female moths producing total flights of different durations on Night 1 were examined for differences between migratory and non-migratory individuals (Fig.3.13). The ranges of moth weights in all groups were comparable, although the mean weight of the moths in the >6h category (79.1mg) was significantly higher than that in the 2-6h category (mean 71.2mg; $d=4.48$, $p<0.01$). Moths in the <30m category were also significantly heavier than those in the 2-6h category ($d=2.87$, $p<0.01$).

FIGURE 3.11.

The distribution of take-off on longer flights of different durations by female moths on Night 1 (n=174).

Flight duration.

• >6h (47 flights).

o 2-6h (87 flights).

× 1-2h (71 flights).

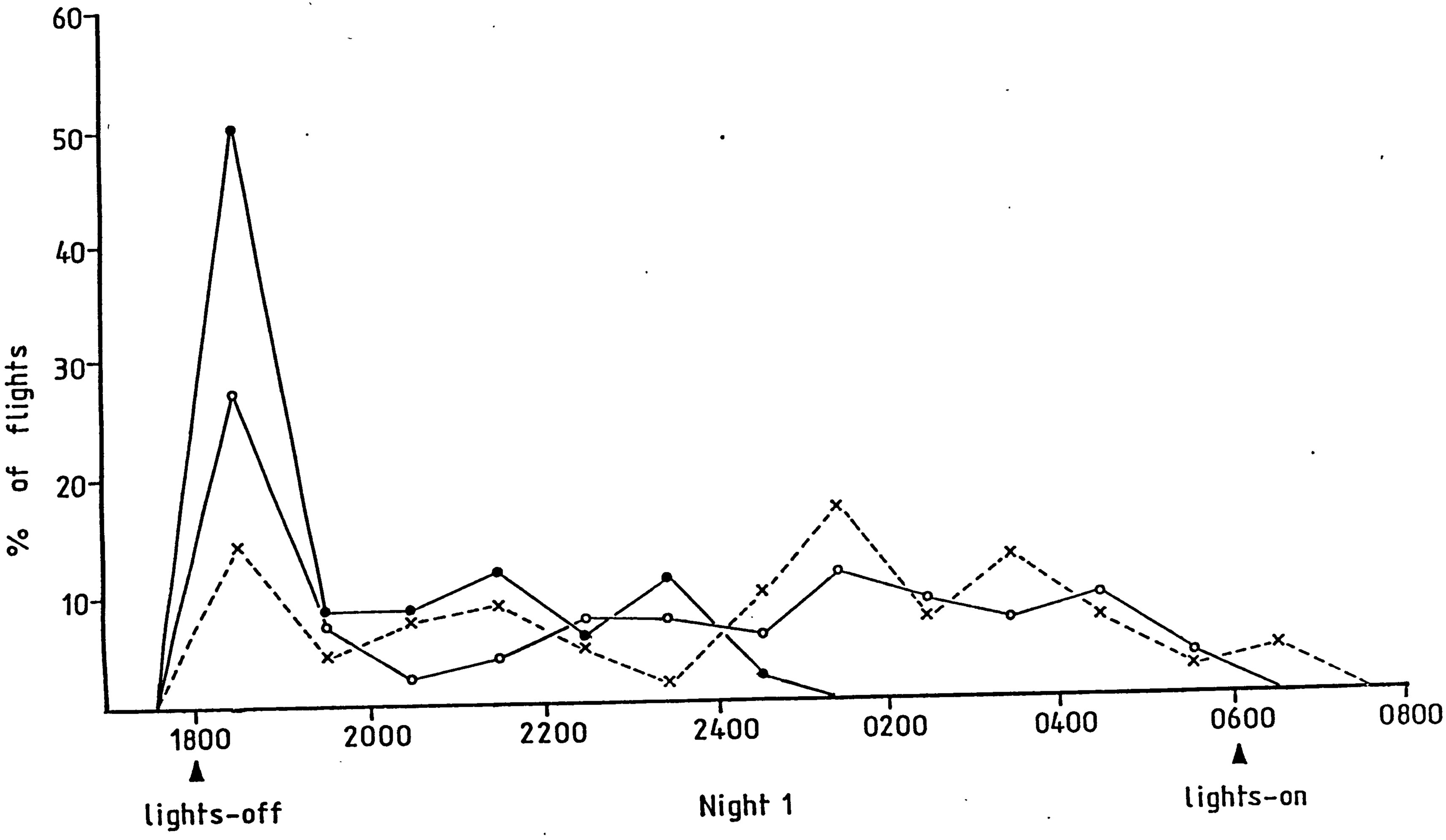

60
50
40
30
20
10
% of flights
1800
2000
2200
2400
0200
0400
0600
0800
lights-off
Night 1
lights-on

FIGURE 3.12.

The distribution of take-off on longer flights of different durations by male moths on Night 1 (n=65).

Flight duration.

• >6h (22 flights).

○ 2-6h (38 flights).

× 1-2h (25 flights).

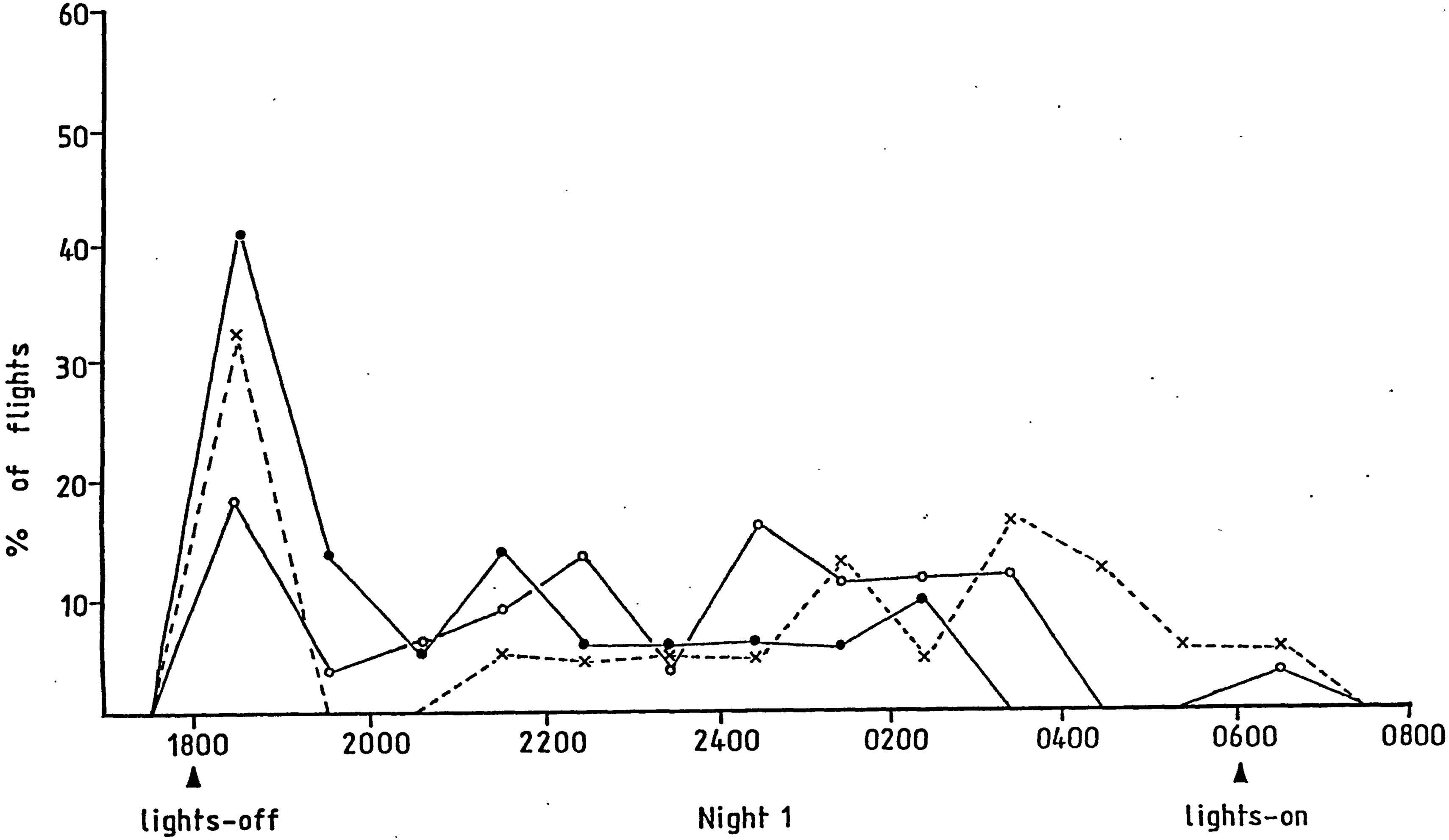
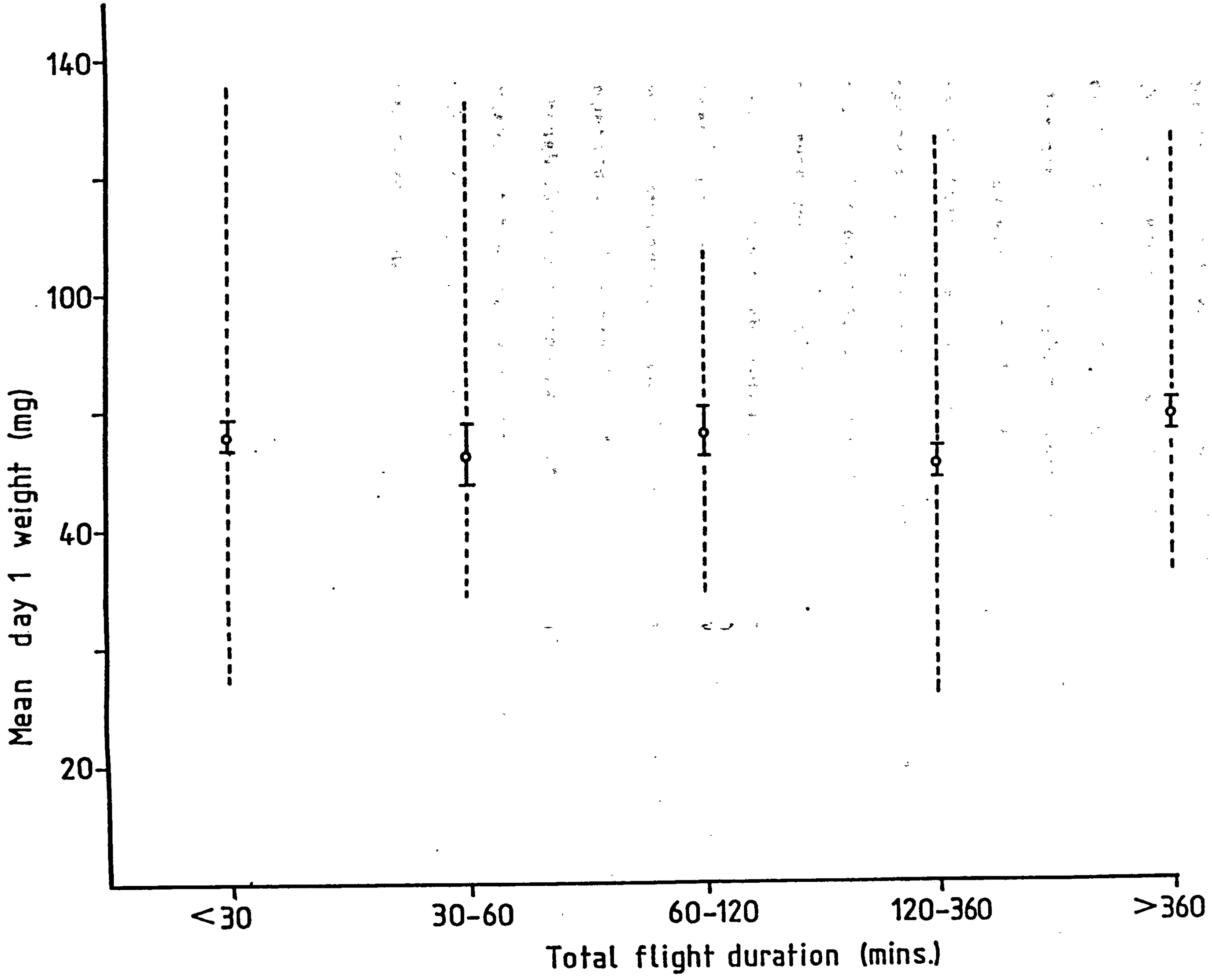

60
50
40
30
20
10
% of flights
1800
2000
2200
2400
0200
0400
0600
0800
lights-off
Night 1
lights-on

FIGURE 3.13.

The Day 1 mean weights (±95% confidence limits) of female moths flying for different durations on Night 1. Dotted lines indicate the range of weight observed in each flight duration category.

Flight duration.	n
<30m	206
30-60m	46
60-120m	49
120-360m	140
>360m	138

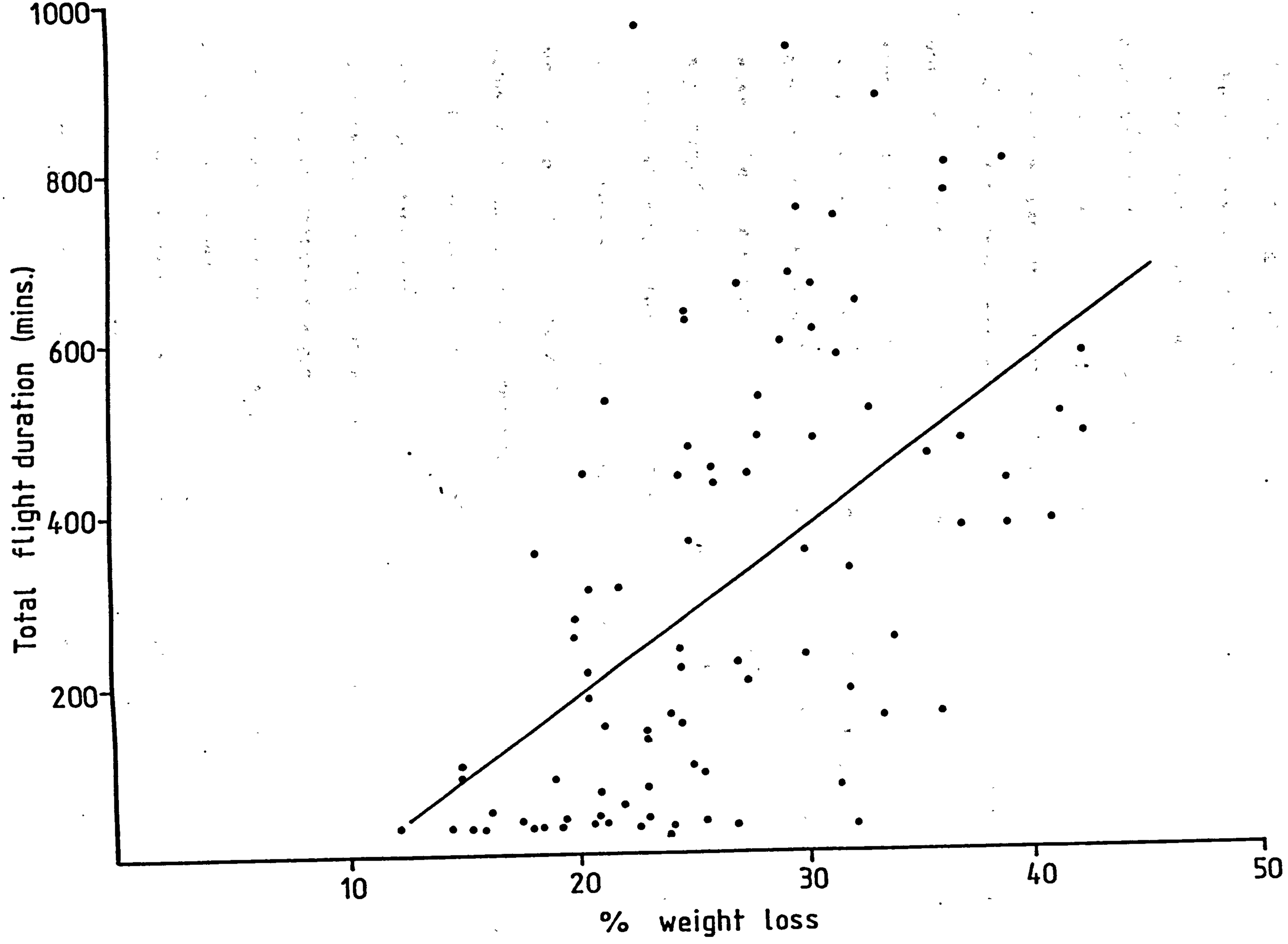

140
100
40
20
Mean day 1 weight (mg)
<30
30-60
60-120
120-360
>360
Total flight duration (mins.)

3.4.3. Weight loss and flight duration on Night 1.

In a further effort to characterize migrant moths on some criterion other than flight (see also Chapter 2), the flight duration of moths on Night 1 was regressed against percentage weight loss of the same moths over the period of the flight test (c. 24h). The results of the preliminary counterweighting experiments in Chapter 2 had shown that weight loss was related to flight duration (Fig.2.2) and it seemed possible that this could be used as a means of assessing flight capacity without the need for elaborate equipment for recording flight duration. For example, a simple static tethered-flight system to which the insect was attached and left for the night would be a useful means of assessing the flight potential of large numbers of moths from field infestations (although all tests would have to be carried out under similar environmental conditions, particularly with respect to temperature). The results of the analysis are shown in Fig.3.14. Although the regression was significant ($b=19.596\pm3.15$, $p<0.001$), the variance of the data was extremely large, with some moths giving no flight >30m losing as much weight as those showing a total flight duration of >10h.

FIGURE 3.14.

The regression of total flight duration on percentage weight loss of female moths during the flight test period; y= -208.19+19.596x (n=91).


1000
800
600
400
200
Total flight duration (mins.)
10
20
30
40
50
% weight loss

4. DISCUSSION

4.1. The response to selection.

The hypothesis that flight capacity is under genetic control, first suggested by the demonstration of a probable genetic association between discrete groups of migrant moths (Fig.3.1), was confirmed by the results from the selection experiments which clearly showed that it was possible to select migrant and non-migrant lines of moths from a given population (Figs 3.2-3.4). Experiments of this type have only rarely been carried out on migrant insects, but when such work has been reported, significant effects have usually been found. Utida (1970) found that the percentage of the active form of Callosobruchus maculatus in a laboratory population could be decreased by selection, and Dingle (1968) raised the percentage of long flying milkweed bugs (Oncopeltus fasciatus) from 20-30% to 60% in one generation; flight activity was maintained at this level for a further three generations by continued selection. Data specific to Lepidoptera is scarce; however Blair (1978), working on the Noctuids Agrotis segetum (Denis and Schiff.) and A.ipsilon Hüfn. was able to demonstrate a significant decrease in flight activity (taken as indicating dispersal power) by selecting for inactive moths over two generations. He also recorded an increase in flight activity by selecting for active moths, although this increase was not significant. Despite the paucity of experimental evidence, other work on Lepidoptera has suggested a genetic contribution to flight capacity. Gilbert and Singer (1973) suggested that differing dispersal rates of individuals in populations of the butterfly Euphydryas editha Boisd. were partly genetically based, and Wellington (1964) reported that "active" moths of Malacosoma pluviale (Dyar) tended to give rise to

active offspring (in terms of range of dispersal) and vice versa.

The results from the selection experiments carried out in this study were generally consistent with those which are obtained when a polygenic (metric) character is subjected to directional selection (Falconer,1981). Selection for migrant moths generally resulted in a rapid increase in the proportion of female migrants in the population, although the response in males was slower in the initial generations of the CK "Migrant" line (Fig.3.3). The response to selection for female migrants in the KF "Migrant" line was also slow (Fig.3.4), and was presumably a reflection of the low frequency of "flight" alleles in the initial stock. When selection for migrant moths was relaxed (e.g. in generations 6 and 14 for females in the CK "Migrant" line, Fig.3.2), the proportion of migrants fell in accordance with theoretical expectations (Falconer,1981), indicating that some degree of genetic heterogeneity was still present in the population, and that fixation had not yet occurred. It is however interesting to note that the high percentage of migrants in the eighth generation in the CK "Migrant" line (males and females) was achieved with selection in only one of the preceeding two generations, presumably because "flight" alleles had already become widespread in this line.

The failure to select out a non-migrant line in the early generations of the CK selection experiments was in marked contrast to the relative success (particularly in the males, Fig.3.3) of selection for non-migrants in the eighth and subsequent generations of the same line. The most plausible reason for this anomaly is that selection in the initial generations was conducted on the hypothesis that the capacity for migratory flight was under the control of a single gene, as postulated for European Lepidoptera by Kettlewell (1952). Because of this, some

non-migrant moths from the "Migrant" line were used for selective breeding in the "Non-migrant" line. As it now seems probable that migratory capacity is a polygenic character, some latent "flight" genes may have been present in these non-migrant moths from the "Migrant" line which were expressed in subsequent "Non-migrant" generations. This tendency seemed to be more marked in the males, where the proportion of migrants in the "Non-migrant" line was actually higher than that in the "Migrant" line in the early generations (Fig.3.3). These relatively high levels of activity shown by both males and females prompted the reduction in larval rearing density mentioned in section 2.4.2, the aim being to suppress the appearance of migrant moths cued by high larval rearing densities (Chapter 2). This had little effect, and the original rearing density was reverted to after one generation. From the eighth "Migrant" line generation, and the equivalent (sixth) "Non-migrant" line generation, only moths originating from the actual line in question were selected for breeding, thus excluding any genetic input from other lines. The effectiveness of this method in increasing the intensity of selection is clearly demonstrated in Figs 3.2 and 3.3.

A further reason for the relative difficulty encountered in selecting for short flight (cf. Blair,1978) may have been due to the fact that moths were only flight-tested on Night 1. Davis (1980) estimated that up to 50% of migrant insects may be missed by flight testing on one day only. A few moths may have given prolonged flights on Night 0 (Appendix 2) but not on Night 1, and some may also give migratory flight on Night 2 (Chapter 4). Consequently some potential migrants may have been used for selective breeding in the "Non-migrant" lines which were not detected by the flight testing procedure.

The slight upward drift in the level of prolonged flight in the unselected CK line (Fig.3.2) was probably due to "flight" genes becoming more evenly distributed within the insect stock as a result of inter-breeding between migratory and non-migratory individuals. Evidence of this "spread" of "flight" genes can be seen in Fig.3.1, where several groups of migrant moths overlapped between Nov.30th and Dec.15th.

4.2. Heritability of flight capacity.

The significant offspring on parent regressions obtained in the calculation of heritability estimates for flight capacity were expected in the light of the results from the selection experiments, and confirmed that flight capacity in S.exempta has a significant additive genetic component. The actual value (0.40) obtained for overall heritability was similar to that reported for flight capacity in Lygaeus kalmii when offspring were regressed against female parent (Caldwell and Hegmann,1969), and was within the range of heritabilities obtained for a number of important life-history traits in Oncopeltus fasciatus (Hegmann and Dingle,1982) and Dysdercus bimaculatus (Derr,1980). However, it should be stressed that the precise heritability values obtained in this study are specific to the particular laboratory stock in question reared under the stated environmental conditions, and it is likely that different field populations would yield different heritability values (Falconer,1981, gives a detailed review of the limitations of heritability estimates). However, in view of the conclusive nature of the results obtained in this work, it would be very surprising if no significant evidence for a genetic contribution to flight capacity was found in field populations.

The significantly lower heritability found for the single offspring on

female parent regressions, when compared to the single offspring on male parent regressions, suggests that male parents contribute a higher proportion of additive genetic variance to flight capacity than female parents. These results contrast with those obtained by Caldwell and Hegmann (1969), who found that the offspring on female parent regression gave a higher heritability (0.4) than the offspring on male parent regression (0.2). High heritability indicates that a fast response to directional selection is likely (Falconer,1981), and in this context it is interesting to note that the response to selection of males in the later generations of the CK line was much more marked than that of the females, particularly in the "Non-migrant" line (Fig.3.3). Characters with low heritabilities tend to be those most closely connected with reproductive fitness (Falconer,1981 p.150). However, the heritability values associated with both male and female parents in this study were found to be high (0.5-0.9), particularly those associated with male parents, and in other insects this is thought to be a reflection of the maintenance of additive genetic variation by uncertain environments (Hoffman,1978; Dingle et al.,1977). It is not clear if the significantly higher heritability values associated with male parents when compared to those associated with female parents has any real importance in terms of field biology, given the relatively small numbers of moths involved and the fact that experiments were carried out on cultured insects.

The flight duration frequencies of individual families of moths (Fig.3.10) were of particular interest not only because the usually highly skewed overall flight duration frequency (Fig.2.4) was substantially altered in most families, but also because it was evident that segregation of "flight" and "non-flight" alleles was occurring in some families, whilst in others only "flight" or "non-flight" genotypes

with no intermediates appeared to be present. This type of segregation suggests that relatively few loci may be involved in the determination of flight capacity. Lamb and MacKay (1979) reported a similar variability in migratory tendency within clones of the pea aphid Acyrthosiphon pisum (Harris) reared under strictly controlled environmental conditions, and concluded that a large genetic component was involved in this variation.

4.3. Characteristics of migrant moths.

The distributions of the times of take-off of male and female moths on longer flights clearly demonstrated a tendency for longer flights to start in the early part of Night 1, particularly at "dusk". The "dusk" peak of take-off noted in the distributions of take-off on all flights >1m (Figs 2.5-2.7) is thus probably closely associated with the onset of migratory flight in many moths. This would be consistent with the field observations of Rose and Dewhurst (1979), who found that within 30m of dusk, moths which had initially congregated in trees had left and moved quickly downwind, and with the laboratory observations of Gatehouse and Hackett (1980), who found that moths regularly took off on long flights at dusk. This behaviour would make maximum use of the available darkness for migration. In the laboratory, moths often took off on long flights during the "daylight" period, but it is not clear if this has any field significance.

In common with the results obtained in Chapter 2, no association could be found between size (in this case in terms of Day 1 weight, Fig.3.13) and migratory capacity on Night 1. These observations lend further weight to the suggestion made in Chapter 2 that environmentally-determined size plays no major role in migration in S.exempta. Because

of the similar ranges of weights of moths in all flight duration categories, there was no possibility of identifying individual migrants or non-migrants on the basis of weight, although on average, short-flying or very long flying moths tended to be heavier. These results contrast with those of Macaulay (1974), who found that the flight duration of tethered Plusia gamma depended on their initial weight, and also with those of Cockbain (1961a), who reported that the flight capacity of 24h old Aphis fabae was directly related to their initial fat content. It has been suggested that between- and within-species size variation in some insects has adaptive significance; larger size confers advantages when resources are temporary because it allows an increased rate of egg production and enhances the chances of surviving migration or diapause (Derr et al.,1981; Dingle et al.,1980). However, there does not appear to be any such association in S.exempta, and it is interesting to note that the closely related Spodoptera littoralis, though a larger moth than S.exempta, is not thought to be a large scale migrant (Campion et al.,1977).

Significant weight loss during migratory flight has been reported in some insects. Cockbain (1961b) found that tethered Aphis fabae lost water (and therefore, presumably, weight) as a result of evaporation and excretion during tethered flight. The brown planthopper Nilaparvata lugens was also found to lose weight during flight (Baker et al.,1980). However, the attempt to identify migrant moths on the basis of weight loss on Night 1 in this study (Fig.3.14) was unsuccessful due to large variations in weight loss between individual moths, even though the overall tendency was towards increasing weight loss with increasing flight duration.

4.4. An hypothesis for migration.

The clear inference from the results described in this chapter is that a complex genetically controlled polymorphism for flight capacity may exist in S.exempta. The crucial question which immediately arises is whether such a polymorphism could be maintained under field conditions. A comprehensive answer to this question is not possible at this stage, as a more detailed knowledge of the precise nature of the genetic basis for flight capacity is required before any firm conclusions can be drawn. However, as a basis for further work, a working hypothesis has been formulated. For the purposes of the present discussion, only a brief outline of the hypothesis is presented here, together with an appraisal of its validity in terms of theoretical models and field and laboratory observations on other insects. The full application of this hypothesis in terms of the field biology of S.exempta is discussed later (see General Conclusion).

The hypothesis envisages the existence of three basic genotypic groups of moths. These are described below.

Type 1: all genotypical (obligate) non-migrants.

Type 2: facultative migrants; may be phenotypically migrant or non-migrant, the switch between the two forms being controlled by the population density experienced by larvae during their development.

Type 3: all genotypical (obligate) migrants.

It should be stressed that within each genotypic group there may be considerable genetic variation depending upon the exact combination of alleles that each individual carries. The major point is that the phenotypic expression of a particular genotype enables any particular moth to be categorized into one of the three groups described above.

Such a situation is similar to that envisaged by Dingle et al. (1982) for the genetic control of life-history traits in the milkweed bug Oncopeltus fasciatus.

In the field, differential selection is seen as operating on these genotypic groups between the wet and the dry seasons. In the dry season, restricted habitat range and low larval density favours the non-flight genotypes, whereas during the rains, selection for emigration is favoured as it allows moths to colonize new habitats. High larval density in outbreaks throws the switch between non-migrant and migrant patterns of behaviour in individuals with Type 2 genotypes.

The mechanisms by which genetic polymorphisms may arise and be maintained is a subject of some controversy, and a number of theories have been expounded (see Falconer,1981 pp.42-45 for a brief review). Of these, the role of heterogeneous environments in maintaining polymorphisms is the one which most closely fits the hypothesis described above. The main theoretical difficulty encountered in cases where genetic polymorphisms are maintained by differential selection in heterogeneous environments is the danger of one or other allele becoming fixed (reviewed by Hendrick et al.,1976). These authors concluded that genetic polymorphisms can be maintained by differential selection, but in many cases they may not be stable. However the model proposed by Haldane and Jayakar (1963) permitted polymorphism when additive gene action is involved (as in this case) where one homozygote (which could correspond to either Type 1 or 3 genotypes) was favoured in one environment and one in another. Further, Roff (1975) described a model involving three genotypes where a stable polymorphism is possible if one homozygote and the heterozygote disperse, or if the dispersal tendency

is a quantitatively inherited trait. These conditions appear to fit the hypothesis outlined above. In general, population models tend to show that polymorphic populations are more "fit", in terms of average rate of increase and population size (Giesel,1976), than monomorphic populations.

Differential selection as a result of varying climatic factors was also suggested as the reason for variation in the rate of reproduction and the level of fecundity in moths of the genus Choristoneura by Campbell (1962), who proposed an X-chromosome model based on experimental evidence to account for this variation. Rose (1972) also suggested that in Cicadulina spp., a balance may be maintained between long-bodied poor fliers adapted for reproduction, and short-bodied forms suited to dispersal when habitats become dry. Studies on other insects have revealed that considerable inter- and intra-specific genetic variation occurs. Oliver (1972), in a study of four butterfly species, made field observations on preferred habitats, food-plants and flight behaviour, and compared phenotypic differences in appearance and physiology in the laboratory, of populations from widely separate locations. Every comparison showed some phenotypic differentiation and genetic incompatibility. Oliver (1979) also reviewed work demonstrating similar differences in other Lepidopteran species. Stock and Robertson (1980), working on selected Choristoneura species, found that intra-specific genetic variation (in terms of response to an organophosphate insecticide) can be as large as inter-specific variation. In some species, genetic differences between populations have been shown to result in a polymorphism in the form of a cline, such as the geographically variable photoperiodic diapause shown by Drosophila littoralis Meigen (Lumme and Oikarinen,1977). However, such a situation

is not likely to occur in S.exempta where wide gene mixing is thought likely to occur over much of the African range of the species (den Boer,1978). Indeed, for the hypothesis to operate satisfactorily (given that a flight polymorphism is postulated), wide gene mixing and continual re-invasion of isolated habitats must occur, or fixation of "non-flight" alleles would occur in isolated, though possibly relatively stable, habitats (although even species in relatively stable habitats may maintain some dispersal tendency, den Boer,1970). Thus for a genetic flight polymorphism of the type envisaged above to be maintained, large scale dispersal of populations must occur during the wet season. Such a situation is consistent with the ideas of Rose (1979,1982-see General Introduction) rather than the idea that populations of moths move in discrete groups (Rainey and Betts,1979).

4.5. Conclusions.

On the evidence presented in this chapter, there can be little doubt that quantitative genetic factors play a major role in the control of migration in S.exempta. The exact nature of these and the ways in which they may operate in the field are not yet clear, although it seems likely that any environmental cues for migration are largely superimposed on these genetic components. The inter-relationship between migration and other related life-history characters is of particular importance, as variations in one character tend to lead to changes in related ones (Hegmann and Dingle,1982; Sokal and Taylor,1976). In this context, the relationship between migration and reproduction is of critical importance in a migrant insect (Dingle,1972), and further work was undertaken to examine this aspect of migration in S.exempta.

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CHAPTER FOUR.

The effect of age, feeding, and mating on flight capacity, and the association between migration and ovarian development in S.exempta.

TABLE OF CONTENTS.

1. INTRODUCTION.

In insect species, migration has evolved in response to the need to find and colonize new breeding grounds. As a result, the relationship between migration and reproduction has come to be seen as crucially important in the development of the life-history strategies of insects (Dingle,1972).

Insect migration is often thought of as being pre- (or inter-) reproductive, as many female insects migrate while the ovaries are immature or in the process of maturing (reviewed by Johnson,1963,1969). Thus a feature common to the migratory strategies of many insects is the relationship between the time available for migration and the rate of ovarian development in the females. Consequently, factors which affect the duration of the pre-oviposition period, and hence the time available for migration, are of major importance in regulating when migration and reproduction occur. Several factors may affect the duration of the pre-oviposition period, but the general tendency is for migrant individuals to have longer pre-oviposition periods. Females of the rice plant skipper butterfly Parnara guttata guttata reared under a 14h photoperiod (which corresponds to adults developing in early autumn) have higher flight activity and a longer pre-oviposition period than those reared under a 16h photoperiod, the conditions experienced by the non-migratory summer adults (Ono and Nakasuji,1980). In unmated but fed females of Dysdercus superstitiosus, flight persisted for two days longer and oviposition began a day later in isolated compared to crowded bugs (Gatehouse and Hall,1976). Conditions of larval development, especially food quality and larval density, may also be of importance in determining the duration of the pre-oviposition period. Such effects

have been studied in detail for locusts and aphids (reviewed by Johnson,1969), but also occur in other species including some Lepidoptera. Poor larval food quality lengthened the pre-reproductive period (defined as the time between emergence and ovarian maturation, as distinct from the pre-oviposition period, which is defined as the time between emergence and first oviposition) in the diamond-back moth Plutella maculipennis (Curtis) (Hillyer and Thorsteinson,1969), and Hackett (1980) suggested that larval conditions and temperature during early adult life were important in determining the length of the pre-oviposition period in Heliothis (cited as Helicoverpa) armigera (Hübner).

The complex relationship between the development of the ovaries, the flight apparatus, and the ancillary systems such as the fat-body has been termed the "oogenesis-flight syndrome" (Johnson,1969). The differential development of these systems in response to environmental factors produces a variety of morphs which ranges from sexually immature but flight-worthy females (migrants) through sexually mature flight-worthy non-migrants and brachypterous adults, to parthenogenetic flightless forms. The presence or absence of males, and the time of mating (if it occurs) also influence the type of behaviour and physiology which the females of any given species show in relation to migration and reproduction (Johnson,1969).

Studies on the neuro-endocrine basis for the relationship between migration and reproduction have shown that Juvenile Hormone (J.H.) titres are important in some insects. In Oncopeltus fasciatus, migratory flight occurred at intermediate J.H. titres, while oviposition did not begin until J.H. titres reached a threshold level above that required for migration. The speed at which J.H. titres rose and hence the time

available for migration was controlled by environmental factors such as host-plant quality (Rankin and Riddiford,1977,1978). J.H. was also implicated in the control of migration and reproduction in the convergent ladybird beetle Hippodamia convergens Guér. (Rankin and Rankin,1980).

Insect migration is also thought to involve the suppression of vegetative responses such as feeding, mating, and oviposition (Kennedy,1961,1975), and this is consistent with the idea (see above) that migration and reproduction are mutually exclusive. Thus in the milkweed bug Oncopeltus fasciatus, flight, mating, feeding, and oviposition are segregated into different times of day (Caldwell and Rankin,1974), and in the garden chafer Phyllopertha horticola (L.), most females emerge ready to oviposit and do not migrate, but those not ovipositing immediately migrate and lay later (Milne,1960).

It should be stressed, however, that not all insects follow this pattern of pre-reproductive migratory behaviour. Females of the spruce budworm Choristoneura fumiferana emerge fully gravid and cannot fly until they have oviposited (Wellington and Henson,1947), and females of the European pine shoot moth Rhyacionia buoliana fly during the oviposition period (Green,1962; Green and Pointing,1962). Flight activity of the Argentine stem weevil Listronotus bonariensis (Kuschel) was also found to be unrelated to sexual activity, as large populations of "migrant" weevils flying in summer contained a high proportion of gravid females (Goldson,1981).

Several intrinsic factors affect the initiation and duration of migratory flight. The most important of these is age. In many species, maximum flight activity is only attained several days after adult

emergence, and then declines with the onset of reproduction. This has been demonstrated for a number of insects including Oncopeltus fasciatus (Dingle,1965), Dysdercus spp. (Dingle and Arora,1973), the mosquito Aedes aegypti (L.) (Rowley and Graham,1968), the Oriental armyworm moth Mythimna separata (Hwang and How,1966), Cicadulina spp. (Rose,1972), Plusia gamma (Macaulay,1972), and the brown planthopper Nilaparvata lugens (Padgham,1983). This delay in the onset of major flight activity is thought to be associated with the teneral period, the time taken for the cuticle (particularly the wing hinges) to harden. Dingle (1965) studied the deposition of cuticular growth rings in Oncopeltus fasciatus and found that maximum flight activity occured at the end of the teneral period. The time taken for the biochemical processes necessary for sustained flight activity to mature (as, for example, demonstrated for the house fly Musca domestica L. by Rockstein and Brandt,1963) may also be a reason for the delay in the onset of flight activity in migrant insects.

The availability of adult food can also have significant effects on the expression of migratory flight. Although some Diptera (Aedes spp. and Simulium spp.) are capable of flying large distances without feeding (Hocking,1953), other insects cannot migrate without feeding. The army cutworm, Chorizagrotis auxiliaris (Grote) only made short flights after eclosion prior to feeding (Koerwitz and Pruess,1964), and the cabbage looper Trichoplusia ni flew better when fed 10% sucrose compared to starved moths or those fed only water (Kishaba et al.,1967). Quality of food may also affect flight performance. An increased proportion of individuals of the Rutherglen bug Nysius vinitor took off on prolonged flight in response to inadequate food (Kehat and Wyndham,1973). In Dysdercus fasciatus Sign. the availability of adult food suppresses

migration and wing-muscle histolysis ensues (Dingle and Arora,1973).

There is little published data on the effect of age, feeding, or ovarian development on the migration of Spodoptera exempta, although the number of nights on which migrant moths could fly has important implications for the potential range over which moths could disperse before breeding. Aidley (1974) flew S.exempta moths fed 10% sucrose solution on a flight mill, and concluded that moths could migrate for more than one night if they had access to nectar. However, Gatehouse (pers. comm.) found that starved moths were capable of long (>3h) flights on Night 2.

Important observations on the rate of ovarian development of female moths have also recently been reported by Page (1982). He found that females developed their ovaries at the same rate up to c. 48h after emergence. At this point, some moths arrested development for a further 1-3 days before maturing, while others continued developing to full maturity without an arrestment stage. This pattern was found in both field and laboratory moths, and appeared to be largely independent of climatic conditions. These findings suggested a possible genetic basis for rate of ovarian development. The interesting possibility thus arose that these differing rates of ovarian development could be directly related to genetically-based differences in flight capacity.

This chapter describes an investigation into the effect of age, feeding, and mating on the flight capacity of four-day old female moths, and examines the possible association between the rate of ovarian development and flight capacity.

2. MATERIALS AND METHODS.

2.1. Insect material.

All moths were reared in the maize culture between May and December 1982. For the experiments on the effect of age, feeding, and mating on flight performance (section 2.3.1) only moths from the stock (CK) culture and the CK "Migrant" line were used. However, for the experiments on the association between migration and ovarian development (section 2.3.2), moths from the CK stock, CK "Migrant", CK "Non-migrant", and KF (Kenyan) lines were used (see Chapter 3 for the origin of these lines).

2.2. Environmental conditions.

These were as described in Chapter 2, section 2.2.2.

2.3. Experimental.

2.3.1.The effect of age, feeding, and mating on flight performance.

A series of experiments was carried out to ascertain the effect of feeding and mating on the subsequent flight performance of female moths which had initially been flight-tested on Night 1 without prior access to food, water, or males. After being subjected to the appropriate experimental treatment (see below), all moths were flight tested again on Night 4. The reason for Night 4 being chosen as the time for the second flight test was because most females were assumed to have reached maturity and mated on or before Night 3 (Khasimuddin,1978; Page,1982). All moths were reared under the standard T20 larval feeding regime described in Table 2.1. The experimental treatments were as follows.

(i) Experiment 1: moths were starved and denied access to males on Nights 2 and 3. On removal from the balances after the Night 1 flight

test, moths were maintained in individual 55ml plastic vials until the second flight test to prevent excessive dehydration. An attempt was also made to mate starved females on Nights 2 and 3, but survival of both males and females was very poor, and the attempt was abandoned as unrealistic. Only moths from the CK stock culture were used in this experiment.

(ii) Experiment 2: in this experiment, only moths from the CK "Migrant" line were used. They were subjected to two treatments. One group of moths was given access to 20% w/v sucrose solution ad lib from Day 2 to Day 4, but were not provided with access to males. Individual moths were kept in a standard 450ml rearing jar with a filter-paper floor and lid. The second group was also provided with sucrose solution as above, but one male of the same age and genetic background (where possible) was also placed in the rearing jar for Nights 2 and 3. The sucrose solution for both groups was checked on Day 3 and replenished if it had dried out. The jars were checked for the presence of eggs on Days 3 and 4. Some of the unmated female moths were dissected on Day 5 (after the second flight test) to determine whether or not mature eggs were present in the ovaries, and all moths in the mated group were dissected at the same time to determine the presence or absence of spermatophores in the bursa copulatrix as a check on their mated status. Any moths found to be unmated were discarded from the analysis. The individual mounting brackets (Chapter 2, section 2.1.3) glued to each moth stayed in place satisfactorily from emergence to Day 5 in the vast majority of individuals, and did not interfere with mating

(iii) Experiment 3: this experiment was exactly similar to Experiment 2, except that only moths from the CK stock culture were used.

All moths in the above experiments were flight-tested on Night 1 using

the standard procedure described in Chapter 2. The Night 4 flight test was carried out using the same procedure as on Night 1, with moths being weighed on Day 4 to ascertain the correct counterweight for the balance. However, mated moths from Experiments 2 and 3 generally laid eggs on the paper drum while attached to the flight apparatus on Night 4, and consequently lost significantly more weight than immature or unmated moths. In order to avoid the possibility of having a large number of moths suspended on the balances as a result of too heavy a counterweight after oviposition, the counterweight given to the mated moths in Experiments 2 and 3 was reduced to 50% of their Day 4 weight. Moths were still able to generate enough lift to operate the equipment with this reduced counterweight.

2.3.2. The association between ovarian development and flight capacity.

In the light of the results obtained by Page (1982-see Introduction), a further series of experiments was carried out to try and establish a link between the rate of ovarian development of individual moths and their flight capacity. These are described below. All moths were starved and unmated, and reared under the standard T20 larval feeding regime (Table 2.1).

(i) Experiment A: this was a preliminary experiment carried out to confirm that the differing rates of ovarian development reported by Page (1982) for female moths from field and laboratory populations in Kenya were also a feature of the cultured moths maintained at UCNW Bangor. The procedure used was similar to that used by Page (pers.comm.) and is as follows.

The emergence times of individual female moths were recorded. A sample of moths was killed at emergence, and further samples were killed at a

series of times between 18 and 80h post-emergence. All moths were killed by immersion in 70% alcohol, and were then stored in individual vials (in alcohol) for at least 5 days. They were then dissected, and the terminal oocyte width in one ovariole was determined using a graticule eyepiece in a light microscope under the low-power objective. As the terminal oocytes were often deformed, the first obviously undistorted oocyte was measured. If ~~only~~ one or more ovariole contained a few mature eggs, the moth was recorded as mature, and the width of the mature egg was recorded. Moths from the CK stock, "Migrant", "Non-migrant", and KF lines were used in this experiment.

(ii) Experiment B: a sample of starved and unmated female moths were flight-tested on Night 1 in the normal way. These included moths from the stock (CK) culture and the CK "Migrant" and "Non-migrant" lines to obtain a sample containing both migrant and non-migrant individuals. The emergence times of these moths were recorded. All were killed at 48h post-emergence (Day 2/Night 2), as by this time the split in the rate of development between individual moths should have become apparent. Moths were stored for later dissection in the manner described for Experiment A.

(iii) Experiment C: this experiment was similar to Experiment B in all respects except that all moths were killed at 72h post-emergence.

(iv) Experiment D: the aim of this experiment was to examine the association between flight capacity on Night 2 and the state of ovarian development on Day 3. Samples of moths from the CK "Migrant" and "Non-migrant" lines were flight tested on Night 2, these moths being kept in individual rearing jars without access to food or males for the duration of Day 1 and Night 1. All moths were killed after flight testing at 62h post-emergence, and were again dissected to determine their state of

ovarian development by the method described in Experiment A.

3. RESULTS.

3.1. The effect of age, feeding, and mating on flight performance.

The detailed results from these experiments are given below:

Experiment 1: the results from this experiment are given in Fig.4.1. A sample of 27 moths were flown, of which only 22% could be classified as migrants (i.e. gave a total flight duration of >120m) on Night 1. The majority of moths (70%) gave no flights of >30m duration. When the same moths were flown on Night 4, none showed a total flight duration of >120m, and again the majority of moths (89%) gave no flight >30m duration. The drop in the percentage of migrants between Nights 1 and 4 was significant (Fisher's exact test, $p=0.011$). In general, survival of moths between Nights 1 and 4 was poor during this experiment, and only c. 50% of moths attached to the balances on Day 4 survived the flight test. All moths which failed to survive the duration of the experiment were excluded from the analysis.

Experiment 2: the results of this experiment are given in Fig.4.2. Of the moths flown on Night 1, the majority (81%) were classified as migrants. Of the remainder, 13% fell into the <30m flight duration category, and 6% into the 30-60m category. However, only 25% of these moths were classified as migrants on Night 4, and this represented a significant decrease over the percentage of Night 1 migrants (Fisher's exact test, $p=0.002$). When the mated and unmated groups were considered separately, a significant decline in the proportion of migrants between Nights 1 and 4 was again recorded in both cases (unmated group, $n=10$, Fisher's exact test, $p=0.038$; mated group, $n=6$, Fisher's exact test, $p=0.030$). Survival of moths in both this experiment and Experiment 3 was considerably better than in Experiment 1, with c. 90% of moths initially

FIGURE 4.1.

The effect of starvation on the flight performance of CK stock line female moths on Night 4 when compared to their flight performance on Night 1. All moths unmated (n=27).

Night 1.

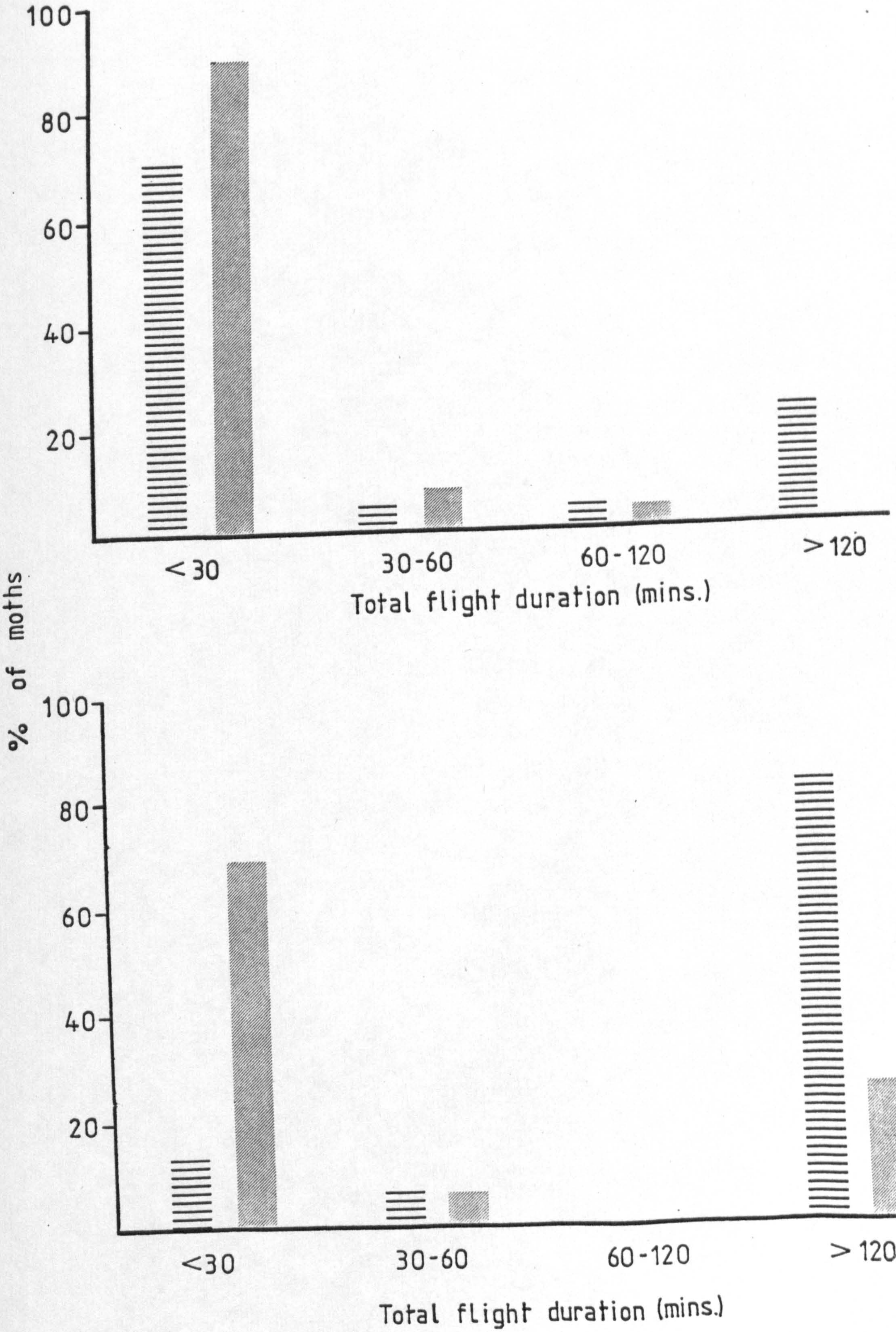
Night 4.

FIGURE 4.2.

The effect of feeding on Nights 2 and 3 on the Night 4 flight performance of CK "Migrant" line female moths when compared to their flight performance on Night 1. Data on mated and unmated moths pooled (n=16).

Night 1.

Night 4.


% of moths
100
80
60
40
20
<30
30-60
60-120
>120
Total flight duration (mins.)
100
80
60
40
20
<30
30-60
60-120
>120
Total flight duration (mins.)

tested on Night 1 surviving until the end of the Night 4 flight test.

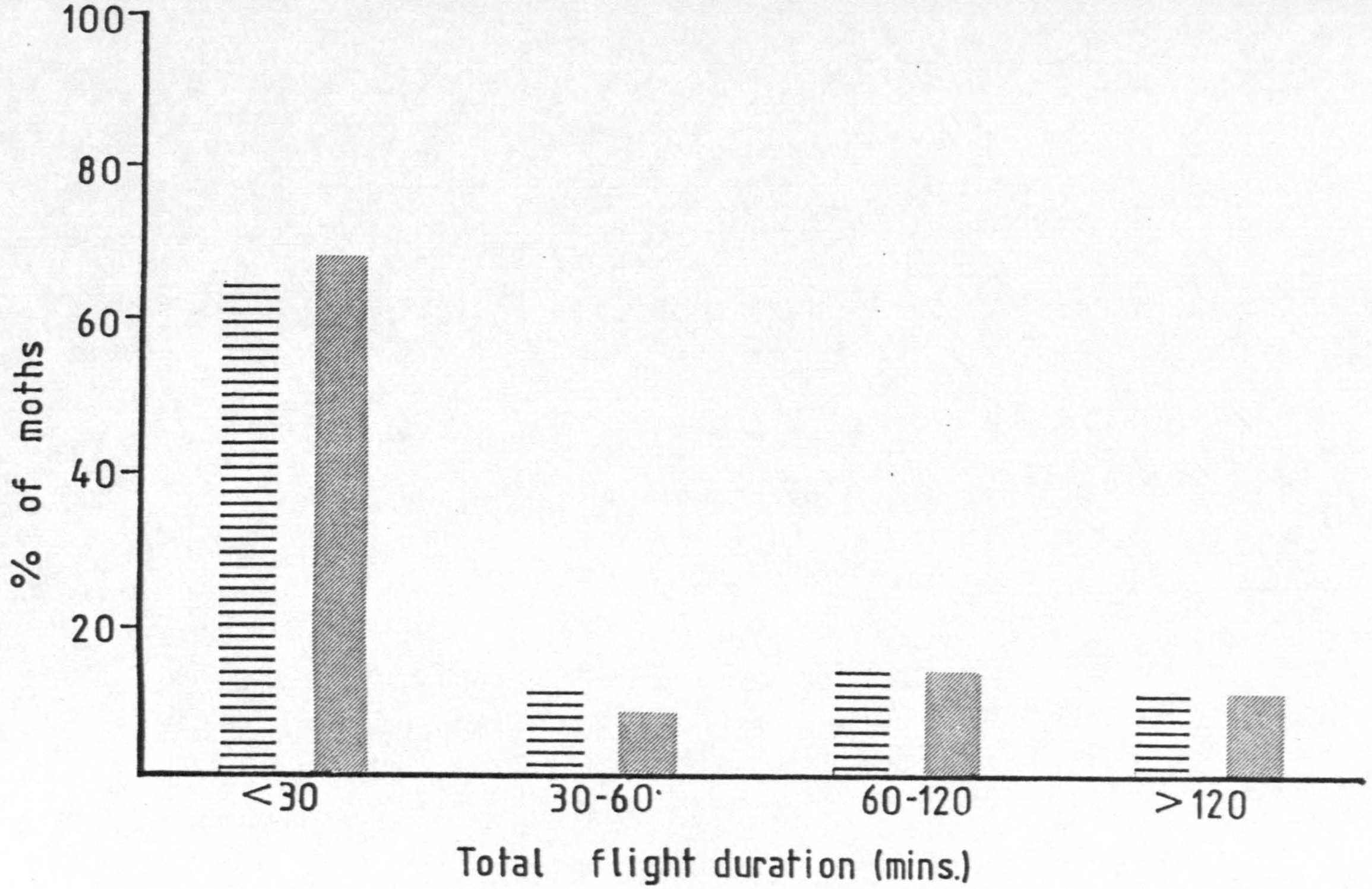
Experiment 3: the results of this experiment (Fig.4.3) contrasted strongly with those from Experiment 2. On both Nights 1 and 4, only 11% of moths could be classified as migrants, and the majority of moths gave total flight of <30m on both nights (64% of moths on Night 1, and 68% of moths on Night 4). There was no significant difference between the proportions of migrants recorded on the two nights in either the unmated group (n=7) or the mated group (n=21).

A feature of the flight records of several mated moths on Night 4 in Experiment 3 was the occurrence of a series of short (10-15m) and apparently weak flights covering a period of 1-2h (no mated moths in Experiment 2 showed this pattern of flight behaviour). The time between the end of one flight and the start of the next could be as short as 30s. This sporadic pattern of activity was qualitatively different from the characteristically unbroken flight activity pattern normally observed on Night 1, and was possibly indicative of "local" flights associated with the search for suitable oviposition sites, and therefore not significant in terms of displacement. Any moth showing this type of behaviour was placed in the <30m flight duration category.

Those mated moths which gave long and uninterrupted flights on Night 4 in both Experiments 2 and 3 (4 individuals, one of which had not given flight >120m on Night 1) all laid fertile eggs on Night 3, and also oviposited on the landing drum on Night 4. In fact, 90% of all mated moths first oviposited on Night 3 (the night prior to the second flight test), and a similar proportion of moths laid eggs on the landing drum of the balance on Night 4. On dissection, 17% of all mated moths contained 2 spermatophores, and the remainder only one. Only two moths did not lay any eggs despite being mated; the presence or absence of

FIGURE 4.3.

The effect of feeding on Nights 2 and 3 on the Night 4 flight performance of CK stock line female moths, when compared to their flight performance on Night 1. Data on mated and unmated moths pooled (n=28).


100
80
60
40
20
% of moths
<30
30-60
60-120
>120
Total flight duration (mins.)

mature eggs in these two moths on Day 5 was unfortunately not recorded.

Fourteen of the 17 unmated moths from Experiments 2 and 3 were dissected on Day 5. The majority of these (93%) were found to contain mature eggs. This included one moth which had given relatively short (total flight of 137m in flights of 44m and 93m) migratory flight on Night 4. However, one moth which had given a single flight of 522m on Night 4 was found to have immature ovaries on Day 5. These were the only unmated Night 4 migrants dissected.

3.2. The association between ovarian development and flight capacity.

Experiment A: the results from this preliminary experiment are shown in Fig.4.4. All moths emerged with immature ovaries, and initiated ovarian development. However, by 40h it was apparent that some moths had already reached maturity (terminal oocyte width >0.45mm), whereas others remained immature up to at least 72h post-emergence. Very few moths were found with terminal oocyte widths in the 0.33-0.45mm range, implying that oocyte development through this size range was rapid. Those moths still immature at 72h would presumably have reached maturity within the next 24-48h.

Experiment B: the results from this experiment are given in Table 4.1 (a). The rationale behind this experiment was that those moths which gave migratory flight on Night 1 were most likely to be those which arrested ovarian development at 40-48h post-emergence, whereas those which gave only short flights were most likely to have reached maturity by the same time. However, the results obtained did not support this hypothesis, as the proportions of mature migrants and non-migrants at 48h were not found to be significantly different (Fisher's exact test, $p=0.43$).

FIGURE 4.4.

The relationship between age (hours post-emergence) and terminal oocyte width (mm) in female S.exempta moths (n=66).

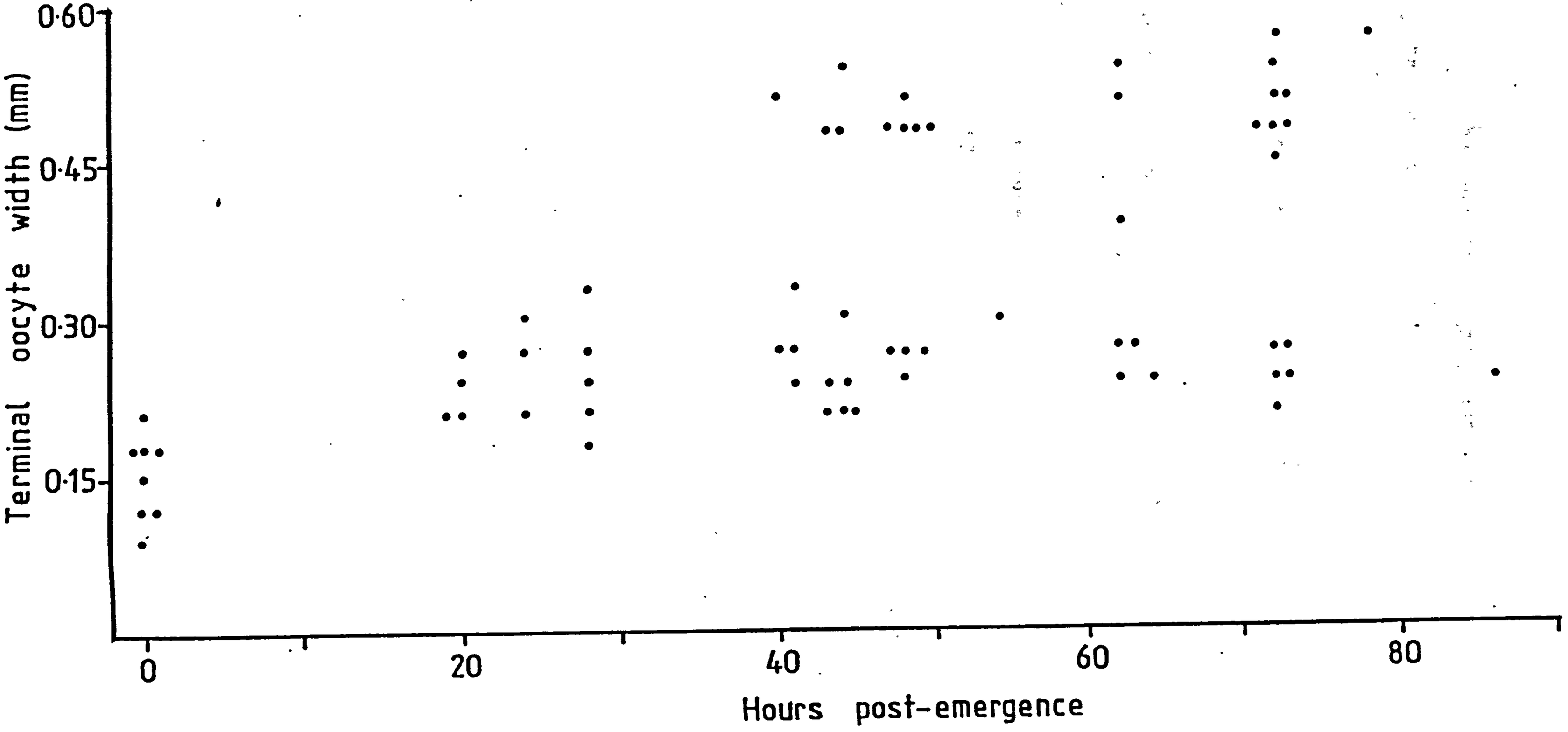

0·60
0·45
0·30
0·15
Terminal oocyte width (mm)
0
20
40
60
80
Hours post-emergence

TABLE 4.1.

The relationship between the flight capacity of female moths on Night 1 and their state of ovarian development at 48h and 72h post-emergence.

(a) 48h.

	% mature	% immature	
Migrants	26.7	73.3	n=15
Non-migrants	14.8	85.2	n=27

(b) 72h.

	% mature	% immature	
Migrants	57.1	42.9	n=7
Non-migrants	20.7	79.3	n=29

Experiment C: this experiment was carried out to ascertain whether any direct association between flight capacity on Night 1 and state of ovarian development only first became apparent in 3-day old moths (Table 4.1b). However the results were similar to those in Experiment B, with no significant difference being found between the proportions of mature migrants and non-migrants at 72h (Fisher's exact test, p=0.076), although overall a higher percentage of moths were mature at 72h than at 48h.

Experiment D: the results obtained from Experiments B and C suggested that if any direct relationship existed between migration and rate of ovarian development, it was more complex than initially envisaged. This experiment was an attempt to clarify the situation. Of the 22 moths which were flight-tested on Night 2 and killed immediately after the flight test, 50% were immature non-migrants, 14% were mature non-migrants, 32% were immature migrants, and 1 individual (4%), which gave two flights of 65m and 96m respectively, was classed as a mature migrant. This latter moth was derived from the CK "Non-migrant line", but all other moths classed as migrants in this experiment were derived from the CK "Migrant" line.

3.3. Variations in ovarian development between culture lines.

The data from the above experiments were re-examined to determine if there were any differences in the proportion of female moths mature at 48 and 72h between the CK stock, CK "Migrant", CK "Non-migrant", and KF (Kenyan) lines. No significant differences were found between the CK stock, CK "Migrant", and CK "Non-migrant" lines (Table 4.2a). However, no mature moths were observed in the Kenyan (KF) line at 48h, and this represented a significant difference when compared to the CK stock

TABLE 4.2.

Differences in percentage maturity at 48h and 72h post-emergence of female moths derived from different culture lines.

(a) 48h.

Line	% mature	% immature	
CK (Stock)	35.3	64.7	n=17
CKA (Active)	43.8	56.2	n=16
CKN (Non-active)	16.7	83.3	n=18
KF (Kenyan)	0	100.0	n=13

(b) 72h.

Line	% mature	% immature	
CK	18.2	81.8	n=22
KF	0	100.0	n=8

TABLE 4.3.

Mean weights (±95% c.l.) of mature and immature female moths at 48h and 72h post-emergence. All weights in mg.

Time killed	Mature	Immature
48h	78.1±10.01 (n=8)	68.7±4.83 (n=22)
72h	80.3±9.69 (n=10)	86.8±11.27 (n=18)

(Fisher's exact test, p=0.02) and "Migrant" (Fisher's exact test, p=0.008) lines, though not when compared to the "Non-migrant" line (Fisher's exact test, p=0.26). Sufficient data on percentage maturity between lines at 72h were only available for the CK stock and Kenyan lines (Table 4.2b). Again, no moths were found to be mature in the Kenyan line at 72h, although the sample of moths was small. Only 18% of the CK moths were mature, and the difference between the two lines was not significant (Fisher's exact test, p=0.55).

3.4. Mean weights of mature and immature moths.

It was apparent from the above experiments that rate of ovarian development was independent of flight capacity per se, and also largely independent of genetic background. There was insufficient time to examine this relationship in detail in this study, but as a preliminary, mean Day 1 weights of starved moths found to be mature or immature at 48h and 72h were examined for possible differences. Only data on moths from the CK stock, "Migrant", and "Non-migrant" lines were used, as moths from these lines had approximately similar rates of ovarian development (Table 4.2). All data on moths from the KF line were excluded from the analysis. The results are given in Table 4.3. Moths immature at 48h had been lighter on Day 1 (mean 68.7mg) than the mature moths (mean 78.1mg), but this was not a significant difference. Moths immature at 72h were slightly heavier on Day 1, but again the difference was not significant.

4. DISCUSSION.

4.1. The effect of age, feeding, and mating on flight performance.

The series of experiments on the effect of feeding and mating on flight capacity of female moths on Night 4 demonstrated that the proportion of moths giving migratory flight declined significantly between Nights 1 and 4, but only when the initial Night 1 sample contained a relatively high proportion of migrant individuals (Experiments 1 and 2). This decline in flight capacity occurred irrespective of whether the moths had fed and/or mated in the intervening period. When the proportion of Night 1 migrants was low (Experiment 3), the proportion of Night 4 migrants was also low. With the exception of two moths in Experiment 3 (one mated and one unmated), all those moths (seven individuals) which did show migratory flight on Night 4 had also been classified as migrants on Night 1. This would suggest the tentative conclusion that only genotypical migrants were capable of sustained flight on Night 4. This decline in flight capacity with age over the first few days of adult life is in marked contrast to the pattern of behaviour shown by many other migrant insects (see Introduction), including some Lepidoptera. Kishaba et al. (1967) found that 3-4 day old moths of the cabbage looper Trichoplusia ni flew more consistently than 0-2 day old moths, and Hackett (1980) found that flight capacity in Heliothis armigera reached its peak on the fourth night after emergence. Tethered flight studies made by Hwang and How (1966) on the Oriental armyworm moth Mythimna (=Leucania) separata indicated that maximum flight duration was attained in 3-4 day old moths; these latter results concur with those of Kanda and Naito (1979), who found that M.separata only became active on the third night after

emergence. This apparent discrepancy between the behaviour of S.exempta and other moths is probably only a reflection of the fact that S.exempta is capable of migration earlier in life than the above species, and also possibly reaches ovarian maturity faster.

Within the context of flight capacity, providing moths with a source of food (20% sucrose solution) on Nights 2 and 3 allowed a greater number of moths to survive until Day 5, and was also necessary for moths to be capable of sustained flight on the flight balances on Night 4 (whether or not the long flights recorded on Night 4 are "migratory" is discussed below). These observations thus provide support for the suggestion made by Aidley (1974) that moths could migrate for more than one night if they had access to nectar, although this probably only applies to flight on Nights 3 and 4 as starved moths were shown to be capable of long flights on Night 2 (Experiment D). If moths were not provided with sucrose (Experiment 1), relatively few moths survived until Day 5, and none gave migratory flights on Night 4 (Fig.4.1). However, the validity of Experiment 1 in terms of field conditions is probably limited. Rose and Dewhurst (1979) reported that moths emerging from outbreaks flew in to trees at dusk or on emergence, where they fed avidly on blossoms (Dewhurst,1982), the inference being that nectar may often be available in the field. Purely in terms of adult survival, water availability may be just as important as the availability of nectar, as dehydration rather than reserve depletion is the main reason for high mortality in moths deprived of adult food (Dr A.Gunn, pers.comm.).

Whether adult food was metabolized into a flight fuel, or whether it simply enabled basic metabolic processes to be maintained is not entirely clear. The types of substrates that migrant insects use as

flight fuel varies (reviewed by Blem,1980), but the available evidence suggests that most Lepidoptera utilize lipid. Van Handel (1974) found that lipid content of Spodoptera frugiperda moths was considerably reduced when they were flown to exhaustion, although this contrasted with earlier results when lipid content did not significantly diminish during flight (Nayar and Van Handel,1971). Some Lepidoptera are thought to convert dietary sugars into lipid for use as flight fuel. This type of pathway was demonstrated in the migratory monarch butterfly Danaus plexippus plexippus L. (Brown and Chippendale,1974) and is also thought to occur in Spodoptera (cited as Prodenia) eridania (Stevenson,1968). This association between carbohydrate and lipid metabolism must be essential to those Lepidopteran species which do apparently need to feed as adults in order to increase their flight capacity (Koerwitz and Pruess,1964; Kishaba et al.,1967-see Introduction). A similar situation may occur in S.exempta, as moths fed 10% sucrose show no drop in total lipid levels (unlike starved moths or those fed only distilled water), indicating that lipid is being synthesized at the same rate as it is utilized (Dr A.Gunn, pers.comm.). However, it should be stressed that adult feeding is not essential for migration on Night 1 in S.exempta (all experiments in Chapters 2 and 3 were carried out using starved moths), and, as mentioned above, the results from Experiment D showed that moths were also capable of migrating on Night 2 without having fed. This suggests that moths utilize reserves carried over from the larval and pupal stages as flight fuel, which are probably principally lipids. It has been shown that flight capacity is directly related to lipid content in some insects. Cockbain (1961) demonstrated this for Aphis fabae, and Atkins (1966) found a positive correlation between the fat

contant of individuals of the douglas fir beetle Dendroctonus pseudotsugae Hopk. and their ability to fly. In insects showing alary polymorphism, macropters also often have higher lipid levels than brachypterous forms. This has been demonstrated for the cowpea weevil Callosobruchus maculatus (Nwanze et al.,1976) and the brown planthopper Nilaparvata lugens (Padgham,1983). However, it is not clear if migrant S.exempta moths have higher lipid levels than non-migrants, or whether lipid levels are significantly depleted during sustained flight.

Mating does not appear to inhibit or enhance migratory flight in S.exempta, as the flight results obtained from the mated and umated groups of moths in Experiment 2 were virtually the same. However, the samples of moths were small. Hackett (1980) also found that mating had little effect on the flight capacity of 4-day old Heliothis armigera moths. However, these results contrast with the behaviour of the European pine shoot moth Rhyacionia buoliana which does not fly until it has mated (Green and Pointing,1962). Mating in S.exempta did have the effect of shortening the pre-oviposition period by stimulating oviposition (though not ovarian development), as virtually all mated moths oviposited on Night 3, and no unmated moths laid eggs on Nights 3 or 4. Similarly, delayed mating extended the pre-oviposition period of Spodoptera littoralis (Ellis and Steele,1982), and Traynier (1983) found that unmated females of the potato moth Phthorimaea operculella (Zell.) laid few eggs compared to mated ones.

The results obtained in the experiments on feeding and mating on flight provided somewhat ambiguous evidence on the question of whether or not migratory flight in female S.exempta moths was terminated when ovarian maturity was attained. The one unmated moth from Experiment 2 which gave migratory flight on Night 4 was found to be immature when

dissected on Day 5, which is consistent with the hypothesis that only immature moths migrate on Night 4, and that ovarian maturity inhibits migration. Similarly, flight duration in Mythimna separata declined rapidly when moths reached ovarian maturity (Hwang and How,1966), although these authors did not report whether isolated individuals continued to give long (migratory) flights. However, one unmated Night 4 migrant from Experiment 2 was found to contain mature eggs on Day 5. Further, and more significantly, all the mated Night 4 migrants from Experiments 2 and 3 had oviposited on Nights 3 and 4, although the number of moths involved was small. The important question is whether those mature moths classified as migrants on Night 4 were indeed bona fide migrants, or whether the flight observed was qualitatively different from that observed on Night 1. Dingle (1965) identified high threshold, long duration migratory flights and short duration, low threshold non-migratory flights in Oncopeltus fasciatus, and found that flight threshold declined with age. Flight patterns also changed with age in females of the Rutherglen bug Nysius vinitor. Flight was migratory in immature bugs, but mature individuals made only short, broken local flights (Kehat and Wyndham,1973). The sporadic pattern of flight activity recorded for some mated moths in Experiment 3 could be considered as reflecting short-range flights of the type mentioned above, although it is possible that this broken flight pattern was due to the fact that the counterweight given to these moths was only 50% of their initial weight rather the the normal 60%, and consequently moths were failing to consistently generate enough lift to keep the foil flag out of the photo-detector. However, those moths classed as migrants on Night 4 all gave unbroken flights typical of those observed on earlier

nights, although again it is possible that this flight was qualitatively different from that observed on Night 1; on Night 1 moths had not begun to oviposit, whereas on Night 4 the majority of individuals were actively ovipositing (and in the field would also possibly be calling for a mate). The flight pattern of moths on the flight balances was almost certainly influenced by this behaviour, and "long" flights may not have been migratory in a behavioural sense. However, significant flight during the oviposition period is not uncommon in insects. Females of the spruce budworm moth Choristoneura fumiferana cannot fly until they have oviposited, and will then fly actively upwards to take advantage of convective transport (Wellington and Henson,1947). The frit fly Oscinella frit L. makes several long flights in the pre-oviposition period as well as between laying batches of eggs (Rygg,1966), and the stilt bug Jalysus spinosus also continues to fly after the onset of oviposition (Elsey,1974). A possible explanation for the behaviour observed in S.exempta is that in the majority of individuals, ovarian maturity does inhibit further migration, since significantly fewer moths classified as migrants on Night 1 gave migratory flight on Night 4, and most were mature by this time. Observations made on light-trap catches of S.exempta moths by Whellan (1958) also suggested that only immature moths migrate; on one occasion, a trap sample contained an even sex ratio of moths, and the females were found to be immature. This was taken to mean that a migrating swarm of moths was passing through the area. However, on another occasion, mainly males were caught when oviposition was occurring in the vicinity of the trap, and it was assumed that mature female moths had finished migrating, were busy ovipositing, and thus not attracted to the light. However, it is possible that a few genotypic migrants may retain the capacity for

sustained flight even after maturity is reached.

The implications arising from the number of nights on which moths have the potential to migrate are of some importance in tracing the origins and possible destinations of moths caught in the light and pheromone trap network in East Africa. Tucker et al. (1982) calculated backward and forward tracks of moths flying within wind-systems on the assumption that female moths would fly for no more than one and a half nights before being caught in light-traps. The results obtained in this study suggest the possibility that some moths may be capable of migratory flight for up to four nights, and more significantly, that some have the potential to fly long distances after the first night of oviposition. However, further work is required to clarify this point.

4.2. The association between ovarian development and flight capacity.

The results obtained in Experiment A on the varying rate of ovarian development in S.exempta were similar to those obtained by Page (1982), and confirmed that arrested ovarian development also occurred in the cultured insects maintained at UCNW Bangor. Arrested ovarian development is a relatively common phemomenon amongst insects, occurring in a number of orders. Sams (1975) reported vitellogenic arrest stimulated by starvation in the cockroach Blatta orientalis L. characterized by cessation of yolk uptake and oocyte growth, and the mosquito Aedes aegypti arrested ovarian development two days after adult emergence unless fed a large blood meal (Lea et al.,1978). The large variation in age at the onset of calling (2-10 days) in the armyworm moth Mythimna (cited as Pseudaletia) unipuncta (Haw.) was also tentatively linked with maturation rate (Turgeon and McNeil,1982), but the association was not demonstrated experimentally. Oosorption can also occur. Females of the

screw-worm fly Chrysomya bezziana Villeneuve mature all their oocytes, but up to 30% may be resorbed if not enough protein is available to develop all eggs (Spradbery and Schweizer,1981). However, it is not known if this occurs in S.exempta. Possible explanations for the observed variation in oocyte development rate in S.exempta are discussed below.

The failure to find any direct association between rate of ovarian development and flight capacity on Night 1 (Table 4.1) was suprising in view of the often close links between migration and ovarian development in insects (see Introduction). The fact that no significant differences could be found in rates of ovarian development between the CK "Migrant" and "Non-migrant" lines (Table 4.2) suggests that a direct genetic link between rate of ovarian development and flight capacity is also unlikely. A study of the genetic co-variance between migration and rate of ovarian development, of the type carried out for various life-history traits of the milkweed bug Oncopeltus fasciatus by Hegmann and Dingle (1982), would help to clarify this point. The fact that a significantly slower rate of ovarian development was observed in the KF line when compared to the CK stock line at 48h but not at 72h is somewhat anomalous, but emphasises the degree of variability in the rate of ovarian development within lines. It also implies that there may be a small genetic component involved in rate of ovarian development which is independent of migration. This type of situation was also envisaged by Hillyer and Thorsteinson (1969), who suggested that some females of the diamond-back moth Plutella maculipennis were genetically programmed to be reproductive at emergence in spite of experiencing larval conditions which normally extended the pre-reproductive period.

The results from Experiment D confirmed that no direct link could be established between rate of ovarian development and flight capacity. All those moths which gave migratory flight on Night 2 were (with one exception) derived from the CK "Migrant" line, and were therefore genotypic migrants. Virtually all these moths (with one exception which again raises questions about the nature of flight in older moths, see above) were immature at 62h (Day 3). Thus these moths had the potential to migrate on Nights 3 and 4 unless migration was inhibited by ovarian maturity. Those moths which had not migrated on Night 2, but which were either mature or immature at 62h, were likely to be genotypic non-migrants, as most were derived from the CK "Non-migrant" line. Taken in conjunction with the results from Experiments B and C, the evidence thus suggests that the main effect of delayed ovarian development in terms of flight capacity is to extend the time available for migration in those moths with the genotypic potential to migrate on several nights.

It is apparent from the above discussion that factors which govern the duration of the pre-oviposition period in S.exempta are likely to be of central importance in controlling the number of nights over which individual moths could potentially migrate. It should also be stressed that these same factors also govern the duration of the pre-oviposition period in non-migrants. Recent work has shown that if moths were not given access to food or water on emerging, then few moths were mature at 48h post-emergence. If, however, those moths were fed either sucrose solution or water, then most matured by 48h (Dr A.Gunn, pers. comm.). It was originally thought that the availability of larval food was also important in determining rate of ovarian development, and this was the reason for the study of Day 1 weights of moths found to be mature or immature at 48 and 72h (Table 4.3). The fact that no significant weight

differences were found between mature and immature moths also indicates that larval food deprivation, despite the fact that it results in significantly reduced adult weights (Fig.2.14), does not result in arrested ovarian development. However, total egg production of moths was reduced when larvae were deprived of food (Dr A.Gunn, pers. comm.). Thus larval conditions and availability of moisture are likely to be the main factors governing the duration of the pre-oviposition period and fecundity in S.exempta. Larval food quantity and quality have been shown to be important in ovarian development in other Lepidoptera. Lukefahr and Martin (1964) found that Heliothis zea reared on cotton squares (which provide poor nutrition) and starved as adults did not lay any viable eggs, and Hillyer and Thorsteinson (1969) found that poor food quality and high larval density extended the pre-reproductive period in Plutella maculipennis. The ovarian capacity and daily egg production of the butterfly Heliconius charitonius (L.) is also probably largely determined by the extent and/or quality of larval nutrition (Dunlap-Pianka,1979). Water availability has been found to be of importance in egg production in other insects. Jacobson (1965) found that the pale western cutworm moth Agrotis orthogonia Morrison laid more eggs when fed sugar solutions or water, and Derr (1980) found that water was necessary for proper egg development in Dysdercus bimaculatus. However, Heliothis virescens laid five times as many eggs when adults were fed on sugar as opposed to water (Lukefahr and Martin,1964). It is not yet clear precisely how much water availability varies in the field, or what quantities moths are required to imbibe in order to complete ovarian development. Dew is often available in the field, but the dew-point may occur at a temperature which inhibits flight, thus preventing moths from

reaching it.

4.3. Conclusions.

The relationships between age, feeding, mating, and ovarian development, and the way in which they relate to migration in S.exempta, are complex and are currently the subject of intense study. Consequently the situation as it is described in this chapter is likely to alter rapidly. However, the evidence so far accumulated allows certain tentative conclusions to be drawn. Essentially, mating and feeding appear to be largely unimportant in determining whether or not migration occurs in older moths, although adult feeding is necessary if moths are to survive for long enough to migrate on Nights 3 and 4. The association between age and flight capacity is almost certainly linked with the state of ovarian development, and there seems little doubt that in those moths with the genotypic potential to migrate, ovarian maturity does inhibit migration leading to a consequent decline in flight capacity with age in the majority of individuals. However, it is not yet clear if this is invariably the case, and it is possible that a few genotypical migrants may retain the capacity to migrate even once ovarian maturity has been attained. The factors which affect the duration of the migratory period in males also require further study, and it is possible that such a study would help to clarify the situation with respect to females.

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GENERAL CONCLUSION.

1. General considerations.

The data accumulated during this study have provided a substantial contribution to the understanding of the factors controlling migration in *Spodoptera exempta*, and the significance of the associations between migration and other aspects of the biology of the armyworm have also been highlighted.

The applicability of the results obtained in this study to the field biology of *S.exempta* is a vital consideration. Comparisons between the laboratory results obtained using cultured insects in this study and similar field observations have generally been favourable (e.g. Chapter 1 sections 4.2 and 4.4, and Chapter 2 section 4.1). Therefore for the remainder of this discussion, it is assumed that the laboratory results obtained during this study are sufficiently representative of field behaviour, providing comparisons are not made too specifically, to allow valid conclusions to be drawn about the field biology of *S.exempta*.

The aim of this General Conclusion is to draw together relevant results from the preceeding chapters, and, within the framework of known field biology, to present a hypothetical model for the control of migration in *S.exempta* with specific reference to the East African region.

2. The control of migration in S.exempta in East Africa.

At the outset, it should be stated that the main thrust of the hypothesis presented below is that the whole life-history strategy of *S.exempta* is adapted to maximizing dispersal in the wet season, and maintaining populations in restricted suitable habitats during the dry

season; consequently the importance of "hidden" low density populations (Rose,1979,1982) in the population dynamics of S.exempta is emphasised.

In order to construct a hypothetical model for migration in S.exempta, several assumptions have been made about the control of migration based on the results obtained in this study. These assumptions are:

(a) flight capacity of any particular moth is ultimately determined by its genotype. Three genotypic groups of moths are envisaged as being distinguishable; genotypic (obligate) migrants, facultative migrants, and genotypic (obligate) non-migrants (Chapter 3);

(b) the only significant environmental component involved in the control of migration is the density experienced by larvae during their development (Chapter 2). High larval density throws the phenotypic switch between migratory and non-migratory patterns of behaviour in facultative migrants. Other environmental cues for migration may exist, but it is difficult to envisage what these might be. The most likely environmental factors which act on the larvae have now been examined (Chapter 2), and as the adults are short-lived and may commence migration soon after emergence, any environmental migratory cue acting directly on this stage has little or no time in which to operate; thus the likelihood of such cues providing an adequate prediction of future environmental trends is slight. It is possible that adult feeding soon after emergence (moths fly into trees and feed before taking off, Dewhurst,1982; Rose and Dewhurst,1979) may have some significance in terms of the control of migration, but since adult feeding in older moths has been dismissed as unimportant in determining whether or not an individual will be a migrant (Chapter 4), this possibility seems unlikely;

(c) migratory flight in females is generally inhibited when ovarian

maturity is attained (Chapter 4), and consequently in those individuals with the genotypic potential for migratory flight, factors which affect rate of ovarian development determine the time available for migration. Variations in rate of ovarian development (Page,1982) occur as a result of varying availability of adult food or water (Dr A.Gunn, pers.comm.). Lack of larval food and high larval densities tend to reduce adult weight, and although this results in reduced fecundity, rate of ovarian development is not affected (Dr A.Gunn, pers.comm.).

Migration in S.exempta is envisaged as occurring on a large scale only during the wet (outbreak) season. During the dry (off) season, populations exist only at low densities in isolated and topographically restricted habitats (Page,1982, Persson,1981,-see General Inroduction), where sufficient green grass remains to support larval development. Larvae in these habitats are in the passive phase, and this is thought to be the usual form of the insect (Khasimuddin,1981; Rose,1975). Because potential habitats are relatively restricted in size and geographically isolated during the dry season, emigration from these areas is strongly selected against because emigrants are unlikely to locate suitable habitats. However, larval densities are not sufficiently high to cue migration in facultative migrants, and so, although there are likely to be a few genotypical migrants in the air at times, the numbers involved are likely to be small. Passive phase larvae are well adapted to surviving predation and disease through their isolation and cryptic colouration and behaviour (Whellan,1954), and, consequently, are inconspicuous even at relatively high absolute densities (Rose,1975). Temperatures are likely to be low in areas where low density populations persist during the off season, leading to slow larval development

(passive phase larvae develop more slowly than active phase larvae anyway, Khasimuddin, 1981) and a consequently low rate of population increase due to the extended generation times.

At the onset of the rains, the potential range of the armyworm undergoes a massive and rapid expansion, as previously dry areas again become suitable for larval development (Rose,1982). The selection pressures acting on migrant moths during the wet season are the reverse of those operating during the dry season, as it is now advantageous to leave the current habitat during the wet season in order to colonize widely available new habitats. Hughes (1979) pointed out that the success of facultative migration is dependent upon the timing of movement. He suggested that the strategy of leaving the initial habitat while its quality is still high is not effective, and it is better to delay movement and, by breeding, increase migrant numbers. However, the habitat range of S.exempta during the rains is so large that any emigrant is likely to be favoured. At the onset of the rains, the general increase in temperature enables faster larval development, and the immediate colonization of newly appeared habitats results in an overall increase in population size, even though absolute larval densities may not increase dramatically. The rate of increase in the population at any particular location dependent, on the abiotic and biotic factors affecting rate of development and mortality in that area (Rose,1982). The increased overall population levels result in more genotypical migrants being airborne, thus resulting in a more rapid rate of habitat colonization. Van der Eijk (1983) found that the beetle Gyrinus marinus Gyll. only took off when weather conditions were favourable, and this emphasises the point that the precise number of potential migrants in any particular local population of S.exempta which

actually take-off on migratory flight, and the duration of this flight, will probably also depend on local climatic conditions, particularly temperature (Brown et al.,1969).

It is apparent from the above discussion that the growth of S.exempta populations at the the start of the wet season results in a dramatic increase in the numbers of migrant moths in the air. The direction of movement and the consequent distribution of these migrants moths is envisaged as being influenced by prevailing meteorological conditions, particularly wind strength and direction, in two ways. The first possible effect, as recent work on the likely distribution of moths from outbreaks in relation to wind direction has shown (Tucker et al.,1982), is for moths to become distributed over a wide area. This type of dispersal enables the topographically extremely extensive suitable habitats which are available during the rains throughout the whole range of S.exempta to be re-colonized by migrant moths. It also ensures that the areas where low density populations survived during the previous dry season receive a fresh input of new genetic material, thus ensuring that "migratory" alleles, which would have been depleted to a certain extent from such populations during the dry season due to the emigration of genotypic migrants, are re-introduced in a completely new population. The genetic heterogeneity of low density populations at the start of the dry season must therefore be sufficient to ensure that any loss of "migratory" alleles from the population during the dry season is not so great as to result in an inability to produce migrants at the start of the next wet season. However, the slow rate of larval (and pupal) development during the dry season means that relatively few generations are required to survive the dry season, and therefore the loss of

"migratory" alleles is not likely to be great.

The second possible effect of wind-systems on population distribution is the concentration of moths by convergent wind-systems. This process has been studied in detail, is well documented (see General Introduction), and is generally accepted to be the mechanism which brings sufficiently large numbers of moths into one area to produce an outbreak. Larval densities in outbreaks are high, and therefore the majority of larvae are in the active phase. This is considered to be the atypical form of the larva (Khasimuddin,1981), and because population densities are so high and larvae so conspicuous, the insects are vulnerable to attack by pathogens and predators. Consequently, the behaviour of active phase larvae is probably adapted to completing their development as rapidly as possible. For example, their black colouration is likely to result in absorption of radiant energy which helps to speed their growth.

In terms of migration, the use of the density experienced by larvae during their development as an environmental cue to trigger facultative migration can be seen to have adaptive significance if the dispersal of high density populations into widely available new habitats is the major consideration rather than the prediction of future environmental quality in situ, as it ensures that a greater proportion of the population leaves the current habitat when population densities are high. A possible reason for the apparent lack of any other environmental cues for facultative migration during the rains may be that the costs of migration, in terms of mortality at least, may be fairly low during the wet season because of the ubiquitousness of suitable habitats.

At the end of the rains; the potential habitat range of S.exempta again begins to shrink, and in many areas low density populations which

became established during the wet season will die out. However, the strategy of dispersing over the entire range during the wet season ensures that populations become established in areas which will remain suitable for development throughout the dry season, and accounts for the wide gene mixing which occurs over the East African range of S.exempta (den Boer,1978). The fact that migration occurs very early in adult life, and that mating is post-migratory, is further circumstantial evidence for the existence of wide gene mixing. The fact that those areas suitable for larval development during one dry season may not necessarily be the same areas which remained favourable during the previous dry season also emphasises the need for wide-ranging dispersal.

A similar situation to the hypothesis described above is also thought to occur in Californian populations of the beet armyworm, Spodoptera exigua. Permanent establishment is restricted to areas with sufficiently mild winters to allow survival, and migration permits re-invasion during favourable periods of areas where overwintering does not occur (Hogg and Gutierrez,1980), although it is not clear if a return migration is necessary in order to maintain the populations in permanent breeding areas. The dispersal strategy of the black swallowtail butterfly Papilio polyxenes Fabr. described by Blau (1980) also has some similarities with the dispersal pattern envisaged for S.exempta during the wet season. Year-round breeding and dispersal by P.polyxenes allows colonization of new ephemeral habitat patches, which provide a continual supply of temporary refuges from natural enemies and high intra-specific population densities. In S.exempta, migration and larval phase variation play a major role in distributing the population into new (though not necessarily ephemeral) habitats, where low density populations might be

expected to evade attack by parasites, predators, and pathogens (see above).

The idea that the East African populations of S.exempta can be regarded as a series of relatively high density, mobile regional populations which are continually re-distributed and re-concentrated by convergent wind-systems (Rainey,1982, Rainey and Betts,1979-see General Introduction) is probably unrealistic, largely because it assumes that passive phase larvae (and hence, by definition, low density populations) play no major role in the population dynamics of S.exempta. This is inconsistent with the data now available which support the existence of widespread low density populations, which are thought to persist in the same locations throughout the year in many areas (Nyierenda,1982; Page,1982; Persson,1981-see General Introduction). The suggestion that populations move in discrete groups also pre-supposes that the majority of moths in a given population invariably migrate. However, the data obtained in this study have shown that many moths are incapable of sustained "migratory" flight, and even in those moths classifiable as migrants, flight capacity between individuals varies considerably. This pattern of behaviour can be viewed as a further mechanism for ensuring that populations (particularly high density ones) are quickly dispersed, as it inevitably means that a certain proportion of the population will remain in the general area of emergence. Differences in flight capacity further ensure that migrant moths originating from one area will, even in the presence of concentrating meteorological factors, often tend ultimately to oviposit in separate locations. The suggestion that low density populations play an important part in the population dynamics of S.exempta also obviates the need to postulate a "return" southward migration (for which the evidence is scanty, Brown et al.,1969) e.g.

from Ethiopia and Somalia into Kenya, Uganda, and Tanzania. A "return" migration of this type is a necessary corollary of the hypothesis that populations move in discrete groups. There is every reason to suppose that low density populations exist in the highlands of Ethiopia, and that the hypothetical model described holds good throughout the main range of the insect.

The outbreaks of S.exempta which occur irregularly in the Yemen could be regarded as a special case, as the climate does not permit year-round survival and the source moths for the outbreaks which do occur have to migrate across the Red Sea from Africa. Under the hypothesis for migration envisaged, Yemeni populations of the African armyworm should therefore contain a high level of "migratory" alleles. An attempt was made during this study to obtain insect material from the Yemen Arab Republic in order to test this hypothesis, but as yet no insects have been received.

It should be stressed that the arguments described above are not intended to denigrate the importance of wind-influenced movement in the population dynamics of S.exempta, as moth movements within wind-systems do play a vital role in the initiation of outbreaks, and aid in the dispersal of moths over a wide area, thus enabling the re-colonization of new habitats during the wet season. Further, as Joyce (1981) inferred, wind-systems may affect the distribution of moths making non-migratory as well as migratory flights. However, the precise function of movements of large numbers of moths within wind-systems does need to be re-evaluated and placed more within the context of current thinking on the biology of S.exempta. For example, the concentration of moths leading to high density populations of active phase larvae could be

regarded as helping to greatly accelerate the rate at which moths were dispersed into new habitats at the start of the wet season; high larval density results in the production of facultative migrants, thus increasing the number of potential colonizers, and the faster rate of development of active phase larvae results in the faster production of these migrants.

The differing rates of ovarian development observed in both laboratory and field populations can also be viewed as adaptations to aid population dispersal. By having an association between the duration of the pre-oviposition (or more accurately the pre-reproductive) period and the availability of nectar or water to the adults, both migrant and non-migrant moths with a range of pre-oviposition periods are produced. In the case of migrants, this means that moths with the genotypic potential to migrate on more than one night will actually fly for a varying number of nights, which again will tend to enhance dispersal further. For non-migrant moths, it means that moths will start ovipositing on different nights, which will tend to de-synchronise the development of subsequent generations in that area.

It should also be stressed that inter-reproductive migration cannot entirely be ruled out on the data currently available. There is no firm evidence either for or against its existence, and further work is necessary in order to ascertain whether or not it occurs. If inter-reproductive migration does occur, it would be an efficient means of effecting further population dispersal.

3. Implications for control operations.

In terms of crop protection against insect attack, immigration of a pest species into a crop, and its reproductive potential on arrival, is

usually more important than its pattern of emigration (Joyce,1981). However, when dealing with a highly mobile pest such as S.exempta, the pattern of emigration is also important, as it may determine the likelihood and/or severity of future attacks. A knowledge of the factors controlling migration in migrant pests is therefore important in determining exactly when and where the pest population is likely to reach damaging levels.

The control of migration in S.exempta appears to be largely independent of environmental factors, and therefore there is little possibility of identifying populations which are likely to produce high proportions of migrants on the basis of past or prevailing climatic and /or other environmental features in that particular location. Nevertheless, it is possible that the identification of a threshold density above which the phenotypic switch for migration is thrown may aid in predicting the level of migratory activity likely to result from a given population, although it seems possible that this might vary between populations. The greater understanding of the factors controlling migration provided by this study may also be of value in helping to improve the accuracy of the armyworm forecasting system.

The current aim of the regional control strategy is to control the first outbreaks of the season in the hope that this will delay the build up and dispersal of armyworm populations, and so reduce the incidence and scale of outbreaks later in the season (Rose,1982-see General Introduction). Thus the current regional control strategy is based on a model of the type described above, and this work therefore provides strong evidence in support of the methods currently being employed to control S.exempta on a regional basis.

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APPENDIX ONE.

1. Vitamin and antiobiotic mixture for the artificial diet.

Weigh out and mix together the following vitamins:

	g
Nicotinic acid	5.0
Calcium pantothenate	5.0
Riboflavine	2.5
Aneurine hydrochloride	1.25
Pyroxidine hydrochloride	1.25
Folic acid	1.25
D-biotin	0.1
Cyanocobalamine	0.01

Next take:

1g of the vitamin mixture

Add 2g of Streptomycin

18g Aureomycin (veterinary soluble powder)

40g Ascorbic acid

Mix well and store in a refrigerator.

2. Procedure for egg and pupal surface sterilization.

1. Submerge in 0.1% sodium hypochlorite for 10 minutes.
2. Rinse in distilled water five times.
3. Submerge in 10% formalin for 40 minutes.
4. Rinse in distilled water five times.
5. Allow the water to evaporate.

For pupal sterilization, only steps 1 and 2 are necessary.

3. Pupal and adult weights (mg) of insects reared on artificial diet.

Pupal weight		Adult weight	
Male	Female	Male	Female
161.5	143.6	87.3	102.6
112.1	100.9	63.5	74.7
105.4	166.1	51.2	122.3
155.1	111.7	106.0	57.7
143.5	150.4	86.4	63.3
119.4	97.9	81.7	115.1
107.4	107.1	145.0	134.8
191.8	169.6	131.1	
189.1	206.3		

Means given in Table 1.2.

4. Mean female pupal and adult weights (mg) of insects in the maize culture; each mean represents insects from one generation.

Average pupal weight	Average adult weight
136.56 (n=13)	88.13 (n=9)
124.39 (n=30)	71.93 (n=24)
118.76 (n=32)	70.24 (n=29)
104.11 (n=40)	63.96 (n=37)
122.03 (n=31)	73.27 (n=27)
140.86 (n=17)	90.21 (n=13)
151.02 (n=56)	89.61 (n=47)
148.65 (n=19)	86.61 (n=17)
132.09 (n=48)	80.69 (n=46)
125.46 (n=28)	76.31 (n=28)
122.39 (n=37)	74.05 (n=31)
148.97 (n=23)	96.35 (n=13)

The above values are plotted in Fig.1.3. The means of the 12 average values are given in Table 1.2.

APPENDIX TWO.

1. Morphometric data on moths reared at different larval densities.

BL=body length, FWL=fore-wing length, FWW=fore-wing width, HWL=hind-wing length, HWW=hind-wing width. All lengths in mm.

(a) Moths reared at 10 larvae/jar (T10 treatment).

Day 1 wt.(mg).	BL	FWL	FWW	HWL	HWW
88.1	13.3	13.6	5.3	10.8	8.8
82.5	14.4	15.1	7.0	11.6	10.3
90.0	13.8	12.8	6.1	10.3	8.3
103.6	13.5	15.7	7.0	11.4	8.0
109.2	13.7	13.9	5.6	11.2	9.3
117.3	15.8	16.2	7.0	12.8	11.3
95.4	14.6	13.9	7.2	11.5	9.9
71.3	14.0	14.3	7.0	11.3	10.3
84.3	13.4	14.0	6.7	11.0	9.7
89.8	13.5	13.9	6.5	10.1	7.2
81.6	14.1	14.2	6.4	11.2	8.0
77.5	13.0	14.6	6.1	10.9	8.9
68.0	13.2	13.8	5.9	10.5	8.0
72.0	13.2	13.9	6.9	10.3	8.8
93.9	14.9	14.0	7.3	11.4	9.8
72.1	14.8	15.0	6.6	11.7	9.7
96.5	14.9	15.0	6.8	11.3	10.0
69.3	13.0	14.8	7.3	11.1	9.9
72.0	13.5	13.1	7.2	9.9	9.7
95.9	13.8	14.5	6.9	11.9	10.2
79.5	13.9	13.8	6.9	10.8	8.4
57.8	12.2	13.0	6.0	10.4	7.2
66.2	13.0	13.9	7.0	10.7	9.0
76.7	12.9	13.8	6.9	10.3	8.8
73.6	13.7	13.9	6.6	10.8	9.9
77.3	13.0	13.9	6.3	10.3	8.1
69.2	13.0	14.1	6.9	11.0	8.9
91.3	14.0	14.2	7.0	11.2	8.5
65.6	12.4	11.9	6.0	9.4	8.0
74.0	12.9	13.0	6.8	10.2	8.9

(b) Moths reared at 20 larvae/jar (T20 treatment).

Day 1 wt.(mg).	BL	FWL	FWW	HWL	HWW
77.8	13.8	14.2	6.9	10.8	9.4
92.8	13.2	14.0	4.9	11.0	7.8
102.1	13.6	15.7	5.9	11.9	6.3
90.0	13.7	15.0	5.3	11.3	9.4
84.1	12.8	14.0	5.7	10.6	10.4
81.5	13.6	14.3	6.2	9.8	7.5
82.5	13.4	15.7	6.5	11.0	9.9
70.8	13.5	14.3	7.1	10.6	9.3
63.8	13.0	14.0	7.2	9.8	9.2
111.0	13.9	15.0	7.3	11.3	11.0
83.4	12.6	13.9	6.9	11.0	10.1
83.4	12.8	13.9	6.1	10.8	9.5
85.0	13.1	14.2	6.8	10.3	9.8
76.9	13.2	14.2	6.7	11.0	8.8
72.5	14.0	14.2	7.3	10.7	9.9
63.4	13.0	14.4	7.6	10.6	9.0
64.8	13.9	14.7	7.8	10.6	10.3
68.9	12.5	14.8	7.8	11.3	8.5
53.4	12.8	12.9	6.3	10.5	9.6
51.9	12.9	13.9	7.0	10.7	9.5
59.0	12.3	14.5	7.2	10.7	9.5
69.4	13.1	14.6	7.5	10.9	9.8
72.1	13.5	14.5	7.0	10.8	10.2
77.7	13.0	14.6	7.7	11.3	10.5
71.1	13.7	13.2	7.0	9.9	8.8
62.2	12.3	12.9	6.9	10.5	8.3
64.5	12.9	12.9	6.8	9.6	7.8
41.5	11.0	11.9	6.3	9.1	7.8
74.7	13.5	13.7	7.2	10.9	9.0
62.4	12.6	13.0	6.7	10.3	8.7
50.7	12.4	12.3	6.9	9.3	8.7
76.0	12.3	14.0	6.9	10.3	7.5

(c) Moths reared at 40 larvae/jar (T40 treatment).

Day 1 wt.(mg).	BL	FWL	FWW	HWL	HWW
66.0	13.8	14.6	6.9	11.0	9.5
73.4	12.8	14.2	5.7	11.2	7.8
54.2	12.2	13.3	7.3	10.1	8.3
55.0	12.9	13.9	5.5	10.6	7.8
61.0	14.0	13.7	6.2	10.9	9.9
43.1	12.9	12.5	5.4	9.6	8.4
53.5	12.9	13.2	7.1	9.8	9.6
56.5	12.5	13.4	6.9	10.1	8.7
52.7	12.4	13.4	6.5	10,2	9.7
57.0	10.4	14.0	6.5	10.7	11.0
72.0	13.7	14.5	7.2	11.2	8.5
73.1	13.0	14.1	7.2	11.3	9.7
82.3	13.1	13.8	7.3	10.7	9.4
68.9	12.5	14.6	6.3	11.4	8.4
58.2	13.5	14.6	6.6	11.0	8.6
57.7	13.3	14.4	6.9	10.5	7.1
84.5	14.4	15.5	8.0	12.0	11.3
53.8	12.8	12.1	5.7	10.1	9.0
76.3	14.6	14.9	7.0	11.4	9.1
54.2	12.1	14.1	6.8	10.0	9.5
75.7	12.4	13.2	7.3	10.3	8.4
44.4	11.6	12.0	6.1	9.5	7.4
61.9	13.3	13.7	7.5	10.8	9.4
45.0	12.7	12.7	6.5	10.8	8.2
59.4	12.0	13.5	7.0	10.2	7.5
53.9	12.1	13.3	6.8	9.8	8.6

2. The percentage of moths classified as migrants (total flight >120m) on Night 0 and Night 1. Data on moths from the larval food deprivation experiments (see Chapter 2, section 2.2.3).

Treatment	Night 0 migrants (%)	Night 1 migrants (%)
8h food deprivation (n=23)	4.4	8.7
Control (n=23)	0	21.8
4h food deprivation (n=20)	5.0	20.0
Control (n=15)	0	13.4
6h food deprivation (n=22)	0	31.8
Overall (n=103)	1.9	19.4

APPENDIX THREE.

1. The percentage of female migrants (total flight >120m on Night 1) in the CK and KF "Migrant" and "Non-migrant" lines (Figs 3.2 and 3.4).

Generation	CKA "Migrant" line	Generation	CKN "Non-migrant" line
2	53.3 (n=15)	P	31.3 (n=16)
3	75.0 (n=12)	1	47.4 (n=19)
4	69.2 (n=13)	2	45.5 (n=11)
5	80.0 (n=20)	3	77.8 (n=18)
6	52.2 (n=23)	4	no selection
7	no selection	5	no selection
8	80.0 (n=21)	6	41.7 (n=24)
9	73.7 (n=19)	7	22.7 (n=22)
10	78.9 (n=19)	8	no selection
11	92.9 (n=14)	9	38.4 (n=13)
12	68.8 (n=16)	10	20.0 (n=15)
13	92.5 (n=26)	11	42.9 (n=7)
14	no selection		
15	42.1 (n=19)		

Generation	KF "Migrant" line	KF "Non-migrant" line
2	22.2 (n=18)	15.0 (n=20)
3	33.3 (n=3)	0 (n=5)
4	72.5 (n=8)	6.7 (n=15)

2. Mid-parent and mean offspring flight durations (minutes) used in the calculation of overall heritability of flight capacity (Fig.3.5).

Mid-parent	Mean offspring	Mid-parent	Mean offspring
294.5	106.2	139.5	190.9
281.0	192.8	411.0	373.3
30.0	181.0	30.0	295.3
115.5	211.6	52.5	183.5
599.0	342.7	32.0	61.5
30.0	61.4	413.5	371.4
363.5	468.3	652.5	248.6
660.0	338.0	347.5	477.0
755.5	401.7	749.0	433.6
30.0	30.0	30.0	48.0
32.0	112.2	31.0	125.8
593.0	283.4	352.0	302.8
576.0	418.0	396.5	278.3
427.0	278.2	457.0	418.8

APPENDIX FOUR.

1. Day 1 weights (mg) of female moths found to be mature at 48 and 72h post-emergence (Table 4.3).

48h	72h
66.1	68.7
87.4	86.3
97.4	65.9
85.6	81.1
76.0	73.2
81.1	89.3
68.6	85.1
62.6	91.1
	103.4
	58.7

2. Day 1 weights (mg) of female moths found to be immature at 48 and 72h post-emergence (Table 4.3).

48h	72h
91.0	120.7
77.3	98.5
75.0	83.2
67.3	69.1
71.0	84.6
74.1	70.8
75.3	61.9
75.4	66.5
67.5	84.7
73.3	115.7
49.3	97.8
67.2	148.4
63.7	83.7
48.0	74.3
50.0	84.2
54.8	82.5
73.0	75.8
82.0	59.3
80.0	
66.9	
63.4	
66.4	

Postscript...