CHAPTER 5

GEOGRAPHIC VARIATION IN FLIGHT CAPACITY AND LIFE HISTORY TRAITS

5.1 Introduction

There are no specific studies of geographic variation in life-history traits for *Helicoverpa* species in Australia. Zalucki *et al.* (1986) document evidence indicating geographic differences in oviposition preference for crop hosts by *H. punctigera* and *H. armigera,* and a study of genetic (electrophoretic) variation of *Helicoverpa* species in Australia by Daly & Gregg (1985) indicated a measure of genetic variation between widely separated populations. Direct comparison of life-history traits among such populations has not been undertaken.

This chapter aims to determine if important life history traits such as flight capacity, time to first reproduction, and body size parameters differ among geographically separated populations of *H.punctigera* and *H.armigera.* Potential differences in life history traits will be broadly related to the long term environmental conditions of each locality.

5.2 Methods

5.2.1 Insect Material

Sample populations of *H. punctigera* larvae were obtained from Warren (32° 30'S, 147° 30'E) and Armidale (30° 30'S, 151° 37'E) in New South Wales, and Windorah (25° 30'S, 142° 30'E) in Queensland. Sample populations of *H. armigera* larvae were obtained from Kununurra (15° 50'S, 128° 45'E) in Western Australia, Emerald (23° 30'S, 148° 6'E) in Queensland, and Wooli (29° 51'S, 153° 16'E) in New South Wales.

All population samples consisted of more than 30 late instar larvae collected during August and September 1989 (*H. punctigera*) and December 1989 and January

1990 (*H. armigera*). Geographical and environmental data for each of these localities are presented in Table 5.1.

Rearing conditions and experimental procedure were the same for each population. The population sample was maintained in a culture room at a constant temperature of 25 °C, a relative humidity of 60 - 70 %, and a 14:10 L:D photoperiod. Larvae were reared on artificial diet (Teakle & Jensen 1985) in 35 ml plastic containers. Pupation occurred in the excess diet and frass.

Newly emerged moths from the sample were randomly paired and placed into 325 ml plastic containers provided with 10 % honey solution, and paper towelling as an oviposition surface. Newly laid eggs were transferred to artificial diet, and the resulting larvae and pupae were maintained as described above. Adults derived from these larvae were used in the experiment.

The following body size and life-history parameters were measured: pharate pupal weight (mg), forewing length (mm) and pre-oviposition period (days). Individual moths were flight tested on each of the first 12 nights following emergence. Tethering and flight testing of moths followed the procedure described in Chapter 3. Only female moths were flight tested.

5.2.2 Statistical analysis

Potential differences between populations for life-history and body size traits were determined using analysis of variance and multiple range testing (Zar 1974). Median flight durations and the distribution of flight durations were compared using the survival function analysis of Dixon (1981).

5.3 Results

Analysis of variance indicated significant differences between pre-oviposition periods ($F_{2,48} = 9.88$, $P < 0.001$), but not pharate pupal weights ($F_{2,48} = 1.2$, $P > 0.1$) or forewing lengths ($F_{2,48} = 0.18$, $P > 0.1$) among *H. punctigera* sample populations. Mean pre-oviposition period of *H. punctigera* females from the Warren sample

population were significantly greater than those from the Windorah or Armidale populations (see Table 5.2).

For *H. armigera,* analysis of variance indicated significant differences between pharate pupal weights ($F_{2,51} = 8.52$, $P > 0.001$) and pre-oviposition periods ($F_{2,51} = 8.33$, $P < 0.001$), but not forewing lengths ($F_{2,51} = 2.19$, $P > 0.1$) among sample populations. Females from the Wooli sample population exhibited the greatest pharate pupal weights, and females from the Emerald population exhibited a significantly longer pre-oviposition period than females from either the Wooli or Kununurra populations (see Table 5.3).

Median flight durations (mins) (± s.e.) of moths from each sample population during the first 12 nights following emergence are shown in Table 5.4 for *H. punctigera* and Table 5.5 for *H. armigera* . Significant differences in median flight durations among sample populations were evident during nights 2 - 4 only for *H. punctigera* moths. The distribution of median flight durations on nights 1 - 4 is shown in Fig 5.1 for *H. punctigera* moths. Examination of Fig 5.1 shows that moths from the Warren population undertook flights of shorter duration on these nights in comparison with females from the Windorah or Armidale sample populations.

For *H. armigera* moths, significant differences in median flight durations were evident on nights 1 and 3 - 7 (see Table 5.5). Between nights 1 - 4 there were no consistent trends in the identity of long flying moths; however, between nights 5 - 7 moths from the Emerald sample population clearly exhibited the greatest median flight durations (Fig 5.2).

5.4 Discussion

The populations of *H. punctigera* and *H. armigera* originating from different geographic localities differed significantly in flight capacity during parts of their adult life and in some life history traits. Population differences in any behaviour may result from either genetic or environmental causes. To distinguish between these two sources of variation it is necessary to hold one of them constant (Dingle 1984). In this experiment

moths were reared under uniform laboratory conditions soon after the cultures were initiated. Hence, the phenotypic differences observed in *H. punctigera* and *H. armigera* must be genetically based. Possible shifts in the parameters of original wild populations through inadvertent laboratory selection of genotypes should have been minimised by adequate population sample sizes (> 15 pairs).

Prior evidence for genetic variance among *Helicoverpa* populations is limited. Daly & Gregg (1985) estimated genetic variation among *H.punctigera* and *H.armigera* populations using electrophoretic techniques. They concluded that, although populations throughout Australia were differentiated from each other, it was likely that considerable gene flow occurred between widely separated regions. This may account for the few significant differences in life history traits detected among populations of *H. punctigera* and *H. armigera* in this study.

Differences in flight capacity among sample populations were more marked for *H. armigera* than *H. punctigera* moths. Though both *H. punctigera* and *H. armigera* are migratory (Farrow & Daly 1987), *H. armigera* is considered to be more sedentary (Wardhaugh *et. al.* 1980). Among the *H. armigera* populations sampled, moths from Kununurra and Wooli represent near coastal and coastal populations respectively, and moths originating from Emerald represent an inland population. Variability of annual rainfall at Kununurra and Wooli is low (see Table 5.1); also Kununurra represents a large irrigated cropping area in which populations may be self contained. Moths originating from Kununurra and Wooli exhibited the shortest pre-oviposition periods and the lowest levels of flight activity (see Table 5.3). Moths originating from the Emerald population exhibited lengthened time to first oviposition and increased flight capacity during the reproductive phase of their lives. These life history traits may reflect the short term suitability of habitats at this locality. There is, however, no direct evidence to suggest that stable populations of either *H. punctigera* or *H. armigera* persist in any of these geographic localities for any extended period of time. Turnover of *H. armigera* populations in cropping regions such as Emerald would be less than *H. punctigera* (Fitt 1989). For such highly mobile insects as *Helicoverpa* spp gene flow between widely

separated regions clearly occurs (Daly & Gregg 1985); this would serve to reduce the likelihood of stable, locally adapted, genotypes persisting in a given locality. The scope of this experiment is insufficient to arrive at any specific conclusions concerning patterns of genetic variation among *Helicoverpa* populations. This experiment has shown that geographic variation does exist among *Helicoverpa* populations for some life history traits; further experiments are required before any conclusions can be drawn regarding the cause of such variation.

5.5 References

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Table 5.1 Climatic data for geographic localities of source population samples. Climatic data from the Division of National Mapping (1986).

Locality	Variability of Annual Rainfall	Temperature °C January Av. Max	Temperature °C July Av. Max.	Rainfall (mm) January Median	Rainfall (mm) July Median
H. punctigera					
Windorah (NSW)	High	39	21	10	5
Warren (NSW)	Low	33	15	50	37
Armidale (NSW)	Low	27	12	150	50
H. armigera					
Kununurra (WA)	Low	36	30	150	0
Emerald (Qld)	Moderate	33	21	100	10
Wooli (NSW)	Low	30	21	100	50

Table 5.2 Life history and body size parameters of *H. punctigera* females. Sample populations obtained from Warren, Windorah, and Armidale, New South Wales. Values represent mean (± s.d.).

	Warren n = 15	Windorah n = 20	Armidale n = 15
Pharate pupal weight (mg)	$250.6^{a} \pm 34.4$	$266.8^{a} \pm 20.1$	$269.1^{a} \pm 34.9$
Forewing length (mm)	$15.8^{a} \pm 1.1$	$16.1^{a} \pm 1.0$	$16.0^{a} \pm 0.9$
Preoviposition period (days)	$3.4^{b} \pm 1.0$	$2.1^{a} \pm 1.0$	$1.5^{a} \pm 0.9$

Values followed by differing superscript differ significantly at $P < 0.05$ (SNK test). Within row comparisons only.

Table 5.3 Life history and body size parameters of *H. armigera* females. Sample populations obtained from Kununurra (Western Australia), Emerald (Queensland) and Wooli (New South Wales). Values represent mean (± s.d.).

	Kununurra n = 17	Emerald n = 18	Wooli n = 18
Pharate pupal weight (mg)	$356.5^{a} \pm 25.9$	$369.2^{a} \pm 39.7$	$399.5^{b} \pm 24.4$
Forewing length (mm)	$18.7^{a} \pm 0.6$	$18.5^{a} \pm 0.9$	$18.0^{a} \pm 1.2$
Preoviposition period (days)	$3.3^{a} \pm 1.8$	$4.5^{b} \pm 1.9$	$2.3^{a} \pm 1.1$

Values followed by differing superscript differ significantly at $P < 0.05$ (SNK test). Within row comparisons only.

Table 5.4 Median flight durations (mins) (± s.e.) for *H. punctigera* females from Warren, Windorah, and Armidale (New South Wales). Individual moths were flown on the first 12 consecutive nights following emergence.

	Geographic locality				
Night after emergence	Warren n = 15	Windorah n = 20	Armidale n = 15	Bresl. Stat. *	P
1	3.2 ± 0.9	5.8 ± 0.7	7.4 ± 2.6	2.0	0.36
2	4.2 ± 0.8	12.7 ± 6.3	9.7 ± 3.1	8.5	0.01
3	5.2 ± 0.9	14.4 ± 7.3	17.3 ± 1.9	10.9	0.004
4	5.7 ± 2.4	27.4 ± 26.6	27.3 ± 2.9	15.1	0.001
5	23.0 ± 4.7	29.0 ± 3.7	34.5 ± 11.6	0.1	0.92
6	36.2 ± 10.9	17.2 ± 6.5	38.3 ± 8.9	3.6	0.17
7	18.6 ± 12.9	24.0 ± 6.1	27.5 ± 8.9	0.5	0.77
8	21.3 ± 20.8	26.2 ± 24.0	18.0 ± 0.9	4.1	0.13
9	24.1 ± 2.9	27.3 ± 8.4	23.4 ± 16.2	0.6	0.74
10	21.4 ± 7.4	18.5 ± 5.7	24.0 ± 9.2	0.2	0.9
11	18.2 ± 1.2	21.0 ± 14.5	12.7 ± 10.2	1.6	0.44
12	16.4 ± 5.9	11.2 ± 2.6	12.7 ± 3.6	1.6	0.44

* Generalised Wilcoxon (Breslow) statistic. Within row comparisons only.

Table 5.5 Median flight durations (mins) (± s.e.) for *H. armigera* females from Kununurra (Northern Territory), Windorah (Queensland), and Wooli (New South Wales). Individual moths were flown on the first 12 consecutive nights following emergence.

	Geographic locality				
Night after emergence	Kununurra n = 17	Emerald n = 18	Wooli n = 18	Bresl. Stat. *	P
1	3.5 ± 0.3	3.2 ± 0.1	12.5 ± 1.1	15.0	0.001
2	10.0 ± 2.9	11.5 ± 3.2	11.7 ± 9.2	0.7	0.68
3	6.5 ± 4.2	11.2 ± 5.3	12.2 ± 21.2	9.1	0.01
4	9.4 ± 0.9	20.6 ± 2.5	30.0 ± 19.1	11.5	0.003
5	21.1 ± 7.0	45.0 ± 0.1	15.5 ± 6.1	7.7	0.02
6	19.7 ± 4.8	67.2 ± 0.2	25.2 ± 12.1	12.0	0.002
7	32.1 ± 22.4	59.0 ± 12.7	14.0 ± 2.4	10.1	0.006
8	22.8 ± 20.9	19.3 ± 19.8	16.0 ± 2.2	1.9	0.39
9	34.0 ± 24.3	23.5 ± 22.8	16.1 ± 8.5	3.7	0.15
10	33.2 ± 5.8	24.1 ± 18.4	15.3 ± 5.0	5.3	0.15
11	27.2 ± 7.5	23.0 ± 4.6	14.1 ± 0.3	4.4	0.11
12	22.8 ± 4.1	25.2 ± 3.6	12.8 ± 0.8	4.9	0.08

* Generalised Wilcoxon (Breslow) test. Within row comparisons only

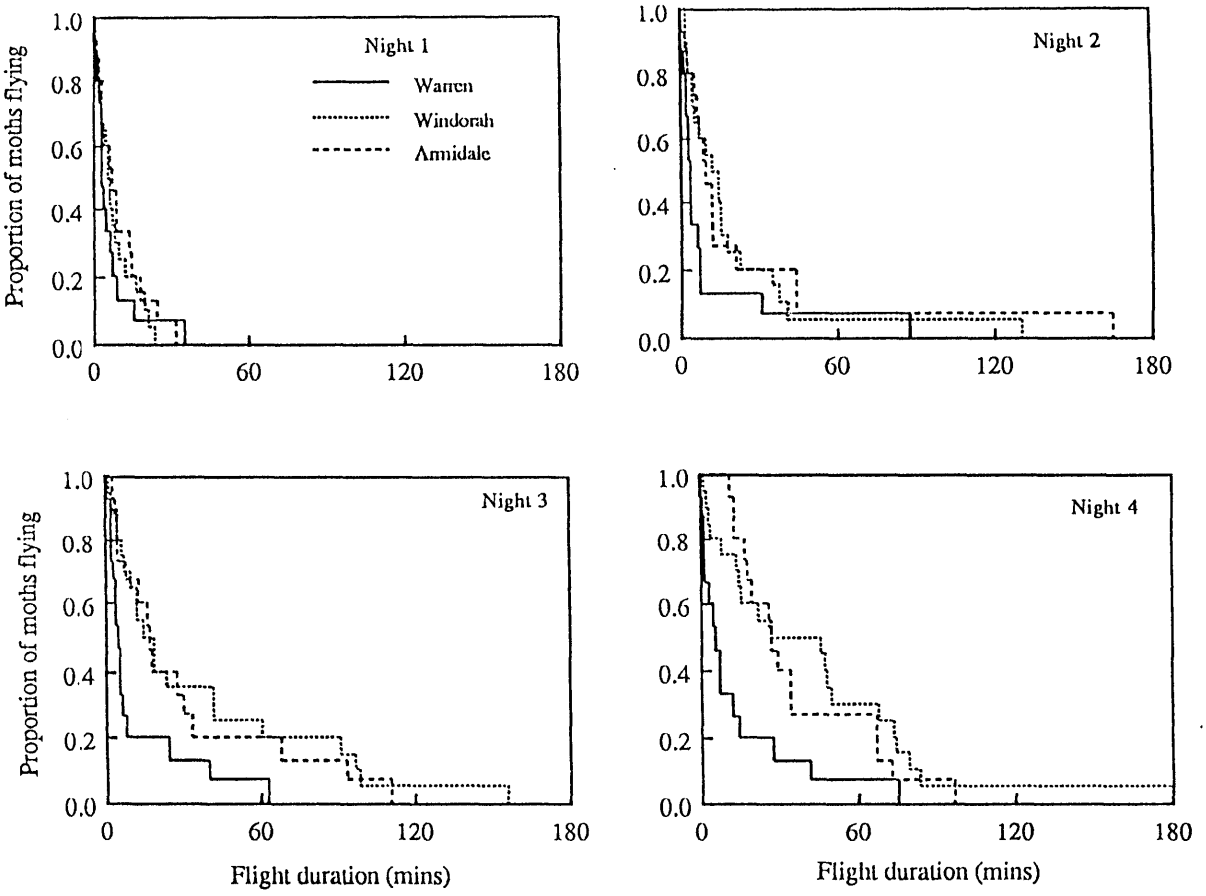

Fig 5.1 The distribution of median flight durations of *H. punctigera* females on nights 1 - 4 after emergence.

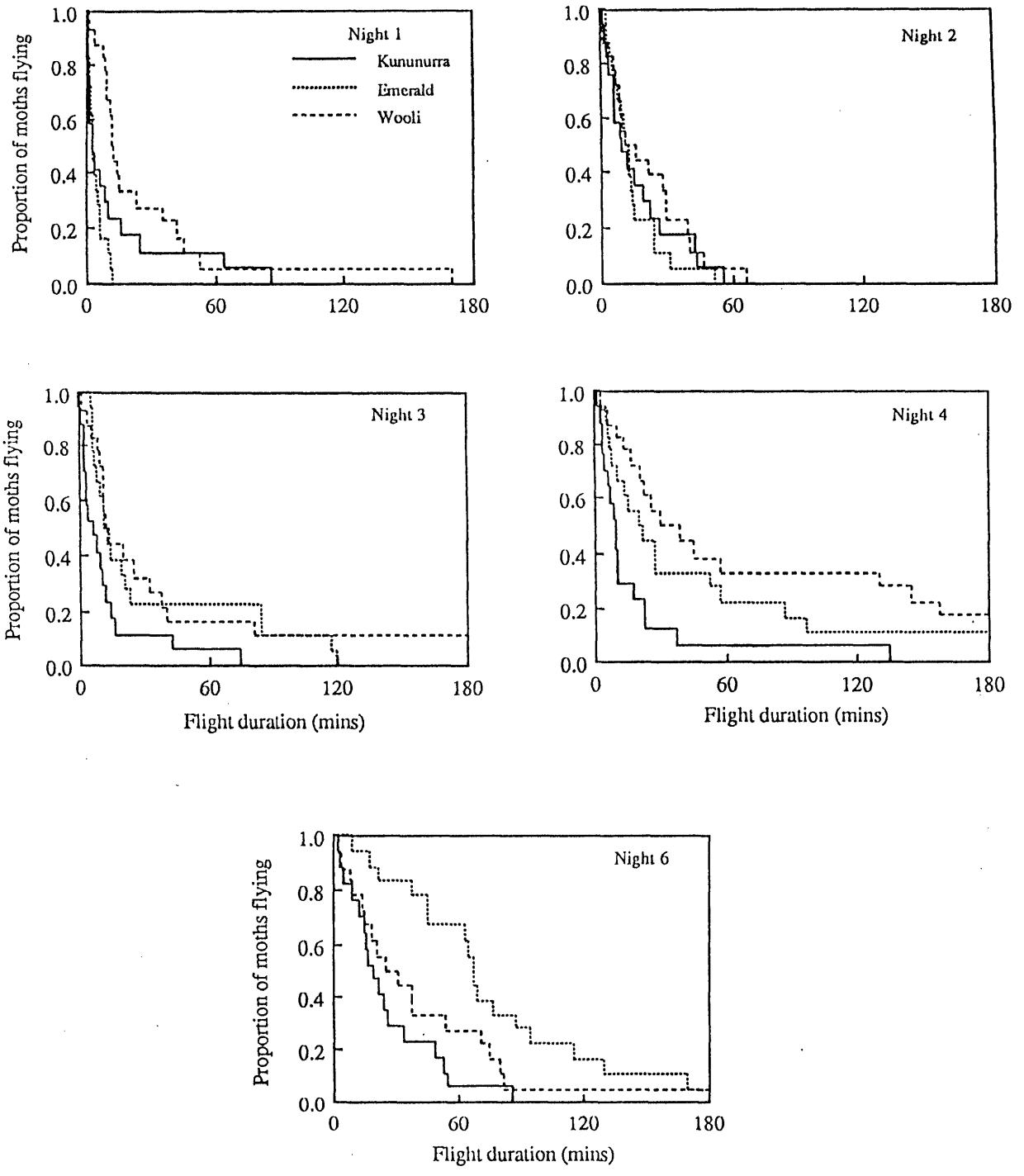

Fig 5.2 The distribution of median flight durations of *H. armigera* females on nights1 - 4 and 6 after emergence.

Chapter 6
THERMOREGULATION AND FLIGHT THRESHOLDS

6.1 Introduction

May (1979) defines thermoregulation as 'the maintenance of body temperature relatively independent of environmental temperature by means of adaptive responses of an organism in its natural environment'. Environmental temperature is of great importance as a factor limiting flight. Its influence, however, is effected largely through an insect's body temperature; all insects having a minimum body temperature below which flight is impossible (Chapman 1982). Many insects are able to regulate body temperature within certain limits by behavioural and/or physiological mechanisms (May 1979, 1985). Studies on thermal biology of moths have shown that thermoregulatory ability within and between taxa is highly variable. Some species regulate body temperature within relatively narrow limits, for example, Sphingidae (3 spp) (Heinrich 1971, Bartholomew & Heinrich 1973), Lasiocampidae (1 spp) (Casey 1981) and certain Noctuidae (10 spp) (Bartholomew & Heinrich 1973, Heinrich 1987), whereas in others, body temperature may vary largely with ambient temperature, for example, Noctuidae (1 spp) and Geometridae (4 spp) (Bartholomew & Heinrich 1973, Casey & Joos 1983). Close regulation of body temperature by insects may confer potential advantages deriving from increased habitat use both temporally and spatially (May 1985) and may enhance important activities such as foraging (Bartholomew & Heinrich 1978, Heinrich 1987) and mate attraction (Hastings & Toolson 1991). Nocturnal moths regulate body temperature solely by means of endothermic mechanisms (see review, May 1985). Heat production may be derived from contraction of flight muscles during pre-flight warm-up and from the high metabolic rate of muscles during flight (Heinrich & Bartholomew 1971). Heat loss from the thorax may subsequently be restricted by insulating scales and hairs (Casey 1981); conversely, excessive heat may be removed by allowing blood to cool in peripheral regions of the body (May 1985).

The aim of this chapter is to investigate the thermoregulatory ability and minimum flight thresholds of *H. punctigera* and *H. armigera*. An understanding of their thermal biology and

flight thresholds is of potential significance in improving our capacity to predict the flight behaviour of these moths.

6.2 Methods

6.2.1 Insect material for laboratory experiments

Cultures of *H. punctigera* and *H. armigera* were established from larvae collected from field crops in the Armidale region. Larval development was completed on artificial diet based on the formula provided by Teakle & Jensen (1985). All insect material was subsequently bred for a single generation under constant rearing conditions prior to experimentation. Larvae were reared individually in 35 ml plastic containers at 25 ± 1 °C and a 14:10 L:D regime. Pupation occurred within the larval rearing container amongst excess diet and frass. Adults were held individually in 275 ml plastic containers and provided with 10 % honey solution as a nectar substitute, which was renewed daily. Adults were maintained under the same conditions of temperature and photoperiod as those for rearing larvae.

6.2.2 Pre-flight warm-up and thoracic temperatures during flight

Moths were anaesthetised with diethyl ether, a small (< 4 mm^2) area of scales was removed from the dorsal surface of the thorax and a small hole (approximately 0.35 mm diam.) was punctured through the tergum with the point of a needle. A 48 gauge (0.08 mm diameter) copper constantan thermocouple was inserted to a depth of 2 mm and fixed in place with melted beeswax. Moths were held at ambient temperatures (T_{amb}) of 20 ± 1.0 °C, 15 ± 1.0 °C or 10 ± 1.0 °C (n = 10 in each case) for a period of 2 hr, to allow recovery from anaesthetisation and stabilisation of body temperature near T_{amb}.

Warm-up behaviour (wing shivering) was initiated by gentle prodding. At the completion of warm-up the thermocouple lead provided a tether from which moths were flown. Moths initiated take-off by their own volition. Moths carried their own body weight during flight and exhibited full amplitude wing beats. Forward motion, however, was restricted by the length of the thermocouple lead. At the completion of flight testing (moths were flown until T_{th} stabilised) moths were returned to holding containers to cool. Thoracic temperature (T_{th}) was

recorded every 10 sec or less during pre-flight warm-up, tethered-flight, and cool-down with a microcomputer. During cool-down changes in T_{th} were recorded until T_{th} stabilised near T_{amb}. Thoracic mass (mg) was recorded for each moth tested.

6.2.3 Duration of pre-flight warm-up, and thoracic and abdominal temperatures during tethered-flight

Two tethered-flight methodologies were used to investigate thermoregulation during flight. Firstly, moths were tested using the static tethered-flight procedure outlined in Chapter 2; in this method, moths were suspended from a vertical rod, did not maintain their own body weight during flight and air flow over the body was minimal. The second method involved tethering moths to lengths of cotton twine. Moths flown in this way carried their own body weight during flight, were permitted greater freedom of movement, and air flow over the body was increased. Details of these methods are given below.

6.2.4 Static tethered flight : *H. punctigera*

Prior to experimentation, moths were anaethetised with diethyl ether. A 5mm length of polyethylene tubing was attached to the dorsal surface of each moth using contact adhesive. This provided a friction fit onto a metal rod which acted as a tethering arm. Following tethering moths were held in temperature controlled cabinets at a series of ambient temperatures ranging from 0 - 35 ^{o}C for a period of 2 hr prior to flight testing. Moths were removed from the constant temperature cabinet, attached to the tethering arm and replaced within the cabinet. Loss of tarsal contact initiated either wing shivering or flight. The elapsed time from removal of the insect from the temperature controlled cabinet, attachment to the tethering arm and replacement in the cabinet was no more than 4 - 5 sec. Moths were flight tested for a period of 5 min. Two measurements of body temperature were taken at the completion of the test period; thoracic (T_{th}) and abdominal (T_{abd}). For each moth T_{th} was taken prior to T_{abd}. Time taken for removal of the moth from the constant temperature cabinet to implantation of a thermocouple in the thorax followed by the abdomen was approximately 2 - 3 and 4 - 5 sec respectively. Moths maintained wing beats throughout the time taken to measure body temperatures. Measurements

of T_{th} and T_{abd} were made to the nearest 0.1 °C using a needle thermocouple (diam = 0.3 mm) attached to a hand held micropcomputer. Individual moths were discarded after one measurement of T_{th} and T_{abd}. Room temperature during tethering and measurement of body temperatures was maintained at 24 ± 1 °C. The relationship of T_{th} to T_{amb} was summarised by linear regression. A slope significantly different from 1 was taken to indicate thermoregulation, the converse indicating no thermoregulation (May 1985).

6.2.5 Tethered to cotton twine : *H. punctigera and H. armigera*

Following anaethetisation with diethyl ether, a 50 cm length of cotton twine was attached to the dorsal surface of the thorax using contact adhesive. Prior to experimentation moths were held for a period of 2 hr in temperature controlled rooms at T_{amb} ranging from 5 - 30 °C. Pre-flight warm-up was initiated by gentle prodding. The duration and nature of warm-up behaviour was recorded. At the completion of warm-up moths initiated flight by their own volition. The cotton twine attachment allowed moths to fly while being restrained by the observer. Moths typically flew to the extent of the twine and maintained their own body weight during flight; forward movement, however, was restricted by the length of the tether. At the completion of 3 - 5 min of continuous flight (T_{th} typically stabilised during this period) T_{th} and T_{abd} were recorded. For each moth T_{th} was taken prior to T_{abd}. The time taken between measurement of T_{th} and T_{abd} was 2 - 3 secs. Moths maintained rapid wing beats while body temperatures were recorded. Individual moths were discarded after one measurement of T_{th} and T_{abd}. Regressions of T_{th} on T_{amb} were interpretated as described above.

6.2.6 Field measurement of thoracic temperature during free-flight

Moths were captured using a hand net either as they flew above vegetation or as they approached lights. Moths were immobilised in a pocket of netting to avoid hand contact. T_{th} was measured within 3 - 4 sec of capture using a hand held copper constantan needle thermocouple (needle diameter = 0.3 mm). The thermocouple was dried and T_{amb} was measured near the site of capture immediately after measurement of T_{th}. Temperatures were read to the nearest 0.1 °C. Regressions of T_{th} on T_{amb} were interpreted as described above.

6.2.7 The role of thoracic scales and hairs as thermal insulators

Freshly killed moths were implanted with a thoracic thermocouple as described above and heated to approximately 40 °C under an incandescent lamp. T_{th} and T_{amb} were recorded to the nearest 0.1 °C at 5 sec intervals with a microcomputer as the thorax cooled. Measurements continued until T_{th} stabilised near T_{amb}. This procedure was then repeated using moths with thoracic scales removed. Scales were removed by lightly rubbing the surface of the thorax with a fine brush. Cooling constants (min^{-1}) were calculated from the slope of the semilog plot of $\ln(T_{th} - T_{amb})$ versus time (Casey 1981). Thoracic mass (mg) was recorded for each moth.

6.2.8 Estimating heat storage relative to heat loss from warm-up and convective cooling

Heat production was estimated from the heat gain (°C min^{-1}) recorded during endothermic warm-up (section 6.2.2) and from convective heat loss (°C min^{-1}) recorded during passive cooling (section 6.2.6). At any given difference between T_{th} and T_{amb} ($T_{th\ excess}$), energy production (J g $thorax^{-1}$ min^{-1}) will be equal to heat gain plus heat loss, multplied by the weight of the thorax (g^{-1}) and its specific heat (3.43 J g^{-1} $°C^{-1}$) (Heinrich & Bartholomew 1971, Casey 1981). Heat production was computed at 2.5 °C intervals during warm-up at T_{amb} of 15 °C and 20 °C, using mean rates of increase in T_{th} (°C min^{-1}) and mean rates of cooling (°C min^{-1}) at each $T_{th\ excess}$.

6.3 Results

6.3.1 Pre-flight warm-up and changes in T_{th} during tethered- flight

For both *H. punctigera* and *H. armigera* endothermic warm-up was signalled by rapid vibration of the wings (wing shivering); the amplitude of wing vibration visibly increased with increasing T_{th}. Moths initiated rapid walking just prior to take-off which was spontaneous at the completion of warm-up. In both species endothermic warm-up was rapid and approximately linear (Fig 6.1). Following an initial decline in T_{th} immediately after take-off, T_{th} typically stabilised during the 3 - 5 min test period in both species. T_{th} declined rapidly

following cessation of flight. If no further activity was undertaken, T_{th} continued to decline until stabilising near T_{amb}.

Mean (± s.d.) rates of endothermic warm-up at 10, 15 and 20 °C are presented in Table 6.1. Analysis of variance indicated significant differences in warm-up rates for both *H.punctigera* ($F_{2,30} = 36.9$, $P < 0.01$) and *H. armigera* ($F_{2,27} = 27.3$, $P < 0.01$). Warm-up rate declined in both species with decreasing T_{amb}. Warm-up rate was positively correlated with thoracic mass (Fig 6.2). The regression of warm-up rate (y) (°C min^{-1}) on thoracic mass (x) (mg) (pooled data for both species) was $y = 0.07x - 0.87$ ($r^2 = 0.55$, $F_{1,19} = 25.3$, $P < 0.001$). At 20 °C, T_{th} at take-off differed significantly ($t_{19} = 2.31$, $P < 0.05$) between species. Mean values (± s.d.) were 27.3 ± 1.4 °C for *H. punctigera* and 29.1 ± 2.2 °C for *H. armigera*.. T_{th} at take-off ranged from 24.7 - 29.5 °C for *H. punctigera* and 25.4 - 31.9 °C for *H. armigera*..

6.3.2 Duration of, and proportion of moths initiating, pre-flight warm-up

Figure 6.3a shows a plot of pre-flight warm-up time (sec) for *H. punctigera* and *H. armigera* versus T_{amb}. Warm-up duration for both species showed a logarithmic increase and increasing variance with decreasing temperature. Log normal transformation of the time data linearised the relationship (Fig 6.3b). Regression lines are as follows: for *H. punctigera*, $\ln(\text{time}) = 8.70 - 0.21T_{amb}$, ($r^2 = 0.86$, $F_{1,75} = 461.0$, $P < 0.001$) and for *H. armigera*, $\ln(\text{time}) = 8.66 - 0.21T_{amb}$, ($r^2 = 0.79$, $F_{1,65} = 243.4$, $P < 0.001$). The regression lines for *H. punctigera* and *H. armigera* are essentially the same.

Proportions of moths initiating pre-flight warm-up at T_{amb} between 3 and 19 °C are shown in Fig 6.4. Proportions of moths initiating warm-up behaviour and continuing through to take-off declined abruptly below 9 °C for both *H. punctigera* and *H. armigera*. No moths were able to initiate warm-up at T_{amb} of 3 °C, or less.

6.3.3 Relationship of T_{th} and T_{abd} to T_{amb} during tethered-flight

6.3.3.1 Static tethered-flight : *H. punctigera*

At T_{amb} in the range 11 - 30 °C all moths (n = 34) exhibited continuous flight (= 5 min duration). For each individual moth tested T_{abd} was lower than T_{th} (see Fig 6.5). At T_{amb} > 30 °C flight was intermittent; all moths (n = 10) frequently ceased flight to hold the fore- and hindwings away from the body. These moths typically resumed flight within 5 - 10 sec of stopping. For each moth, T_{abd} was consistently lower than T_{th}. At T_{amb} in the range 6 - 11 °C most moths exhibited continuous flight, a proportion (37 %) were observed to fly intermittently. These moths alternated between flight and wing shivering. Moths alternating between wing shivering and flight exhibited T_{abd} higher than T_{th}. Moths displaying continuous flight, however, consisently exhibited T_{abd} lower than T_{th}. The lowest T_{amb} at which continuous flight was observed was 6.5 °C. At T_{amb} < 6 °C no moths (n = 10) exhibited continuous flight; all moths alternated between low amplitude wing beats and wing shivering. Table 6.2 gives proportions of moths exhibiting continuous flight, wing shivering and cool-down behaviours in the range of T_{amb} < 6, 6 - 11, 12 - 30 and > 30 °C. Mean thoracic excess (T_{th} - T_{amb}) was greatest at T_{amb} 11 - 30 °C and lowest at T_{amb} > 30 °C (Table 6.3). The regression of T_{th} on T_{amb} ($T_{th} = 10.3 + 0.96T_{amb}$, $r^2 = 0.92$) did not differ significantly from 1.0 ($t_{1,42} = -1.02$, $P > 0.1$).

6.3.3.2 Tethered to cotton twine : *H.punctigera* and *H.armigera*

For both *H. punctigera* and *H. armigera* , T_{th} and T_{abd} lie above, but largely track T_{amb} (Figs 6.6a and b) during tethered-flight. Regressions of T_{th} on T_{amb} were significantly different from 1 for both *H. punctigera* ($t_{1,63} = -3.28$, $P < 0.01$) and *H. armigera* ($t_{1,57} = -5.39$, $P < 0.001$). Regression lines were, $T_{th} = 0.84T_{amb} + 12.5$ ($r^2 = 0.82$) for *H. punctigera*, and $T_{th} = 0.63T_{amb} + 17.6$ ($r^2 = 0.58$) for *H. armigera* . In both *H. punctigera* and *H. armigera* thoracic excess ($T_{th.exc}$) (T_{th} - T_{amb}) was greater at low T_{amb} than at high T_{amb}. T_{abd} was consistently lower than T_{th} in all moths tested. In both species, T_{abd} approached the isothermal line with increasing T_{amb}.

6.3.4 Relationship of T_{th} to T_{amb} during free flight

Plots of T_{th} versus T_{amb} during free flight for *H. punctigera* and *H. armigera* are shown in Figs 6.7a and b respectively. *H. punctigera* moths were active (flying) over T_{amb} of 9 to 23 °C and in *H. armigera* at T_{amb} from 12 to 25 °C. At low T_{amb} (10 - 12 °C), T_{th} averaged 16.3 ± 2.4 °C above T_{amb} for *H. punctigera* and 13.5 ± 3.1 °C above T_{amb} for *H. armigera*. At T_{amb} of 20 °C or more, T_{th} averaged 7.8 ± 1.1 °C above T_{amb} for *H. punctigera* and 6.9 ± 1.7 °C above T_{amb} for *H. armigera*. Regressions of T_{th} on T_{amb} were significantly different from 1.0 for both *H. punctigera* ($t_{1,41} = -9.12$, $P < 0.001$) and *H. armigera* ($t_{1,35} = -4.74$, $P < 0.001$). Regressions of T_{th} on T_{amb} were: for *H. punctigera*, $T_{th} = 22.8 + 0.28T_{amb}$ ($r^2 = 0.23$), and for *H. armigera*, $T_{th} = 18.8 + 0.48T_{amb}$ ($r^2 = 0.34$).

6.3.5 Thoracic scales as thermal insulation

Removal of scales from the thorax significantly increased thermal conductance for both *H. punctigera* ($t_{17} = -4.91$, $P < 0.001$) and *H. armigera* ($t_{10} = -11.97$, $P < 0.001$) (Fig 6.8). Mean thoracic cooling constants (°C min^{-1} $°C^{-1}$) were 0.33 ± 0.03 (scales removed) and 0.27 ± 0.02 (scales intact) for *H. punctigera* and 0.22 ± 0.01 (scales removed) and 0.16 ± 0.01 (scales intact) for *H. armigera*. Thermal conductance of *H. punctigera* was on average higher than that of *H. armigera*.

6.3.6 Heat storage relative to heat loss

Estimates of heat production (J g $thorax^{-1}$ min^{-1}) at T_{amb} of 15 and 20 °C, calculated from the sum of the heat gain during endothermic warm-up and from the heat loss during convective cooling are presented in Table 6.4 for *H. punctigera* and Table 6.5 for *H. armigera*. At a T_{amb} of 15 °C, heat production, calculated from the temperature change during endothermic warm-up, increased slightly in *H. punctigera* and remained relatively constant in *H. armigera* as the difference between T_{th} and T_{amb} (T_{th} excess) increased. Heat loss via convective cooling, however, also increased as the thoracic excess increased. The sum of these two components (the estimate of total heat production) is given in column 4 of each table. During warm-up a progressively greater proportion of this total is lost via convective cooling.

For example, during warm-up from 15 °C, *H. punctigera* moths with a thoracic excess of 10 °C produce approximately 348 J g thorax^{-1} min^{-1}, however, they are losing 180.5 J g thorax^{-1} min^{-1} due to convection (Table 6.4). Hence, approximately 52 % (180 / 348) of the heat produced is lost through convection without increasing T_{th} (Table 6.4). This similarly applies to *H.armigera,* where approximately 55 % (150 / 275) of the heat produced is lost (Table 6.5). During endothermic warm-up from 15 °C neither species elevated T_{th} more than 10 °C above ambient, and at warm-up from 20 °C neither species elevated T_{th} more than 7.5 °C above ambient.

6.4 Discussion

As with other nocturnal moths (May 1979, 1985), *H. punctigera* and *H. armigera* utilise a pre-flight warm-up behaviour (wing shivering) to elevate T_{th} to a level at which flight is possible. *H. punctigera* and *H. armigera* maintain T_{th} in flight of between 21 to 32 °C. Wing shivering was observed in both species at ambient temperatures of less than 28 °C, whereas, at ambients of more than 28 °C flight was spontaneous. Only a small proportion of *H. punctigera* (< 20 %) and *H. armigera* (< 5%) were capable of warming from a T_{amb} of 5 °C, and none from 3 °C (Fig 6.4). The majority of moths, however, were unable to warm-up from a T_{amb} of less than 9 °C. These results are consistent with results for other noctuids of similar body size. Taylor & Shields (1990) recorded a minimum ambient temperature of 4 °C from which the armyworm, *Pseudaletia unipuncta,* was able to initiate wing shivering, with the majority of individuals only capable of initiating warm-up at 10 °C or above. Similarly, Casey & Joos (1983) demonstrated a minimum threshold of approximatley 10 °C for *Heliothis obsolita.*

Warm-up rates (°C min^{-1}) (Table 6.1) in *H. punctigera* and *H. armigera* are positively correlated with T_{amb}, as in other Lepidoptera (Heinrich & Bartholomew 1971). At the three ambients at which T_{th} was recorded during endothermic warm-up (10, 15, and 20 °C) *H. armigera* exhibited a consistently higher rate (means of 1.81, 2.65, and 3.73 °C min^{-1}) than *H. punctigera* (means of 1.23, 2.11 and 2.25 °C min^{-1}). These differences appear to be a function of the greater thoracic mass of *H. armigera* (Fig 6.2). Previous studies relating the

rate of endothermic warm-up to body mass in insects have shown either no relationship (Heinrich & Bartholomew 1971) or a positive correlation (Morgan & Heinrich 1987).

In *H. punctigera* and *H. armigera*, T_{th} varies largely with T_{amb} during flight (Figs 6.5 - 6.7). During static tethered flight (*H. punctigera* only) the slope of the regression line (0.96) was essentially the same as 1.0, indicating passive variance of T_{th} with T_{amb}. Further, T_{th} excesses were similar at all ambients (Fig. 6.5). Regressions of T_{th} on T_{amb} during flight while tethered to cotton twine (slopes of 0.84 for *H. punctigera* and 0.63 for *H. armigera*) and during free flight (slopes of 0.28 for *H. punctigera* and 0.48 for *H. armigera*), however, were significantly different from 1.0 for both species. Further, T_{th} excesses were consistently larger at low T_{amb} than at high T_{amb}. Independance of T_{th} from T_{amb} and narrowing of T_{th} excesses with increasing T_{amb} are indicative of active thermoregulation (May 1985, Bartholomew & Heinrich 1973, Heinrich 1987). Failure of *H. punctigera* moths to display 'normal' thermoregulatory behaviour during static tethered flight is most likely a consequence of the insect not supporting its full body weight. The progressively lower value for the slope of the regression line (and hence the increasing independance of T_{th} from T_{amb}) during flight while tethered to cotton twine and during free flight, further indicates the negative impact of tethering on thermoregulatory ability. Heinrich (1974), in presenting differing plots of T_{th} versus T_{amb} for sphinx moths during tethered flight and free flight, also concluded that tethering had a large effect on the physiology of thermoregulation. These findings strongly suggest that inferences of thermoregulatory ability drawn from tethered flight studies should be treated with caution unless accompanied by field data showing body temperatures during free flight.

Thermoregulation during flight depends on a balance between the rates of heat production and heat loss. Minimum rates of heat loss depend primarily on body size (McCrea & Heath 1971, Casey 1976). As body size decreases, surface to volume ratio increases, thereby facilitating higher mass-specific thoracic cooling. The greater thoracic cooling rates recorded for *H. punctigera* relative to *H. armigera* (cf. Tables 6.4 and 6.5, Fig. 6.8) are clearly a function of its smaller thoracic mass (see Fig. 6.2). The capacity of the moths to be active at low T_{amb} depends in part on their ability to retain the heat generated by warm-up and

by flight. Thoracic cooling rates increased by approximately 22 % for *H. punctigera* and 37 % for *H.armigera* after removal of scales (Fig. 6.8). Thoracic scales clearly act as an effective barrier to heat loss. Though not measured directly, there appeared to be no obvious difference in the length or density of scales between the two species.

Rates of energy expenditure by *H. punctigera* and *H. armigera* during pre-flight warm-up are clearly dependent on T_{th} excess (see Tables 6.4 and 6.5). This is consistent with data for other moths (Heinrich & Bartholomew 1971, Casey 1981, Heinrich 1987). Despite the increased levels of energy expenditure recorded during pre-flight warm-up, T_{th} increases linearly with time for both *H. punctigera* and *H. armigera* (Fig. 6.1). This is a consequence of the increased rates of convective cooling that occur as the difference between T_{th} and T_{amb} increases (Tables 6.4 and 6.5). This may also explain the logarithmic form of Fig. 6.3a (warm-up time versus T_{amb}). Lower rates of endothermic warm-up ($^{o}C\ min^{-1}$) (Table 6.1) at low T_{amb}, coupled with increasing rates of convective cooling as the T_{th} excess increases, result in the lengthened time required to elevate T_{th} to the 20 - 30 ^{o}C required for flight by both species (see Fig. 6.7).

As *H. punctigera* and *H. armigera* maintain greater thoracic excesses (T_{th} - T_{amb}) during flight at low T_{amb} than at high T_{amb} (see section 6.3.4 and Fig. 6.7), warm-up from low T_{amb} will be energetically more expensive than warm-up from high T_{amb} (see tables 66.4 and 66.5). For *H. punctigera* and *H. armigera* to warm-up and fly on a regular basis at low T_{amb} implies an increased requirement for food sources (nectar) to replenish energy reserves. Nectar flow of many plants, however, has been shown to be positively correlated with ambient temperature (Free 1970). The possibility of decreased nectar yields at low ambients may, therefore, serve to limit the frequency of flight for these two species at low T_{amb}.

Summary

Nocturnal activity by *H. punctigera* and *H. armigera* is dependent on their ability to shiver and raise thoracic temperature to a level at which flight is possible. Endothermic warm-up by both species is possible from ambient temperatures as low as 3 ^{o}C; the majority of either species, however, are not capable of warming from below 9 ^{o}C. Both species maintain thoracic temperatures of 20 - 30 ^{o}C during flight at ambients of 10 - 25 ^{o}C. Tethered flight

methodologies are intrusive on normal thermoregulatory balance, manifested as increased dependence of T_{th} on T_{amb}. Warm-up for both species is energetically more expensive from low ambients than high ambients. The increased energy requirements for warm-up at low ambient temperatures may limit the frequency of warm-up and flight.

6.5 References

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Table 6.1 Endothermic warm-up ($^{o}C\ min^{-1}$) by *H. punctigera* and *H. armigera* at T_{amb} of 10, 15 and 20 ^{o}C. Values followed by differing superscripts differ significantly at $P < 0.05$ (SNK test) (within row comparisons only). .

		Ambient temperature (^{o}C)		
		10	15	20
H. punctigera	x	1.23^{a}	2.11^{b}	2.25^{b}
	s.d.	0.45	0.31	0.38
	range	0.93 - 1.66	1.47 - 2.52	1.93 - 2.88
	n	11	12	10
H. armigera	x	1.81^{a}	2.65^{b}	3.73^{c}
	s.d.	0.33	0.65	0.69
	range	1.30 - 2.31	1.74 - 3.93	2.51 - 4.41
	n	10	10	10

Table 6.2 Proportions of *H. punctigera* moths exhibiting continuous flight (> 5 min duration), wing shivering and passive cool-down during static tethered flight at T_{amb} in the ranges < 6 °C, 6 - 10 °C, 11 - 30 °C, and > 30 °C.

	Ambient temperature (°C)			
	< 6	6 - 10	11 - 30	> 30
Continuous flight	0	0.63	1.0	0
Wing shivering	1.0	0.37	0	0
Cool down	0	0	0	1.0
Number of moths	10	16	34	10

Table 6.3 Mean thoracic (T_{th} - T_{amb}) and mean abdominal (T_{abd} - T_{amb}) excess (± s.d.) of *H. punctigera* moths during static tethered flight at T_{amb} in the ranges < 6 °C, 6 - 10 °C, 11 - 30 °C, and > 30 °C.

	Ambient temperature (°C)			
	< 6	6 - 10	11 - 30	> 30
n	10	16	34	10
T_{th} excess	7.7 ± 0.9	8.4 ± 2.3	9.5 ± 1.9	5.3 ± 1.6
T_{abd} excess	8.3 ± 1.8	7.8 ± 1.5	3.2 ± 1.7	0.7 ± 0.5

Table 6.4 Energy expenditure (J g thorax^{-1} min^{-1}) by *H. punctigera* calculated from the sum of the heat gain during warm-up and from the heat loss during passive convective cooling. Values given for thoracic excess (T_{th} - T_{amb}) at 2.5 °C intervals and warm-up at T_{amb} of 15 °C and 20 °C. Convective heat loss is also expressed as a percentage of the estimated total heat production.

	J g thorax^{-1} min^{-1}			
T_{th} excess °C	Warm-up	Convective loss	Total produced	% loss
T_{amb} - 15 °C				
2.5	137.5 ± 49.2	20.9	158.4	13.2
5.0	144.7 ± 62.0	57.3	202.0	28.4
7.5	160.2 ± 46.0	137.4	297.6	46.2
10.0	167.9 ± 30.7	180.5	348.4	51.8
T_{amb} - 20 °C				
2.5	187.5 ± 59.3	20.9	208.4	10.0
5.0	212.8 ± 45.5	57.3	270.1	21.2
7.5	153.1 ± 48.2	137.4	290.5	47.3
10.0	-	180.5	-	-

Table 6.5 Energy expenditure (J g thorax^{-1} min^{-1}) by *H. armigera* calculated from the sum of the heat gain during warm-up and from the heat loss during passive convective cooling. Values given for thoracic excess (T_{th} - T_{amb}) at 2.5 °C intervals and warm-up at T_{amb} of 15 °C and 20 °C. Convective heat loss is also expressed as a percentage of the estimated total heat production.

	J g thorax^{-1} min^{-1}			
T_{th} excess °C	Warm-up	Convective loss	Total produced	% loss
T_{amb} - 15 °C				
2.5	124.8 ± 33.6	9.5	134.3	7.1
5.0	118.8 ± 49.5	25.3	144.1	17.5
7.5	110.6 ± 54.8	74.1	184.7	40.1
10.0	124.7 ± 38.7	150.1	274.8	54.6
T_{amb} - 20 °C				
2.5	231.0 ± 50.4	9.5	240.5	3.9
5.0	219.1 ± 62.2	25.3	244.4	10.3
7.5	221.9 ± 82.9	74.1	296.0	25.0
10.0	-	150.1	-	-

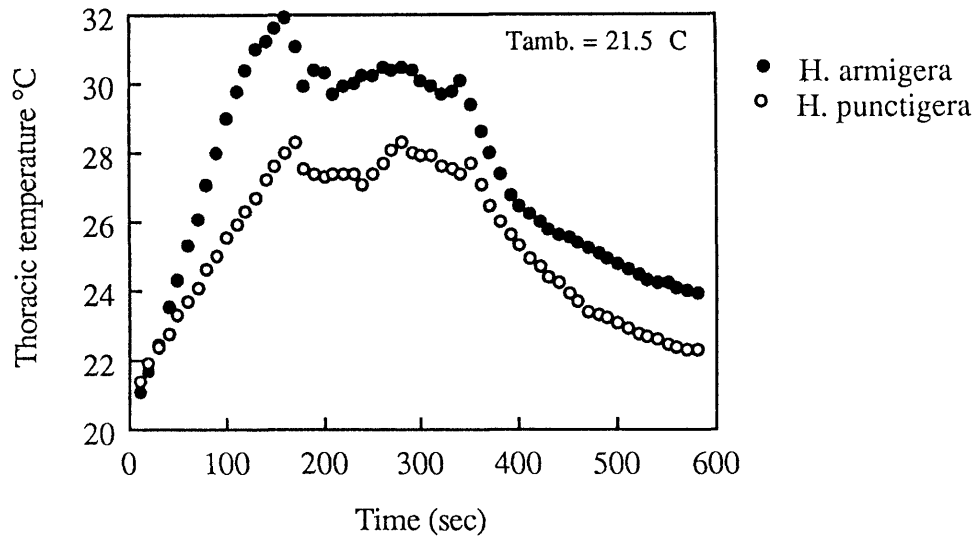

Fig 6.1 Plots of thoracic temperature (°C) versus time during endothermic warm-up, tethered flight and cooldown for a single *H. armigera* female(thoracic mass 68 mg) and a single *H. punctigera* female (thoracic mass 47.5 mg) at an ambient temperature of 21.5 °C.

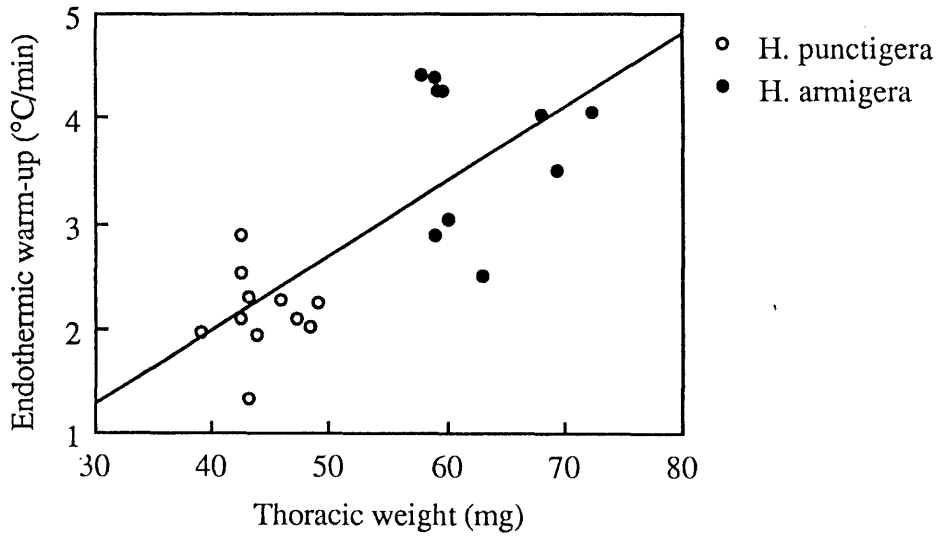

Fig 6.2 The relationship of endothermic warm-up rate ($^{o}C\ min^{-1}$) to thoracic weight (mg) for *H. punctigera* and *H. armigera* at $20 \pm 1\ ^{o}C$.

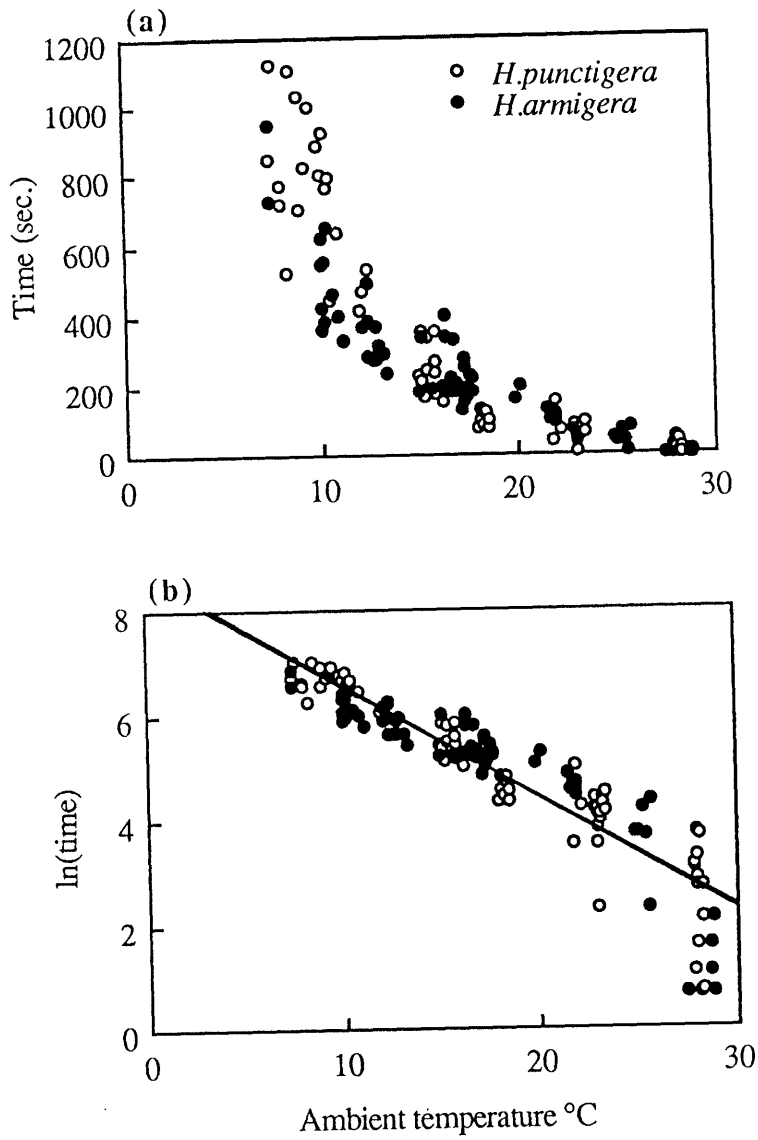

Fig 6.3 Plots of a) pre-flight warm-up time (sec) versus ambiemt temperature (°C) for *H. punctigera* and *H. armigera,* and b) plots of the same data with lognormal transformation of the time data.

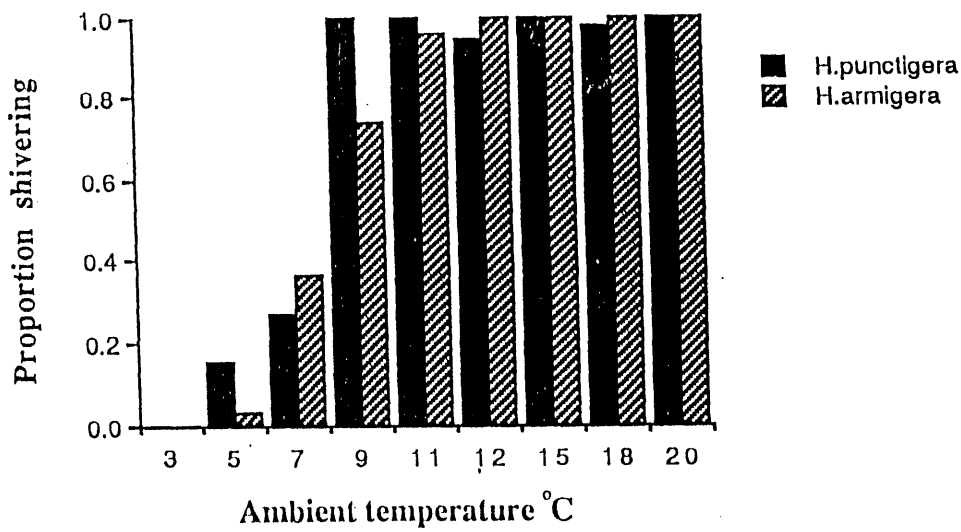

Fig 6.4 Proportions of *H. punctigera* and *H. armigera* moths initiating pre-flight warm-up (wing shivering) and continuing through to take-off as a function of ambient temperature (°C).

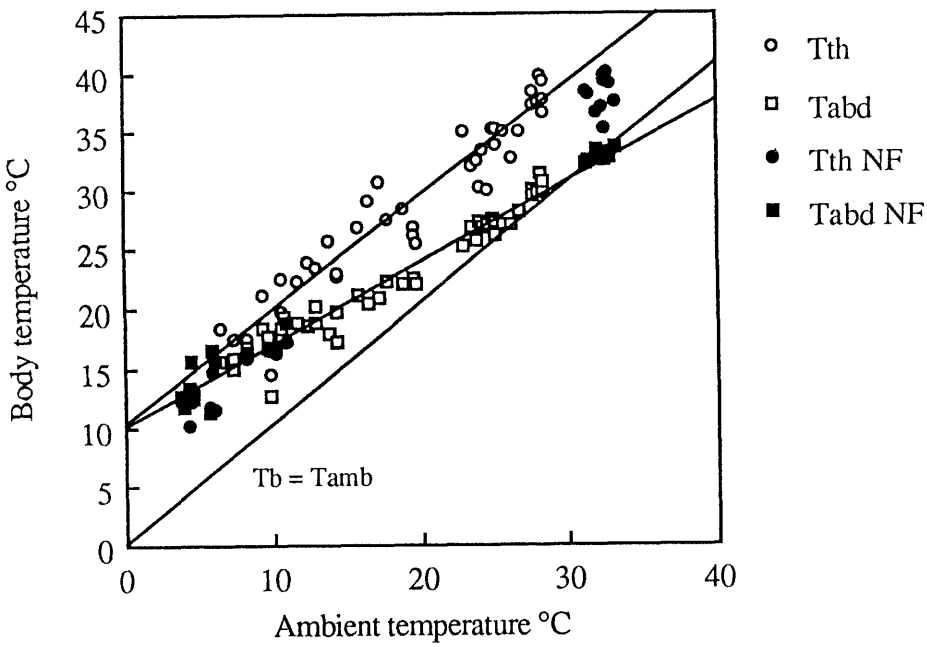

Fig 6.5 The relationship of thoracic and abdominal temperature to ambient temperature (°C) for *H. punctigera* during static tethered flight. Moths unable to maintain continuous flight (> 5 min duration) are classified as non-fliers (NF).

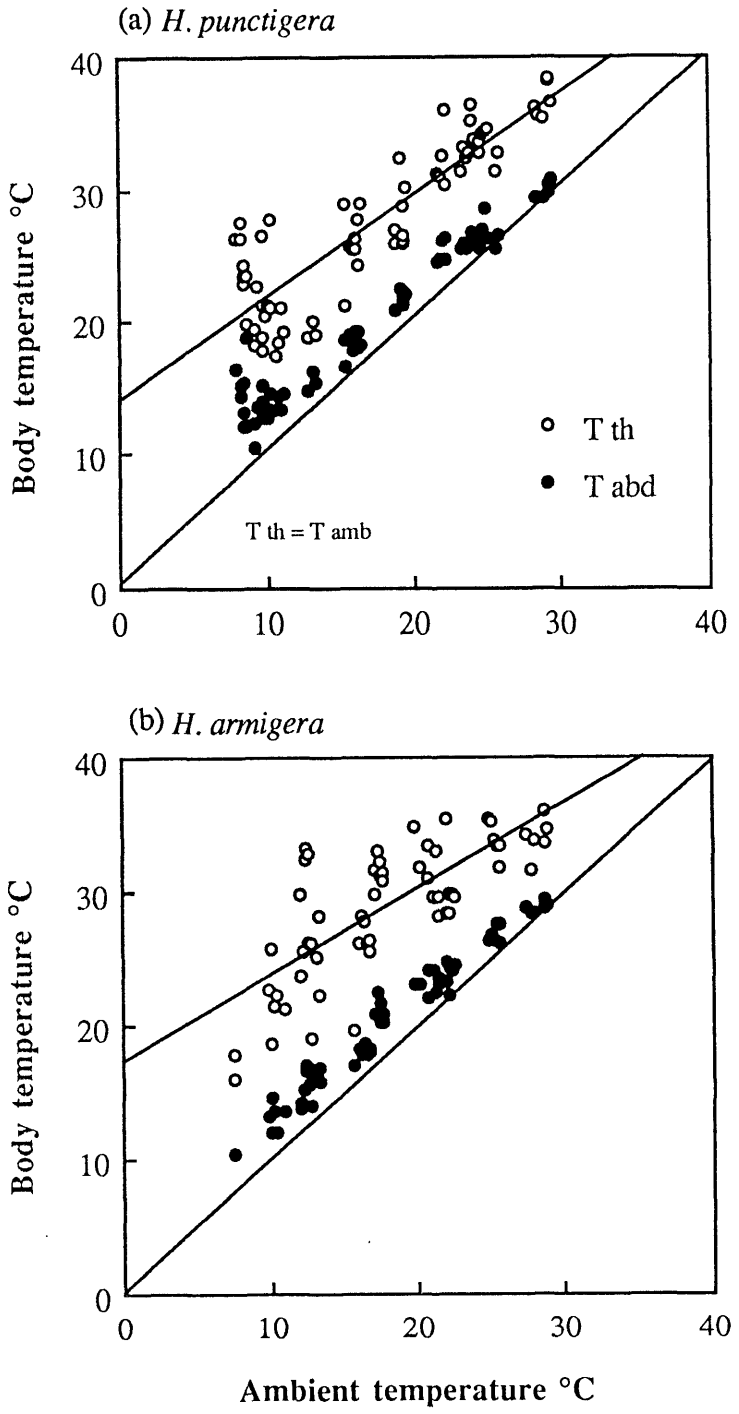

Fig 6.6 The relationship of thoracic and abdominal temperature to ambient temperature (°C) during tethered flight (attached to cotton twine) for a) *H. punctigera* and b) *H. armigera*.

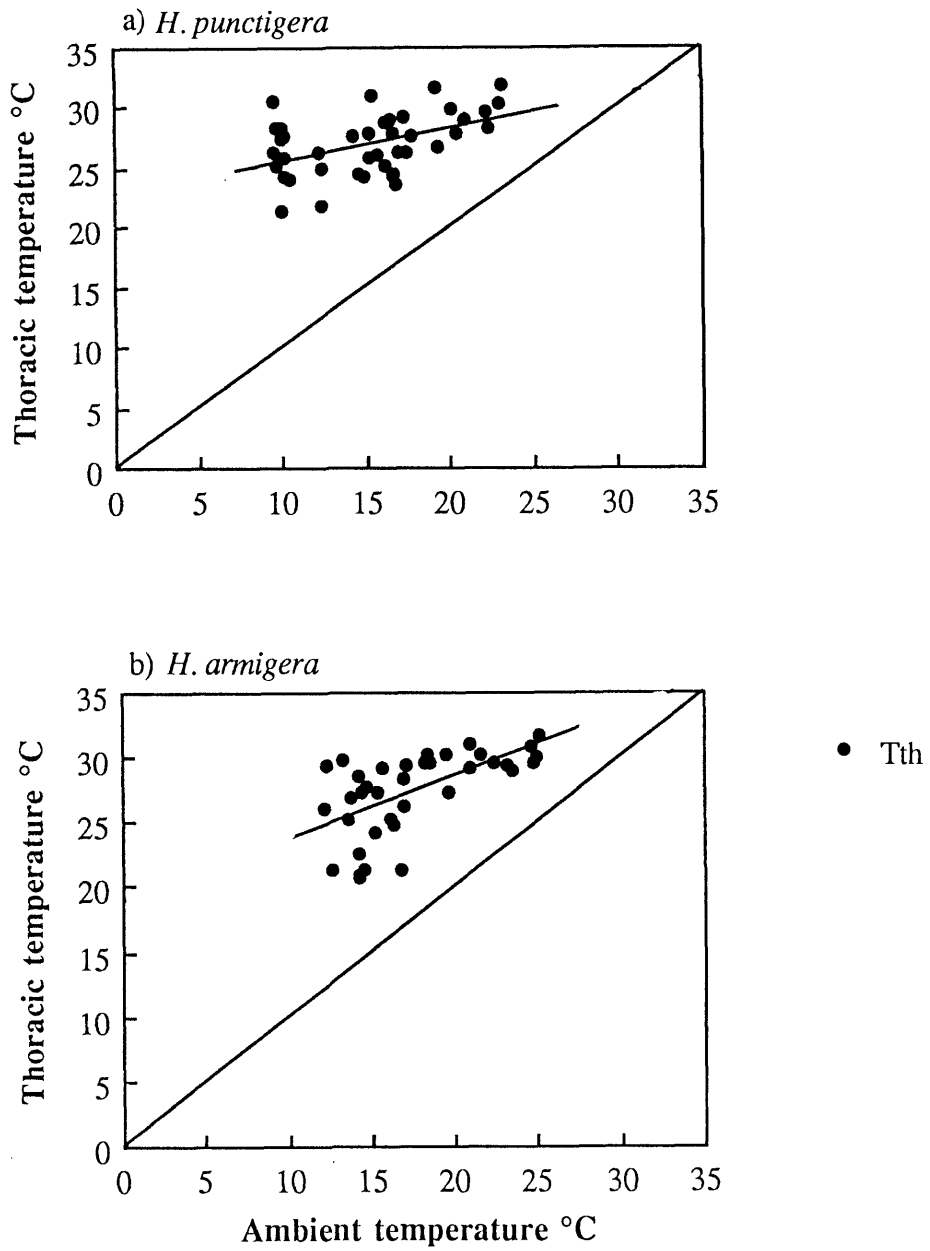

Fig 6.7 The relationship of thoracic temperature to ambient temperature (°C) based on moths caught during free flight for a) *H. punctigera* and b) *H. armigera.*

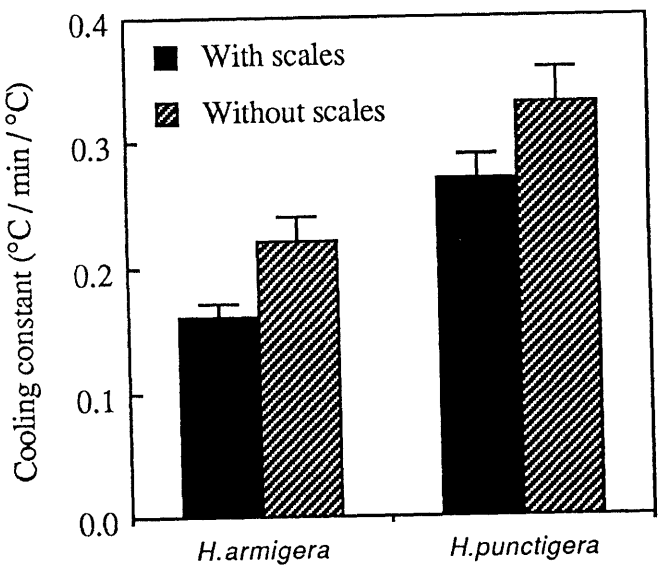

Fig 6.8 Thoracic cooling ($^{o}C\ min^{-1}\ ^{o}C^{-1}$) before and after removal of thoraic scales for *H. punctigera* and *H. armigera.*