I dedicate this work to Lisa, who was such an important part of my life in the first year of this project, and to Jenny, who picked up the pieces so wonderfully in the third. I would also like to recognise the more lasting influence of my parents, and in particular my mother. Her willingness to drop everything in order to copy or reinvent all the paraphenalia associated with my interest in insects and other living things over the years, usually at ridiculously short notice, is something I appreciate very much (particularly in a physicist) and should praise more often than I do.

ENDOTHERMY AND THERMOREGULATION IN SOLITARY BEES.

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Queen's College, January 1990.

"Every naturalist is aware that many species of insects, particularly hymenopterous insects, which live in society, maintain a degreee of heat in their dwellings considerably above that of the external atmosphere, but no one, I believe, has hitherto demonstrated the interesting fact that every individual insect when in a state of activity maintains a separate temperature of body considerably above that of the surrounding atmosphere, or medium in which it is living, and that the amount of temperature varies in different species of insects, and in different states of those species."

George Newport 1837.

"There was an Old Man in a tree,
Who was horribly bored by a bee;
When they said, 'Does it buzz?'
He replied, 'Yes, it does!'
It's a regular brute of a bee!"

Edward Lear 1846.

ABSTRACT

Graham Stone
D.Phil. Thesis

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This thesis examines the roles of endothermy and body size in the thermal biology of solitary bees (Hymenoptera: Apoidea) within the species *Anthophora plumipes* (Anthophoridae) *Amegilla sapiens* (Anthophoridae) and *Creightonella frontalis* (Megachilidae), within the genus *Anthophora*, and over the Apoidea as a whole.

The effects of body size, climate and sexual interactions on the biology of *Anthophora plumipes* were investigated in Oxford between 1987 and 1989. Both ambient temperature and body size had a significant effect on females' ability to forage, what time they initiated foraging in the morning, and the type and mass of provisions collected. The behaviour of males was also strongly dependent on ambient temperature, which affected not only when they emerged from their nest tunnels, but also how long they spent basking, when and where they fed, and whether they showed courtship behaviour.

The activity patterns and behaviour of male and female *A. plumipes* over time were shown to correlate with a complex array of factors. Activity patterns of females depended on the quality of floral resources available at foraging sites, body mass, ambient temperature, the position of the female in her nest-provisioning cycle, and levels of male interference at foraging sites. Male behaviour not only depended on body size and ambient temperature, but also on which other bees (particularly male and female conspecifics) were encountered while patrolling food sources and at the nest site.

Endothermy in bees is much more widespread than previously thought, and warm-up before flight was present to some degree in all the species examined. Levels of thermoregulation achieved, however, varied considerably between species. Warm-up rates in bees, and thoracic temperatures in free and tethered flight, are shown to depend on ambient temperature and body mass within a species (for temperate and tropical examples), across members of the genus *Anthophora* and across the Apoidea as a whole. The persistence of these relationships over a range of comparative levels suggests that they are of fundamental importance. The form of these relationships differs between families in the Apoidea, and significant patterns only emerge when a comparative technique controlling for phylogeny is applied. Furthermore, body temperatures may also depend, in at least some cases, on sex and there may be differences within a group of related species between provisioning and parasitic forms. The interaction of all these factors is complex, and the predictive value of a variable such as body mass does not always emerge unless sophisticated techniques are used to control for other variables.

The errors associated with two common methods in the measurement of insect body temperatures have often been loosely discussed but rarely quantified. This thesis examines (a) the magnitude and possible effects of errors in 'grab-and-stab' measurement of body temperature, and (b) the errors in measurement of body temperature using fixed sensors linked by thermally conducting leads to measuring devices. In neither case do the demonstrated errors preclude use of the technique, but care with interpretation is required. In both cases, measurement of thoracic temperature in small bees involves the largest errors, and this is the most serious obstacle to comparisons of endothermic and thermoregulatory abilities over the full range of body sizes found in the Apoidea.

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Chapter 1 Introduction.

1.1 A brief review of insect endothermy.

Thermoregulation in animals implies that variation in body temperature (T_b) is less than variation in ambient temperature (T_a) over the same range of environmental conditions. T_b is affected both by external heat sources (most importantly, solar radiation, and potentially, geothermal heat) and by internal generation of heat. The former is termed ectothermy, and the latter endothermy.

Animals may be both ectotherms and endotherms. All animals are to some extent ectothermic in that even the best endothermic thermoregulators also utilise solar radiation under some conditions. It is also true that because heat is a by-product of most metabolic processes, all animals produce heat and are in some sense endotherms. In practise, however, the term endothermy is generally used to refer to generation of substantial quantities of heat above background metabolic levels.

Irrespective of whether they are principally ectothermic or endothermic, most terrestrial animals show some degree of thermoregulatory ability, which may involve both behavioural and physiological mechanisms. Behavioural mechanisms are preeminent in both ectotherms and endotherms, though in endotherms physiological processes become increasingly important. The role of behavioural thermoregulation in endotherms becomes particularly marked when the limits to physiological regulation are reached.

Endothermy alone need not imply regulation of body temperature. Maintenance of a constant temperature difference between body and environment through endothermy does not necessarily imply thermoregulation. While endothermy can be shown by measurement of elevated T_b in the absence of external heat sources at a single T_a, thermoregulation can only be demonstrated by examination of T_b over a range of T_a.

The smallest animals to regulate their body temperature by internal generation of heat (endothermy) are insects. Unlike larger endothermic animals, such as most mammals and birds, no endothermic insects regulate T_b at all times (homoiothermy), but do so only during certain types of activity (heterothermy). Each period of the activity dependent on elevated T_b is preceded by periods of warm-up and followed by cooling. Unlike heterothermic birds and mammals, endothermic insects generally do not become torpid as their body temperatures approach that of their environment. Many potentially endothermic insects can walk around, feed, mate and lay eggs in this state (Heinrich 1981).

The body temperature of an endotherm is determined by the relationship between how rapidly heat is generated and how rapidly it is lost. Heat loss occurs over the surface area of an

animal, and for a given body form, surface area to volume ratio increases with decreasing size. Therefore, unless they can insulate themselves more efficiently, smaller animals must generate heat at a higher rate than larger ones to maintain the same elevated body temperature. If physical considerations determine warm-up rates and the levels of regulated T_b then there should be strong relationships between these characters and body mass. How much variation in thermoregulatory strategies is determined not by physical laws but by phylogenetic and ecological differences between species? If the roles of these factors are to be assessed, sophisticated techniques of comparative analysis must be applied.

The study of insect body temperatures is well established (reviewed by Heinrich 1981). In the early 1700's, René Réaumur observed and recorded beehive temperatures that were substantially higher than those of their surroundings, and generation of heat by honeybee colonies must have been familiar to bee keepers long before this. Davey (1826) and Newport (1837) used small mercury-in-glass thermometers to demonstrate the ability of individual insects to generate heat. The thermometers then available were far too large to allow accurate or prolonged measurement of internal T_b, and a major breakthrough was achieved in 1831 when Leopoldo Nobili and Macedonio Melloni used thermocouples for the first time. In 1899, Bachmetjer used thermocouples to show that heat was generated by the thoracic flight muscles of insects, and not throughout the body. Thermocouples and thermistors have remained the basic tools of insect thermal physiology.

The study of endothermy has advanced considerably since the 1950s. Regulation of thoracic temperature (T_{th}) by insects in flight was first suggested and convincingly demonstrated by Adams and Heath (1964; Heath and Adams 1965) for the hawkmoth *Hyles lineata*. Heinrich (1970) demonstrated that another hawkmoth, *Manduca sexta*, is capable of regulating its T_{th} between 39 and 42°C over the T_a range 17-30°C. A series of detailed studies by Heinrich showed bumblebees (*Bombus* spp.) to be excellent thermoregulators (Heinrich 1972a-c, 1974, 1975, 1976). Thermoregulatory ability is usually expressed in terms of the gradient of the best fit regression of T_b (T_{th} in the case of insects) on T_a; the lower the gradient, the greater the degree of thermoregulation. For queens of *Bombus vosnesenskii*, a good thermoregulator, the gradient of this regression is 0.27 (Heinrich 1975), and the poorer the thermoregulator, the closer this gradient is to 1. Many subsequent studies have discussed the ability of different insects to raise their T_{th} at rates dependent on T_a, and to regulate their T_{th} over a range of T_a. Endothermy has now been recorded in at least 8 insect orders: Odonata, Orthoptera, Neuroptera, Hemiptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera. Although in most cases elevated T_{th} is associated with flight, endothermy is also associated with other activities such as brood incubation by bumblebees (Heinrich 1974), ball-rolling in some dung beetles (Bartholomew and Heinrich 1978), and singing in katydids (Heath and

Josephson 1970). Heinrich (1981) has suggested five general advantages to endothermy in insects: broadening of the thermal niche, accelerating rates of egg and larval development (e.g. Seeley and Heinrich 1981), advantages in scramble and contest competition (Bartholomew and Heinrich 1978; Heinrich and Bartholomew 1978), escape from predators (Fletcher 1978) and combating disease (e.g. Watanabe and Tanada 1972).

In 1928 Dotterweich linked rapid vibration of the wings, and thus activity of the flight muscles, with an increase in T_{th} before flight in hawkmoths. Krogh and Zeuthen (1941) repeated and substantiated his results. Citing the discovery by Nielsen (1938) that heat generation and muscular work rate are correlated in man, they suggested that heat production was an unavoidable by-product of muscular activity, and that the rate of heat production depended directly on the rate of work of the thoracic flight muscles. Of all modes of animal locomotion, flapping flight is the most energetically demanding per unit time and the flight muscles of insects, which occupy most of the thorax in actively flying species, are the most metabolically active of all known tissues (Kammer and Heinrich 1974). Because the efficiency of muscle in terms of contractile work is only 20%, with the rest of the expended energy being liberated as heat (Bartholomew 1981), active flight may lead to the generation of high T_b. In all insects studied to date, warm-up correlates with high levels of muscular activity, usually of the thoracic flight muscles (e.g Heinrich and Kammer 1973), although the tymbal muscles in the first abdominal segment of cicadas are an exception (Josephson and Young 1979). When an insect warms up, the thoracic flight muscles responsible for raising and lowering the wings contract more or less in phase, rather than alternately as in flight. In some species, such as bumblebees, the opposing phasic contractions are simultaneous, and no wing movement is visible. In others, such as many endothermic butterflies, moths and dragonflies, the muscle contractions are not exactly synchronous, leading to rapid, low amplitude vibration of the wings. This visible wing movement has given rise to the term 'wing-whirring', used by some authors to describe warm-up in such insects. If it is accepted that endothermy involving muscle activity has evolved as a consequence of muscle activity patterns associated with flight, then it is unlikely that any endothermic systems involving tonic muscle contractions have evolved. No cases of such a phenomenon in insects have yet been reported.

Another endothermic mechanism, proposed by Crabtree and Newsholme (1972) and Newsholme *et al.* (1972), has received little acknowledgement in publications on insect endothermy. These studies analysed the levels of activity of two enzymes involved in the glycolytic pathway and found in insect flight muscle: fructose diphosphatase (FDPase) and phosphofructokinase (PFKase). PFKase converts fructose-6-phosphate to fructose diphosphate. FDPase carries out the reverse of this process, and shows only 10% or less of the activity of PFKase

in the flight muscles of most insects (Newsholme *et al.* 1972; Newsholme and Crabtree 1973). The usual role of FDPase is thought to be regulation of the rates of glycolysis through the regulation of FDPase activity by AMP (Newsholme and Crabtree 1970). Newsholme *et al.* (1972) demonstrated levels of FDPase activity in six bumblebee species that are ten times higher than levels recorded from the flight muscles of some other Hymenoptera. Because the activity of FDPase in bumblebees is not affected by AMP, an alternative function for this pair of enzymes has been proposed (Crabtree and Newsholme 1972; Newsholme *et al.* 1972; Newsholme and Crabtree 1973). These workers have suggested that in bumblebees these two enzymes form a 'substrate cycle', or 'futile cycle', interconverting two substrates at equal rates, releasing energy from ATP and producing heat as a "waste product", decoupled from flight activity. The heat thus generated would then be available for warm-up. At a muscle temperature of 35°C, activity of this futile cycle could produce $0.07 Wg^{-1}$ muscle, or about 25% of the power generated during warm-up in a Queen *Bombus vosnesenskii* (Newsholme *et al.* 1972; Heinrich 1975a). Because activity of this futile cycle inhibits glycolysis, which is necessary for the provision of fuel for flight in bumblebees (Newsholme *et al.* 1972), such a mechanism is proposed to contribute to warm-up only when the bee is not flying. The rate of activity of the FDPase/PFKase cycle in bumblebees has been shown to correlate negatively with ambient temperature (Clark *et al.* 1973; Clark 1976), a result predicted if this system is involved in thermoregulatory processes. The extent of this phenomenon remains little known, and substrate cycling between enzymes other than FDPase and PFKase is possible. The importance of such systems remains controversial, and it is probable that in insects which do show high levels of muscle activity during warm-up this type of endothermic mechanism, if it does occur, contributes a small proportion of the total heat generated. The only endothermic insects in which heat generation has not been shown to correlate with intense muscular activity are the larvae of social wasps in the genus *Vespula* (Ishay and Ruttner 1971). Although muscular activity may be involved, no vigorous activity by the larvae is recorded, and generation of heat by alternative systems (such as futile cycling) seems possible. Futile cycling is discussed further in Chapter 7.

The established view is that heat generated during flight muscle activity cannot be controlled (Kammer 1981) (although some insects, such as dragonflies, can regulate the heat produced by interspersing gliding and powered flight) and that any thermoregulation demonstrated by flying insects occurs through regulation of heat loss over the body surface. Mammals and birds are often able to regulate the flow of heat across their integuments by regulating the thickness of the insulating air layer trapped against their bodies through mechanisms such as piloerection. Although many endothermic insects are covered in insulating layers of hairs or scales, there is no evidence that they actively control their thickness. The most general mechanism used by insects to regulate heat

loss is the control of heat circulation via the blood. In hawkmoths and bumblebees thermoregulation is achieved by restricting heat to the thorax at low T_a, and releasing heat, via the blood, to other parts of the body when the thorax overheats (Chapter 4).

With the growing awareness of the wide taxonomic distribution of insect endothermy have come advances in our understanding of the metabolic processes involved. The neural control of endothermy has been studied (Hanegan and Heath 1970; McCrea and Heath 1971; Hanegan 1972; Esch 1988), and its metabolic cost has been extensively investigated in the laboratory through the use of oxygen consumption data (Kammer and Heinrich 1974; Heinrich 1975a; Bartholomew *et al.* 1981; Casey 1981a; Bartholomew and Barnhart 1984). The energy supplies and stores used in warm-up have also been examined (Hudson 1958; Clegg and Evans 1961; Joos 1987). The adaptive value of endothermic regulation has been discussed in terms of muscle kinetics and efficiency: below certain temperature maxima, the rate of muscle activity increases with temperature, and thermoregulation allows the evolution of narrower temperature optima for enzymes and finer control of the many complex enzyme reaction equilibria which constitute muscular and neural activity (Barnett *et al.* 1975; Josephson 1981).

Some field studies have used endothermic insects as a convenient biological system in which to investigate more general phenomena, such as osmoregulation and foraging (e.g. Willmer 1986, 1988). Others have attempted to link thermoregulatory abilities to specific aspects of species ecology, such as male courtship behaviour (Chappell 1982, 1984; Stone *et al.* 1988), or to morphological differences between taxa (Bartholomew and Heinrich 1973; Bartholomew and Casey 1978; Casey and Joos 1983). Many recent studies apply the original investigations of Heinrich and others to new species (e.g. Baird 1986; Heinrich and Buchman 1986; Heinrich 1987; Morgan 1987). The existence of clear general patterns in endothermic parameters (such as warm-up rate) across members of a species, or across species in a taxon, remains to be demonstrated. Because most field studies of insect thermal physiology provide only a 'snapshot view' of body temperatures under a particular set of conditions, the roles of thermoregulation and endothermy within a broader ecological context are known only for very few species. In fact, the only insects for which such information is available, due largely to almost 20 years of work by Heinrich and colleagues, are bumblebees (*Bombus* spp.) (Heinrich 1972a-d; Heinrich and Heinrich 1983) and the honeybee, *Apis mellifera* (Heinrich 1979; Cooper *et al.* 1985; Dyer and Seeley 1987). More detailed studies of the interaction between ecology and thermal physiology are required.

1.2 The aims of this thesis.

This thesis examines endothermy in a single insect taxon, the bees (order Hymenoptera,

suborder Apocrita, division Aculeata, superfamily Apoidea [but see Chapter 7]), and has two major objectives. The first is to describe the ecology, behaviour and thermal physiology of a single bee species in considerable detail, making possible a discussion of the importance of endothermy in all aspects of its adult life. In particular I assess the effects of T_{th}, body mass and sex on behaviour and activity patterns, both in the field (Chapters 3 and 4) and in the laboratory (Chapter 4).

The second objective is to establish how widely endothermy is found in the Apoidea. Data on endothermic ability for a diverse range of bee taxa are examined in order to establish whether there are any general patterns over the Apoidea. I examine the importance of body mass, phylogeny and the thermal environment inhabited by a species for predicting warm-up rates and T_b in flight (Chapters 5 and 6).

The methods used to measure insect T_b have remained almost unchanged and unquestioned in the literature (Chapter2). The commonest field technique involves insertion of a thermocouple into the body of an insect as soon as possible after capture ('grab-and-stab'). The temperature reading obtained is assumed to equal that of the insect at the time of capture. The behaviour of the insect after capture, and the time delay between capture and insertion of the thermocouple may be critical to the difference between the recorded temperature and the insect's actual T_{th} at the time of capture. In Chapter 2 I examine the changes in T_{th} that occur at the end of tethered flight in bees and assess their magnitude and the effect that errors in 'grab-and-stab' techniques may have on estimates of thermoregulatory ability in insects (Stone and Willmer 1989a). In the laboratory there are measurement errors inherent in the continuous recording of T_b using electrical sensors due to heat loss through the sensor leads, but there has been no specific discussion of their importance and magnitude. The assumption has been that these errors are small except for small insects and that the warm-up rates obtained for such small insects are underestimates of true values (Prof. G. Bartholomew, personal communication). It is therefore possible that such errors might seriously bias any analysis of the role of body mass in determining warm-up rates across a taxon including such small species. A simple treatment of such errors is presented (Chapter 2), and I demonstrate that the probable effect of these errors is too small to explain the relationships between warm-up rate and body mass that are described.

Much variation in intra- and inter-specific patterns of warm-up remains to be explained. Any investigator who has examined endothermy in insects in the laboratory must be aware not only of changes in the form of warm-up curves over time within individuals, but also of differences between individuals of the same species. There is no discussion of the former, and little of the latter, in the literature. Factors such as exhaustion of fuel supplies, habituation to the stimuli used by investigators to induce warm-up, or differences in the location of temperature measuring devices

between study insects may be significant sources of experimental variation. The effects of such factors should be investigated and, where possible, controlled for. In Chapter 4, I examine the importance of exhaustion and feeding of tethered insects for determining their endothermic performance. A large number of bees was used in an attempt to minimise the effect of individual variation in the establishment of general hypotheses, and to provide data in which individual differences may be analysed.

As well as consideration of within-species variation, there are good reasons to expect the existence of interesting patterns across species within endothermic insects. One relationship yet to be investigated fully is that which exists between body mass and warm-up rate. Across the range of body sizes shown by heterothermic vertebrates, warm-up rate decreases with increasing body size (Bartholomew *et al.*1957; Morrison 1960; Lasiewski and Lasiewski 1967). Inasmuch as insects are generally smaller than vertebrates, and warm up more rapidly than vertebrates, the generalisation that rate of warm-up in heterothermic animals is inversely related to mass can be extended to include heterothermic insects (Heinrich and Bartholomew 1971; Bartholomew 1972). May (1976a) has suggested that because rates of metabolic heat production cannot compensate for the increasing rates of heat loss experienced by very small endotherms, warm-up rate should be positively correlated with body size. The relationship between these variables for endothermic insects is unclear: studies to date have either indicated no relationship or a non-significant positive correlation (May 1976a), suggesting that at very low body masses the relationship between warm-up rate and body mass may be reversed. The analyses performed to date (e.g. May 1976a) have not taken into account the complicating effects of phylogeny in cross-species comparisons (reviewed by Pagel and Harvey 1988). There is also a shortage of methodologically compatible data. It therefore remains unclear whether purely physical relationships dominate the thermal ecology of the smallest endotherms. Resolution of this problem requires the generation of a large data set from a group of species whose phylogeny is well established, and its analysis using statistically valid comparative methods. It is then possible to investigate the relationships between other factors related to thermal physiology, such as ecological and behavioural differences between species and sexes within a species.

As an endothermic group the bees represent an ideal taxon within which to investigate both the detailed thermal ecology of a few key species and patterns across species. Bees have been the subject of many of the existing studies on thermal physiology in insects. To date there has been a particular bias towards certain groups: as well as the work on honeybees and *Bombus* spp. introduced earlier, there have been several studies on euglossine 'orchid bees' (Inouye 1975; May 1976a; May and Casey 1983) in the family Apidae, and the large carpenter bees of the genus *Xylocopa* in the family Anthophoridae (Chappell 1982; Nicolson and Louw 1982; Louw and

Nicolson 1983; Baird 1986; Heinrich and Buchmann 1986; Willmer 1988). An increasing body of knowledge on the natural history of bees, particularly on variation within the taxon in foraging and mating strategies, has yet to be analysed in the light of advances in thermal physiology. The most detailed work to date has been on social bees, with detailed investigations not only of individual physiology but also colonial strategies, particularly those of honeybees. The thermal physiology of solitary bees is less well known, and their ecology differs in several important respects from that of social bees. All female solitary bees provision their cells independently: the thermal strategies of solitary bees are thus the results of interactions between bees and their environment unbuffered by thermal or energetic assistance from other bees. Unlike the non-breeding female workers of social species such as *Apis mellifera*, all female solitary bees are available for mating, sometimes more than once. Females may be concentrated or dispersed in space and time, leading to a wide diversity of male mating systems. Most studies on thermoregulation in social bees have discussed the physiology and foraging strategies of workers. Thermal aspects of the sexual interactions which are a major component of the daily lives of many solitary bees, particularly males, have been little studied (e.g. Chappell 1982, 1984). This thesis investigates the importance of sexual differences in endothermic ability in more aspects of the behaviour of a single species than past studies have done.

Bees are well-suited to a cross-species analysis of the effects of body size, ranging in weight from a few milligrammes to more than a gramme. There are also some published studies with which new data may be combined to allow analyses spanning several families within the Apoidea. Bees are abundant and diverse insects in many parts of the world, allowing the collection of data for many species from a wide variety of habitats. Bees also appear to withstand the procedures required to obtain continous measurements of T_{th} very well, and can usually be released alive at the end of experiments.

The species chosen for detailed study in order to achieve the first objective is the solitary bee *Anthophora plumipes*, (family Anthophoridae). In Britain *A. plumipes* flies in the spring when weather conditions and T_a fluctuate widely. This variation creates a situation in which some degree of endothermic thermoregulation, allowing continued activity, has advantages over activity that is governed solely by dependence on unpredictable environmental conditions. Preliminary studies revealed that *A. plumipes* is indeed highly endothermic, and thus a good candidate for study. A large study population allowed individuals to be removed without apparent disturbance to the population. *A. plumipes* is one of the largest species of solitary bee found in large numbers in Britain, and is of a size (100-220mg) allowing laboratory investigation of T_b without lasting damage to the bee. *A. plumipes* is also so passive that living individuals can easily be handled in the field. There is a considerable literature on the phylogeny and general biology of the genus *Anthophora*,

allowing for comparisons with other species in this genus.

For the second objective, I have carried out two comparative analyses of warm-up rates and T_b across the Apoidea. The first uses only my own data for members of the genus *Anthophora*. Data for most of the species in this analysis were gathered in Israel from February to March 1989. The second analysis includes data on species in Papua New Guinea investigated in September and October 1987, as well as species studied in Britain and Israel and data gathered from the existing literature. These studies revealed that endothermy is far more widespread in the Apoidea than had previously been demonstrated. The collection of data for bees inhabiting a wide variety of environments and from a variety of families has allowed me to analyse the importance of phylogeny and habitat as well as body size in predicting warm-up rates and T_b across the Apoidea, and to resolve the relationships between these variables.

Chapter 2: Methods, materials and sites.

2.1 Research Sites

Comparative studies required gathering data on as many species in as wide a range of habitats as possible. As well as visiting British sites, oportunities arose to work in Israel at semi-arid and true desert study sites, and in Papua New Guinea in humid tropical habitats varying from coastal to sub-montane rainforest. The site at which each species was studied is given in Table 2.1 and each of the study sites mentioned for each country in Table 2.1 is described below. The names used for the bumblebees are *sensu* Kloet & Hincks (1978) for the European species, and *sensu* Krombein *et al.* (1979) for the North American species discussed in Chapter 6. Unnamed species are currently being identified by Chris O'Toole at the Hope Department of Entomology at the University Museum, Oxford.

A. British Study Sites.

The largest *Anthophora plumipes* nest site studied in Oxford was in a south-facing wall in Merton College car park, with between 200 and 300 occupied nests in an area of old stone wall 18m by 2m. Observations of feeding behaviour were made at two sites identified as important foraging areas for this species, shown in Fig.2.1. The closest was a garden in University college of which the nest site wall formed the southern boundary, where the main forage source for *A. plumipes* was comfrey. The second major site was the Botanic Gardens, the walls of which also support a small population of *A. plumipes*. To observe the effects of differences in microclimate on bee foraging behaviour, two areas of lungwort (Chapter 3) in different locations in the garden were chosen. The sun site faced south, with a large open area to the east. On a clear day it received almost continuous sun from sunrise onwards, and experienced the highest T_a. The shade site was shaded by trees, and by a high wall 3.5m to the south. This site received direct sunlight only in the evening, and on a sunny day was always cooler than the sun site. Both sites covered an area of approximately $1m^2$ and the numbers of flowers available to foragers at each site were similar, with approximately 250 in each site over the period for which the sites were compared (see below).

Bees were studied at three other British study sites.

1) Dry Sandford Pit Reserve is a small reserve run by the Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust (BBONT) at Dry Sandford, near Oxford. The reserve has a large area of exposed sand cliffs populated extensively by, amongst other species, *Colletes daviesanus* and *Osmia leaiana* (Table 2.1).

2) The Bee Research Unit, University College, Cardiff specialises mainly in research on honeybees (*Apis mellifera*). In 1988 a research programme was also being carried out on the nesting

Table 2.1 Study sites and dates of study for the species in this thesis.

Species	Family	Study location	Period of study
Species studied in the U.K.			
Apis mellifera L.	Apidae	Zoology dept. Oxford	2.87-5.87, 1988.
Bombus terrestris (L.)	Apidae	Botanic Gardens, Oxford.	1988.
B. pascuorum (Scop.)	Apidae	Botanic Gardens, Oxford.	4.87-6.87, 1988.
B. lucorum (L.)	Apidae	Botanic Gardens, Oxford.	5.88-8.88.
B. lapidarius (L.)	Apidae	Botanic Gardens, Oxford.	1988.
Psithyrus vestalis (Geoffroy)	Apidae	Botanic Gardens, Oxford.	6.88-9.88.
Anthophora plumipes (Pallas)	Anthophoridae	Botanic Gardens, & Merton and Univ. Colleges, Oxford.	3.87-6.87, 3.88-6.88, 3.89-6.89.
A. quadrimaculata (Panzer)	Anthophoridae	Botanic Gardens, Oxford.	6.88-7.88.
Melecta albifrons (Fab.)	Anthophoridae	Merton College, Oxford.	as for *A. plumipes*.
Osmia rufa (L.)	Megachilidae	Merton College, Oxford.	4.87-6.87, 4.88-6.88.
Osmia leaiana (Kirby)	Megachilidae	Sandford Pits BBONT reserve, near Oxford.	5.88-7.88.
Megachile willoughbiella (Kirby)	Megachilidae	Botanic Gardens, Oxford.	5.87-7.87, 5.88-7.88.
Megachile centuncularis (L.)	Megachilidae	Botanic Gardens, Oxford.	5.88-7.88.
Colletes cunicularius (L.)	Colletidae	Kenfig N.C.C. reserve, near Cardiff, Wales.	3.88-5.88.
Colletes daviesanus Smith	Colletidae	Sandford Pits BBONT reserve, near Oxford.	5.88-7.88.
Andrena clarkella (Kirby)	Andrenidae	Bee Research Unit, University College, Cardiff.	2.88-4.88.
Andrena fulva (Mulleer in Allioni)	Andrenidae	Bee Research Unit, University College, Cardiff, and Oxford.	3.87-5.87, 3.88-4.88.
Andrena nigroaenea (Kirby)	Andrenidae	Merton College, Oxford.	4.88-6.88.
Lasioglossum smeathmanellum Kirby	Halictidae	Botanic Gardens, Oxford.	6.88-7.88.
Species studied in Papua New Guinea (see text for description of sites).			
Amegilla sapiens (Cockerell)	Anthophoridae	Baiyer River Sanctuary; Kobum plantation; Madang; Kuk Research Station.	7.86-8.86, 8.87-10.87.
Xylocopa (*Koptortosoma*) spp.	Anthophoridae	Baiyer River Sanctuary.	9.87.
Thyreus quadrimaculatus (Rad.)	Anthophoridae	Baiyer River Sanctuary; Madang.	as for *A. sapiens*.
Creightonella frontalis (Fab.)	Megachilidae	Baiyer River Sanctuary; Kuk Research Station; Madang.	8.87-10.87.

Table 2.1 (cont.)

Species	Family	Study location	Period of study
Megachile spp.	Megachilidae	Baiyer River Sanctuary; Kuk Research Station.	9.87.
Megachile spp.	Megachilidae	as above.	9.87.
Coelioxys spp.	Megachilidae	Madang.	8.87-10.87.
Nomia spp.	Halictidae	Kuk Research Station.	9.87.
Nomia spp.	Halictidae	Kuk Research Station.	9.87.
Species studied in Israel in February and March 1989.			
Genus *Anthophora*	**Subgenus**		
Anthophora plumipes (Pallas)	*Anthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. senescens Lepeletier	*Anthophora*	Avdat Research Farm, Negev Desert.	
A. fulvitarsis Brullé	*Anthophora*	Avdat Research Farm, Negev Desert.	
A. erschowi Fedtschenko	*Pyganthophora*	Avdat Research Farm, Negev Desert.	
A. libyphaenica Gribodo	*Pyganthophora*	Avdat Research Farm, Negev Desert.	
A. nigriceps Morawitz	*Pyganthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. rubricus Dours	*Pyganthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. sergius (Nurse)	*Pyganthophora*	Avdat Research Farm, Negev Desert.	
A. sp. aff. *sergius*	*Pyganthophora*	Avdat Research Farm, Negev Desert.	
A. vernalis Morawitz	*Pyganthophora*	Avdat Research Farm, Negev Desert.	
A. biciliata Lepeletier	*Lophanthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. caelebs Gribodo	*Lophanthophora*	Avdat Research Farm, Negev Desert.	
A. dispar Lepeletier	*Lophanthophora*	Avdat Research Farm, Negev Desert; Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. disparilis Friese	*Lophanthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. hispanica (Fabricius)	*Lophanthophora*	Avdat Research Farm, Negev Desert.	
A. rutilans Dours	*Lophanthophora*	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	
A. priesneri Alfken	*Petalosternon*	Avdat Research Farm, Negev Desert.	
A. wegelini Friese	*Petalosternon*	Avdat Research Farm, Negev Desert.	
Other genera			
Xylocopa cyanescens Brullé (=*X.iris* Christ of authors)	Anthophoridae	Har Gilo, Jerusalem; The Hebrew University, Jerusalem.	

biology of bees in the genus *Andrena.*

3) Kenfig Pool and Dunes S.S.S.I. is a small N.C.C. reserve in an area of sand dunes near Pyle on the southern coast of the Gower peninsula, Glamorgan. Permission from the N.C.C. was obtained before working at this site.

B. Study Sites in Papua New Guinea.

All work at Madang, Kuk and Kobum was carried out in September and October, 1987.

1) Baiyer River Sanctuary, situated in an area of low montane rainforest (altitude 1160m) in the Baiyer River valley near Mount Hagen, Western Highlands Province, is a reserve operated by the P.N.G. government's Department for the Environment and Conservation. This site was first visited during an undergraduate expedition in July, August and Sptember 1986 (Stone *et al.* 1988), and again in October, 1987.

2) Kuk Agricultural Research Station (altitude 1550–1600m), located just outside Mount Hagen, is operated by the P.N.G. government's Department of Primary Industry. Most of the station is covered in plots of a variety of crops, including cardamom.

3) Madang is a major town on the northern coast of P.N.G. Physiological work was carried out in the laboratories of the Christensen Research Institute (C.R.I.), located 10km to the north of Madang town. Field work on the solitary bee *Creightonella frontalis* was carried out at Siar coffee plantation, planted with 102 hectares of *Coffea canephora* Pierre ex Froehner (syn. *C. robusta*) (Willmer and Stone 1988). The nests of this species were constructed in the walls of artificial drainage ditches, 25-90cm in depth. The walls of the ditches were usually bare earth, but with up to 30% vegetation cover in places.

Fieldwork on the Madang population of the solitary bee *A. sapiens* was carried out within the grounds of the C.R.I.

4) Kobum (altitude 1000m) is the site of a large new cardamom plantation in an area originally covered in lower montane rainforest, in the mountains to the south of Madang, Madang Province.

C. Study sites in Israel.

Work in Israel was carried out between 1.2.89 and 19.3.89.

1) Most of the work in Israel was carried out at the Botany Department of the Hebrew University, Givat Ram, Jerusalem. Bees were collected from an artificial mediterranean plant community established at Har Gilo (Beit Jala) in the Occupied Territories of the West Bank to the south of Jerusalem.

2) In the Negev Desert bees were collected and studied at Avdat experimental farm, an arid agriculture research station run jointly by Be'er Sheba University and the Hebrew University. The farm is located in the high Negev, to the south of Sede Boqer, in an area of stony desert (strictly in

part of the Irano-Turanian steppe-desert transition zone). Natural vegetation is concentrated in wadis (water runoff channels), and is augmented at Avdat by almond orchards.

All other data used in comparative studies were collected from the literature, or were personal communications from Dr. Pat Willmer (see Chapter 6).

2.2 Statistical techniques.

When analysing the affects of more than one continuous variable (such as T_a and body mass) on another continuous variable (such as T_{th}), multiple regression has been used. Utilisation of this technique is only valid if all the data are statistically independent. When each data point comes from a different individual (e.g. bee), this assumption is probably justified. When each individual contributes a different number of values to the data set, as in examinations of warm-up rates, flight temperatures or abdominal pumping rates (see below) it may not be. If there are differences between individuals (such as damage due to insertion of a thermocouple) which are not due to the variables being examined, individuals for which more data were obtained will bias the analysis towards this unknown variable. To control for this effect, each individual must contribute the same weight to the analysis. To achieve this mean values of the variables being investigated were obtained for each individual, and are referred to an individual means (e.g. individual mean warm-up rate, etc.). These values are then used in a normal multiple regression analysis. When giving the results of such analyses rather than simply the correlation coefficient R I have given R^2, which is the proportion of the total variance in the data accounted for by the regression. Sample sizes (n) and p values are also given.

When I have analysed the affects of variables which are not continuous but categorical (such as differences due to three different populations, Chapter 5) I have used Anova. When the categorical variable has onlt two levels (such as sex) I have entered it as a continuous variable in multiple regression. In such a situation multiple regression and Anova are equivalent. Statistical tests were carried out using the Glim and Statview statistical packages for the Apple Macintosh, or following techniques described in Sokal and Rohlf (1981).

Where simple regression lines have been fitted on figures, the equation for the regression is given in the figure legend, together with the value of R^2. When mean values have been used, the sample size for each mean is given by each point, and error bars indicating ± 1 standard error are fitted.

2.3 Field techniques.

A. Foodplants: nectar and pollen sampling.

Chapter 2. Methods, materials and sites

Nectar was collected from flowers using glass micropipettes with volumes of 1,2 and 5 µl (Camlab, U.K.). The volume of nectar collected was calculated from the length of the fluid column. In the cases of the flowers of *Pulmonaria saccharata* and *Symphytum orientale* (Chapter 3), and of *S. jamaicensis* (Chapter 5), nectar accumulated in a deep corolla tube. Gentle pulling of the flower allowed removal of the entire corolla without unduly disturbing the nectar. Nectar was visible as small droplets inside the base of the corolla, which could then be collected with a micropipette. Each flower was therefore only sampled once, and removed from the plant. Nectar was collected from flowers that were exposed to insect visitors, the aim of measurements being to assess the pattern of variation in the nectar resources available to foragers. 20 flowers of each species were sampled sequentially at each sampling time. For both of the foragers whose flower visitation is discussed in detail, the length of the tongue should allow access to any nectar in the corolla of the plants studied, and the volume recorded is assumed to approximate the volume available to foragers.

Nectar concentration was measured immediately after collection with a pocket refractometer modified by the maker to accept volumes of fluid down to 0.5µl (Bellingham and Stanley Ltd., U.K.). The volumes of nectar available in the three plant species listed above were very small and the concentrations high under some climatic conditions (Chapter 3, 5). Because of the small volumes involved, and the high viscosity of some nectars at high sucrose concentrations, available nectar under such conditions may not be collectable using the techniques described (Corbet 1978a). Furthermore, it is possible that the bees discussed in this thesis were able to collect small volumes of concentrated nectar by diluting it with saliva, as observed for *Apis mellifera* (Simpson and Reidel 1964). If this is true, flowers recorded as empty may still represent a sugar source for foragers. Despite the undoubted existence of such errors, it is assumed that they are relatively small.

The flowers of lungwort (*Pulmonaria saccharata*), which is an important forage source for *A. plumipes* (Chapter 3), continued to contain nectar for up to three days. During this period there were clear changes in the colour and form of the corolla which indicated flower age. The potential value of measuring nectar supplies at a forage source in explaining the behaviour of foragers will depend on the similarity between the subset of the total available flowers sampled by animal and experimenter; if nectar measurements are to be useful, experimenter and bee must visit the same sort of flowers. Because bees may differentiate between flowers containing different quantities of nectar (Marden 1984; Corbet *et al.* 1984; Wetherwax 1986), and the rates of nectar secretion by flowers may vary with age (Boetius 1948, in Corbet 1978a), it was necessary to establish whether either nectar supplies or the behaviour of *A. plumipes* were related to flower age. The corolla of lungwort consists of a cylinder of 5 or 6 fused petals forming a corolla tube which opens out 9-11mm from the base of the corolla to produce a flower 14-16mm across. The age of the flower was scored on the

basis of changes in the colour of the petals, summarised in Table 2.2.

Table 2.2 Scoring of flower age in lungwort on the basis of changes in corolla form and colouration.

Score.	Description of flower.
1	Immediately after the opening of the flower bud, with uniformly pink petals which remained partly folded (flower diameter 5-7mm) for several hours.
2	Flowers a uniform pink, and fully opened, giving a corolla diameter of 14-16mm.
3	Flowers with blue pigmentation of the vessels in the petals; anthers at entrance to corolla tube not yet shrivelled.
4.	Flowers with extensive blue colouration of the petal surface; anthers shrivelled.

Scoring of flowers that were sampled for nectar was carried out by two observers (G.N.S., and Dr. Pat Willmer). The affect of flower age on nectar standing crop was investigated at three lungwort sites in the Botanical Gardens in Oxford on 12.4.87, including the sun and shade sites described above. The third site was in an east facing bed 60m from the shade site, with a diurnal pattern of climatic change very similar to that of the sun site. At 11 times at the shade site, and at 6 times at each of the two others, between 06.30h and 17.30h, each observer collected 10 flowers, collecting any nectar and ageing them on the basis of the scale given in Table 2.2. At each time interval and for each site a mean nectar concentration, volume and flower age were calculated. The results of this study are discussed in Chapter 3.

When recording the foraging behaviour of *A. plumipes* flowers were designated as either pink (scores 1 and 2) or blue (scores 3 and 4), in order to keep up with the rapid foraging flight of this bee. The effects of flower age on nectar and foraging in *A. plumipes* are discussed in Chapter 3. It is also possible that *A. plumipes* selected flowers on bases other than colour; bumblebees, for example, have been shown to avoid recently visited flowers (Corbet *et al.* 1984; Kato 1988). Although different individual *A. plumipes* were observed to visit the same flower within periods of 10s or less, the possibility that a similar discriminatory ability exists in this species cannot be excluded. In the light of the results discussed in Chapter 3, estimates of standing crop for lungwort were obtained by sampling 10 blue and 10 pink flowers at random.

Pollen was collected using a mixture of glycerol and the dye Saffranin Red. Short sections of wooden splints were attached to the insides of the lids of small tubes, so that they hung down inside the tube without touching its walls. The splint was dipped into warmed Saffranin Red/glycerol mixture, which was allowed to dry as a sticky semi-solid drop at the end of the splint.

When taking a pollen sample from a bee in the field, the prepared tip of the splint was pressed against the bee's scopa (or other area, as required), and covered in pollen; the serum tube was then closed. In this way the time for which the splint was exposed to other contaminating sources of pollen was minimised. Only a single sample was collected on each splint, and to avoid contamination, new sections of splint were attached each time a tube was used, and the tube thoroughly washed. In the lab, the dying mixture and pollen were removed with a scalpel, and placed on a microscope slide. The slide was then warmed until the mixture melted, and a coverslip applied. As well as samples from the bees themselves, samples were also taken from the anthers of plant species on which the bees were observed to feed, forming a reference collection. In cases where identification proved difficult I received considerable help from Mrs M. Harley at the Palynology Unit of the Royal Botanic Gardens, Kew.

Large insects were excluded from flowers of *Stachytarpheta jamaicensis* in order to examine the loss of corollas from the flowers in the absence of disturbance by foragers (Chapter 5). Flower spikes were enclosed within a netting cage made from mosquito netting supported on a light wire frame. Because of the effects that bagging is known to have on the microclimate of flowers (Corbet and Willmer 1981; Corbet and Delfosse 1984), the results obtained are used only as an indication of what might happen in the absence of the mechanical effects of foragers.

B. Climate

Ambient temperature was generally measured using a mercury thermometer placed in the shade such that the bulb was at least 6 inches above the ground to reduce the effect of ground temperatures on the recorded air temperature. This measure of air temperature was found to be a satisfactory approximation to a more sophisticated measure usd in other studies of thermal physiology (see below). During 'grab-and-stab' measurements of bee body temperature (see section 2.4 below), air temperature was measured as soon as possible after the measurement had been made using the dried thermocouple held in a shaded position where the bee was captured. A second measure of ambient temperature was used for *Amegilla sapiens* (Chapter 5; Stone *et al.* 1988). A copper-constantan thermocouple 0.5mm in diameter was inserted into the body of a dried specimen. Readings were taken with the bee positioned 2cm from a *Stachytarpheta mutabilis* flower, on which this species had been feeding (Stone *et al.* 1988), in sun and in shade, with a thermocouple thermometer (P.I.8013, Portec, U.K.). This temperature measure corresponds to what Chappell (1982) called the 'effective' or 'operational environmental' temperature. As he stated, dried bees have the same size, shape and colour as living animals, and hence react similarly to air temperature, air movement and solar radiation, though possessing a far lower heat capacity. Mercury thermometer readings in nearby shade were regressed against simultaneous thermocouple readings,

giving a highly significant positive correlation (n=23, R=0.85, p<0.001), suggesting that the thermometer readings were a good approximation to operative environmental temperature.

Humidity was measured using a Vaisala HMI 31 humidity and temperature probe. When studying nectar supplies humidity and temperature were recorded with the sensor immediately next to flowers on the plant being examined. Light levels were measured using a dome lightmeter (Unwin 1980) manufactured by the workshops at the Dept. Zoology, Oxford University, and calibrated in Lux against a photographic lightmeter.

C. Bee Activity.

1. Activity at the *A.plumipes* nest site.

Levels of activity at the nest site were estimated by counting the numbers of bees flying across a vertical area of the wall containing a portion of the nest site over a 10 minute period. The same area of wall ($4m^2$) in the same part of the nest aggregation was used for all estimates of activity. The same method was used to assess levels of activity at the nest site *Creightonella frontalis* (Chapter 5), where activity was recorded over a given area of nest site. In the case of females entering nest tunnels in the observed area, each arrival/departure was counted as a separate event. This means that the data obtained for males (which generally flew in front of the wall) and females (which generally flew into or out of the wall, are not identical measures of activity. It is also inevitable that some bees will have been observed repeatedly. The aim of the measurement is to describe changes in the relative levels of flight activity within a sex. This method was not used to measure population sizes of either sex; the only reliable way to obtain such estimates was to capture and count marked bees (see below).

Males typically departed from nest tunnels after a period at the nest entrance, which made observation relatively simple. Provisioning females, however, left the nest entrance immediately after moving up the nest tunnel, giving no time for recognition of marks as they departed. Small nets (diameter 6-8cm, height 6-8cm) were placed over the tunnel entrances to capture departing females. The nets were held in place with elastic stretched around a masonry nail embedded in the wall either side of the nest entrance. Nets were placed over 40 entrances at least 30 minutes before dawn on each morning that departure was recorded (Chapter 3). The nests selected were as far as possible evenly distributed along the wall, although because I wanted to look at the effect of female mass on departure time the nests of females of known unladen mass (see below) were selected. Departing females flew straight into the nets, and remained in the apex of the cone. They were very visible and often buzzed audibly in the nets, from which they were released as rapidly as possible.

When examining the depths of male *A. plumipes* in nest tunnels (Chapter 3), absolute depths could only be measured while the bees were in a dormant, inverted state before morning

emergence, at which time the depths of other features in the tunnels were recorded. When the males began to move towards tunnel entrances prior to flight their depth was measured by eye with reference to these features. More accurate measurement was impossible due to the reaction of the bees; close approach by an observer lead to retreat within the tunnels.

2. Foraging at nectar sources:

Levels of forager activity were assessed by counting the number of foraging visits to a given food plant, or to part of a larger foraging area, over a given time period. Where the number of foragers per unit time was high, the time period used was typically ten minutes. When activity levels were low, the site was observed continuously throughout the flight period shown by the species observed. Where different types of flight activity have been distinguished, the basis of differentiation is described in the text.

The total duration of nectar forages at a given nectar site was measured with a stopwatch from the time at which the bee first fed from a flower to the time at which the individual left the site being observed. The number of flowers visited during this time was counted with a standard 'click-counter', and the mean time per flower (the time required to handle each flower and reach the next) obtained by dividing the total forage duration by the number of flowers visited. When measuring the mean number of flowers per inflorescence visted by bees foraging on comfrey, two counters were used to record the total number of flowers, and the total number of inflorescences, visited on a foraging trip. The mean number of flowers visited per inflorescence was obtained by dividing the latter by the former.

D. Bee marking, and field determination of body mass and payload.

Individuals of *Anthophora plumipes*, *Amegilla sapiens*, and *Creightonella frontalis* were marked at some point to allow me to follow the fate of individuals. The bees were captured, and narcotised briefly using carbon dioxide from a small hand-held source (Sparklets). Queen *Apis mellifera* marking discs (2.5mm in diameter and shaped to fit the dorsal surface of the thorax; E.H.Thorne [Beehives] Ltd.) were attached to the pubescence of the thorax with thick superglue (Loctite, U.K.). By the time the bee recovered from carbon dioxide narcosis the marker was securely attached in such a way that it appeared neither to hinder mobility of the thorax nor motion of the wings. Marking did not seem to have any long-term detrimental effects; in 1988 the first males of *A. plumipes* to emerge were marked, and some of these were amongst the last to be seen at the end of the season. The combination of 5 colours and 100 numbers allowed the marking of a relatively large population.

To measure the masses of pollen and/or nectar being carried by the bees it was necessary to establish their unladen mass. If the abdomen of a narcotised bee was gently depressed

dorsoventrally between finger and thumb, the mouthparts extended and any nectar carried by the bee was usually regurgitated as a drop either at the base or at the tip of the glossa (e.g. Willmer 1986). Dissection of 4 individuals fed nectar and then treated in this way revealed that very little nectar remained in the honey crop. A small percentage of bees known to contain nectar would not regurgitate and this technique must therefore have associated with it an error of unknown magnitude. It provides, however, the only indication of nectar supplies carried by bees which are also carrying pollen, and my assumption is that the errors involved do not significantly affect comparisons between bees or days. This treatment also seemed to cause no lasting harm, and the same bee could be emptied of its nectar load many times. When bees returned without pollen loads, comparison of the weights ontained after removal of nectar in this way shows that the although not all of the nectar may be regurgitated, the method is at least highly repeatable. Body mass estimates obtained for a single female *A. plumipes* recaptured returning only with nectar 9 times gave a range of unladen body masses between 190 and 200mg, with a mean of194.5±0.8mg.

Using this technique it was found that *A. plumipes* (both male and female) captured immediately after emergence from their nest tunnels in the morning carried very little nectar. They also carried no pollen. This was taken as the 'unladen' condition. Masses were determined using a portable battery powered balance (Unwin 1980). Bees returning to their nests without any visible pollen load were weighed, and any change in mass assigned to a nectar load. Bees returning with pollen were narcotised and weighed. They were then 'milked' for nectar, as described above. The volume of nectar removed, and its concentration, were recorded. The bee was then reweighed. The difference between this weight and the bee's unladen weight was assigned to its pollen load. It is assumed that any changes in the mass of the bee itself during flight, due to fuel utilisation or defaecation, were negligible compared to the mass of pollen and nectar carried. Using this technique, the nature of pollen loads, and the mass, volume and concentration of nectar carried could be studied over time.

2.4 Laboratory techniques.

A. Measurement of body temperatures and warm-up rates.

Bees used in experiments were captured and cooled to about 10°C before being restrained in a styrofoam-padded vice on a cooled steel stage. A hole was made mid-dorsally in the thorax with a tungsten needle mounted in a micromanipulator, so that the depth of penetration of the needle could be precisely controlled. A constantan-steel thermocouple with an external diameter of 0.2 mm, also mounted in a micromanipulator, was inserted 1 mm into larger species, and as shallowly as possible in smaller species so as to minimize damage to internal structures. For the smallest bees investigated

(less than 75mg, including *Lasioglossum smeathmanellum* [Halictidae], 10mg: see Chapter 6) a finer copper-constantan couple (diameter 0.1mm) was used. The thermocouple was secured to the dorsum of the thorax using a minimal amount of adhesive (Copydex, Unibond-Copydex Ltd, UK), which held the bee securely enough to allow the thermocouple to be the bees' sole support during tethered flight in even the largest species examined (Fig.2.2). A discussion of errors in the measurement of warm-up rates due to heat loss down sensor wires is given in Appendix 1 at the end of this chapter. The appendix shows that the errors involved are probably small, and unlikely to seriously or systematically affect my data or analysis. After the adhesive had hardened, the bee was released from the clamp and allowed to warm passively to room temperature before the experiment commenced. In the cases of *C. frontalis* and *A. sapiens* the same individual was investigated at more than one ambient temperature by moving the apparatus between rooms regulated at different temperatures. This procedure was necessary in order to obtain sufficient data from the relatively small populations of these species. After movement to a new room, each bee was allowed to equilibrate with room temperature before warm-up was stimulated. During warm-up, thoracic temperatures were recorded continuously using a thermocouple thermometer and a chart recorder (L6512, Linseis, West Germany). Bees often initiated warm-up without stimulation, but in some cases gentle tapping with forceps was necessary. Bees were allowed to grip a small piece of styrofoam to prevent flight attempts before warm-up was completed (Fig.2.2). Typically, bees warmed to a thoracic temperature at which the styrofoam was dropped and tethered flight initiated. As soon as wing movement ceased, the styrofoam was reintroduced to the bee's tarsi. Usually the bee then cooled to room temperature before attempting a further warm-up.

The temperature at which a bee initiated tethered flight is referred to as its voluntary flight temperature (VFT). After flight for a period of 30 seconds or so, body temperature stabilised, and this temperature is termed the bee's stable flight temperature (SFT) (Stone and Willmer 1989a). Not all periods of flight were long or uniform enough to give a SFT reading. By stimulating the bee to warm-up in a number of rooms maintained at different temperatures it was possible to obtain SFT and VFT data for a range of ambient temperatures from a single insect. Here SFT values are taken to represent approximate thoracic temperatures in free flight. Once suspended flight was initiated, wingbeat frequencies measured using an optical tachometer (Unwin and Ellington 1979) did not differ significantly from those measured in field conditions. For example, Unwin and Corbet (1984) report wingbeat frequencies in the field at an ambient temperature of 22°C for foraging *Bombus pascuorum* of c.220Hz. Tethered *B. pascuorum* showed a wingbeat frequency, measured with the same apparatus, of c.200Hz. Because a tethered insect in flight does not have to generate sufficient lift to counter its own weight it is probable that metabolic output and associated heat generation are

lower during tethered flight than during free flight; Kutsch and Stevenson (1981) found that the mean wingbeat frequency of locusts in free flight was 20% higher than during tethered flight. Tethered flight can therefore only be an *indication* of the situation in free flight.

B. Rates of passive cooling.

It is difficult to control the cooling rate of a living bee capable of endothermy. Even if the bee appears inactive, its pattern of heat loss over time may be changed by physiological control of heat distribution within the body (Chapter 4), or warming through activity of the thoracic flight muscles without obvious wing movement. For this reason, cooling constants were obtained for freshly killed dead bees (e.g.*A. plumipes*), and where cooling constants are given for live bees apparently cooling passively, this is stated. This was sometimes unavoidable where small populations of species made it important to release as many individuals as possible alive.

The dead bees were weighed on a sensitive balance (Mettler AE 160) at the beginning and end of each experiment as a check against evaporative water loss. After sealing in a thermocouple (see above) the bee's thorax was heated with a microscope lamp (48watt, Vickers, U.K.) situated 10cm from the bee. Heating of head and abdomen was minimised by shading them with pieces of polished sheet steel, acting both as shading screens and heat sinks. The bee was enclosed in a perspex chamber, whose air temperature was monitored, to minimise the cooling effects of air currents in the room. When the bee's thorax had been warmed to the required temperature, the lamp was switched off and removed. The bee was then allowed to cool passively until it had equilibrated with room air temperature. Each bee was warmed and allowed to cool three times. After reweighing, head, legs, wings and abdomen were removed to determine thoracic mass.

C. Feeding bees while attached to the thermocouple.

Short-tongued bees such as *Colletes cunicularius* or *Andrena fulva* were fed *ad libitum* with a paintbrush holding a drop of 40-50% sucrose solution placed against the retracted mouthparts. The bee generally responded rapidly by extension of the tongue, which required no change in its body position relative to the styrofoam held in its feet. Feeding long-tongued bees such as *Anthophora* spp. was much more difficult. Because of the length of the mouthparts it was difficult for the bee to swing them forward to reach the nectar while maintaining a normal resting position with the feet on the styrofoam. I have never seen these species feed on a flat surface in the wild, and it is clearly difficult for them to do so. A second problem was that the dense pubescence on the ventral surface of these species hid the mouthparts, so that it was often difficult to reach them. To prevent wetting of the pubescence with sugar solution, a very small volume of nectar on the head of a pin was applied to the tip of the tongue. This required considerable patience, but was generally successful in inducing feeding.

2.5 'Grab-and-stab' measurement of body temperature.

'Grab-and-stab' measurement of insect body temperatures, using a thermocouple usually mounted inside a hypodermic needle, has become a standard field technique. A review of the techniques used, and a detailed examination of potential sources of error associated with the method, is presented in Appendix 2 at the end of this chapter. Alternatives have yet to be developed to a point where they are generally practicable in the field. For example, there is considerable interest in the use of thermal imaging for measurement of insect body temperatures; equipment is now available that can give accurate images of heat distribution within particular areas of an insect's body (Heinrich 1987; Stabentheiner and Schmaranzer 1987; Schmaranzer and Stabentheiner 1988). As yet, these techniques can only be used where the insect whose temperature is to be measured is restrained in, or is frequently located within, the relatively small area within the focal range of the sensing device (e.g. Cena and Clark 1972) and are not yet suitable for measuring the temperatures of rapidly moving insects.

Field measurements of body temperature were made using the 'grab-and-stab' technique on bees freshly caught in a fine gauze net. A copper-constantan thermocouple mounted inside a fine hypodermic needle was used (diameter 0.6mm) connected to a Portec thermocouple thermometer. Values were obtained for both thoracic and abdominal temperatures, the order in which the two parts were sampled switching for alternate bees. Both measurements were completed within 6 seconds through the net while the bee was held using the pressure of the net against a small block of styrofoam. Measurements were always carried out in shade, and T_a was measured immediately afterwards using the dried and shaded thermocouple as close as possible to the site of capture. In general, captured bees struggled and buzzed audibly, both activities ceasing as soon as the thermocouple was inserted.

Body temperatures thus obtained have been plotted against the ambient temperatures at the time and site of capture to obtain a regression of thoracic (T_{th}) or abdominal (T_{ab}) temperature on ambient temperature (T_a). For an animal that regulates its body temperature, the gradient of the best fit regression for the data over the range of T_a in which regulation occurs must be less than 1: the lower the gradient, the better the level of thermoregulatory ability. Discovery of data showing this relationship is considered '*prima facie* evidence for regulation' of body temperature (May and Casey 1983), although such a relationship is only an indicator of thermoregulatory ability (Morgan and Heinrich 1987)



Fig.2.1 A map of central Oxford, showing important areas in this study of *A. plumipes* (red circles). Key to abbreviations: **UCMG** University College Master's Garden, rich in comfrey and horse chestnut. **NS** Main nest site. **1-3** Lungwort sites in the Botanical gardens: **1** Sun site, **2** Shade site, **3** unnamed lungwort site.

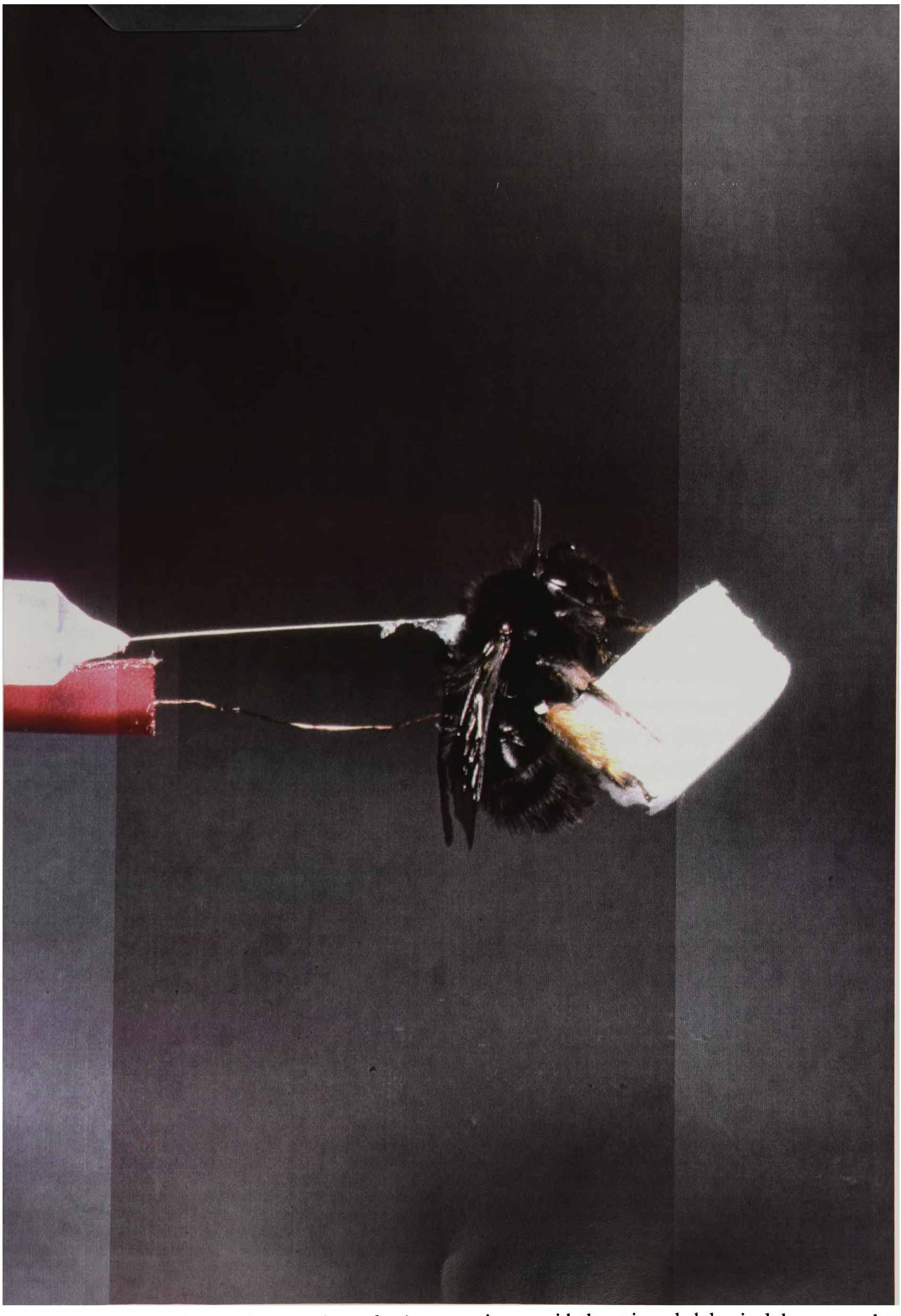


Fig.2.2 A female *Anthophora plumipes* warming up with thoracic and abdominal thermocouples inserted.

Appendix 1.

The discussion which follows is relevant mainly to the work discussed in Chapters 4,5 and 6. As with the discussion of "grab-and-stab" techniques in Appendix 2, the following section requires use of data and reasoning not introduced in full until later chapters. However, the points raised are sufficiently general to the continuous measurement of insect body temperatures that they should be discussed here. I am greatly indebted to both Professor George Bartholomew and Dr Charles Ellington for their assistance in considerations of these errors. First the depth to which the thermocouple should be inserted is discussed, and then the problem of heat loss along sensor wires.

1. Depth of insertion of the thermocouple. In any measurements of insect body temperatures it is necessary to make an unavoidable compromise between two types of error. Measurement error, associated with the insertion of the thermocouple, is reduced by deep insertion into the thorax. A second error, which might be termed physiological and behavioural error, stems from disturbance to the bee's normal behaviour and patterns of endothermy by damage to the tissues associated with insertion. It is my experience that the deeper the thermocouple is inserted, the more 'distressed' the bee becomes. Warm-ups become partial and disturbed, the bee making frequent attempts to pull itself free from the thermocouple, and rarely resting. Deep insertion frequently leads to slow death on release from the thermocouple. Warm-up rates and sustained thoracic temperatures recorded are higher, more uniform and easier to reproduce when measured using shallow insertion. No bee was retained on the thermocouple for longer than 1 hr, and all were released alive and seen to fly away. Data for any bees severely harmed by insertion of the thermocouple were discarded. This procedure has proved sufficiently 'gentle' to allow warm-up rates in one recaptured bee (a marked male *Anthophora plumipes*, body mass 165mg) to be examined three times over the period of a month. The technique is similar to those used by the other workers whose results are cited in Chapter 6.

I have assumed that shallow insertion of the thermocouple has not lead to misleading results. The thermal conductivity of the thoracic tissue is at least 10-20 times that of the surrounding air. The thorax is almost completely occupied by the flight muscles, and in the absence of evidence to the contrary it is assumed that thermogenesis is occurs evenly throughout these tissues. The thoracic volume is small, and insulated by the cuticle, the head and abdomen and, in many cases, also by pubescence. It is unlikely, for these reasons, that there is a significant temperature gradient within the tissues of the thorax. It is therefore probable that most of the temperature gradient between warm bees and their environment exists at the body surface, so that shallow body temperatures can be replaced by core temperatures with little error. For the reasons described above,

deeper insertion was avoided.

2. The problem of heat loss to the sensor and along its leads.

I am not aware of any discussion of the magnitude of these problems in the published literature, and what follows is an attempt at a very simple 'order of magnitude' investigation of them (Stone and Willmer 1989b). The warm-up rate of a bee at any time will be the result of the following: (metabolic heat production + heat uptake from any ectothermic source) minus (convective and other body surface area heat losses) minus (heat losses via sensor and wires). This appendix shows that heat losses via wires are small compared with other cooling effects, and that losses via wires are small compared with the total power generated by the bee's endothermic mechanism. Although heat losses are predicted to vary with bee body mass, this analysis suggests that the observed effects are not of sufficient magnitude to explain the correlations between warm-up rate and body mass discussed in Chapters 4 and 6.

The bee's power output required to heat the thermocouple can be estimated from the heat capacities of bee and sensor and the energy required to heat the bee and thermocouple through the same unit temperature increase. The heat capacity of a body is given by its mass multiplied by its specific heat capacity. The sensor in my experiments consisted of a steel tube 0.2mm in external diameter through which was threaded a constantan wire < 0.1mm in diameter. Wire and tube were soldered at the tip (see section 2.3A). For the sake of approximation the thermocouple is considered to consist of solid steel wire 0.2mm in diameter. Both the thermal conductivity and the specific heat capacity of the thermocouple are thus overestimates. The thermocouple is mounted in a resin block such that 1.0cm of wire is exposed between the bee and the resin. The resin is considered to be an infinite heat sink at ambient temperature. Returning to heat capacities:

heat capacity of wire = mass x specific heat capacity of steel,

mass= $\pi r^2 h$ x density = 3×10^{-6}kg ($r=10^{-4}$m, $h=10^{-2}$m, density=10^4 kgm^{-3}),

specific heat capacity of steel = 0.5 J kg^{-1}K^{-1},

$\therefore$ heat capacity of wire = 1.5×10^{-6} J K^{-1}.

Consider an isolated bee thorax of mass 100mg (10^{-4}kg):

specific heat capacity = 3.4 J kg^{-1} K^{-1} (Heinrich, 1975a),

$\therefore$ heat capacity of bee =3.4×10^{-4} J K^{-1}.

The heat required to warm the thermocouple is only 0.4% of that required to warm a bee of thorax mass 100mg. Even for a bee of thorax mass 25mg (heat capacity is 8.5×10^{-5} J kg^{-1}) the heat required to warm the sensor is only 2% of the heat required to warm the bee. Now we need to consider heat loss by conduction along the wire.

A Simple Model.

Consider the bee as a sphere, suspended by the thermocouple. Heat is assumed to be evenly distributed throughout the sphere's volume and the physiological impact of thermocouple insertion is assumed not to vary with body size, all bees being equally stressed. The specific heat capacity of all bees is assumed to be the same at 3.4 J kg^{-1} K^{-1}. Because I want to use data for power generated from Heinrich's (1975a) work on *Bombus vosnesenskii*, the same temperature conditions will be used. All bees are assumed to have a 10°C temperature excess over ambient (for *B. vosnesenskii* the figures were thoracic temperature 31°C and ambient temperature 20°C). It is necessary to consider the fraction of the total power generated by the bee that is lost down the wires. This requires estimates of the power generated by bees of different masses at this ambient temperature. Here it is necessary to use approximations in the absence of precise data. Heinrich (1975a) states that a *B. vosnesenskii* queen maintaining a steady temperature excess under the conditions given above expends energy at a rate of 23J g^{-1} min^{-1}, or 400W kg^{-1}.

Data presented in Chapter 6 show that small bees warm up more rapidly per unit mass than large bees. Thus small bees must generate a higher power per unit mass than larger bees. Heat production is assumed to be proportional to mass raised to a power between 0.6 and 0.85 (Prof. G.A. Bartholomew, personal communication), taken as 0.7. Then mass-specific power outputs are proportional to mass raised to the power -0.3. From this relationship the mass–specific and total power outputs for hypothetical *Bombus* species with a range of body masses have been calculated (Table 2.3). Total outputs for bees of the given thoracic masses are obtained by multiplying the mass-specific power outputs by the mass of the thorax.

The thermocouple

Where the thermocouple wire enters the resin block it is assumed to be at ambient temperature, so that the gradient from bee temperature (30°C) to ambient temperature (20°C) occurs along the 10mm of exposed steel. Significant heat loss through the wire is also assumed to occur by conduction along it, and convective loss from the wire to be minimal by comparison. Given the low conductivity of air, the small surface area of exposed wire, the low temperature excess involved, and the absence of forced convection, this appears a reasonable assumption.

Heat loss via the wire (Q_{wire}) in this simplified model is then given by:

$$Q_{wire} = KA\ dT/dx,$$

where K is the conductivity of steel ($60 Wm^{-2}K^{-1}$),

A is the cross-sectional area of the wire; $A = \pi r^2 = 3x10^{-8} m^2$.

dT/dx is the temperature gradient along it (Km^{-1}). dT/dx is 10K in 1cm = 1000K m^{-1}.

∴ $Q_{wire} = 1.8x10^{-3}$ W, whatever the size of the bee.

Table 2.3 shows the estimated power generated by bees of given mass using the assumptions given above, and the fraction of this power that is lost along the wire.

Table 2.3 Power generation per unit mass, total power generated and the proportion of that power lost *via* the thermocouple for hypothetical *Bombus* species over a range of body masses.

Thorax mass (Kg)	Power output per unit mass (W/Kg)	Total power generated (W)	% total power lost via sensor
2.5×10^{-5}	788	0.020	9
5.0×10^{-5}	640	0.032	5.6
7.5×10^{-5}	567	0.043	4.2
1.0×10^{-4}	519	0.052	3.5
2.0×10^{-4}	422	0.084	2.1
2.4×10^{-4} (B.vosnesenskii)	400	0.096	2.0

The proportion of heat generated that is lost down the wires in this simplest model increases with decreasing thorax mass, and at a thoracic mass of 25mg reaches about 9% of the total heat generated. In the case of the *B. vosnesenskii* being studied by Heinrich (1975a), the bee was in thermal equilibrium, and thus the vast majority (97.9% in this model) of the generated heat must have been lost by body surface effects, mostly by convection. The change in the proportion of generated heat that is being converted into a rise in thoracic temperature and being dissipated by other means by this model is from 91% at a thoracic mass of 25mg to 98% at a thoracic mass of 240mg. It is unlikely, even if these assumptions are only approximately acceptable, that errors due to heat loss along sensor wires could explain any more than a small proportion of the correlation between warm-up rate and body size discussed in Chapter 6. Cooling down sensor wires could, however, become significant at the smallest body sizes investigated. If data for the smallest bees examined (<50mg total body mass) are eliminated, the observed relationship between body mass and warm-up rate across the Apoidea described in Chapter 6 remains significant.

Appendix 2.

ENDOTHERMY AND TEMPERATURE REGULATION IN BEES: A CRITIQUE OF 'GRAB AND STAB' MEASUREMENT OF BODY TEMPERATURE

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Summary

'Grab and stab' methods have become standard in the measurement of insect body temperatures. The gradient of the best-fit regression of body temperature on ambient temperature is often used as a measure of the thermoregulatory ability of a species. The temperatures recorded are commonly accepted as slight underestimates of actual values prior to capture due to passive cooling between capture and insertion of the thermocouple. Here we present laboratory experiments involving tethered flight which show that bees often warm up on cessation of flight, and that errors due to warm-up over the time interval typically associated with 'grab and stab' sampling may be significant. More importantly, the errors due to warm-up in two species are shown to change with ambient temperature, thus affecting the form of the relationship between ambient and body temperatures. We compare laboratory and field data to illustrate the way in which warm-up errors may exaggerate apparent thermoregulatory ability, and we urge greater caution in the interpretation of 'grab and stab' data.

Introduction

'Grab and stab' measurement of insect body temperatures, using a thermocouple usually mounted inside a hypodermic needle, has become a standard field technique. The insect whose body temperature is to be measured is typically captured in a net and held using insulating gloves or forceps (e.g. Heinrich, 1979; Louw & Nicolson, 1983; Cooper *et al.* 1985; Heinrich & Buchmann, 1986; Dyer & Seeley, 1987), or restrained against an insulating material such as styrofoam (e.g. Chappell, 1982, 1984; Baird, 1986; Stone *et al.* 1988). The thermocouple is then inserted into the thorax or other body tagma, and the maximum temperature recorded. The assumption has always been that as soon as the insect is captured it starts to lose heat. As Baird (1986) stated in his work on the bee *Xylocopa virginica*, 'individuals in the net either became agitated or calm, presumably cooling passively'. Some workers have tested the accuracy of the technique by

Key words: bees, thermoregulation, endothermy.

examining the cooling characteristics of either freshly dead, heated insects or tethered insects over the period between capture of the insect and insertion of the thermocouple (e.g. Heinrich, 1979, 1986; Rawlins, 1980; May & Casey, 1983). The conclusions in all cases are (a) that body temperature measurements are underestimates, and (b) that errors are of the order of 1°C and, once acknowledged, can be disregarded.

Because of these assumptions, the aim of all workers has been to minimize the time between the capture of the insect and insertion of the thermocouple. The values obtained are then taken as accurate indications of what the body temperature was during the activity immediately preceding capture, generally flight. Readings obtained more than a certain time after capture are discarded as inaccurate. Maximum tolerated handling times vary between studies, and often depend directly on how easily the insect can be restrained, in the net or otherwise, in a suitable position for insertion of the thermocouple. Tolerated handling times vary from 3 s (Cooper *et al.* 1985; Heinrich, 1986; Morgan & Heinrich, 1987) to 10–12 s in cases where several tagmata are to be sampled (e.g. Baird, 1986), with the average tolerable handling time around 5–7 s (e.g. Chappell, 1982; Louw & Nicolson, 1983). This method is often but not always (e.g. Rawlins, 1980) fatal to the insect.

One method of calculating the errors in 'grab and stab' measurements is to 'grab and stab' an insect whose body temperature is being measured continuously. However, we do not know of any ideal study that involves both continuous measurement of body temperatures during and after flight and 'grab and stab' measurement after restraint in a net. Insects captured in a net should probably not be regarded as flying (since their wing movement is generally restricted) but as physiologically prepared for flight and escape at the earliest possible opportunity. Furthermore, it is known that attacking honeybees elevate their body temperatures above usual levels for flight (Heinrich, 1979), and it seems reasonable to suspect that other endothermic bees may continue to de so when agitated within the confines of a net.

Body temperatures thus obtained are plotted against the ambient temperatures at the time and site of capture to obtain a regression of thoracic (T_{th}) or abdominal (T_{ab}) temperature on ambient temperature (T_a). For an animal that regulates its body temperature, the gradient of the best-fit regression for the data over the range of T_a in which regulation occurs must be less than 1: the lower the gradient, the better the level of thermoregulatory ability. Discovery of data showing this relationship is considered '*prima facie* evidence for regulation' of body temperature (May & Casey, 1983). Although authors often correctly state that such a relationship is only an indicator of thermoregulatory ability (e.g. Morgan & Heinrich, 1987), it has been tempting to regard a gradient of less than 1 for the regression of T_{th} on T_a as sufficient evidence of thermoregulatory ability, even when the range of T_a over which the data were obtained has been very narrow (e.g. May & Casey, 1983, for *Eulaema cingulata*, where N is small and the T_a range only 22–26°C). It has been assumed not only that the errors in the 'grab and stab'

technique are small, but also that they are constant as a function of T_a and therefore do not affect the form of the T_{th}/T_a relationship.

In this paper we investigate changes in body temperatures at the end of tethered flight in several bee species, and compare detailed laboratory and field data for three species. We show that 'grab and stab' errors may not in fact lead to underestimates of body temperature, but to overestimates; and that these errors can be important in conclusions concerning thermoregulatory ability.

Materials and methods

Field measurements of body temperature were made using a copper–constantan thermocouple mounted inside a fine hypodermic needle (diameter 0·6 mm). Values were obtained for both thoracic and abdominal temperatures, the order in which the two parts were sampled being switched for alternate bees. Both measurements were completed within 6 s through the net while the bee was held using the pressure of the net against a small block of styrofoam. Measurements were always carried out in shade, and T_a was measured immediately afterwards using the dried thermocouple as close as possible to the site of capture. In general, captured bees struggled and buzzed audibly, both these phenomena ceasing as soon as the thermocouple was inserted.

For measurement of body temperatures during tethered flight, bees (always females unless specified otherwise) were first narcotized using carbon dioxide, and placed in a styrofoam clamp on a cooled stage. A small hole was made in the dorsum of the thorax with an entomological pin. A thermocouple consisting of 40 gauge copper wire inside steel syringe tubing with an external diameter of 0·25 mm was inserted <1 mm into the aperture, and sealed in place using adhesive (Copydex, Unibond-Copydex Ltd, UK). Once the adhesive had dried, the bees were released from the stage and allowed to warm to room temperature, which for the interspecific comparisons was always 21·5–22·5 °C. Experiments were all carried out in still air. Bees were given a piece of styrofoam to hold in their feet. The bee was tethered only by the thermocouple, and thus did not support its own weight. The importance of this experimental limitation is considered in the Discussion. Warm-up was initiated voluntarily, or induced by gentle tapping of the abdomen with fine forceps. Flight was also initiated voluntarily, the bee releasing the styrofoam, and prolonged when necessary by continued tapping. When body temperature during tethered flight had stabilized, the styrofoam was reintroduced to the bee's feet and flight and wing movement ceased. Thoracic temperatures were recorded continuously using a Portec P.I.8013 digital thermocouple meter and Linseis chart recorder. All bees were released with no apparent harmful effects.

The temperature at which the bee dropped the styrofoam and initiated tethered flight is referred to as the voluntary flight temperature (VFT), and the stable temperature resulting after continuous flight at a given ambient temperature the stable flight temperature (SFT).

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Creightonella frontalis (Fabricius) (Megachilidae) and *Amegilla sapiens* (Cockerell) (Anthophoridae) were investigated in August and September 1987 at the Christensen Research Institute, Madang, Papua New Guinea. *Anthophora plumipes* (Pallas) (Anthophoridae) and *Bombus terrestris* (L.) (Apidae) were studied between March and July 1988 at the Zoology Department, Oxford University, and *Colletes cunicularis* (L.) (Colletidae) at the Bee Research Unit, University College, Cardiff in April 1988.

Results

After establishment of a stable flight temperature, cessation of flight led to three different types of body temperature response in the bee species examined; (a) passive cooling (Fig. 1B), (b) a brief rise in temperature followed by passive cooling (Fig. 1A,C,D), and (c) rapid prolonged temperature increase due to endothermic activity (Fig. 2).

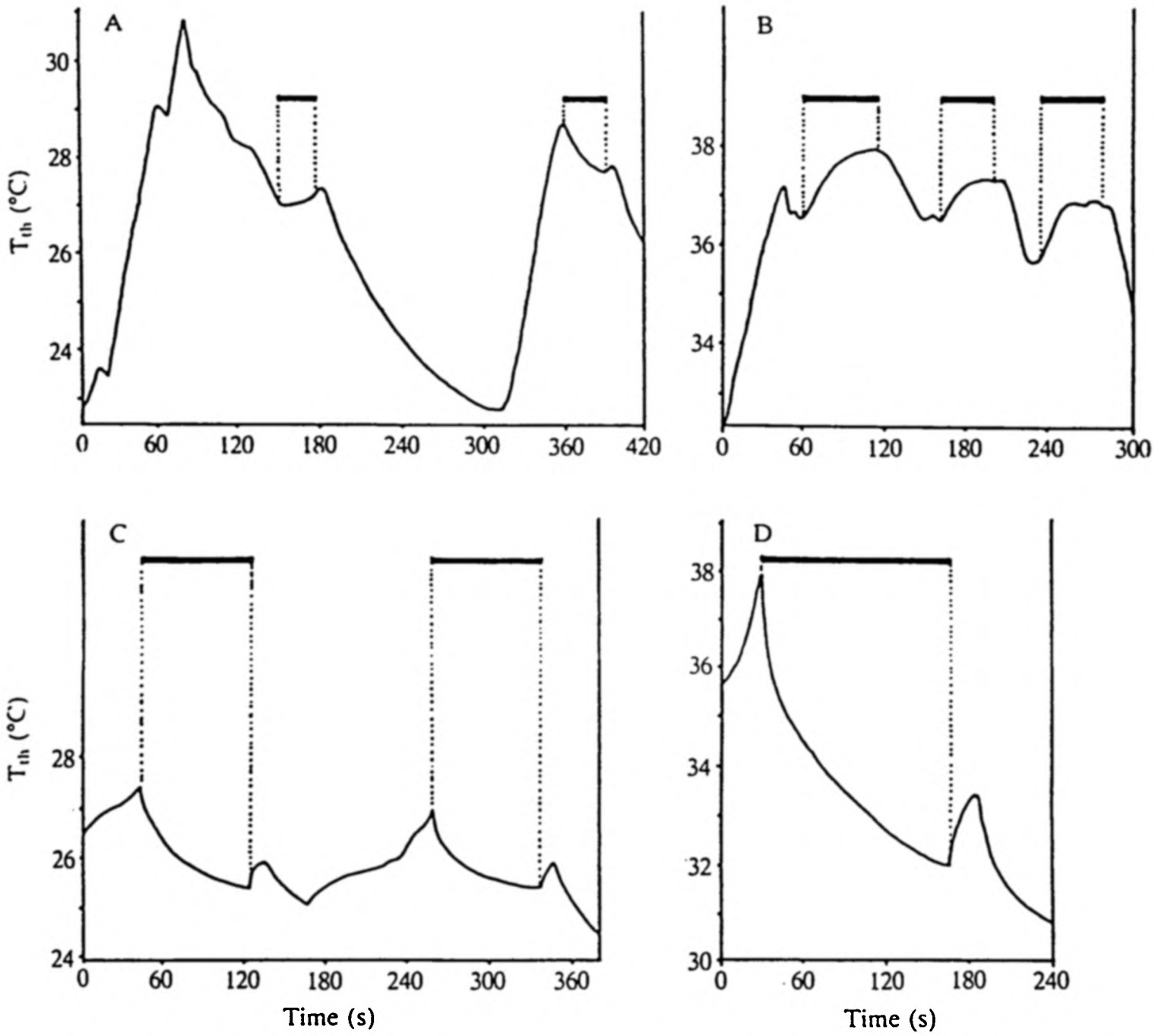


Fig. 1. Typical changes in body temperature at the end of tethered flight at $T_a = 22°C$ in four bees. Flight is indicated by solid bars. (A) *Colletes cunicularis*. (B) *Anthophora plumipes*. (C) Male *Creightonella frontalis*. (D) *Creightonella frontalis*.

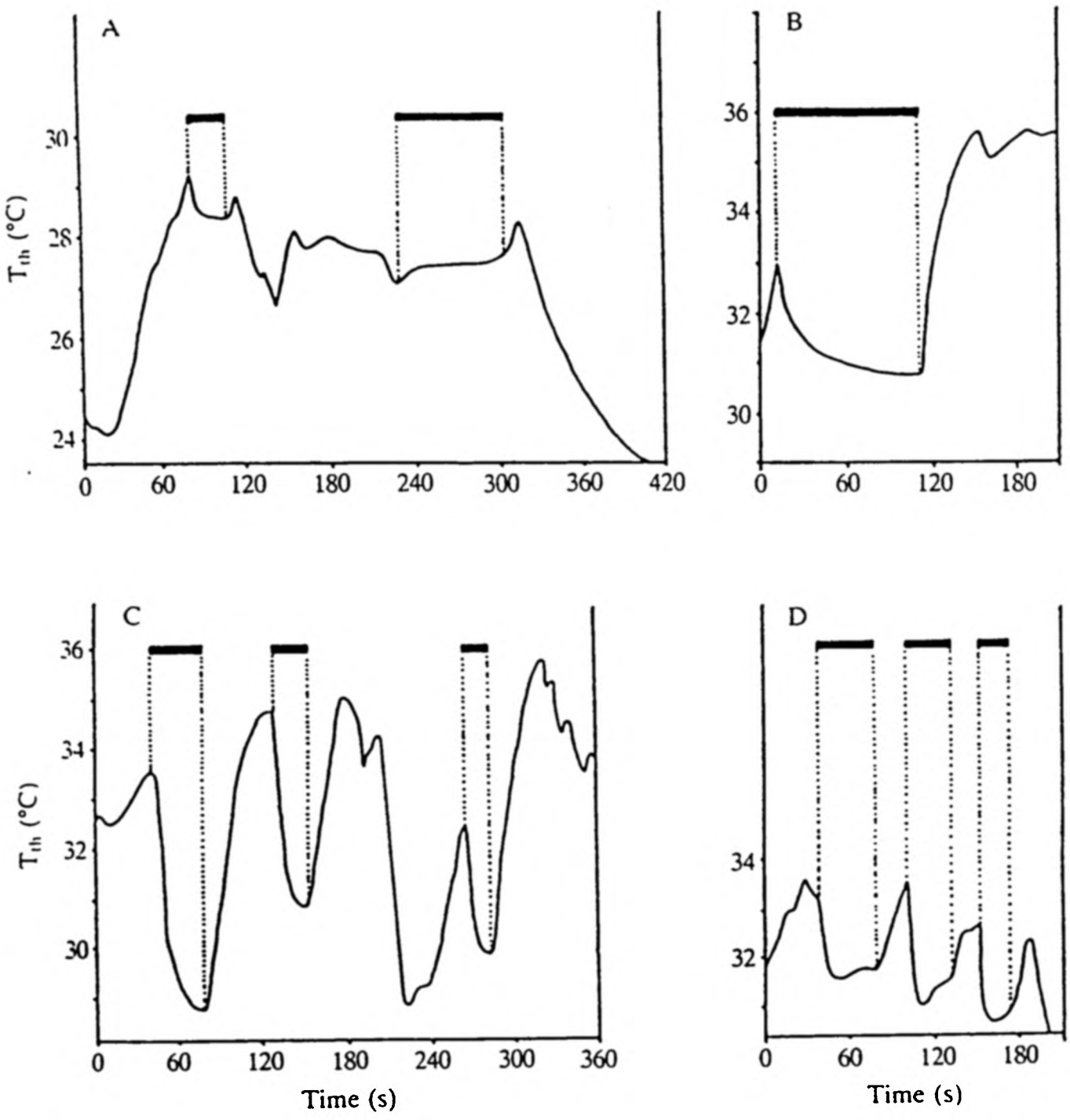


Fig. 2. Four cases of post-flight warm-up. (A) *Colletes cunicularis*. (B) *Creightonella frontalis*. (C) *Anthophora plumipes* at 10°C. (D) *A. plumipes* at 22°C.

Where cessation of flight was followed by passive cooling, were the tethered bee to be sampled using 'grab and stab' the errors would be typically approximately 0·5°C after 5 s and 0·9–1·3°C after 10 s (Table 1). This is true whether the bee flying at a T_a of 22°C has a high SFT, such as *A. plumipes*, or a lower SFT, such as male *C. frontalis*, and irrespective of whether the bee has a high mass, such as female *Creightonella*, or a lower mass, such as female *Colletes cunicularis*. Although small bees, with their higher surface area to volume ratio, should lose heat more rapidly than larger bees for a given temperature excess, larger bees typically have a higher SFT excess above T_a than small bees (e.g. 8·1°C for *C. frontalis*, 4·4°C for *Amegilla sapiens*). The greater the temperature excess, the more rapid the cooling will be. In the time involved in 'grab and stab' sampling, these two counteracting effects on rates of temperature change seem to balance each other out. In fact, cooling errors do not change significantly with changing T_a

Table 1. *Summary of data for body mass, stable flight temperature and end of flight changes in body temperature*

Species	Mean body mass (mg)	T_a (°C)	SFT (°C)	Mean temperature change over 5 and 10 s (°C) Prolonged warm-up 5 s	Prolonged warm-up 10 s	Brief temperature rise 5 s	Brief temperature rise 10 s	Cooling 5 s	Cooling 10 s
Anthophora plumipes	185 ± 3 (N = 15)	22	35·1 ± 0·3 (N = 52)	0·65 ± 0·1	1·1 ± 0·25	0·2 ± 0·1	−0·1 ± 0·1	−0·6 ± 0·05	−1·33 ± 0·1
Anthophora plumipes	185 ± 3 (N = 4)	10	29·4 ± 1·0 (N = 9)	1·55 ± 0·05	2·4 ± 0·2				
Bombus terrestris	295 ± 35 (N = 8)	22	33·3 ± 0·3 (N = 20)	0·9 ± 0·1	1·65 ± 0·1	0·0 ± 0·1	−0·4 ± 0·1	−0·6 ± 0·1	−1·05 ± 0·15
Colletes cunicularis	112 ± 4 (N = 7)	22	28·6 ± 0·3 (N = 56)	0·66 ± 0·05	1·65 ± 0·05	0·13 ± 0·05	−0·3 ± 0·1	−0·3 ± 0·05	−1·0 ± 0·1
Creightonella frontalis female	305 ± 13 (N = 10)	22	30·1 ± 0·3 (N = 22)	1·4 ± 0·1	2·05 ± 0·1	0·65 ± 0·2	0·4 ± 0·2	−0·4 ± 0·1	−0·9 ± 0·2
Creightonella frontalis male	113 ± 7 (N = 6)	22	25·3 ± 0·3 (N = 15)						
Amegilla sapiens	117 ± 4 (N = 10)	22	26·4 ± 0·5 (N = 24)	1·2 ± 0·1	1·65 ± 0·15	0·43 ± 0·05	0·15 ± 0·1	−0·5 ± 0·1	−1·05 ± 0·15

Errors are ± 1 S.E.

SFT, stable flight temperature; T_a, ambient temperature.

for either *A. sapiens* or *C. frontalis*. Our estimates of these errors agree well with published estimates and, if cooling were the general case for insects netted in the field, the errors could probably be ignored as small and systematic.

A common observation on cessation of tethered flight, particularly in certain species, was a brief rise in temperature of variable magnitude followed by passive cooling. Typical examples of these phenomena are shown in Fig. 1A,C,D. These rises in temperature are rapid enough to give a noticeable error in 'grab and stab' measurements within the usual 5 s handling time, but in all cases the magnitude of the error would be small. In female *C. frontalis*, for example, thoracic temperatures had risen $0{\cdot}65 \pm 0{\cdot}2$°C above SFT after 5 s and had fallen to $0{\cdot}4 \pm 0{\cdot}2$°C above SFT after 10 s. For both of the species with the highest SFT excesses (*A. plumipes* and *B. terrestris*, Table 1), these brief rises in temperature were rare and of negligible size, expressed more in terms of a delay in cooling of a few seconds. Errors were often smaller after 10 s than after 5 s, but in all cases were smaller than errors due to cooling, and again can probably be ignored. The probable origin of this phenomenon is considered in the Discussion.

However, some bees of all species showed immediate rapid warm-up on cessation of tethered flight, generally leading to further flight. Here the errors involved are larger. Warm-up after flight is identifiable by a sustained increase in T_{th} with time whose gradient compares well with prolonged periods of warm-up preceding flight. They may be brief (*Colletes cunicularis*, Fig. 2A) or sustained (*Creightonella frontalis*, Fig. 2B). *C. frontalis* females showing warm-up achieved, on average, 1·4°C above SFT after 5 s and 2·1°C after 10 s (Table 1) at an ambient temperature of 22°C. *Bombus terrestris* warmed up, on average, by 0·9°C after 5 s and by 1·6°C after 10 s at the same air temperature.

The magnitude of predicted 'grab and stab' errors due to warm-up ('warming error') is a function of ambient temperature in at least two of the species investigated. Thus, at 22°C *A. plumipes* raised T_{th} by 0·6°C after 5 s and 1·1°C after 10 s. whereas at an ambient temperature of 10°C these values became 1·5 and 2·2°C, respectively. Fig. 3 shows the significant negative correlation between the magnitude of warming errors and T_a for *C. frontalis*.

When SFT is plotted as a function of T_a for female *C. frontalis*, the gradient of the best-fit regression is 0·83 ($N = 45$, $r = 0{\cdot}92$, $P < 0{\cdot}0001$) (closed symbols on Fig. 4). If the errors due to warm-up after 5 or 10 s from Fig. 3 are added to the real SFT values, the result must be a line whose gradient is less than 0·83, giving a false indication of greater thermoregulatory abilities. To illustrate this point, T_{th} after 10 s as a function of T_a for all female *C. frontalis* tested is shown as a dashed line in Fig. 4. Although only 53 % of female flights were followed by warm-up, the net population effect on the relationship between T_{th} and T_a due to increased warm-up errors at low T_a is a reduction in the gradient from 0·83 to 0·74 ($N = 45$, $r = 0{\cdot}85$, $P < 0{\cdot}0001$), or an apparent increase in 'thermoregulatory ability'. The gradient of the best-fit regression for T_{th} after 5 s is 0·76 ($N = 45$, $r = 0{\cdot}86$, $P < 0{\cdot}0001$), and most of the apparent increase in 'thermoregulatory ability' for this species thus occurs within the accepted handling time. Were all individuals to

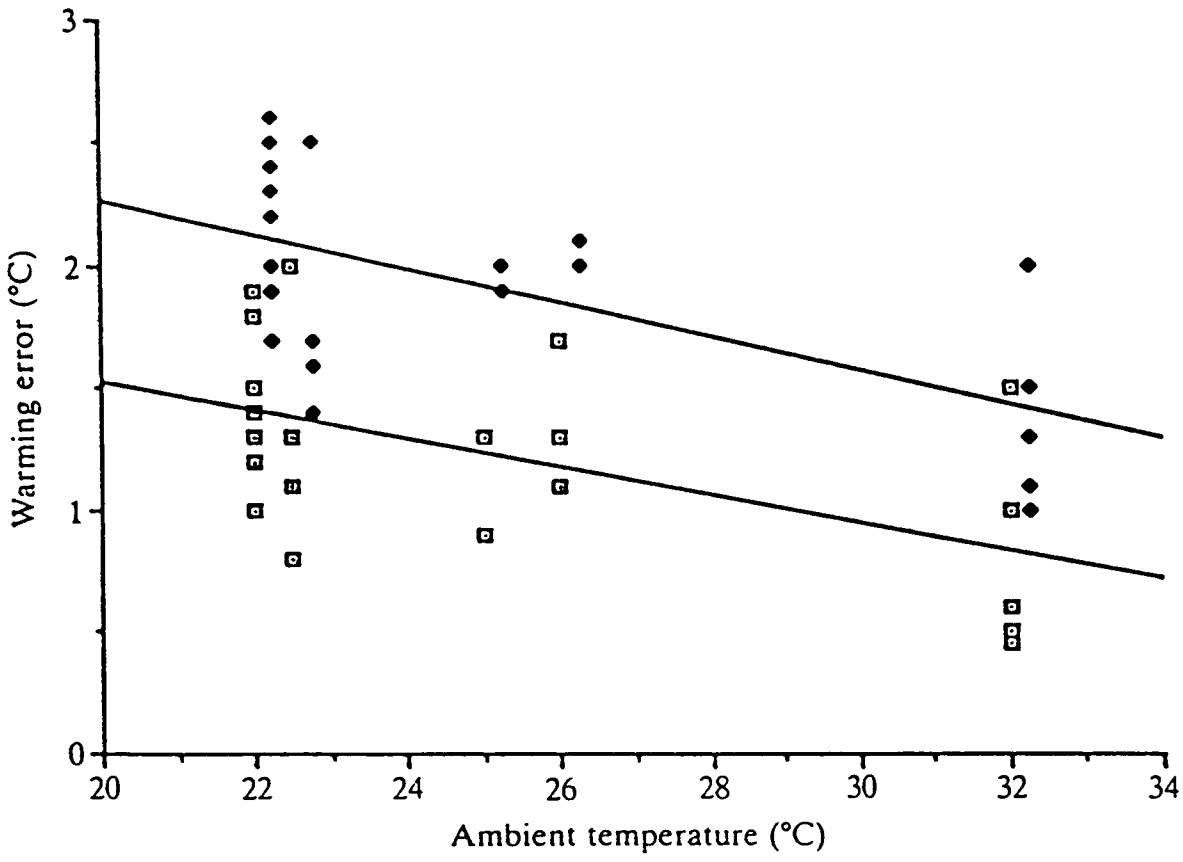


Fig. 3. The relationship between warming errors after 5s (⊡) and 10s (◆) and ambient temperature for females *Creightonella frontalis* showing post-flight warm-up. Best-fit regressions: after 5s $y = 2{\cdot}7 - 0{\cdot}06x$, $r = 0{\cdot}56$, $P < 0{\cdot}01$; after 10s $y = 3{\cdot}6 - 0{\cdot}07x$, $r = 0{\cdot}62$, $P < 0{\cdot}01$.

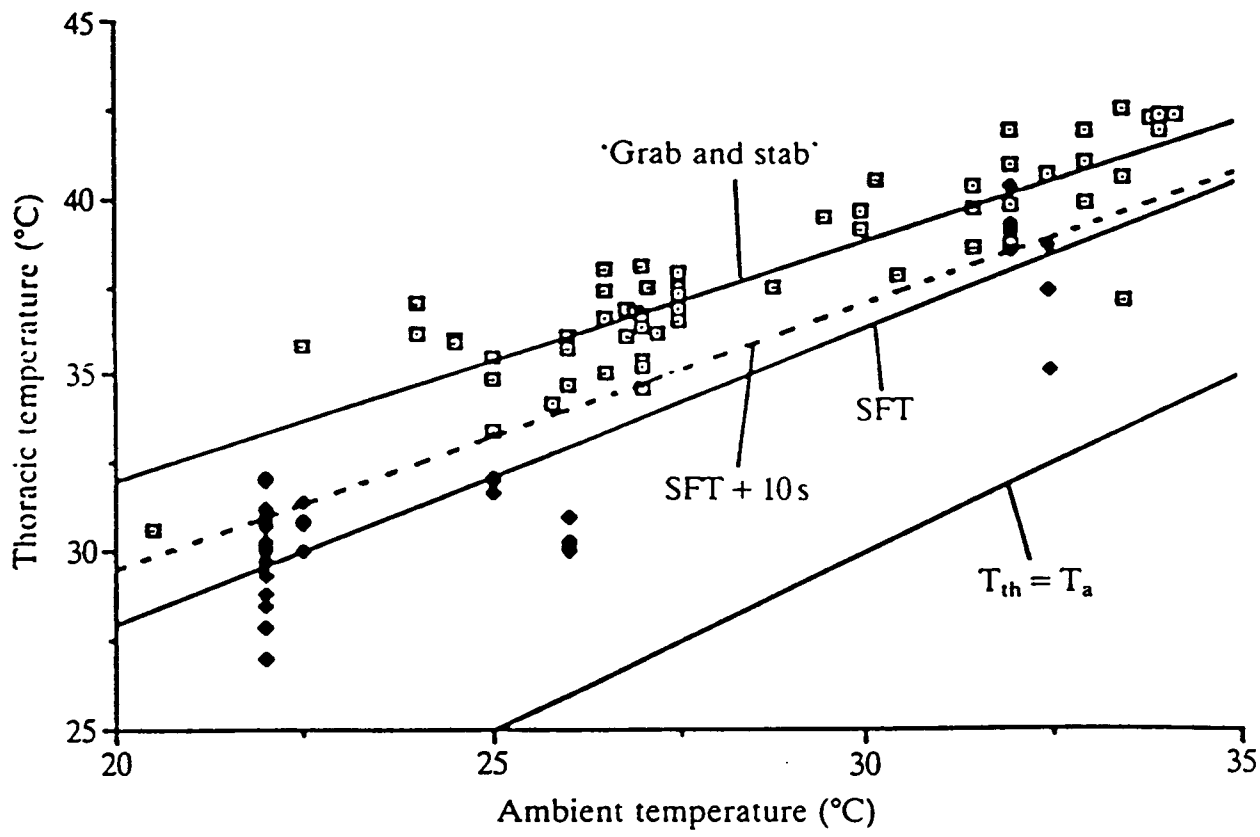


Fig. 4. Thoracic temperatures (T_{th}) measured using 'grab and stab' (⊡) and stable flight temperature (SFT) (◆) as functions of ambient temperature (T_a) for female *Creightonella frontalis*. The dashed line indicates temperatures 10s after cessation of tethered flight (individual points not shown). Best-fit regressions: 'grab and stab' data $y = 18{\cdot}5 + 0{\cdot}68x$, $r = 0{\cdot}88$, $P < 0{\cdot}001$; SFT data $y = 11{\cdot}3 + 0{\cdot}83x$, $r = 0{\cdot}92$, $P < 0{\cdot}001$.

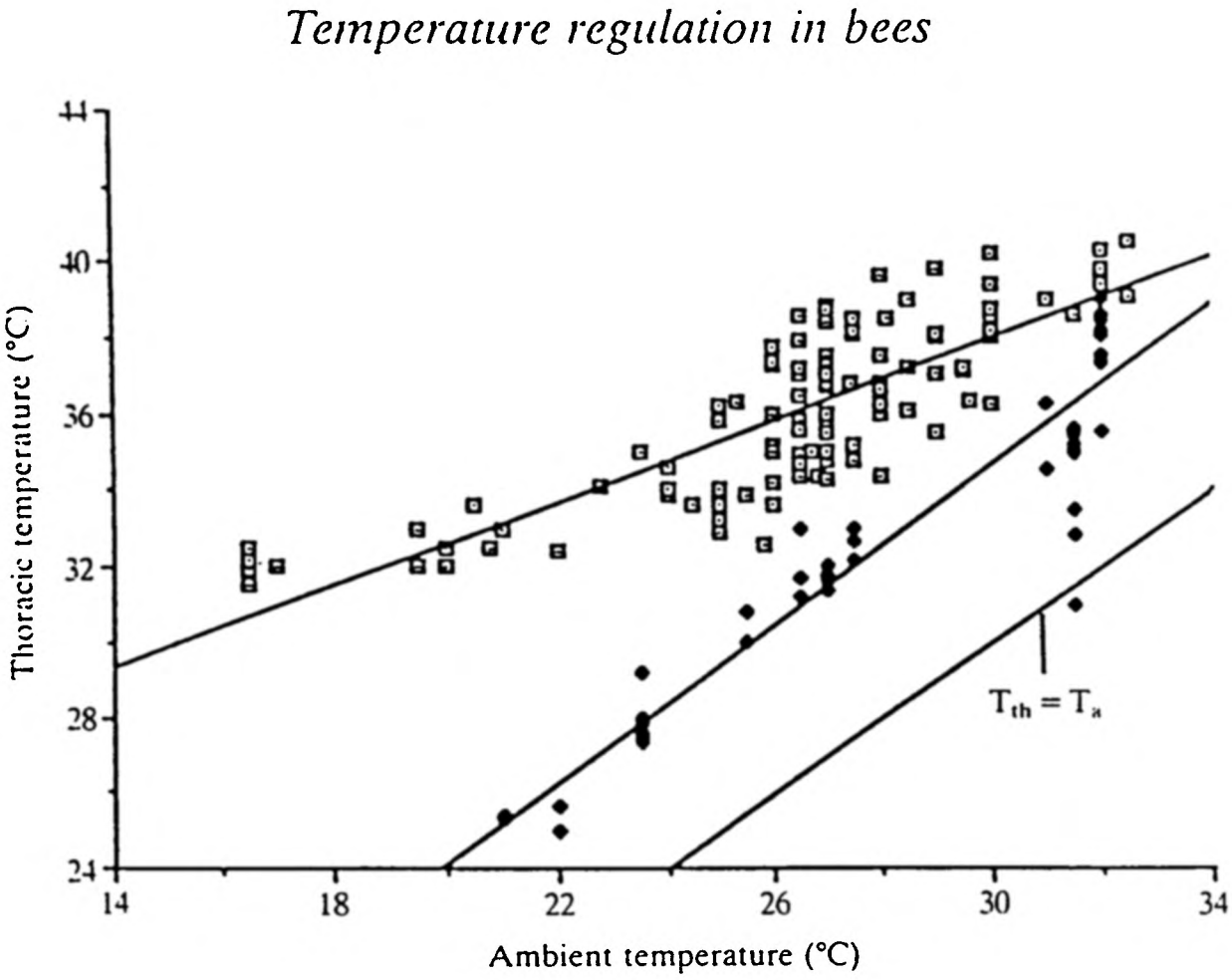


Fig. 5. Thoracic temperatures (T_{th}) measured using 'grab and stab' (⊡) and stable flight temperature (SFT) (◆) as functions of ambient temperature (T_a) for *Amegilla sapiens*. Best-fit regressions: 'grab and stab' data $y = 21{\cdot}8 + 0{\cdot}55x$, $r = 0{\cdot}88$, $P < 0{\cdot}001$; SFT data $y = 3 + 1{\cdot}05x$, $r = 0{\cdot}94$, $P < 0{\cdot}001$.

warm up after capture, and perhaps at a higher than usual rate owing to agitation within the confines of a net and/or the insulating properties of the net and the experimenter's gloved hand, these errors could only increase in magnitude. Field data for body temperatures in *Creightonella frontalis* females are also shown in Fig. 4 (open symbols). The gradient of the best-fit regression for the relationship between T_{th} and T_a is 0·68 ($N = 57$, $r = 0{\cdot}88$, $P < 0{\cdot}0001$). This is significantly lower ($F_{1,110} = 4{\cdot}4$, $P < 0{\cdot}05$; Sokal & Rohlf, 1981) than the gradient for the regression of SFT on T_a (0·83, above), but lies within the error margin that we would predict if most captured bees show endothermic behaviour during the period between capture and sampling (see the SFT + 10 s line in Fig. 4).

Field data for 'grab and stab' measurements of T_{th} in *Amegilla sapiens* are shown in Fig. 5 (open symbols). Again the gradient of the best-fit regression of T_{th} on T_a (0·55) is substantially less than 1 ($N = 100$, $r = 0{\cdot}88$, $P < 0{\cdot}0001$). However, a plot of SFT as a function of temperature has a gradient of 1 ($N = 47$, $r = 0{\cdot}94$, $P < 0{\cdot}0001$) (closed symbols, Fig. 5). Again the gradients of these two lines are significantly different ($F_{1,159} = 52{\cdot}74$, $P < 0{\cdot}001$). If we accept that SFT is a valid approximation to body temperatures during flight (see Discussion), then the accuracy of the 'grab and stab' data, particularly at low T_a, should perhaps be reassessed. It is our contention that warm-up by insects trapped in the net may well be a more serious source of error in thermoregulatory studies than has previously been appreciated.

G. N. Stone and P. G. Willmer

Discussion

Recommendations for possible modifications of 'grab and stab' procedures clearly depend on which of the observed patterns of temperature change on cessation of flight actually occurs in the net in the field. Bees which are inactive as soon as they are captured, and do not struggle in the net, will cool passively, and should be sampled as quickly as possible. Bees which show a brief temperature rise before cooling passively show greater errors after 5 s than after 10 s, but in either case the errors are small. However, bees which go into warm-up on capture are likely to show greater errors, and in a previously unappreciated direction. In our experience, bees often do struggle energetically for the time interval between capture and sampling.

If errors in 'grab and stab' measurement of body temperatures do involve warming rather than cooling, some important assumptions about the technique must be revised. If cooling is more important, then it is true that smaller bees, with their higher surface area to volume ratios, will cool more rapidly than larger bees, other things being equal. This has led to the general assumption that errors involved in measuring body temperatures of large bees are smaller than those in small bees (e.g. May & Casey, 1983). The use of insulating gloves to hold the bee while the thermocouple is inserted will also reduce cooling by reducing the effective body area over which heat loss can occur.

However, if errors are generally due to warming these predictions are reversed. Owing to their lower surface area to volume ratios, larger bees will, other factors being equal, warm more over the sampling time interval than small bees (G. N. Stone & P. G. Willmer in preparation). Since gloves insulate the body against heat loss, their use will increase the magnitude of errors due to warming. These errors alone may generate more effective 'thermoregulation' in large bees than in small bees.

The relationship between the size of warming errors and ambient temperature is a predictable one where the bee does, in fact, have thermoregulatory ability. At its stable flight temperature, the bee's heat gain and heat loss are in equilibrium. Heat gain is due to muscular activity, and heat loss to two components; (a) passive cooling due to the SFT excess over T_a, and (b) forced convective cooling due to wing movement and, in the field, forward motion. Both passive and forced convective cooling increase with increasing SFT excess. At a given temperature excess, cooling due to forced convection will be greater than that due to passive cooling, the difference increasing with increasing speeds of air flow over the body (Chappell, 1982). In field situations during flight it must be reasonable to assume that, in maintaining a given SFT excess, most heat production is necessary to counter this forced convective heat loss. When wing movement ceases, as it is forced to do in a net, the bee's heat production will still be pitched to maintaining the SFT at that T_a. In the absence of forced convective cooling, and probably augmented by the agitated state of the bee in the net, this will lead either to a rapid rise in temperature of the brief type (Fig. 1A,C,D) or to prolonged warm-up (Fig. 2). The higher the SFT excess the bee was maintaining before capture, the

greater the thermogenic output of the flight muscles, and the more rapid this post-flight warm-up will be. Thus, the greater the SFT excess, the greater the magnitude of the warming error. Hypothetical big bees which are genuinely good thermoregulators with a high SFT, for which errors due to cooling are assumed to be small, will thus show the highest errors due to warm-up. At 22°C, *C. frontalis* females maintain an SFT excess of 8°C, whereas at a T_a of 32–32·5°C they maintain an SFT excess of 5°C. Warm-up within 5 s of cessation of flight is correspondingly greater at a T_a of 22°C than at 32°C. If, as is probable, power output during tethered flight is an underestimate of power output in free flight, the true forced convective cooling which the bee's thermogenic ability is normally countering will be greater, and the warm-up when this cooling is removed more rapid and of greater magnitude. Thus, our errors are almost certainly underestimates of the effect in the field.

The errors discussed here do not seriously challenge demonstrations of endothermy in many good regulators. Insects such as *Bombus vosnesenskii* (Heinrich, 1975) have such high temperature excesses at low T_a that even were the effects of potential post-flight warm-up errors taken into consideration a considerable 'genuine' temperature excess would remain. However, use of the gradient of the best-fit regression for the relationship between T_{th} and T_a as sufficient evidence of thermoregulation, particularly where the sampled range of T_a is small, should be discouraged. Some authors are at pains to use statistics only from large data sets with significant regressions (e.g. Morgan & Heinrich, 1987). As these authors state, 'Clearly the accuracy of this test depends on how well the data set represents the thermal relationships of the insect'. Nevertheless, in some cases, gradients from best-fit lines are used as indications of thermoregulation (e.g. May & Casey, 1983), when even the best fitted regressions are very poor approximations to the real data (the best-fit regressions to the data have non-significant values of r). Use of the gradient from such data sets is misleading.

A potential alternative and simple measure of body temperature in flight is the stable flight temperature used here. Manufacture of the necessary thermocouples, and their insertion without causing obvious stress to the animal, is relatively simple. In the field, temperatures can be dictated to a continuously running cassette recorder. In the laboratory, the same bee, given time to stabilize at different ambient temperatures, can give a whole series of SFT data. Thus it is possible to gain an indication of the thermoregulatory abilities in a species from a far smaller number of individuals, and without killing any of them. In field situations where the population of the study species is small, or where the natural range in T_a is limited, this technique has advantages. Although non-lethal techniques for measurement of body temperatures in the field have been developed (e.g. Willmer, 1986), these are subject to the same kinds of errors inherent in 'grab and stab', and open to other errors, such as variation in positioning of the thermocouple and lack of direct contact between thermocouple and flight musculature.

An obvious criticism of the use of SFT lies in the use of tethered flight as an

indicator of heat generated in true, free flight. It is our experience that once suspended flight is initiated, wingbeat frequencies measured using an optical tachometer (Unwin & Ellington, 1979) do not differ significantly from those measured in field conditions. For example, Unwin & Corbet (1984) report wingbeat frequencies in the field at an ambient temperature of 22 °C for foraging *Bombus pascuorum* of about 220 Hz. Tethered *B. pascuorum* showed a wingbeat frequency, measured with the same apparatus, of about 200 Hz. Flight power output depends not only on the frequency of wing movement, but also on its amplitude. Tethered flights were in all cases only accepted where the volume of sound produced approached levels in the field, and considerable draught was produced. An ideal apparatus would involve flight in a wind tunnel where both air flow and lift generated could be measured, as used by Esch (1976), but such sophisticated analysis is impracticable for most environmentally related studies of insect thermal biology. We suggest that a gradient of less than 1 for a regression of SFT on T_a may be a safer indicator of thermoregulatory ability. The assumption here is that the proportion of power used in free flight actually used in tethered flight is not itself a function of ambient temperature, and thus the error which no doubt exists does not affect the form of the relationship. This assumption remains to be tested.

We are not suggesting that 'grab and stab' be abandoned as a technique – it is and will probably remain the simplest and most accurate method of measuring body temperatures in the field. Nor are we recommending changes in technique – whether the bee cools or warms actively the best policy is to measure the bee's temperature as rapidly as possible, preferably without holding it. It will normally be clear to each field worker whether the captured insect is warming up in the net (audible buzzing and rapid abdominal pumping are good potential indicators of endothermic activity) and thus whether particular figures recorded for T_{th} are likely to be under- or overestimates. Thus what we advocate is a more thoughtful approach to the way 'grab and stab' data are interpreted.

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Chapter 2. Methods, materials and sites

Chapter 3. The ecology and behaviour of *Anthophora plumipes*.

3.1 Introduction.

This chapter introduces the general biology of the genus *Anthophora*. I then describe the phenology, ecology and behaviour of *Anthophora plumipes* in Oxford, observed from 1985 to 1989. The aim throughout the chapter is to describe the ecology of *A. plumipes*, and to show how its courtship and foraging behaviour depend on ambient temperature (T_a) and body mass. Differences in behavioural change with temperature between males and females, and between females in different phases of the nesting cycle, are described. I also consider the importance of nectar supply and sexual interactions in determining the activity patterns of *A. plumipes*.

Anthophora is a large genus of fast-flying, robust bees occurring on all of the continents except Australia and South America. Bees of this genus are well represented in early studies (e.g. Say 1837; Walsh 1868; Frison 1922; Rau 1929; Linsley and MacSwain 1942), most of which concerned the structure of the nest tunnels and the arrangement of cells within them, the flowers exploited by the adults, and the morphology of the life history stages. Recently there has been considerable interest in the use of glandular secretions in these and closely related anthophorine bees as pheromones or anti-bacterial cell-lining agents (Hefetz *et al.* 1979; Batra 1980; Norden *et al.* 1980; Hefetz 1983). Although many of these studies contain partial, often anecdotal, observations of flight activity, behavioural responses to ambient climatic conditions, foraging behaviour and intraspecific interactions, very few detailed studies exist (e.g. Esmaili 1963; Norden 1984). There is therefore a limited amount of information on this genus to act as a background against which my studies of *Anthophora plumipes* can be presented. All of the studies listed above concern North American species. There has been very little published work on the biology of any of the European species and even less on species from other parts of the world.

The members of the genus *Anthophora* vary in mass from about 50mg (*A. quadrimaculata,* Britain) to over 500 mg (*A. hispanica*, Israel), and all members of the genus appear to be solitary. They are often very furry, prompting comparison with the bumblebees of the genus *Bombus* (Apidae),and have very long tongues for their body size in comparison with most other solitary bees (Percival 1965; Procter and Yeo 1973; Michener and Greenberg 1980) (Fig.3.1). Some studies have shown that individual species visit a relatively wide variety of flowers for both pollen and nectar (e.g. Mayer and Johanssen 1976; Norden 1984), while others show particular populations to rely mainly on a single or very few dominant pollen and nectar sources (e.g.Esmaili 1963; Thorpe 1969).

In most species, males emerge earlier in the season than females (protandry), and there is

usually only one generation a year (e.g. Linsley and MacSwain 1942; Esmaili 1963; Thorpe 1969; Norden 1984), although some species are bivoltine (Mayer and Johanssen 1976). Mating usually occurs soon after emergence of the females, and although individual males may mate with more than one female, studies to date suggest that each female is mated only once (Stephen *et al.* 1969; Norden 1984). The only study (Esmaili 1963) to have looked at this point in detail, however, suggests that females may mate several times through the course of the season. He found considerable variation in the amount of sperm present in spermathecae taken from young female *Anthophora occidentalis*, and suggested that the small size of the spermathecae may result in the need to replenish supplies through the season. His conclusions are, however, only tentative. Mating may occur at the nest site (Esmaili 1963; Mayer and Johanssen 1976) or at flowers (Norden 1984). Male *Anthophora* show a very distinctive flight pattern when in search of females at nectar sites. They fly rapidly from flower to flower on a plant frequented by females, briefly inspecting individual flowers. When a female is located, the male hovers behind or above her for a few seconds before 'pouncing' onto her (Esmaili 1963; Norden 1984). At nest sites females are intercepted as they hover in front of tunnel entrances, and male behaviour on detecting a female is similar to that shown at nectar sites (Mayer and Johanssen 1976). If the female is receptive, she remains passive while the male completes mating. Unreceptive females are generally able to reject a male by falling to the ground, struggling and buzzing loudly.

Within a species, females are generally bigger than males, and there may be considerable intraspecific size variation within the male sex (Norden 1984). Batra (1978) and Norden (1984) report that males often chase and intercept each other; Norden regards this as a case of mistaken identity, implying that the males are searching only for females. Although males may show apparently aggressive interactions during the day, the males of at least some species roost together during the night (Linsley 1962a; Mayer and Johanssen 1976). Detailed studies of two species suggest that males are generally short lived, with a maximum lifespan ranging from 20-25 days (Frison 1922; Rau 1929; Esmaili 1963; Norden 1984). Because males emerge before females, the population sex ratio changes through the season. Frison (1922), Rau (1929) and Norden (1984) report male-biassed sex ratios of about 2.5:1, although Mayer and Johanssen (1976) report a sex ratio of 1:1 for the bivoltine species *Anthophora urbana*. There is no mention in any of these studies of the stage in the season at which these sex ratios were established.

Many species nest gregariously where suitable substrates occur. Favoured nest sites are often vertical clay or sandstone banks (Linsley and MacSwain 1942; Michener and Lange 1958; Esmaili 1963; Torchio and Youssef 1968; Thorpe 1969; Norden 1984), although open areas of bare soil may also be colonised (Torchio and Youssef 1968; Thorpe 1969; Mayer and Johanssen 1976).

It is probable that most species will nest singly or in small groups if suitable nesting substrates only occur in very small areas, or are extensive and abundant. Females commencing a nest tunnel seem to be attracted to areas of current nest-building activity by nest odours (Norden 1984), giving a clumped distribution of nests where the substrate allows. There have been no studies to date concerning the relatedness of females nesting together. Nests are attacked by a variety of parasites, including other bee species of the genus *Melecta* (Anthophoridae). Female *Anthophora* will generally fly towards such bees aggressively and chase them away (Batra 1978). Sometimes several unrelated females will simultaneously attack *Melecta* (Thorpe 1969).

5 species of *Anthophora* are found in Britain (*A. plumipes, A. retusa, A. furcata, A. quadrimaculata* and *A. bimaculata*) of which *A. plumipes* is by far the commonest. Both male and female *A. plumipes* have a uniformly black cuticle (except for the face of the male), modified by the coloration of the dense pubescence which covers much of the body surface, including the face, coxae and femora (Fig.3.2). Female *A. plumipes* are a uniform black, except for the scopae of the hind femora, which are orange-brown (Fig.3.3). Young males have a rust-brown pubescence on the dorsal surface of the thorax, and the abdomen is brown at the front and darker at the tip. The thorax of older males fades to a paler sandy brown. The undersurface of the male is covered with white pubescence. Males have a distinctive bright yellow face, which is covered in young individuals with white pubescence, and have distinctive brushes of hairs on the tarsi of the second pair of legs (Fig.3.2), giving rise to the name *plumipes*. This characteristic is present in some form in the males of many species of *Anthophora*. The body length of this species (measured from the clypeus to the posterior of the last abdominal tergite) ranges from 17mm in the largest females to 10-11mm in the smallest males. In 1988 females ranged in mass from 155-243 mg, with a mean mass of 198.2±1.0mg (n=221). Males ranged in mass from 98-205mg, with a mean mass of 161.8±1.5 mg (n=145). Frequency distributions for male and female masses in 1988 are shown in Fig.3.4.

3.2 Patterns over the season in *Anthophora plumipes:* emergence, longevity, and body mass.

A. plumipes is a spring bee, and is strongly protandrous (Fig.3.5); in 1987, 1988 and 1989 the first males emerged before the end of March. In 1989, after an exceptionally mild winter, male *Anthophora plumipes* had emerged by the end of February. Throughout the emergence period as many bees as possible were marked and weighed, as described in Chapter 2. Recently-emerged males were easy to recognise due to their bright rust-red colouration, and complete coating of pubescence. As the bees aged, much of the pubescence on the face, legs, underside of the body and dorsum of the thorax was gradually lost, and these changes, in conjunction with wing wear, made it

possible to avoid confusing newly-emerged males with those which had emerged earlier. The time between emergence and marking was harder to estimate for females. All unmarked females were captured and marked over long periods of observation on each day in order to minimise the probability that newly emerged females were missed. The markers used (Chapter 2) enabled me to tell subsequently both when and where the bee was marked. In 1988 male emergence peaked around the beginning of March, but although the numbers of new males appearing fell away rapidly after this period, freshly emerged males continued to arive at the nest site through to late May, 60 days after the emergence of the first males. Female emergence showed a later peak than male emergence in 1988, with greatest rates of appearance of new females at the nest site occurring from mid-April to late May (Fig.3.5). The flight season in 1988 lasted just over 10 weeks, the last *A. plumipes* being seen at the end of the first week in June.

A. plumipes males are extremely long-lived when compared with the other species described in section 3.1. Two males marked on the 31.3.88 and two marked on 6.4.88 were still flying on 5.6.88, by which time all females at the nest site had disappeared. This gives males a maximum lifespan of 9-10 weeks. Many of the marked males resident at the nesting aggregation lived over 6 weeks. Female lifespan, based on observations of marked females, was approximately 5-7 weeks (see section 3.6).

Over the course of the season there was no change in the mean unladen mass of recently-emerged females (Fig.3.6). Recently-emerged males, however, showed a significant decrease in mean unladen body mass (Fig.3.6; $y=174.2-0.75x$, $R^2=0.884$, $p<0.01$). The mean mass of males emerging in the first few days of the season was 171.0±4.1 mg (n=26), and the mean mass of the few males emerging towards the end of the season had fallen to 143.5±6.8 mg (n=11). Possible disadvantages of large body mass in males are discussed in section 3.9.

3.3 Flowers used by *A. plumipes*.

In 1985 and 1987 both male and female *A. plumipes* exploited a sequence of nectar plants over the season. In March, April and early May *A. sapiens* visited Fumitory (*Corydalis solida* (L.) Schwarz), *Aubretia deltoidea* (L.) DC. and lungwort (*Pulmonaria saccharata* L. and *P. officinalis* L.). Between late April and the beginning of June these plants ceased flowering, and both males and females shifted their foraging activities to comfrey (*Symphytum orientale* L.), dead nettle (*Lamium album* L. and *L.purpureum* L.), alkanet (*Pentaglottis sempervirens* (L.) Tausch ex L.H.Bailey), borage (*Borago officinalis* L.) and Solomon's seal (*Polygonatum multiflorum* (L.) All.). From mid-May onwards, a major nectar source was horse chestnut (*Aesculus hippocastanum* L. and *Aesculus* x *carnea* Hayne), particularly during hot, dry weather (see section 3.7). In 1988 and

1989 all of these foraging sources (with the exception of horse chestnut) were available more or less simultaneously, due to exceptionally mild winters. The clear favourites for *A. plumipes* were then shown to be lungwort and comfrey.

An important pollen source early in the season was wallflower (*Cheiranthus cheiri* L.), and pollen was also collected from lungwort, comfrey, alkanet, horse chestnut and cherry (*Prunus* spp.).

Sites for all these plants are as discussed in Chapter 2.

3.4 Male-male interactions in *A. plumipes*.

The first males to emerge spent periods of suitable weather in University College garden, which contained extensive areas of lungwort, comfrey and Fumitory. Each male was usually found in a particular area of the garden, within which it either flew around nearby foodplants or rested at locations characteristic of the individual within this area. The resting places were always exposed to morning sun, and located in depressions in the ground or vegetation, giving shelter from wind. On The selection of these sites may be important in behavioural thermoregulation (section 3.8c). At intervals males flew further afield and interacted with other males. The commonest interactions were chasing flights, in which the intruder was usually repelled. Males sometimes grappled in flight and fell to the ground. When these flights involved only inspection of other foodplant areas, without feeding, I have referred to them as patrolling flights. While patrolling, males frequently landed on projecting twigs and leaves and held onto them with their mandibles. They then drew the tarsi of the mesothoracic legs over the substrate and it is probable that they were depositing pheromones. Such scent-marking behaviour is common among male anthophorine bees (Velthuis and De Camargo 1975; Raw 1975; Alcock 1978; Coville *et al.* 1986).

At the onset of foraging in the morning, and just before the cessation of flight activity in the evening, males were not aggressive towards each other, and showed intensive foraging behaviour (see section 3.7). Despite the intensity and apparent aggression of male-male interaction during much of the flight period, male *A. plumipes* often roosted together (Fig.3.7). Unlike the females, which preferred deep tunnels (>5cm) for nest cell construction, males often slept in shallow tunnels (3cm or so deep). The long-lived males mentioned in section 3.3 lived together in the same tunnels for their entire lives. As many as 7 males shared the same tunnel entrance together, and although the arrival of each individual triggered aggressive or defensive wing-buzzing and leg-waving in those already present, the incoming male was accepted. Introduction of a male not usually resident in the tunnel produced a more aggressive reaction from resident males, and the unfamiliar male usually left the tunnel and returned to its own. The only time when this changed was during sudden rainstorms,

or abrupt cooling of the air and clouding over. Males then rapidly returned to the nest-site, and entered any available hole until the poor conditions improved (see section 3.8). Only then would they find their own burrow.

3.5 Mating behaviour and courtship.

Males chased females during their foraging trips and during the periods for which females searched for nest sites (Section 3.6). Females were intercepted in flight in front of their nest entrances, or while hanging from or hovering in front of flowers. Over the course of this study, male *A. plumipes* were observed many times to intercept, and even pounce on, foraging bumblebees (particularly the predominantly black *Bombus lapidarius* L. and *B.ruderarius* Muller). On occasion, males even flew towards blackbirds and black cats. When a male encountered a female, he hovered 5-10 cm behind her and followed her in flight (Fig.3.8). He then 'pounced' on her, knocking the female down into vegetation. In cases where no copulation was achieved, the female was easily able to reject the male by raising her legs over her back, and curving her abdomen forward beneath her body. In such cases the females flew off at high speed, leaving the male behind. The vast majority of the many hundreds of attempts observed ended in this way. When copulation was achieved, the male held himself against the female's back, and waved the brushes on the mesothoracic legs in front of the female's face or moved them over her head (Fig.3.9). While waving his second pair of legs, the male frequently drew the tarsal brushes through his mandibles, and it is possible that secretions from the mandibular glands were being utilised during this display.

If a female was seen by several males, she was then followed by a line of them. The first male would attempt to copulate, observed by the other males, which hovered in close attendance (Fig.3.8). If the female rejected the first male, these other males followed her when she flew off. If the pair assumed a copulatory position, the other males would usually attempt to dislodge the male, head-butting him, or hovering near him and attempting to pull him away with their legs. Most of the copulations observed in 1988 occurred between 25 and 35 days after the emergence of the first male, but new females continued to emerge after this time, and copulations must then have occurred.

3.6 Female activity at the nest site: the roles of climate and body mass in provisioning behaviour.

For two to three days after emergence, females showed a behaviour pattern referred to as 'searching': slow zig-zag flight back and forth across the front of the wall, inspecting depressions and tunnels. Once located, each potential nest entrance was thoroughly inspected. Old spiders' webs and other debris were pulled away using the mandibles and front legs. The wall held a large

population of spiders of several species, including *Amaurobius ferox* Walckenaer, *Amaurobius fenestralis* Stroem and *Tegenaria atrica* C.L.Koch. The presence of many remains of female *A. plumipes*, even in fresh webs, suggested that these predators took a very heavy toll. It is probable that most of the female mortality due to this particular set of predators occurred during the searching phase of the female nesting cycle. Searching females often took shelter in nest tunnels in weather conditions in which males and provisioning females continued to fly (section 3.8).

A 50cm x 50cm grid placed against the nest area of the wall allowed me to map the location of all nest entrances in use by females. Observations on marked females revealed that once a nest entrance had been selected, the female provisioned cells at that nest for 7-15 days (Table 3.1). The female then searched again, until a second nest site had been located. Each female provisioned 3-5 nests with different tunnel entrances during her life (Table 3.1).

Table 3.1 A summary of nesting data for 7 females observed in 1988. Number of days per nest entrance includes the time required to search for a new nest site between nest provisioning periods.

Female	Date marked	Last date seen	Number of nest entrances used	Total days observed at nest site	Number of days per nest entrance
A	10.4.88	24.5.88	4	45	11.25
B	12.4.88	30.5.88	5	49	9.8
C	12.4.88	15.5.88	5	34	6.8
D	15.4.88	26.5.88	5	42	8.4
E	15.4.88	29.5.88	3	45	15
F	17.4.88	20.5.88	3	34	11.3
G	23.4.88	1.6.88	4	40	10

When the weather was sufficiently good to allow foraging from dawn to dusk (for a detailed discussion of the role of climate, see sections 3.7 &3.8 below), female provisioning behaviour had a clear daily pattern. The first trips in the morning were long, and the females returned without pollen but carrying nectar (nectar collected from females returning from their first morning forage on 21.4. and 5,6,7,9,11 and 24.5.88 gave a mean load volume of 29.3±1.4µl, and a mean concentration of 37.8±1.4 % sucrose. n=49). Observation of marked individuals confirmed that many of the females were collecting nectar from comfrey and lungwort less than 20m from the nest site in University College gardens. After 4-5 trips of this type, the females began to collect pollen. Pollen collection continued until the evening, and was followed by 3-4 nectar collecting trips of short duration just before the end of flight activity. Under suitable conditions, foraging began before dawn, and finished after dusk. The longest continuously observed period of foraging was

from 04.50 to 21.30 on 5.5.88. After flight activity ceased, females were often heard to continue nest excavation and cell construction as late as 23.00h. Patterns of time allocation between provisioning flights and nest occupation for 3 females on 23.4.87 are shown in Fig.3.10a-c.

The provisioning behaviour of the females was heavily dependent upon weather, and under poorer conditions females foraged for as little as 2-3 hours per day (see section 3.8a). Under such conditions some females foraged and others did not (see below). Temperature and light levels in particular determined whether a female would forage at all. These factors also determined how rapidly pollen and nectar sources visited by the females were depleted by males and other females, and thus how many flowers had to be visited to obtain a load of given mass. Furthermore, because the mate searching activity of males was also temperature dependent, and their attentions disrupted female foraging (see section 3.7), temperature also indirectly determined the efficiency of female foraging in a manner independent of resource availability.

On cold cloudy days when T_a was between 4 and 12°C, few males were active, and only the largest females provisioned their cells with pollen. The role of body mass in determining activity at low temperatures in *A. plumipes* is discussed in full in Chapter 4. None of the females flying at T_a below 6-8°C collected pollen, and sampling of the nectar loads of returning females (see Chapter 2) revealed that they were collecting large volumes of dilute nectar. The same females continued to collect only nectar for as long as such weather conditions persisted, leading to the conclusion that female *A. plumipes* may establish a supply of nectar in their nests over such periods. Under conditions in which some of the largest females collected pollen, and were therefore completing the provisioning of cells, many of the smaller females were restricted to nectar foraging, and therefore cannot have been completing cells. Over long periods of bad weather, marginal for flying, body mass must therefore have had a direct effect on female reproductive success.

The analysis which follows is a population-level indication of the importance of climatic factors and body mass in foraging. There is also an effect of body mass on foraging at the individual level, which is discussed in Section 3.8. Because of the time and care required to measure the masses of the components of the load carried by each female, it was not possible to gather a sufficiently large data set to analyse the relationships between body mass, climate and load carried at the individual level on a given day. Instead I present an analysis of means for these variables gathered on 14 days between 21.4.88 and 28.5.88. Air temperature and light levels used for each day are the means over the period of activity of the bees. The days varied in climatic conditions from cold and cloudy (22.4.88, mean T_a=10.7°C) to hot sun (17.5.88, mean T_a=24.5°C).

There are clear relationships between the mass of females provisioning their cells, the nature of the resource gathered, and T_a. Having controlled for the effect on forager mass of light

levels using multiple regression, there was a significant negative correlation between the mean unladen mass of pollen-foraging females and T_a (Fig.3.11a) ($n=13$, $R^2=0.460$, $p=0.023$); low T_a prevented smaller females from foraging. There was a significant positive correlation between the percentage of active females collecting pollen and T_a (Fig.3.11b) ($n=14$, $R^2=0.730$, $p<0.001$); on days with higher mean T_a more females collected pollen as well as nectar. This relationship was caused by a shift from nectar foraging to pollen foraging with increasing T_a by smaller females, resulting in the decrease in the mean mass of pollen foragers described above (Fig.3.11a). The mean total load carried by females (both pollen and nectar), having controlled both for the different masses of females active and for light levels, increased with T_a (Fig.3.11c) ($n=14$, $R^2=0.425$, T_a $p=0.05$). The same was true for the mass of pollen carried by pollen foragers ($n=14$, $R^2=0.680$; T_a $p=0.008$; log light levels $p=0.035$; forager mass $p=0.18$), and the mass of nectar carried by nectar foragers ($n=14$, $R^2=0.600$; T_a $p=0.01$; log light levels and body mass $p>>0.05$) (Figs.3.11d,e). The mass of pollen or nectar that could be carried was thus strongly dependent on climate; the mean total load collected by females (both pollen and nectar) increased from a minimum of 21.4 mg (nectar only) at $T_a=12°C$ to a maximium of 70.1mg (nectar and pollen) (or 36% of the average female body mass) at a T_a of 25°C. Fig.3.11e shows that pollen foragers, on average, carried less nectar in their crops than nectar foragers, indicating that the total load carried by pollen foragers was probably not limited by honey-crop volume, but by the total mass the bees can carry.

Even having accounted for the effect of climate there was still considerable variation in pollen loads carried. An interesting point is that having controlled for T_a and for light intensity there was no significant correlation between the mass of the load carried by a female and female body mass over all 14 days, whether pollen carriers ($n=58$, $R^2=0.053$, $p=0.086$) or nectar carriers ($n=114$, $R^2=0.065$, $p=0.295$) are considered. Although bigger bees would be expected to carry bigger loads, large females returned with large or small loads, and it is clear that factors other than lifting power (and thus body mass), such as pollen and nectar availability, distance to the foraging site, or the time interval that the female left the nest unguarded, must also have affected the payload carried.

3.7 Foraging behaviour in *A. plumipes*; the effects of climate and interactions between individuals.

In this section the effects of climate, nectar supply and sexual interaction on the activity patterns and the behaviour shown by *A. plumipes* are discussed. Because I was unable to locate a site at which females were foraging for pollen in numbers, I have no activity pattern data for pollen collection. On fine days pollen collection was concentrated in the middle of the day (section 3.6,

Fig.3.10), and nectar collection in the morning and evening. The factors investigated below go some way to explaining why this bimodality in nectar foraging should exist.

A. Patterns of foraging activity at lungwort and comfrey.

The foraging behaviour of *A. plumipes* on lungwort was investigated in the Botanic Gardens during the spring of 1987, at two sites of similar flower density but different patterns of climatic change over the day (the sun and shade sites described in Chapter 2). Bee activity was scored at the two sites by recording the number of foragers visiting flowers at the site over 10 minute observation periods. Nectar volume and concentration in the lungwort flowers was also recorded using the methods described in Chapter 2. To illustrate the effects of climatic differences between sites I have chosen representative days showing particular patterns of climatic change.

On days when the climate of the two sites differed little there was very little difference in the patterns of bee activity. On a cool and cloudy day (14.4.87) the patterns of temperature change and bee activity at the two sites were similar (Fig.3.12a,b). In contrast, on 16.4.87, a hot, sunny day, climate and bee activity patterns differed markedly between sites (Fig.3.12c,d,e). From 11.00h to 16.00h the shade site was, on average, 3-4°C cooler than the sun site (Fig.3.12c). Activity by male and female *A. plumipes* at the shade site had a single peak in the latter part of the afternoon, with a broad high activity period from 11.00h to 18.00h (Fig.3.12d). In contrast, at the sun site activity showed a bimodal pattern over time. This pattern was also shown by two bumblebee species active at this site (*Bombus lucorum* L. and *B.pascuorum* Scop.; Fig.3.12d). In all three species the period of low activity during the middle of the day stretched from 12.00h to 16.00h, the period over which temperatures at this site were highest (23-26°C). This type of activity pattern difference between sites suggested a preferred temperature range which was exceeded at the sun site during the middle of the day. Fig.3.13 is a plot of numbers of both males and females observed foraging at lungwort as a function of T_a. Peak activity appears to coincide with T_a of 19-24°C, with a rapid decrease in activity at higher temperatures. These data are for males and females, and the population response to temperature illustrated in Fig.3.13 does not take account of the sexual differences in temperature-dependent changes in levels of activity illustrated in section 3.8a. Nevertheless, activity of both males and females fell at lungwort above 23-4°C, suggesting that there is indeed an upper maximum preferred T_a for foraging by *A. plumipes*.

The mean unladen mass of female *A. plumipes* foraging at the sun site changed over the day on 16.4.87. From 07.00-09.00h the mean mass of marked bees active at the site was 194.0±2.1 mg (n=24), falling to 169.0±3.7 mg (n=10) from 12.00-14.00h, while at the shade site the mass of foragers was unchanged (196.7±3.4 mg, n=9). On 14.4.87, a day on which there were

no significant differences in climate between the sun and shade sites, there was no difference in the mass of foraging females between sites or over time. It appears that only smaller females continued to forage at the hotter sun site after most other females had departed for other foraging sites. The continued activity of the smaller females at this site over this period may have been due to physiological ability (such as possible superior avoidance of heat stress [Willmer 1983]) or to some other difference in their cost/benefit function for foraging (such as greater ability to remove nectar under these conditions) relative to larger females. The relevance of the former, in terms of the effects of body mass on heating and cooling rates (and thus on avoidance of overheating) is discussed in Chapter 4. A factor which potentially could differentially affect the nectar foraging ability of large and small females is discussed in the next section.

Differences between the activity patterns of males and females were investigated in detail for foraging on comfrey (*Symphytum orientale*). The site studied was one which was insolated for all but the last hour of sunlight during the day, situated to the north west of a large open area in the University College Master's garden (see Fig.2.1); data are presented for 24.5.87, which showed patterns typical of a warm, fine day in May. Similar activity patterns were recorded for 27 and 29.5.87 (section 3.7c below, Fig.3.25). Female activity was bimodal, while male activity showed a single peak (Fig.3.14a). Analysis of the relationship between T_a and activity for each sex on 24,27 and 29.5.87 revealed a linear increase in flight activity with temperature for males ($n=33$, $R^2=0.670$, $p<0.0001$) and a curvilinear relationship for females (Fig.3.14b). Polynomial regression shows that both the positive correlation between activity and temperature and the decrease in female activity at high T_a are significant ($n=48$, $R^2=0.390$, $p(x)=0.0001$, $p(x^2)=0.0001$). On comfrey, the afternoon drop in female activity occurred at a relatively low T_a - about 15-16°C on 24.5.87. These temperatures appear too low for the drop in female activity to have been due solely to thermal considerations (Chapter 4).

B. The effects of nectar concentration and volume on foraging by provisioning female *A. plumipes*.

1. Foraging at lungwort.

A factor other than temperature which might have determined which of the lungwort sites described above was visited by *A. plumipes* is nectar availability. First I discuss the effects of flower age on nectar supplies and foraging by *A. plumipes*, and then the relationship between nectar supplies and activity patterns.

The flowers of lungwort underwent a pronounced colour change as they aged, and the effects of flower age on nectar volume, composition and forager behaviour were investigated for the

reasons, and using the methods, discussed in Chapter 2. For flowers sampled on 12.4.87 there was no significant correlation between flower age and either of mean volume (n=23, R^2=0.086, p=0.18) or mean concentration (n=23, R^2 =0.102, p=0.15).

The foraging of *A. plumipes* from longwort was examined in detail on 16.4.87 (section 3.7c, below). On this day there were totals of 260 blue and 234 pink flowers at the sun and shade sites. Having controlled for the effects of ambient temperature and time, foraging *A. plumipes* showed no preference for either flower type - each foraging bee visited a mean proportion of 0.52±0.02 (n=185) pink flowers during a single visit to a flower patch (n=370, R^2 =0.062: ambient temperature p<0.001; time of day p=0.31; flower type p=0.15). These results suggest that for the period over which the following results on foraging activity were obtained, flower age was not a significant factor in flower choice by *A. plumipes*. These results are used as a justification for the assumption that, given the reservations discussed in Chapter 2, the estimates of standing crop obtained for lungwort do adequately represent the values encountered by foraging *A. plumipes*.

Studies of the lungwort nectar on 12.4.87 and 16.4.87 showed that while nectar concentration was on average slightly higher at the sun site (Fig.3.15a,b), nectar volume was significantly higher at the shaded site (Fig.3.15c,d). Calculation of the mean total sucrose per flower, using methods described by Bolten *et al.* (1979), shows that on 16.4.87 the shade site had significantly more sucrose available to foragers than the sun site (2 group unpaired t test: 20d.f., t=-3.63, p=0.0017; shade site mean sucrose per flower 0.44±0.05 [0.30-0.81]mg, sun site mean sucrose per flower 0.22±0.03 [0.1-0.44]mg; each mean is the mean of the means at each time interval) (Fig.3.15e). On a sugar reward availability basis, foragers should always have foraged at the shade site. Activity at the shade site increased when T_a at that site exceeded about 15°C on 16.4.87 (10.00-10.30h, Fig.3.12d), considerably before the decrease in visitation observed at the sun site (Fig.3.12e). This suggests that while there was some movement of the same marked individual bees between the two sites, the patterns of visitation at the two sites were largely independent phenomena. The data suggest that visitation at the sun site declined when T_a rose above preferred levels, while visitation at the shade site increased when T_a reached minimum favoured levels. Because there was a variety of sites available for foragers, these transition temperatures need not represent very accurately the critical lower and upper temperature for foraging activity in this species. Critical temperatures for flight are discussed in Chapter 4.

The lapping efficiency of long-tongued bees decreases above a certain nectar concentration due to increased nectar viscosity (Harder 1986), and long-tongued bee species avoid concentrated nectars utilised by shorter-tongued species (Percival 1965; Corbet 1978a). Tongue length increases with body size in bumblebees (Harder 1982), and a similar relationship probably holds for *A.*

plumipes. Because the nectar concentrations over the middle of the day at the sun site on 16.4.87 were relatively high (Fig.3.14b) it is therefore possible that large female *A. plumipes* were unable to collect nectar still available to smaller females, leading to the observed change in the mean mass of females foraging at this site on 16.4.87. The effect of nectar concentration on nectar foraging may be complicated by dilution of concentrated nectar with saliva to facilitate its collection, as described by Simpson and Reidel (1964) for *Apis mellifera*. Whether *A. plumipes* responds to concentrated nectar in this way is unknown.

2. Foraging at comfrey and horse chestnut.

In 1987 the middle of May was warm and dry. The comfrey in the University College garden was a frequently-visited nectar source in the morning and evening, but female visitation declined markedly during the middle of the day as described in the previous section. Fig.3.16a shows visitation data for 11.5.87. Maximum T_a over this part of the season were 17-21°C (e.g. Fig.3.16b for 11.5.87), and it is unlikely that the comfrey was abandoned by females in the middle of the day for thermal reasons. A putative reason for the midday decrease in female activity concerns changes in the nature of the resource collected.

Although *Symphytum orientale* has a relatively deep corolla tube (11.4±0.1mm, n=35), which will protect the nectar to some extent from changes in climate occurring outside the flower (Corbet 1978b; Corbet *et al.* 1979a), the large changes in relative humidity over the day occurring over this part of the season had a considerable effect on the comfrey nectar. The changes in comfrey nectar volume and concentration on 11.5.87 are shown in Fig.3.17a,c. The mean nectar volume showed little change over the day, varying between 0.09 and 0.16 µl per flower (Fig.3.17a). Mean nectar concentration rose from 32% sucrose at 07.30h to 59% sucrose at 14.45h, falling thereafter to reach a mean of 39% by 19.30h (Fig.3.17c). These changes in nectar concentration correlated with changes in relative humidity (Fig.3.17d).

Nectar supplies were also monitored at a large horse chestnut tree (*Aesculus* x *carnea*) less than 20m from the nest site. This species has far larger individual flowers with more nectar than comfrey. On 11.5.87 flowers contained a mean over the day of 14.7±1.1µl of nectar (n=100, range 5.5-22.0 µl), with a mean concentration of 27.8±1.4% sucrose (range 20.0-38.5%). Over the day, nectar concentration was less strongly affected by changes in ambient relative humidity than comfrey nectar (Fig.3.17c,d). The nectar concentrations recorded for *Aesculus* x *carnea* form an interesting contrast to the range reported by Percival (1965) for the closely related *A. hippocastanum* (33-74% sucrose).

Females returning to their nests were captured, and the sources of their nectar load

determined by observing the pollen coating their bodies. Female *A. plumipes* that had collected nectar at *Aesculus* x *carnea* were readily identified by the coating of bright red or orange pollen caught on the body hairs while forcing their way into the flower. Many females also had a small pollen load on their scopae, which made identification easy: comfrey has pale cream-coloured pollen, which is easily distinguished from the red horse chestnut pollen. Over 200 pollen samples were taken from returning bees at this time and identified using the methods described in Chapter 2, and no red pollen other than horse chestnut was collected by *A. plumipes*. The nectar load carried by the bees was collected, and its volume and concentration determined. In this way I intended to find which nectar source the population was favouring, and whether the favoured source changed over the day. It is assumed that no significant change in the concentration of the nectar collected occurred between collection and return to the nest. Both the comfrey and horse chestnut sources were so close to the nest site that this assumption appears justified.

Over the day the proportion of returning females carrying horse chestnut nectar increased, from only 10% at 08.30h to 80% by 18.30h (Fig.3.18a). During the middle of the day, despite the low levels of activity by females on comfrey (Fig.3.16a), nectar collecting activity continued from horse chestnut. The midday drop in activity at comfrey is therefore not necessarily indicative of an overall fall in female activity, but reflects a population shift from one forage source to another. Marked individuals were observed to change their nectar source, so the phenomenon occurred also at the individual level. Because of the nature of the *Aesculus* nectar source (a large tree), it was very difficult to gauge levels of female activity. Although observations of a particular 3m x 3m area of flowering branches on the outside of the tree, and near its base, showed that female activity on 11.5.87 peaked during the middle of the day (Fig.3.18b), I do not know how representative such a small sample was of the entire volume of the tree being foraged.

Fig.3.17d shows the relationship between relative humidity and the mean concentration of the nectar present in *Aesculus* x *carnea* and comfrey flowers, and the nectar carried by returning foragers. In all cases, the concentration of the nectar correlates negatively with relative humidity (comfrey: $n=15$, $R^2=0.610$, $p<0.001$; horse chestnut: $n=14$, $R^2=0.510$, $p=0.003$; returning foragers: $n=15$, $R^2=0.675$, $p<0.001$). At high relative humidities (c.80%), comfrey nectar was more concentrated than *Aesculus* x *carnea* nectar (Fig.3.17d), and the nectar carried by returning foragers most closely matched the concentration of the comfrey nectar. At lower relative humidities, however, the concentration carried by the female population was closer to the mean concentration of horse chestnut nectar. That the mean concentration collected by foraging females throughout the day was higher than the mean horse chestnut concentration (Fig.3.17d) was due to the fact that some females continued to visit some other nectar source. Examination of pollen samples collected on that

day showed that a small proportion of returning females continued to visit lungwort and comfrey. Since the volume per flower of both sources changed relatively little over the day (indeed, the volume per flower for comfrey increased, if anything; Fig.3.17a), it appears that the shift from one foraging source to the other occurred as a result of changes in nectar concentration. On 11.5.87, the number of female *A. plumipes* foraging on comfrey correlated negatively with comfrey nectar concentration (Fig.3.19a: n=6, R^2=0.550, p=0.09). On seven days over this period, the mean nectar concentration for *Aesculus* x *carnea* flowers was determined between 14.00h and 16.00h, and the proportion of returning females carrying *Aesculus* x *carnea* nectar determined. These results yielded a strong positive correlation between nectar concentration and the preference of the female population for this nectar source (Fig.3.19b: n=14, R^2=0.450, p=0.006). An important question remains to be answered - was it the high concentration of comfrey nectar during the middle of the day that drove the females away from this nectar source, or was it the low sucrose concentration of the horse chestnut nectar at high relative humidities that rendered it unattractive to foraging females when alternatives were available? Bees are known to avoid collecting the nectar of otherwise favoured flowers when it is dilute (e.g. Vansell *et al.* 1942; Corbet and Delfosse 1984), so the latter is obviously possible. Clearly factors other than thermal considerations determined the activity patterns shown by females, and observations at a single nectar source could therefore give a misleading view of female activity patterns.

It is possible that patterns of pollen availability may also have affected female choice of forage source. Percival (1965) states that flowers of *A. hippocastanum* show anther dehiscence throughout the day, and it is therefore probable that the shift between forage sources was not caused by a change in *Aesculus* pollen availability.

C. The effects of temperature on foraging behaviour and male interference with female foraging.

Male *A. plumipes* showed a characteristic change in their flight behaviour with changing temperature. At a feeding site, males showed two types of flight: 'foraging' flight involved feeding from flowers, and 'patrolling' flight involved flight around the flowering patch, inspecting individual flowers for the presence of females. Patrolling males only rarely stopped to feed, and often interrupted feeding to pursue other bees. These two flight types were at the extremes of a continuum of flight activity types. The transition from foraging to patrolling involved a decrease in the number of stops at flowers per unit time in flight, a decrease in the time spent feeding at each flower and an increase in flight speed. Data on foraging from lungwort on 16.4.87 at the sun and shade sites in the Botanic Gardens illustrate these relationships and are summarised in Table 3.2.

Table 3.2 A summary of the mean ambient temperatures 15cm above the ground, mean total number of flowers visited on each visit to the flower patch, mean total duration of the forage at the flower patch and mean time per flower over time for male *A. plumipes* at lungwort at the sun and shade sites on 16.4.87. Data are given as the means±1 standard error, with the range in parentheses.

Time of day interval	n	T_a (°C)	Total flowers visited per forage	Total forage duration (s)	Mean time per flower (s)
07.00-09.00h	10	11.5±0.4	15.8±1.6 (8-25)	51.4±7.7 (23-106)	3.2±0.2 (2.2-4.5)
09.01-12.00h	19	16.1±1.2	11.5±1.7 (1-26)	42.3±9.1 (8-130)	4.0±0.6 (1.5-12.0)
12.01-14.00h	14	22.3±0.9	5.1±1.0 (1-15)	11.8±2.3 (1-27)	2.3±0.3 (1.0-4.1)
14.01-16.00h	47	19.3±0.6	4.0±0.3 (1-12)	8.9±1.0 (2-35)	2.2±0.1 (1.0-5.3)
16.01-18.00h	35	19.1±0.4	4.8±0.6 (1-19)	10.2±1.6 (1-44)	2.0±0.1 (0.3-4.4)
18.01-20.00h	60	15.2±0.3	10.1±1.3 (1-48)	29.2±4.0 (1-150)	2.8±0.1 (1.0-6.0)

From initiation of foraging in the morning until mid-afternoon forage duration decreased ($n=185$, $R^2=0.240$, $p<0.001$), the total number of flowers visited on each visit to the patch by males decreased ($n=145$, $R^2=0.200$, $p<0.001$) and the mean time per flower decreased ($n=185$, $R^2=0.200$, $p<0.001$), (Figs.3.20a,b,c). When males abandoned patrolling behaviour in the evening and foraged briefly in the evening similar changes occurred in reverse. These changes correlated strongly with changes in T_a; with increasing T_a forage duration decreased ($n=185$, $R^2=0.240$, $p<0.001$), the total number of flowers visited on each visit to the patch by males decreased ($n=145$, $R^2=0.200$, $p<0.001$) and the mean time per flower decreased ($n=185$, $R^2=0.200$, $p<0.001$) (Figs.3.20d,e,f). It is possible that the recorded changes in male foraging behaviour were induced not by changes in T_a but by changes in nectar availability. Fig.3.15 shows how nectar volume and concentration changed over time at this site on 16.4.87. Male behaviour changed bimodally over time, whereas nectar supply changes were unidirectional. It is therefore difficult to explain the changes in male behaviour on the basis of changes in nectar supply alone. The changes in male behaviour more closely match changes in T_a (Fig.3.12c). I do not suggest that T_a alone determined these aspects of male behaviour. Generally it is true that in the morning and evening, when male foraging was intense, flower processing times were higher, forage duration was longer, and the number of flowers visited was higher than in the middle of the day, regardless of site.

Male behavior was observed in greater detail on comfrey. Unlike the lungwort favoured in the early part of the season, which is a low-growing plant, comfrey grows to 30-40cm or more above the ground. Some flowers are sheltered within this growth, while others are accessible from the outside without penetrating among the leaves and stems. Foragers are therefore able to choose

between the more accessible outer flowers and the less accessible, sparser inner flowers. In the following discussion, outer flowers are defined as those on the outer surface of the plant, and inner flowers are those hidden from the plant surface by leaves. The latter could only be reached by flying within the plant. As observed at lungwort, males foraged intensely in the morning and evening, during which periods their male-male aggression was markedly less intense than during the middle of the day. On cold days (10-12°C or less) in the absence of warm sun, males did not become aggressive; they foraged for nectar, rested, or remained in their nest entrances and other sleeping places. Females were then able to forage without any attention from males. During warm days, after a period of feeding in the morning, the males' behaviour changed in a very similar way to that described for lungwort. The following is a detailed account for 24.5.87.

When males first arrived at the comfrey, the mean distance between successive flowers visited was approximately 30cm (Fig.3.21a). Initial flights were of short duration, and involved frequent rests and basking. As intense foraging developed, the distance flown between flowers decreased to about 20cm. As male-male aggression began, and males began to respond towards passing females, males moved further and further between flower inspections, reaching a metre or more from 1300h -1600h. Males then patrolled the comfrey, only rarely stopping to feed. In the evening there was a brief period of foraging with short distances between feeds before males dispersed for the night. To obtain an estimate of how much nectar was carried by males, 37 were captured on 21.5.87 and the nectar in their honey crops sampled as described in Chapter 2. The volumes carried varied from undetectable traces up to 16.5µl, and never reached the large volumes carried by provisioning females. The volume present in males increased on average through the day (Fig.3.22a; $n=37$, $R^2=0.380$, $p<0.001$), despite the apparent concentration of foraging behaviour in the morning. The concentration of the nectar carried by males followed closely that of the nectar available at the same time in comfrey flowers (Fig.3.22b). This suggests either that the males were concentrating the nectar they had imbibed earlier in the day, or that they were taking as much nectar as they required from flowers throughout the day.

Comfrey has a complex inflorescence; the flowers open as an unwinding spiral, older flowers lower down the stem, with immature flowers still coiled within the spiral. Any one flower stem typically had two flowers that had opened that morning, and two flowers that opened the previous day. The latter often still contained nectar. The bees could thus vary the number of flowers per flower head that they attempted to feed from. The number of flowers per flower head visited by males rose from a mean value of 1.44±0.21 (n=11) at 07.00h to 2.75±0.30 (n=11) near 10.00h during the peak male foraging period (Fig.3.21b). During male-male chasing and courtship of females, the number of flowers visited at each head fell, rising again during the brief period of

foraging in the evening (Fig.3.21b).

During the middle of the day the sensitivity of males to the presence of other flying insects, particularly black bees, appeared much higher than it was either in the morning or the evening. Sight of a black flying object caused males to break from the usual flightpath in pursuit. This change in male flight behaviour was easily observed. Although the number of females available during the middle of the afternoon was lower than it was earlier in the day (Fig.3.14a), the number of breaks in the usual flight pattern peaked at this time (Fig.3.21c). The number of pounces by patrolling males (see sections 3.1, 3.5) on female *Anthophora* also peaked in the afternoon (Fig.3.21d). The most active males at the peak of their patrolling activity reached rates of 11 pounces per minute!

Male pouncing had a significant deleterious effect on the foraging of females visiting flowers on the outer, exposed surface of the comfrey plants. Males rarely flew into the interior of the plant, and females foraging there were rarely intercepted. The more outer flowers a female visited, the higher the mean rate of male pouncing she could expect to receive during the middle of the day ($n=8$, $R^2=0.520$, $p<0.02$) (Fig.3.21e). There was a significant positive correlation between the frequency of pounces experienced and the handling time required by a female to feed from a flower ($n=36$, $R^2=0.260$, $p=0.003$). From 4.5±0.2s/flower (90 flowers visited by 9 females, range 3.7-5.5s/flower) without male hindrance, female handling time increased to 9.0±1.0s/flower (25 flowers visited by 4 females, range 7.5-11.0s/flower) with a male pounce rate of once every 3 seconds. The peak pounce rate experienced by a female pursued by two males was once every 2.5 seconds as long as she stayed at the flower, although this was exceptionally high.

Over time during the day, females showed an increasing tendency to visit flowers within the comfrey plant rather than on the exposed outer part (Fig.3.21f) when compared to males. This change in female foraging behaviour could have been in response to male interference, or due to better supplies of nectar in unvisited inner flowers than those on the exterior. Comparison of nectar volume and concentration between inner and outer comfrey flowers on 24.5.87 revealed that there was no significant difference in the volume of nectar per flower, and significantly lower nectar concentrations in the inner flowers (16 samples of 20 flowers each; multiple regression of concentration on time and flower type: $n=16$, $R^2=0.690$, $p<0.001$). To compare the number of flowers of each type visited per unit time, the time taken to visit at least 8 flowers of one type was recorded for females on 24.5.87. A mean value of the foraging time per flower was obtained from each forage and the mean of all the forages taken to obtain a population mean foraging time per flower for inner and outer flower types. Forages of both types were observed throughout the day. The time taken to visit inner flowers (9.1±2.0s, 18 forages) was considerably higher than that required to forage from outer flowers (5.0±0.3s, 42 forages), due to the longer flight and manoeuvre

time between inner flowers. This suggests that the rate of nectar collection achieved from inner flowers was lower than that obtainable from outer flowers. If females did switch from outer to inner flowers for reasons other than male interference, then this same behavioural change should occur in the absence of this factor.

The effect of male interference was determined by a simple experiment. On 26.5.87 all males encountered in the garden were captured and restrained in a 1.0 x 1.0 x 0.5m netting cage over potted flowering lungwort plants. The foraging behaviour of females in the absence of males was observed on 26 and 27.5.87. Any males which arrived in the garden during this period were captured as quickly as possible and put into the cage. The males were released on the morning of 28.5.87, and male and female behaviour observed again on 29.5.87. Data were collected for bees foraging from the same comfrey plants observed on 24.5.87. To detect whether removal of males had a significant effect on available nectar supplies, nectar volume and concentration were measured on 26 and 29.5.87. On both 26 and 27.5.87 female foraging showed a change from the pattern recorded on 24.5.87. Females continued to forage from outer flowers throughout their flight period (Fig.3.23a). On 29.5.87, female foraging reverted to the pattern seen on 24.5.87, with males patrolling outer flowers (Fig.3.23b) The temperature and nectar volume and concentration did not differ significantly between days over the period of the experiment (Figs.3.24a,b,c), and it is unlikely that differences in these variables caused the observed changes in female behaviour. The activity pattern of foraging females on 27.5.87, in the absence of males, still showed the bimodality observed in the presence of males (Fig.3.25). Thus, although male interference did correlate with a change in female behaviour, this experiment failed to show that male interference also significantly affects the female activity pattern, causing a bimodality in activity at relatively low T_a. I would like to have been able to test the hypothesis that over a longer time period female activity patterns would become less bimodal in the absence of males.The bimodality in nectar foraging by females must also be due in part to the shift from nectar collection to pollen collection over the middle of the day (section 3.6 and Fig.3.10); under climatic conditions which permit the provisioning of a cell, part of the bimodality in female nectar collecting activity must be independent of T_a.

3.8 Behavioural responses to changing ambient temperatures in *A. plumipes*.

This section describes the effects of temperature on flight activity at the nest site, departure from nest tunnels in the morning and male basking behaviour.

A. Activity Patterns at the nest Site.

Data for the levels of flight activity at the Merton Wall nest site were gathered from before

dawn until after dark on 4 days; 27.4, 31.4, 3.5 and 5.5.87. Accompanying climatic data were also collected. Light levels were recorded with a light meter at 45°, facing south, at a height of 1.5m. The three measures of temperature used were intended to incorporate the three thermal environments typically experienced by *A. plumipes*: they were air temperature in the shade 1.5m above the ground (the height at which the majority of nests were located), the temperature of the wall's surface (wall temperature), and the temperature of the wall 3-5 cm into nest tunnels (tunnel temperature), both at the same height. Wall surface temperature depended not only on the T_a at the time, but also on levels of solar radiation. Because of the large thermal inertia of the wall, tunnel temperature was principally determined by past thermal conditions. If a warm, bright day was followed by a cool cloudy day, the air temperature in nest tunnels was often considerably higher than the air temperature outside. The days differed considerably in T_a; 27.4.87 was a hot day, with T_a exceeding 20°C from 14.00 to 17.00h, 31.4.87 was mild, with T_a of around 14°C throughout the day and 3.5.87 was a cold day, with T_a only briefly exceeding 10°C. Patterns of bee activity also differed between the days. Some general conclusions can be made from the data (Figs.3.26a,b,c).

1. The activity period shown by females was variable and depended on T_a. On 27.4.87, a warm day, female activity commenced at 05.45h and continued until 20.30h, giving 14.75 hours of activity. On 3.5.87, a cool day, activity commenced at 07.00h and had ceased by 17.30h, giving only 10.5 hours of activity. Activity patterns and changes in temperature over the day for these two days are shown in Figs.3.26a,b,c.

2. On all days, provisioning female flight activity showed some degree of bimodality, with a period of reduced flight in front of the wall during the middle of the day. On 27.4.87 and 3.5.87, the bimodality was pronounced, and on 31.4.87 and 5.5.87 the midday drop in flight activity was less clear. This drop in female flight activity coincided with the long waits inside the nest which may be associated with egg-laying (see Fig.3.10).

3. Provisioning females maintained flight activity even during poor conditions, including low temperatures and light levels, strong winds and light rain. That females were able to leave the nest tunnels and forage at air temperatures as low as 5-6°C (see Table 3.4 below) before dawn, and thus without the possibility of basking, is strongly indicative of endothermic ability. Males and searching females showed a far more variable flight activity pattern than provisioning females. The total length of activity was rather shorter than that of provisioning females, and concentrated during the warmer part of the day. Activity by males and searchers was strongly dependent on temperature and light

levels (see number 4, below), and both these groups rapidly took shelter in their sleeping tunnels during periods of heavy cloud or rain. Only when T_a was high was there any indication of a bimodality in male flight activity over time (27.4.87, Figs.3.26a,b). There was never any observed indication of bimodality in the flight activity of searching females. Searching females showed a much higher threshold temperature for flight activity than provisioning females - very similar, in fact, to that of males. They were also the first to return to their tunnels in poor weather. Chapter 4 shows that there is no apparent physiological difference in the endothermic abilities of searching and provisioning females. Thus although females are capable of considerably greated independence of T_a at low T_a when acting as provisioners, such endothermic expenditure is avoided while nest searching. This gives rise to the interesting speculation that male activity may also yield an inaccurate underestimate of male endothermic ability.

One possible explanation for the midday peak of searching female activity is that searchers are minimising their chances of meeting established females. Established females will often attack searching females which fly close to their nest entrances. Another is that by flying only under the most benign conditions, with their flight abilities at their best and metabolically least costly, searching females maximise the probability that they will be able to escape effectively from predators.

4. The activity of all of the bees flying at the wall (in terms of numbers observed per unit time), including parasitic *Melecta albifrons*, was strongly dependent on temperature. The measure of temperature which most accurately predicted levels of activity varied between groups. For searchers and males, activity during the day was divided between occupation of nest tunnels, resting on the wall's surface, and flight. By comparison, occupation of tunnels and resting on the wall formed relatively smaller parts of the time budget of provisioning females. Thus for bees which spend part of their time inside the tunnels and part of their time in free flight, both tunnel and T_a may have independent effects on flight activity. Results for the following multiple regression analyses (each of which correlates flight activity with air, wall and tunnel temperature, and light levels) are presented in Table 3.3. (below).

The activity of provisioning females correlated positively with both tunnel and T_a. The same was true for searching females. Furthermore, activity of searching females, which spent much of their time on cool days basking on the wall, also correlated positively with wall temperature. Male activity showed no significant correlation with T_a, but did correlate positively with both tunnel and wall temperature. Activity of the nest parasite *Melecta albifrons* correlated positively with air and tunnel temperatures, but not with wall temperature.

Table 3.3. Multiple regression summaries for the relationships between bee activity and temperature at the nest site.

Group	Sample size	R^2	p values		
			air temperature	wall temperature	tunnel temperature
Anthophora plumipes					
Provisioning females	100	0.22	<0.005	N.S.	<0.030
Searching females	100	0.58	<0.050	<0.001	<0.001
Males	100	0.50	N.S.	<0.003	<0.001
Melecta albifrons	61	0.58	<0.010	0.09	<0.001

The most general result is that for all of the active bees other than provisioning females, temperatures associated with their resting sites (tunnel temperature, wall temperature) are better predicitors of activity levels having controlled for other climatic variables than T_a. These measures of temperature are not strictly independent of each other: wall surface temperature is dependent both on the deep tunnel temperature and on T_a . The absence of any correlation between male activity and T_a having controlled for the effects of tunnel and wall temperature thus does not mean that male activity is not dependent on T_a, but that it is better predicted by a more complex function of T_a than by T_a alone. Having controlled for these measures of temperature, there was never any significant correlation between activity and light levels. Since all of the bees active at the nest site other than foraging females essentially 'tunnel hop' (periods of flight activity in front of the wall followed by periods of rest in their tunnels or basking on the wall) the dependence of their activity on tunnel and wall temperatures is to be predicted. Similarly, the activity of foraging females, which spend at least 50% of their time in flight or foraging at sources distant from the nest site, is predictably dependent on T_a.

Figs.3.27a and b show the mean numbers of provisioning females, searching females and males observed at a given air (Fig.3.27a) and tunnel (Fig.3.27b) temperature. These figures clearly show that provisioning females were active in numbers at much lower temperatures than males and searchers. The numbers of provisioning females correlated positively with T_a up to 13-14°C, and then remained independent until T_a exceeded 24°C, when activity declined (Fig.3.27a). A similar pattern relates provisioning activity and tunnel temperature (Fig.3.27b). For both measures of temperature, male and searching female activity remained at very low levels below a threshold temperature. From the figures these appear to be 17°C (tunnel) and 15°C (air), although some males

and searching females were active at considerably lower values. Minimum T_a at which males and searching females were observed to fly were 3 and 9°C respectively. At higher temperatures male activity increased with temperature more rapidly than in searching females. Male activity declined at T_a above 23-24°C.

B. Departure from tunnels in the morning.

1. Females: Time of morning departure from nest tunnels was dependent on climate, nest position on the wall, and female body mass, although not all of these factors were important on all mornings. Unlike the males (see below), females flew directly from within their nest tunnels at considerable speed, and observing female departure over the whole length of the wall was only made possible by placing small conical nets over the tunnel entrances (see Chapter 2). The time at which a female was observed in the net was recorded as her time of departure. Female departure was recorded in detail for 8 days: 31.4.87, and 3,6,7,10,12,15, and 20.5.87. Data for these days are summarised in Table 3.4. To allow comparison between days, the mean time of departure (t_{md}) for all the bees trapped was calculated for each day. To give a measure of the air temperature experienced each day by the bees at the earliest potential start to their activity period, just before dawn, I have used air temperatures in front of the nest site measured at 06.00h.

Tunnel temperature was the best predictor of t_{md}: the cooler the tunnels, the later the females departed (Fig.3.28a). Multiple regression revealed that having controlled for the effects of tunnel temperature there was no significant correlation between t_{md} and either T_a at 06.00h or light levels. For example, on 10.5.87, despite T_a at 06.00h of only 5.4°C, t_{md} was 06.40h due to high tunnel temperatures (mean 15.6°C). On 20.5.87, despite similar mean T_a (5.7°C) and brighter sun (Table 3.4), t_{md} was delayed until 08.07h due to lower mean tunnel temperatures (12.0°C). On 3.5.87, which showed both low mean T_a (4.0°C) and tunnel temperatures (8.9°C), departure was delayed further still to a mean of 09.32h.

The main nest site faced south, and as the sun rose, nests on its western end were insolated, and warmed, before those at the eastern end. On 7.5.87 there was a significant correlation between the time of departure of individual females and the position of the nest entrance in the grid. Females in tunnels whose entrances were insolated earlier emerged earlier (Fig.3.28b). On both this day and on 20.5.87 there was a significant negative correlation between time of emergence of an individual female and her body mass: larger females emerged before small females (Fig.3.28c,d). I would predict both body mass (see Chapter 4) and tunnel position to be significant determinants of emergence time under poor conditions. Clearly climate is an important determinant of emergence time, and body mass and tunnel position may also be important under certain conditions.

2. Males: Males showed a characteristic sequence of behaviours associated with departure from their nest tunnels in the morning leading to flight. At night they remained 30-50mm down the tunnels, often upside down or on their sides. The first sign of renewed activity was the resumption of an upright position. Males moved forward until they sat in the tunnel entrance, tipped forward with the dorsum of the thorax exposed to any available sunlight. Sometimes males left the tunnel entrance and basked on the wall before flight, but departure from the tunnel entrance was generally direct.

The awakening and departure behaviours preceeding flight activity were highly dependent on climate. Cold wind, or sudden clouding over, led to retreat by all males into the tunnels. The behaviour of males in their tunnels was quantified by looking at the relationship between the depth at which the males chose to rest in the tunnels and microclimatic factors. Depths were measured from the plane of the nest entrance. The microclimatic factors recorded were T_a, light intensity at the tunnel entrance and tunnel temperature 3cm into the wall. Male positions are mean depths for all the males observed in tunnels at the time the observation was made. Data were gathered on 6, 8, 9, 10, 15, 21 and 27.4.87. Having controlled for all of these factors, T_a was of dominant importance ($n=52$ $R^2=0.520$, $p<0.0001$): the higher the T_a, the closer the males came to the nest entrance until flight occurred at an T_a of 10-14°C (Fig.3.29a). Having controlled for T_a and light levels, tunnel temperature was also significantly correlated with male depth ($p=0.018$); the higher the wall temperature, the lower the T_a required for flight. Having controlled for these measures of temperature, there was no corelation between male position and light intensity. The lack of any significant correlation between male position and light levels does not mean that males do not respond to changes in light levels. Male movement up and down the tunnels often appeared to correlate with changes in the level of insolation of the wall. It seems to be the case that males responded to the temperature changes in their immediate surroundings resulting from changes in levels of sunlight: having controlled for the effects of changing air and tunnel temperatures no significant effect of light levels remains.

During the period before the appearance of females at the start of the season, or on days with marginal flying conditions, males resident at the nest site made sorties from their tunnels to feed, returning to occupy their tunnels for periods of time before flying again. The durations for which different males rested in their tunnels were recorded. Tunnel occupation durations depended on both T_a and body mass. Male tunnel occupation times at each T_a have been divided into data for large males (160-200mg) and small males (<160mg). Having controlled for the other factor using multiple regression, tunnel occupation duration correlates negatively with both T_a and body mass ($n=67$, $R^2=0.394$, body mass $p<0.001$, T_a $p<0.0001$) (Fig.3.29b). That the tunnel occupation

times for smaller males were so much longer than those for larger males suggests that there will be a T_a at which only larger males can continue to fly in search of females. Thus under poor environmental conditions, body mass may also directly affect male reproductive success. This role of body mass is consistent with the limitations on thoracic temperature imposed by body size and endothermic ability discussed in the Chapter 4.

Tunnel occupation by both large and small males ceased at T_a over 16°C, to be replaced by continuous flight activity and periods of basking on the wall and vegetation.

C. Male basking behaviour.

Although on a sunny morning males were able to fly at air temperature as low as 5°C (e.g. 10.5.87), in the absence of solar heating their minimum air temperatures for flight were rather higher at 10-12°C. Although behavioural thermoregulation was claerly of major importance to males, flight activity at these ambient temperatures is nonetheless suggestive of endothermy, and is near the lower temperature limits for flight activity in some other known endothemic insects, including honeybees (Heinrich 1979; Cooper, *et al.* 1985) and bumblebees (Heinrich 1972b). Males spent a large proportion of their time resting in the sun. I refer to resting in the sun as 'basking'. Demonstration of a relationship between the duration of these rests, and the location chosen for resting, and climate (see below) is used to infer their thermoregulatory purpose. Bask duration was measured with a stopwatch from the time of landing to the time of flight.

Males basked on the wall just after leaving their tunnels, and on the ground or on vegetation during both foraging and patrolling behaviour. Rather than holding their wings over the abdomen in such a way that the wings overlapped each other, basking males (and females) held their wings so that they did not meet, and the abdomen was exposed to the sun. Basking was particularly frequent and prolongued during foraging behaviour at low T_a. The duration of basks by male *A. plumipes* while patrolling for females at lungwort feeding sites in the Botanic Gardens correlated negatively with both light intensity and T_a. Multiple regression revealed that, having controlled for light intensity, T_a and not light intensity is the major determinant of bask length ($n=31$, $R^2=0.400$, T_a $p<0.001$, light intensity $p=0.24$) (Fig.3.29c).

To examine the ability of *A. plumipes* to raise their body temperature solely by basking, I inserted 0.25mm diameter copper-constantan thermocouples into the thoraces of 2 freshly dead males and 2 females. These bees were positioned on a piece of wood covered in manilla-coloured paper, designed neither to reflect nor to absorb all the incident sunlight. The bees were placed in the sun, and their thoracic temperatures allowed to stabilise. Legs and wings were in a life-like basking attitude, with the wings held flat along the side of the body. Light levels in the sun and air temperatures in the shade were measured simultaneously. Analysis of the resulting data reveals that

there is a strong positive correlation linking both light levels and T_a to thoracic temperatures reached by the dead bees (Figs.3.30a,b), once one of them has been controlled for, but no significant difference between the sexes (n=50, R^2=0.930; T_a $p<0.0001$, light levels $p<0.0001$, sex $p=0.195$). The highest thoracic temperatures reached by these dead 'basking' bees were over 39°C in bright sun at a T_a of 19.5°C. While basking early in the morning, particularly during days with low T_a, males selected sites facing directly into the sun, but surrounded on all sides by high vegetation. This behaviour allowed selection of sites with local T_a up to 5°C higher than more exposed sites, and may also have helped to conceal the bees from predators. Provisioning females were seen basking much less often than males, and they were often active under conditions (before dawn, heavy cloud or light rain) which made basking impossible. On cold, clear days, however, some females basked on a dark east-facing wall immediately after morning emergence from their nest tunnels.

3.9 Discussion.

There have been many studies of the factors affecting the behaviour and activity patterns of nectar foraging insects. Nectar supply, and thus caloric reward, has long been implicated as a major controlling factor in foraging behaviour and activity patterns (e.g. Butler 1945). The importance of physical climatic variables has also been emphasised (Szabo and Smith 1972; Gerling *et al.* 1983; Willmer 1982, 1985a, b). It is only relatively recently that studies in the field of both these sets of variables have been made. In some, caloric reward has been implicated as a major determinant (e.g. Corbet 1978b; Corbet *et al.* 1979b; Louw and Nicolson 1983) and in others, limitations of temperature (Willmer 1986). Interaction between the two has also been stressed (e.g. Heinrich 1975b, 1976b; Schaffer *et al.* 1979; Willmer and Corbet 1981; Stone *et al.* 1988). Willmer (1983) pointed out that the relative importance of nectar supplies and climatic conditions in determining insect activity patterns depends on the thermal biology of the species concerned, and particularly on whether the species is primarily ectothermic or endothermic. Environmentally determined changes in forager behaviour have also been quantified (e.g. Schlising 1970; Willmer 1982, 1983). My work on *Anthophora plumipes* confirms that several factors affect the activity patterns and behaviour of this species, and that the factors which determine behaviour independently may interact to produce new effects.

There have been no published studies on the thermal biology of *Anthophora*, but several of the characteristics of the behaviour of members of this genus are indicative of endothermy. *Anthophora* spp. have been recorded visiting desert flowers before dawn when ambient temperatures are below 10°C (e.g. Linsley *et al.* 1963 for *Anthophora neglecta* and *A. affabilis* in Arizona) and foraging in light rain and cloud (e.g. Norden 1984 for *A. abrupta* and Esmaili 1963

for *A. occidentalis*). Female *A. plumipes* are able to forage at ambient temperatures as low as those recorded for the most hardy bumblebees (Heinrich 1972a, 1975a), long regarded as the quintessential endothermic insects, and this is strongly suggestive of endothermy.

To what extent is it true to say the the behaviour of *A. plumipes* was determined by temperature? Temperature correlated with the time at which both males and females emerged from their nest tunnels in the morning; if air and tunnel temperatures were sufficiently high, females foraged before dawn and after dusk. T_a had a strong effect on the provisioning behaviour of females, determining not only which females within the population were able to provision their cells, but also the nature and mass of the load collected. Male behaviour was also highly dependent on T_a. For males at the nest site temperature determined whether or not the bees flew or rested in their tunnels, and how long they basked between flights. The transition from foraging to patrolling behaviour at nectar sources also correlated with increasing T_a, and at low T_a no patrolling behaviour occurred. The behaviour of *A. plumipes* is clearly strongly dependent on T_a. The behaviour of searching females showed also that the sensitivity to temperature of two different behavioural phases (searching and provisioning) may be radically different. This is an important result. The change in male behaviour with increasing temperature had a profound effect on female foraging. Both the nectar collected per unit time and the location of the flowers visited were affected by male behaviour. In this way temperature indirectly affected the behaviour of females well within the bounds of their own physiological thermoregulatory ability (Chapter 4).

Desertion of foraging sites by both males and females at high temperatures may also in some cases have been in response to excessive T_a. Both nectar foraging and flight activity at the nest site decreased markedly above 24-25°C (Figs.3.13, 3.27). Restriction of foraging to low ambient temperatures, resulting in bimodality in activity patterns at high T_a such as those recorded for endothermic bumblebees (Willmer 1983), has been recorded for other *Anthophora* species as well as for *A. plumipes* (e.g. Linsley *et al.* 1963; Wainwright 1978). In the absence of other constraints on foraging activity, such bimodal activity patterns are suggestive of considerable heat generation in flight. *A. plumipes*, like other anthophorids (e.g. Bohart 1958, for *Anthophora* spp.), often hovers while inspecting flowers during foraging. Flight between flowers involved sustained hovering within a single patch. This type of flight may not be energetically more demanding than forward flight (Ellington 1984), but it is likely to impose greater thermal stress due to decreased convective cooling (Chappell 1982, 1984). On bright sunny days, when passive heating alone in still air can generate considerable thoracic temperature excesses in the absence of strong breezes (Fig.3.30), such feeding flight may be thermally intolerable. Males were able to continue activity at nectar sites at higher temperatures than females. Fast forward flight, characteristic of patrolling

males active during the warmest part of the day, must generate greater convective cooling than hovering foraging flight, and may thus reduce the thermal loading of the bee. Some other studies have reported a predominance of fast forward flight rather than hovering at high T_a in bees (e.g. Hurd and Linsley 1975; Chappell 1982) and the potential for regulation of body temperature through adjustment of flight speed has been suggested by several workers (e.g. Heinrich and Buchmann 1986). Why males should remain active at a site from which females have departed is open to debate. Females often returned to such nectar sites in the evening, in which case continued activity by males may have increased their chances of mating relative to males that abandoned the site altogether.

Nectar supply was also an important determinant of foraging behaviour. Males stocked up on nectar early in the day, and maintained their supplies by feeding occasionally during their patrolling period during the middle of the day. Despite changes in the volume and concentration of nectar available to them for much of their flight period male activity appeared essentially independent of changes in nectar quality or quantity. They were able to maintain their patrolling activity while carrying nectar of a wide range of concentrations, reaching 60% sucrose. Provisioning females, however, must collect nectar volumes beyond their own requirements, of a concentration suitable for mixing with pollen in their cells, and must therefore respond to changes in nectar supply at food sources.The shift of the female population from comfrey to horse chestnut showed that females do respond to changes in nectar supply. Since nectar volumes did not change appreciably at the two sites, this site selection may have been on the basis of nectar concentration. Male interference had a considerable effect on female foraging, and may also affect their activity patterns. These findings show that activity patterns and foraging behaviour in *A. plumipes* are strongly dependent on temperature, but that a wider knowledge of the effects of other factors, such as changing nectar supplies and interactions between the sexes, is necessary to achieve a fuller understanding of changes in activity over time.

I know of no other study of solitary bees suggesting that females establish nectar stores whilst not provisioning their cells with pollen. When T_a is low, many female *A. plumipes* collect only nectar, and may therefore establish a nectar reservoir. Whether this behaviour results in a store of nectar which is used in cell provisioning when pollen foraging is resumed, or is used by the female as fuel for warm-up, remains to be established. It is possible that the nectar accumulated is used to provision several cells simultaneously, although I know of no evidence which suggests that members of the genus *Anthophora* do provision more than a single cell at a time, and suspect that this is unlikely. Use of a 'honey pot' energy reserve is common in social bees, and is also shown by 'solitary' queen bumblebees (*Bombus* spp.) while incubating their first brood cells (e.g. Heinrich

1972d, 1974). Chapter 4 shows that the cost of warm-up at low T_a is considerable, and the establishment of an energy reserve may enable female *A. plumipes* to continue to provision cells at marginal air temperatures. It is also possible that the reserve is used to fuel continued nest-building activities inside the wall during poor weather.

Body size has been shown to be important in several aspects of the thermal ecology of *A. plumipes*. Body mass determined which females could fly at low temperatures, and which could collect pollen under poor conditions. Large females could not only collect pollen under poorer conditions than small females, but by so doing also increased the total proportion of their cells stocked under conditions in which the nest parasite *Melecta* was inactive. In some instances, larger females were able to emerge from their nest tunnels earlier in the morning than smaller females. Body mass was also important in predicting which females could continue to forage at sites exposed to high T_a - large females were the first to leave (c.f. Willmer 1983). Male activity was also affected by body mass. Larger males were able to spend less time in their nest tunnels and more time in flight in poor weather than smaller males. For both males and females, large mass correlated with activity at low temperatures, and lower mass with activity at high temperatures. In general, females are larger than males, and, as foragers, able to fly at lower T_a than they are. This is consistent with extension of the relationships within each sex to a comparison between the sexes. Possible thermoregulatory reasons for these results are discussed in detail in Chapter 4.

This chapter demonstrates that although *A. plumipes* is able to sustain activity over a wide range of ambient temperatures, the ability of an individual to sustain activity at a given T_a is strongly dependent on body mass. Only the largest females are active at the lowest air temperatures, and only the smallest males at the highest. Ambient temperature has considerable effects on the size and type of load gathered by females, and also on the behaviour of males. The provisioning behaviour of females is shown to depend not only on T_a and body size, but also on changes in the availability of floral resources, and on the interfering effects of patrolling males. Aspects of the activity patterns and behaviour of this species are strongly suggestive of endothermy, a possibility investigated in Chapter 4.



Fig.3.1 An anaesthetised male *Anthophora fulvitarsis* from Avdat, Israel (see Chapter 6), showing the extremely long mouthparts typical of members of the genus *Anthophora*.

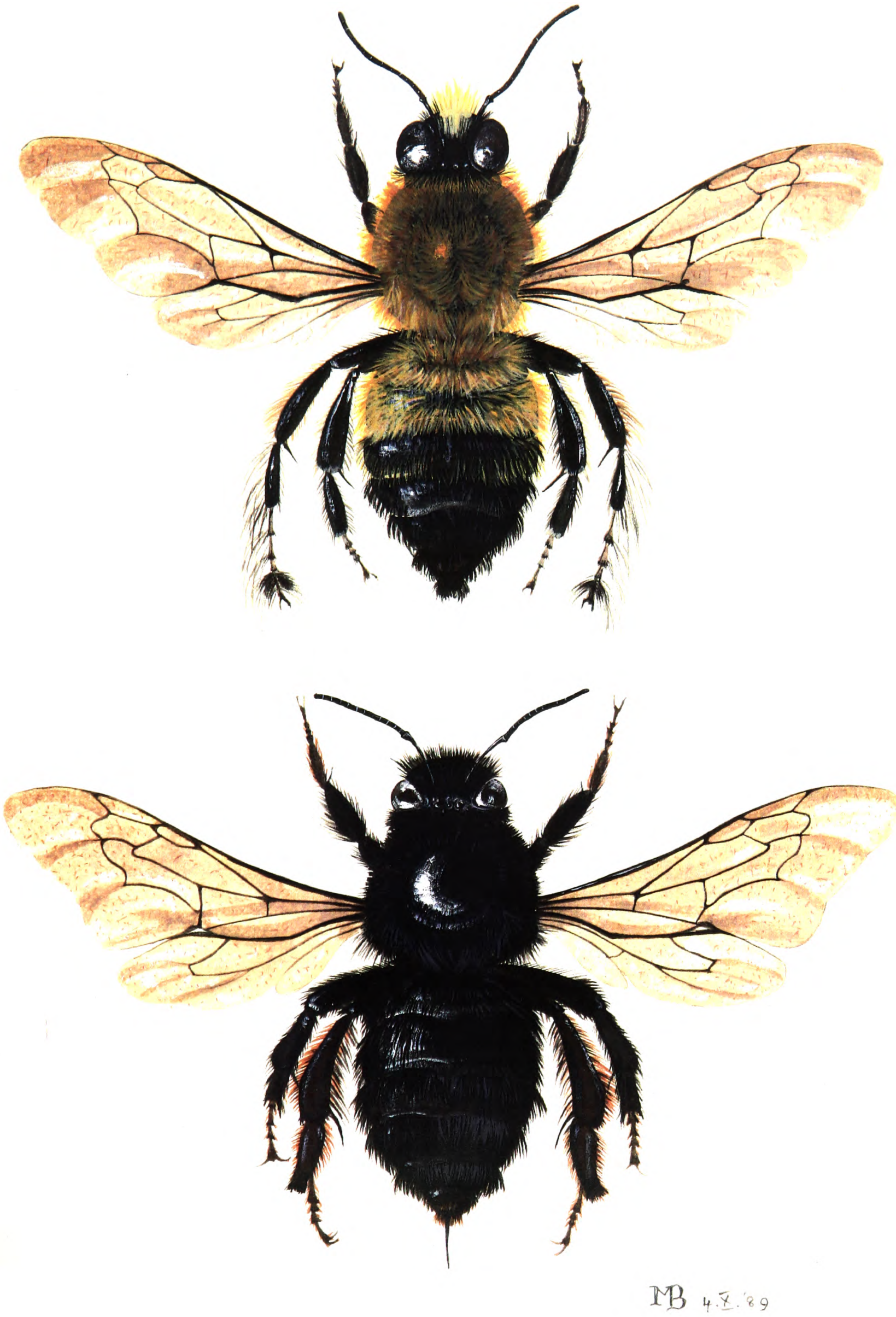


Fig.3.2 Male (upper) and female (lower) *Anthophora plumipes*, shown approximately 5 times life size.



Fig.3.3 A female *Anthophora plumipes* at the entrance to her nest tunnel.

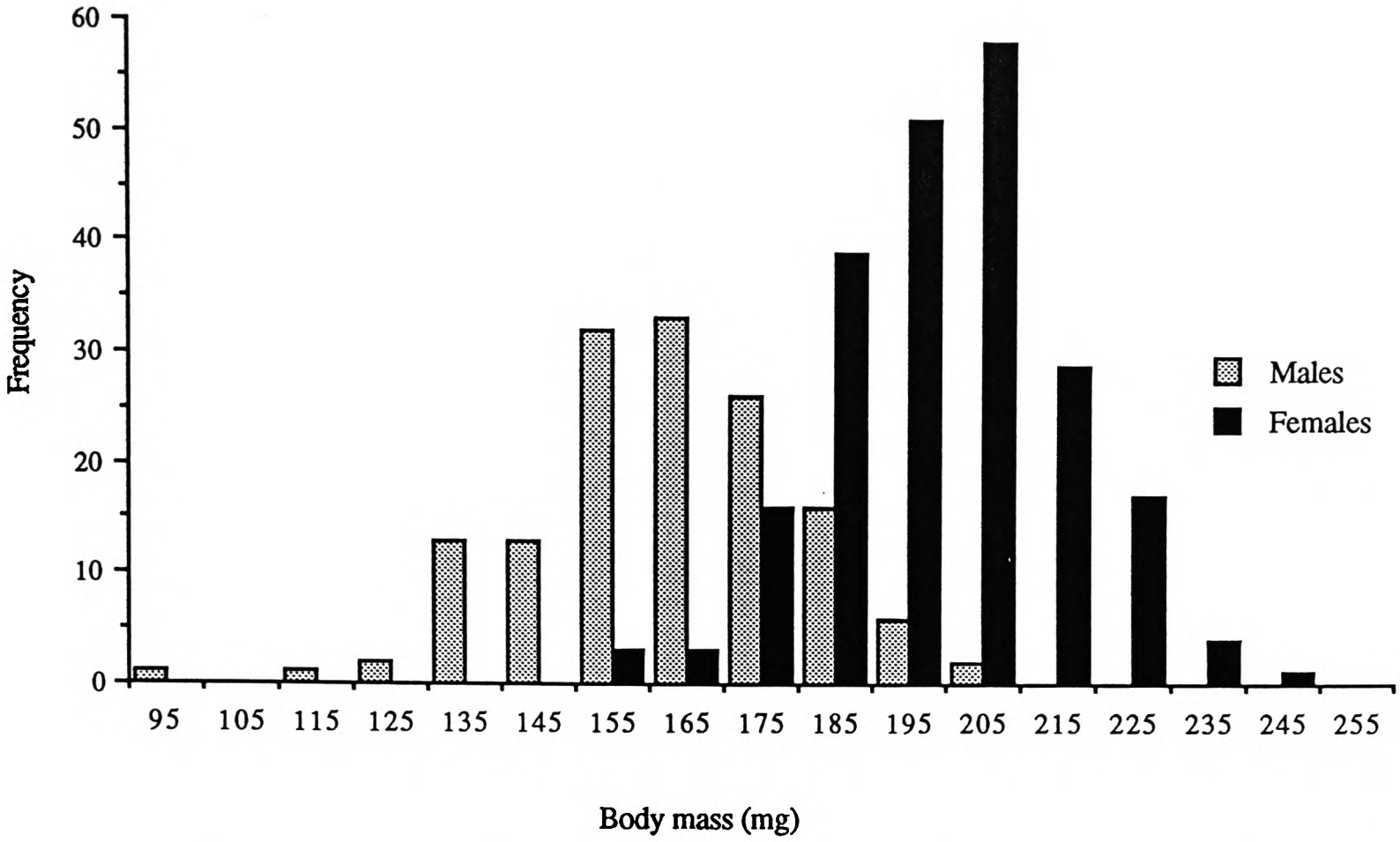


Fig.3.4 Frequency distributions of ♂ and ♀ body masses for *A. plumipes* marked in 1988.

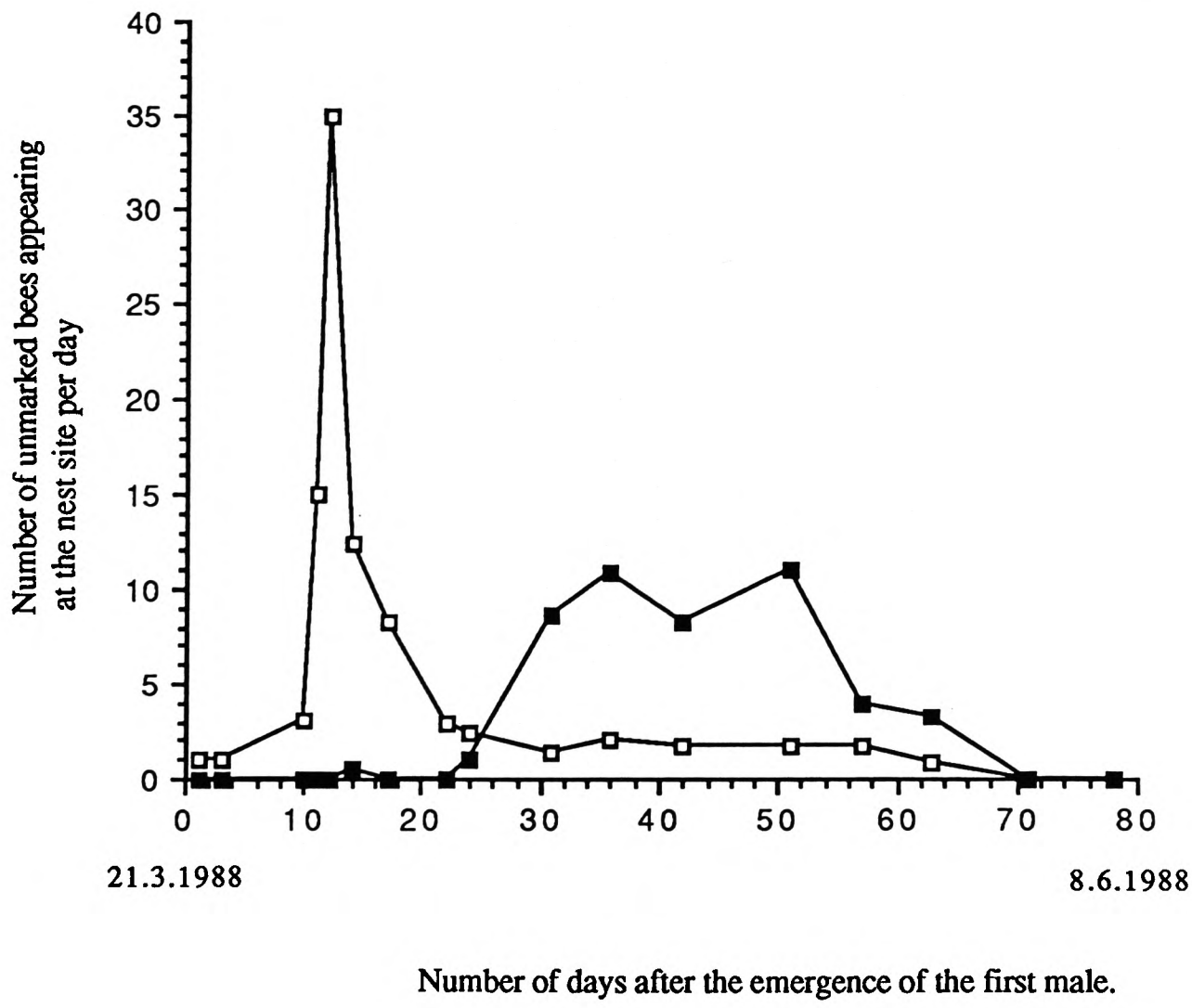


Fig.3.5 The daily rates of appearance of fresh ♂ (□) and ♀ (■) *A. plumipes* at the nest site through the 1988 season.

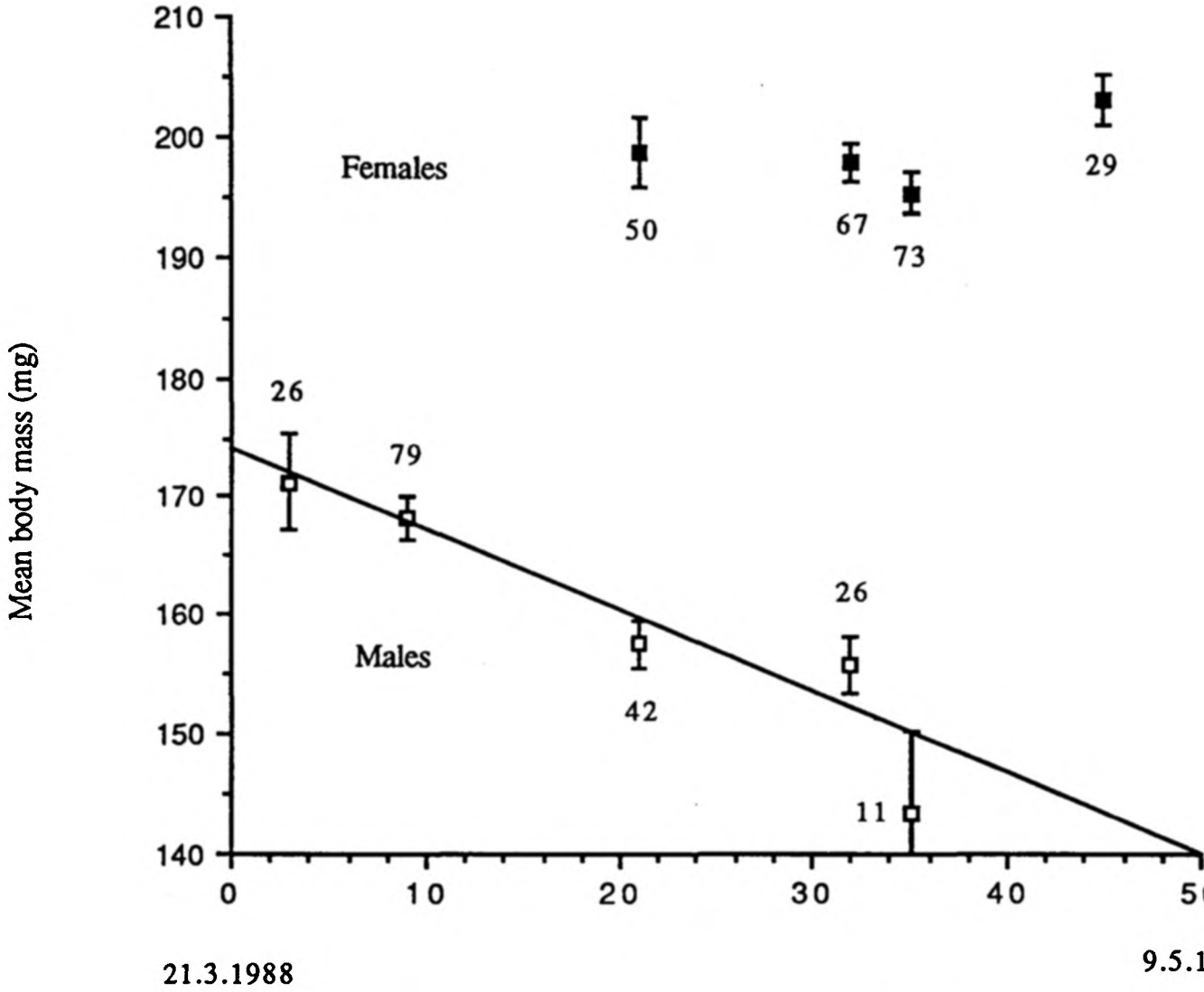


Fig.3.6 Mean body masses of ♂ (❐) and ♀ (■) *A. plumipes* through the season in 1988. (Males: $y=174.2-0.8x$, $R^2=0.889$)



Fig.3.7 Three male *Anthophora plumipes* (including one marked individual) sharing a tunnel entrance before morning emergence.



Fig.3.8 Two male *A. plumipes* hovering behind a female which has landed on a comfrey plant (*Symphytum orientale*).



Fig.3.9 A copulating pair of *A. plumipes*. The second pair of legs of the male are raised in front of the female during courtship display.

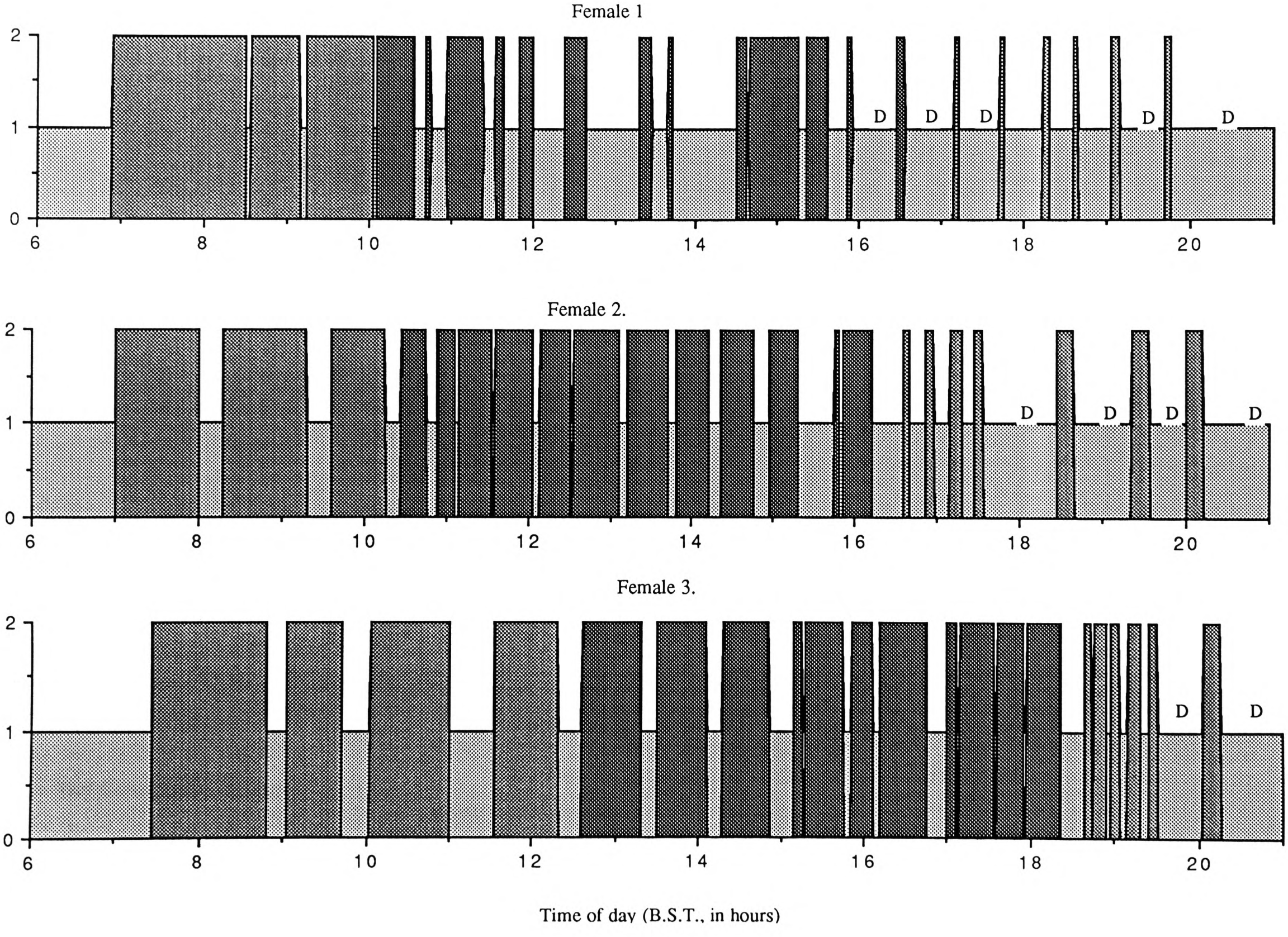


Fig.3.10 Division of time between different activities through the day for three ♀ *A. plumipes* on 23.4.87. **Palest** shading indicates time spent in the nest. A **D** above such shading indicates that the female was observed to be excavating during this period. **Darker** shading indicates absence of the female from the nest after which she returned without a visible pollen load. **Darkest** shading indicates absence of the female from the nest after which she returned with a visible pollen load on her scopae.

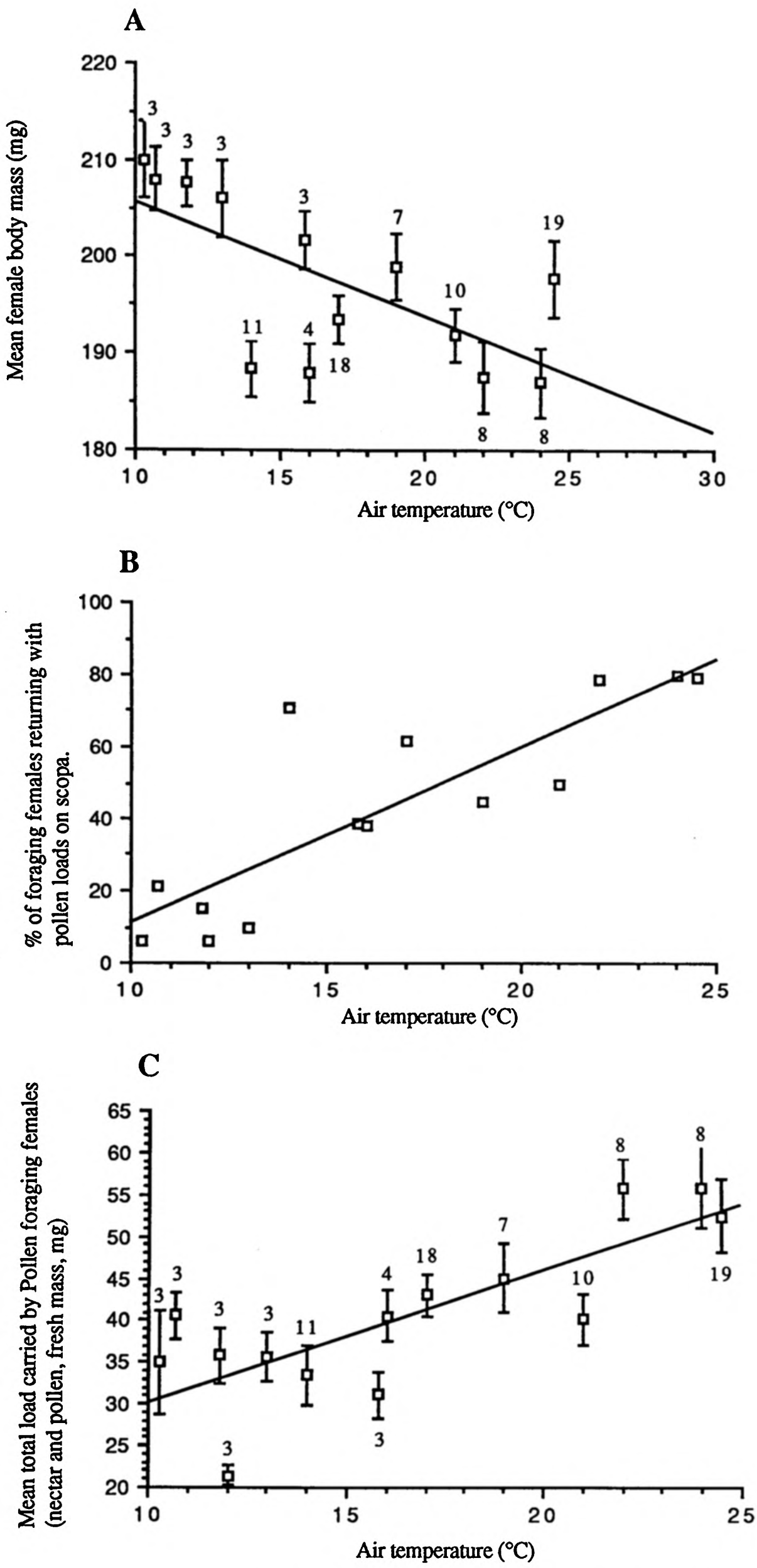
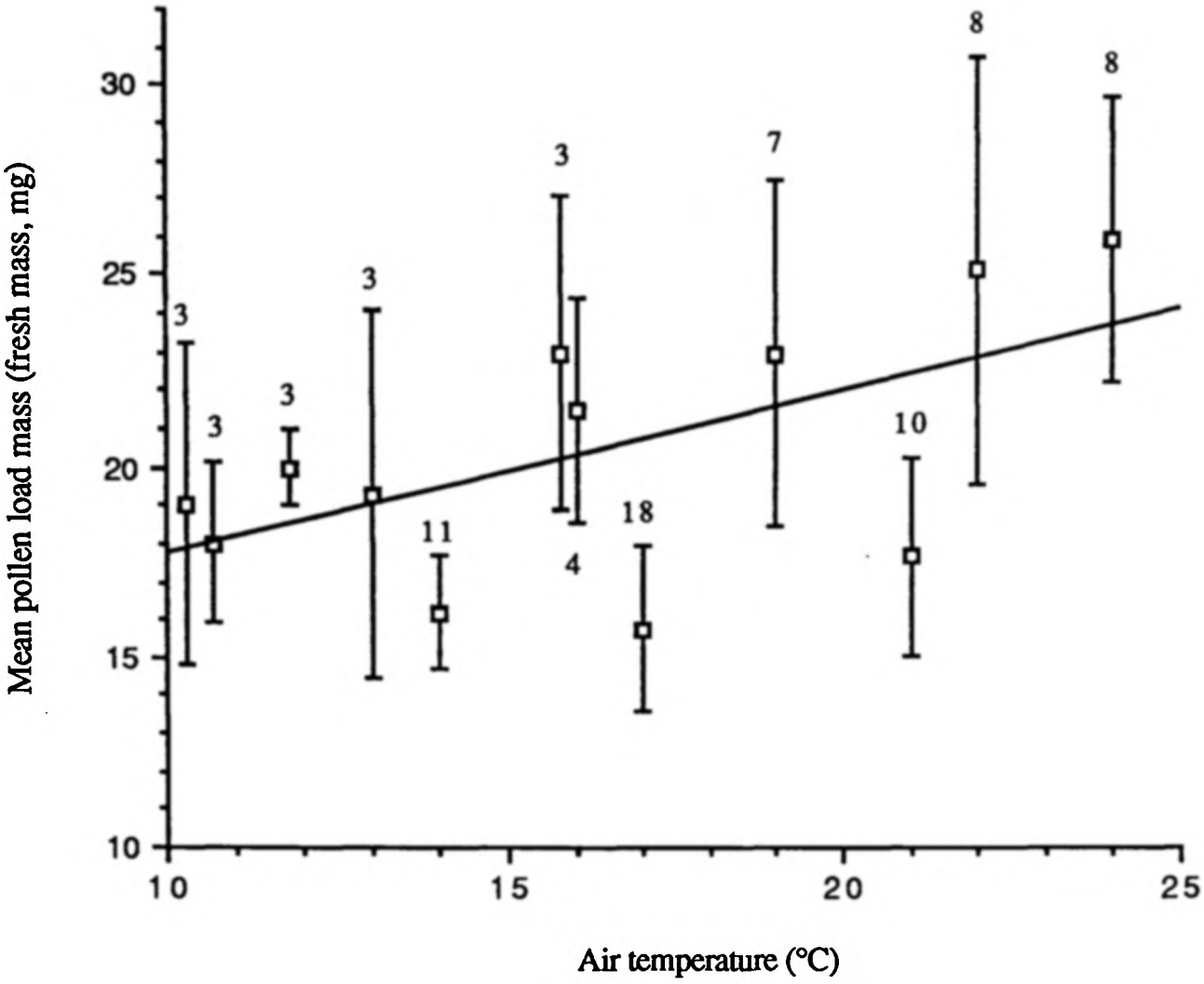


Fig.3.11 **(a)** The mean mass of pollen-foraging females as a function of mean air temperature over the foraging period for 13 days in the spring of 1988 ($y=217.6-1.2x$, $R^2=0.460$). **(b)** The % of females carrying visible pollen loads on their scopae as a function of air temperature over the same days ($y=-37.6+4.9x$, $R^2=0.720$). This is irrespective of whether females were also carrying nectar. **(c)** The mean total pollen and nectar load carried by females carrying pollen as a function of air temperature over the same days ($y=14.5+1.56x$, $R^2=0.624$).

D



E

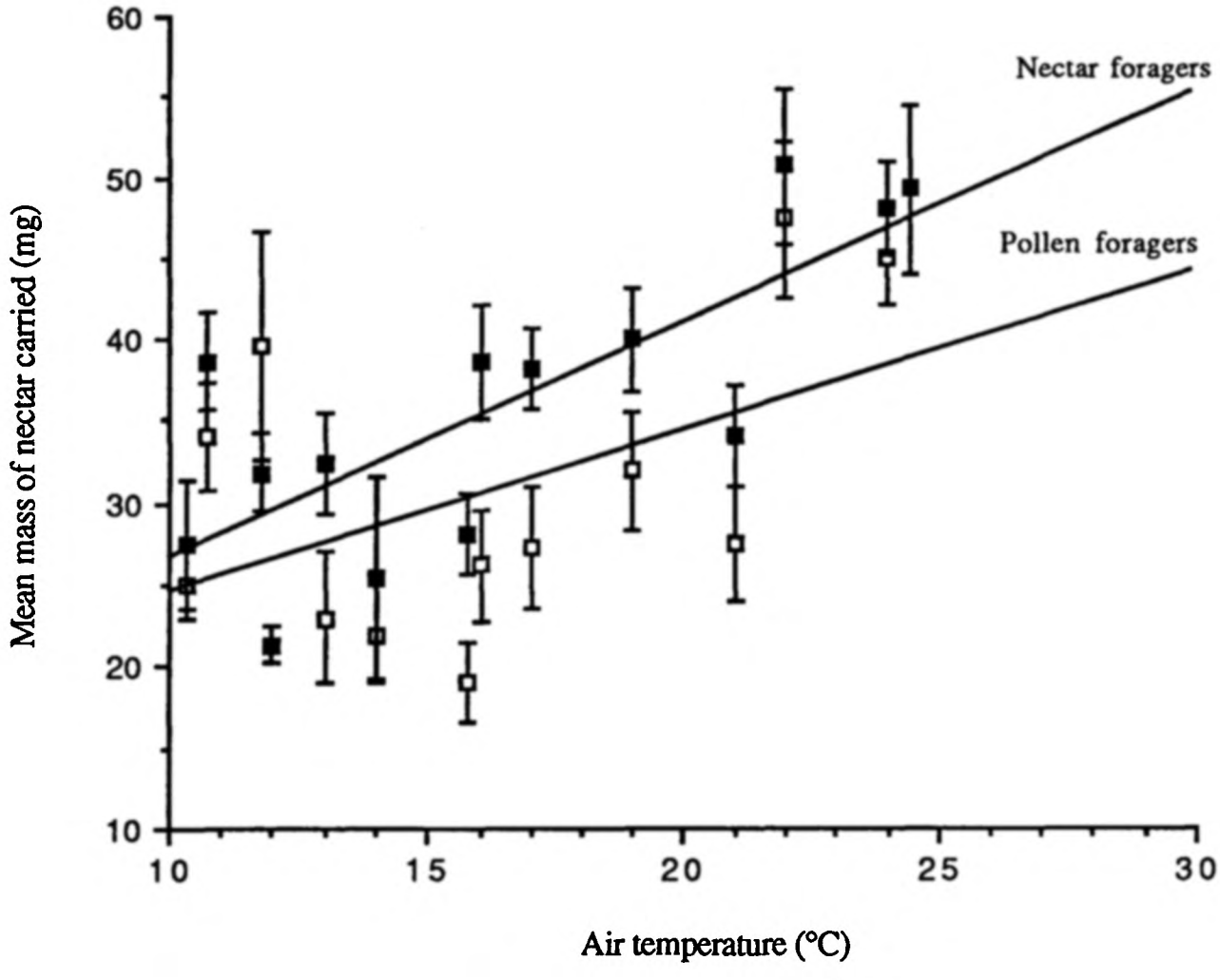


Fig.3.11 (cont.) (d) The mean pollen loads carried by returning foragers as a function of mean air temperature over the foraging period for 13 days in the spring of 1988 ($y=13.4+0.43x$, $R^2=0.336$). **(e)** Mean nectar loads for pollen (❐) and nectar (■) foragers as functions of mean air temperature for the same days (nectar foragers: $y=12.45+1.43x$, $R^2=0.593$; pollen foragers: $y=14.4+1.0x$, $R^2=0.230$).

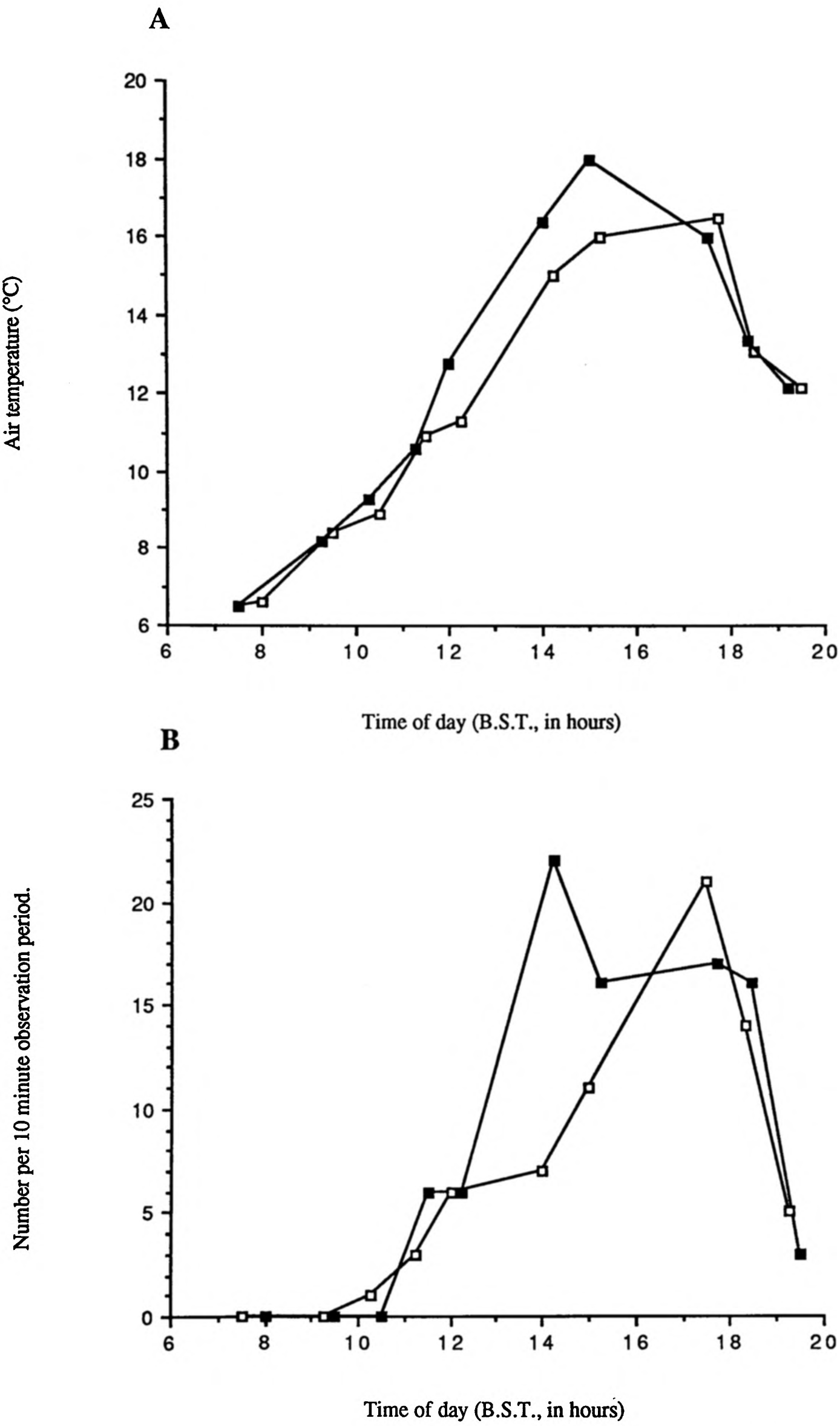
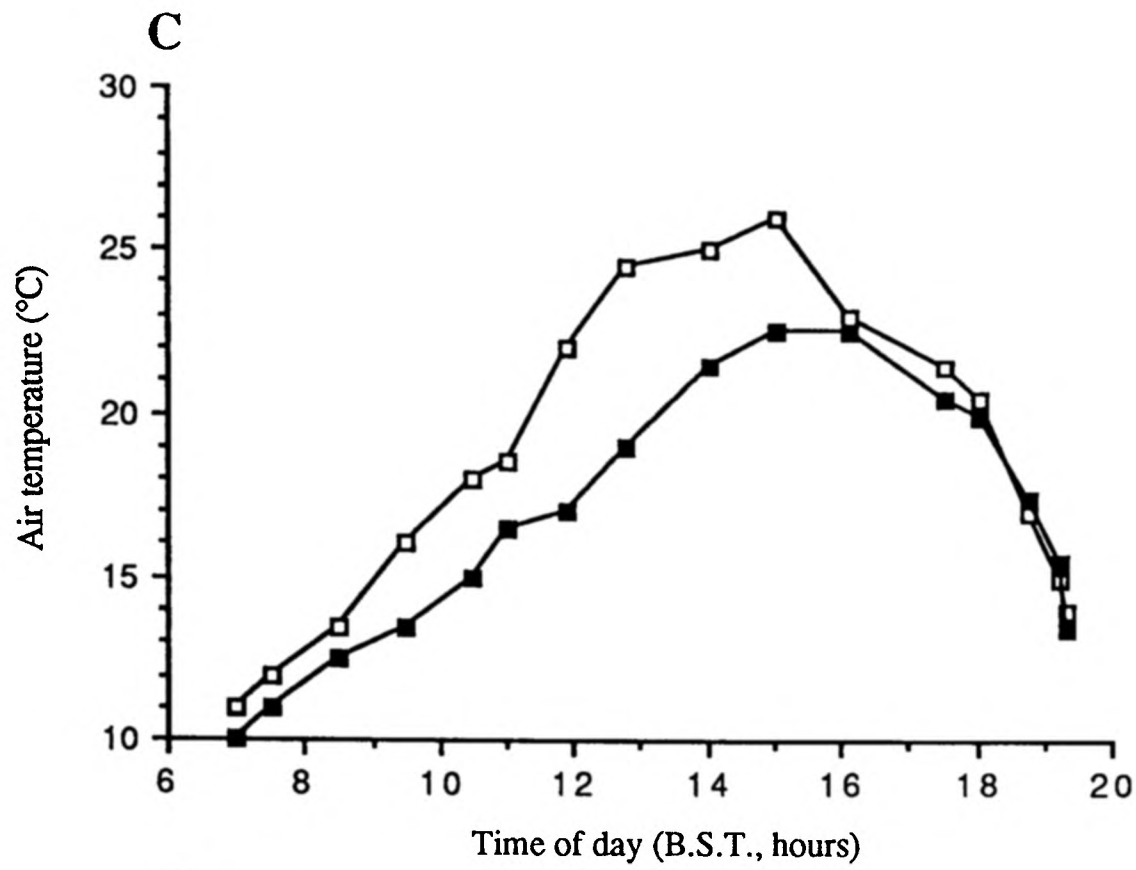
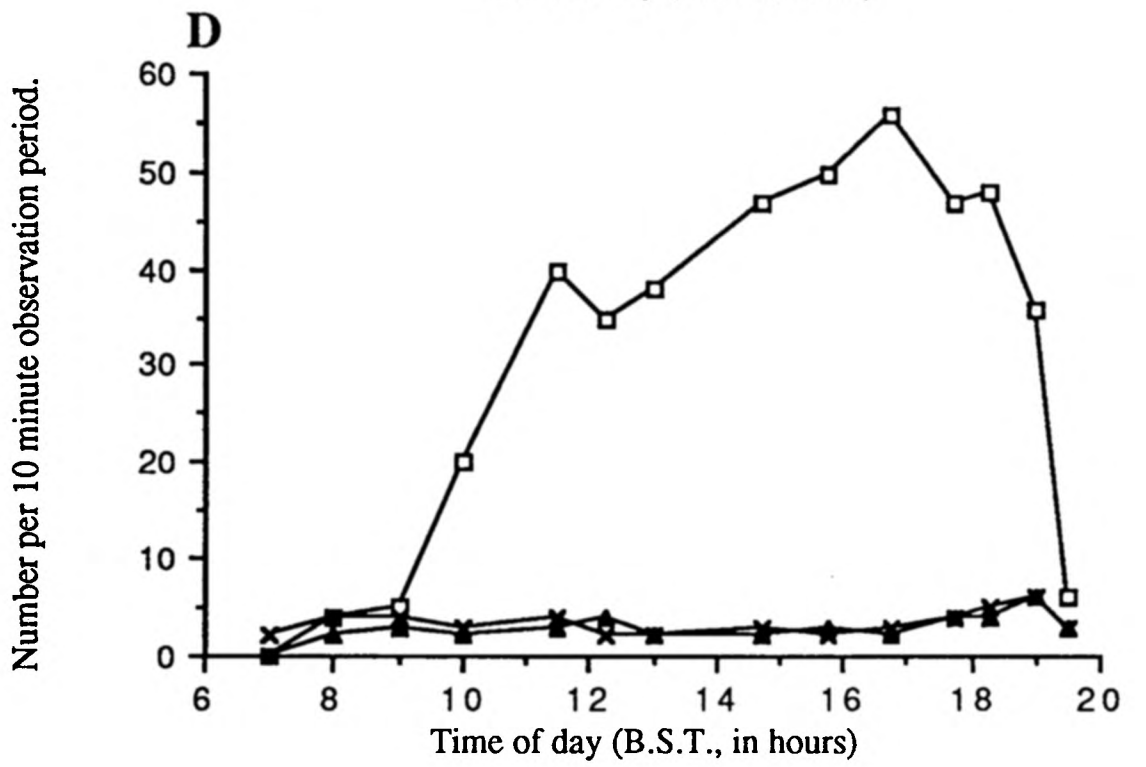


Fig.3.12 **(a)** Air temperature over time at the sun (■) and shade (❐) lungwort sites in the Botanical Gardens on 14.4.87. **(b)** Total flight activity of ♀ and ♂ *A. plumipes* at the sun (■) and shade (❐) sites on 14.4.87.





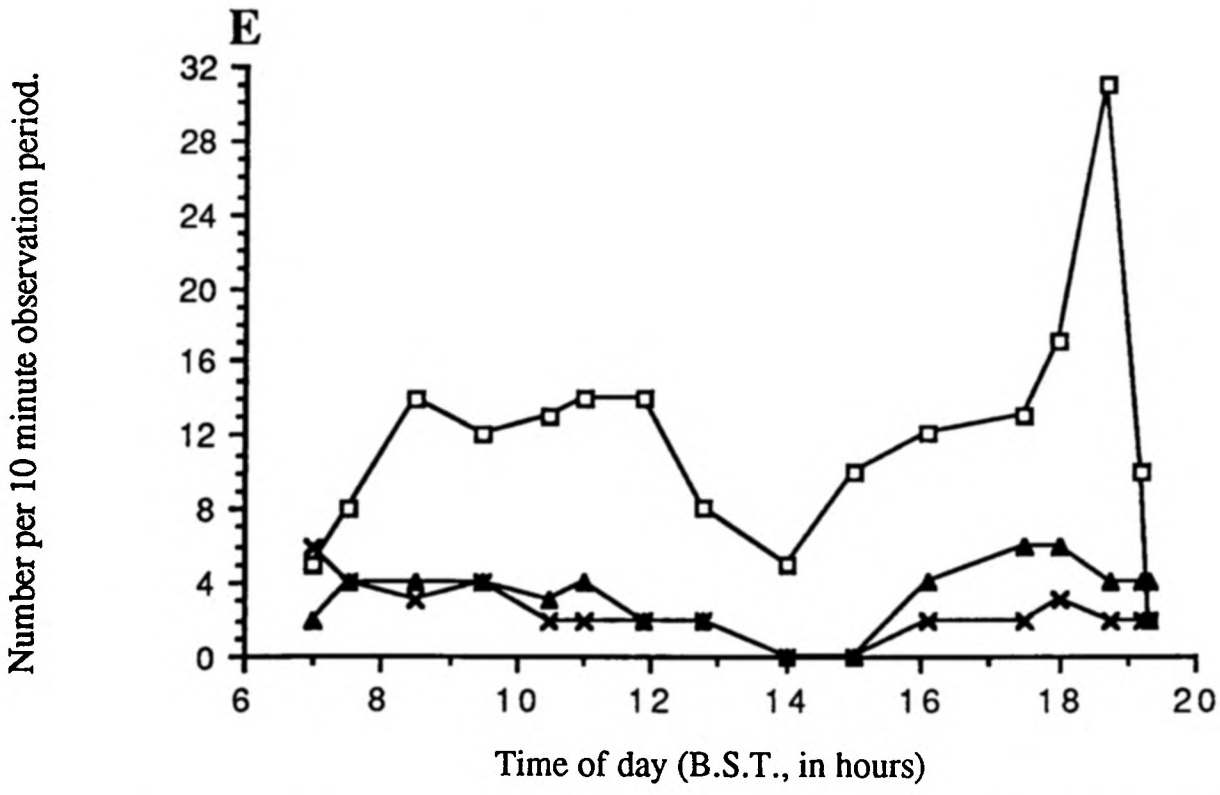


Fig.3.12 (cont.) **(c)** Air temperature over time at the sun (❐) and shade (■) lungwort sites in the Botanical Gardens on 16.4.87. **(d)** Flight activity of ♂ and ♀ *A. plumipes* (❐), *Bombus lucorum* workers (X) and *B.pascuorum* workers (▲) over time at the shade site on 16.4.87. **(e)** Flight activity of ♂ and ♀ *A. plumipes* (❐), *Bombus lucorum* workers (X) and *B.pascuorum* workers (▲) over time at the sun site on 16.4.87.

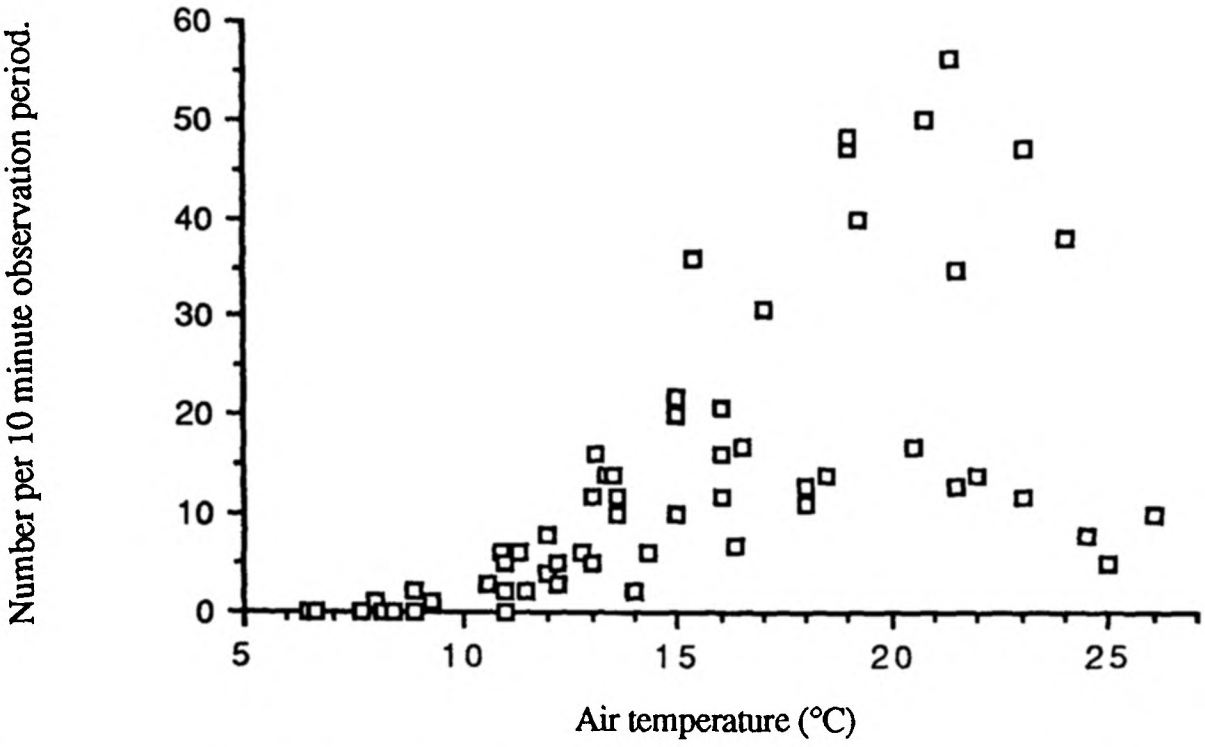


Fig.3.13 Total ♂ and ♀ *A. plumipes* foraging at sun and shade lungwort sites as a function of ambient temperature.

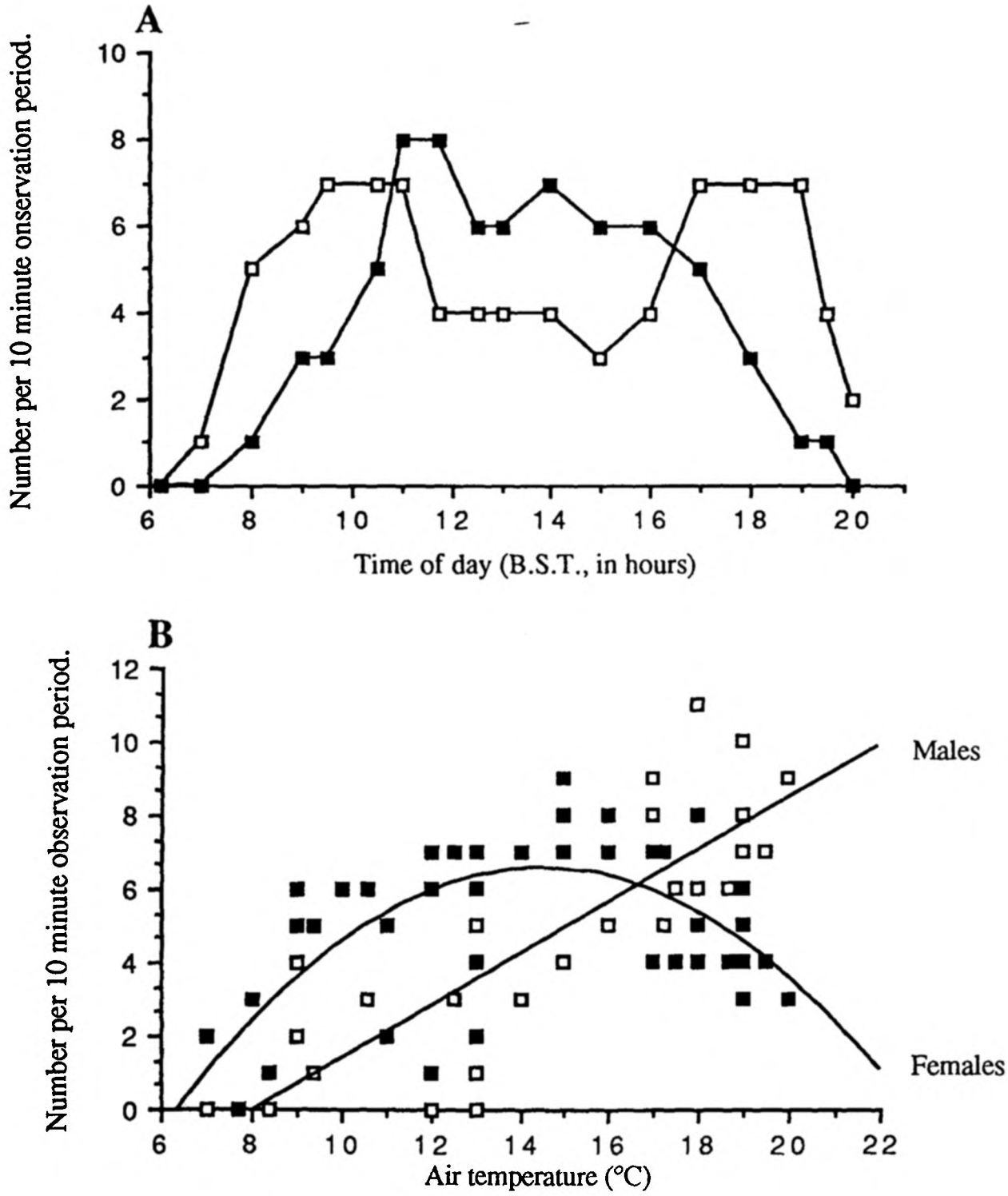
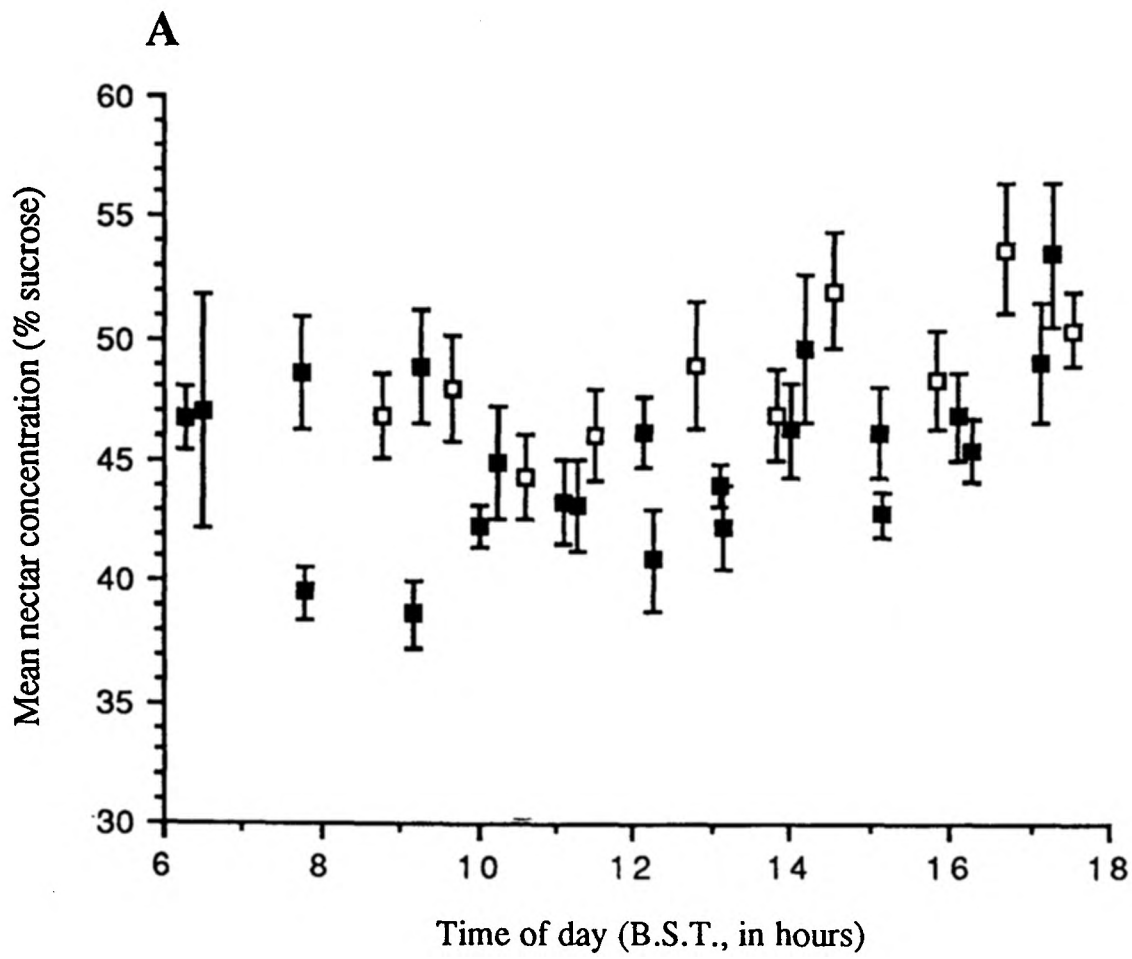


Fig.3.14 **(a)** Flight activity of ♀ (■) and ♂ (□) *A. plumipes* over time at comfrey in the University College gardens on 24.5.87. **(b)** Numbers of ♀ (■) and ♂ (□) *A. plumipes* active at this site over all days of observation as a function of ambient temperature (males: $y=-5.74+0.71x$, $R^2=0.656$; females: $y=-14.16+2.85x-0.10x^2$, $R^2=0.384$).



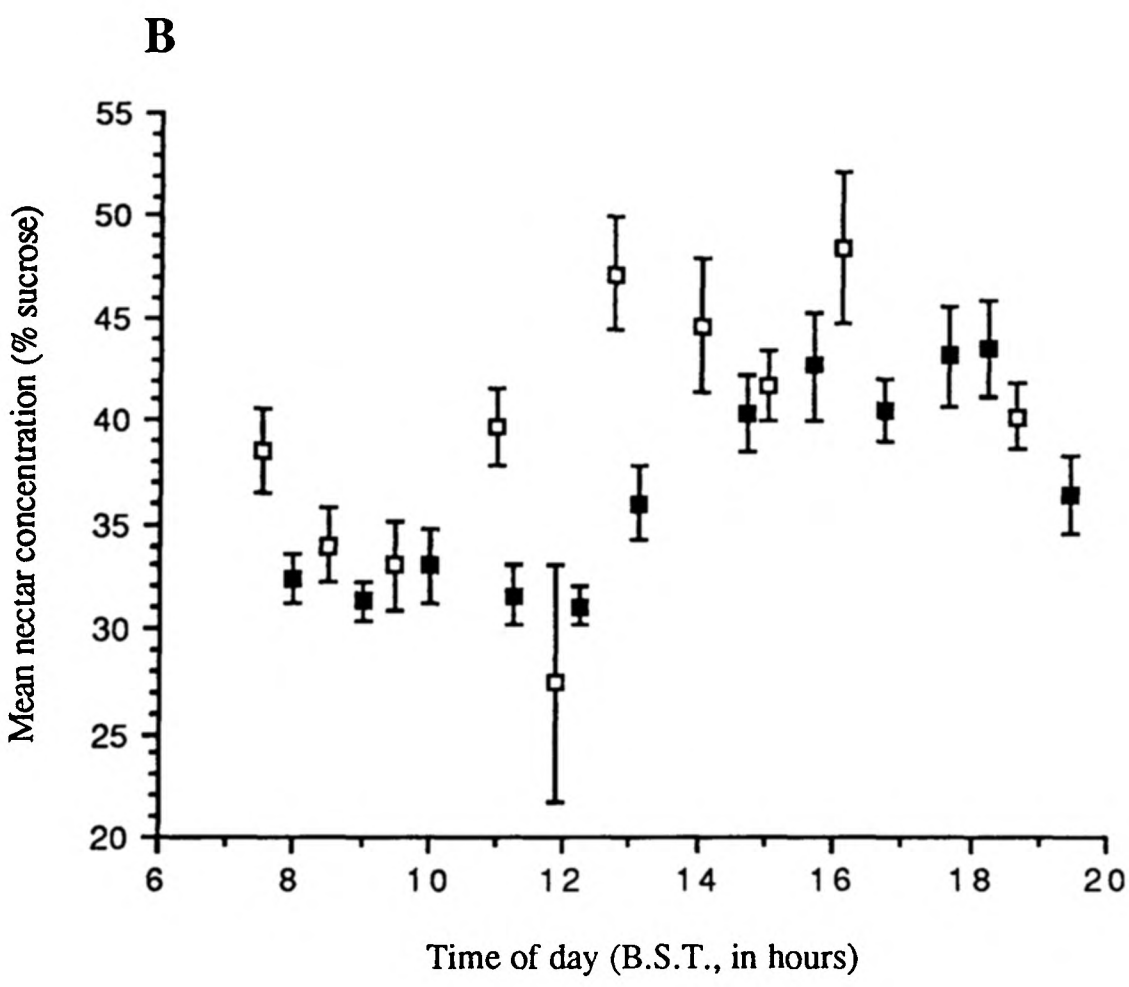
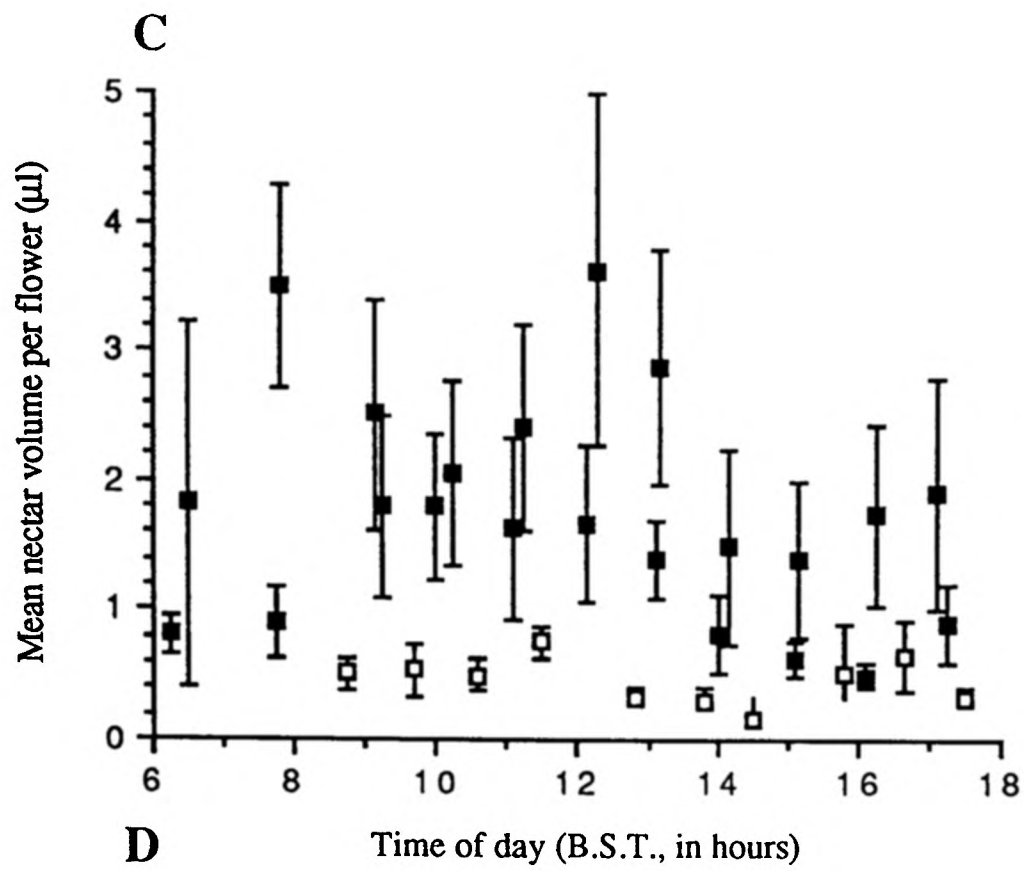
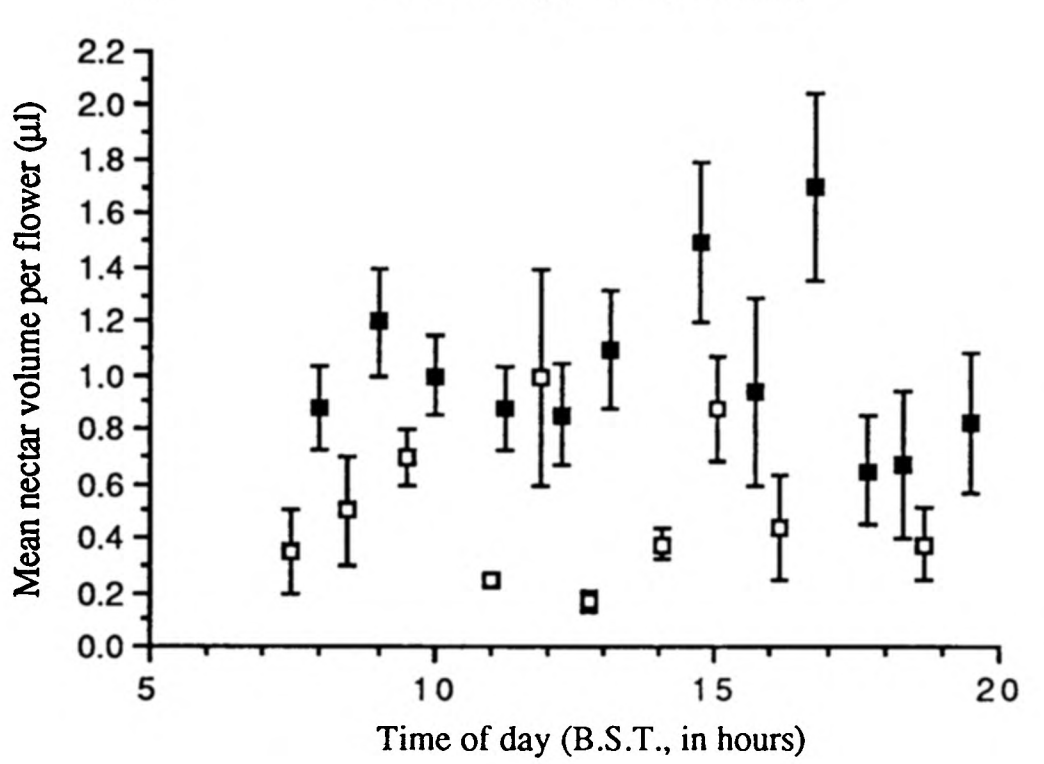


Fig.3.15 (a) Mean nectar concentration over time for sun (❒) and shade (■) sites on 12.4.87. **(b)** Mean nectar concentration over time for sun (❒) and shade (■) sites on 16.4.87. Sample sizes for each mean are 20 flowers.





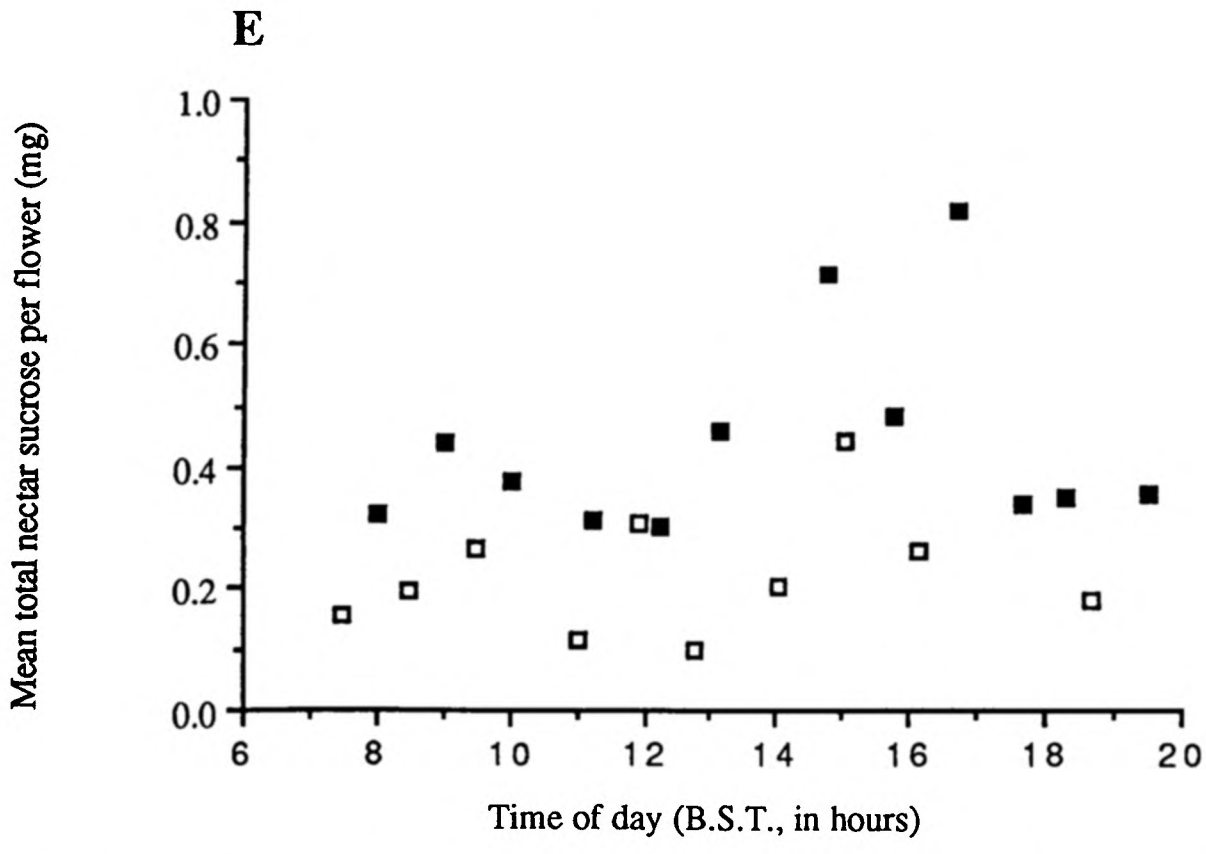
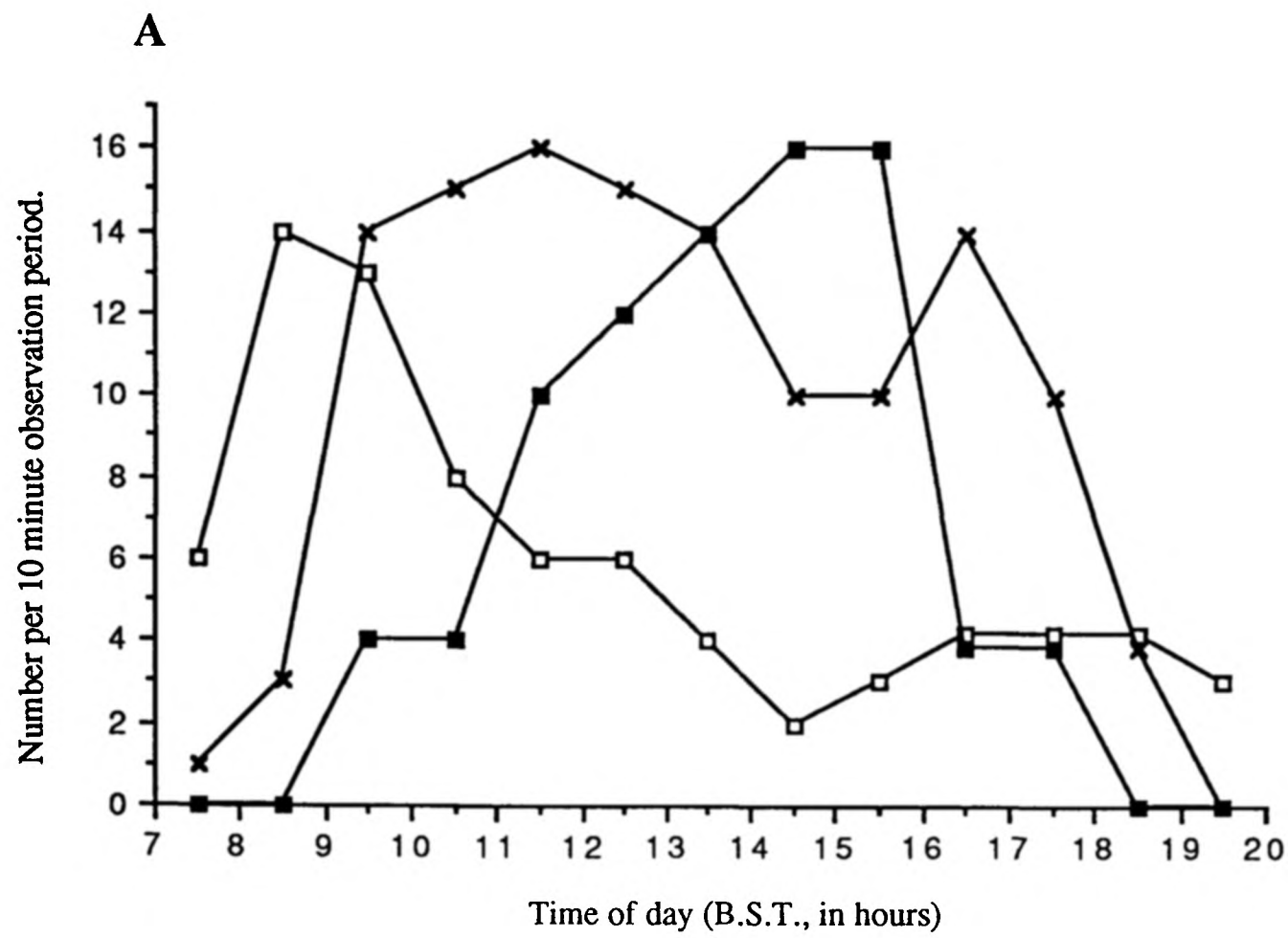


Fig.3.15 (cont.) (c) Mean nectar volume over time for sun (❒) and shade (■) sites on 12.4.87. **(d)** Mean nectar volume over time for sun (❒) and shade (■) sites on 16.4.87. Sample sizes for each mean are 20 flowers. **(e)** Mean total sugar per flower over time for sun (❒) and shade (■) sites on 16.4.87. Sample sizes for each mean are 20 flowers.



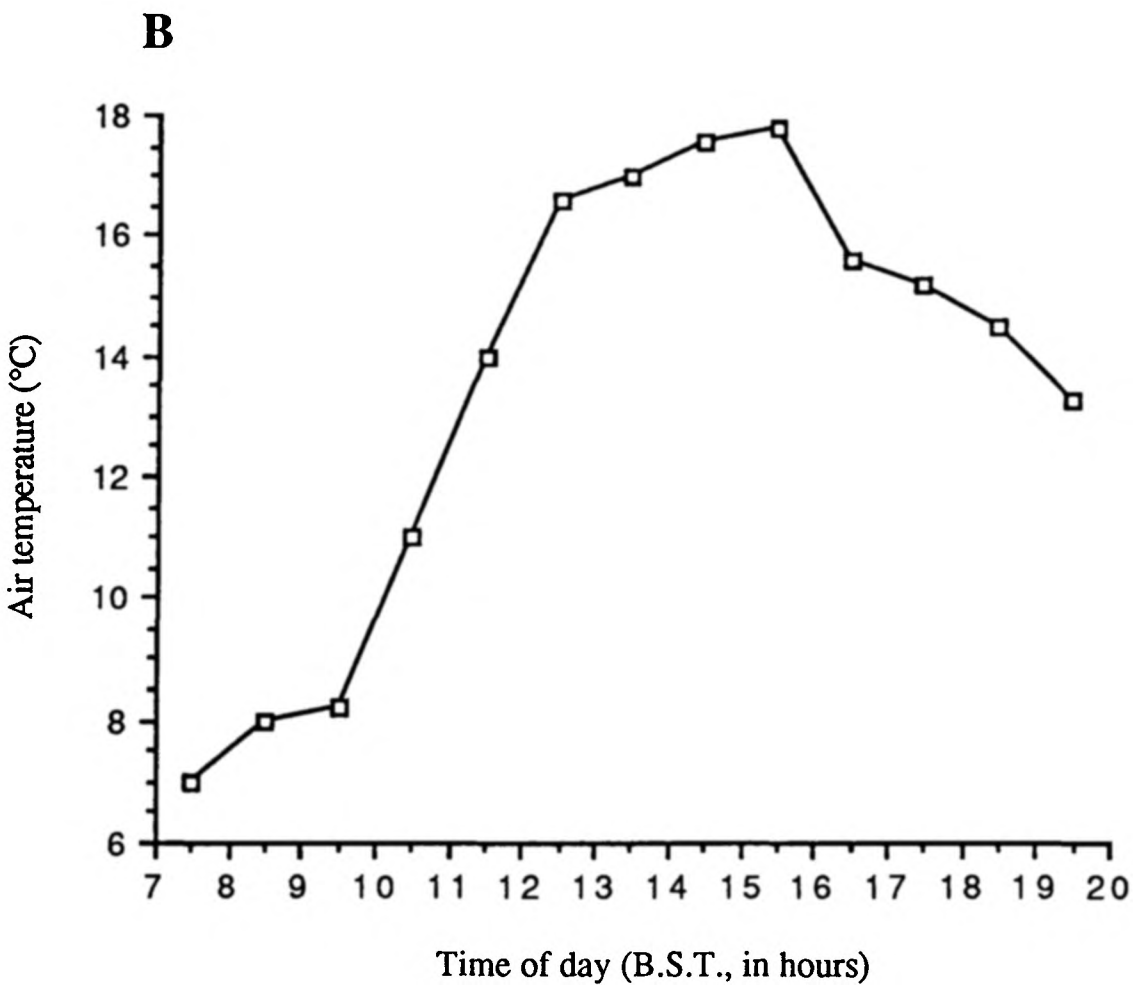
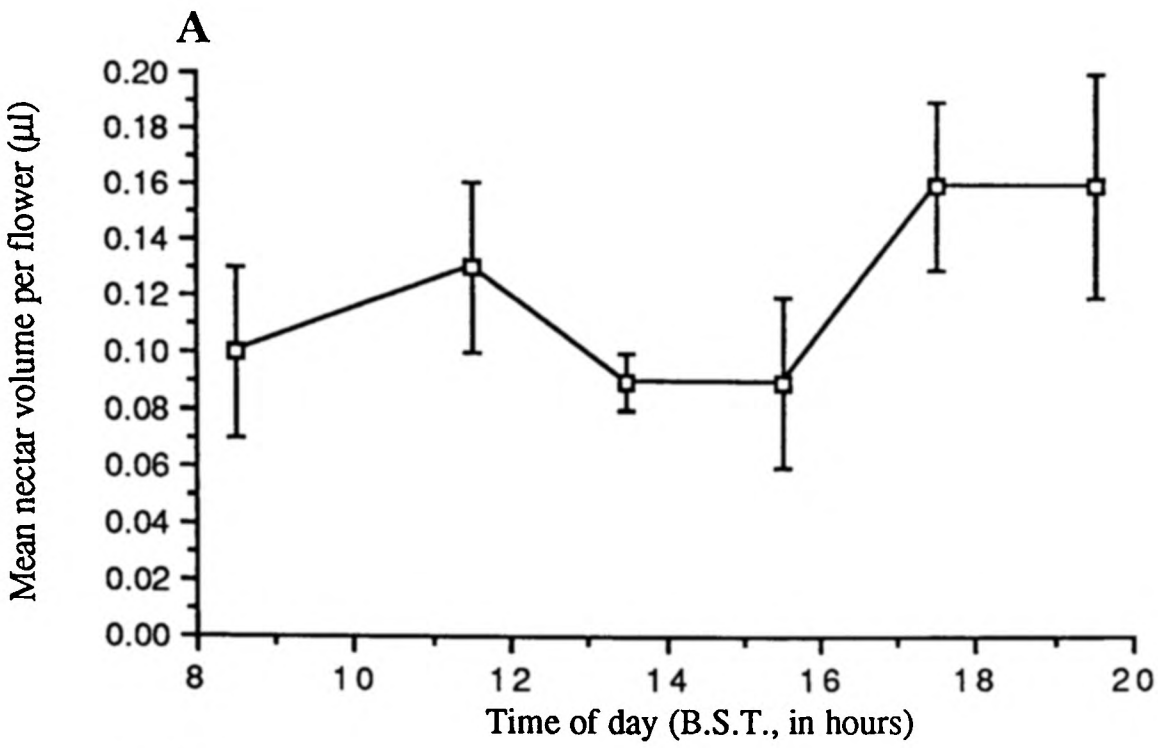
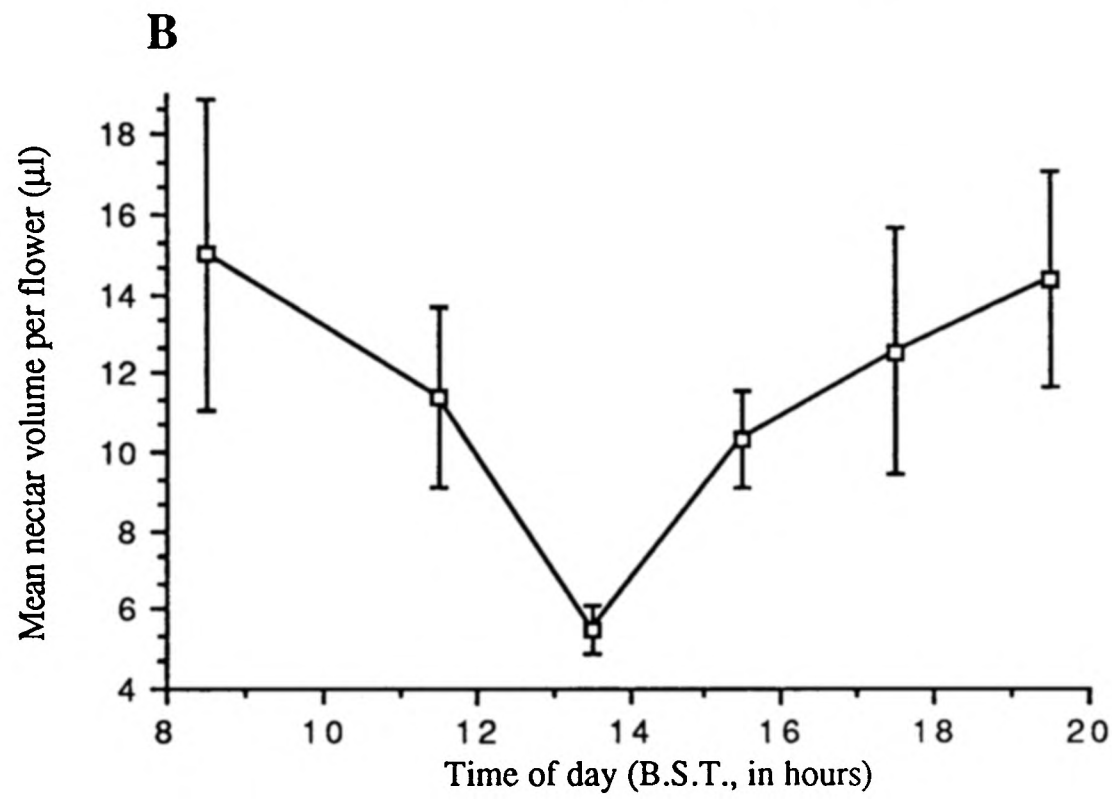


Fig.3.16 (a) Flight activity of foraging females (❐), foraging males (X) and patrolling males (■) over time at comfrey in the University College gardens on 11.5.87. **(b)** Air temperature over time at the same site on 11.5.87.





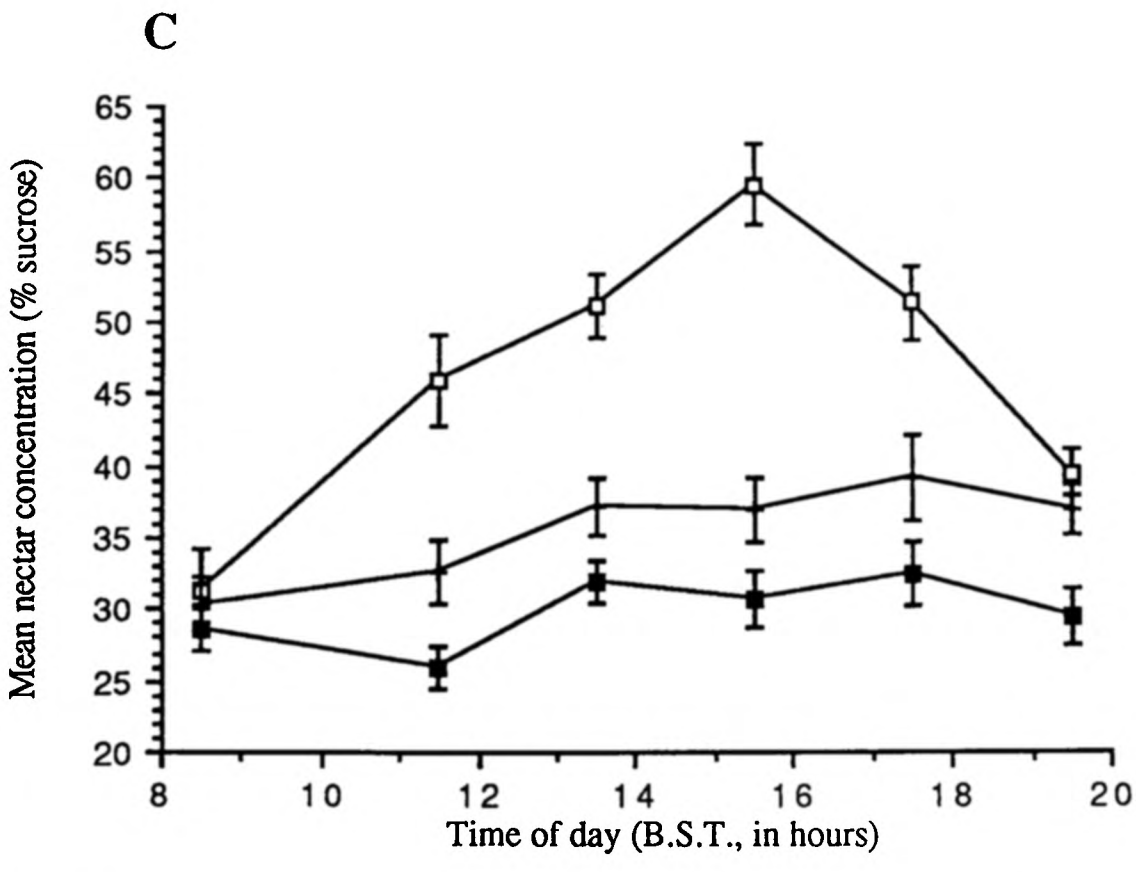


Fig.3.17 (a) Mean nectar volume per flower over time for comfrey, and **(b)** Mean nectar volume per flower over time for horse chestnut 11.5.87. **(c)** Mean nectar concentration over time for comfrey (❐), horse chestnut (■) and ♀ *A. plumipes* returning to the nest site (+) on 11.5.87. All means are for a sample size of 20.

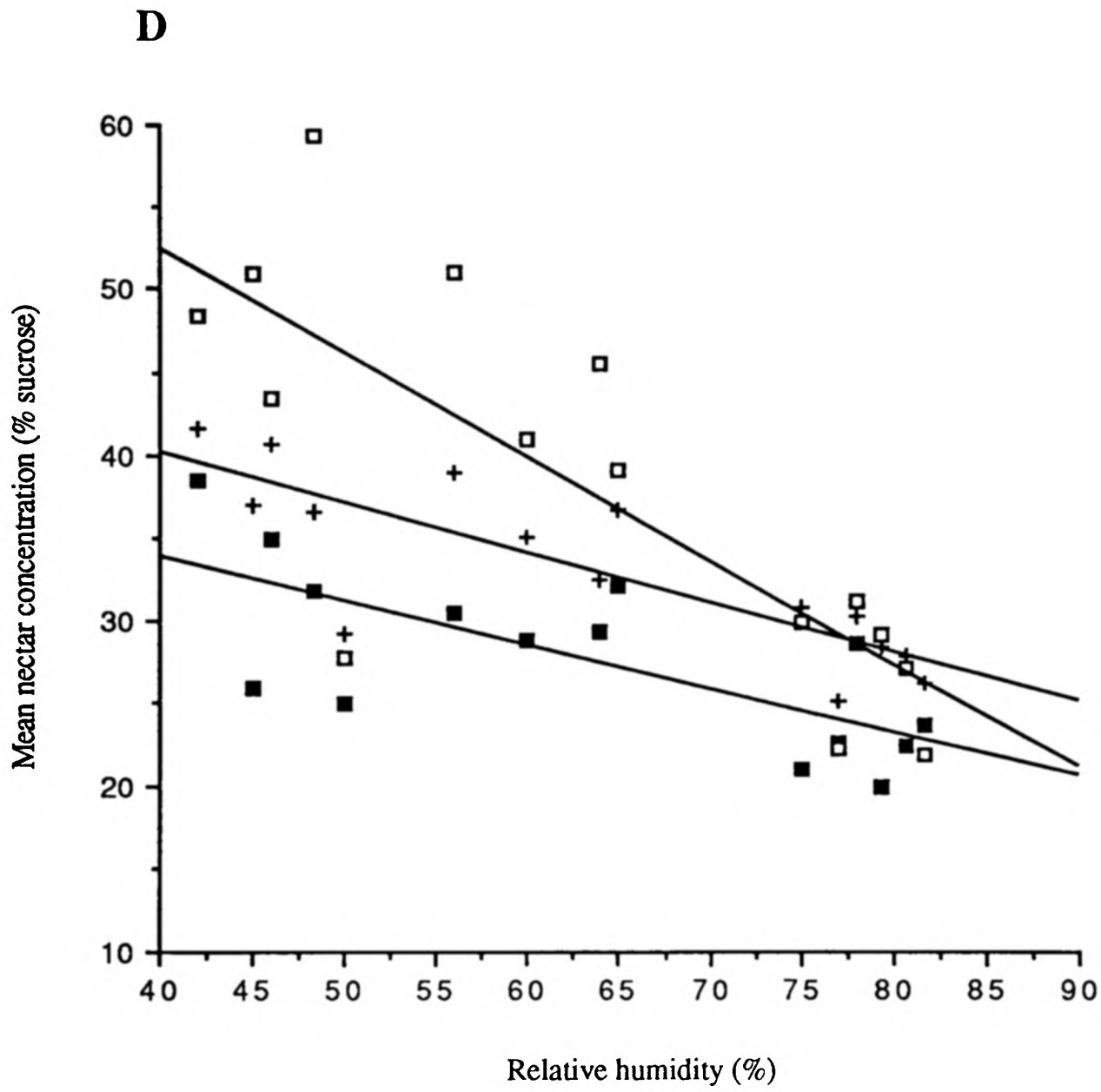


Fig.3.17 (cont.) (d) Mean nectar concentration for comfrey (❐), horse chestnut (■) and ♀ *A. plumipes* returning to the nest site (+) on 11.5.87 as functions of relative humidity (comfrey: $y=77.4-0.62x$, $R^2=0.608$; horse chestnut: $y=44.4-0.26x$, $R^2=0.504$; females: $y=51.93-0.30x$, $R^2=0.672$).

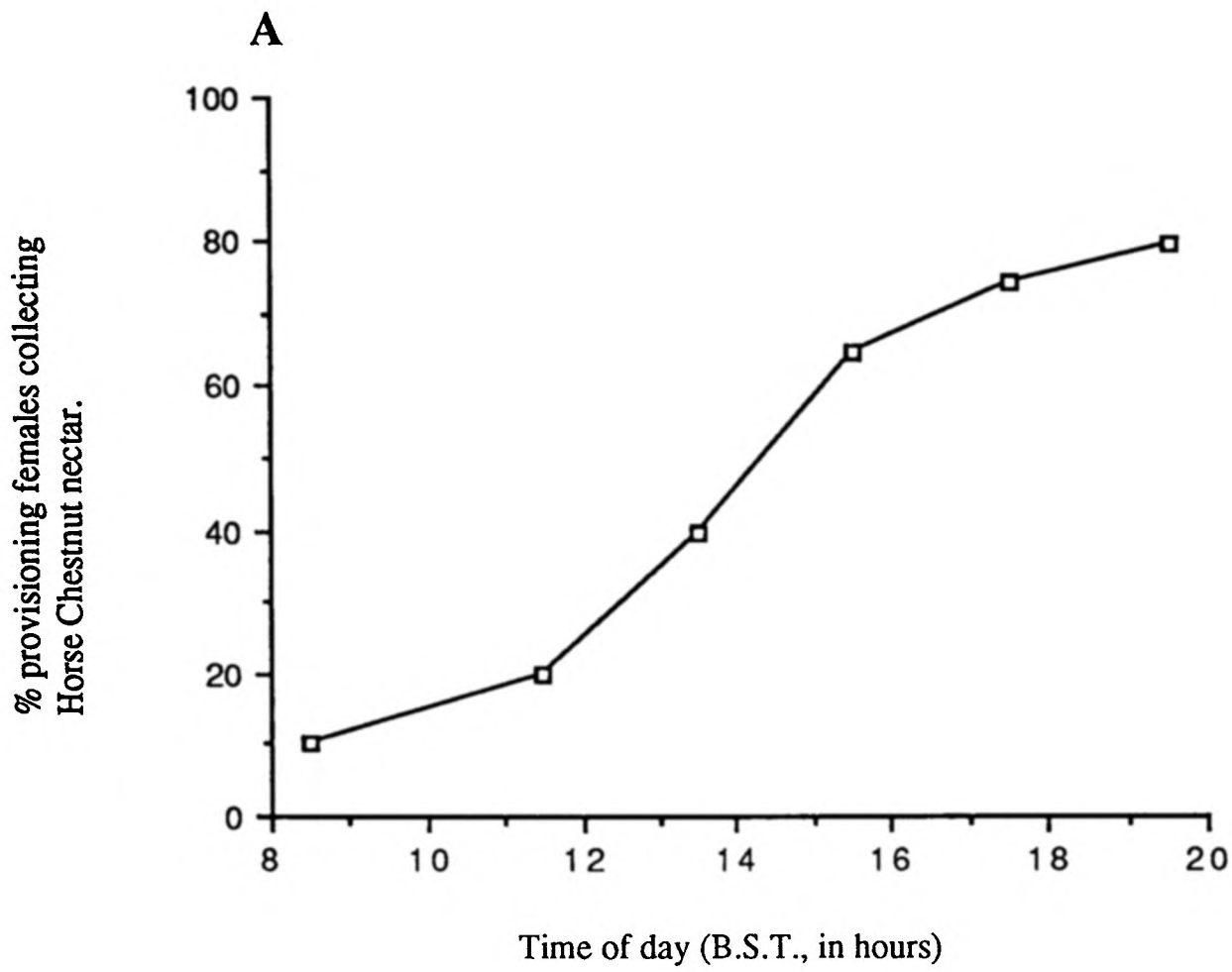


Fig.3.18 (a) The % of ♀ *A. plumipes* returning to the nest site with loads of horse chestnut nectar over time on 11.5.87. **(b)** Numbers of ♀ *A. plumipes* active over time in a 3m x 3m area on the exterior of a flowering horse chestnut tree on 11.5.87.

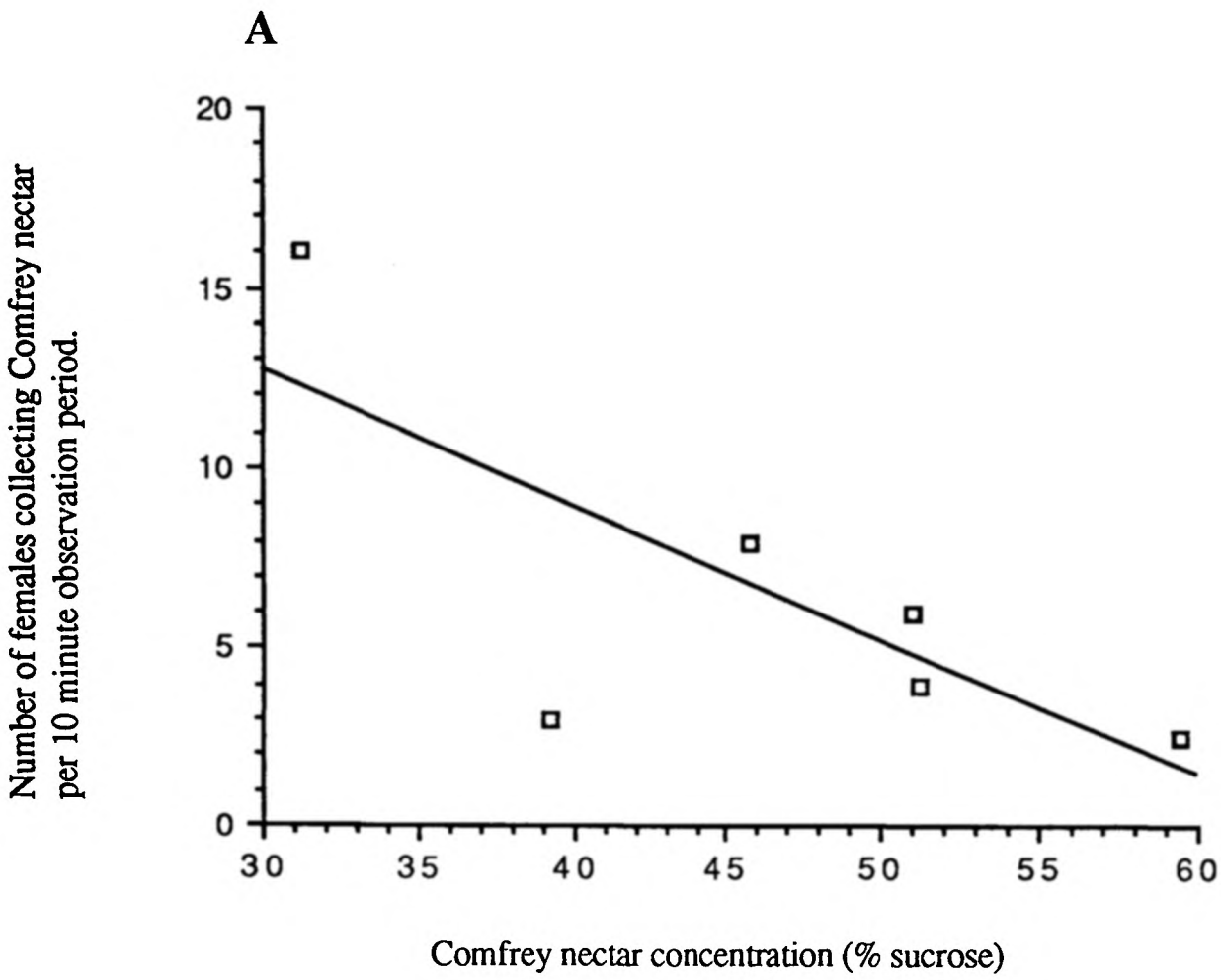


Fig.3.19 **(a)** The number of ♀ *A. plumipes* collecting comfrey nectar as a function of mean comfrey nectar concentration on 11.5.87 ($y=24.1-0.38x$, $R^2=0.548$). **(b)** The % of ♀ *A. plumipes* returning to the nest site with horse chestnut nectar as a function of horse chestnut nectar concentration over 7 days in May, 1987 ($y=-39.6+3.0x$, $R^2=0.449$).

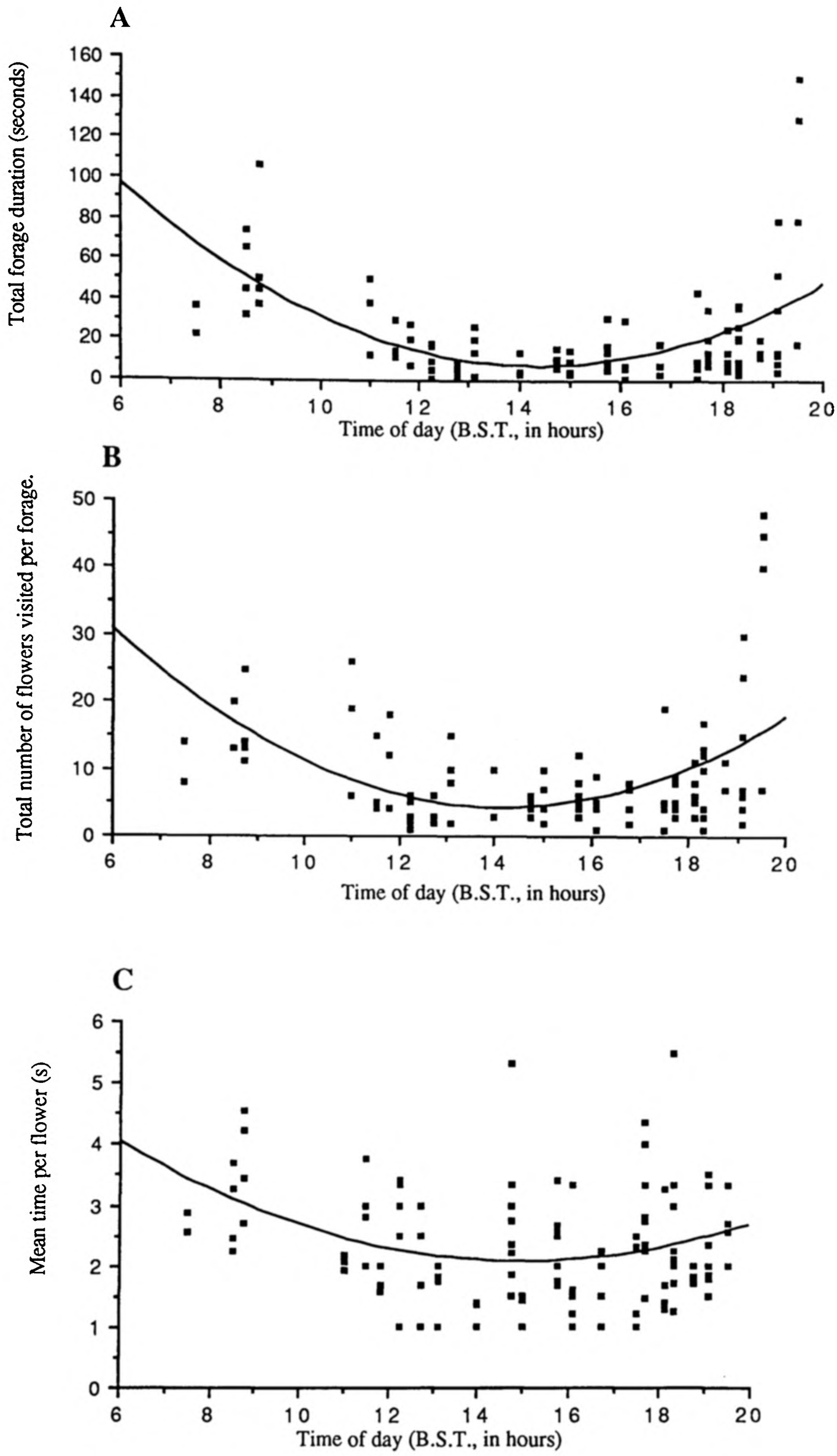


Fig.3.20 **(a)** Total forage duration, **(b)** The total number of flowers sampled per forage at the flower patch and **(c)** mean time per flower as functions of time of day for ♂ *A. plumipes* foraging at sun and shade lungwort sites in the Botanical Gardens on 16.4.87 (Total forage duration: $y=271-36.7x+1.3x^2$, $R^2=0.314$; total flowers: $y=84.5-11.33x+0.4x^2$, $R^2=0.250$; mean time per flower: $y=7.6-0.74x+0.025x^2$, $R^2=0.102$).

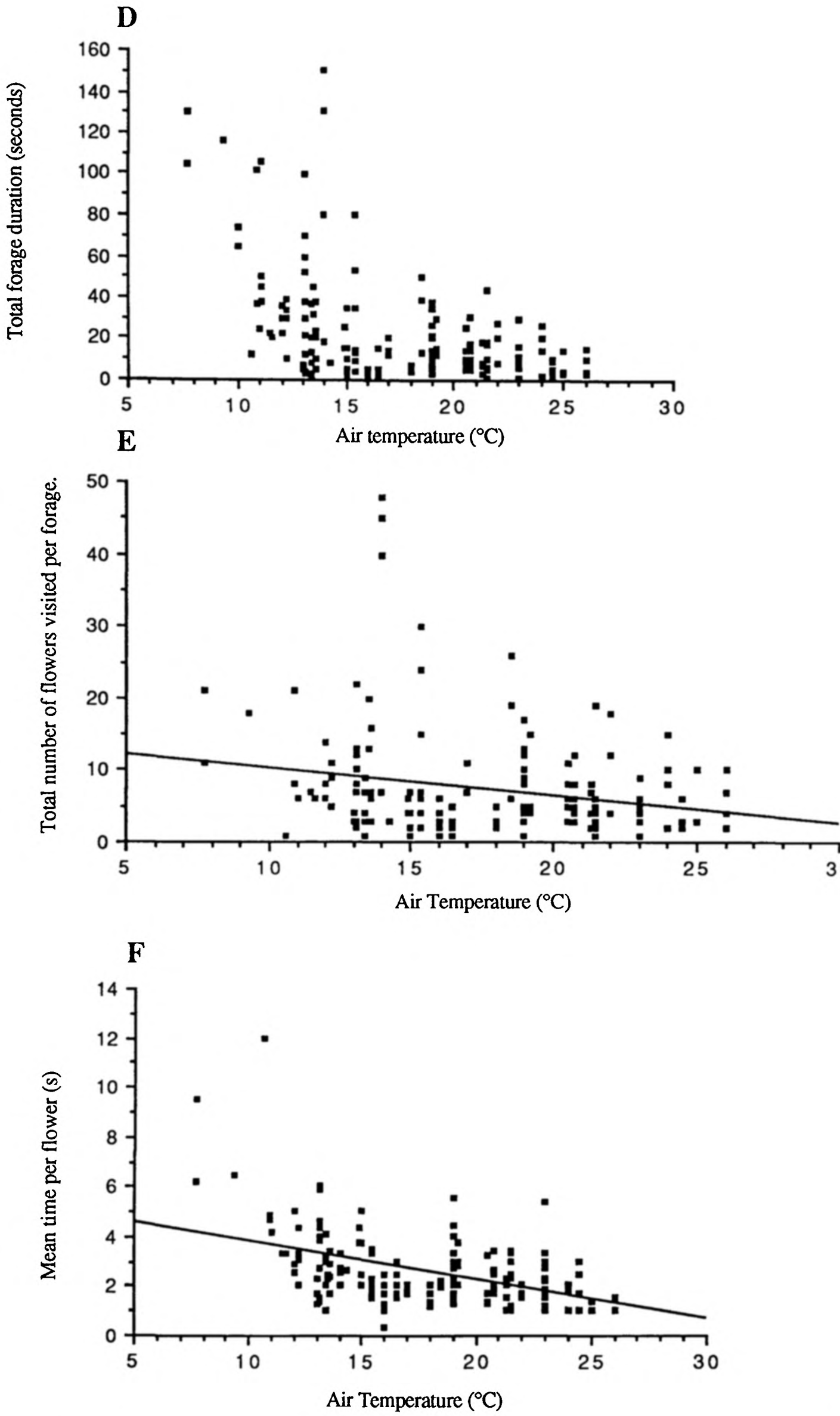


Fig.3.20 (cont.) (d) Total forage duration, **(e)** total number of flowers sampled per forage at the flower patch and **(f)** mean time per flower as functions of ambient temperature for ♂ and ♀ *A. plumipes* foraging at sun and shade lungwort sites in the Botanical Gardens on 16.4.87 (total flowers: $y=13.95-0.38x$, $R^2=0.050$; mean time per flower: $y=5.33-0.16x$, $R^2=0.202$).

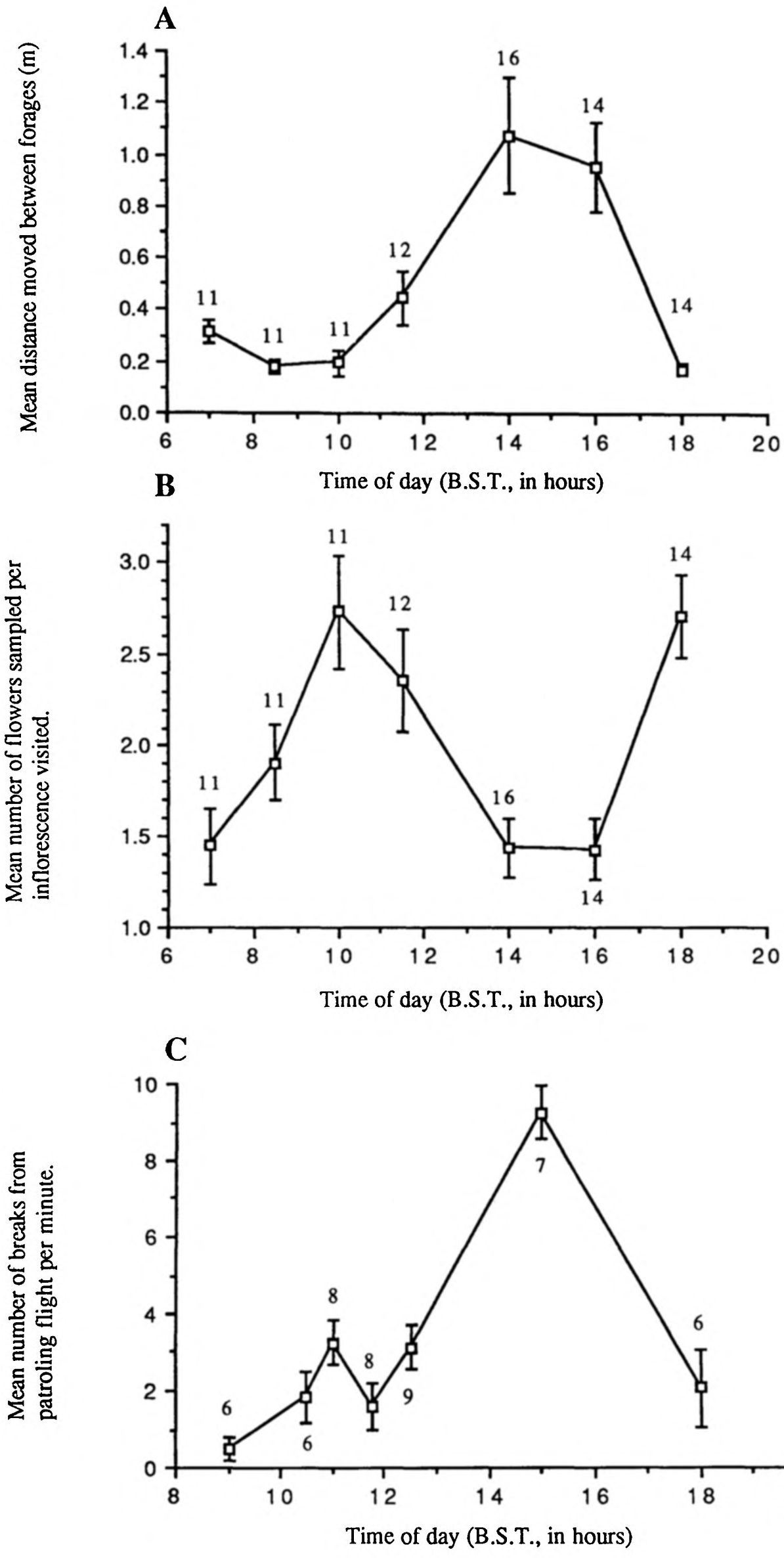


Fig.3.21 **(a)** The mean distance moved between inflorescences visited, **(b)** the mean number of flowers per inflorescence visited, and **(c)** the mean number of breaks from foraging flight in pursuit of other insects over time by ♂ *A. plumipes* on 24.5.87.

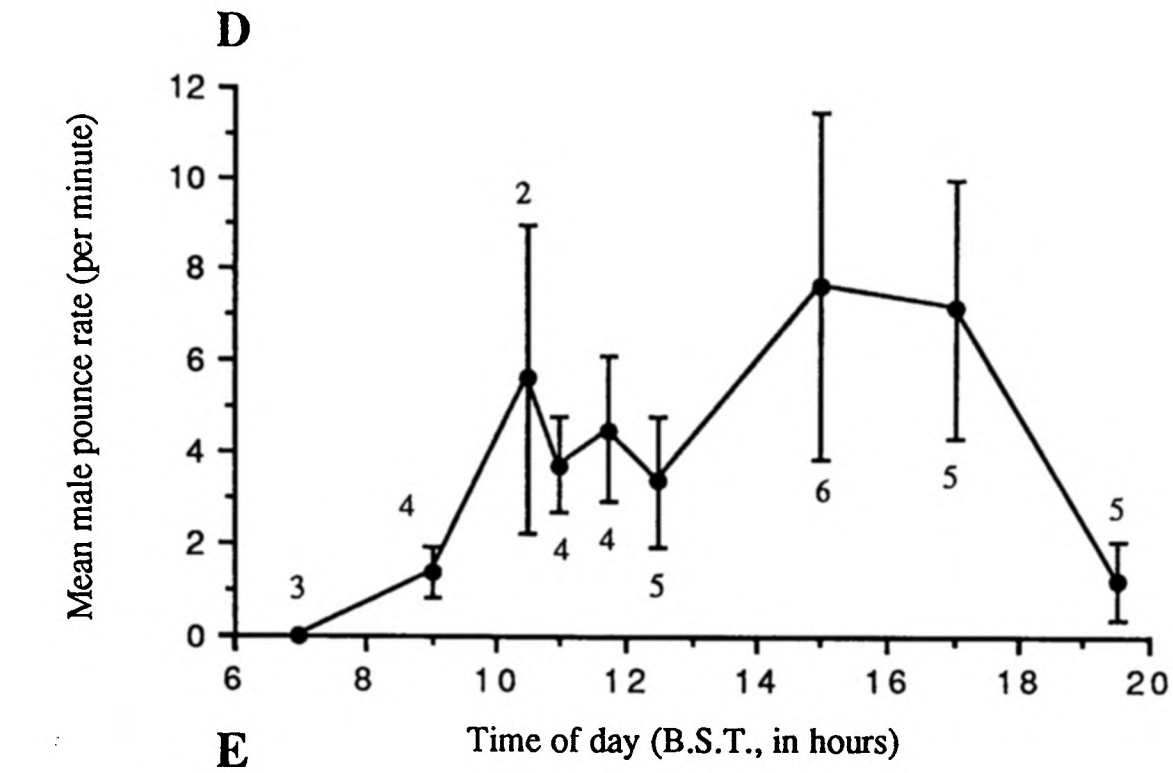


Fig.3.21 (cont.) (d) The mean ♂ *A. plumipes* pounce rate on females as a function of time on 24.5.87. **(e)** The ♂ pounce rate experienced by ♀ *A. plumipes* on 24.5.87 as a function of the proportion of the total flowers visited by the ♀ which are on the exterior of the comfrey plant ($y=-2.44+13.7x$, $R^2=0.504$). **(f)** The change in the proportion of outer flowers visited by males and females foraging at comfrey over time on 24.5.87.

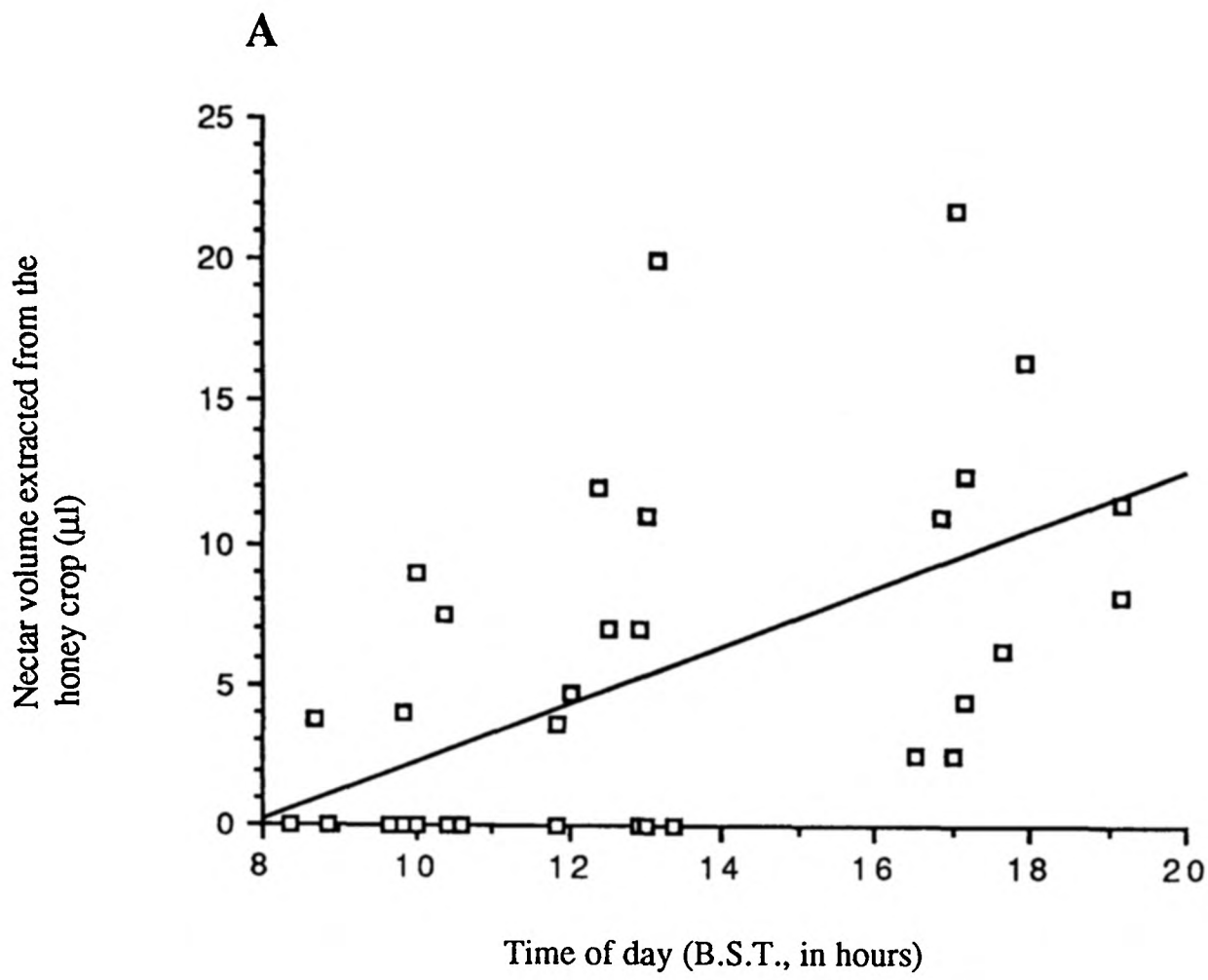


Fig.3.22 (a) The nectar volume obtained by gentle squeezing of the honey crop of ♂ *A. plumipes* as a function of time on 21.5.87 ($y=-8.35+1.05x$, $R^2=0.336$). **(b)** The concentration of nectar in comfrey flowers (❐) and in the crop of male *A. plumipes* (■) as a function of time on 21.5.87. The sample size for each mean is 20.

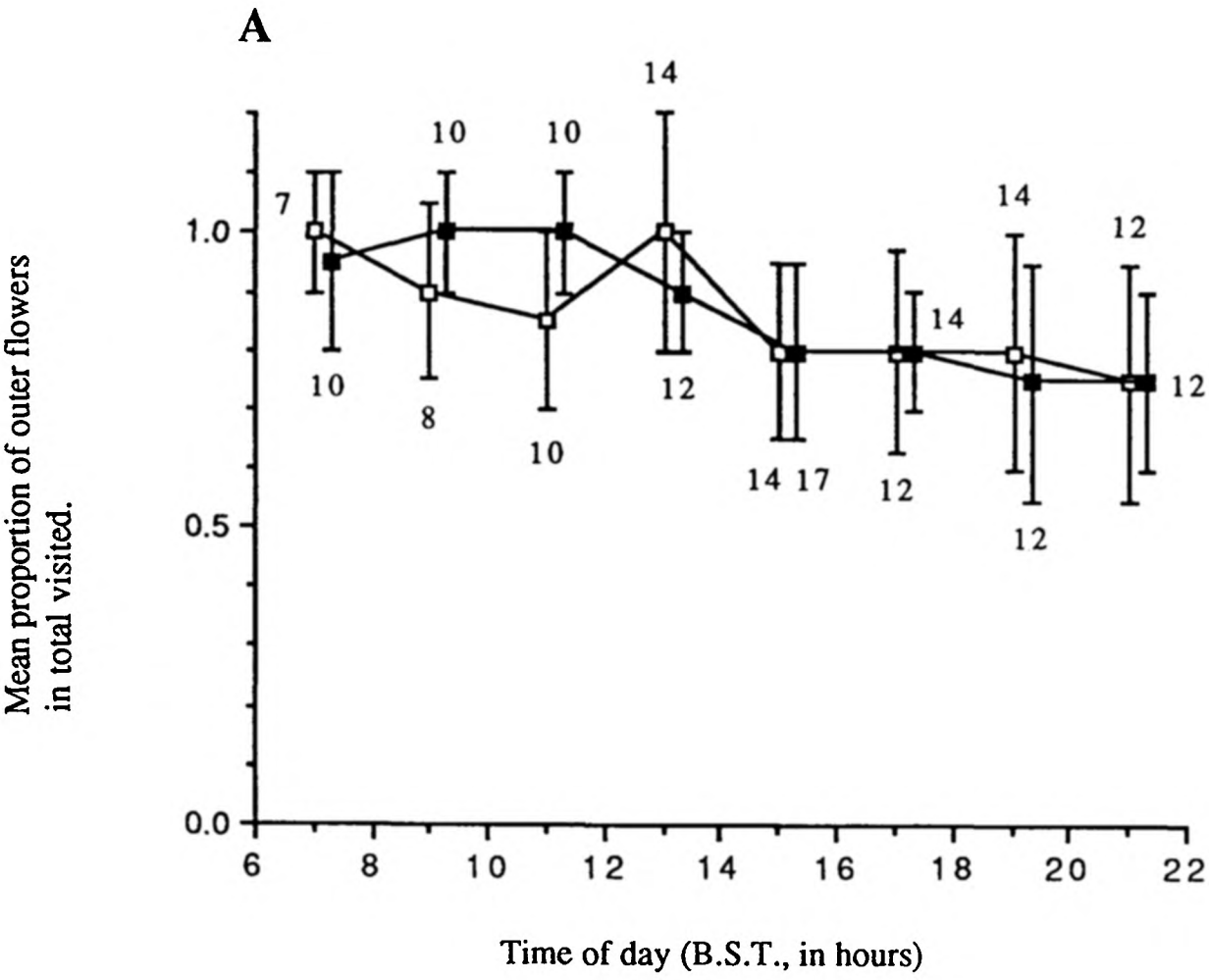


Fig.3.23 (a) The proportion of outer comfrey flowers visited by ♀ *A. plumipes* over time on 26.5.87 (❐) and 27.5.87 (■), in the absence of ♂ interference. **(b)** The proportion of outer flowers visited by ♀ (❐) and ♂ (■) *A. plumipes* over time on 29.5.87.

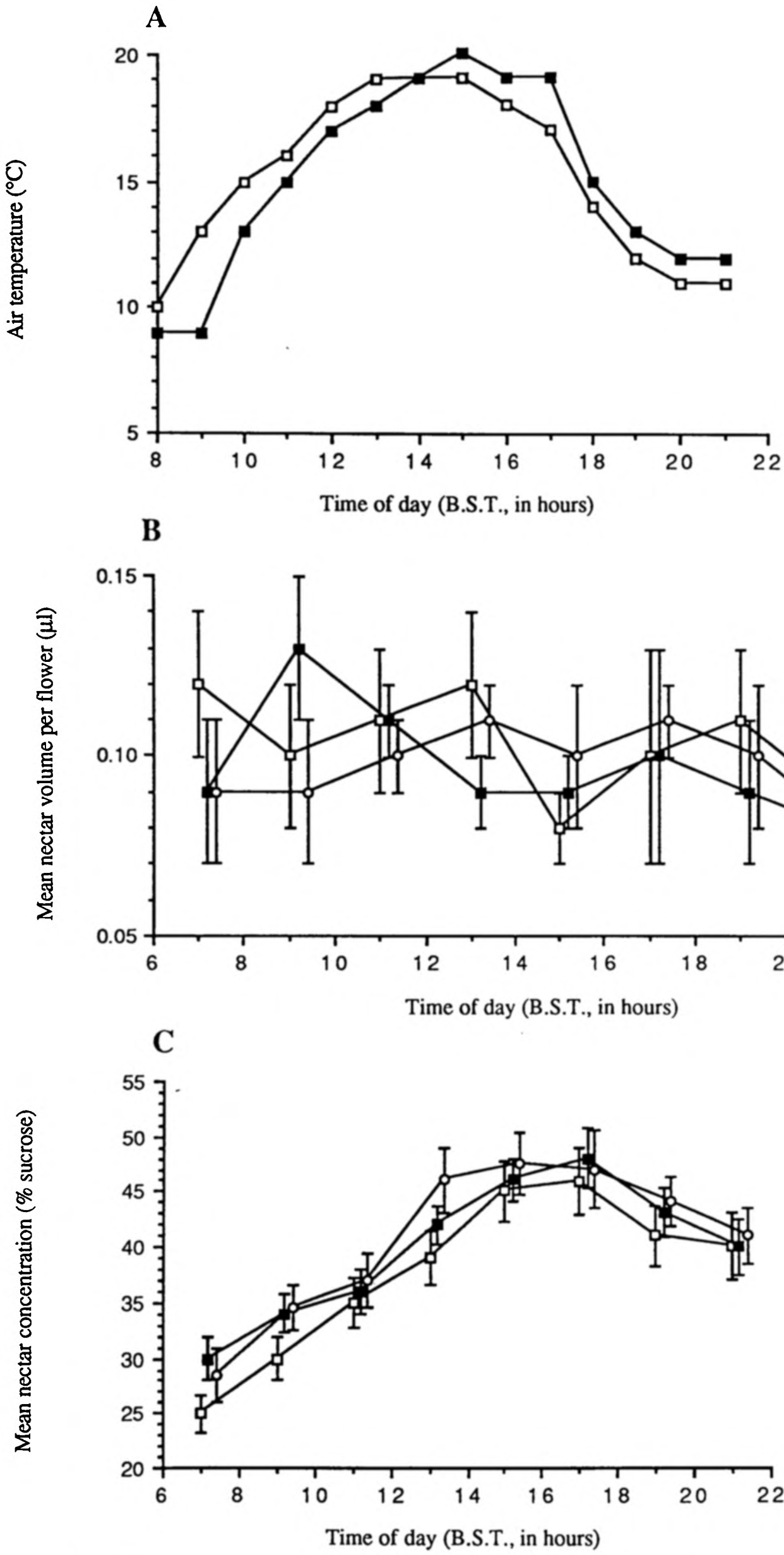


Fig.3.24 (a) Ambient temperature measured 15cm above the ground over time for the comfrey foraging site on 27.5.87 (❐) and 29.5.87 (■). **(b)** Mean nectar volume per flower as a function of time for outer comfrey flowers on 24.5. (❐), 25.5. (■) and 29.5.87 (O). **(c)** Mean nectar concentration as a function of time for outer comfrey flowers on 24.5. (❐), 25.5. (■) and 29.5.87 (O). The sample size for each mean is 20 flowers.

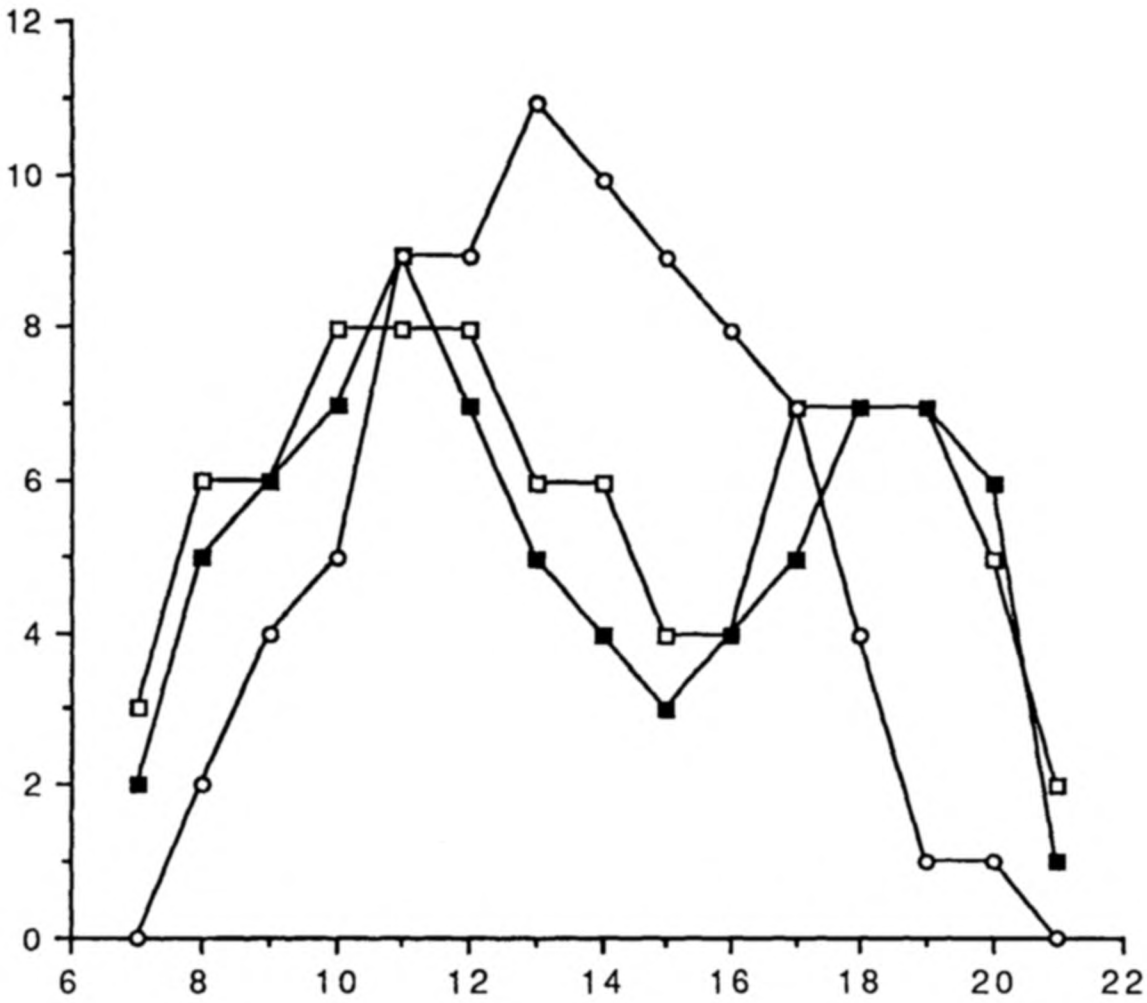


Fig.3.25 Flight activity of ♂ (O) and ♀ (■) *A. plumipes* over time at comfrey on 29.5.87, and of females (❐) on 27.5.87.

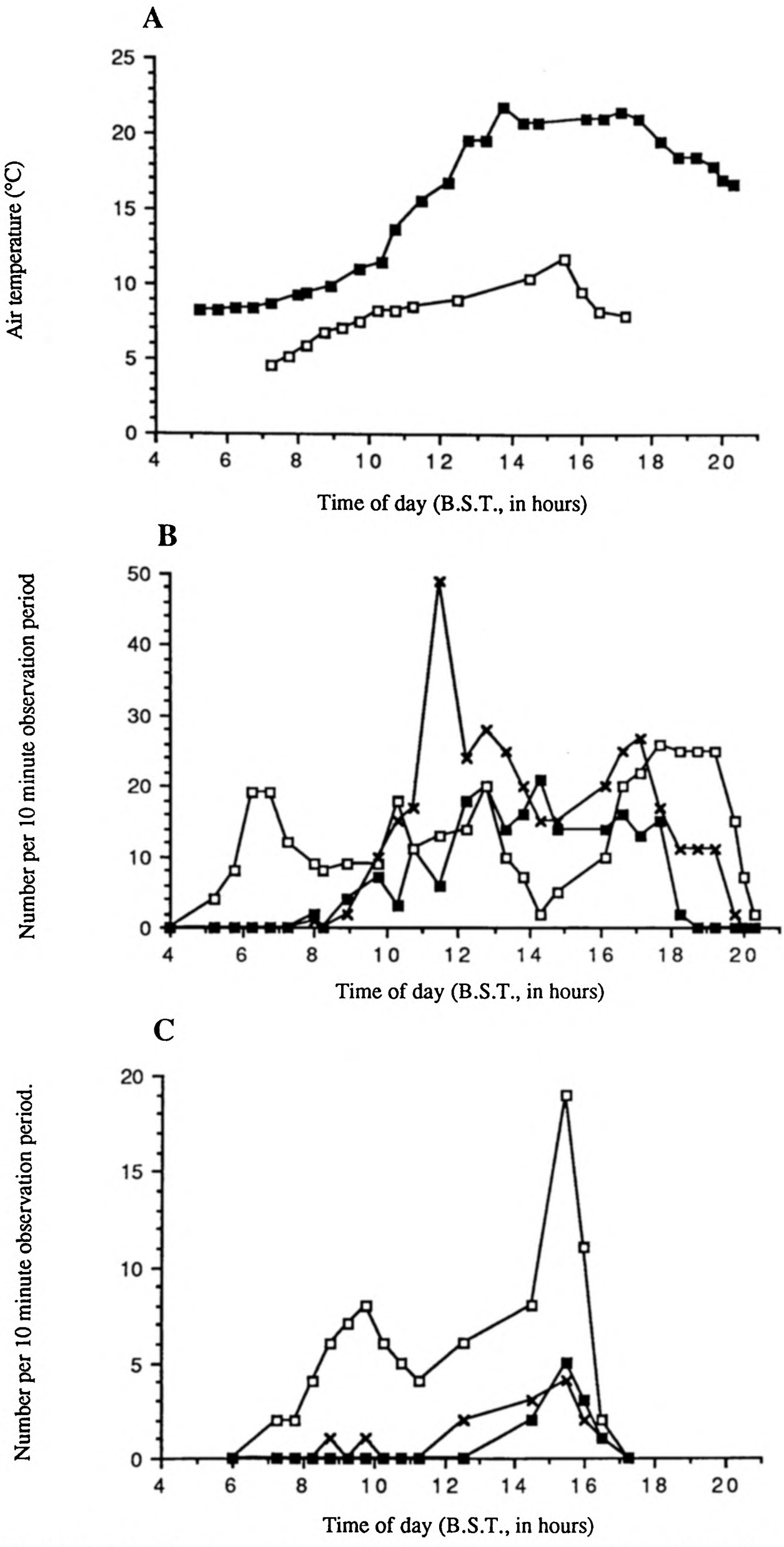
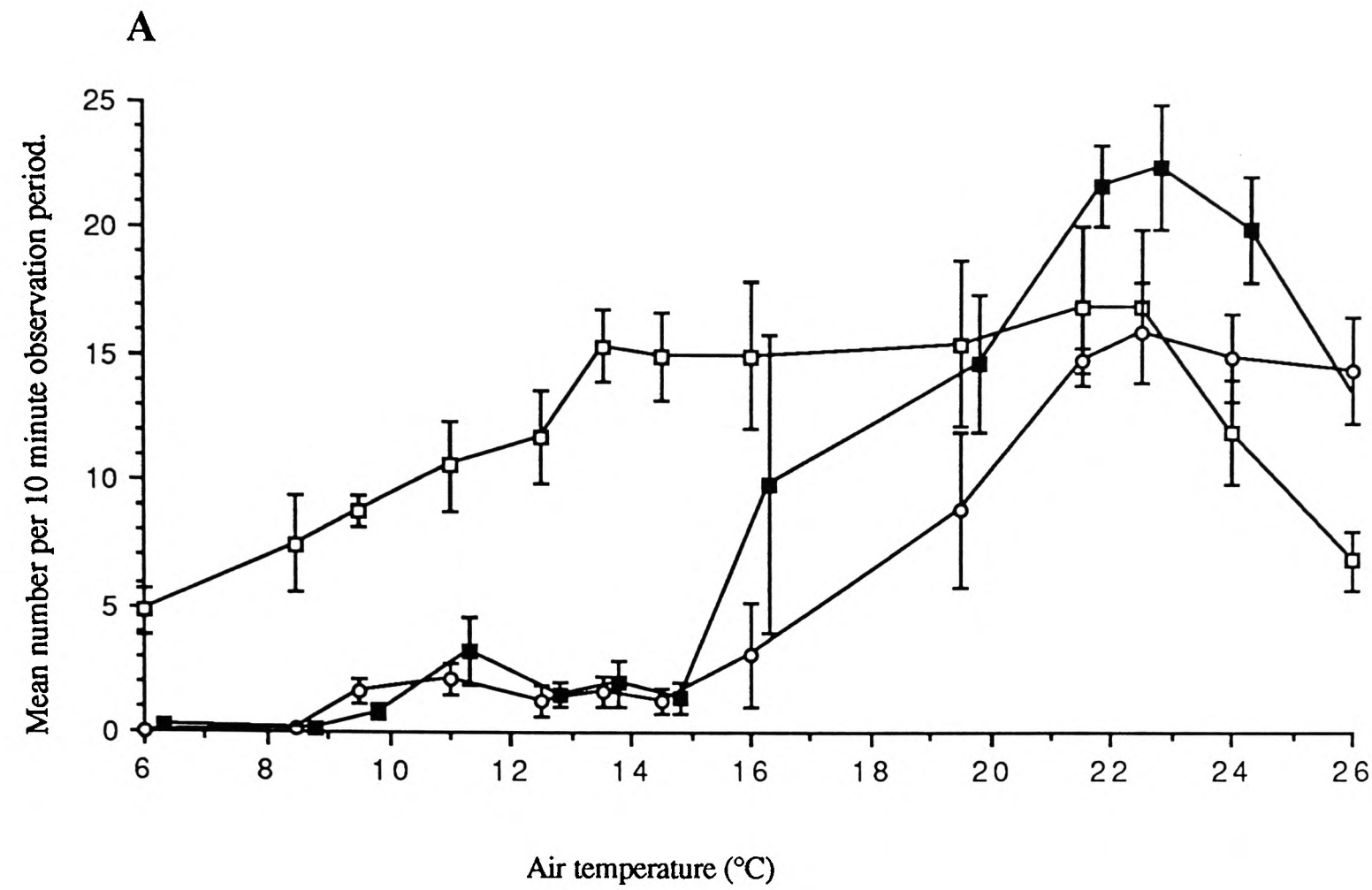


Fig.3.26 **(a)** Air temperature over time at the nest site on two days of contrasting climate: 27.4.87 (■) and 3.5.87 (❐). **(b)** Numbers of males (**x**), provisioning females (❐) and searching females (■) seen over time at the nest site on 27.4.87. **(c)** Numbers of males (**x**), provisioning females (❐) and searching females (■) seen over time at the nest site on 3.5.87.



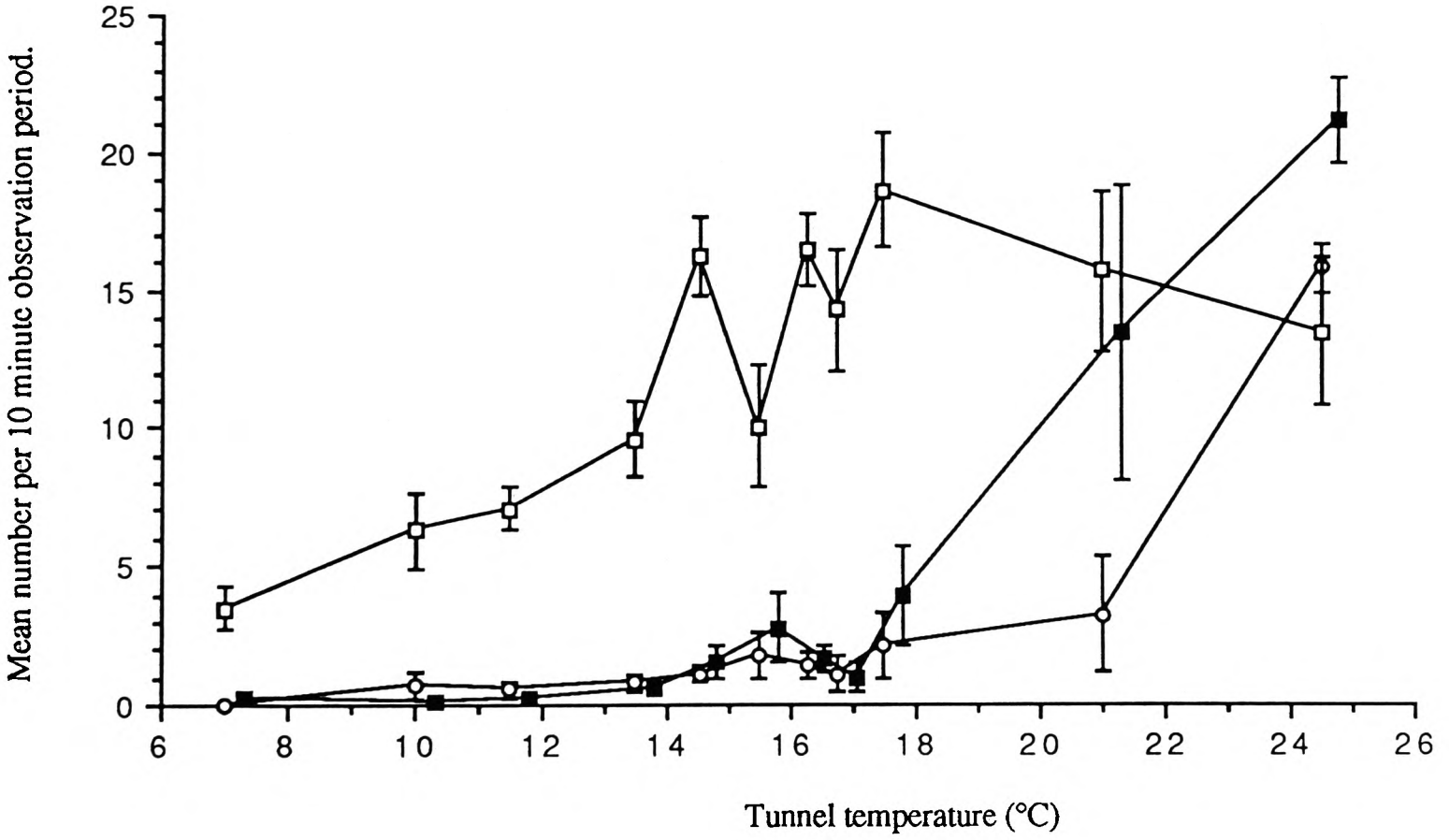
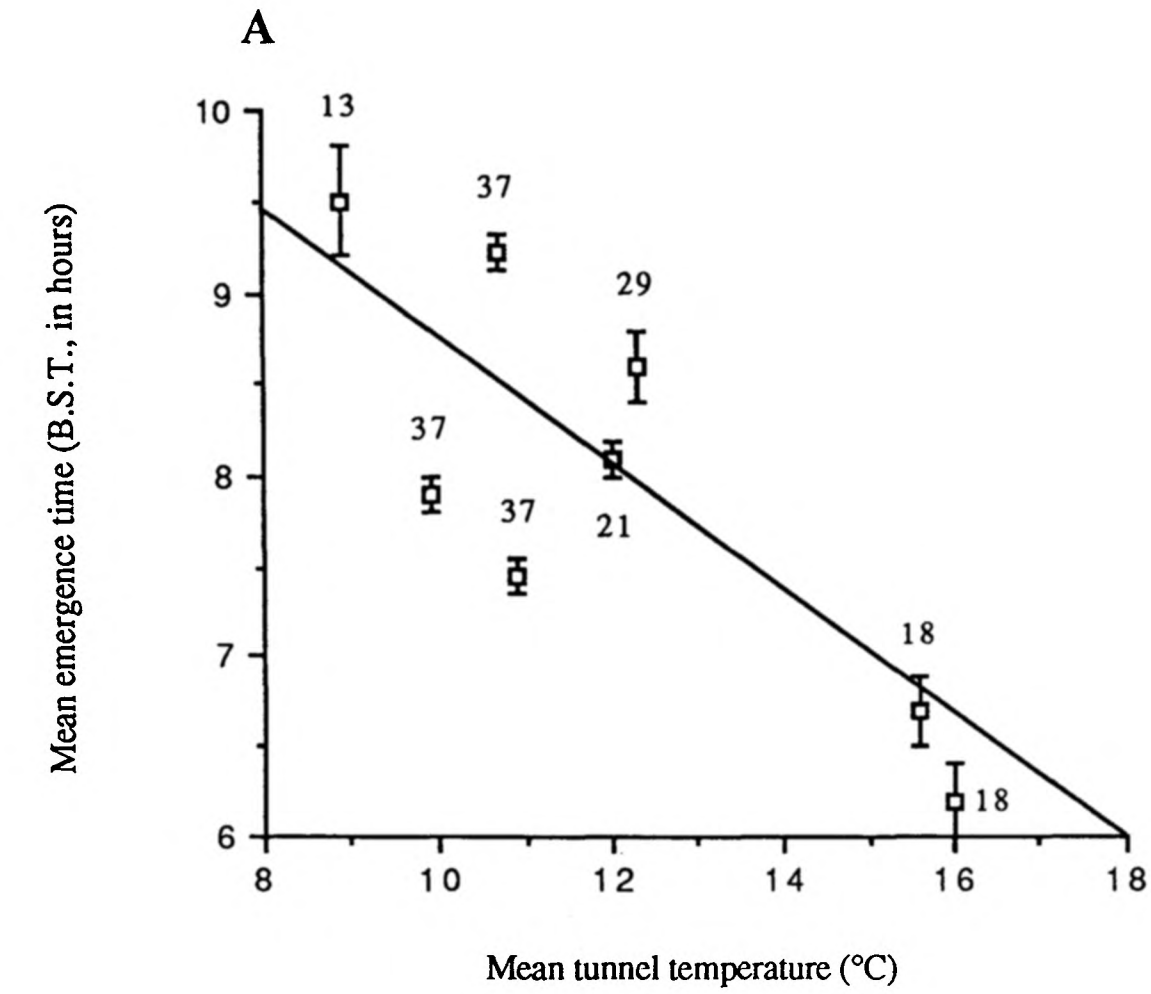


Fig.3.27 (a) The mean number of provisioning females (❐), males (■) and searching females (O) seen as a function of air temperature at the nest site over all days of observation. **(b)** The mean number of provisioning females (❐), males (■) and searching females (O) seen as a function of tunnel temperature at the nest site over all days of observation.



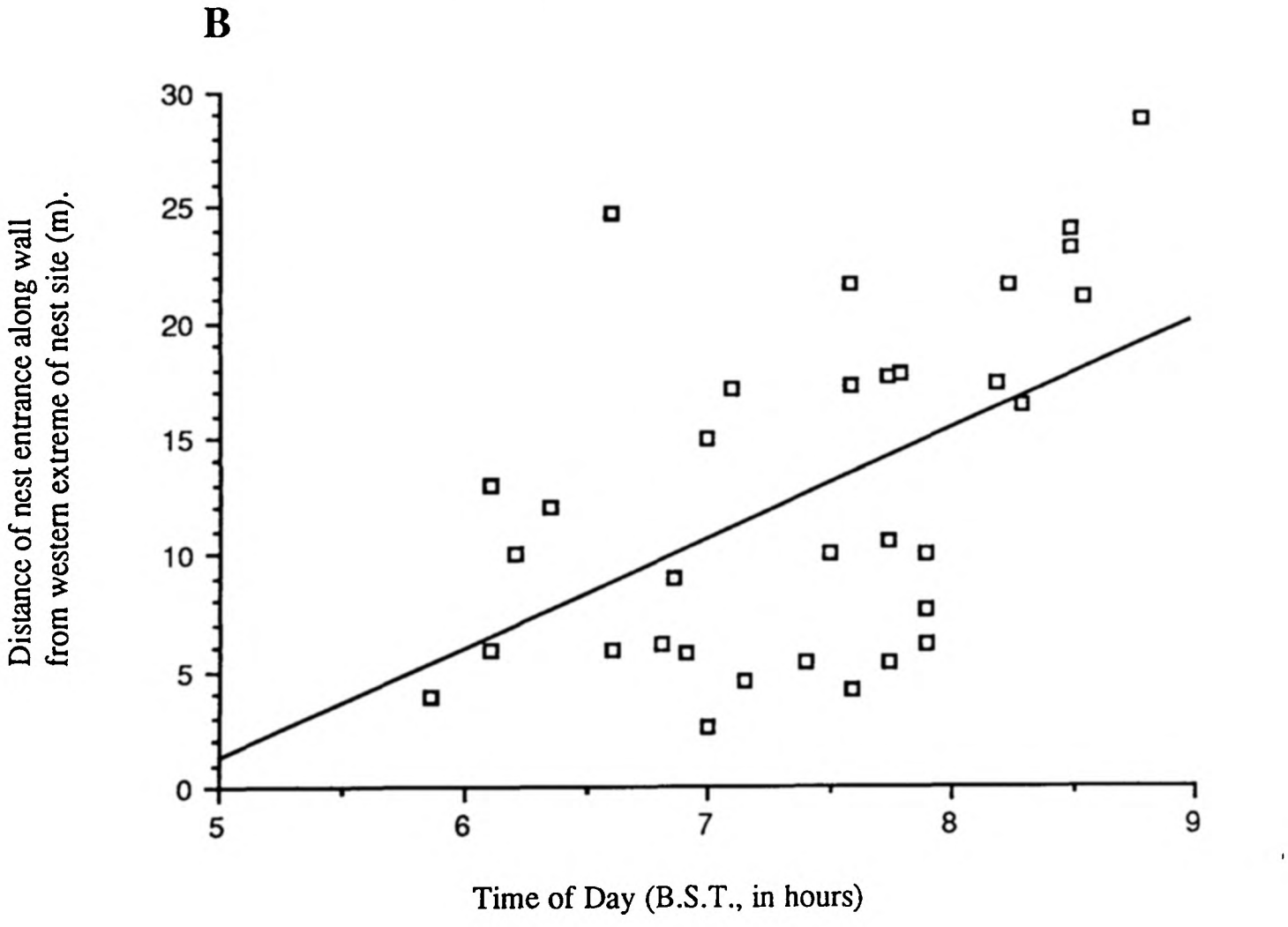
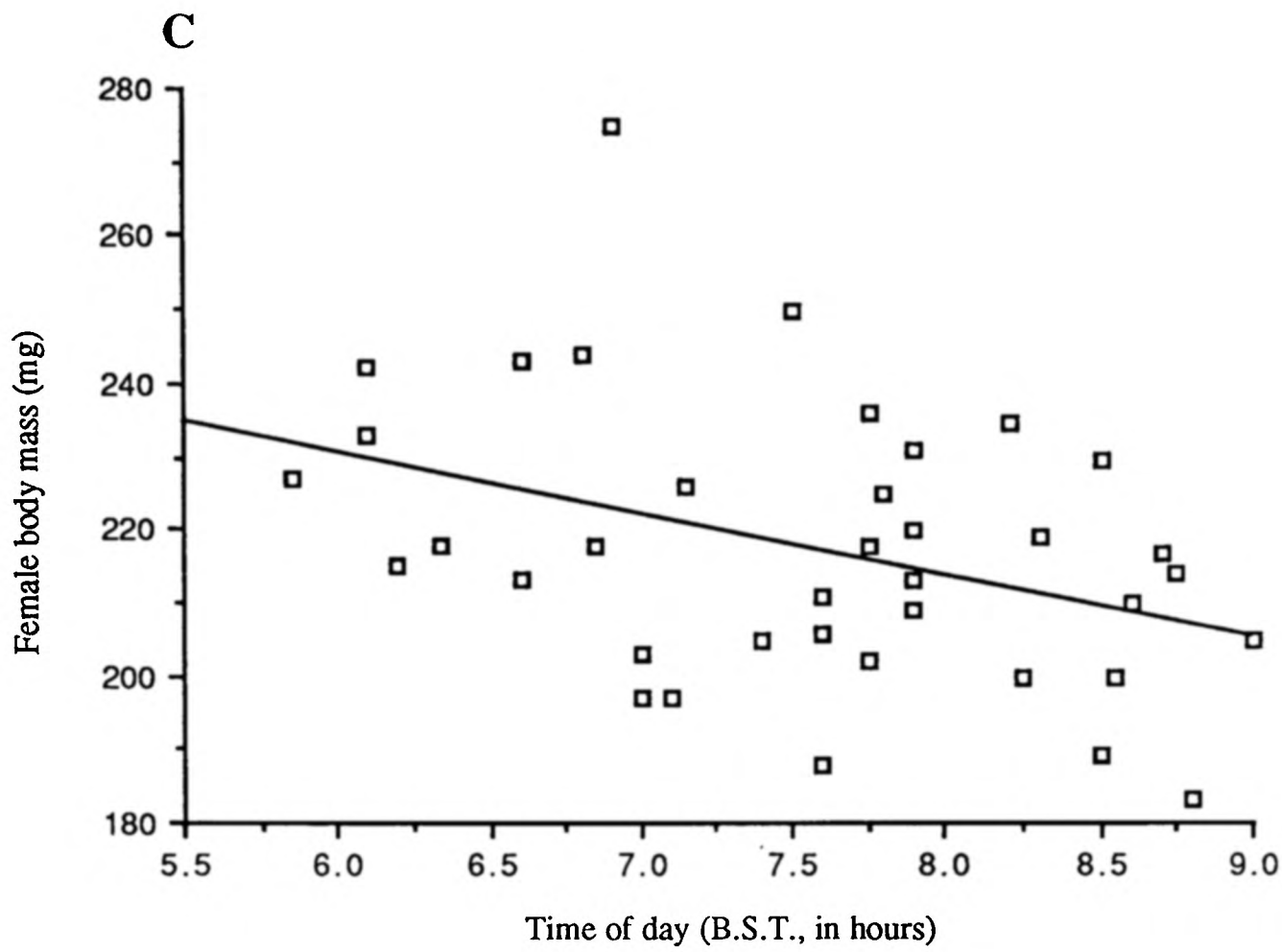


Fig.3.28 (a) The mean time of morning emergence for ♀ *A. plumipes* at the nest site as a function of mean tunnel temperature (y=12.44-0.37x, R^2 =0.689). **(b)** The relationship between morning emergence time for ♀ *A. plumipes* and position of the nest entrance on 7.5.87 (y=-22.5+4.74x, R^2 =0.260).



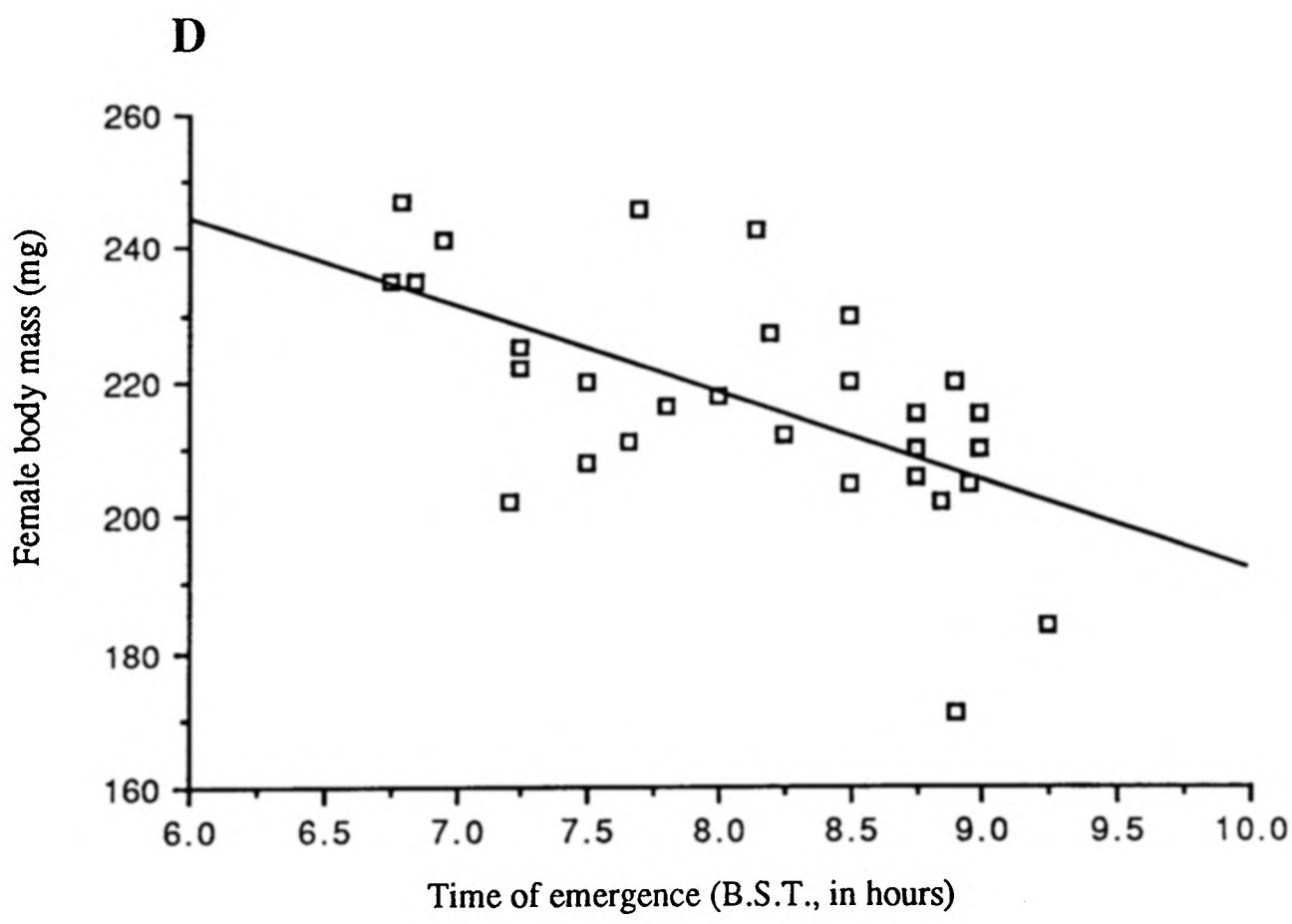


Fig.3.28 (cont.) The relationship between ♀ body mass and morning emergence time on **(c)** 7.5.87 ($y=281.2-8.43x$, $R^2=0.144$) and **(d)** 20.5.87 ($y=323.0-13.1x$, $R^2=0.360$).

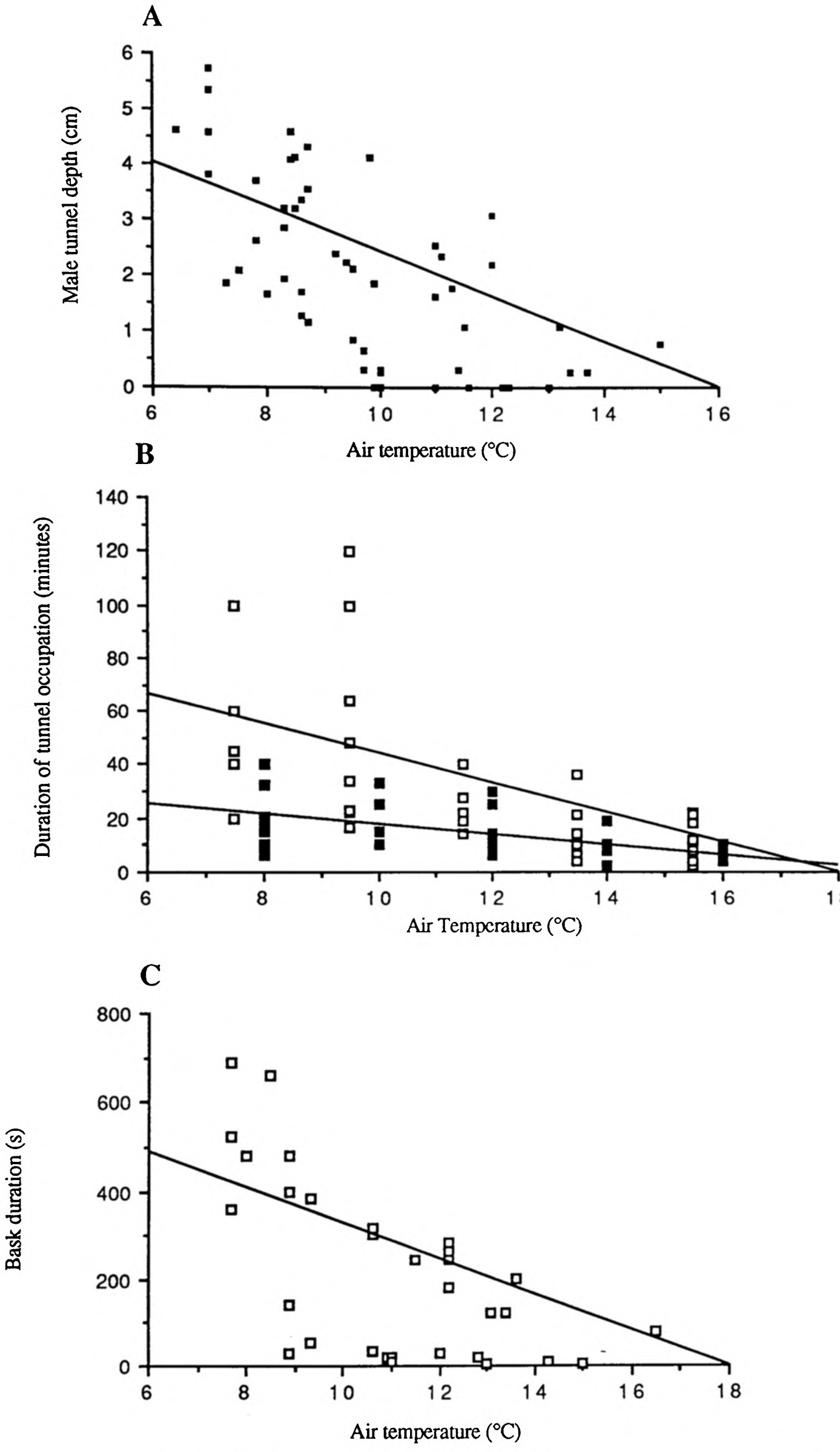


Fig.3.29 **(a)** The depth of ♂ *A. plumipes* within tunnel entrances as a function of air temperature (y=7.26-0.53x, R^2 =0.422). **(b)** The duration of rests by ♂ *A. plumipes* in tunnel entrances as a function of air temperature, divided between large (body mass 160-200mg) (■) and small (body mass<160mg) (❐) males (large males: y=36.8-1.9x, R^2 =0.325; small males: y=104.5-6.3x, R^2 =0.410). **(c)** Bask length for ♂ *A. plumipes* at lungwort forage sites in the Botanical Gardens as a function of air temperature (y=822.0-55.5x, R^2 =0.397).

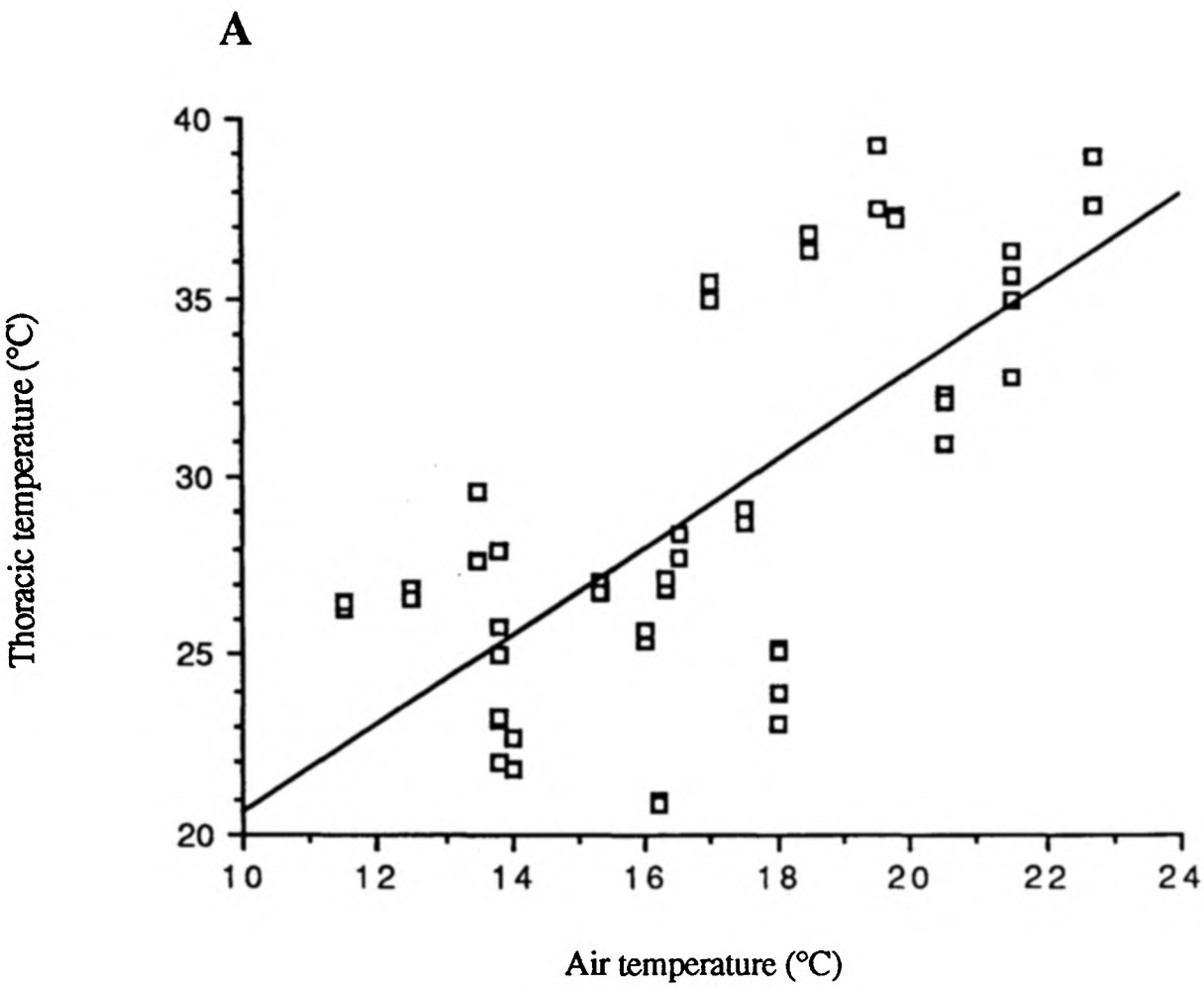


Fig.3.30 Thoracic temperatures achieved by 4 freshly-killed *A. plumipes* (2♂, 2♀) in the sun as a function of air temperature **(a)** and light intensity **(b)** (air temperature: $y=8.3+1.23x$, $R^2=0.504$; log (light): $y=-31.0+14.1x$, $R^2=0.490$).

Chapter 4 Physiological thermoregulation in *Anthophora plumipes.*

4.1 Introduction

Whatever the mechanism of heat generation used by endothermic insects (Chapter 1), regulation of body temperatures over narrow bounds requires that more heat must be generated at low ambient temperatures (T_a) than at higher T_a. At high T_a, the heat generated by flight activity may lead to overheating, and under such conditions thermoregulation can occur only through regulation of heat loss.

Thermoregulatory ability can be measured using the gradient of the relationship between body temperature and T_a. In the case of endothermic insects it is generally the thorax whose temperature is being regulated, and so the relationship between thoracic temperature (y) and ambient temperature (x) is used. The lower the gradient of this relationship (i.e. the lower the dependence of thoracic temperature [T_{th}] on ambient temperature [T_a]), the better the thermoregulation. However, care must be taken when comparing the thermoregulatory abilities of animals in this way (Stone and Willmer 1989a; Chapter 2).

Large bumblebees are very good thermoregulators, being able to raise their body temperatures to around 36°C before and during flight above a minimum T_a of 5°C. At T_a near 25°C and higher, active flight leads to generation of excessive heat in the thorax, and mechanisms of active heat removal from the thorax are required. The temperature of the thorax is controlled by regulation of heat transfer from the thorax in the form of hot haemolymph passing down the petiole into the abdomen (Heinrich 1972c, 1976a). At low T_a, *Bombus* minimises heat loss from the thorax to the abdomen by operation of a haemolymph counter-current heat exchange system in the petiole (Heinrich 1976a). Operation of this system maintains a high $T_{dif.}$ at low T_a. Continuous haemolymph flow between abdomen and thorax is required because the amount of trehalose (the transport sugar predominantly used by insects) present in the thorax is minimal (Professor Andreas Bertsch, personal communication), and more must be supplied continuously through mobilisation of glycogen or lipid reserves in the abdomen if flight or warm-up are to continue (Clegg and Evans 1961; Joos 1987). At high T_a, active flight leads to very high T_{th}, and to avoid dangerous overheating the petiole counter current is replaced by pulses of hot blood flowing into the abdomen from the thorax, alternating with pulses of cooler blood in the other direction (Heinrich 1976a). Blood flowing into the abdomen is pumped by the ventral diaphragm over the hairless lower surface of the abdomen, where it cools. The flow of cooled blood forward into the thorax lowers T_{th}. As a consequence of this active regulation and within the T_a limits over which thermoregulation is possible, the higher the T_a, the lower the thoracic temperature excess (T_{ex}), and the lower the

temperature difference between abdomen and thorax ($T_{dif.}$).

Activation of this thermoregulatory mechanism produces a characteristic pattern of change in T_{th} and T_{ab} as functions of T_a within an individual. Below that T_a at which the bee begins to shunt heat to the abdomen, T_{th} as a function of T_a is commonly a straight line with a gradient of less than 1. In the absence of other heat sources T_{ab} is determined by passive heat conduction from the thorax and is thus also a linear function of T_a, with a gradient greater than that of T_{th} on T_a. When the bee begins to pulse warm blood into the abdomen at higher T_a the gradient of T_{th} on T_a decreases and the gradient of T_{ab} on T_a increases (e.g. Kammer 1981, Fig.2). These changes in gradient, obtained by sampling T_{th} and T_{ab} over a population using 'grab-and-stab' techniques, suggest that an abdominal countercurrent exchange system of the type described for *Bombus* may be in operation. However if there is considerable variation between bees in the temperature at which the petiole counter current becomes an alternating flow of blood, it is possible that a population utilising this thermoregulatory mechanism would not demonstrate the described individual changes in T_{th} and T_{ab} with sufficient clarity for use of this mechanism to be evident. In this case an alternative technique can be used (see section 4.6a below).

In this chapter data on body temperatures in *A. plumipes* obtained both in the lab and using the 'grab-and-stab' technique are presented. First 'grab-and-stab' data are discussed, which suggest that not only is *A. plumipes* endothermic, but also that it is able to thermoregulate. I then discuss laboratory experiments at several T_a which substantiate these suggestions. Evidence is presented which suggests that *A. plumipes* has a mechanism for accentuating heat loss from the abdomen which operates at high T_a, producing cooling of the thorax similar to that observed for *Bombus*. A discussion of the morphological and anatomical characteristics of *A. plumipes* which are apparent adaptations to endothermic regulation follows. In an extended Discussion section estimates are made of the power generated by *A. plumipes* at a given T_{th} and T_a, and of the total energy required to warm up at a given T_a. Data for *A. plumipes* are compared to results obtained for other endothermic insects.

4.2 'Grab-and-stab' measurement of abdominal and thoracic temperatures.

A. Body temperatures and ambient temperature.

'Grab-and-stab' measurements were made in the Botanical Gardens, Oxford, and in the grounds of Merton College and University College during 1987, 1988 and 1989. T_{th} and T_{ab} as functions of T_a for *A. plumipes* are shown in Fig.4.1. T_{th} increased from an average of c.25°C at T_a=5°C to 39°C at T_a=26°C. Over the three years during which this work was undertaken, T_{th} and T_{ab} were measured over the full range of T_a at which this species was observed to fly (0-29°C). It is

thus probable that 25°C is very close to the minimum T_{th} at which this species flies, and 39°C close to the maximum. These conclusions are supported by laboratory data given in section 4.3. The gradient of the least squares regression of T_{th} on T_a is 0.62. (R^2=0.93, n=81, p<0.0001) and as T_a increases, T_{ex} decreases (Fig.4.2). This strongly suggests that *A. plumipes* is able to regulate the temperature of its flight muscles (May 1976a). The gradient of the least squares regression of T_{ab} on T_a is 0.96 (R^2=0.92, n=81, p<0.0001), and T_{ab} does not appear to be regulated. As T_a increases, the gradients of both T_{ab} and T_{th} on T_a decrease (Fig.4.1). The significance of this effect is tested by examining (the residuals obtained by regression of T_{th} on T_a) as a function of T_a, having controlled first of all for the effect of body mass. If the change in gradient is significant, the residuals should be a significant curvilinear function of T_a. Both effects are highly significant (T_{th}: n=80, R^2=0.250, p<0.001; T_{ab}: n=80, R^2=0.214, p<0.001). While a decrease in the gradient of T_{th} on T_a predicted if *A. plumipes* thermoregulates using a similar system to *Bombus*, the same is not predicted for T_{ab}. That *A. plumipes* may have an abdominal blood shunting system to assist with heat loss from the thorax requires laboratory confirmation and this is discussed in section 4.6a.

B. Body temperatures and body mass.

T_{th} does not only depend on T_a: body mass is also an important predictor of T_{th} once the strong correlation between T_{th} and T_a has been controlled for using multiple regression (R^2=0.96, n=80; mass p<0.0001, T_a p<0.0001). Larger bees have a higher T_{th} at a given T_a than smaller bees, and therefore have a higher T_{ex}. If it is true that all individuals of *A. plumipes* (both males and females) have a similar minimum T_{th} for flight and a similar upper lethal temperature, then this result suggests that smaller bees should be restricted in their ability to fly at low T_a, and larger bees in their ability to fly at high T_a (Willmer 1983, 1985a). This result goes some way to explaining the differences in activity patterns between large and small males and large and small females discussed in the previous chapter.

4.3 Warm-up rates, voluntary flight temperatures and stable flight temperatures.

Elevated body temperatures alone do not confirm the presence of endothermy. Body temperatures must be shown to increase over time in the absence of any exogenous source of heat, such as solar radiation or convective heating from a warm substrate. To investigate thermogenesis in *A. plumipes* T_{th} and T_{ab} were recorded in the laboratory using the techniques discussed in Chapter 2 at four different T_a; 9,16,21 and 29°C. 9°C was selected because it lies within the range at which, in the absence of solar heating, most but not all *A. plumipes* will fly in the field; females and some of the larger males will fly, and the smaller males remain in their tunnels. It was hoped that experiments at this T_a would replicate these observations and give a sounder predictive rule for bees

which would or would not fly. The highest natural T_a at which this species was observed to fly was 29°C. T_a reached 27-29°C in the shade in late May, 1989 and only small males were then observed in flight over shaded foraging sites. Experiments at this T_a were intended to reveal what occurs when *A. plumipes* experiences thermal stress, and 16°C and 21°C were chosen as convenient intermediate temperatures. Patterns of warm-up and flight observed at 21°C are discussed first, followed by warm-up at 9°C and 29°C. The results obtained at an ambient temperature of 16°C showed patterns similar to those obtained at 21°C, and are therefore discussed only when examining the effect of T_a on warm-up rates and body temperatures. Warm-up rates and body temperatures obtained at the different ambient temperatures are summarised in Table 4.1.

Table 4.1 Warm-up rates, voluntary flight temperatures and stable flight temperatures (see section 4.3d) in male and female *A. plumipes*. Each value given is the mean of the individual means obtained for each sex at each T_a±1 standard error, with the number of individual means in parentheses.

Ambient temperature (°C)		Mean warm-up rate ($°Cmin^{-1}$)		Voluntary flight temperature (°C)	Stable flight temperature (°C)
T_a=9°C	Males	3.4±0.2	(17)	31.6±0.5 (15)	27.5±0.5 (5)
	Females	4.2±0.3	(14)	32.7±0.4 (8)	29.9±0.6 (4)
T_a=16°C	Males	7.5±0.9	(4)	31.5±0.3 (4)	29.0±0.2 (4)
	Females	9.9±1.0	(4)	34.0±0.3 (4)	32.2±0.2 (4)
T_a=21°C	Males	8.8±0.4	(21)	32.3±0.5 (17)	30.8±0.3 (20)
	Females	12.3±0.6	(21)	34.4±0.6 (11)	34.6±0.6 (12)
T_a=29°C	Males	12.3±0.5	(6)	34.8±0.3 (3)	37.4±0.3 (3)
	Females	15.2±0.4	(3)	36.5±0.3 (3)	38.6±0.2 (3)

A. Warm-up rates at an ambient temperature of 21°C.

Acceptable warm-up traces were obtained from 20 male and 28 female *A. plumipes* at T_a=21°C. After release from the cooled stage and passive equilibration with room temperature, many bees initiated warm-up without stimulation. Others were encouraged to do so by tapping of the antennae or abdomen with fine forceps. A typical warm-up trace is shown in Fig.4.3. In almost all cases, the first warm-up from T_a was markedly curvilinear, warm-up rate increasing with T_{th}, sometimes decreasing slightly just before flight temperatures were reached. Thereafter warm-ups became more linear due to increased rates of warm-up at low T_{th}, but the positive correlation

between warm-up rate and T_{th} persisted. The possible significance of this change in the form of warm-up with time is discussed in section 4.9d. Each bee demonstrated a slightly different relationship between T_{th} and warm-up rate, and more data points were obtained for some bees than for others. It was therefore necessary to prevent biasing of any analysis across all bees by individual bees for which large data sets were obtained (see Chapter 2). The mean warm-up rate and mean T_{th} were obtained for each bee, and are referred to as individual means. The relationship between individual mean warm-up rate and individual mean T_{th} for both male and female *A. plumipes* is shown in Fig.4.4a; the positive correlation is highly significant ($n=48$, $R^2=0.72$, $p<0.0001$). The overall relationship between warm-up rate and T_{th} is shown in Fig.4.4b. Again the positive correlation is highly significant, ($n=342$, $R^2=0.5$, $p<0.0001$), although because all data points are included, this figure should be taken only as an indication of the relationship between these two variables. This demonstrates that as the bee warms, increased rates of heat generation more than compensate for the increasing rates of heat loss experienced as it generates a growing T_{ex}. There is also a strong positive correlation between body mass and individual mean warm-up rate ($n=48$, $R^2=0.43$, $p<0.0001$, Fig.4.4c). Multiple regression reveals, however, that once the strong correlation between T_{th} and individual mean warm-up rate has been controlled for, there is no significant relationship between warm-up rate and body mass ($n=48$, $R^2=0.021$, $p>0.05$). It is probable, however, that body mass determines the higher mean T_{th} reached by larger bees, and thus contributes to their higher individual mean warm-up rates; there is a strong positive correlation between mean T_{th} and body mass ($n=48$, $R^2= 0.46$, $p<0.0001$) as shown in Fig.4.4d. Having controlled for the relationship between body mass and mean T_{th}, T_{th} still remains a significant predictor of warm-up rate ($n=48$, $R^2=0.3$, $p<0.0001$). To prove an effect of body mass on warm-up rate other than that caused through T_{th} would require manipulation of the thoracic masses of individual bees, which was beyond the scope of my work. These analyses suggest that warm-up rate is a function of both body mass and T_{th}, and while T_{th} has an effect on warm-up rates independent of body mass, body mass effects warm-up rates via T_{th}. The relevance of body mass in determination of warm-up rates is discussed more fully in Chapter 6.

There is no significant difference between warm-up rates in males and females once the effects of T_{th} and body mass have been controlled for. It is worth noting that at 21°C at high T_{th}, the warm-up rates in the largest females reach more than 19°C per minute, the highest rates recorded in any organism.

B. Rates of abdominal pumping at an ambient temperature of 21°C.

An indication of the metabolic effort required for thermogenesis is provided by the rate of

abdominal pumping. 'Abdominal pumping' describes the antero-posterior concertina-like movements of the abdominal segments associated with movement of air in and out of the abdominal tracheal system, and thus the supply of oxygen to the tissues (Sotavalta 1954, Weis-Fogh 1967; Bartholomew and Barnhart 1984). My measurement of abdominal pumping frequency did not include estimates of the volume of air transported with each movement, and so the relationship between pumping rate and oxygen supplied to the tissues can only at best be approximate. It is possible to measure by eye pumping rates up to 6 or 7 times a second. At high T_{th}, and at T_a=21°C, female *A. plumipes* show rather higher rates than this, and the maximum rates of abdominal pumping are estimated to have exceeded 10 per second. There is a strong positive correlation between the rate of abdominal pumping and T_{th}, as shown in Fig.4.5. Within the relationship between T_{th} and abdominal pumping rate, further variation in pumping rates is explained both by body mass, the sex of the bee, and significant differences between individuals independent of mass, sex and body temperature. The results given below have been analysed in such a way that such differences between individuals have been controlled for (Chapter 2). At a given T_{th}, smaller bees have higher rates of abdominal pumping, and at a given temperature and body mass, males have lower rates of pumping than females (multiple regression n=236, R^2=0.67, T_{th} $p<0.0001$, body mass $p<0.0002$, sex $p<0.0004$).

C. Rates of heat loss at an ambient temperature of 21°C.

The methods used to determine cooling rates were discussed in Chapter 2. The rate at which a body cools depends on how rapidly heat is lost per unit area from its surface (its conductance, C), and on the temperature difference that exists between the body and its surroundings (T_{ex} in the case of a bee's thorax). This relationship is expressed algebraically as: $dT_{th}/dt \propto CT_{ex}$. C is the rate of heat loss from a bee per unit temperature excess per unit area, and is high for bodies that lose heat quickly, and low for bodies that cool slowly. C can be calculated by multiplying the cooling constant [the gradient of a regression of cooling rate (y) on T_{ex} (x)] determined by analysis of the cooling curve of each bee (Fig.4.6a) by the specific heat capacity of tissue (taken as 0.8 cal $g^{-1}°C^{-1}$, or 3.4J$g^{-1}°C^{-1}$; Heinrich 1975a). In this way, values of C were obtained for 14 *A. plumipes* (8 males and 6 females). The mean cooling constant for *A. plumipes* is 0.53 °C $min^{-1}°C^{-1}$, giving a mean thermal conductance of 0.03 W$g^{-1}°C^{-1}$, or 0.42 ca $min^{-1}g^{-1}°C^{-1}$. These mean values are compared to the conductances of other endothermic insects in the Discussion at the end of this chapter. As expected, the cooling constant (and therefore the thermal conductance) of *A. plumipes* decreases significantly with increasing thoracic mass; small bees lose heat more rapidly than large bees (Fig.4.6b. n=14, R^2=0.527, p=0.01). If total body mass is used rather than thoracic mass,

then sex becomes a significant predictor of rates of heat loss; at a given body mass, males lose heat more slowly than females (n=14, body mass p=0.002, sex p= 0.01). It may therefore be true that, perhaps through better insulation of the thorax, males require less heat to raise the temperature of the thorax over a given temperature rise than females do. Some evidence that males are indeed better insulated than females is given in section 4.8.

D. Voluntary flight temperatures and stable flight temperatures at an ambient temperature of 21°C.

Tethered flight was initiated when the bee released the styrofoam held in its tarsi and began vigorous, loud wing movement, during which the legs were held close to the body, as in free flight. At T_a=22°C, the temperature at which flight was initiated by the bee (voluntary flight temperature, or VFT) was somewhat higher than the temperature the bee sustained during tethered flight, and over a period of 1-2 minutes during flight T_{th} fell to a lower stable temperature (stable flight temperature, or SFT) which was sustained until flight ceased (see Fig.4.3). A similar sequence of changes in T_{th} during flight is described by Louw and Nicolson (1983).

There is considerable variation within the species in VFT and SFT at T_a=21°C. In both male and female *A. plumipes*, VFT is predicted well by body mass. As with the warm-up rates given above, mean values for both variables are used for each bee. When all bees are considered, larger bees warmed to a higher T_{th} before flight was initiated than smaller bees (n=43, R^2=0.51, p<0.0001) (Fig.4.7). Overall there is also a positive correlation between individual mean SFT and body mass - larger bees had a higher SFT than smaller bees (n=32, R^2=0.18, p=0.015, Fig.4.8). These results support the conclusion from 'grab-and-stab' measurement of body temperature that body mass is important in predicting body temperatures.

E. Exhaustion and endothermy

Before going on to discuss the effects on the various parameters of warm-up in *A. plumipes* at different T_a, it is worth mentioning another cause of variation. Generally after 3 or 4 warm-ups over the full range of T_{th} from T_a to VFT, bees showed an increasing tendency not to complete further warm-ups, a slight lowering of warm-up rates, and weak, uncoordinated 'flight'. This apparent fatigue could be dramatically 'cured' by feeding the bee with a solution of sucrose. The tongue was touched with a pinhead carrying a small drop of 45% sucrose solution. If the bee was fatigued it generally lowered its mouthparts and swung them forward, extending the glossa. If this was met with a paintbrush carrying a drop of similar sucrose solution, the bee would usually feed. Shortly after feeding was initiated, there was a marked increase in abdominal pumping, and a

rapid increase in T_{th}. The bee warmed to levels in excess of those in previous warm-ups, and ceased feeding shortly before flight. This increase in apparent thermogenic ability remained for several subsequent warm-ups. The major effect of feeding was an increase in VFT and in the power of tethered flight. Two examples of this effect are shown in Fig.4.9. Possible reasons for this observation are considered in the Discussion at the end of this chapter.

This effect on warm-up is not easy to control for. As described in Chapter 3, both males and females collect nectar to the exclusion of all other flight activities during the early part of their flying period. As far as was possible, bees were therefore collected during the latter stages of this period to minimise the probability that they might be limited during warm-up by low levels of nectar in their crops.

The following sections characterise warm-up patterns in *A. plumipes* at the lowest and highest T_a investigated, and then summarise the general relationships between T_a and warm-up parameters in this species.

4.4. Warm-up at an ambient temperature of 9°C.

Initial rates of warm-up by all bees at this T_a were very low (see Heinrich 1987), with associated low rates of abdominal pumping. At a T_{th} of 9°C, visible responses to external stimuli, such as tapping or movement within the bee's field of vision, were very slow. A typical warm-up under these conditions is shown in Fig.4.10. Of those bees which warmed up and initiated tethered flight 17% of female warm-ups (n=6) and 38% of male warm-ups (n=13) showed a shoulder in the warm-up curve, as shown in the figure, where warm-up rate decreased for a period before the final warm-up to flight temperature. The shoulder occurred at a T_{th} of 16-21°C, and flight at 28-32°C.

As was the case at T_a=21°C, there is a strong positive correlation between individual mean warm-up rate and individual mean T_{th} over the period of warm-up (Fig.4.11a), and overall between warm-up rate and T_{th} (Fig.4.11b). Multiple regression reveals that once the relationship between mean T_{th} and mean warm-up rate has been controlled for, body mass is not a significant predictor of warm-up rate (n=31, R^2=0.58, mean T_{th} $p<0.0001$; mass p=0.18). As at T_a =21°C there was a positive correlation between body mass and T_{th} at T_a=9°C (), and body mass may therefore affect warm-up rate through an effect on T_{th}. There is also a positive correlation between individual mean VFT and body mass (n=23, R^2=0.21, p=0.028). T_{th} fell on initiation of flight, giving a mean SFT of 27.5±0.5°C (n=5) for males, and 30.0±0.6°C (n=4) for females. The small sample size precludes any meaningful analysis of the effect of mass on SFT, although overall, mass and individual mean SFT are positively correlated (n=9, R^2=0.71, p=0.017), suggesting that the same positive correlation between these two variables found at T_a =21°C exists at T_a=9°C. Even brief interruption

of flight by the bee, leading to cooling of the thorax by more than 2-3°C, made it impossible for flight to be resumed without warm-up. This suggests that the minimum T_{th} for flight in this species is near 25-27°C, supporting the conclusion reached using 'grab-and-stab' techniques.

Four out of 14 females, and 9 out of 17 males, failed to reach T_{th} high enough for flight at T_a=9°C. There was a strong positive correlation between ability to warm up to flight temperatures and body mass (Fig.4.12) (n=6, R^2=0.77, p=0.021). Despite low initial warm-up rates, *A. plumipes* did generate a T_{ex} of 22-24°C at this T_a (individual mean male excess 22.6±0.5°C, [n=15]; individual mean female excess 23.7±0.4°C [n=8]), 5-7°C more than the average T_{ex} at take-off at T_a=21°C.

4.5. Warm-up at an ambient temperature of 29°C.

Warm-up rates at T_a=29°C were uniformly high - females individual mean warm-up rate averaged 15.2±0.4 °C per minute (n=3), and male individual mean warm-up rate 12.3±0.5 °C per minute (n=6), but the maximum recorded warm-up rate (19.2 °C per minute for a 201mg female) was not higher than the maximum recorded at T_a =21°C. This suggests that this rate is near the maximum possible in this species, under ideal conditions of T_{th} and minimal rates of heat loss. The T_{ex} at which those *A. plumipes* which could be induced to fly released the styrofoam was only 7-8°C, giving a mean over individual mean VFT's of 34.8±0.3 °C (n=3) for males and 36.5±0.3 °C (n=3) for females (Table 4.1). These VFT's are close to those at T_a=21 °C, and suggest that despite the undoubted ability of the bees to generate far higher T_{th} at this T_a, further increase in T_{th} is disadvantageous. When tethered flight was initiated, T_{th} rose rapidly. Although T_{th} did approach an asymptotic SFT value, in only 3 males and 4 females were flights at full power of over 15 seconds in duration recorded. These flights gave the following mean individual mean 'SFT' values; males 37.4±0.2°C (n=3), females 38.6±0.2°C (n=4), although it is certainly true that prolonged flight would lead to the generation of far higher T_{th}. In all cases, flight ceased once T_{th} approached 38-40°C, and even stimulation with forceps could not cause resumption of flight. Three large females (masses 210, 215 and 235mg) showed hardly any warm-up at all, and although vigorously defending themselves against stimulation (using forceps) with their legs and jaws, no rise in T_{th} beyond 34-36°C could be obtained. The results of these experiments clearly show that exposure to high T_a caused excessive heat generation during flight, and confirmed that the largest *A. plumipes* would not even attempt flight under conditions under which smaller males will fly.

4.6 Regulation of thoracic temperature by regulation of blood flow to the abdomen.

A. Evidence for regulation of heat flow to the abdomen in *A. plumipes*.

Chapter 4. Physiological thermoregulation in *A. plumipes*

The basic mechanism by which T_{th} may be regulated by control of blood flow between thorax and abdomen was discussed in section 4.1 for *Bombus*. The pattern of Tth and Tab over Ta expected when such a system is in operation was discussed in section 4.1. No clear pattern of this type was obtained for *A. plumipes* (Fig.4.1). However, failure to detect this pattern over a population does not necessarily mean that such a system is absent. An alternative method of detecting this mechanism, if present, requires simultaneous measurement of T_{th} and T_{ab}, as described in Chapter 2. When this type of thermoregulatory system is in operation, a characteristic plot of T_{th} and T_{ab} over time is obtained (e.g. Heinrich 1976a for *Bombus*, or May 1976b for the dragonfly *Anax junius*). During warm-up, T_{th} rises more rapidly than T_{ab}, and both level out with T_{th} higher than T_{ab}. When flight ceases in *Bombus*, active export of heat from the thorax to the abdomen is revealed by a simultaneous rapid fall in T_{th} and rapid rise in T_{ab}. Whether or not this pattern is observed depends not only on whether such a system exists in the species examined, but also on whether at the T_a being used the bee needs to utilize such a system. We should only expect such a thoracic cooling mechanism to be used if accelerated cooling of the thorax at the end of flight is required. In such a large endothermic insect T_{th} will usually fall with cessation of flight muscle activity (but see Chapter 2, Appendix 2), though the pattern of heat flow in the bee will change once the convective draught of flight ceases. More heat may flow into the head once flight ceases, and at high T_a active heat loss to the abdomen may be a mechanism to avoid overheating of the head. If T_a is low, and T_{th} is well below the bee's upper lethal temperature, the bee will not be in danger of overheating and can cool passively without needing to shunt warm blood to the abdomen. If the bee is to maintain a readiness for flight (if, for example, the bee has landed briefly to feed from a flower), then heat losses to the abdomen should be minimised, and no rapid increase in T_{ab} at the end of flight is expected.

To confirm with *A. plumipes* that the thermocouples used were correctly placed to record T_{ab} and T_{th}, these were recorded in a queen of *Bombus pratorum*, during warm-up and tethered flight at T_a=21°C. This allowed comparison of the technique used with other authors' published results (e.g. Heinrich 1975). My results are shown in Fig.4.13. In this case, as indicated on the figure, cessation of flight was accompanied by rapid fall in T_{th}, and an increase in T_{ab}. However, no *A. plumipes* tested at this T_a showed a similar pattern. Fig.4.14 shows a trace for a male similar to those obtained for all 5 *A. plumipes* examined. To test whether the absence of any apparent abdominal blood shunt was due to genuine lack of this ability, or to the fact that the *Anthophora* tested were not under thermal stress, the experiments were repeated at T_a=29°C. At this T_a, T_{th} rise rapidly during flight to 38-40°C, and at the end of flight the typical abdominal shunting pattern appears, as shown in Fig.4.15. Thus *A. plumipes* does have a thermoregulatory mechanism

involving regulation of heat flow between thorax and abdomen, the effects of which only become apparent at relatively high T_a.

For some of the larger female *A. plumipes* at T_a=21°C, and for both males and females at T_a=29°C, cessation of flight led to an unusual pattern of cooling of the thorax. Periods of rapid cooling alternated with brief periods of slower cooling, occurring at regular intervals (Fig.4.16a). The same phenomenon was observed for large *Bombus terrestris* at T_a=21°C, and was particularly marked in some of the large *Anthophora* species (200-500mg body mass) discussed in Chapter 6 (Fig.4.16b). It is tempting to suggest that the abrupt drops in T_{th} correspond to heat transfer to the abdomen. Certainly no such pattern is observed in the cooling of dead bees.

B. The limits to thermoregulation in *A. plumipes*.

It is clear from Fig.4.15 that the ability of *A. plumipes* to regulate T_{th} by heat loss to the abdomen is somewhat limited even at this high T_a. Over the course of the experiment T_{ab} rose steadily and after a time further thermoregulation through control of heat loss to the abdomen would be impossible: continued flight would have lead to continued increase in both T_{th} and T_{ab}. It is relevant that once T_{th} approached T_{ab}, the bee illustrated in Fig.4.15 could not be stimulated into further tethered flight. If the bee is to avoid critical overheating it has no option but to cease flight and to allow itself to cool passively. The observations on the behaviour of *A. plumipes* in the field given in Chapter 3 suggest that the maximum T_a at which this species can maintain flight for long periods without overheating is 24-26°C, depending on body mass. One male *A. plumipes* continued to warm up at T_a=29°C (for reasons which remain unclear) beyond the temperatures at which most bees ceased endothermy. Without any external stimulation, the bee warmed to a T_{th} of 49°C; its movements became uncoordinated at 47°C, and shortly after the bee reached its maximum T_{th} it died. This suggests that the lethal upper limit of T_{th} for *A. plumipes* is around 46-49°C. The maximum T_{th} at which full recovery is possible may be somewhat below these. At the highest T_a at which this species was observed to fly in the field, T_{th} approached 38-39°C, suggesting that this species normally ceases flight activity well before dangerous T_{th} are reached.

4.7 The effect of T_a on the warm-up of *A. plumipes*. (Can this bee really thermoregulate)?

A. Warm-up rates.

Once mass and T_{th} have been controlled for, it is clear that warm-up rates correlate strongly with T_a. The lower the T_a, the higher the T_{ex} the bee will have to maintain for a given T_{th}, and the higher the rate of heat loss with which it will have to contend. Demonstration of the existence of a

relationship between warm-up rate and T_a requires amalgamation of all the warm-up rates of all the bees tested, including variation due to differences in body mass and T_{th}, into a single statistic at each T_a. I have taken the mean of all the individual mean warm-up rates to obtain a single value for males and females at each T_a, referred to as the mean warm-up rate (MWR). The relationship between mean warm-up rate and T_a is shown in Fig.4.17. MWR shows a strong positive correlation with T_a. Bearing in mind the strong positive correlation between T_{th} and warm-up rate, and the fact that T_{th} reached also correlate with T_a, the relationship between warm-up rate and T_{th} must be controlled for before it is possible to say whether or not there is a significant independent effect of T_a. Multiple regression of all the data on warm-up at T_a's of 9°C and 21°C reveals that even after the non-significant effects of sex and the significant effects of T_{th} and mass have been controlled for, warm-up rate is significantly positively related to T_{ex} (559 data points from 84 bees, R^2=0.765; T_{th} $p<0.0001$, body mass $p<0.001$, sex $p=0.09$, T_{ex} $p<0.0001$). At a given T_{th}, warm-up rates at T_a=9°C are lower than those at T_a=21°C (Figs.4.4b and 4.11b). At a given T_{ex}, warm-up rates depend on T_{th} (Fig.4.18).

B. SFT and VFT.

Relationships between VFT, SFT and T_a give an indication of the thermoregulatory ability of *A. plumipes* independent of the conclusions based on 'grab-and-stab' data. In either case, a positive correlation with a gradient of <1.0 is an indication of thermoregulation, as discussed in Chapter 2. Fig.4.19a shows the relationship between VFT, SFT and T_a. VFT is consistently higher than SFT until high T_a's are reached, and the gradients of both as functions of T_a are less than 1, indicating physiological thermoregulation. The laboratory data thus corroborate the results obtained using 'grab-and-stab'. The SFT curve should show an increase in gradient as T_a reaches the levels at which thermoregulation becomes impossible, and had prolonged flights been maintained at T_a=29°C, the graph would undoubtedly show this effect.

C. Time required to complete warm-up.

The total time required to complete warm-up to flight temperature in *A. plumipes* is also dependent on T_a, as shown in Fig.4.19b. The higher the T_a, the lower the T_{ex} which must be generated before flight, and the higher the rate of heat generation at the start of warm-up. It is therefore not surprising that time required to complete warm-up shows a negative correlation with T_a. While at T_a=21°C warm-up took a mean of 1.6±0.1 minutes (n=10 for each) for both females and males, warm-up at T_a=9°C took a mean of 11.2±0.6 minutes (n=11) for males and 11.7±0.6 minutes (n=9) for females; when warm-up occurs at such low T_a outside the protection of the nest tunnel, then the importance of behavioural thermoregulatory mechanisms, such as basking, in

minimising the time for which the bee is exposed to predators without being able to fly, is made clear.

Larger bees warm up more rapidly than small bees (which reduces warm-up time), but also warm up further than small bees (which tends to extend warm-up). Thus whether large bees take longer or shorter periods of time to complete warm-up depends on the balance between these effects. At T_a=21°C these effects seem to cancel each other out - there was no significant effect of individual mean warm-up rate, body mass or individual mean VFT on warm-up duration (multiple regression, n=20, R^2=0.157: body mass p=0.55; warm-up rate p=0.18; VFT p=0.55). At T_a=9°C, however, there is a significant negative correlation between warm-up duration and warm-up rate (Fig.4.20) (n=16, R^2=0.445: body mass p=0.26; VFT p=0.4; MWR p=0.014), and it is probable, therefore, that body mass also affects warm-up duration at this T_a due to its effect on warm-up rate.

4.8 The structure and distribution of pubescence in *A. plumipes*.

Many endothermic insects have on the outer surface of their cuticles an insulating layer of hairs or scales. The presence of this insulating layer has been shown in at least some cases to reduce heat loss to the surrounding air (Church 1960; Bartholomew and Epting 1975; Casey 1981a; Heinrich 1987; Morgan 1987; Kukal *et al.* 1988). The thermal conductivity of the cuticle of *A. plumipes* is very similar to that of most other endothermic insects for which figures are available (Table 4.1), although it is noticeably lower than that of bumblebees of the same total body mass (Heinrich and Heinrich 1983), and somewhat higher than values for sphingid and saturniid moths of the same total body mass (Bartholomew and Epting 1975). In terms of rates of heat loss across all endothermic and partially endothermic birds, mammals and insects, *A. plumipes* gives values very close to those predicted for its mass (Bartholomew and Epting 1975). The pile of this species, while not as effective as the dense scales of some Lepidoptera, is thus nonetheless an effective insulating layer.

I know of no published study on the structure and distribution of insulating pile in bees. For this reason several bumblebees (*Bombus hortorum*) were examined using scanning electron microscopy to allow some basic comparison with *A. plumipes* to be made. *Bombus hortorum* has two main types of seta over the body surface. Much of the head, thorax and abdomen are covered by long, relatively thick 'guard hairs', which at the junction between thorax and abdomen reach over 2 mm in length, and vary from 10-15 μm in diameter. Short (5-7μm), unbranched spines emerge from the surface at regular intervals along the hair's length (Fig.4.21a), and their distribution and form appear regular throughout the hair's length. A second type of seta underlies the guard hairs on the head and thorax, and, to a much lesser extent, the abdomen. These are shorter (up to 1mm) and

finer (3-5μm in diameter) (Fig.4.21b). The spines projecting from the main axis of the seta are much longer than those of the guard hairs (50-75 μm) and the hairs mesh closely together among the guard hairs (Fig.4.21c). By counting the number of setal sockets on the surface of the cuticle, it is possible to estimate that hairs of both types occupy the surface of the thorax at a density of approximately 1000 per mm^2.

The distribution and relative lengths of the setae on the surface of a male *A. plumipes* are shown in fig 4.22. The frons of the head is thickly covered and the back of the head is surrounded by a collar of setae which mesh closely with forward facing setae on the front of the thorax. The top of the thorax is thickly covered with hairs ranging in length from 1.0-2.0mm. The ventral surface of the thorax is also covered with setae, though these are shorter than those on the dorsal surface, and the covering appears less dense (see below). Insulation of the ventral surface of the thorax must be improved by the dense setae on the ventral surfaces of the femora of the legs which in flight are held closely aginst the body. The longest and densest pubescence is found at the back of the thorax, meeting equally long setae projecting forwards from the anterior dorsal part of the abdomen. The air space between thorax and abdomen and the hairs projecting into it appear to be well adapted to insulation of the thorax from heat losses to the abdomen and the surrounding air other than via the petiole. The dense pubescence around the petiole must also serve to reduce the heat losses which would occur were this large surface area exposed to moving air. The dorsal part of each abdominal segment is coated with setae, but the ventral surface bears only sparse, shorter setae, and is effectively naked (Fig.4.22), resembling in this respect the abdomen of bumblebees (Heinrich 1972d, 1976a). As section 4.6A showed, the lower surface of the abdomen may function as a 'thermal window' for loss of excess thoracic heat, as has been shown for *Bombus* (Heinrich 1976a). Male *A. plumipes* generally have longer and denser pubescence than females of the same size, particularly over the dorsal surface of the thorax. This may contribute to the lower rates of heat loss from males than from females shown in section 4.3c.

Scanning electron microscopy reveals that rather than the two distinct types of seta found in *Bombus hortorum*, *A. plumipes* has only a single type, differentiated along its length in such a way that the single hair may simultaneously perform the two apparent functions of the two *Bombus* hair types. Long, fine spines (up to 35μm) project from the lower regions of the setal shaft (Fig.4.23a), and far shorter spines (10-15μm) are borne near the tip (Fig.4.23b). The hairs are up to 2mm in length, and are 4-6μm in diameter, covering the thorax at a density of approximately 700 per mm^2 on the top of the thorax, and at higher densities nearer the petiole joining thorax and abdomen. Church (1960) states that it is the density of insulating hairs rather than their length which contributes most of the insulation provided by fur in insects, and in this respect the insulating layers of *A. plumipes*

and *Bombus hortorum* are similar. The setae on the ventral surface of the abdomen are similar in structure, but apart from a margin of hairs towards the posterior end of each sternite, this area is covered only sparsely with very short, unfeathered setae (Fig.4.24). In structure the hairs of *A. plumipes* closely resemble those of the anthophorid carpenter bee *Xylocopa capitata* (Louw and Nicolson 1983).

4.9 Discussion.

In this section the data obtained using 'grab-and-stab' and laboratory techniques of temperature measurement are first compared. Then the patterns of warm-up shown by *A. plumipes* are compared to those in other endothermic insects. The power required for warm-up and the total energy used by *A. plumipes* are compared to data for other endothermic insects, particularly bumblebees.

A. Comparison of 'grab-and-stab' and laboratory data.

How similar are the estimates of body temperature in flight obtained using these two techniques? Table 4.2 shows mean SFT values at the T_a's used in the lab together with mean 'grab-and-stab' estimates taken from Fig.4.1.

Table 4.2 A comparison between SFT and 'grab-and-stab' data. SFT are means values at the ambient temperatures used in the lab, and 'grab-and-stab' estimates are taken from Fig.4.1.

Ambient temperature (°C)	9	16	21	29
Stable flight temperature (°C)	28.5	31	32	38
'Grab-and-stab' estimate (°C)	28	33	35	39

The two methods agree well in their estimation of T_{th} in flight at high and at low T_a, although at moderate T_a 'grab-and -stab' estimates are higher than the corresponding SFT values. The disparity between the two values is not a surprising one. The reasons why SFT values are probably under-estimates and 'grab-and-stab' over-estimates of true T_{th} have already been discussed in Chapter 2. The two methods give sufficiently similar results, even taking the different sources of error associated with each of them into account, for conclusions on the thermoregulatory ability of *Anthophora plumipes* reached using both simultaneously to be considered sound.

B. Power generation during warm-up.

During warm-up, energy is expended both in movement of the musculature and also in generation of heat. Here only the thermal power generated is considered, and this will be an

underestimate of the actual power generated. Bartholomew (1981) cites a figure of 20% for the efficiency of muscle in converting chemical energy into kinetic energy, and as an approximation the thermal power could therefore be multiplied by 1.25 to obtain an estimate of the total power generated.

The thermal power generated at a given T_{ex} is the sum of the rate of heat storage in the thorax and the rate of passive heat loss. This can be expressed simply as: rate of heat generation at a given T_{ex} = (warm-up rate + passive cooling rate at that temperature excess) x (specific heat capacity of tissue) x (thoracic mass), if we neglect for the moment heat losses from other body tagmata (Heinrich and Bartholomew 1971). If we assume that during warm-up, all the heat generated is sequestered in the thorax, then rates of passive heat loss depend only on T_{ex} (as long as there is no significant variation in factors such as air movement around the bee). It is therefore possible to calculate such rates of heat loss at any T_{ex} for any T_a from best fit regressions of rate of cooling as a function of T_{ex}, such as those shown in Fig.4.6a. As shown in sections 4.3A and 4.4, there are strong correlations between warm-up rate and T_{th} at a given T_a (Figs.4.4b, 4.11b). From these relationships it is possible to calculate the mean warm-up rate for *A. plumipes* at a given T_{th} at a specific T_a, and thus at a given T_{ex}. The specific heat capacity of insect tissue is commonly assumed to be a constant of 3.4 $Jg^{-1}\ °C^{-1}$, or 0.8 Cal $g^{-1}\ °C^{-1}$ (e.g. Heinrich 1975a). The mean thoracic mass for males is 56.5mg, and for females, 65.5mg. From the relationships and quantities described above the power generated at a specified T_{th} and T_a can be calculated. Table 4.3 shows steps in the calculation of the rate of heat production during warm-up for male *A. plumipes* at 9 and 21°C, the T_a's for which my data are most comprehensive. Power output for males and females as a function of T_{ex} is shown in Fig.4.25.

Power output increases linearly with T_{ex}, and, at a given T_{ex}, power output at 21°C is higher than that at 9°C. The power generated during warm-up is strikingly similar to that generated by queen bumblebees (Heinrich 1975a). At a T_{ex} of 20°C, queen *Bombus vosnesenskii* produce 4-5 cal g^{-1} thorax min^{-1}, or 0.28-0.35 watts g^{-1} thorax (Heinrich 1975a). Table 4.3 shows that male *A. plumipes* produce 0.26-0.56 watts g^{-1} thorax, depending on the T_a. The maximum power output produced by *A. plumipes* (1.55 watts g^{-1} thorax, or 22.1 cal g^{-1}thorax min^{-1} at a T_{th} of 40°C at T_a=21°C) is somewhat higher than the maximum rate reported by Heinrich (1975a) for *B. vosnesenskii* (1.05 watts g^{-1}thorax, or 15 cal g^{-1} thorax min^{-1}) or for the cuculiine moths he studied in 1987 (maximum rates almost identical to *B. vosnesenskii*). Using the common approximation that 1ml of oxygen liberates 20.1J (e.g. Weis-Fogh 1967; Casey *et al.* 1981), a simple calculation reveals that *A. plumipes* uses 4.6 ml oxygen per gram per minute when warming up at its maximum rate, over 1.5 times the value found for bumblebees in flight (Heinrich 1975a). In fact, this rate of

Table 4.3. Calculation of rates of heat generation in the thorax by male *Anthophora plumipes*. The following regression equations (all significant at $p<0.0001$) are used to calculate rates of warm-up and cooling at the given thoracic temperatures and thoracic temperature excesses.
Males: cooling rate ($°Cmin^{-1}$) = 0.591[thoracic temperature excess(°C)]-0.025. Warm-up rate at T_a=21°C ($°Cmin^{-1}$)=0.508 [thoracic temperature (°C)]-5.89. Warm-up rate at T_a=9°C ($°Cmin^{-1}$)= 0.259[thoracic temperature (°C)]-2.49. Females: cooling rate($°Cmin^{-1}$) = 0.508[thoracic temperature excess(°C)]-0.459. Warm-up rates for females are given by the same expressions as those for males.

Ambient temperature (°C)	Thoracic temperature (°C)	Thoracic temperature excess (°C)	Warm-up rate ($°C\ min^{-1}$)	Cooling rate ($°C\ min^{-1}$)	Power generated (watts g^{-1} thorax)
21	21	0	4.80	0.00	0.265
21	24	3	6.30	1.75	0.45
21	27	6	7.80	3.50	0.63
21	30	9	9.35	5.30	0.81
21	33	12	10.90	7.10	1.00
21	36	15	12.40	8.85	1.18
21	39	18	13.90	10.60	1.37
21	42	21	15.45	12.40	1.55
9	10	1	0.10	0.57	0.04
9	12	3	0.60	1.75	0.13
9	15	6	1.40	3.50	0.27
9	18	9	2.17	5.30	0.42
9	21	12	2.95	7.10	0.56
9	24	15	3.73	8.85	0.70
9	27	18	4.5	10.60	0.84
9	30	21	5.3	12.40	0.99
9	33	24	6.1	14.16	1.13

oxygen consumption is higher than those recorded for many other endothermic insects during warm-up (e.g. Kammer and Heinrich 1974; Bartholomew *et al.* 1981; Casey 1981; Casey *et al.* 1981; Morgan 1987). Rates calculated for *A. plumipes* are similar to those reported for hovering bees; for example, the euglossine bee *Exaerete frontalis* (body mass 732mg) uses 3.1 ml oxygen g^{-1}thorax min^{-1} while hovering and *Euglossa imperialis* (body mass 170mg) 5.45 ml oxygen g^{-1}thorax min^{-1} (May 1976a; Casey *et al.* 1985). This illustrates the fact that, in terms of metabolic power output, high rates of warm-up are compatible with one of the most energetically costly forms of flight.

Estimates of oxygen consumption.

Estimation of how much air must pass through the bee's tracheal system to allow extraction of the required volume of oxygen requires an estimate of the levels of oxygen remaining in expired air, and thus of the percentage of oxygen removed from inspired air. Bartholomew and Barnhart (1984) reported that the partial volume of oxygen in the air sacs of some endothermic cicadas fell from 21% to 6% during warm-up, indicating removal of almost 75% of the oxygen present in the air sac. This reduction in oxygen content establishes a steep diffusion gradient which draws oxygen, via the spiracles, into the insect's body. In contrast, during flight in the same cicadas, active ventilation of the tracheal system due to activity of the flight muscles (autoventilation) generated a tidal flow of air in the body, and the partial volume of oxygen fell to 16%, indicating removal of only 25% of the oxygen present in the air sacs. Warm-up in *Anthophora plumipes* was always associated with abdominal pumping, and it is reasonable to assume that air is pumped through the spiracles during warm-up. If we assume that *A. plumipes* obtains most of the oxygen it requires through abdominal pumping (Weis-Fogh 1967), and that oxygen is extracted per unit volume in a manner similar to other insects in flight (Weis-Fogh 1967) resulting in the removal of 25% of the oxygen per unit volume of air taken in, then it is possible to estimate how much air is being moved by abdominal pumping. Because bees and cicadas in flight may remove different amounts of oxygen from the air during flight, the following should be regarded only as an 'order of magnitude' treatment. Oxygen makes up 21% of the air by volume, thus for each ml of air the bee is able to extract approximately 0.05ml oxygen. In this example the bee would have to pump 4.6/0.05 = 92 ml air per gram per minute through its body. This is considerably greater than the 8.15 ml g^{-1} total body mass min^{-1} (about 12.2 ml air per gram thorax per minute) shown by flying locusts (Weis-Fogh 1967). Several important approximations have been made in this example, which is intended only to show why the observed pumping rates are so high at the highest metabolic rates shown by *Anthophora plumipes*. The actual volume will depend on how much oxygen the bee can remove from the air, and how much of the gaseous exchange occurs by diffusion through the thoracic spiracles. Whatever the

actual volume, the reasons for the very high rates of abdominal pumping seen during warm-up at high T_{ex} are clear.

C. The total energy required for warm-up.

At both of the T_a's shown in Fig.4.25, the power output for males is higher than that of females. This is because although males have a lower rate of heat loss per unit body mass than females, since rates of heat loss correlate negatively with body mass (Fig.4.6b), and males are smaller than females, their net rates of heat loss are higher. It is also true that at a given T_{ex}, metabolic rates at 21°C are higher than those at 9°C. This result parallels the findings of Heinrich (1987) for cuculiine moths. The reason for this result is that although the rate of heat loss depends only on the T_{ex}, the rate of heat generation depends on T_{th} (Fig.4.26), as shown by Heinrich (1975a, 1987). The following example illustrates the importance of the relationship between the power generated by the thorax and T_{th}. At T_a=9°C (Table 4.3) and with a T_{th} of 15°C (a T_{ex} of 6°C) the mean power generated by *A. plumipes* is 0.27 watts, and the rate of heat loss 0.19 watts. 70% of the estimated generated heat is lost through passive cooling. At T_a=21°C and with a T_{th} of 27°C (the same T_{ex} of 6°C), while the rate of passive heat loss is still 0.19 watts, the total power generated is 0.63 watts. At the higher T_a, passive heat loss accounts for only 30% of the total power generated.

To obtain the total energy required during warm-up it is useful to express the rate of heat generation in another way (Heinrich 1975a):

$$dH_p/dt = [WS\ dT_{th}/dt] - [CAT_{ex}] \qquad \text{equation 4.1}$$

where dH_p/dt is the rate of heat production, and

W is the mass of the thorax,

S the specific heat of tissue,

dT_{th}/dt the warm-up rate at a given T_{th},

C is the conductance of the cuticle,

A the surface area of the thorax exposed to heat losses, and

T_{ex} simply the thoracic temperature excess.

A is difficult to measure alone, and it has been suggested (Heinrich 1975a) that it is best to calculate CA using known estimates for the other variables. When a bee is maintaining its T_{th} at a steady value, then the rate of heat generation equals the rate of heat loss. Thus $[WS\ dT_{th}/dt] = [CAT_{ex}]$, and CA = $[WS\ dT_{th}/dt]$ divided by T_{ex}. W,S and T_{ex} are easy to measure, and dT_{th}/dt is the rate of passive cooling at the T_{ex} that is being maintained, determined from Fig.4.6a. For a female *A. plumipes*, thermoregulating at 36°C at T_a=21°C, CA = [0.065g x 0.8calg^{-1}°C^{-1} x 7°C min^{-1}] ÷ (36-

21°C) = 0.024 cal (or 0.1J) $°C^{-1}$ min^{-1}, slightly lower than the value given by Heinrich (1975a) for queen *Bombus vosnesenskii* (0.044 cal (or 0.18J) $°C^{-1}$ min^{-1}).

The total energy expended during a warm-up is given by the integral of equation 4.1 over the total time required for warm-up, t_f. Thus

$$Hp = WS(VFT-T_a) - CAt_f + \int_0^{t_f} T_{th}dt. \text{ (the area under the warm-up curve)}$$

With an estimate of CA, the total energy reuired to warm-up at any T_a can be calculated once the area under the warm-up curve has been calculated. Table 4.4 (below) gives the values required to calculate the total energy required to warm-up at four T_a's. The results for *A. plumipes* are compared with data obtained in the same way by Heinrich (1975a) for queen *Bombus vosnesenskii* in Fig.4.27. It is clear from Table 4.4 and Fig.4.27 that the lower the T_a, and the longer it takes to raise T_{th} to flight temperatures, the more expensive the warm-up becomes. The total energy required by a female *A. plumipes* is rather less than that required by queen *B. vosnesenskii*. This is due at least in part to the large difference in the masses being warmed. The mean mass of the thorax of queen *B. vosnesenskii* is 0.21g, over three times greater than that of female *A. plumipes*, and the bumblebee therefore needs to expend more energy in order to raise the temperature of its thorax each °C than *A. plumipes* does. *B. vosnesenskii* is also a better thermoregulator before flight than *A. plumipes* is - at low T_a's, the former generates a rather higher T_{ex} than the latter and reaches a T_{th} of near 36°C whatever the T_a (Heinrich 1975a). *A. plumipes* could be said to reduce the total energy required to warm-up by sacrificing its ability to regulate T_{th} within such narrow bounds.

Table 4.4. Calculation of the total energy required to complete warm-up at four ambient temperatures. The figures given are values for an average female (body mass 190-200mg). The specific heat capacity of tissue is taken as 0.8cal$g^{-1}°C^{-1}$ (3.4J$g^{-1}°C^{-1}$), and the thoracic mass as 0.065g. CA is a constant of value 0.024 cal$°C^{-1}min^{-1}$.

Ambient temperature (°C)	VFT (°C)	Time taken to warm from T_a to VFT (min)	Area under warm-up curve (min°C)	Total energy for warm-up (W[cal])
9	31.7	10.75	87.2	14 [3.06]
16	34	3.80	43.5	9.0 [2.15]
21	36	2.10	29.77	6.1 [1.44]
29	38	0.42	5.94	2.6 [0.62]

The inability of some of the smaller *A. plumipes* to warm-up at T_a=9°C indicates that the period of time for which the required metabolic expenditure must be sustained, and the total T_{ex}

which must be generated, clearly place constraints on the conditions under which flight is possible. The danger of being caught in cold spells without sun, or during rain, can also be thought of in terms of the time and energy required for warm-up. In cool cloudy conditions, a wetted *A. plumipes* could not possibly generate sufficient heat both to evaporate the water from its cuticle and to raise T_{th} to the levels required for flight.

D. Patterns of warm-up compared: *A. plumipes* and other endothermic insects.

A. plumipes shows strong positive correlations between warm-up rate and each of T_a, T_{th} and body mass. The positive correlation between warm-up rate and T_a is well documented in other studies on endothermic insects (Heinrich and Bartholomew 1971; Heinrich and Casey 1973; Bartholomew and Casey 1973; Casey *et al.* 1981; Heinrich & MacClain 1986), and the relationship between T_{th} and metabolic rate during warm-up has also been reported (Heinrich and Bartholomew 1971; Heinrich 1975a; Bartholomew *et al.* 1981; Casey *et al.* 1981; Heinrich 1987). To my knowledge no previous workers have shown that T_{th} and T_a are significant independent predictors of warm-up rate, although there are good reasons why this should be so.

The relationship between body mass and warm-up rates has been discussed in the literature, but left unresolved. Most studies to date have been interspecific (e.g. May 1976a; Morgan and Heinrich 1987), and have demonstrated a weak (May 1976a) or strong (Morgan and Heinrich 1987) positive correlation between the two variables. Warm-up rates in *A. plumipes* increased significantly with body mass (Fig.4.4c; a more comprehensive treatment is given in Stone and Willmer 1989b). Body mass correlates positively with mean T_{th} during warm-up, and T_{th} has a clear and well-documented effect on warm-up rate (see above). It is therefore possible that in looking for an effect of body mass only having controlled for T_{th} there is a risk of losing sight of the fundamental importance of body mass.

A notable trend in the warm-up of *A. plumipes* is the change from curvilinear warm-up at low T_a to linear warm-up at higher T_a. This change has also been documented for other endothermic insects (Heinrich 1975, 1987); those which are able to warm up from very low T_a have initial warm-up rates at these T_a's that are very low, as shown for *A. plumipes* (Heinrich 1975, 1987). No other studies have shown the peculiar warm-up pattern (Fig.4.10) that most *A. plumipes* showed at T_a=9°C. The purpose of a pause during warm-up is unclear. It is possible that this form of warm-up curve is an artifact of the pattern of stimulation used to initiate warm-up. This seems unlikely in view of the fact that no similar patterns were seen at any of the other T_a's used, and a constant pattern of stimulation was applied at each T_a. It is also possible that the pause acts as a resting phase before the final metabolic expenditure to flight temperatures - the total energy expenditure during

warm-up at this low T_a is considerably higher than that at higher T_a. During flight in blowflies carbohydrate is utilised more rapidly than it is liberated from the fat body (Clegg and Evans 1961), leading to a fall in haemolymph trehalose concentrations. The drop in warm-up rate in *A. plumipes* may allow liberation of trehalose from the fat body to allow completion of warm-up.

There is also a change in the form of the warm-up curve over time at T_a=21°C in *A. plumipes* from a curvilinear first warm-up to more or less linear warm-ups thereafter (section 4.3a). This change suggests that during the first warm-up the bee's endothermy is in some way modified, resulting in higher metabolic rates at lower T_{th} during subsequent warm-ups. It is possible that such an effect might be caused by release of a modulating hormone such as octopamine. Octopamine levels have been shown to rise during the first few minutes of flight in locusts (Goosey and Candy 1980) and cockroaches (Bailey, Martin and Downer 1983), resulting in increased rates of carbohydrate metabolism (Candy 1978; Whim and Evans 1988) and increases in the force generated by the flight muscles (Whim and Evans 1988). Endothermy occurs in the flight muscles, and involves very high rates of metabolism, and octopamine release during the first warm-up could produce the observed changes in patterns of warm-up. I know of no study of the role of this modulator in endothermy.

E. Abdominal pumping and cooling rates.

The importance of abdominal pumping in supplying oxygen to the flight muscles during warm-up in Hymenoptera has long been known (Sotavalta 1954; Weis-Fogh 1967). While positive correlations have been demonstrated during warm-up between T_{th} and, for example, the frequency of neural 'spikes' in the flight muscles (Heinrich and Kammer 1973) and the rate of activity of the ventral abdominal diaphragm (Heinrich 1976a) in bees (*Bombus*), I know of only one other study that has reported such a correlation between the rate of abdominal pumping and T_{th} (Morgan 1987, for scarab beetles [*Plecoma* spp].). This relationship is easily explained: the greater the T_{ex} which the bee is maintaining over T_a, the higher its rate of heat production must be to counter heat losses (see above), and the greater the required rate of oxygen supply to the tissues. The latter must correlate positively with the rate of abdominal pumping. The negative correlation between body mass and pumping rate can also be explained. It has been demonstrated that at a given T_{ex} a smaller bee, with a higher surface area to volume ratio, will suffer higher rates of heat loss (see section 4.3c above) and therefore require higher rates of oxygen consumption and supply per unit mass per unit time than a larger bee (Bartholomew and Casey 1978).

Having controlled for T_{th} and body mass, males showed lower rates of abdominal pumping than females (section 4.3b). It has also been demonstrated that rates of heat loss from

males, having controlled for body mass, are lower than those from females. If it is assumed that the link between abdominal pumping rates and rates of heat generation is similar for males and females, then these results imply that males require a lower input of heat to warm a unit mass of thorax than females do, other factors being equal. It is also possible that the correlation between abdominal pumping rate and the actual volume of air pumped may vary between individuals due to variation in the volume within the abdomen available for gas exchange. Individuals might vary due to differences in the relative volume of the abdomen occupied by fat body or eggs (P.Miller, personal communication). It is therefore possible that males could be pumping the same volume of air through their spiracles as females whilst telescoping the abdomen at a lower rate.

The lower rates of cooling in males are probably due, at least in part, to the more developed pubescence on the dorsal thorax and abdomen of males discussed in section 4.8. Unlike females, male *A. plumipes* spend much of their time on cool days settled on vegetation or other objects around the nesting site or feeding sites (Chapter 3), only taking off when females arrive at the site or to intercept rival males. Take-off is more or less instantaneous, suggesting that these settled males are maintaining the temperature of the thorax at or near flight temperatures for relatively long periods of time without heat production through active flight. The advantage of lower rates of heat loss in reducing the cost of sustained endothermy at low T_a is clear. Females, which fly for more prolonged periods while foraging, appear to face problems of overheating at higher T_a later in the season (Chapter 3). Since the insulating properties of the pelage of bees cannot be controlled (for instance by the piloerection systems shown by birds and mammals), females must be able to lose heat sufficiently rapidly to enable continued flight during this period, possibly contributing to the evolution of a shorter coat of hairs.

The significant differences between abdominal pumping rates of individuals once T_{th}, sex and body mass have been controlled for remain unexplained. It is possible that there are genuine differences between individuals in the relationship between T_{th} and body mass, or that factors which were not measured (such as crop nectar contents) affected warm-up rate. It is also possible that such differences are artifacts due to variation in my experimental techniques - such as the degree of damage inflicted on the bee by insertion of the thermocouple.

In general the thermal conductance of the cuticle of *A. plumipes* is very similar to that of other highly endothermic insects (Table 4.5).

Table 4.5. Cooling rates and thermal conductance during passive cooling in endothermic insects.

Study animal	Source	Cooling constant ($Ca\ g^{-1}min^{-1}°C^{-1}$)	Conductance ($°Cmin^{-1}°C^{-1}$).(	Conductance $Wg^{-1}°C^{-1}$)
Moths				
Cuculiine moths	Heinrich (1987)	0.55	0.031	0.44
Sphingids & Saturniids	Bartholomew & Epting (1975)	0.35	0.019	0.28
Noctuids & Geometrids	Casey & Joos (1983)	0.50	0.028	0.4
Beetles				
Plecoma spp.	Morgan (1987)	0.48	0.027	0.39
Bees				
Anthophora plumipes	**this thesis**	**0.53**	**0.03**	**0.42**
Euglossine bees	May (1976a)	0.46	0.026	0.37
Xylocopa varipuncta	Heinrich & Buchmann (1986)	0.4	0.023	0.32
Xylocopa capitata	Chappell (1982)	0.42	0.024	0.34
Centris pallida	Chappell (1984)	0.6	0.034	0.49
Bombus spp.	Heinrich & Heinrich (1983)	1.25	0.070	1.00

F. Exhaustion and endothermy.

The effect of feeding on warm-up in *Anthophora plumipes* is not surprising. Radiolabelled glucose appears in the blood of blowflies less than 30 seconds after ingestion (Clegg and Evans 1961). Absorption of the sugars from the gut is not active, but requires a concentration gradient between gut and haemolymph (Treherne 1957). Absorbed sugars are rapidly incorporated into trehalose (Treherne 1957; Clegg and Evans 1961; Friedman 1967). Wigglesworth (1949) implicated substrate depletion as a cause of flight exhaustion in blowflies, and feeding with glucose rapidly restores flight ability in blowflies flown to exhaustion (Hudson 1958; Clegg and Evans 1961). Although the fuel for flight and endothermy in several insects has been shown to be carbohydrates mobilised from the fat body (Ziegler and Schultz 1986; Joos 1987), some insects are thought to use carbohydrates from the gut during flight (Van Handel and Nayar 1972; Surholt and Newsholme 1983; Ziegler and Schultz 1986). It is therefore quite possible that the response shown by *Anthophora plumipes* to feeding was due to availability of renewed energy sources, and that poor performance prior to feeding may have been due to low energy supplies. This is thus a direct observation of the effects of energy limitation on thermogenesis. The rapid release of ingested sugars for flight is also relevant to the observation that male *A. plumipes* carry so little nectar in their

crop (see Chapter 3). As long as floral nectar is available to them, they need not expend unnecessary energy in carrying it in their crops.

G. How good a thermoregulator is *A. plumipes*?

If the gradient of the regression of T_{th} on T_a is used as the indicator of thermoregulatory ability, as discussed in Chapter 2, then it is clear that the thermoregulatory ability of *A. plumipes* depends on whether T_{th} before or during flight is used. The gradient of the regression of VFT on T_a for female *A. plumipes* is 0.24, and the gradient for SFT data 0.49. 'Grab-and-stab' data give a gradient of 0.62. Comparison of VFT on the one hand and SFT and 'grab-and-stab' results on the other indicates that regulation of T_{th} is better prior to flight than it is during flight. Table 4.6 summarises data for a selection of endothermic bees, moths and beetles. In every case where T_{th} both before and during flight have been recorded, thermoregulation before flight is far better than it is during flight. This result shows that while these endothermic insects are clearly able to regulate T_{th} in still air before flight, they are unable to compensate entirely either for the high rates of convective cooling during flight at low T_a, or the heat generated by flight muscle activity at high T_a. Some insects, such as bumblebees (Table 4.6), are good thermoregulators both before and during flight, while others such as *A. plumipes* only regulate well before flight. Why should an insect maintain thoracic temperatures above those it can sustain in flight? At any rate heat production, because of convective cooling experienced in flight, a bee must have a higher T_{th} before take-off than in free flight. Higher thoracic temperatures than can be maintained in flight may have independent selective advantages. If higher muscular power and levels of sensory coordination are required for take-off than for normal flight, then higher T_{th} at take-off may be selected for regardless of the cooling that follows. It is also possible that higher T_{th} in non-flying *A. plumipes* at low T_a have advantages independent of flight; they may contribute to a greater ability to detect mates or predators in resting males, or to digging ability while excavating nests in females. No data on the body temperatures of non-flying *A. plumipes* in the field were obtained.

Comparison of the gradients of best-fit regressions of T_{th} on T_a for insects sampled during flight shows that, compared to other endothermic insects, *Anthophora plumipes* is an average thermoregulator. It cannot match the levels of thermoregulation shown by bumblebees (Heinrich 1972a,b, 1975; Table 4.6), or by the much larger carpenter bees in the genus *Xylocopa* (Heinrich and Buchmann 1986; Baird 1986; Willmer 1988; Table 4.6). *A. plumipes* shows a similar gradient to *Apis mellifera* (Cooper *et al.* 1985; Heinrich 1979), and to the euglossine bees investigated by May and Casey (1983). *A. plumipes* nonetheless manages to fly over a wide range of T_a, and particularly down to very low temperatures. This is at least in part due to the low minimum T_{th} this

Table 4.6 Gradients of regressions of thoracic temperature on ambient temperature, at take-off and/or during flight, in endothermic insects. All the gradients stated are from statistically significant regressions. In the cases indicated, gradients have been estimated from figures. Data for SFT and VFT in *A. plumipes* are gradients of the mean of each statistic over T_a for males and for females at the 4 T_a used, giving a sample size of 8. Data for SFT and VFT in *A. sapiens* and *C. frontalis* (Chapter 5) are gradients of mean values (see Chapter 5) at each T_a over T_a. Bee families and authorities are as in Tables 6.1 and 6.2.

Species	Source		Pre- or during	gradient	sample size	R^2	p	Given regression or estimate.
Bees								
Anthophora plumipes	**this thesis**	(VFT)	**pre**	**0.20**	**8**	**0.941**	**<0.001**	
	this thesis	(SFT)	**during**	**0.52**	**8**	**0.960**	**<0.001**	
	this thesis	(G+S)	**during**	**0.62**	**81**	**0.930**	**<0.001**	
Amegilla sapiens	this thesis (Chapter 5)	(VFT)	pre	0.61	4	0.997	<0.001	
	this thesis (Chapter 5)	(SFT)	during	0.98	4	0.924	<0.020	
	this thesis (Chapter 5)	(G+S)	during	0.54	115	0.671	<0.001	
Creightonella frontalis ♀	this thesis (Chapter 5)	(VFT)	pre	0.46	5	0.878	<0.050	
	this thesis (Chapter 5)	(SFT)	during	0.87	5	0.988	<0.001	
	this thesis (Chapter 5)	(G+S)	during	0.68	84	0.774	<0.001	
Thyreus quadrimaculatus	this thesis (Chapter 5)	(G+S)	during	0.74	28	0.571	<0.001	
Xylocopa pubescens	Willmer (1988)		during	0.20	19	0.194	<0.05	given
Xylocopa sulcatipes	Willmer (1988)		during	0.31	24	0.748	<0.001	given
Xylocopa virginica	Baird (1986)		during	0.23	126		<0.0001	given
Centris pallida	Chappell (1984)		during	0.40	163		sig.	estimate
Apis mellifera	Cooper *et al.* (1985)		pre	1.00	398		sig.	estimate
	as above		during	0.37	452		sig.	estimate
Apis mellifera	Heinrich (1979)		pre	1.00	31		sig.	estimate
	as above		during	0.55	84		sig.	estimate
Bombus edwardsii (w)	Heinrich (1972c)		during	0.13	40		sig.	estimate
Bombus terricola (w)	Heinrich (1972b)		during	0.14	72		sig.	estimate
Bombus vosnesenskii (Q)	Heinrich (1975a)		during	0.27	21		sig.	estimate
Euglossa spp.	May & Casey (1983)		during	0.50	20	0.640	<0.001	given

Table 4.6 (cont.) Gradients of regressions of thoracic temperature on ambient temperature, at take-off and/or during flight, in endothermic insects. All the gradients stated are from statistically significant regressions. In the cases indicated, gradients have been estimated from figures. Bee families and authorities are as in Tables 6.1 and 6.2.

Species	Source	Pre- or during	gradient	sample size	R^2	p	Given regression or estimate.
Euglossa imperialis	as above	during	0.71	36	0.422	<0.001	given
Euglossa nigrita as above	as above	during	0.40	24	0.152	<0.050	given
Eulaema meriana as above	as above	during	0.53	13	0.330	<0.050	given
Moths							
Cuculiine moths	Heinrich (1987)	during	0.29			sig.	estimate
Malacosoma americanum	Casey *et al.* (1981)	pre	0.11	22		sig.	estimate
	Casey (1981a)	during	0.60	24		sig.	estimate
Manduca sexta	Heinrich & Bartholomew (1971)	pre	0.10	36		sig.	estimate
Beetles							
Pachnoda sp.	Heinrich & McClain (1986)	pre	1.00			sig.	estimate
	as above	during	0.70		0.884	<0.001	given
Geotrupes sp.	Krogh & Zeuthen (1941)	pre	0.10			sig . .	estimate

species will tolerate in flight. Minimum T_{th} in flight of about 25°C are well below the minimum T_{th} for flight recorded for *Apis mellifera* (Cooper *et al.* 1985) and the bumblebee species investigated to date (e.g. 30°C for *Bombus edwardsii*, Heinrich 1975a). The cuculiinine moths of the genera *Lithophane* and *Eupsilia*, which are able to warm-up from temperatures near 0°C and have a similar body mass to *A. plumipes*, maintain a minimum T_{th} of around 30°C in flight at T_a=0°C (Heinrich 1987). It appears that through evolving a low minimum T_{th} for flight, *A. plumipes* has compromised on the high cost of maintaining a high T_{ex} at low T_a. A reduction in flight performance would be expected at these low T_{th}, but *A. plumipes* appeared to fly faster, and demonstrated a greater sensitivity to moving objects (other bees, birds, cats) than bumblebees (which were probably maintaining a higher T_{th}) did at similar low T_a.

This chapter demonstrates that *A. plumipes* is an extremely endothermic bee, with rates of warm-up and levels of metabolic heat generation exceeding those of most other insect endotherms. That endothermic ability need not correlate with thermoregulatory ability is shown by the fact that *A. plumipes* is only capable of moderate regulation of T_{th} during flight. Thermoregulatory ability is also shown to depend on which measurement of T_{th} (SFT, VFT and 'grab-and-stab') is regressed on T_a. As well as confirming the predicted role of T_a in determining warm-up rates and body temperatures, individual body mass is also shown to be of considerable importance in the thermal biology of *A. plumipes.*

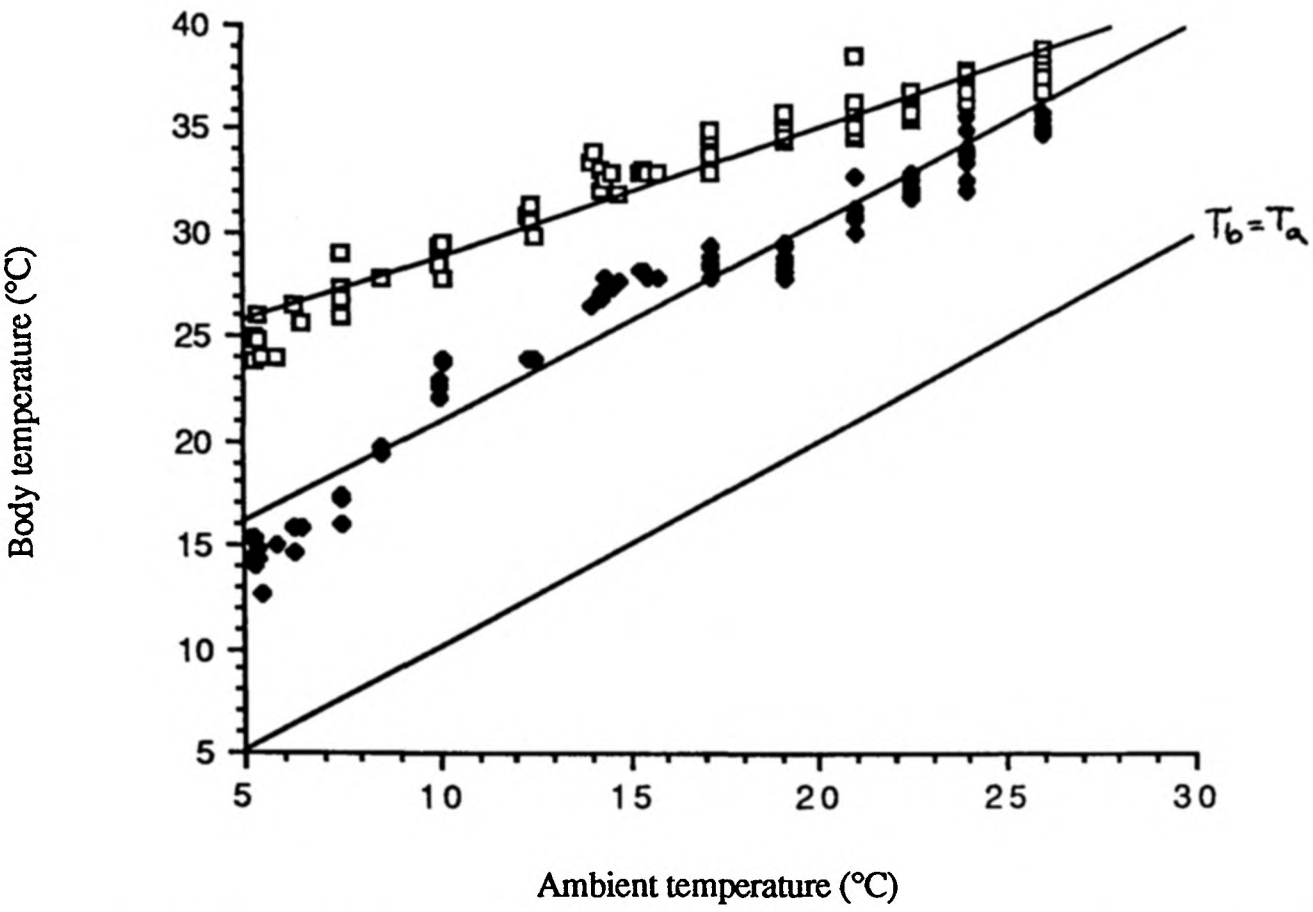


Fig.4.1 Thoracic (❐) and abdominal (◆) temperatures in male and female *A. plumipes* as functions of ambient temperature (T_{th}: y=22.7+0.62x, R^2 =0.940; T_{ab}: y=11.15+0.96x, R^2 =0.940).

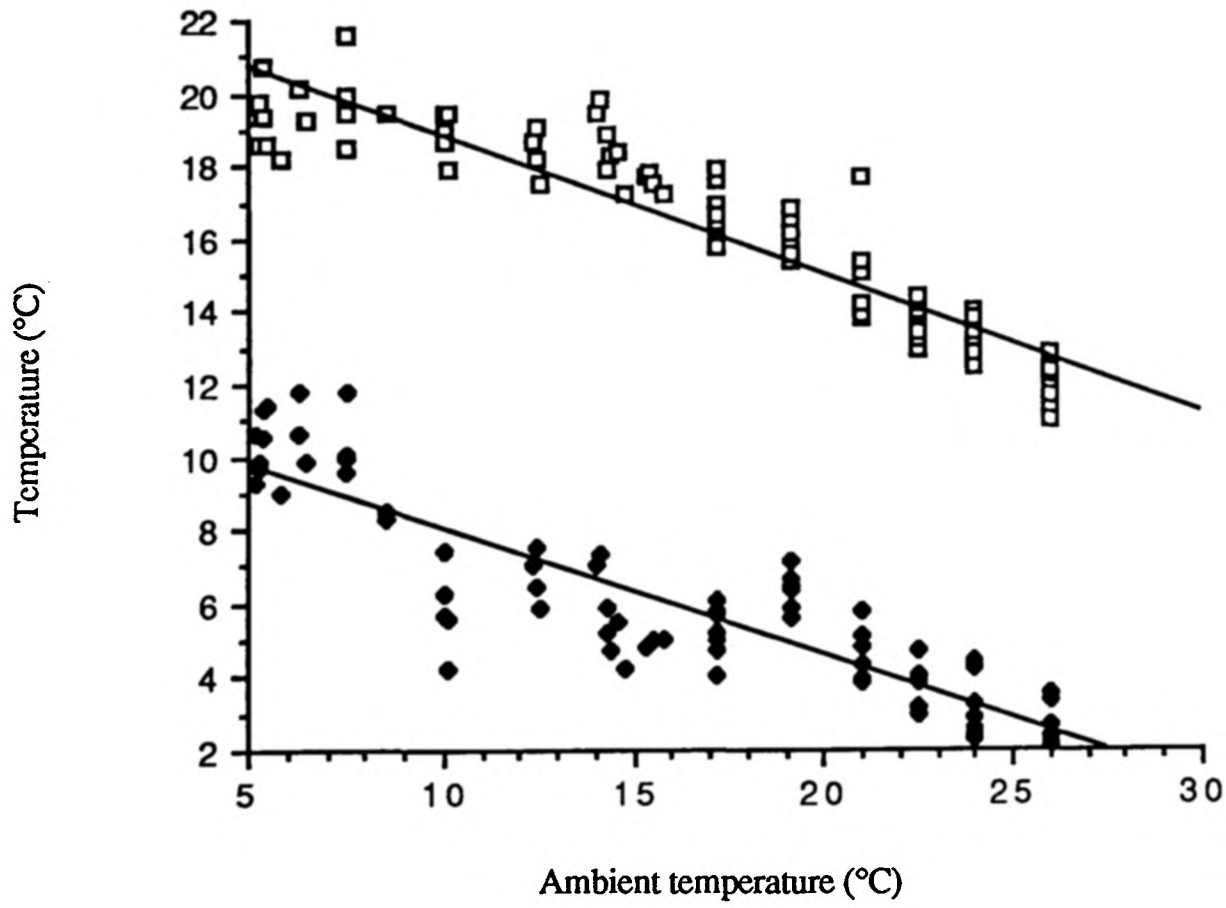


Fig.4.2 Thoracic temperature excess (❐) and the temperature difference between thorax and abdomen (◆) in male and female *A. plumipes* as functions of ambient temperature (T_{ex}:.y=22.66-0.38x, R^2 =0.860; T_{dif}: y=11.5-0.34x, R^2 =0.792).

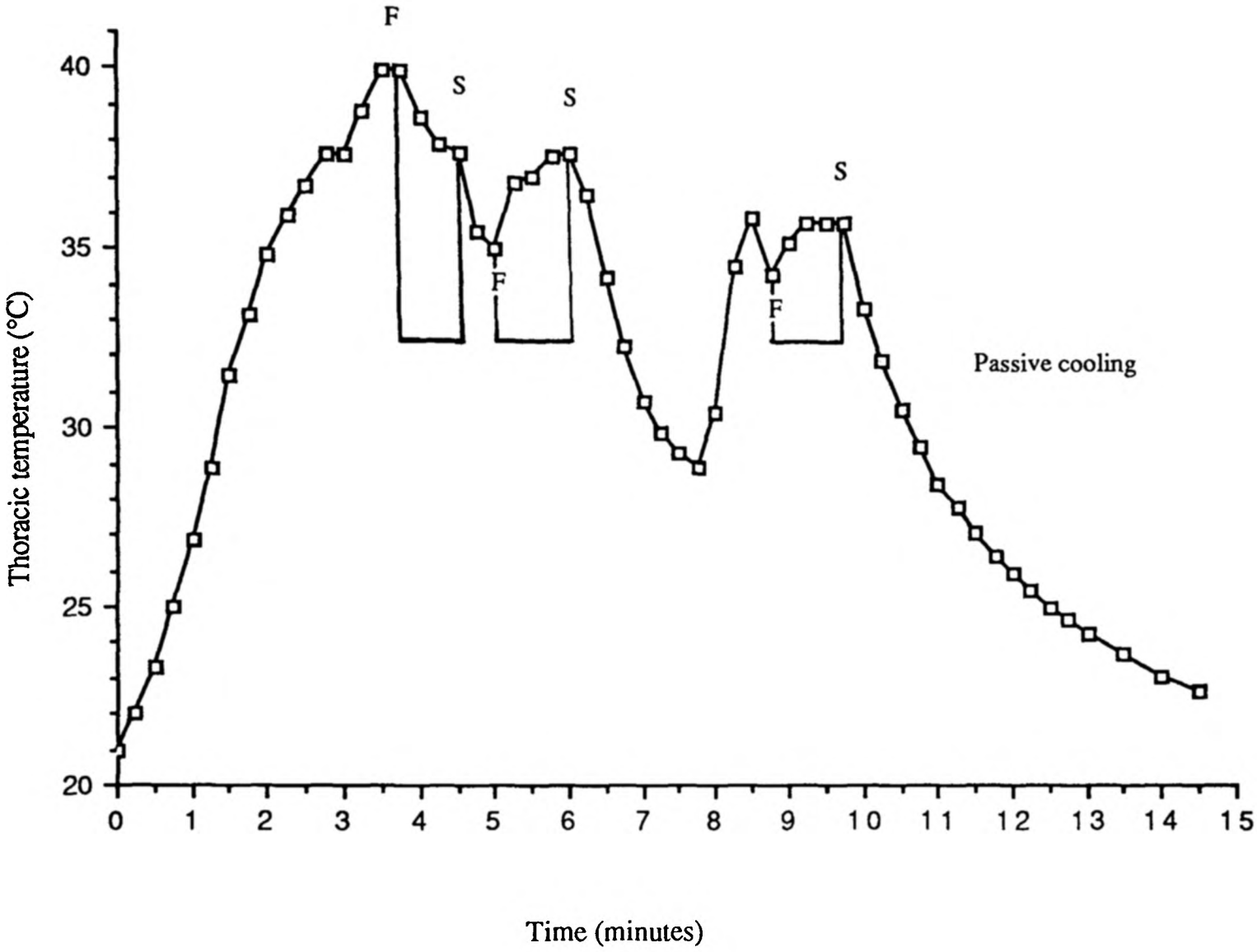
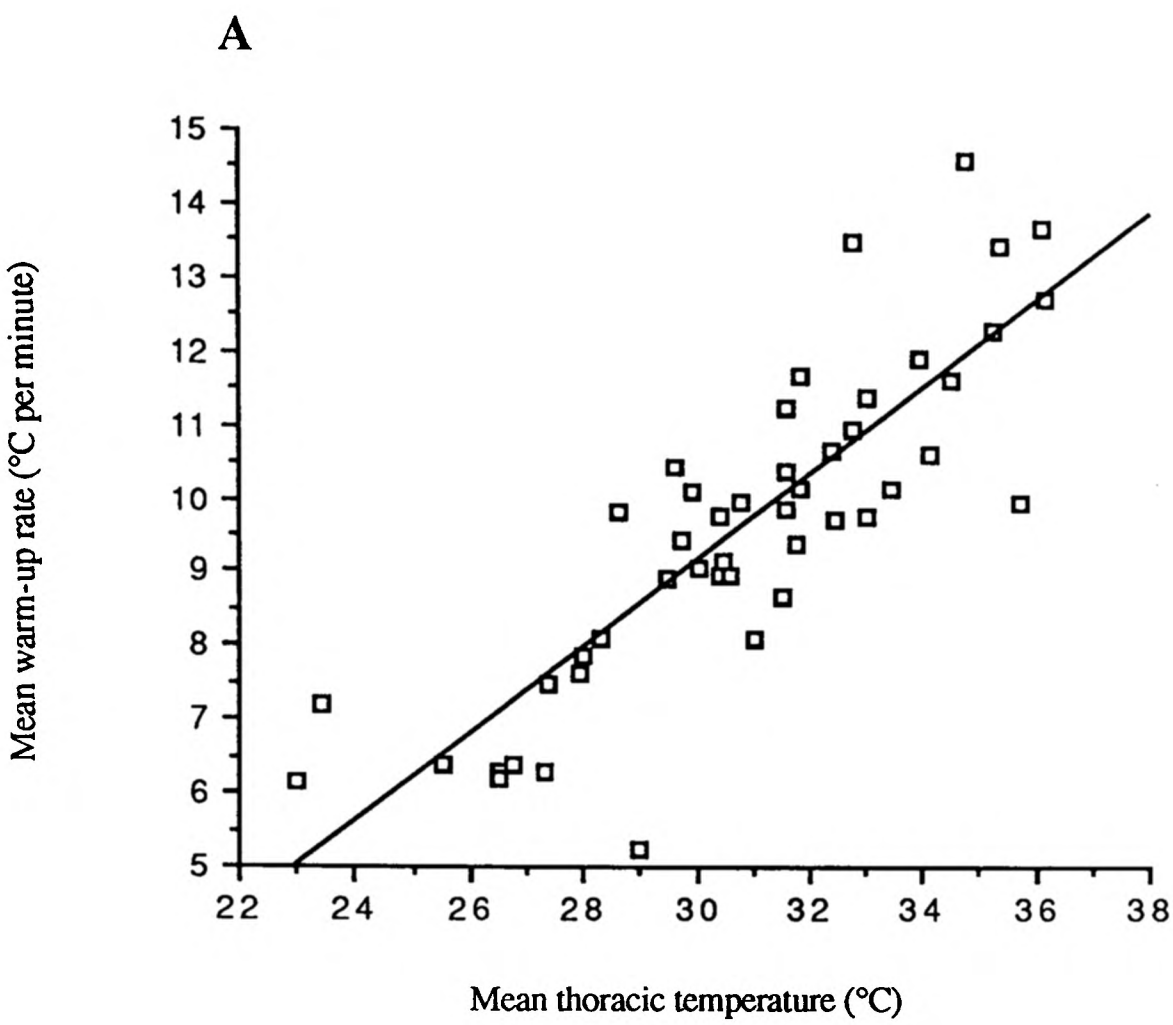


Fig.4.3 Thoracic temperature over time for a female *A. plumipes* during warm-up at T_a=21°C. Initiation of flight is indicated by 'F', and cessation of flight by 'S'.



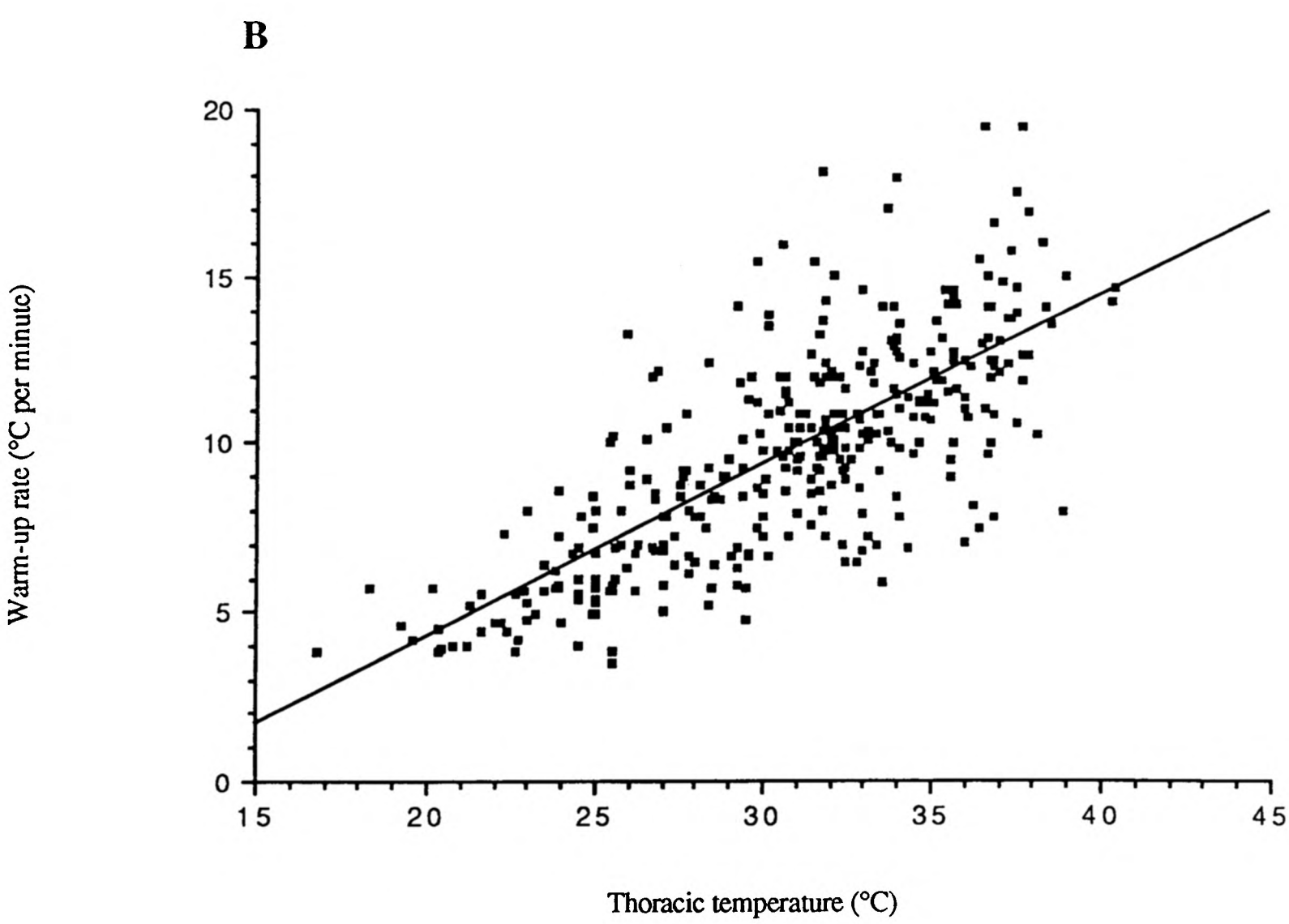
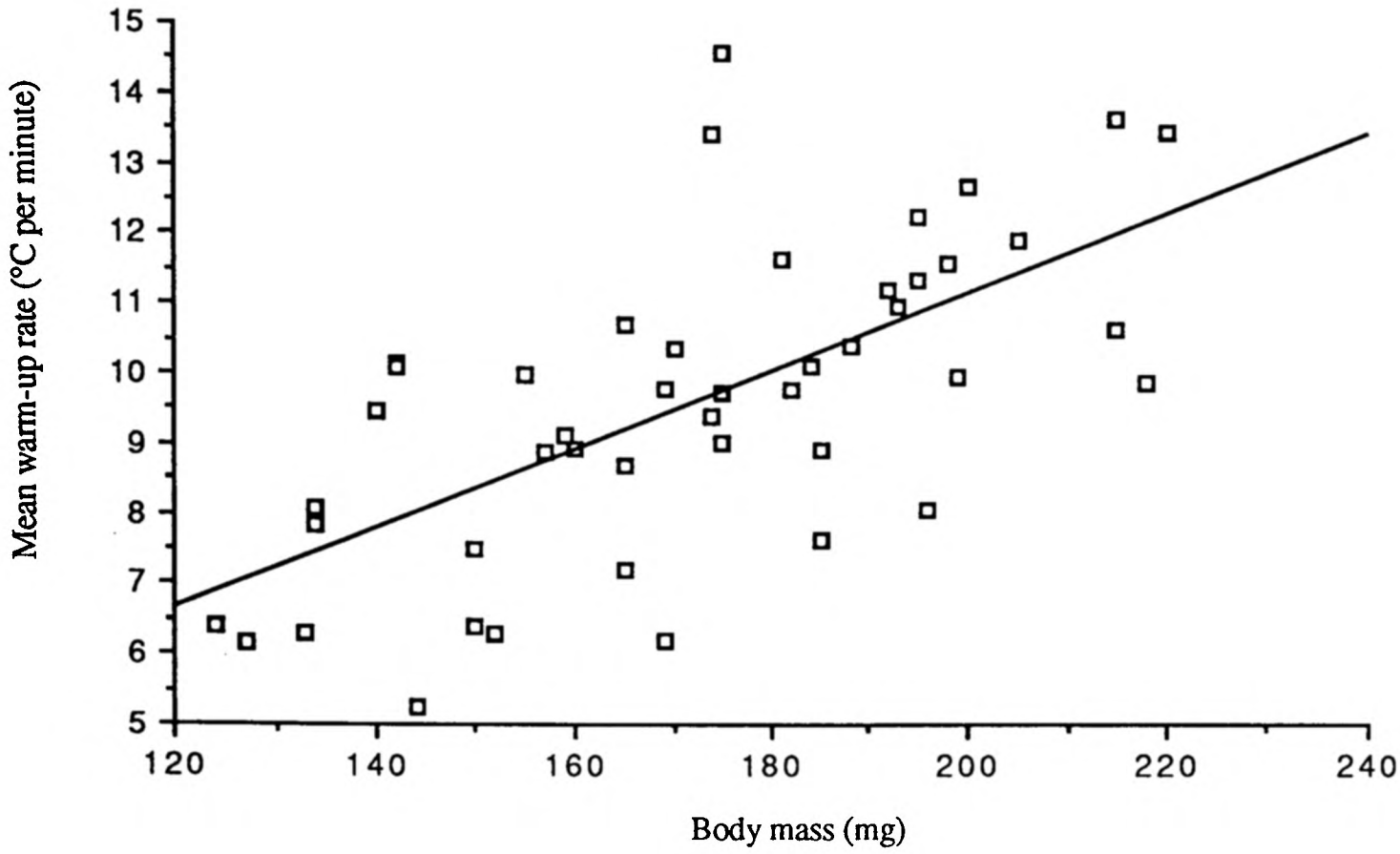


Fig.4.4 (a) Individual mean warm-up rate as a function of individual mean thoracic temperature in *A. plumipes* at T_a=21°C (y=-8.6+0.59x, R^2 =0.722). **(b)** Warm-up rate as a function of thoracic temperature in *A. plumipes* at T_a=21°C (y=-5.9+0.51x, R^2 =0.548).

C



D

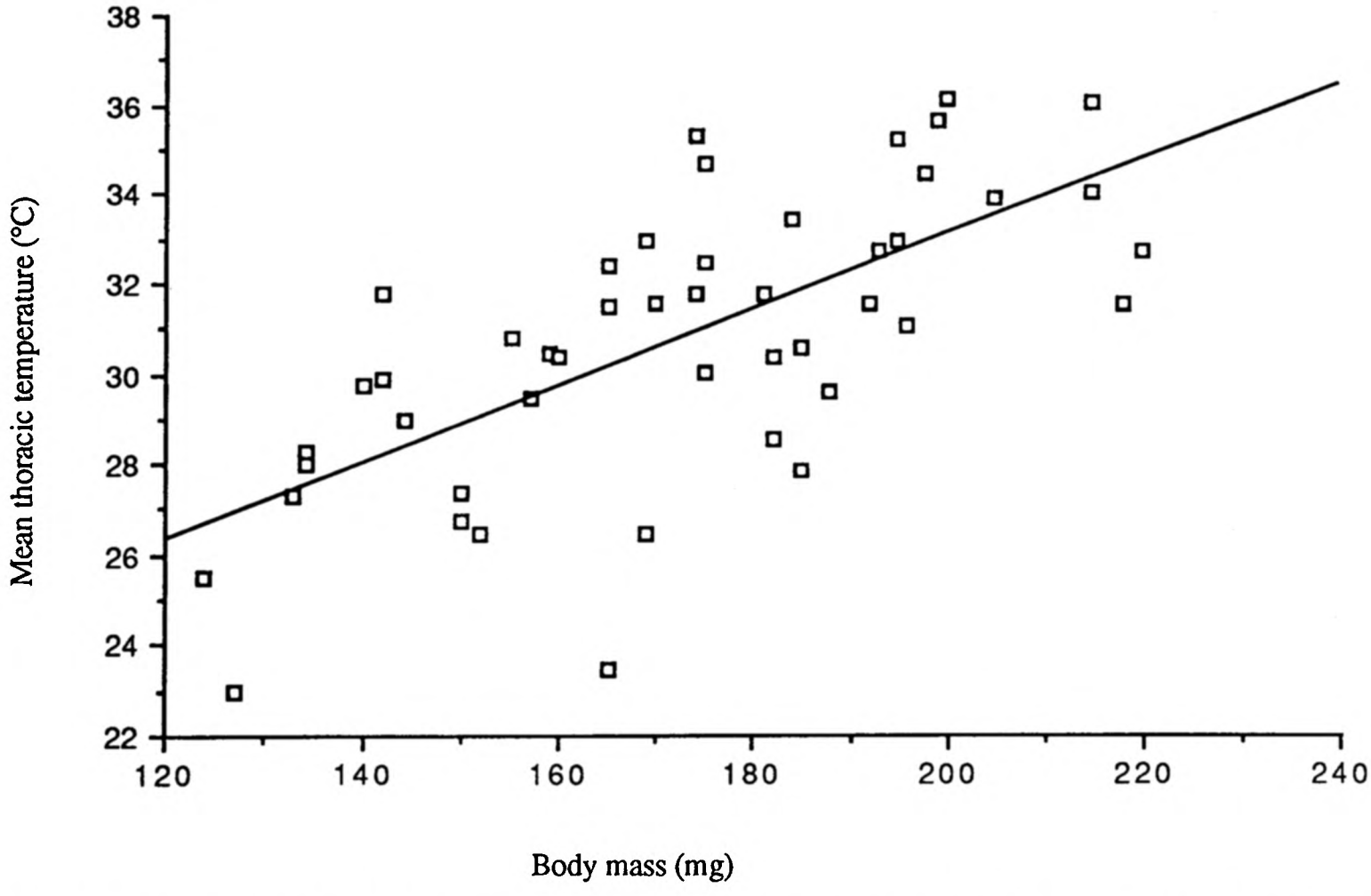


Fig.4.4 (cont.) **(c)** Individual mean warm-up rate as a function of body mass for *A. plumipes* at T_a=21°C (y=-0.21+0.06x, R^2 =0.422). **(d)** Individual mean thoracic temperature as a function of body mass in *A. plumipes* at T_a=21°C (y=16.13+0.08x, R^2 =0.462).

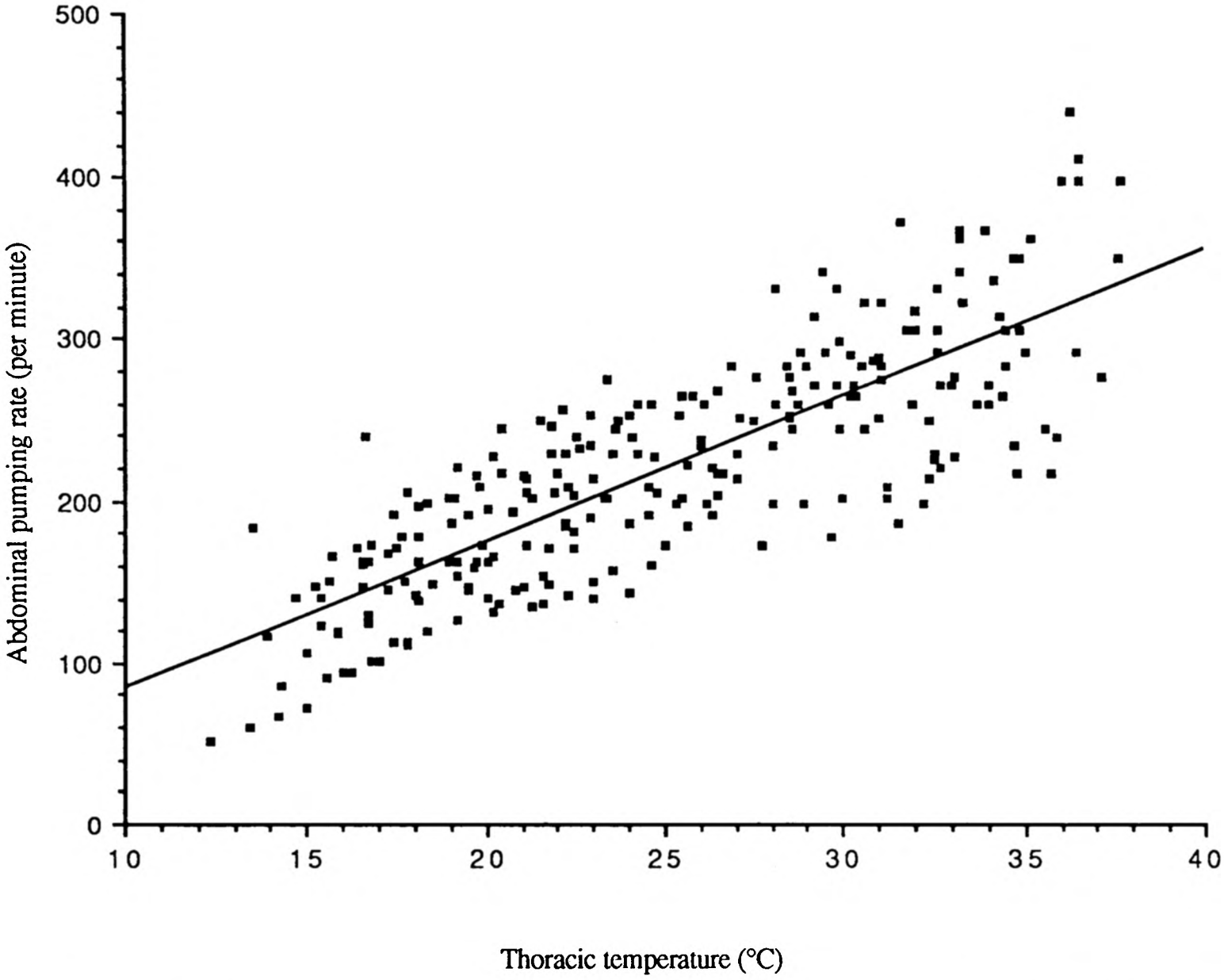


Fig.4.5 Abdominal pumping rate as a function of thoracic temperature in male and female *A. plumipes* at T_a=9°C and T_a=21°C (y=-7.93+9.17x, R^2 =0.673).

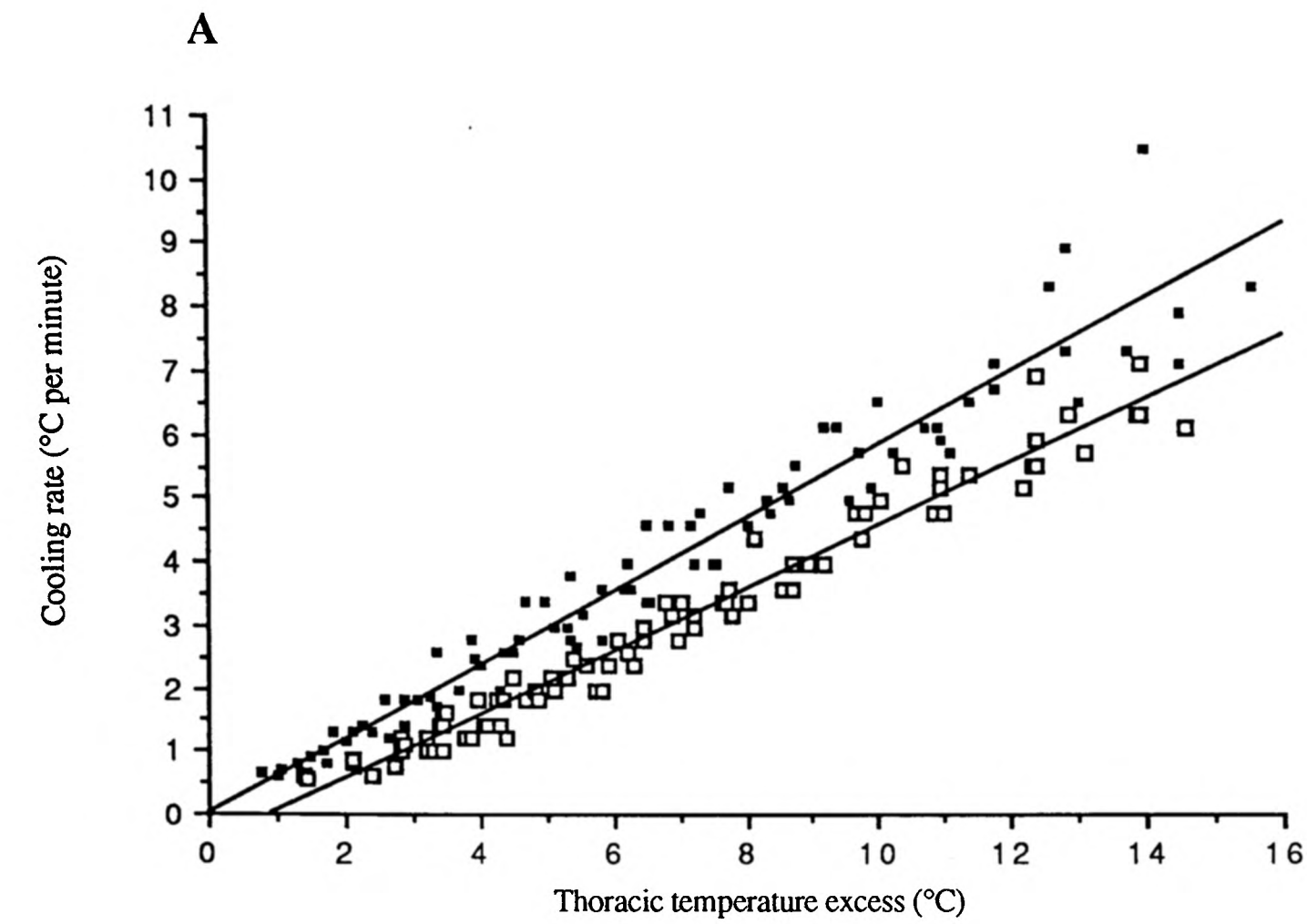


Fig.4.6 (a) Cooling rate as a function of thoracic temperature excess in 2 female (190, 194mg) (❐) (y=-0.46+0.51x, R^2 =0.960) and 3 male (100,115,121mg) (■) (y=-0.025+0.6x, R^2 =0.941) *A. plumipes*. **(b)** The cooling constant as a function of thoracic mass in *A. plumipes* (y=0.82-0.005x, R^2 =0.410).

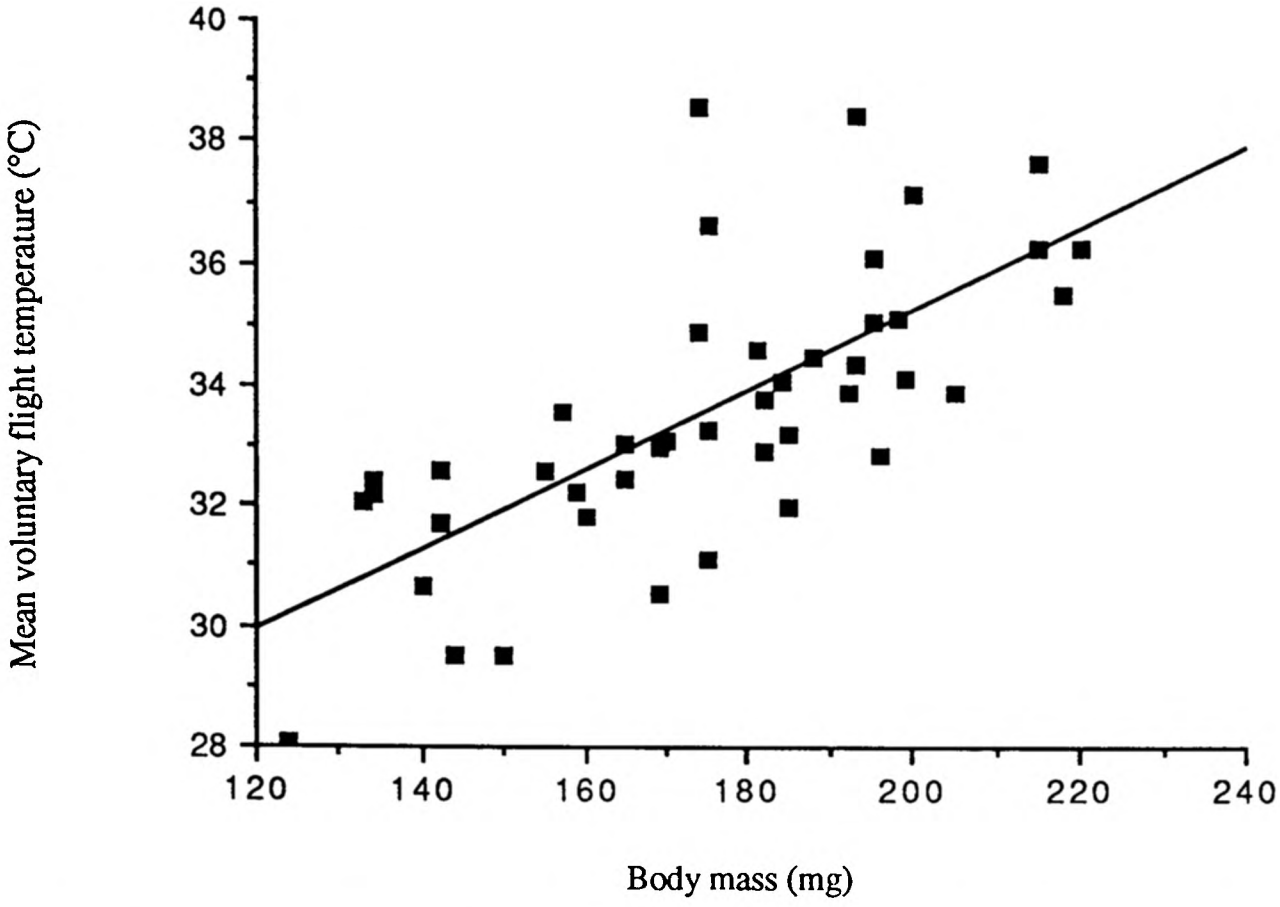


Fig.4.7 Mean individual VFT as a function of body mass in *A. plumipes* at T_a=21°C (y=21.9+0.07x, R^2 =0.518).

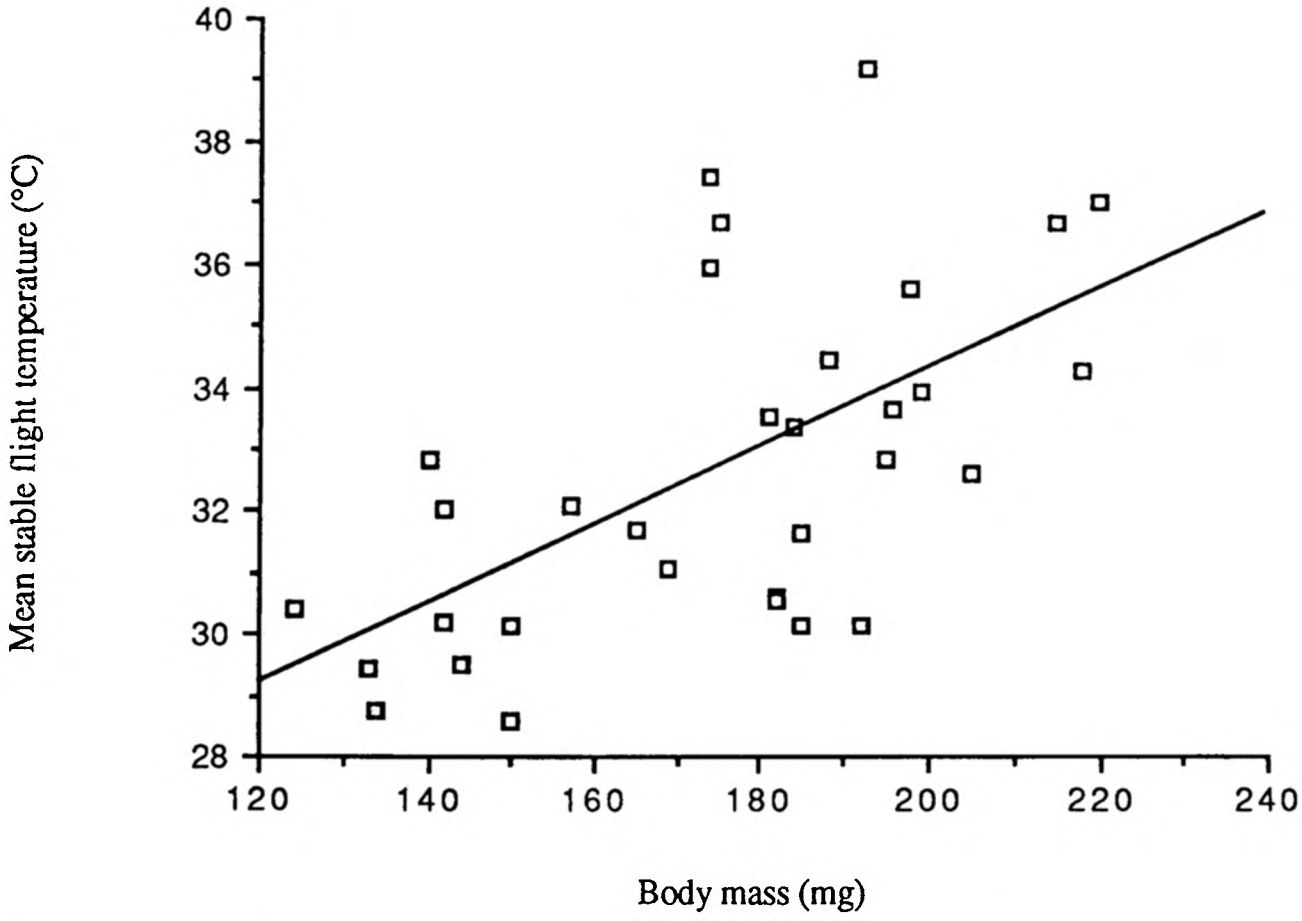
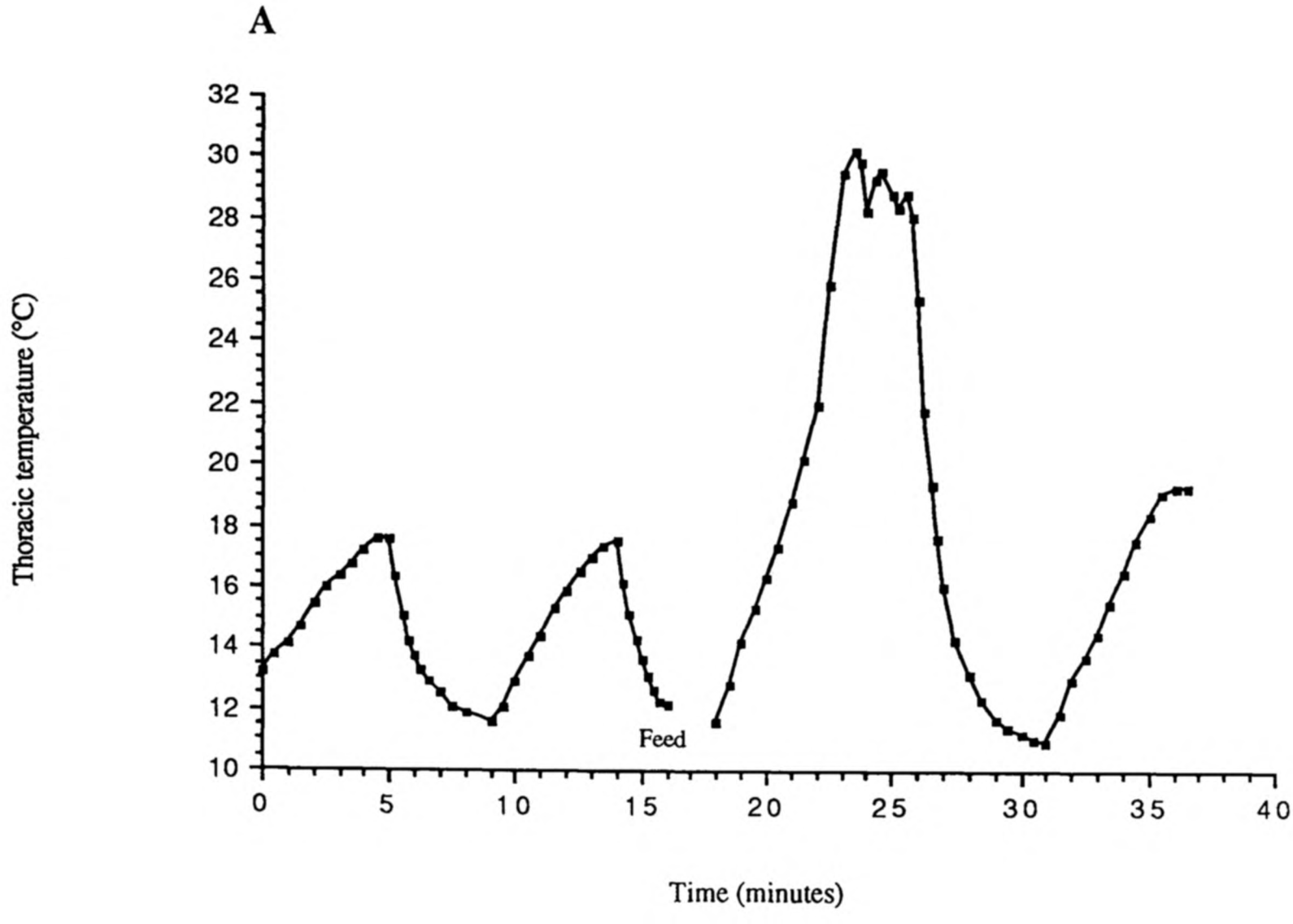


Fig.4.8 Mean individual SFT as a function of body mass in *A. plumipes* at T_a=21°C (y=21.6+0.06x, R^2 =0.360).



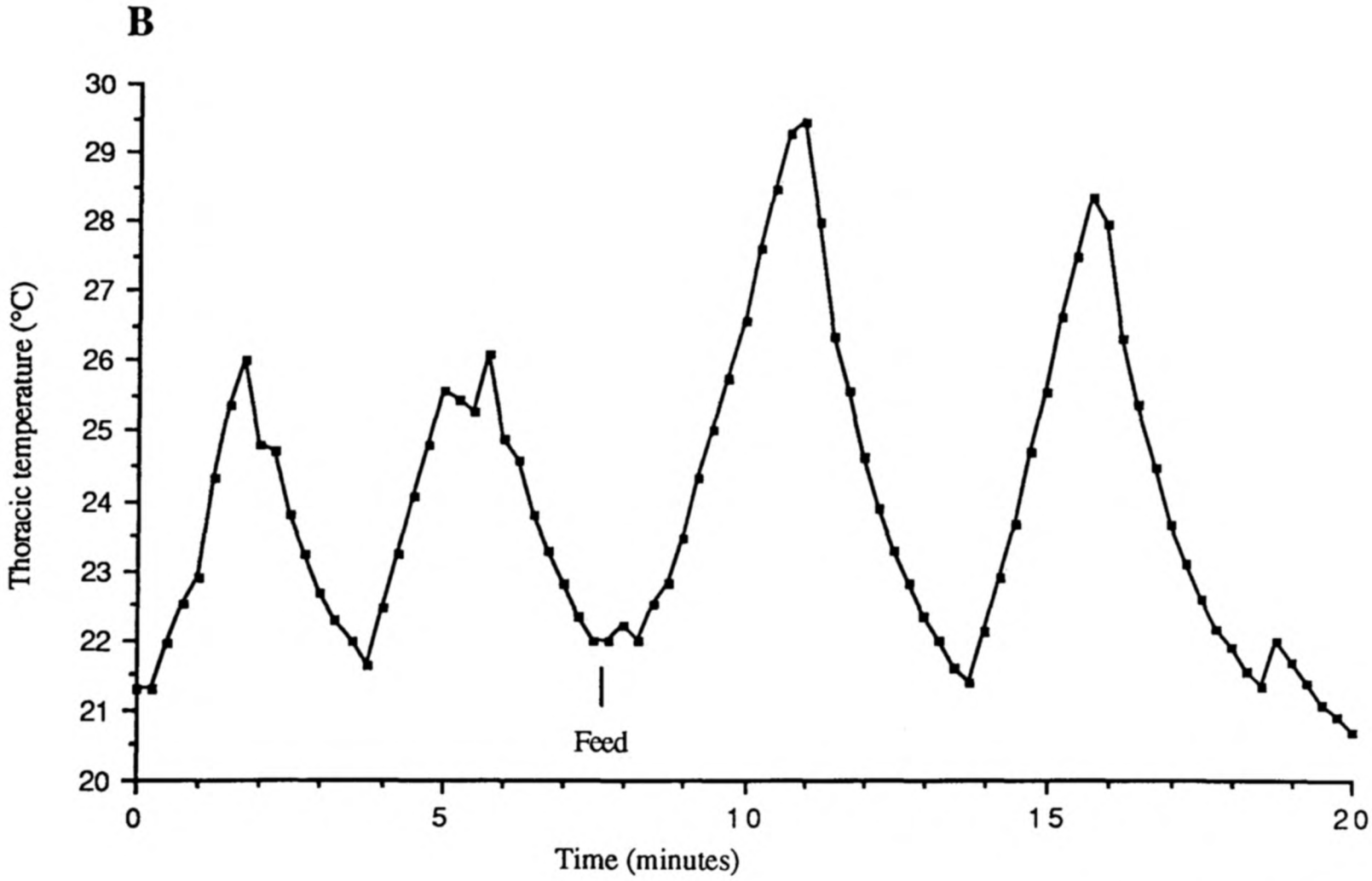


Fig.4.9 Thoracic temperature over time during successive warm-ups and coolings before and after *ad libitum* feeding with sucrose solution in (a) a 135mg male *A. plumipes* and (b) in a 121mg female *Andrena fulva* (Andrenidae).

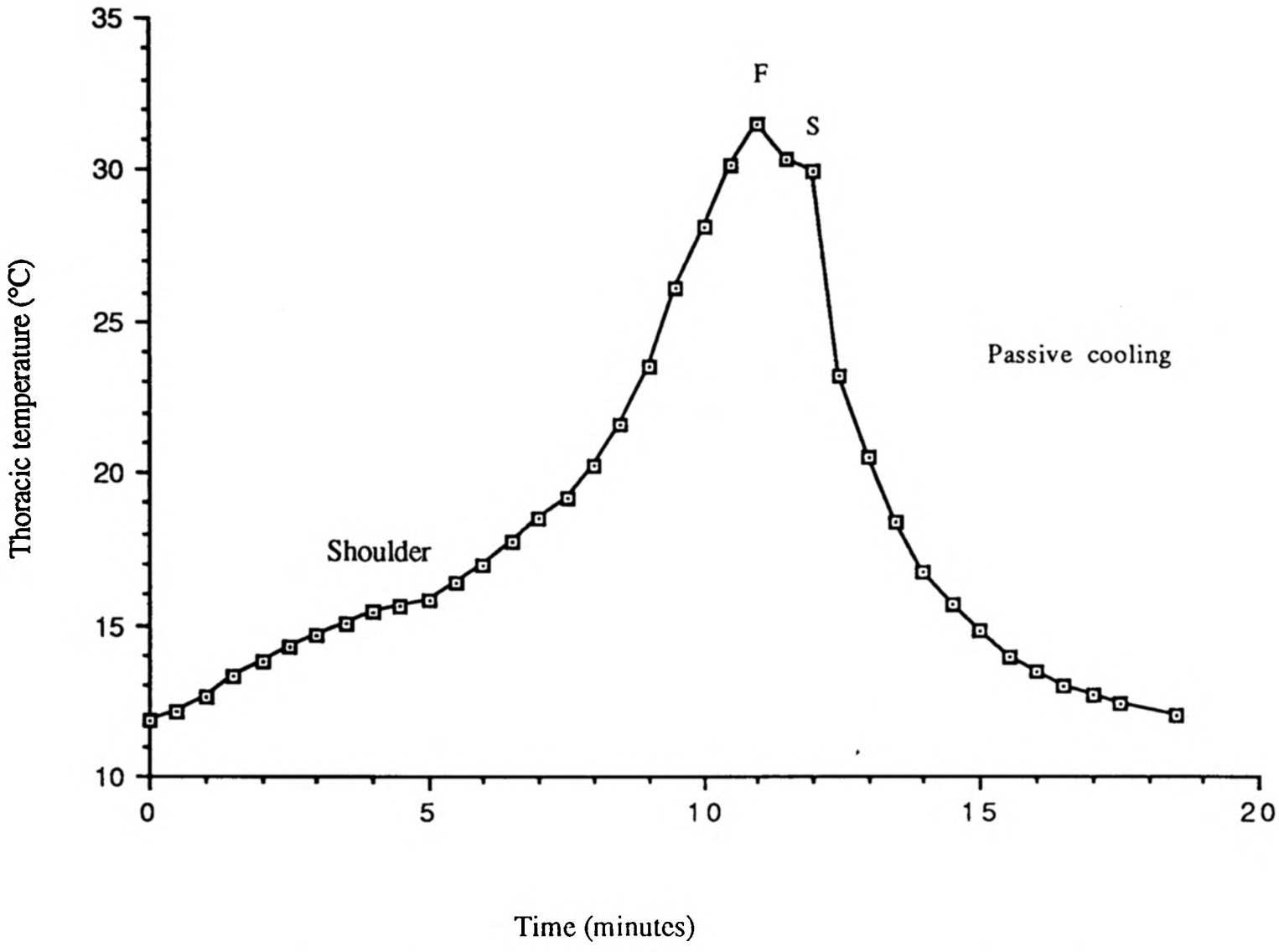


Fig.4.10 Thoracic temperature over time for a female *A. plumipes* during warm-up at T_a=9°C. Initiation of flight is indicated by 'F', and cessation of flight by 'S'.

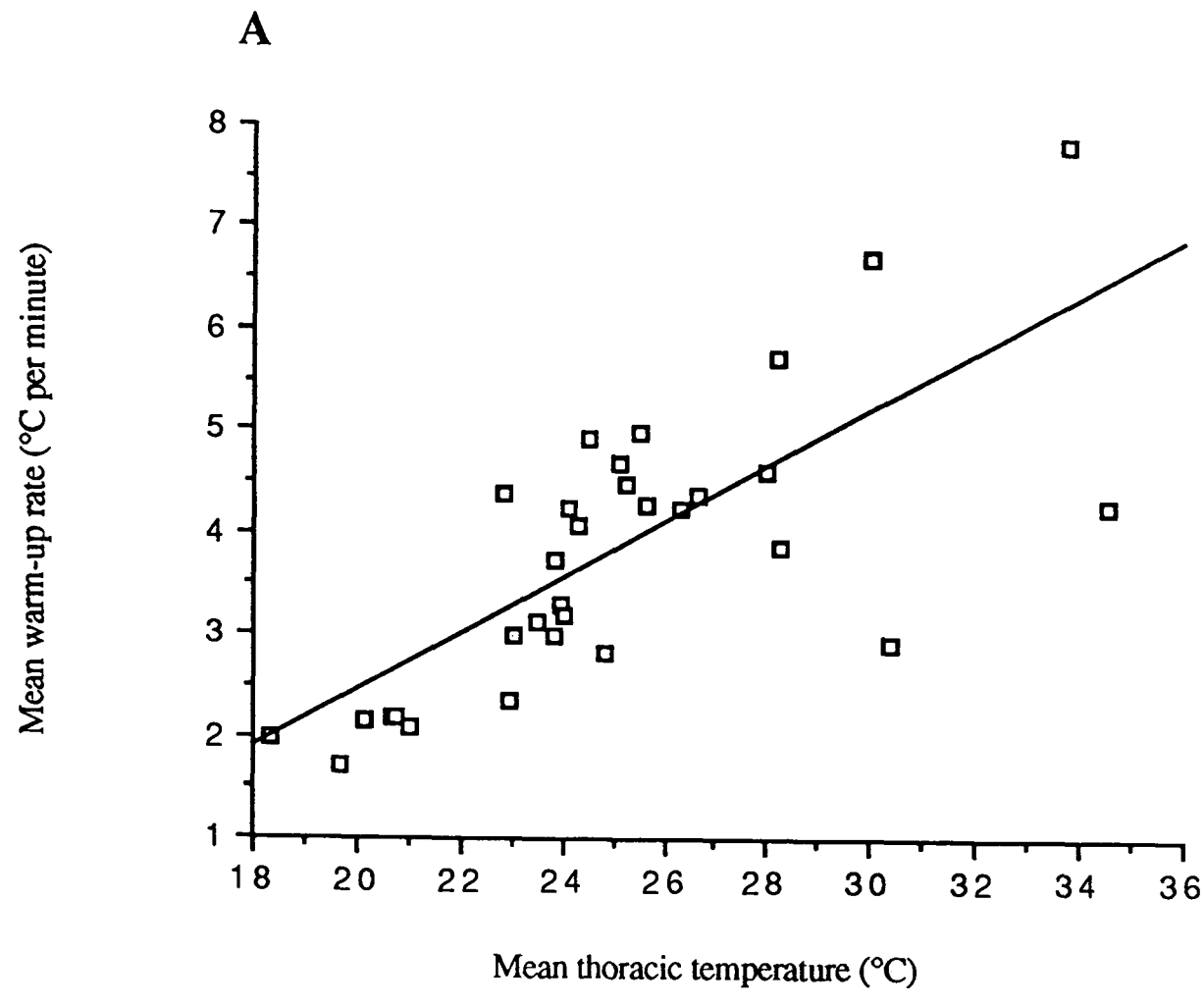


Fig.4.11 (a) Individual mean warm-up rate as a function of individual mean thoracic temperature in *A. plumipes* at T_a=9°C (least squares regression: y=-3.14+0.28x, R^2 =0.548).(**b**) Warm-up rate as a function of thoracic temperature in *A. plumipes* at T_a=9°C (y=-2.5+0.26x, R^2 =0.706).

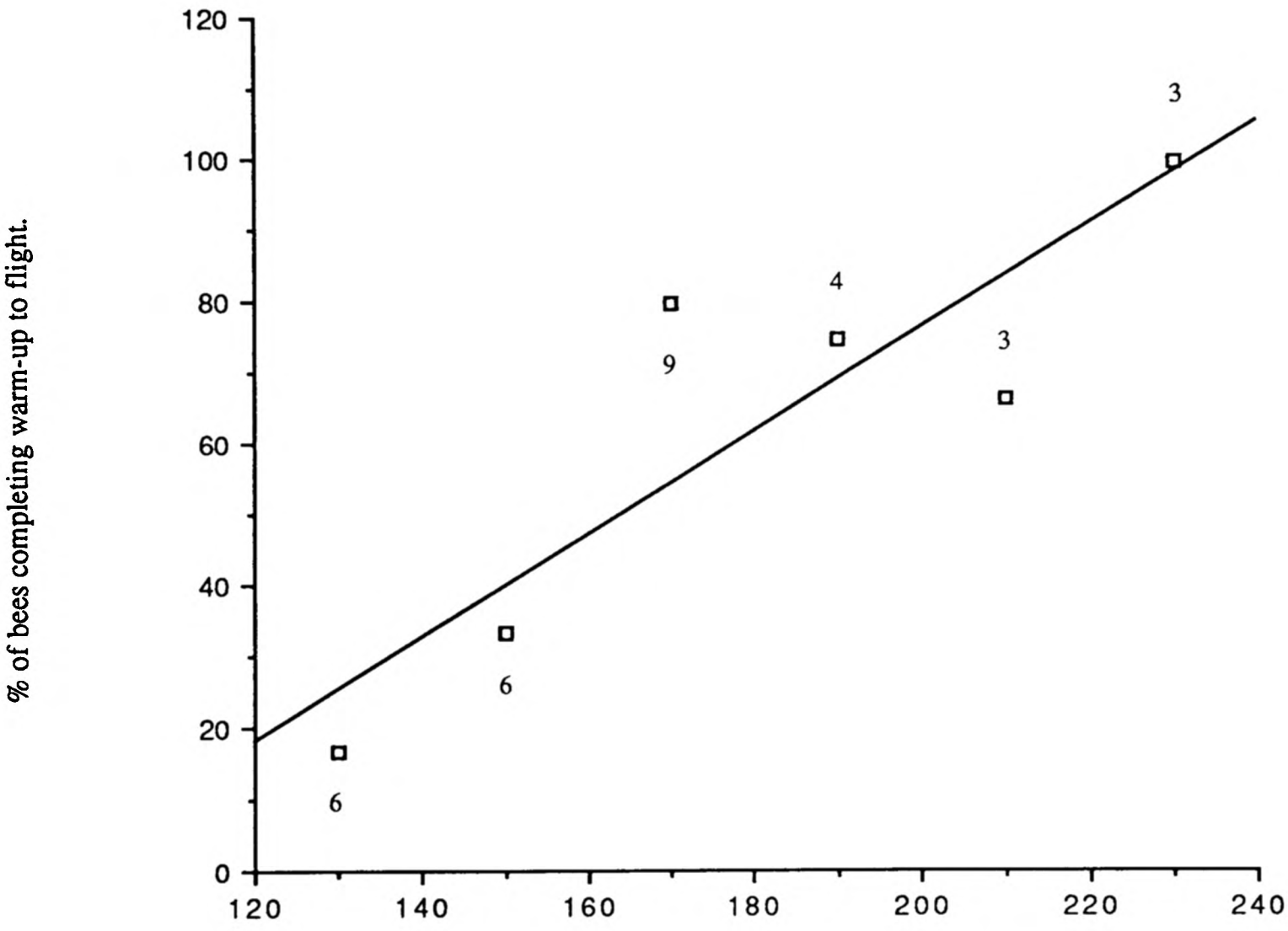


Fig.4.12 The percentage of *A. plumipes* attempting warm-up which reach flight temperatures at T_a=9°C as a function of body mass (y=-69.6+0.73x, R^2 =0.774). The number of bees in each mass interval is shown next to the data point.

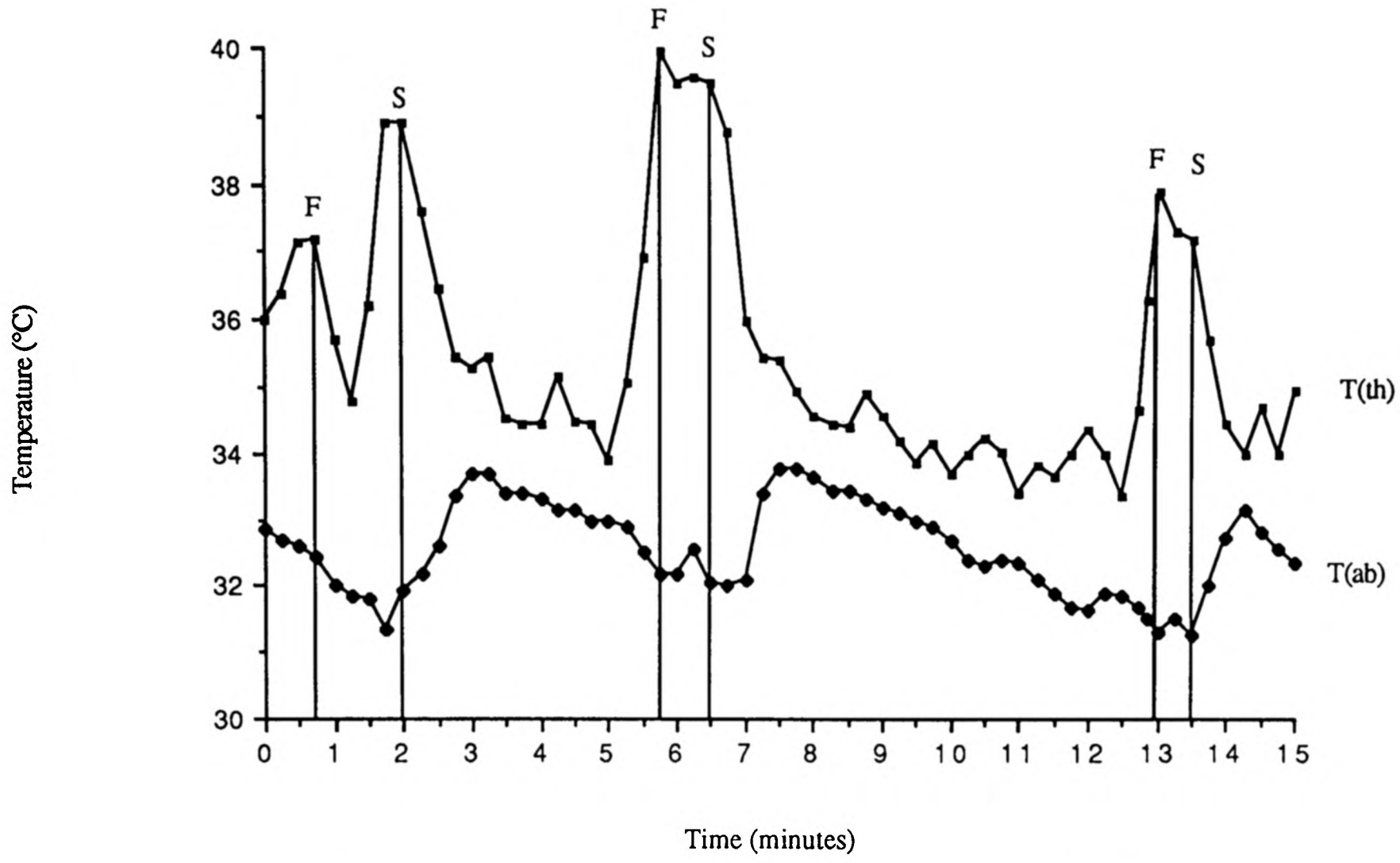


Fig.4.13 Thoracic and abdominal temperatures over time for a queen *Bombus pratorum* at T_a=21°C. Solid bars indicate the duration of tethered flight.

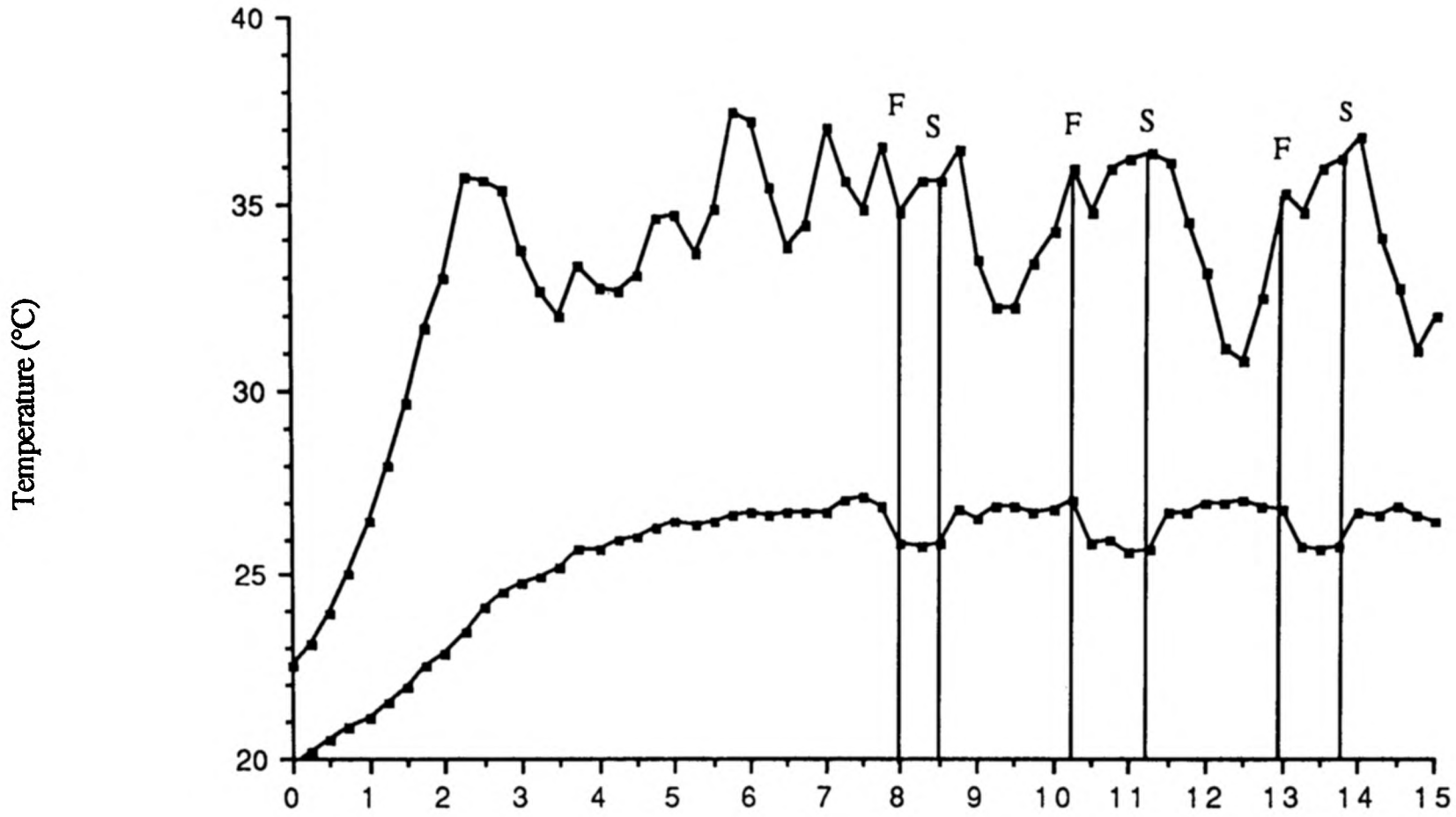


Fig.4.14 Thoracic and abdominal temperatures over time for a male *A. plumipes* at T_a=21°C. Solid bars indicate the duration of tethered flight.

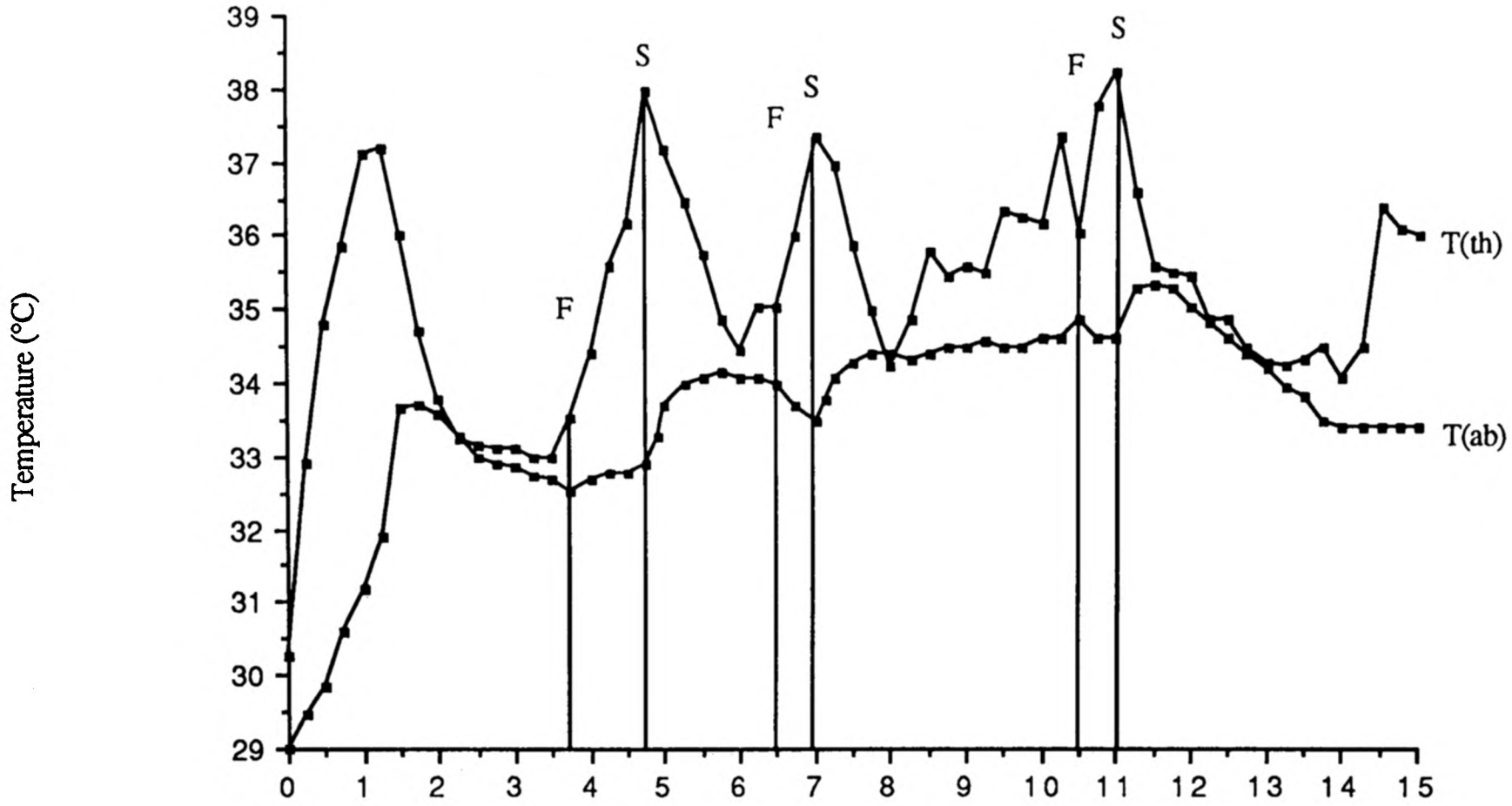


Fig.4.15 Thoracic and abdominal temperatures over time for a female *A. plumipes* at T_a=29°C. Solid bars indicate the duration of tethered flight.

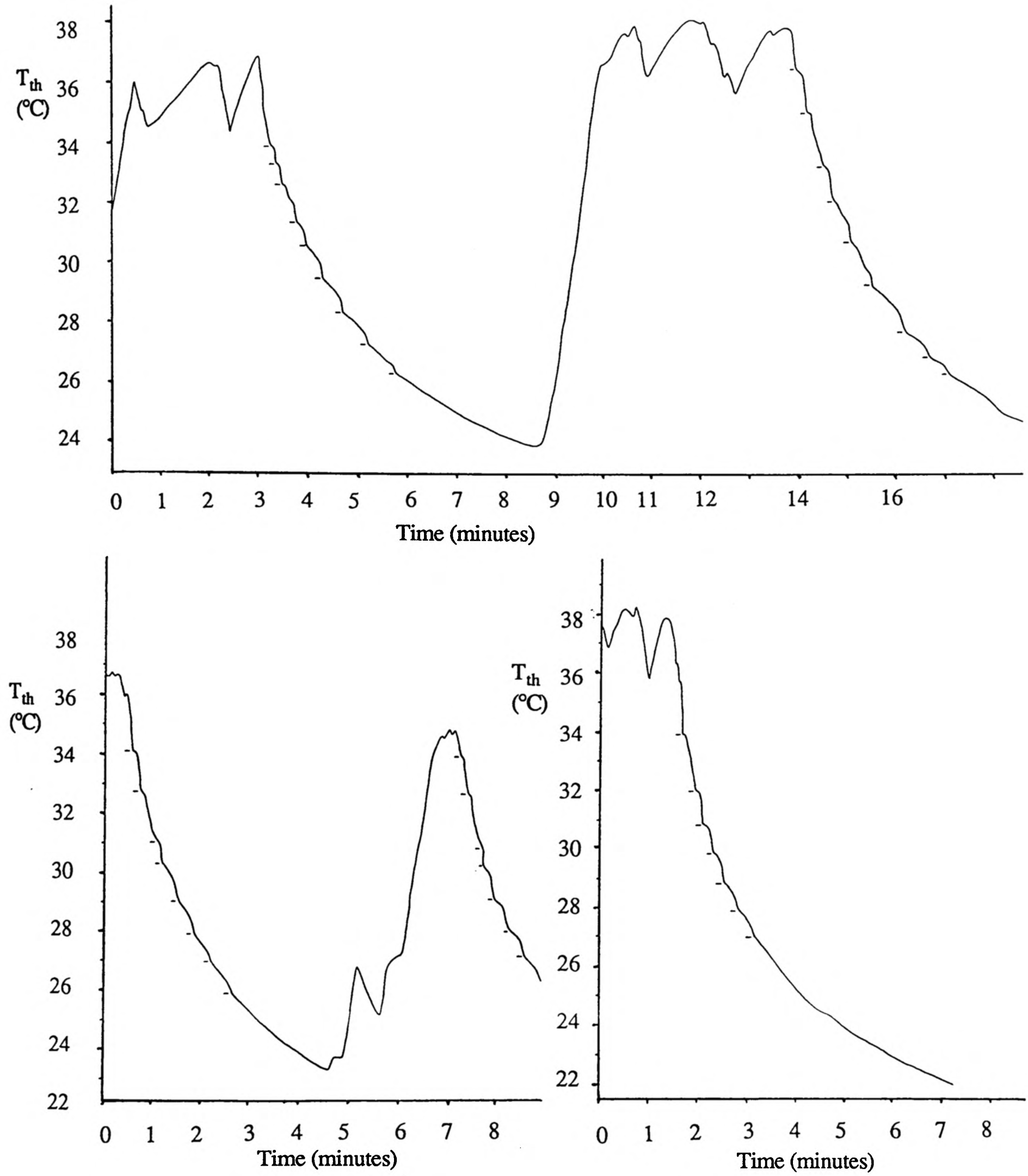


Fig.4.16 Stepped cooling after flight; (a) in a female *Anthophora libyphaenica* (body mass 255mg) at Avdat, Israel; (b) in 2 female *A.plumipes*; upper trace body mass 220mg, lower trace body mass 215mg; All warm-ups were obtained at an ambient temperature of 21°C.

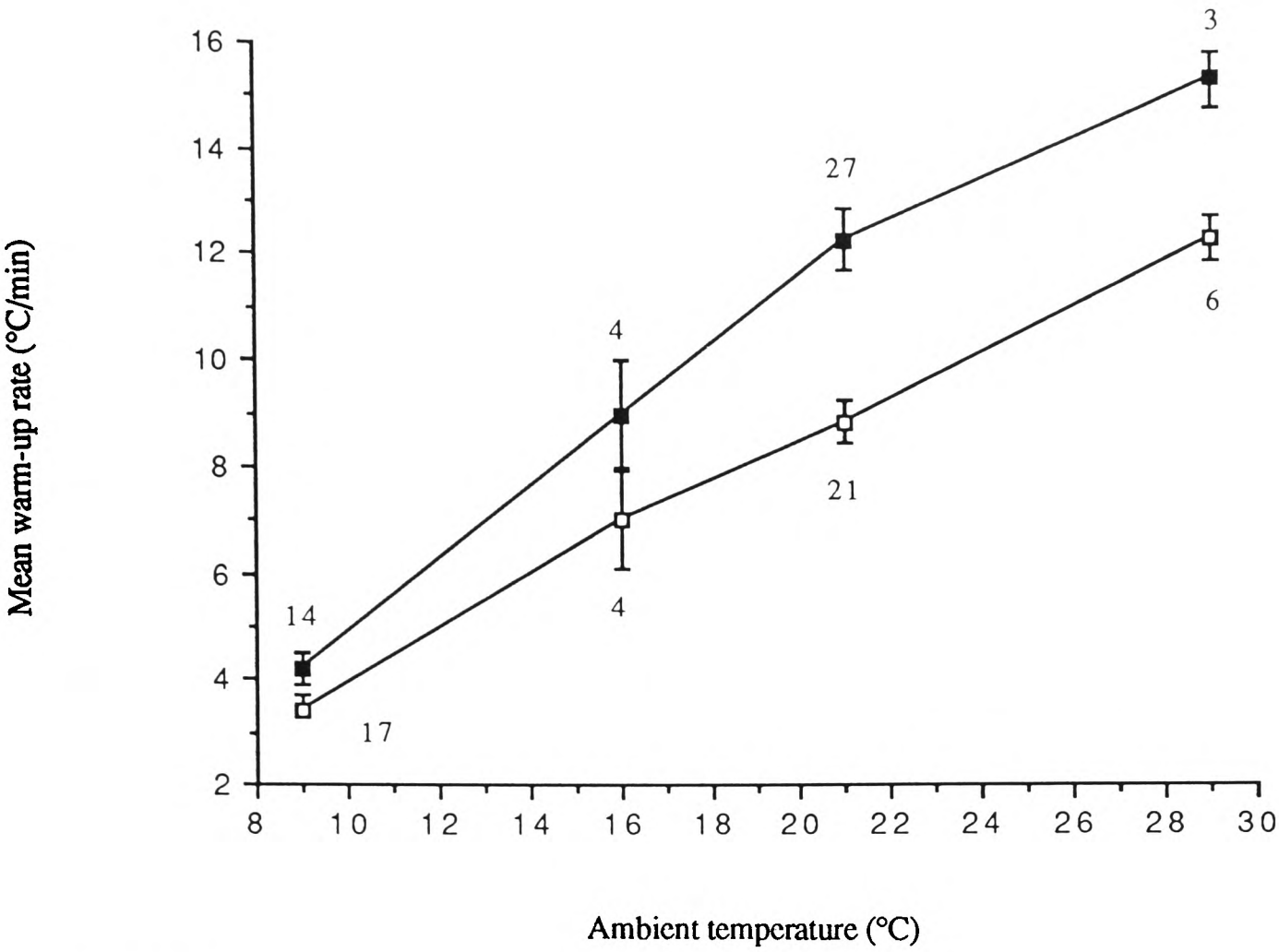


Fig.4.17 Mean warm-up rate as a function of ambient temperature for male (❐) and female (■) *A. plumipes*. The number of individual mean warm-up rates contributing to the overall mean at each ambient temperature is shown by each data point.

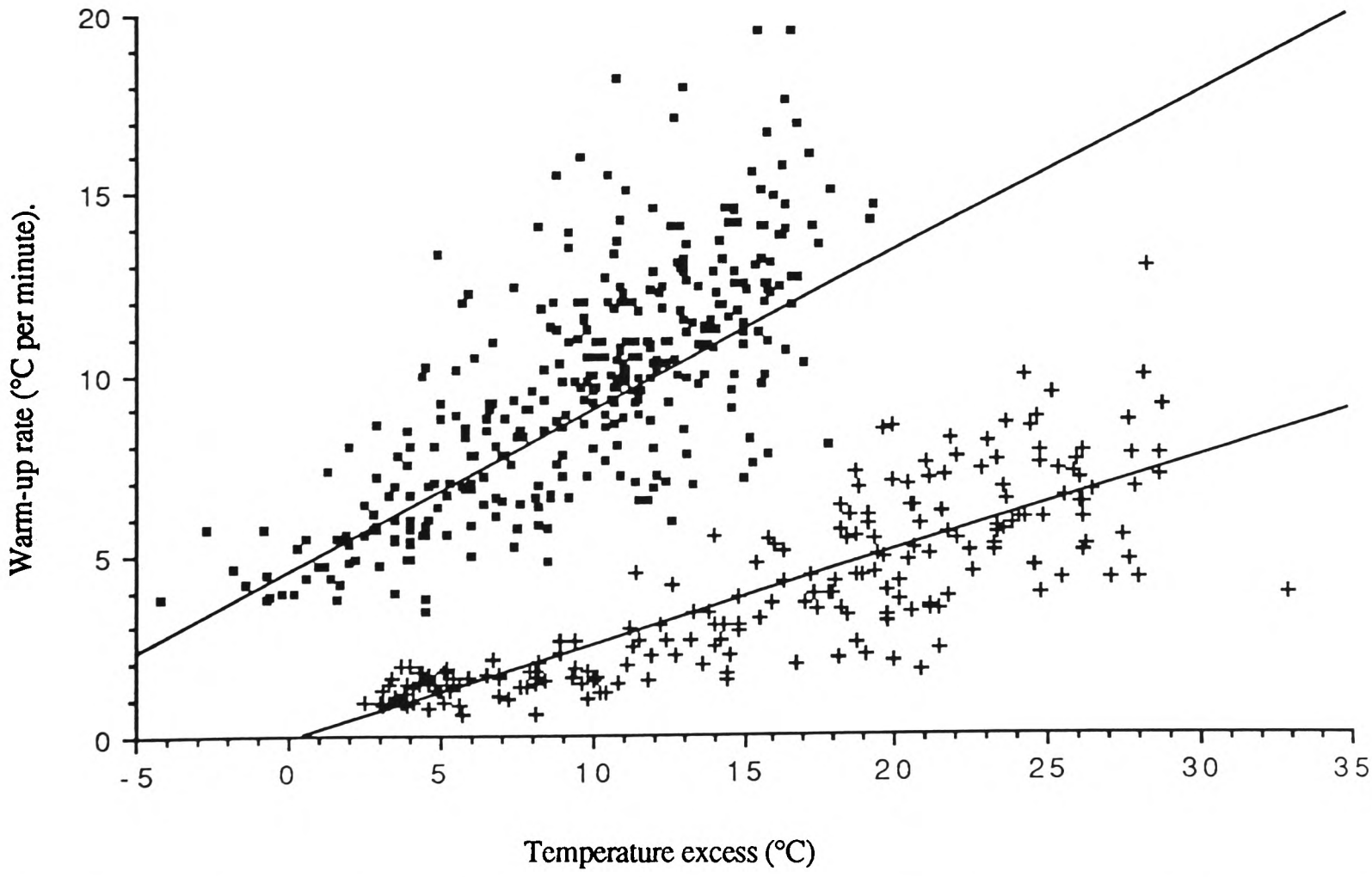


Fig.4.18 Warm-up rate as a function of thoracic temperature excess in *A. plumipes* at T_a=9°C (+) (y=-0.15+0.26x, R^2 =0.706) and T_a=21°C (■) (y=4.8+0.5x, R^2 =0.548).

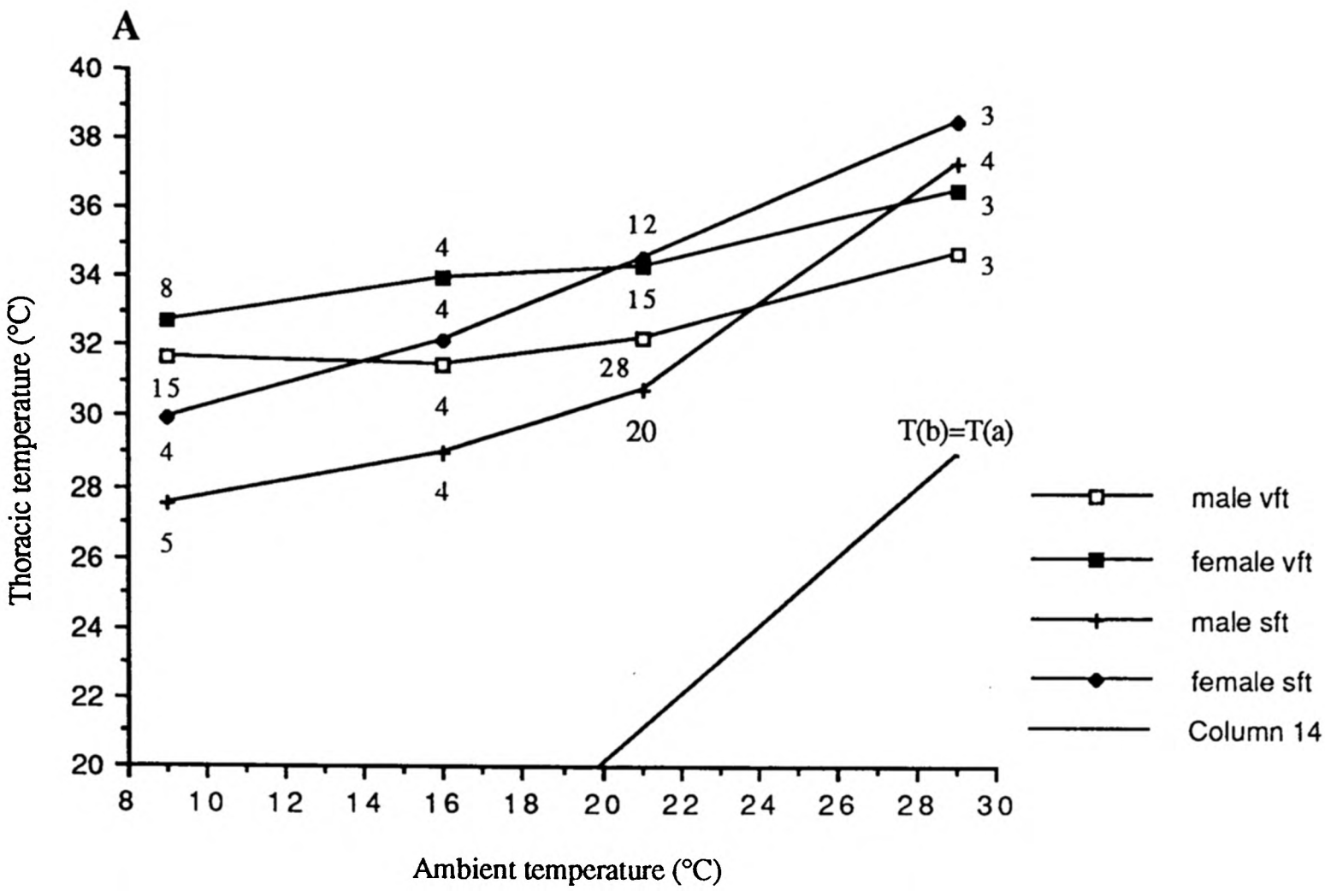


Fig.4.19 (a) VFT and SFT as functions of ambient temperature for female and male *A. plumipes*. (b) Mean duration of warm-up from ambient temperature to VFT as a function of ambient temperature for female (■) and male (❐) *A. plumipes*. In both figures the number of individual means contributing to the overall mean at each ambient temperature is shown by each data point.

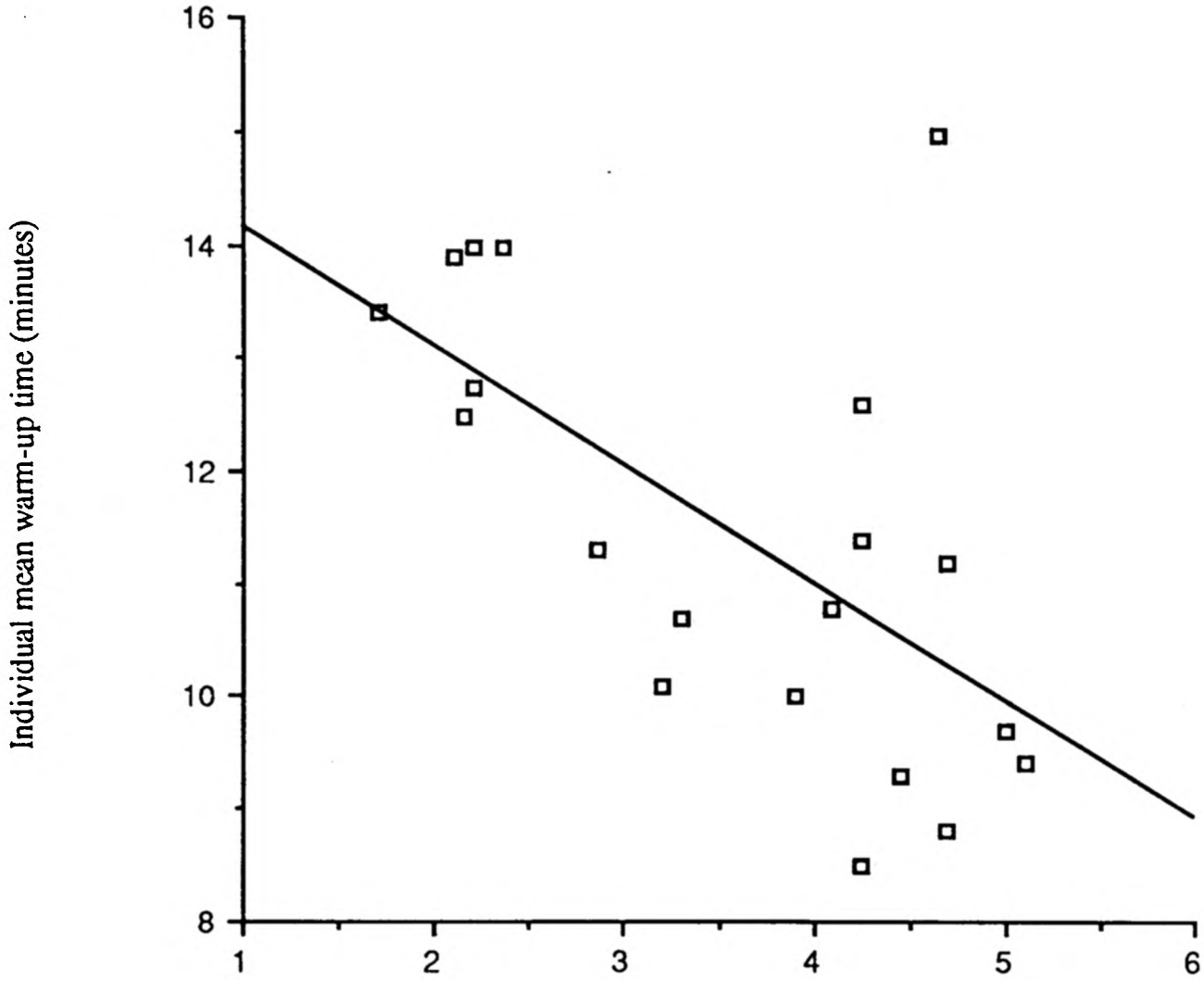


Fig.4.20 Duration of warm-up from ambient temperature to VFT as a function of individual mean warm-up rate in *A. plumipes* at T_a=9°C (y=15.2-1.0x, R^2 =0.372).

A



B



Fig.4.21 Scanning electron micrographs of thoracic pubescence on *Bombus hortorum*. The lengths of each unit on the scale bars on all figures is 0.1mm: **(a)** outer, stout 'guard' hairs. **(b)** inner 'down' hairs.

C



Fig.4.21 (cont.) Scanning electron micrograph of thoracic pubescence on *Bombus hortorum*: **(c)** intermeshing of 'down' hairs beneath a protective 'coat' of guard hairs. Each unit of the scale bar is 0.1mm in length.

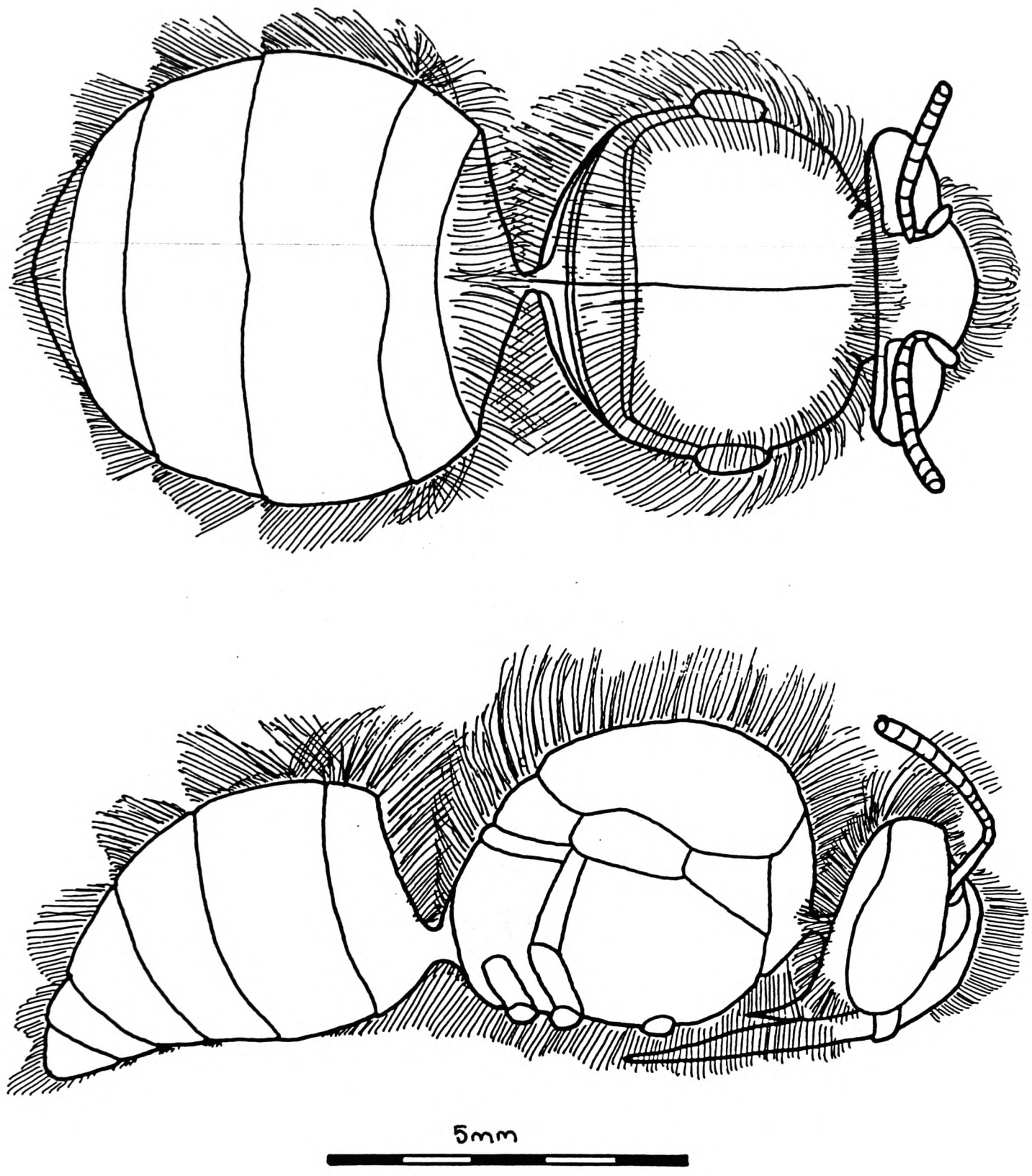
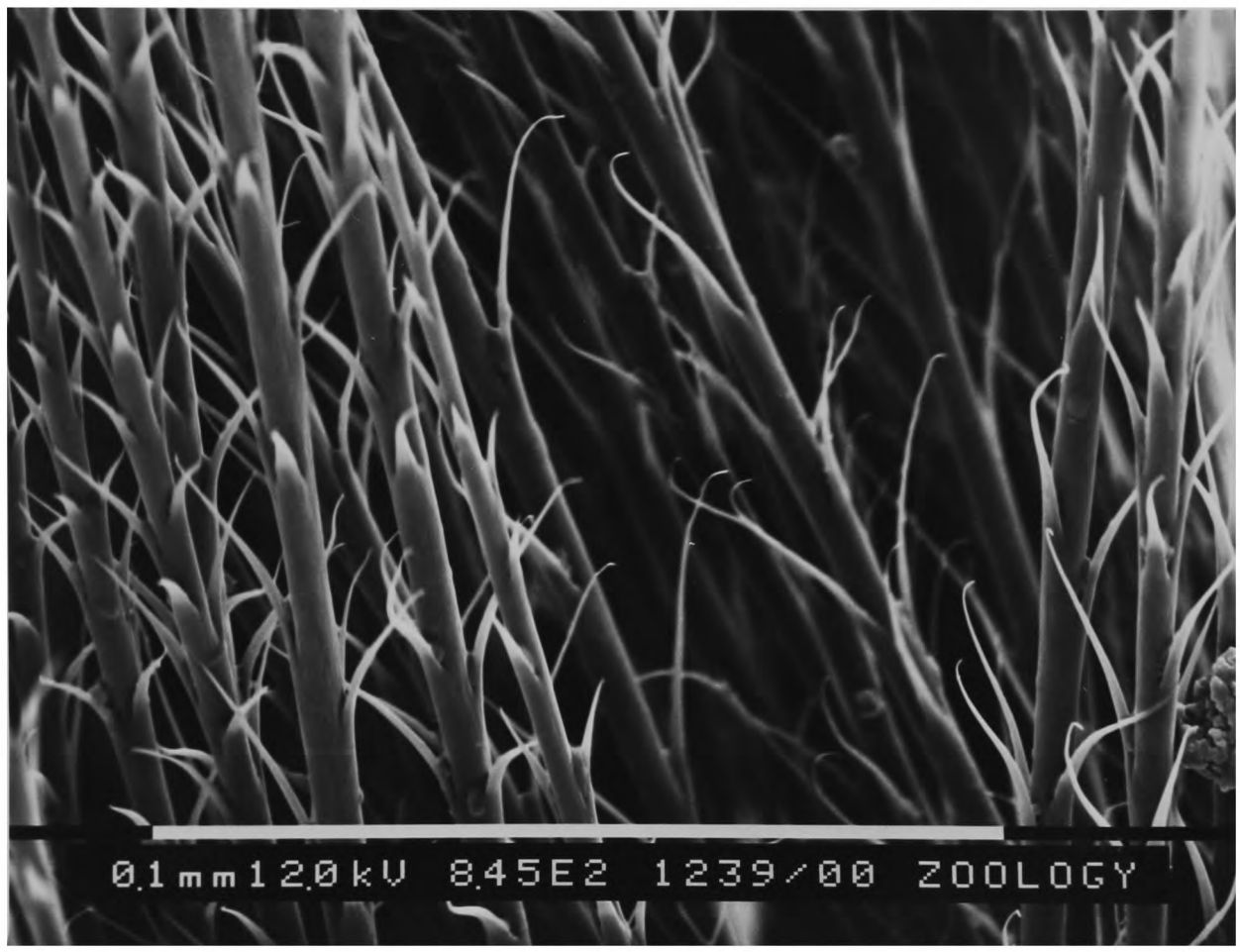


Fig.4.22 The distribution and relative lengths of setae on the body of a male *A.plumipes* seen in longitudinal section.

A



B



Fig.4.23 Scanning electron micrographs of thoracic pubescence on a male *A. plumipes*. The lengths of each unit on the scale bars on all figures is 0.1mm: **(a)** basal plumescent region of setal shaft; **(b)** distal 'guard' region of setal shaft.

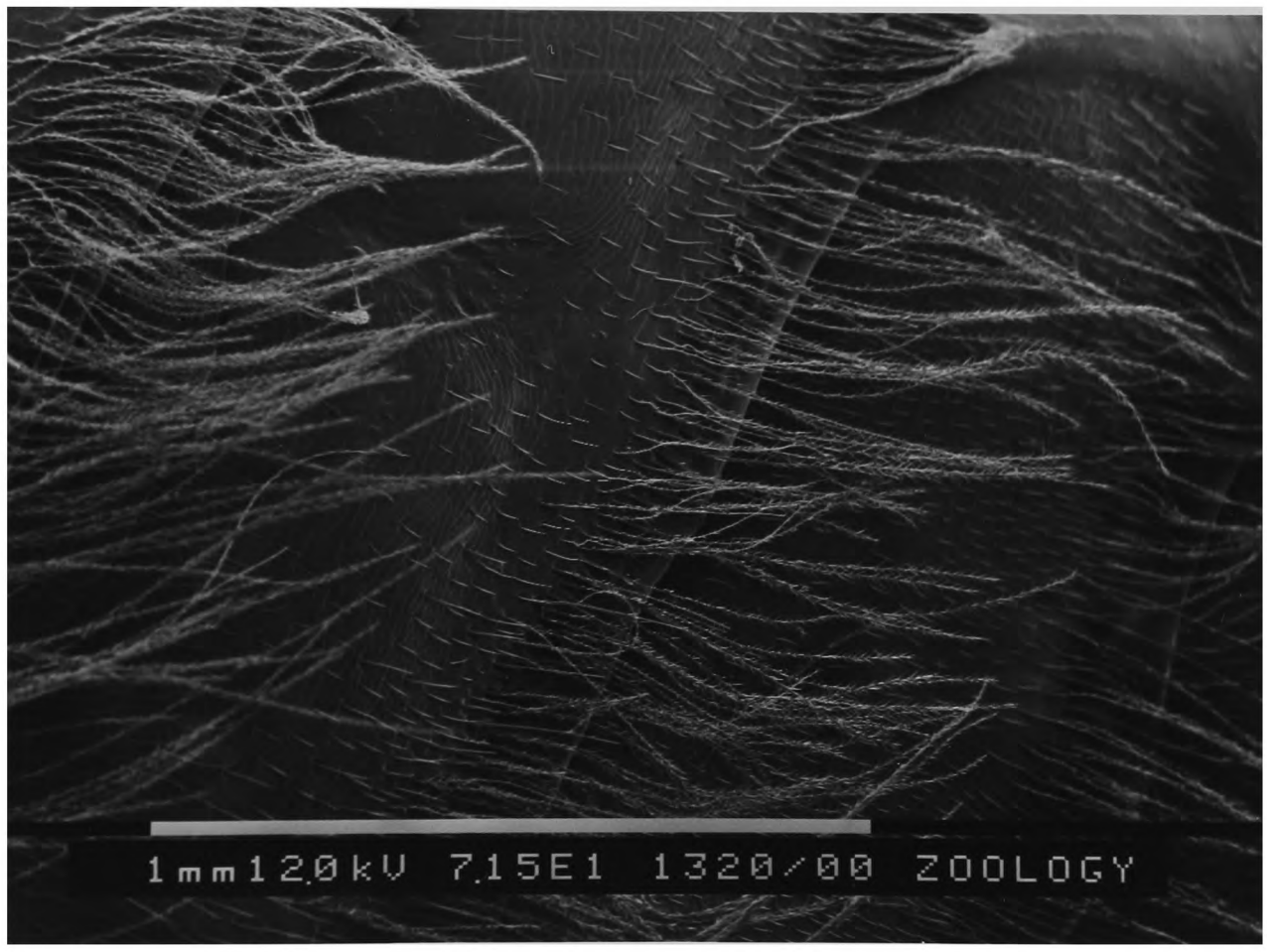


Fig.4.24 Scanning electron micrograph of setae on the ventral surface of the abdomen of a male *A. plumipes*. The length of each unit on the scale bar on the figure is 1mm. Note the lack of pubescence from most of the area of the sternites.

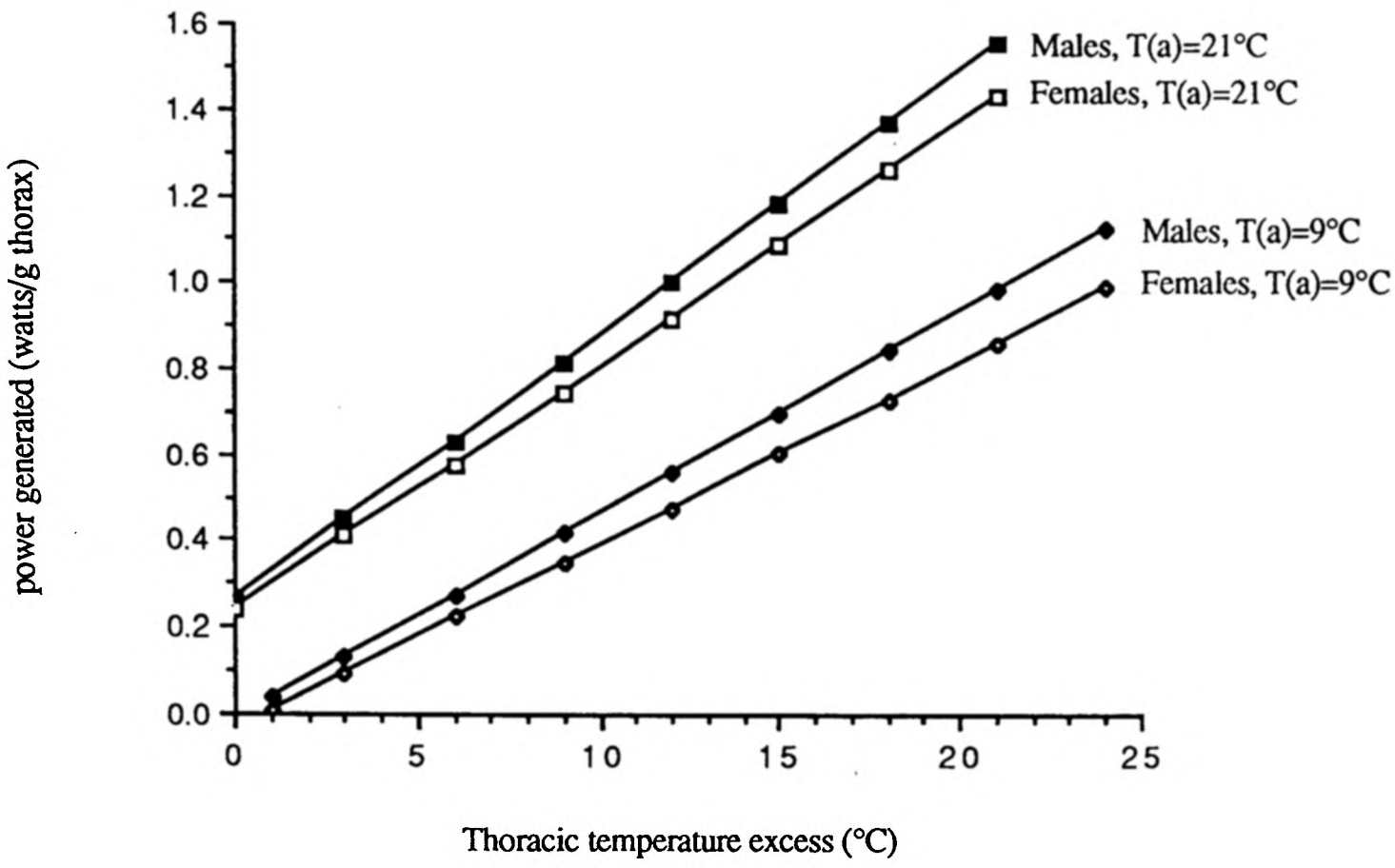


Fig.4.25 Power output as a function of thoracic temperature excess and ambient temperature in male and female *A. plumipes*.

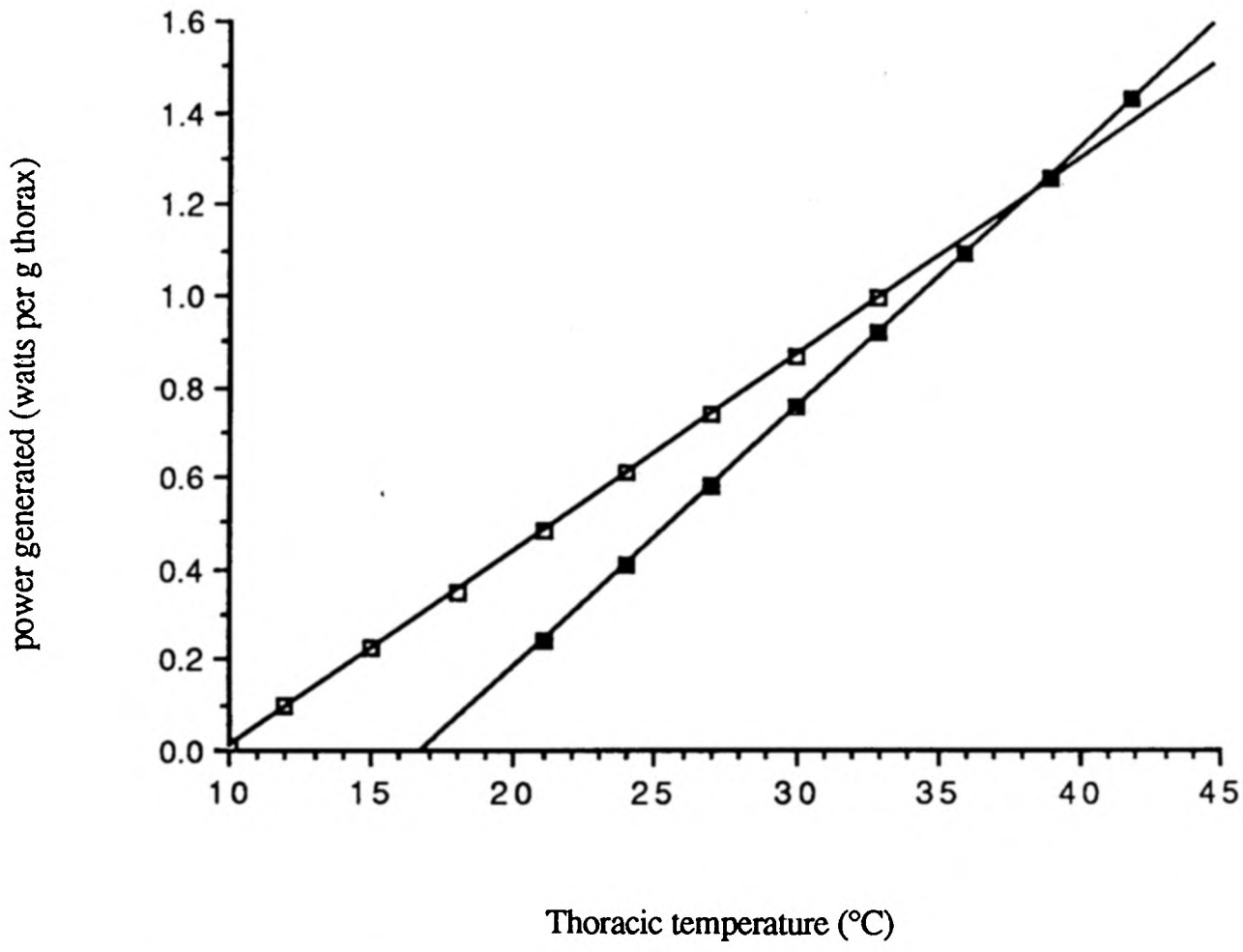


Fig.4.26 Power output as a function of thoracic temperature for female *A. plumipes* at T_a=9°C (❐) and T_a=21°C (■).

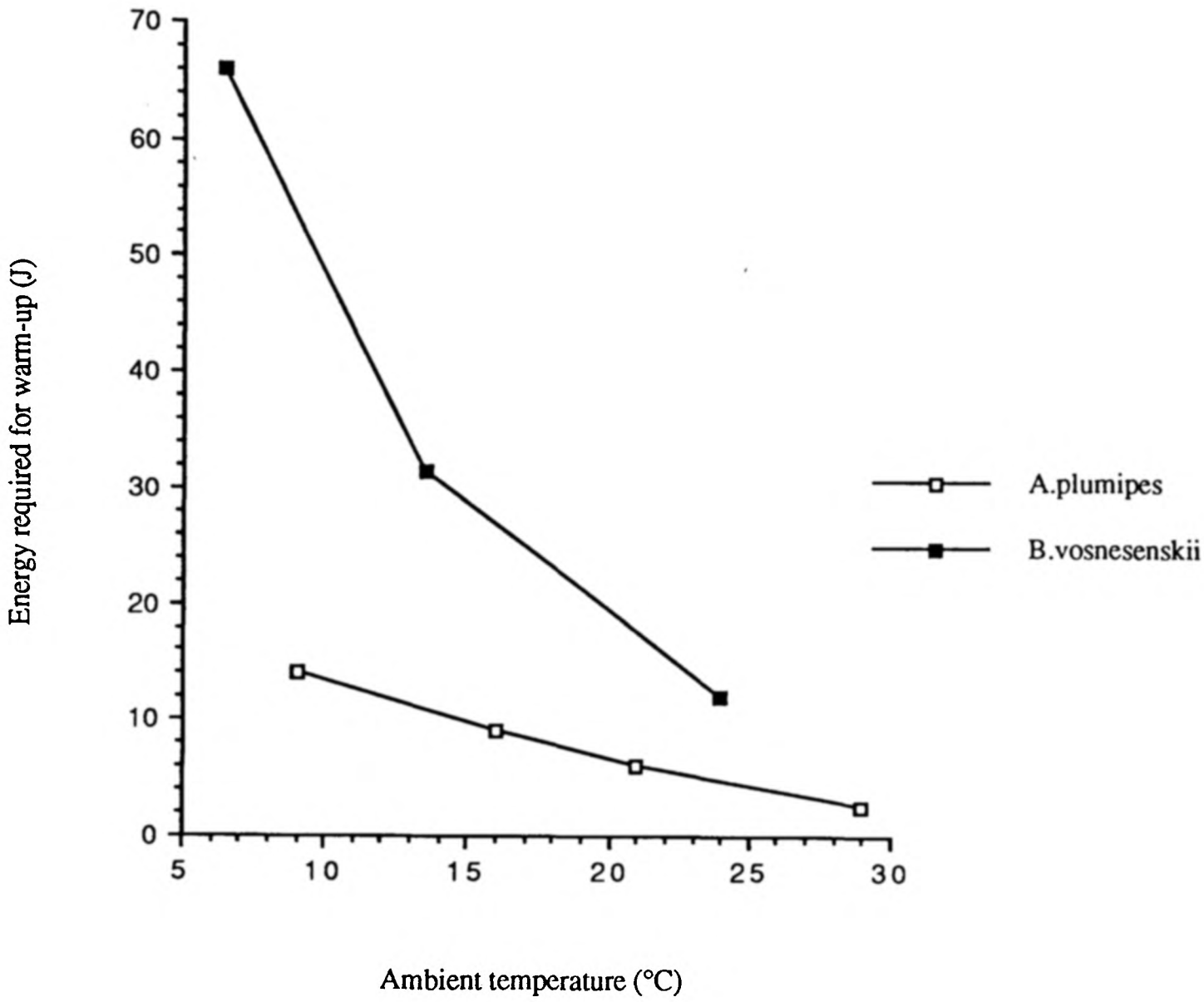


Fig.4.27 The total energy required to complete warm-up from ambient temperature to VFT in *Bombus vosnesenskii* and female *A. plumipes* as functions of ambient temperature.

Chapter 5. Endothermy and thermoregulation in three tropical solitary bees: *Amegilla sapiens, Thyreus quadrimaculatus* and *Creightonella frontalis.*

5.1 Introduction.

Chapter 3 demonstrated correlations between body mass and some aspects of the behaviour of male and female *A. plumipes*. Chapter 4 revealed correlations between body mass and both warm-up rate and body temperatures before and during flight in the same species, and showed the importance of T_a and T_{th} in determining warm-up rates. This chapter examines the effects of body mass and T_a on the body temperatures and warm-up rates of three tropical bee species active under thermal conditions very different to those experienced by *A. plumipes*, and asks whether the relationships described above apply also to these species.

In *A. plumipes*, a highly endothermic bee, behavioural thermoregulation in the form of male basking at low T_a is of considerable importance (Chapter 3). An environment with very high air temperatures should select for behavioural adaptations which avoid thermal stress. Behavioural thermoregulation of this type is described below for male *Creightonella frontalis* flying in an environment where air temperatures reach more than 50°C.

Chapter 3 also showed that as well as T_a, factors such as nectar supply, mass and sex may be important determinants of activity patterns at floral nectar sources. The roles of these factors in the activity patterns of one tropical species (*Amegilla sapiens*, see below) are discussed are detail.

The three solitary bee species discussed in this chapter were studied in Papua New Guinea at sites described in Chapter 2. *Amegilla sapiens* (Cockerell) (Anthophoridae) is a relatively small, robust bee which in overall body proportions very closely resembles *Anthophora*. The genus *Amegilla* is very closely related to *Anthophora*, and was first described by Friese (1897) as a subgenus within *Anthophora*. *Amegilla sapiens* was selected for study because it represents the closest relative to *Anthophora plumipes* that I was able to study in hot, humid conditions, and because it is abundant in P.N.G. *Creightonella frontalis* (Fab.) (Megachilidae) is a large leafcutter bee, common in P.N.G., whose mating system is radically different from that exhibited by *A. plumipes* and *A. sapiens*. Both species are found over a wide range of habitats from hot, dry coastal areas to montane rainforest, and were studied at three sites of differing altitude and climate to investigate the effects of these factors on warm-up rates and body temperatures in different populations within each species. *A. sapiens* and *C. frontalis* are also of economic interest as pollinators of crops (Willmer and Stone 1988; Stone and Willmer 1989c). *Thyreus quadrimaculatus* (Rad.) (Anthophoridae) is a nest parasite of *A. sapiens* encountered at all the sites at which *A.*

sapiens was studied.

For both *A. sapiens* and *C.frontalis*, as for *A. plumipes*, multiple warm-up, VFT and SFT data were obtained for each bee tested. In order to control for the non-independence of data generated by the same bee ('controlling for the effect of individual'), mean warm-up rates, VFT's and SFT's have been calculated for each bee tested (Chapter 2). This reduces each data set to the number of bees tested, and the small sample sizes mean that even apparently strong relationships are statistically insignificant. Regression results for analyses including all the data for each individual have therefore been included to illustrate results that would be predicted from a larger data set.

For all species both laboratory and field measures of body temperature, and estimates of male *C.frontalis* activity, were obtained using the methods described in Chapter 2. During observation of male behavioural thermoregulation, temperatures at the *C.frontalis* nest site were recorded continuously with a thermocouple thermometer data logger (Tempmentor, U.S.A.) which recorded air temperatures in the shade at three sites every 10 seconds. The *C.frontalis* nest site is described in Chapter 2 and in full in Willmer and Stone (1988).

5.2. Physiological thermoregulation in *Amegilla sapiens*.

A. 'Grab-and stab' data.

The three sites at which *A. sapiens* was studied differ significantly in climate over the year (Anon. 1974). Kuk, which is the site at greatest altitude (Chapter 2) is also the coolest site (Fig.5.1), while Madang, at sea level, is considerably warmer at all times of the year (Fig.5.3). At all three sites there is a sexual dimorphism in body mass, males being of significantly lower mass than females (Table 5.1).

Table 5.1 Body masses in *Amegilla sapiens*.

Category	Mean Body mass (mg)± 1 S.E.	Range	Sample size
All females	132.0±2.1	85-183	100
All males	91.8±4.6	68-120	16
Madang females	119.9±3.0	85-160	39
Baiyer River females	127.8±2.5	112-162	22
Kuk females	147.1±2.8	105-183	39

There is also a significant difference in the mean masses of females between sites, with the heaviest *A. sapiens* at the highest altitude (Anova: sex $F_{1,114}=41.0$, $p<0.001$. Site $F_{2,114}=8.10$,

p<0.001) (Table 5.1). 'Grab-and-stab' data were obtained for all three populations: I first describe the relationships between body temperature and T_a within the species, and then discuss differences between populations. Laboratory examinations of warm-up rates, SFT and VFT were obtained only for the Madang population.

Fig.5.2a shows T_{th} and T_{ab} as functions of T_a for *A. sapiens* sampled using 'grab-and-stab' techniques at all three P.N.G. field sites - Madang, Baiyer River and Kuk. The gradient of T_{th} on T_a is 0.54±0.03, while the gradient of T_{ab} on T_a is 0.84±0.04, suggesting that T_{th} is regulated (T_{th}: n=115, R^2=0.671, p<0.001; T_{ab}: n=115, R^2=0.791, p<0.001). Within the species there is considerable variation in T_{th} at a given T_a, particularly at high T_a (Fig.5.2a). A summary of the results of analyses of 'grab-and-stab' data for *A. sapiens* is given in Table 5.2. Multiple regression shows that having controlled for the effects of T_a and site (see below), there is a significant positive correlation between T_{th} and body mass, and no significant effect of sex (Table 5.2).

Table 5.2 Summary of multiple regression analysis of 'grab-and-stab' data for *A.sapiens*.

A. For males and females at Kuk, Baiyer River and Madang. Each analysis involves regression of the y variable on sex, site, air temperature and body mass.

y variable	p values for x variable having controlled for other x variables.						
	Site	Sex	Body mass	mass x site	Ta	n	R^2
Thoracic temperature	0.040	0.520	<0.001		<0.001	115	0.795
Abdominal temperature	0.001	0.900	<0.001		<0.001	115	0.894
Thoracic temperature excess	0.040	0.520	<0.001		<0.001	115	0.550
Thoracic temperature excess	0.930	0.300	0.020	0.058	<0.001	115	0.575
$T_{dif.}$	<0.001	0.610	0.190		<0.001	115	0.750

Having controlled for the effect of T_a there were significant differences in thoracic temperature between the Kuk and Baiyer River populations of *A. sapiens* (n=75, R^2=0.8: T_a p<0.001; site p<0.001) and even more pronounced differences between the Kuk (high altitude) and Madang (low altitude) populations (Fig.5.2b). Because all the body temperature variables in question correlate with body mass, these between-site differences are at least partly explained by the significant differences in body mass between the populations compared (Table 5.1). However, site (analysed as a continuous variable in order of ascending altitude) remains a significant correlate of all the body temperature variables when the effects of mass, sex and T_a are controlled for (Table 5.2): T_{th}, T_{ab}, and T_{ex} increase with altitude. T_{dif} decreases significantly with increasing altitude, having controlled for other variables (Table 5.2).

T_{ex} and T_{dif} as functions of C for all *A. sapiens* are shown in Fig.5.3. From T_{th} approaching ambient at high T_a the T_{ex} generated increased to 15-16°C at T_a =17°C, indicating regulation of T_{th} at low T_a. Multiple regression reveals that both T_{ex} and T_{dif} correlate negatively with T_a, and positively with mass, when sex and site have been controlled for (Table 5.2).

B. Laboratory investigation of body temperatures.

Warm-up rates were obtained from 11 female *A. sapiens*, with a maximum warm-up rate of 13.8°Cmin^{-1} being obtained for a 96mg female at T_a=32°C. A summary of warm-up rates, VFT and SFT for *A. sapiens* at different T_a is shown in Table 5.3. Rather than examining exclusively different individual bees at each T_a, as was the case for *A. plumipes*, in some cases the warm-up of the same individual *A. sapiens* (and *C. frontalis*, below) at different T_a was examined by moving the experimental apparatus between rooms maintained at different temperatures (Chapter 2). This was necessessitated by the relatively smaller populations of both these species available for examination. In calculating the mean figures given in Table 5.3 (and Table 5.5 for *C. frontalis*), a mean has been calculated for each individual at each T_a, and the mean of all these means obtained at a given T_a taken to give the mean value at that T_a. These values have been calculated for the purpose of illustration only, and should not be confused with individual mean warm-up rates etc used in statistical analyses, which are mean values over **all** the T_a at which an individual was examined.

Table 5.3 Mean warm-up rates, voluntary flight temperatures and stable flight temperatures at different ambient temperatures for *Amegilla sapiens*. Values were calculated using the methods described above. Errors are ±1 standard error, and the number of individuals contributing to each mean is given in parentheses.

Ambient temperature	Mean warm-up rate (°Cmin^{-1})	VFT (°C)	SFT (°C)
21.0	2.15 (1)	27.4 (1)	25.8 (1)
23.5	2.8±0.4 (7)	29.2±2.1 (2)	31.3±1.3 (7)
26.5	4.9±0.5 (7)	31.1±0.8 (4)	32.3±0.4 (8)
31.5	6.7±1.4 (8)	33.9±1.5 (6)	31.5±0.5 (8)

Although warm-up rate correlated positively with both T_{th} alone (Fig.5.4a), and with T_a, only the correlation with T_{th} remained significant when the effects of individual, body mass and T_a had been controlled for (n=11, R^2=0.83: T_a p=0.85; body mass p=0.77; T_{th} p<0.001). However, because T_{th} was strongly dependent on T_a (n=44, R^2=0.395, p<0.001), it is probable that T_a affected warm-up rates through T_{th}.

VFT correlated positively with T_a when all data were used (n=44, R^2=0.24: T_a $p<0.001$; body mass p=0.26), but there was no significant correlation when individual means were used (Fig.5.4b) (n=8, R^2=0.32: body mass p=0.70; T_a p=0.14). The use of individual means is an extremely conservative test, and Fig.5.4b suggests that with a larger sample size a strong effect of T_a on VFT would become apparent.

Individual mean SFT correlated significantly with both T_a (Fig.5.4b) and body mass (n=83, R^2=0.94: body mass $p<0.001$; T_a $p<0.001$). For *A. sapiens* the gradient of the regression of VFT on T_a (0.61, Fig.5.4b) was lower than that for SFT (0.98, Fig.5.64). These results suggest that both body mass and T_a are important predictors of body temperatures and warm-up rates in *A. sapiens*.

5.3. Physiological thermoregulation in *Creightonella frontalis*.

A. 'Grab-and stab' data.

Creightonella frontalis shows considerable sexual dimorphism in body mass: females collected at the same three sites had a mean mass of 252±7 mg (n=59, range 135-350mg), and males 115±4 mg (n=25, range 80-160mg). Unlike *A. sapiens, C. frontalis* showed no significant difference in mass between sites (Anova, 83 d.f. sex $p<0.001$; site p=0.09).

A summary of the results of analyses of 'grab-and-stab' data for *C. frontalis* is given in Table 5.4.

Table 5.4 Summary of multiple regression analysis of 'grab-and-stab' data for *Creightonella frontalis* males and females at Kuk, Baiyer River and Madang. Each analysis involves regression of the y variable on sex, site in order of increasing altitude, air temperature and body mass.

y variable	p values for x variable having controlled for other x variables.					
	Site	Sex	Body mass	T_a	n	R^2
Thoracic temperature	0.011	0.047	<0.001	<0.001	84	0.850
Abdominal temperature	0.210	0.580	0.034	<0.001	79	0.860
Thoracic temperature excess	0.011	0.047	<0.001	<0.001	84	0.800
$T_{dif.}$	0.500	0.100	0.120	<0.001	79	0.530

T_{th} and T_{ab} correlated strongly with T_a (Fig.5.5), with T_{th} ranging in females from 35 °C at a T_a of 25 °C to 40-42°C at a T_a of 33°C. T_{th} and T_{ab} also correlated strongly and positively with body mass, and males had significantly lower T_{th} than females (Table 5.4) when mass, site and T_a had been controlled for. There was no significant effect of sex on T_{ab}. The slopes of the best fit regressions for T_{th} on T_a are less than one for both males (0.79±0.07) and females (0.68±0.05)

(Fig.5.5), indicating that both are capable of some degree of thoracic thermoregulation (females: n=58, R^2=0.782, p<0.001; males: n=26, R^2=0.851, p<0.001). The gradient of the best-fit regression of female T_{ab} on T_a (1.00±0.05) indicates that T_{ab} was not regulated.

As for *A. sapiens*, both T_{ex} and T_{dif} correlated negatively with T_a (Fig.5.6) and T_{ex} also correlated positively with mass (Table 5.4). T_{th} and T_{ex} increased significantly with altitude when other effects had been controlled for, but there were no other significant effects of site (Table 5.4). This suggests that there were some qualitative differences in the thermogenic abilities of the Kuk and Madang populations not explained by T_a or body mass differences, and in a direction paralleling those found in *A. sapiens*.

B. Laboratory investigation of body temperatures.

Sixty five measurements of warm-up rate were obtained from 12 female *C. frontalis* over a range of T_a. Maximum warm-up rates of 10.2°Cmin^{-1} were obtained at T_a=32°C from two females weighing 255 and 310mg. A summary of warm-up rates, VFT and SFT for *C. frontalis* at different T_a is shown in Table 5.5.

Table 5.5 Mean warm-up rates, voluntary flight temperatures and stable flight temperatures at different ambient temperatures for female *Creightonella frontalis*. Values were calculated using the methods described above. Errors are ±1 standard error, and the number of individuals contributing to each mean is given in parentheses.

Ambient temperature	Mean warm-up rate (°Cmin^{-1})	VFT (°C)	SFT (°C)
22.0	5.4±0.5 (7)	33.2±0.8 (3)	29.5±0.6 (7)
26.5	6.9±0.4 (5)	34.3±0.1 (2)	31.6±0.4 (5)
31.5	8.75±0.3 (5)	36.7±1.4 (3)	37.6±0.3 (6)
35.0	*	37.1 (1)	39.8 (1)
38.0	*	41.5 (1)	43.3 (1)

Although warm-up rate correlated positively with T_a, mass and T_{th} when all the data were used (multiple regression. n=65, R^2=0.62: T_a p<0.001; body mass p<0.001; T_{th} p<0.001) (Figs.5.7a,b,c), only T_a remained significantly correlated with warm-up rate using individual mean values (multiple regression. n=12: T_a p=0.008; body mass p=0.100; T_{th} p=0.500).

The relationships between VFT , SFT and T_a are shown in Fig.5.8. VFT correlated positively with both body mass and T_a when all data were used (n=55, R^2=0.451: body mass p=0.01; T_a p<0.001), but there were no significant correlations when individual means were used (n=6, R^2=0.83: body mass p=0.11; T_a p=0.35). As for *A. sapiens*, the data in Table 5.5 and

Fig.5.8 suggest that with a larger sample size a strong effect of T_a on VFT would become apparent.

Individual mean SFT correlated positively with both mass and T_a (Fig.5.8) (n=11, R^2=0.974: body mass $p<0.001$; T_a $p<0.001$). Thus the general results obtained for *A. sapiens* are repeated for *C. frontalis*. For this species, as for *A. sapiens*, the gradient of the regression of VFT on T_a (0.46, Fig.5.8) was lower than that for SFT (0.87, Fig.5.8). This extends the generalisation made in Chapter 4 - that thermoregulation before flight is better than that achieved in flight - to two more species.

5.4 'Grab-and-stab' investigation of body temperatures in *Thyreus quadrimaculatus*.

Although in the same family as *A. sapiens* and *A. plumipes*, the behaviour of this bee is radically different. Its flight, like that of *Melecta albifrons*, another nest parasite (Chapter 3), is typically slow, and has none of the fast, precise darting and hovering shown by the other two species. The minimum T_a at which this species was observed to fly was 25°C at both sites, and flight activity ceased very soon after clouding over or shading of a foraging site at which this species had previously been active. The behaviour of *T. quadrimaculatus* is consistent with that of an insect dependent to a large degree on behavioural thermoregulation, and without great endothermic ability. The mean warm-up rate of this species in the laboratory at T_a=22°C was only 1.75°Cmin^{-1} , which is low for a bee of its body mass (100.0±4.7mg, n=14; Chapter 6). 'Grab-and-stab' data were obtained to allow comparison with the highly endothermic, and closely related, *A. sapiens*.

The relationship between T_{th} and T_{ab} and T_a for *T. quadrimaculatus* is shown in Fig.5.9. The gradients of the best-fit regressions of these variables on T_a are 0.74±0.12 (n=28) and 0.78±0.12; both regressions are significant (T_{th} n=28, R^2=0.571, $p<0.001$; T_{ab} n=27, R^2=0.610, $p<0.001$), indicating some thermoregulatory ability. A summary of the results of analyses of 'grab-and-stab' data for *T. quadrimaculatus* is given in Table 5.6.

Table 5.6. Summary of multiple regression analysis of 'grab-and-stab' data for *Thyreus quadrimaculatus*.

y variable	p values for x variable having controlled for other x variables.			
	Body mass	Ta	n	R^2
Thoracic temperature	0.640	<0.001	14	0.813
Abdominal temperature	0.700	<0.001	14	0.771
Thoracic temperature excess	0.640	0.150	14	0.060
T_{th}-T_{ab}	0.900	0.430	14	0.183

Having controlled for the effect of T_a, there is no significant correlation between mass and any of T_{th}, T_{ab}, T_{ex} or T_{dif}, in contrast to the results obtained for *C. frontalis* and *A. sapiens*. This suggests that the thermoregulatory ability demonstrated by this species is not dependent on body size, a result which would not be expected were heat generation by the thoracic flight muscles an important contributing factor (Chapter 4). It is probable that *Thyreus quadrimaculatus* thermoregulates behaviourally, by limiting flight activity to locations and periods of time for which suitable ambient temperatures allow maintenance of required thoracic temperatures without need for endothermy or reduction of T_{th} at higher T_a. *T. quadrimaculatus* is a nest parasite, and thus has no need to maintain foraging activity during poor weather in order to stock cells with pollen. The relatively brief periods of activity this strategy allows, relative to physiological thermoregulation, are therefore sufficient. This idea is further developed in Chapter 6.

5.5. Activity patterns in *A. sapiens*: limitations imposed by nectar sources.

Activity patterns in *A. plumipes* have been shown to be affected by climate, nectar supply and sexual interaction (Chapter 3). In this section I discuss the factors which affect activity patterns in *A. sapiens*. The courtship behaviour of *A. sapiens* and nectar foraging from *Stachytarpheta mutabilis* (Jacq.) Vahl. (Verbenaceae) have been studied in detail at Baiyer River (Stone *et al.* 1988) as well as its foraging behaviour at Cardamom (*Elettaria cardamomum* [L.] Maton, Zingiberaceae) at Kuk (Stone and Willmer 1989c). Foraging in this species from *Stachytarpheta jamaicensis* (L.) Vahl. at Madang is the subject of the following section.

The mating system shown by *A. sapiens* at Baiyer River (Stone *et al.* 1988) was similar to that demonstrated by *A. plumipes*. Male *A. sapiens* patrolled areas of foodplants visited by females, and attempted to mate with them as they fed. The males, like those of *A. plumipes*, switched from foraging behaviour in the morning to courtship behaviour in the afternoon, followed by a brief spell of feeding in the evening. Foraging behaviour by *A. sapiens* at *Stachytarpheta mutabilis* at Baiyer River was affected both by climate and by nectar supply. When activity began in the morning, foraging occurred at those sites first warmed by the sun, and not at those in the shade. In the evening all three *S. mutabilis* sites studied had similar temperatures and light levels, and foraging occurred at the site with the highest nectar volumes per flower (Stone *et al.* 1988).

Foraging at *S. jamaicensis* showed a quite different pattern. Like *S. mutabilis*, *S. jamaicensis* has flowers arranged in groups of 2-4 on a vertical flower spike. The corollas are 10-12 (mean 10.5±0.2, n=25) mm in diameter, and the flower has a corolla tube 8-10 (mean 9.2±0.5, n=25) mm in depth. Foraging and courtship activity at this source were observed in September and October, 1987, at Madang, P.N.G. Data for two representative days of detailed observation, 16 and

17.9.87, are presented. Flowers opened before dawn from about 04.00h (local time) onwards, and were visited by several species of sphingid moth until just after sunrise (about 05.30h). The first bees to arrive were female *A. sapiens*, which arrived from 05.30h onwards (Fig.5.10a,b). Males appeared from 06.00h onwards, and male activity at *S. jamaicensis* always peaked at a later time than female activity (Fig.5.10a,b). Intensive foraging by this species correlated with a rapid drop in the volume of nectar available per flower (Fig.5.11a). Temperature rose rapidly at the study site due to insolation, from 25°C at 05.30h to 34-37°C by 12.00h (Fig.5.12a), with a rapid decrease in relative humidity (Fig.5.12b). Nectar concentration in *S. jamaicensis* flowers rose significantly from a mean of 26.1±1.1% sucrose (n=40) at 06.30h to 35.2±2.0% sucrose (n=40) at 09.30 (Fig.5.11b), by which time foraging by female *A. sapiens* was already declining (Fig.5.10). Over the period of forager activity the corollas of the flowers became loose in the calyx, and from 08.00h onwards corollas began to fall from the flower spikes, reducing the number of flowers visited by foragers. By 11.30-12.00h, only 12.5-20% of the number of flowers originally on flower spikes in a given patch remained (Fig.5.13). By 12.00h-12.30h female activity at the site had ceased. Males remained active at the site for longer than females, but did not forage. Males inspected other insects visiting the few remaining flowers, and any female *A. sapiens* were pounced upon in a manner very similar to that described for *A. plumipes* in Chapter 3.

Foraging by *A. sapiens* at *S. jamaicensis* is unlikely to have been directly limited by temperature. Although cessation of foraging did correlate with high T_a at the study sites, foraging on 17.9.87 continued at T_a at which foraging had ceased on 16.9.87 (Fig.5.10a,b). A more significant limitation to foraging must have been the dropping of flowers by the plant. When foragers were excluded from 10 flower spikes using fine nylon netting, all the corollas had fallen by 13.30h (Fig.5.13), indicating that it was not merely foragers which caused the change in the number of available flowers. This situation is quite different to that in *S. mutabilis*, in which flowers persisted on the flower spike for up to two days, allowing foragers more time over which to make choices between foraging sites (Stone *et al.* 1988).

5.6. Behavioural thermoregulation in male *C. frontalis*.

Creightonella frontalis nests in earth banks and ditches, digging tunnels in areas of bare soil (Willmer and Stone 1988) (Chapter 2). In some areas of suitable substrate these nests formed aggregations with nest entrances reaching a density of 25 m^{-2}. Females made their first provisioning trips of the day from 06.30-07.00h (local time) (Willmer and Stone 1988) and males appeared at nest aggregations from 07.30h onwards (Fig.5.14). Males at the study site flew back and forth along a section of ditch in which most of the nest entrances were concentrated

approximately 5m in length. One flight back and forth along this stretch is termed a single patrol tour. Observations of marked males suggested that the length of the ditch which was patrolled was highly consistent for a given male, although this was not tested. Females returning to their nests were only occasionally intercepted by males, which gathered around particular tunnel entrances in groups of up to 5 individuals. Females which emerged from such entrances rapidly became the centre of a ball of males which competed for mating opportunities. It is probable that these females were virgins to which males may have been attracted by olfactory cues (reviewed by Thornhill and Alcock 1983). The successful male which achieved copulation was harassed by other males until the pair were concealed by vegetation or leaf litter. Males and females were both observed foraging for nectar from weeds near the nest site, including *Passiflora foetida* L. (Passifloraceae), *Mimosa invisa* Mart. ex Colla (Leguminosae) and Pigeon pea, *Crotalaria retusa* (L.) (Leguminosae), but aggregation of males around females, and pairs *in copula*, were seen only at the nest area. No prolonged patrolling of the nectar sources of the type described for male *A.plumipes* (Chapter 3) or *A.sapiens* (Stone *et al.* 1988) was observed for male *C.frontalis*.

The behaviour of male *C.frontalis* showed clear changes over the day which correlated with changes in the microclimate of the nest aggregation area. The first males to arrive at the site patrolled the aggregation area, and between patrolling flights rested for periods of 2-3 minutes on the bank above the ditch (Fig.5.15). The bank warmed more rapidly than the nest entrance area (Fig.5.16), and it is probable that males were basking between periods of flight in the shaded ditch, insolated only when the sun had reached sufficient elevation from 09.00h onwards. Both the time spent basking between flights, and the time taken to complete a single patrol tour of the aggregation area by marked males, decreased significantly with increasing T_a (Figs.5.17a,b) (Bask duration $n=18$, $R^2=0.55$, $p<0.001$; patrol tour duration $n=7$, $R^2=0.86$, $p<0.001$). The resting site preferred by males became the nest entrance area rather than the ditch bank from 09.00h until the hottest part of the day (Fig.5.15).

Male activity at the nest site reached maximum levels between 08.30 and 11.00h, declining to very low levels for the hottest part of the day (Fig.5.14). Temperatures on the ditch bank and near the nest entrances reached maxima of 50°C or more (Fig.5.16), and it is probable that continuous flight activity at such high T_a would be thermally intolerable. During the period of low male activity at the nest site the only males observed were seen resting on coffee bushes 1–2m above the ground (Fig.5.15), where maximum air temperatures were 20°C lower than those in the ditch area (Fig.5.16). Other males were recovered during this part of the day when nests were excavated, and it is therefore possible that some sheltered from high surface temperatures up to 0.5m (Willmer and Stone 1988) below ground.

Males were again active at the nest site in numbers in the evening, reaching a second peak of activity from 16.00-17.00h. Air temperatures fell rapidly in the evening, and most male rests between sites at this time occurred in the ditch around nest entrances (Fig.5.15).

The activity pattern at the nest site, the change in bask length, and the change in the sites favoured all suggest behavioural thermoregulation by male *Creightonella frontalis*. With a diurnal temperature range of 24-36°C 1.5m above the ground surface, elevation of body temperatures must be less of a selective pressure than tolerance of high T_{th}. Female *C. frontalis*, which forage throughout the day, tolerated T_{th} up to 48°C without apparent harm, and males reached T_{th} near 40°C at T_a=35°C. The behavior of males suggests that the period for which mate searching in the ditch is tolerable was severely limited by T_a, and activity all but ceased when T_a around nest entrances in the ditch approached 40°C.

5.7. Discussion.

Amegilla sapiens and *Creightonella frontalis* are both clearly endothermic species, showing that tropical bees inhabiting warm, predictable climates may still exhibit endothermy. The warm-up rates these species show at a given T_a, and the T_a range over which they show warm-up behaviour, are rather different to those obtained for *Anthophora plumipes*. All three species have considerable powers of endothermy and thermoregulatory ability, but demonstration of such abilities depends critically on T_a. Although both *Amegilla sapiens* and *Creightonella frontalis* have high maximum warm-up rates, these were achieved only at very high T_a's of 31-32°C. At such T_a *A. plumipes* did not demonstrate warm-up and could not be induced to commence tethered flight. At T_a=20-22°C, the mean warm-up rates for females of both P.N.G. species are modest (3.75°Cmin^{-1} [n=19] for *A. sapiens*, and 5.8°Cmin^{-1} [n=29] for *C. frontalis*) when compared to those obtained for *A. plumipes* females (12.3°Cmin^{-1}). At the lowest temperatures at which warm-up was observed in *A. plumipes*, both of the tropical species were relatively unresposive to tactile stimuli and did not exhibit warm-up behaviour.

A. plumipes inhabits a very different thermal environment to *A. sapiens* and *C. frontalis*, and it is probable that the endothermic abilities of each species have evolved to suit the range of ambient temperatures experienced by that species, within the constraints imposed by other factors. Differences in the climates inhabited by these species may explain the differences in their behaviour and body temperatures at a given T_a. A test of this hypothesis would have to take into account the effects of body mass described in Chapter 4, as well as possible effects of evolutionary history (phylogeny), and forms the subject of Chapter 6.

The results obtained for *A. sapiens* and *C. frontalis* reiterate the positive correlations

obtained for *A. plumipes* between body mass and T_{th} in flight, in the field and in the laboratory. This implies that, if a minimum T_{th} for flight is required in *A. sapiens*, larger individuals will be able to maintain this temperature at a lower T_a than smaller individuals. If *A. sapiens* also has an upper tolerated T_{th} for flight, then the bees of lower mass should be able to remain flying at higher T_a than heavier bees. The mass differences between populations of *A. sapiens* may therefore have evolved in response to the differing thermal regimes of their habitats, the Kuk population having evolved a higher mean body mass to allow warm-up and maintainance of T_{th} at low T_a, and the Madang population, with a lower mean mass, to sustain flight at the high T_a common in their habitat. Although data for only three populations of *A. sapiens* are discussed here, if this situation were true for this species in general it would constitute an intraspecific insect example of Bergmann's Rule - that, within an endothermic lineage, body size tends to be greater in cold climates than in warm climates (Bergmann 1847).

The significant differences in body temperatures between different populations of *A. sapiens* and *C. frontalis* that are independent of body mass suggest that there are physiological and/or morphological differences between populations that have evolved as a result of differences between the sites. *A. sapiens* at higher altitude had higher T_{th} and T_{ab} at a given T_a than would be predicted on the basis of their greater mass alone, suggesting either differences in the thermogenic abilities per unit mass between populations, or differences in factors affecting rates of heat loss, such as the thermal conductivity of the cuticle, or both. The pubescence of the specimens brought back from P.N.G. is unfortunately too damaged to allow a comparison of the hairiness of the two populations.

If it is assumed that *A. sapiens* regulates T_{th} by controlling blood flow to the abdomen, as discussed in Chapter 4, some indication of the upper temperature limits for physiological regulation of T_{th} can be obtained from the relationship between T_{dif} and T_a. The upper thermal limit of this type of thermoregulation is that T_a at which T_{th} and T_{ab} are equal. Madang *A. sapiens* of a given mass at a given T_a maintained a greater temperature difference between thorax and abdomen than those at higher altitude, implying that they should be able to fly at higher T_a before the limits to physiological thermoregulation are reached. These results suggest that as well as possible adaptive differences in body mass between populations there has also been qualitative physiological adaptation to the different thermal regimes.

The study of *A. sapiens* foraging from *S. jamaicensis* in a tropical climate illustrates a situation quite different from that described in a temperate situation for *A. plumipes* in Chapter 3. Although foraging activity in *A. sapiens* can depend on T_a (Stone *et al.* 1988), the direct effect of T_a in determining the activity patterns observed at *S. jamaicensis* was small. *A. sapiens* foraged

from dawn until corollas fell from the flowers, and throughout this period ambient temperatures were within the range tolerated by this species. Such long periods of moderate ambient temperatures, in which limitations on activity are imposed predominantly by food sources, must be more frequent in a tropical than in a temperate environment. This continuous activity of potential pollinators over the day may be related to the observed transience of many tropical flowers (Dr. Pat Willmer, pers. comm.).

The sex related differences in T_{th} in *C. frontalis* (Megachilidae) were not found in *A. plumipes* and *A. sapiens* (Anthophoridae). In *C. frontalis*, all other things being equal, males had lower T_{th} than females, while in *A. plumipes* and *A. sapiens* males and females were qualitatively similar. It is tempting to suggest that these differences in the endothermy of males relative to females are due to the different selective pressures which have acted on male bees employing different mating strategies. Males of both *A. plumipes* and *A. sapiens* are active patrollers of foraging sites, and are able to fly at minimum ambient temperatures quite close to those of females. In comparison, male *C. frontalis* are very sluggish fliers, and did not appear to show any of the complex territorial behaviour shown by the anthophorids. In 1987 and 1988 I observed males and females of another megachilid - *Megachile willoughbiella* - in Oxford. Male *M. willoughbiella* waited for females at nectar and pollen sites rather than at nest sites and, like male *C. frontalis*, were sluggish fliers. It is possible that the differences in male strategies and levels of endothermy between the anthophorid and megachilid bees described here are due to phylogeny and not to any difference in selective pressures. A test of this hypothesis would be to examine the endothermic ability of the males of many species in many families, including megachilids, that demonstrate an *Anthophora*-like mating strategy. An absence of any significant difference in the endothermic abilities of males and females, or higher levels of endothermy in males than in females, would support the suggestion that mating systems have coevolved with endothermic abilities in male bees. A suitable megachilid for study is *Anthidium manicatum* (L.), which I studied in the Botanical Gardens at Oxford in July and August 1987. I had hoped to obtain more data in 1988 and 1989, but no *A. manicatum* were found flying at any known sites in either of these years. For this reason my results are preliminary, and the aims of this work will only be briefly outlined here. The males of this species are larger than the females, and defend nectar resources (commonly *Stachys lanata* Jacq.) aggressively against other bees in order to attract females (Severinghaus *et al.* 1981). Although female *A.manicatum* only fly at relatively warm ambient temperatures (with a minimum T_a for flight of approximately 20°C), if males are to guard a nectar supply effectively they must be capable of vigorous flight activity at ambient temperatures at which their nectar competitors are active. In the Botanical Gardens the main competitors for nectar were *Bombus lapidarius* L. and *Bombus terrestris* L., which foraged at air

temperatures above 12°C. Male *A. manicatum* were active at air temperatures above 14-15°C, and in this type of mating sytem we might predict higher levels of endothermy in males than in females. Because I was unable to obtain any warm-up or 'grab-and-stab' data for this species, this prediction remains untested. The role of mating systems in the evolution of endothermic ability should be easy to analyse comparatively when sufficient data are available, and remains, I think, a particularly interesting question in this field.

In both *C. frontalis* and *A. sapiens* SFT and VFT measurements give differing indications of thermoregulatory ability. As for *A. plumipes*, both species appear to be able to regulate T_{th} at the onset of flight rather better than in flight. This underlines the point that apparent thermoregulatory ability depends on the type of measurement made.

The example of male *C. frontalis* illustrates that, as was the case for male *A. plumipes*, behavioural thermoregulation, independent of endothermy, may be of great importance, particularly at T_a's where thermoregulation in a species is near its limits.

This chapter shows that while warm-up rates exhibited by two tropical species are substantial, they are somewhat lower than those shown by *A. plumipes*. Once again, body mass and T_a are shown to be significant predictors of warm-up rates and body temperatures, and thermoregulatory ability is shown to depend on the measure of T_{th} that is used. Furthermore, behavioural thermoregulation is shown to be of fundamental importance to tropical species, particularly male *C. frontalis*, as well as to temperate bees such as *A. plumipes*. The importance of availability of floral resources in determining activity patterns in the absence of restrictions imposed by T_a (a situation which may be common in some tropical environments) is illustrated.

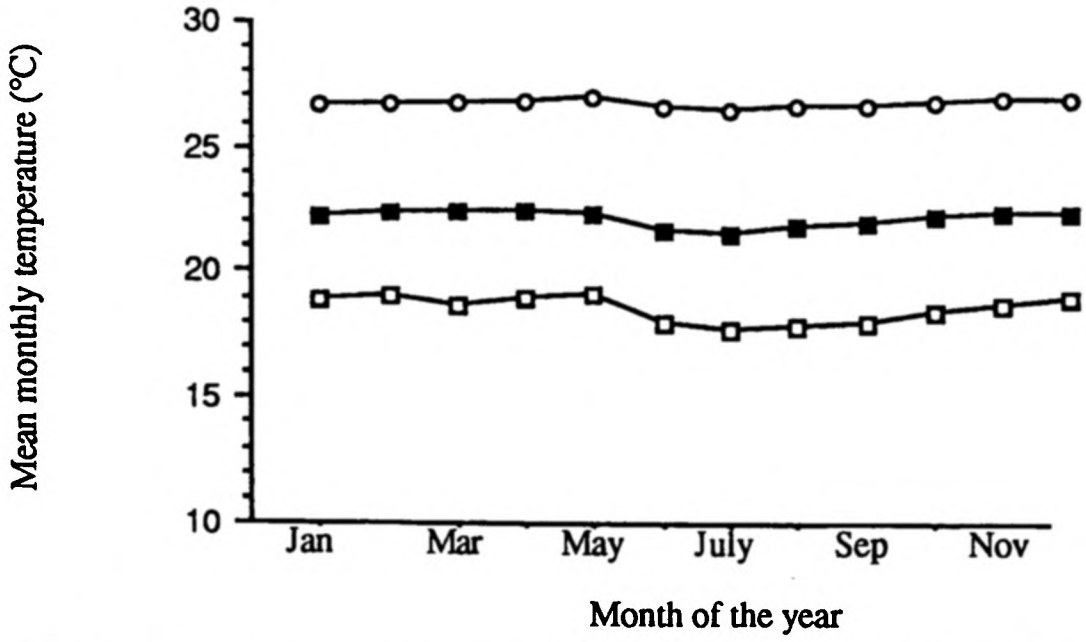


Fig.5.1 Mean monthly temperatures over the year for Madang (O), Baiyer River (■) and Mount Hagen (❐) (From the Papua New Guinea Gazeteer (1974), Central mapping bureau, Dept. of Lands, Surveys and Mines, Port Moresby. Pub. Papua New Guinea Place Names Committee).

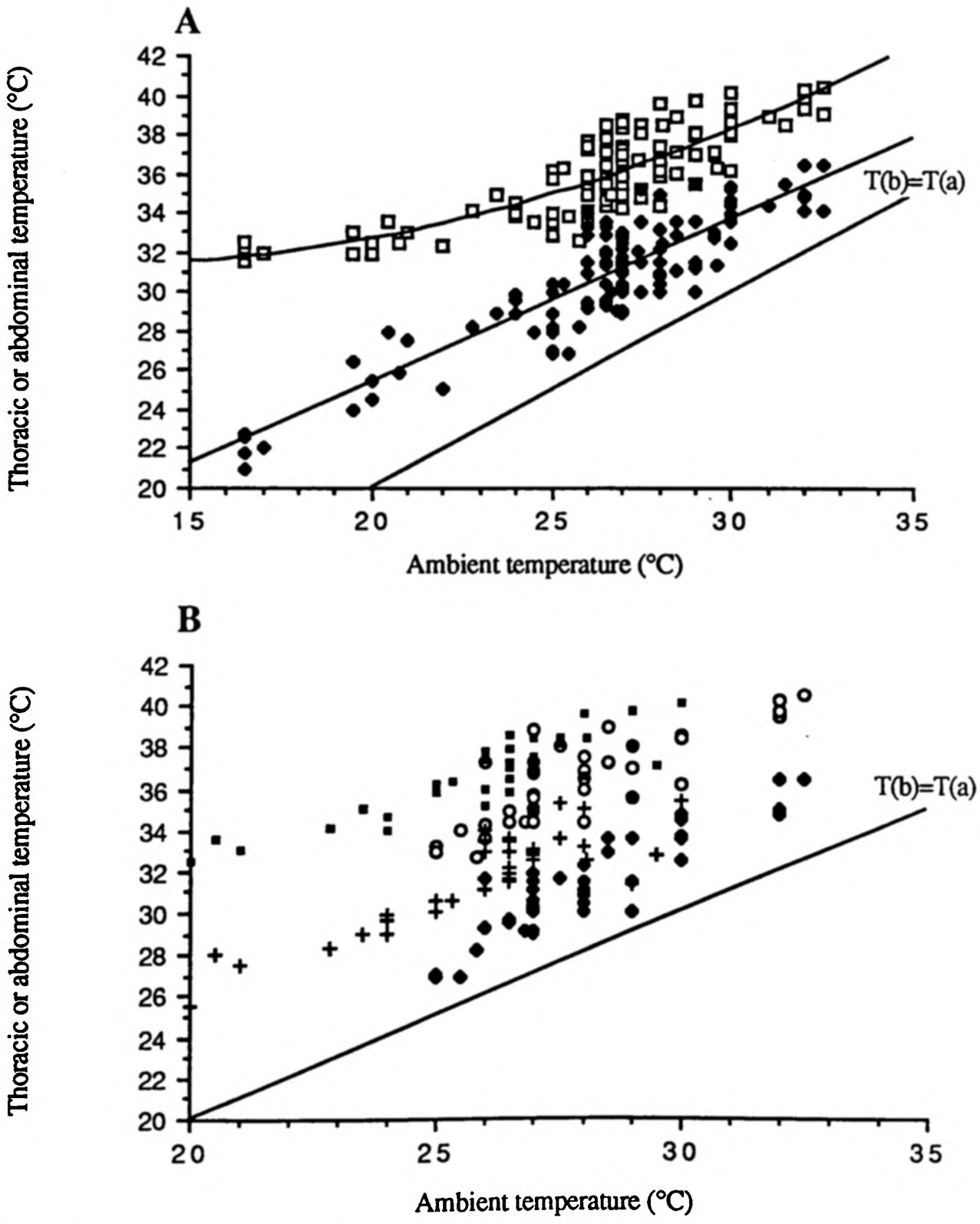


Fig.5.2 **(a)** Thoracic (❐) and abdominal (◆) temperatures of female *A. sapiens* from all sites as functions of ambient temperature (T_{th}: $y=34.1-0.49x+0.021x^2$, $R^2=0.706$; T_{ab}: $y=8.6+0.84x$, $R^2=0.792$). **(b)** Thoracic and abdominal temperatures of *A. sapiens* as functions of ambient temperature, divided between Kuk and Madang populations: Kuk T_{th} (■), Kuk T_{ab} (+), Madang T_{th} (O), Madang T_{ab} (◆).

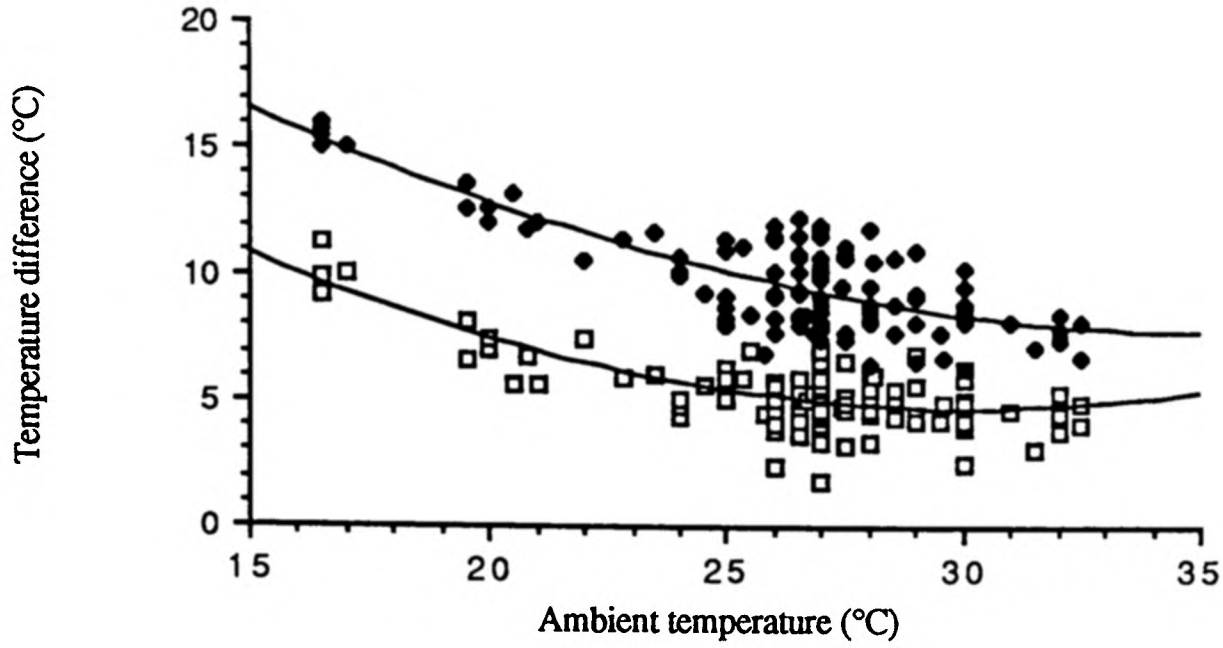


Fig.5.3 T_{ex} (◆)and T_{dif}.(❐) as functions of ambient temperature for *A. sapiens* from all three sites (T_{ex}: y=34.1-1.49x+0.021x2, R^2 =0.624; T_{dif}: y=29-1.62x+0.027x2, R^2 =0.578).

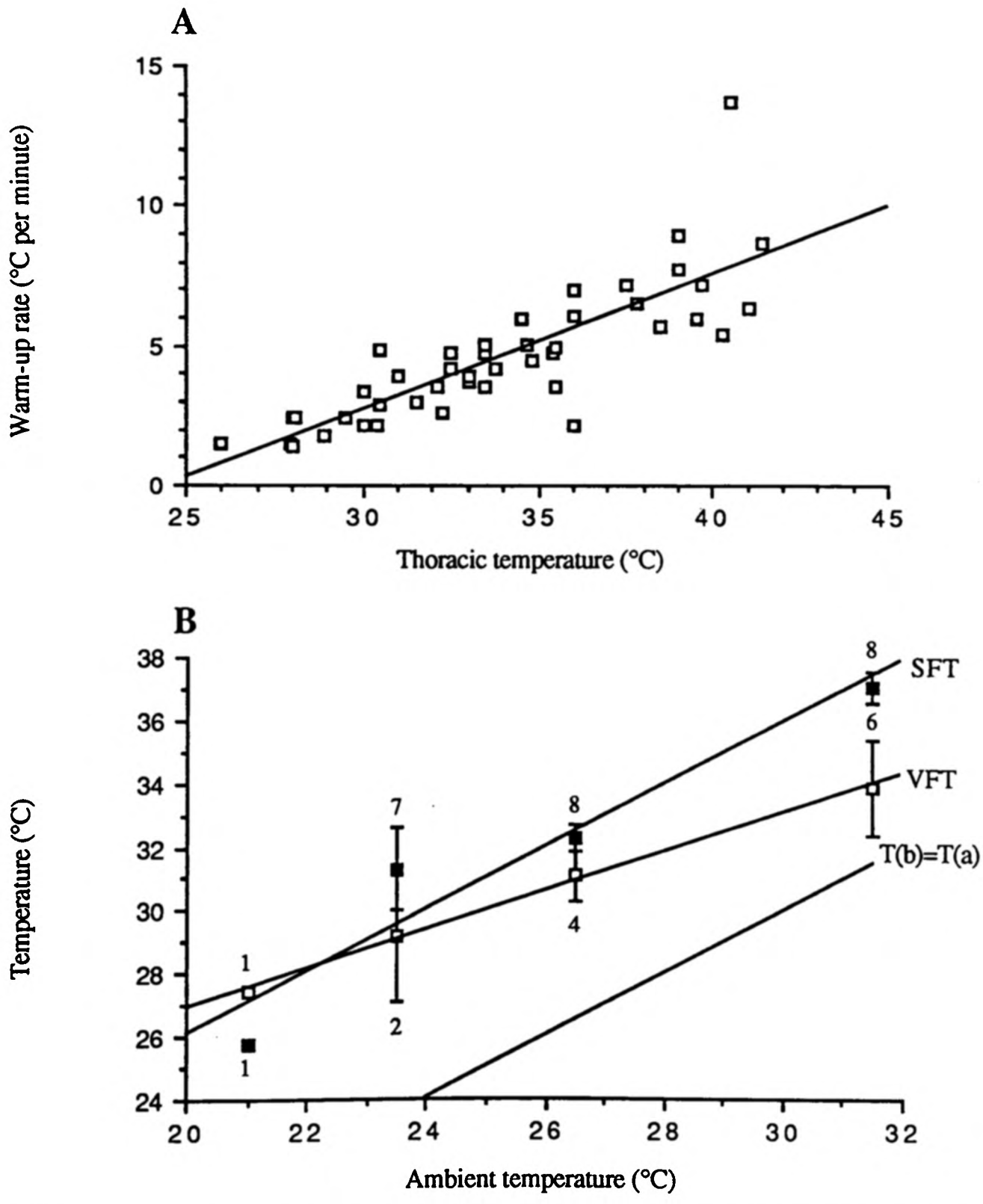


Fig.5.4 **(a)** Warm-up rate as a function of thoracic temperature in *A. sapiens* (y=-11.9+0.49x, R^2=0.672). **(b)** SFT (■) and VFT (❐) as functions of ambient temperature for *A. sapiens* (SFT: y=6.3+0.98x, R^2 =0.924; VFT: y=14.6+0.61x, R^2 =0.997).

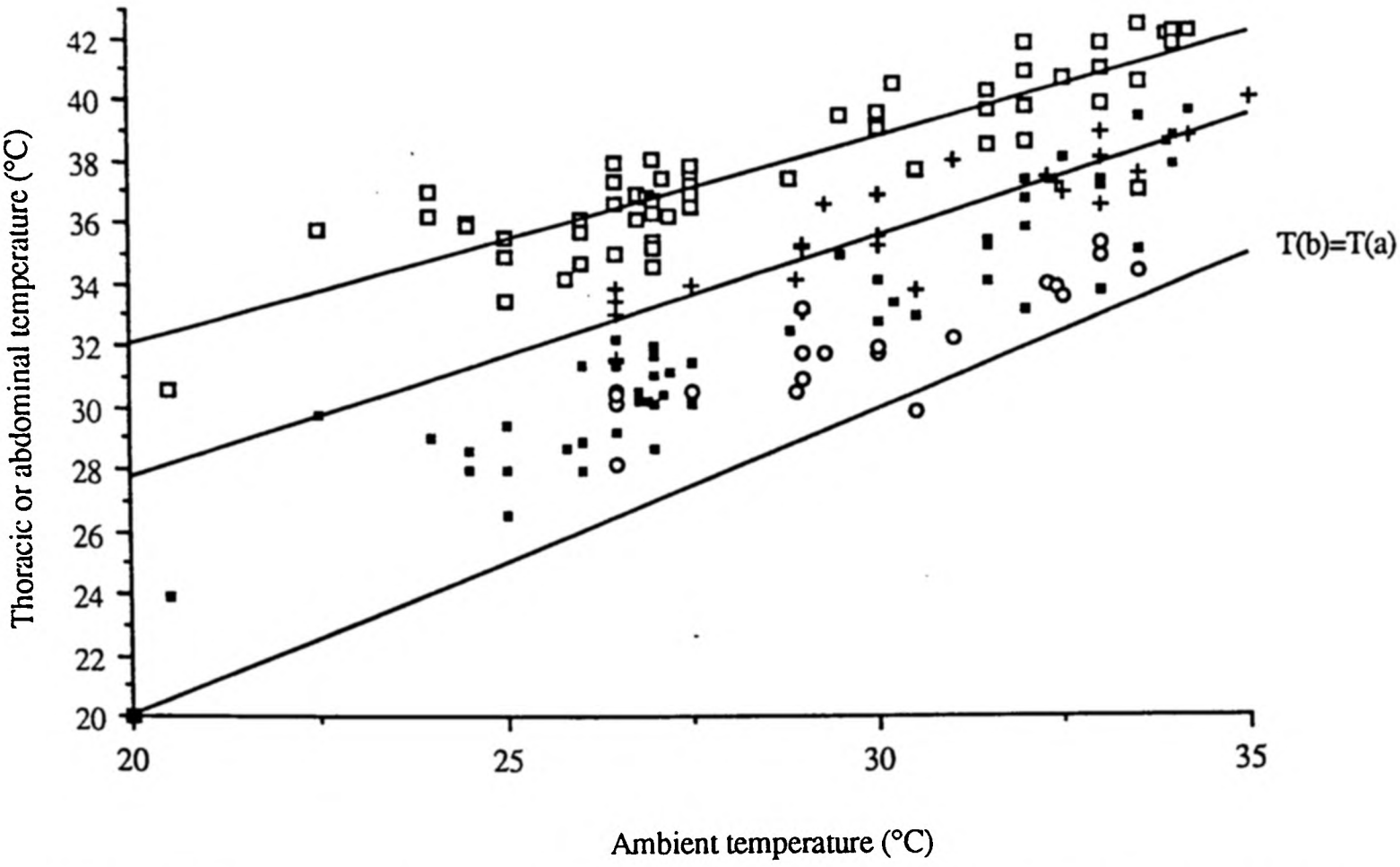


Fig.5.5 T_{th} and T_{ab} as functions of ambient temperature for ♂ and ♀ *C. frontalis* (♀ T_{th}: y=18.5+0.68x, R^2 =0.774; ♀ T_{ab}: y=3.8+1.0x, R^2 =0.865; ♂ T_{th}: y=11.9+0.8x, R^2 =0.846). Key t symbols: male T_{th} (+); female T_{th} (□); male T_{ab} (O); female T_{ab} (■).

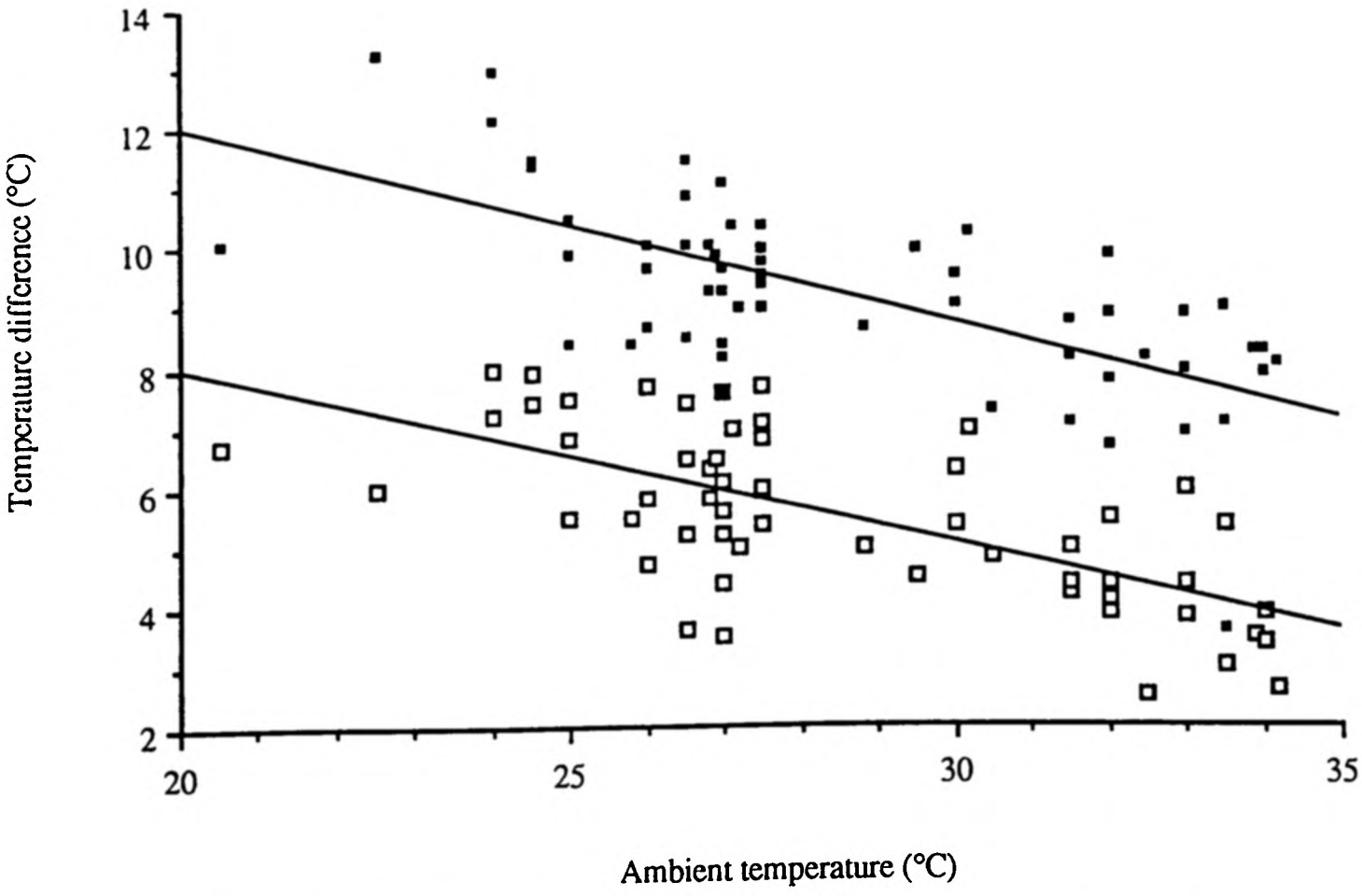


Fig.5.6 T_{ex} (■) and T_{dif}.(□) as functions of ambient temperature for ♀ *C. frontalis* (T_{ex}: y=18.5-0.32x, R^2 =0.449; T_{dif}: y=13.8-0.29x, R^2 =0.462.

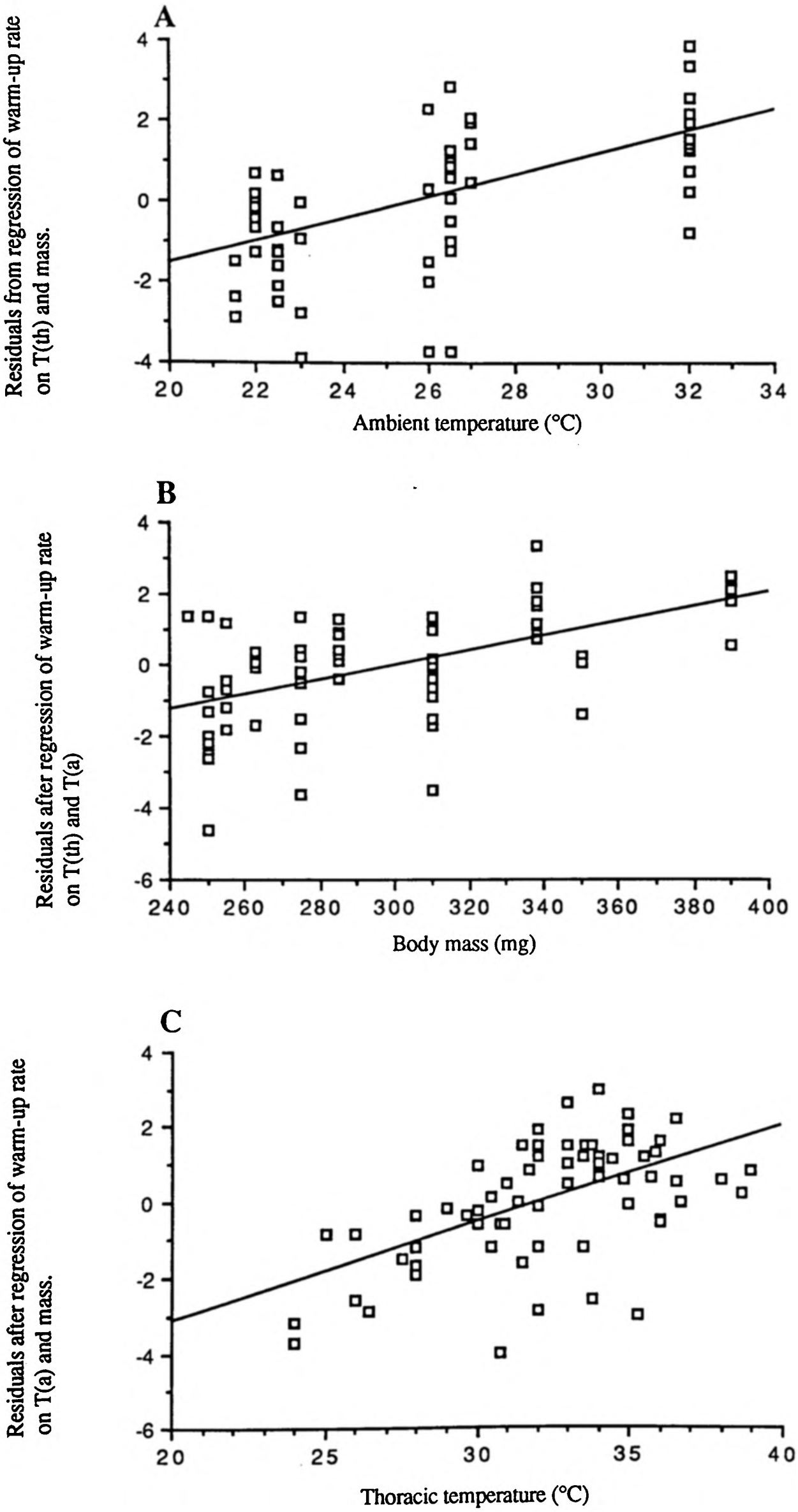


Fig.5.7 (a) Residuals after regression of warm-up rate on T_a and body mass as a function of T_{th} in ♀*C. frontalis* (y=-8.25+0.25x, R^2 =0.302). **(b)** Residuals after regression of warm-up rate on T_{th} and T_a as a function of body mass in ♀*C. frontalis* (y=-6.1+0.02x, R^2 =0.302) **(c)** Residuals after regression of warm-up rate on T_{th} and body mass as a function of T_a in ♀*C. frontalis* (y=-7.0+0.27x R^2 =0.372).

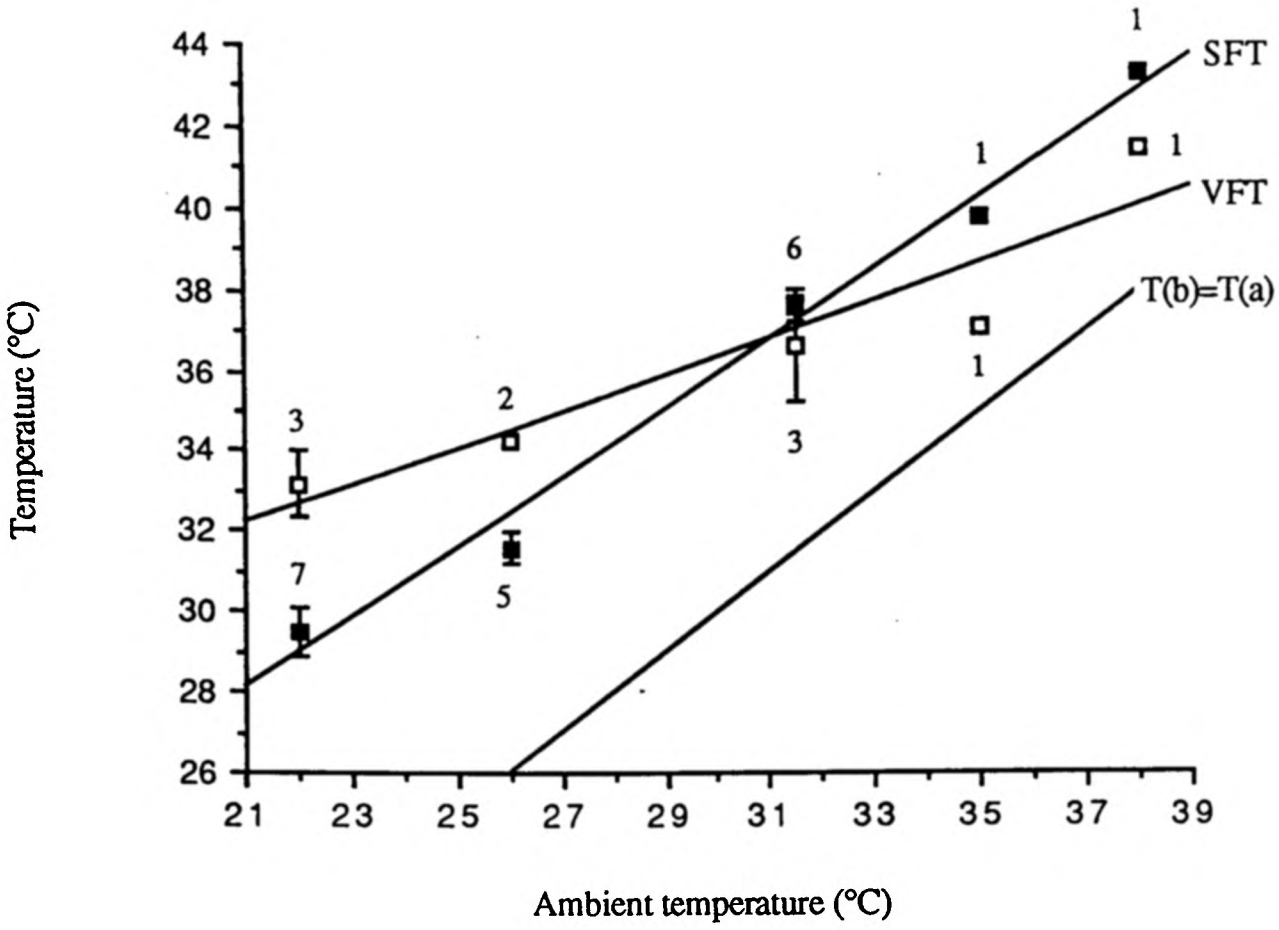


Fig.5.8 SFT (■) and VFT (❐) as functions of ambient temperature for ♀ *C. frontalis* (SFT: y=9.7+0.87x, R^2 =0.988; VFT: y=22.5+0.46x, R^2 =0.878).

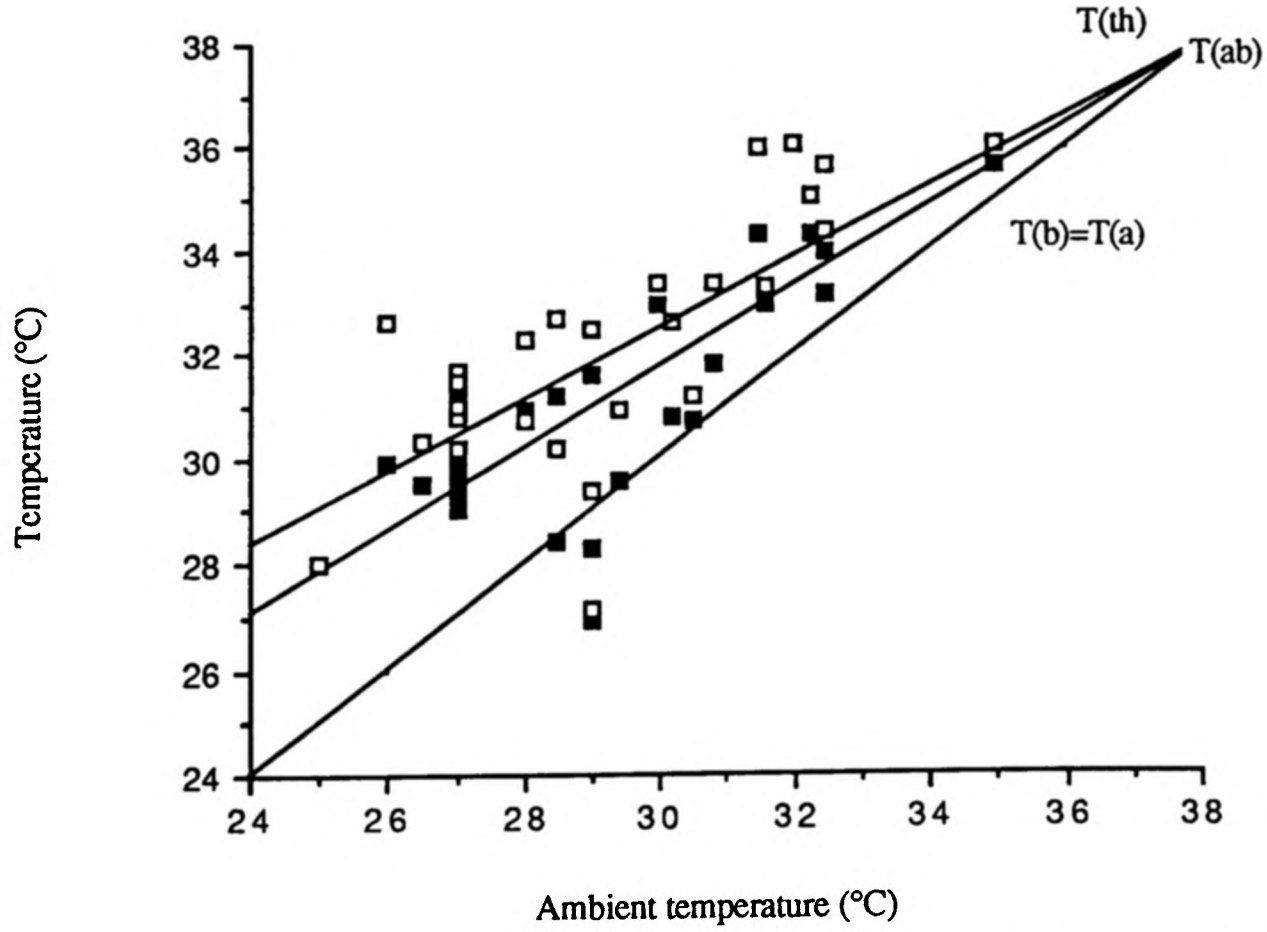


Fig.5.9 T_{th} (❐) and T_{ab} (■) as functions of ambient temperature for *Thyreus quadrimaculatus* (T_{th}: y=10.7+0.73x, R=0.76; T_{ab}: y=8.43+0.78x, R=0.78).

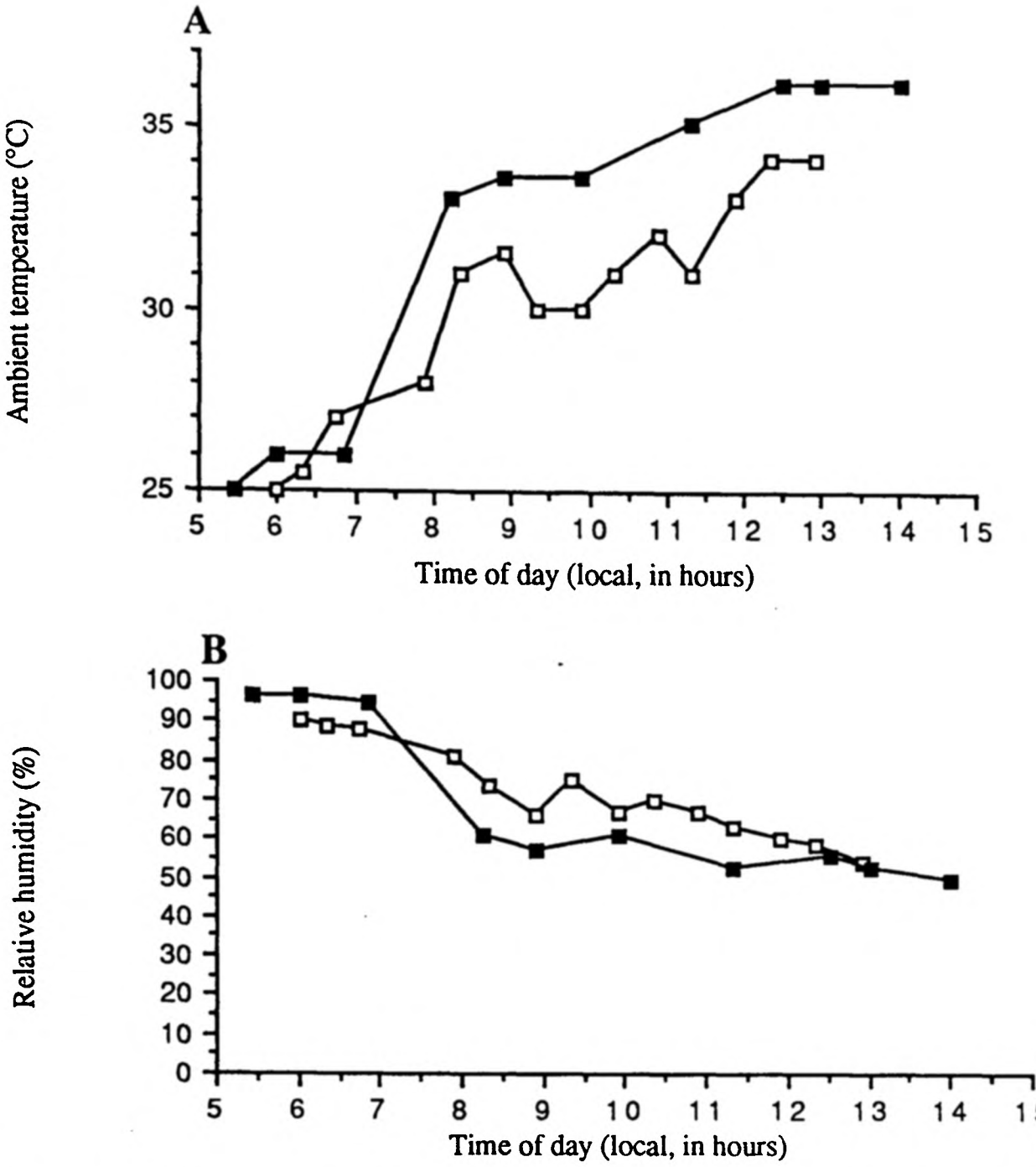


Fig.5.12 (a) Ambient temperature and **(b)** relative humidity as functions of time of day at the *S. jamaicensis* study site on 16.9.87 (❐) and 17.9.87 (■).

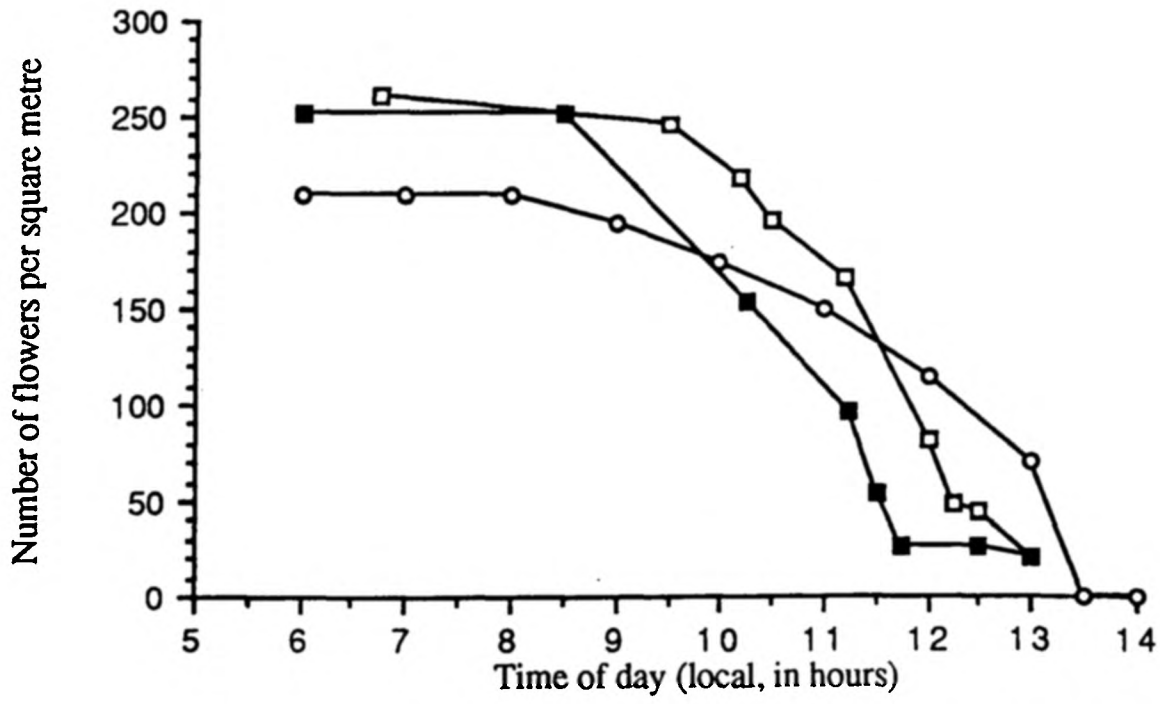


Fig.5.13 The number of available flowers per square metre as a function of time of day for exposed flowers on 16.9.87 (❐) and 17.9.87 (■), and for netted flowers on 17.9.87 (O) at the *S. jamaicensis* study site.

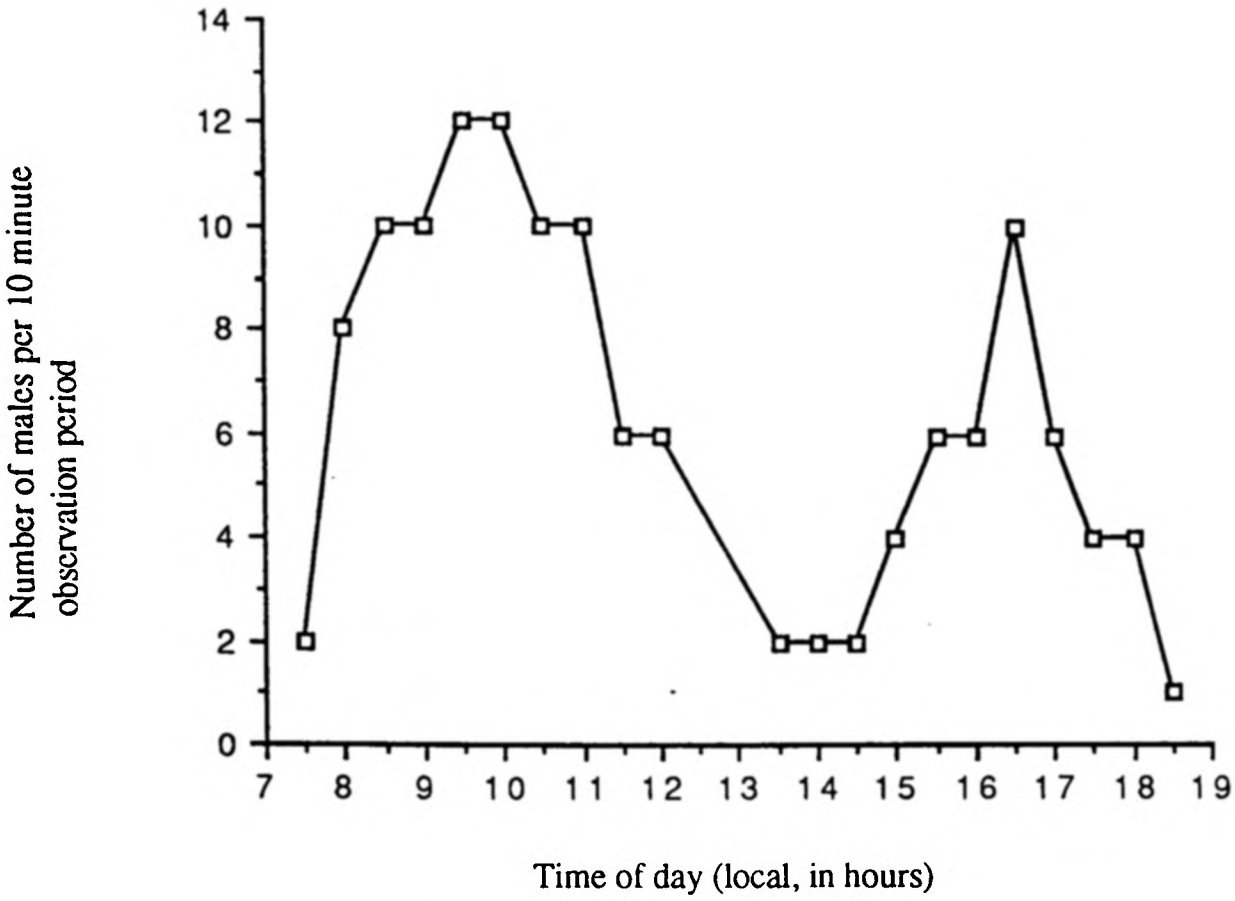


Fig.5.14 Activity over time of ♂ *C. frontalis* at the nest site on 17.10.87. Male activity was scored by counting the number of marked males flying within the 5m length of ditch where nesting females were concentrated. The observer walked up and down the road adjacent to the nesting aggregation during each ten minute observation period.

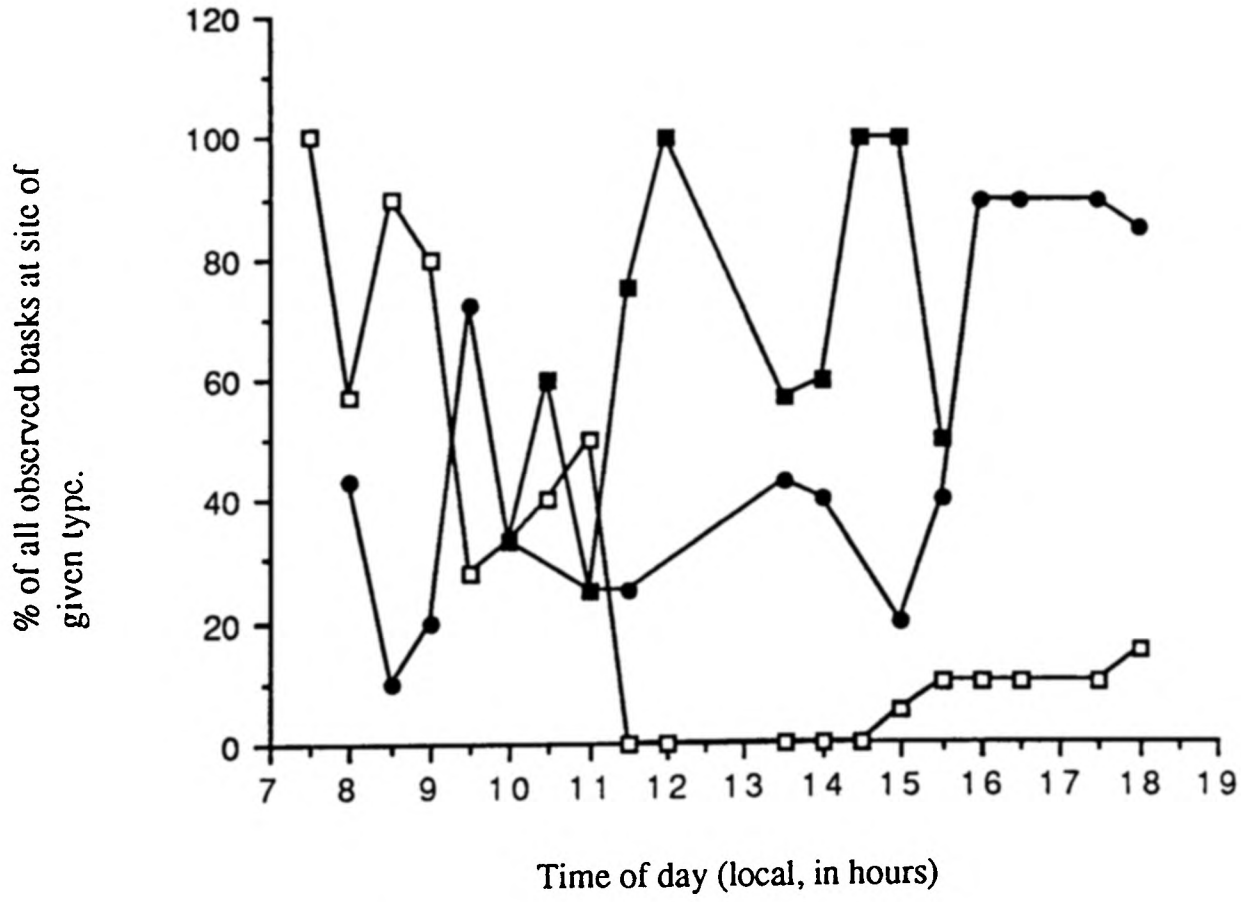


Fig.5.15 Preference of ♂ *C. frontalis* at the nest site for different basking sites over time: nest entrance area in ditch (●), soil bank above ditch (❐), and coffee bushes (■).

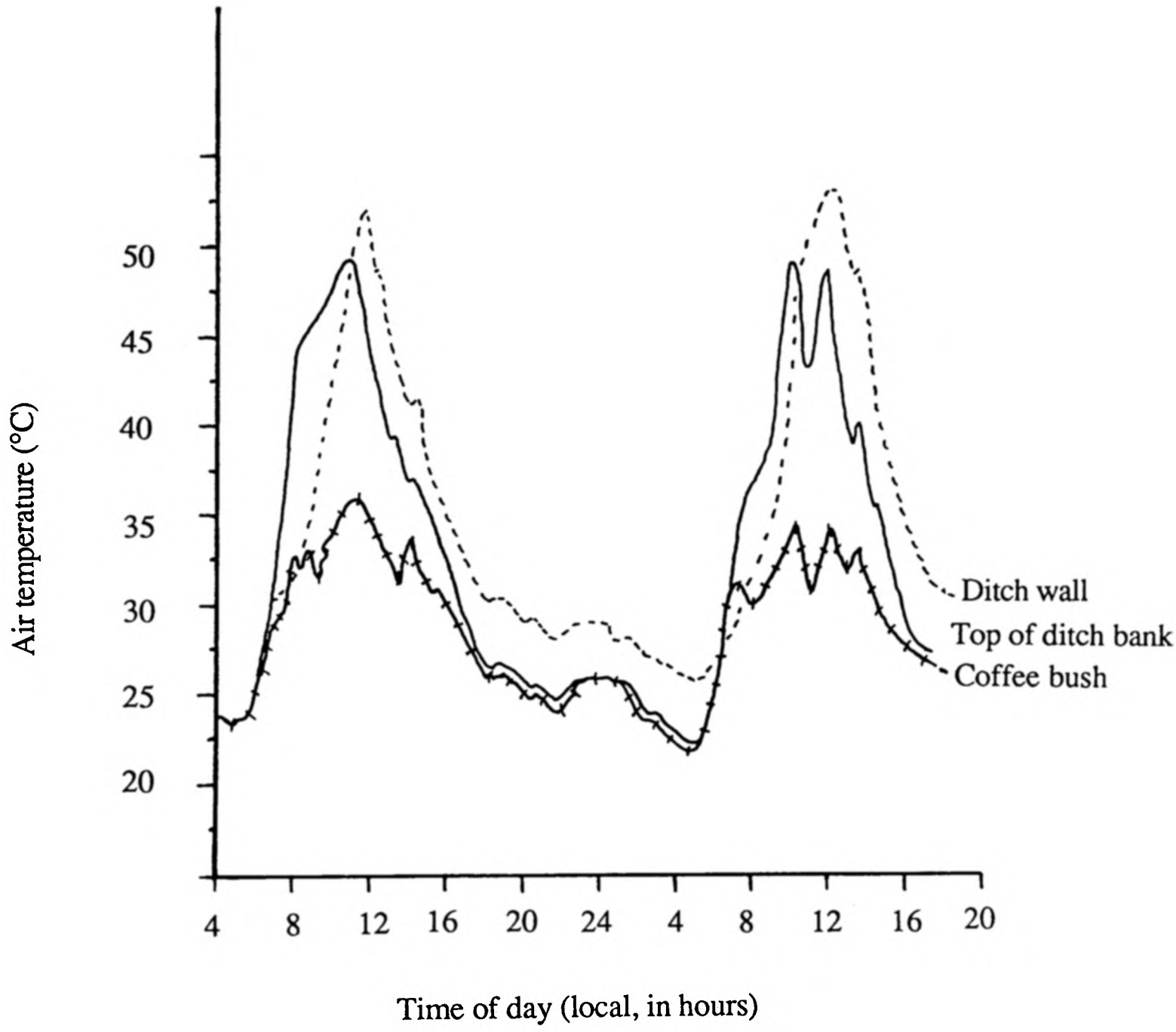
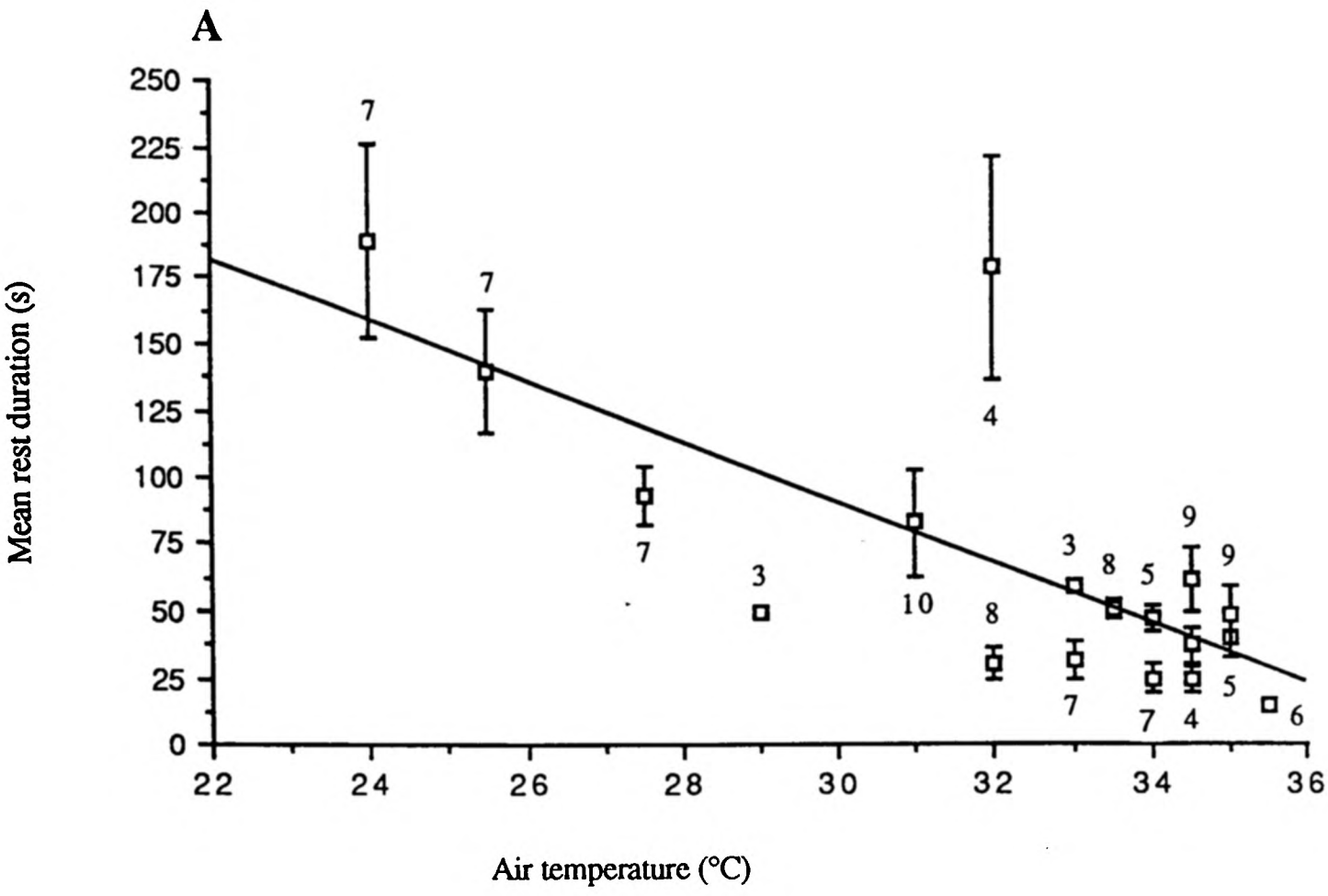


Fig.5.16 Changes in air temperature over a 36 hour period at the three basking site types in Fig.5.15. The thermocouple in the coffee bush was located 1.5m above the soil surface. The foliage of the bush was very open, and the sensor was therefore probably exposed to direct solar heating some of the time.



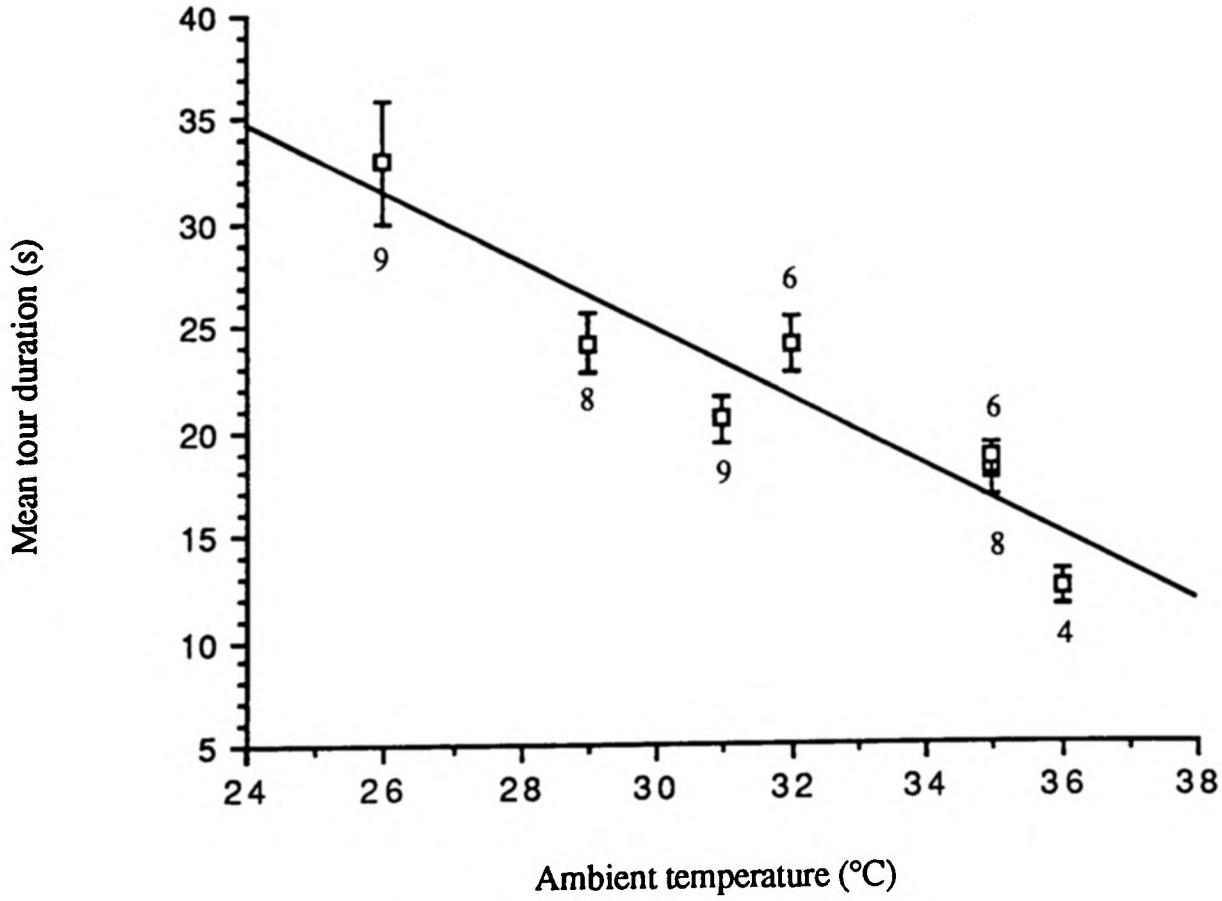


Fig.5.17 (a) Mean bask duration and **(b)** mean patrol tour duration as functions of air temperature fo: ♂ *C. frontalis* at the nest site (bask duration: y=430.5-11.3x, R^2 =0.547; patrol duration y=74.1-1.64x, R^2 =0.865). Data were obtained at the same time intervals as those in Fig.5.17. A unimodal change in air temperature through the day (Fig.5.18) results in more than one reading at some air temperatures

Chapter 6. Comparative analyses of the role of body mass in determining warm-up rates and body temperatures in the Apoidea.

6.1 Introduction.

In small endotherms, the physical limitations of size and shape must constrain endothermic ability (reviewed by Bartholomew 1981). In this Chapter I ask how closely cross-species analyses of four measures of endothermic ability in bees agree with the hypothesis that body size limits warm-up rates and the levels of sustained body temperatures. The results discussed below suggest that within the constraints imposed by body size there is considerable 'leeway' within which other selective pressures can act on endothermic parameters. As well as body mass (Chapter 4), the ambient temperatures that the species experiences while it is active (or 'thermal regime') are shown to be of considerable importance in determining warm-up rates and the levels of sustained thoracic temperatures in bees. Thermal regime (defined fully below) differs significantly between subtaxa (families, genera) within the Apoidea, which means that unless this taxonomic effect is controlled for, relationships between endothermic parameters and body mass are obscured (Stone and Willmer 1989b). The possibility that differences in the endothermic abilities of bees represent different strategies of body temperature regulation within this essentially endothermic group is discussed. The possible form that the relationship between warm-up rate and body temperature might take, based on some simple (but important) assumptions, is discussed below. Similar reasoning can then be applied to the other laboratory measures of endothermic ability introduced in Chapter 4: voluntary flight temperature (VFT) and stable flight temperature (SFT). Field data for 'grab-and-stab' measurements of T_{th} are also analysed.

Two analyses are presented: the first uses data for 20 species within the genus *Anthophora*, including 19 species studied in Israel in the spring of 1989, and 2 British species, in which taxonomic effects are assumed to be small. The second, carried out in collaboration with Dr. Pat Willmer, uses data for 69 species in 24 genera over 6 families, and controls for taxonomic effects; an earlier form of this work has already been published (Stone and Willmer 1989b). In the Discussion the results obtained in these analyses are compared with those presented for *Anthophora plumipes* in Chapters 3 and 4.

Warm-up rates are determined by the balance between heat generation and loss. These two factors are differentially affected by changes in body mass.

(a) If it is assumed that thermogenic power output per unit mass of thoracic muscle is constant, and the mass of the thoracic musculature is a constant proportion of total body mass, then total thermogenic power output is a linear function of body mass. That this may not in fact be the

case is discussed in section 6.5.

(b) If heat loss occurs mainly by convection over the animal's surface, then, since for a given body form surface area to volume ratios increase with decreasing size, specific rates of heat loss (measured in terms of conductance: joules per unit time per degree celsius above ambient temperature per unit body mass) increase with decreasing body mass. The negative correlation between conductance and body mass is well known for both vertebrate (McNab 1970; Bradley & Deavers 1980; Bartholomew 1981) and insect endotherms (Bartholomew & Epting 1975; May 1976a; Bartholomew 1981). This relationship is also shown to hold within a sample of British bee species below.

The relationship between warm-up rate and body mass will be determined by the relative strengths of these two relationships (a and b above). I have assumed that body mass correlates well with the linear dimensions of the thorax across the sample of bees to be compared. It is possible that one bee species might have many more airsacs in its thorax than another of the same size, and thus have a lower mass and the same surface area . Within a single genus of morphologically very similar bees (*Anthophora*, below) this assumption would appear to be sound. Across the Apoidea in general it is possible that such variation may contribute to the differences found between families or genera. If variation in the relationship between linear dimensions and body mass is taxonomically distributed, then it is controlled for using the comparative methods used in this chapter.

Among heterothermic vertebrates, metabolic rates per unit mass of tissue are not constant, but increase with decreasing body mass (Bartholomew 1981). The greater thermogenic ability per unit mass of the smaller heterothermic vertebrates more than compensates for their higher rates of heat loss. Thus, for vertebrate heterotherms undergoing daily torpor (e.g. hummingbirds; Lasiewski & Lasiewski 1967; Schuchmann *et al.* 1983), the relationship between body mass and warm-up rate is a highly significant negative correlation (Bartholomew 1981), smaller endotherms warming more rapidly than large ones. Although warm-up rates for endothermic insects are compatible with an extension of the relationship for vertebrates to lower body masses (Heinrich & Bartholomew 1971; Bartholomew 1981), the form of the relationship within the insects remains uncertain. Some studies suggest that these two variables are not simply related (Heinrich & Bartholomew 1971; Heinrich & Casey, 1973; Bartholomew & Epting, 1975; Dyer & Seeley, 1987), while other studies have found a positive correlation between the two (May 1976a; Morgan & Heinrich 1987). May (1976a) and Bartholomew (1981) have suggested that, in the size range of endothermic insects, rate of heat loss will be the most important factor determining warm-up rates. If this is so, then larger insects, with lower surface area to volume ratios, should be able to warm up more rapidly and to sustain a higher T_{ex} at a given ambient temperature. This assumes that, within the sample of species

compared, heat loss is dependent simply on surface area and is not differentially modified between species by, for example, pubescence or insulating airsacs within the body. It also assumes that, mass for mass, the thermogenic abilities of the tissues concerned are the same for the species compared, although there is some indication that these are relatively greater in smaller insects within an endothermic group (May 1976a; Bartholomew 1981). Perhaps most importantly, it assumes that these purely physical characteristics are limiting for insect warm-up.

Although there are several interspecific comparisons of T_{th} in flight in endothermic insects, no general conclusions on the importance of body mass have been established (see Discussion). The roles of several other factors in determining body temperatures independent of body mass have been investigated, particularly wing loading (Bartholomew & Heinrich 1973; Bartholomew & Epting 1975; Bartholomew & Casey 1978; Casey 1981a; Casey *et al.* 1981). In bees, wing loading is generally very high (Byrne *et al.* 1988) and here it is assumed that the role of wing loading in determining differences in flight temperatures between species is relatively small in comparison with the roles of body mass and thermal environment (see below) (Bartholomew & Heinrich 1973). It is, however, important to realise that once the factors investigated here have been controlled for, wing loading is likely to prove a further useful factor in the prediction of warm-up rates and body temperatures. If the assumptions used above are true, and heat loss is a limiting factor in the body temperatures of small endotherms, then a positive correlation between body temperatures in flight and body mass is predicted, other things being equal. Indeed, because flight in bees always involves greater convective cooling of the body than warm-up (due to wing movement), the relationship should be more apparent than that which exists between warm-up rate and body mass. This is tested in analyses both of field 'grab-and-stab' data and of laboratory SFT data.

Voluntary flight temperatures are closely linked to warm-up rates. For any bee, rates of heat loss increase with the T_{ex} generated above ambient temperature. At a given T_{ex}, small bees should experience greater rates of heat loss than large bees due to their higher surface area to volume ratio. This means that with their thermogenic systems operating at full power, larger bees will reach a higher temperature before rates of heat loss equal rates of heat generation than smaller bees, other things being equal. If it is true that all bees get as warm as they can, or warm until a certain maximum percentage of heat generated is being lost before flight, then VFT should increase with body mass. If bees take flight at body temperatures well within the limits of their endothermic ability, then a 'noisy' relationship, with much variation due to other factors, would be expected.

Although there is a considerable and growing literature on the effects of climate on body temperature in vertebrate endotherms (e.g. Chappell 1980; Chappell & Bartholomew 1981), I know of no study that has incorporated the effects of both body mass and the thermal regime to which the

species is adapted on warm-up rates and body temperatures in insects. Such studies are hindered by the biassed taxonomic distribution of previous studies, which focus on a few insect orders, and on a few families within these orders (see below). In the past, interest has focused on particular families within the Apoidea, particularly bumblebees of the genus *Bombus* (e.g. Heinrich 1972a,b,c,d, 1974; Heinrich & Heinrich 1983), the honeybee *Apis mellifera* (e.g. Heinrich 1979; Cooper *et al.* 1985; Dyer & Seeley 1987) and euglossine 'orchid bees' (May 1976a; May & Casey 1983) in the family Apidae, and the large carpenter bees of the genus *Xylocopa* in the family Anthophoridae (e.g. Louw & Nicolson 1983; Nicolson & Louw 1982; Chappell 1982; Baird 1986; Heinrich & Buchmann 1986; Willmer 1988).

6.2 Methods.

A. Physiological methods.

The methods used to obtain the physiological variables used in the following analyses are those described in Chapter 2. Two important considerations when gathering data from a variety of sources on bees of a variety of sizes are the possibility of significant heat losses from the bee down the sensor leads, and variation in the depth of insertion of the thermocouple sensor. These are also discussed in full in appendices to Chapter 2. The terms used in this chapter (MWR, SFT, VFT) are as defined in Chapters 2 and 4 unless specifically modified (see below).

Because warm-up rates and body temperatures within a species depend on ambient temperature (Chapter 4, and Discussion, below), it is necessary to standardise the ambient temperature at which the data are gathered. All the data used in my comparative analyses were obtained at an ambient temperature of 20-22°C in still air in the absence of any source of radiant heat. This choice of ambient temperature allows incorporation of data from several other studies (Table 6.2). A major point in the subsequent analysis is that such an arbitrary standard temperature for comparison disregards interspecific differences in both the temperature range the bees are 'used to', and in the minimum T_{th} required for flight. The thermal regime to which the bee is adapted is incorporated in the measurement of MTA (see below). When comparing the abilities of different species to fly at low T_a, it is important to consider the minimum T_{th} necessary for flight. This determines the minimum T_{ex} the bee must generate over T_a, and is thus an important determinant of the energetic cost of warm-up. An absolute value is not easy to obtain. Thoracic temperatures at which bees cease to fly in the field are probably greater than the minima at which they will initiate tethered or free flight in the laboratory with sufficient provocation. Minimum T_{th} for flight are important in the energetics of warm-up and it is therefore necessary to know about them in order to relate the endothermic ability of a species to its environment. At present there is insufficient

methodologically compatible data to allow a comparative analysis. However, a preliminary analysis of other variables is nonetheless revealing.

B. Comparative analysis.

Several studies have mentioned the considerable variation in warm-up rates in endothermic insects of similar body masses (e.g. May 1976a; Casey *et al.* 1981; Bartholomew 1981), even among closely related species (Bartholomew 1981). Warm-up rate and body temperature may well be affected by factors other than body mass (such as body shape or pubescence), and the relationships between warm-up rates and these other factors may well vary among taxa. When making interspecific comparisons of this type there is a clear risk of generating spurious across-species patterns due to differences between taxa that are independent of the variables being examined.

Several methods have been used to minimize such taxonomic artefacts (see Pagel & Harvey 1988 for a recent review). One way of minimising the errors involved is to look at patterns within a closely related group of species. The assumption is that other factors affecting warm-up rates (or other characteristics to be compared) do not differ significantly between the species concerned, and that any relationships with body mass that do emerge are real. This type of 'naïve' approach (Grafen 1989) has been used for the analysis within the genus *Anthophora*. Where the species concerned are not closely related enough for this assumption to be acceptable, more complex methods of analysis must be applied.

Covariance in characters of fundamental biological importance within species, and the resulting interspecific relationships between these characters, should have arisen many times during the course of evolution (Ridley 1983; Felsenstein 1985; Huey 1987; Pagel & Harvey 1988). Thus among daughter taxa (families, genera, species) evolving from a single common ancestor in a higher taxon (order, family, genus), biologically important patterns should be repeated. Each time daughter taxa separate, the relationship between the variables concerned is free to evolve independently.

The statistical analysis used in section 6.4 is phylogenetic regression, developed by Grafen (1989), which uses multiple regression to analyse correlations between variables among all the daughter taxa of a higher taxon for all the higher taxa in a phylogeny. In this respect, phylogenetic regression is very similar to a model proposed by Felsenstein (1985). The relationships between the variables in each radiation from a higher taxon [rather than across all species as in the 'naïve' (Grafen 1989) interspecific analysis described above] become data points in the analysis. Each intra-taxon comparison is independent of the others because each taxon has only a single common ancestor, and an interspecific difference leading to the relationship in question has to re-evolve in each taxon. It is

not necessary to know the exact phylogeny to use this analytical method. All that is necessary is that none of the groups used is polyphyletic. Were the groups polyphyletic, the discovery of a relationship between the variables concerned within the group need not indicate a genuine relationship, but could instead indicate a difference between the monophyletic or paraphyletic groups within the polyphyletic assemblage. All the species within a genus are assumed to form a monophyletic group, and the same is assumed for genera within families and families within the superfamily. The degrees of freedom for the F ratios in the text are given by the number of within-taxon analyses contributing to the phylogenetic regression. In this analysis, Grafen's method has several advantages over alternative approaches (Pagel & Harvey 1988). It allows maximal use of a limited data set, can control and test for as many variables as required, and does not require precise knowledge of the true binary phylogeny of the species concerned. To allow comparison with existing studies, 'naïve' (Grafen 1989) simple and multiple regression analyses have been included where useful. When illustrating in multiple regression analyses the significance of one x variable having controlled for another, residuals of the y variable after simple regression on the first x (controlled) variable have been plotted as a function of the second (test) x variable. When preliminary analysis indicates a logarithmic relationship between two variables, I have used logarithmically transformed data. Complete data sets are not available for all the species discussed here, leading to different sample sizes for each analysis.

When there is more than one source for a species, as for *A. mellifera*, mean values from the available studies have been used. This by no means implies that legitimate variations within a species should be ignored. My assumption, implicit in all interspecific comparisons, is that the intraspecific variation is considerably less than the interspecific variation. The names used for the bumblebees in Table 6.2 are *sensu* Kloet and Hincks (1978) for the European species, and *sensu* Krombein *et al.* (1979) for the North American species. The taxonomy and phylogeny of the genus *Anthophora* follows Brooks (1988).

C. Definition of terms.

1. Thermal Regime.

It is impossible to use a single value to describe the average thermal conditions under which a species is active, and any value used will necessarily be an approximation open to criticism. An ideal measure would be similar to the standard operative temperature (T_{es}) used by Bakken (1976), Chappell (e.g. 1982) and others, which integrates the effects of air temperature (T_a), convection and radiation on body temperature, and would be integrated over the period during which the species is active. Although attractive, such a measure is probably impracticable. What interests

me here are the differences between bees active in warmer or colder thermal regimes. One limit to a bee's endothermic ability can be estimated by the minimum temperature at which it is able to forage. Ideally, again T_{es} minima should be used, but in this study minimum air temperatures (MTA) are used as an approximation. In tropical or subtropical species with warm thermal regimes minimum air temperatures for foraging are generally encountered in morning or evening. Minimum air temperatures for foraging in species active in low-temperature regimes may be obtained during sunny periods on cold days. Under such conditions the error between T_a and T_{es} would be more important for bees in cool than in warm thermal regimes (see Chappell & Bartholomew 1981). For species for which I have collected data, MTA values are given for overcast days. When using data from published studies the minimum quoted T_a at which foraging was recorded have been used. During fieldwork work in Israel, MTA values were obtained over only four weeks of observation, and such values are probably over-estimates of the true MTA for each species. I have assumed that there is no systematic bias in the error involved. Clearly the use of such data is far from ideal, but the strength of the resulting relationships despite this source of error suggests that this approximation is justified.

2. Mean warm-up rate (MWR)

This measure is identical in principal to the mean warm-up rate introduced in Chapter 4, and the methods used to record warm-up rates are as described in Chapter 2. In incorporating data from other studies this definition has had to be widened slightly in order to obtain a single measure of warm-up rate for each species to be used in my analysis.

How the value of the mean warm-up rate was obtained depended on the form of the warm-up curve shown by the species. Some species typically showed linear warm-up at the T_a used in this analysis (20-22°C), the warm-up rate seemingly independent of changes in body temperature. In such cases, the mean gradient was calculated for each bee, and the mean of these values calculated to obtain a mean for the species. For all species, data for males and females have been treated separately. In other species, warm-up rate increased with increasing body temperature and body temperature increased exponentially with time until flight temperatures were reached. For such bees, gradients for linear approximations to 3°C sections of the warm-up curve were obtained and means calculated for each bee. The mean of these individual means was then used as the MWR for the species.

Each bee used in the analysis performed at least several warm-ups, and where warm-ups were not simply linear care was taken to ensure that measured warm-up rates were not biassed to relatively low or relatively high T_{th}. I assume that my measurements of average warm-up rate have not been seriously or systematically biassed by differences between species in the form of the warm-

up curve. This is not to say that the form of the warm-up curves is unimportant (see Chapter 4), simply that while warm-up rate may depend on T_{th} (Chapter 4), I am testing here whether it depends also on body mass and thermal regime. Warm-up rates given in the literature, unless referred to as peak rates, are assumed to be mean rates. In two cases where warm-up rates have not been quoted, but figures showing a typical warm-up are presented, approximate rates have been calculated from the figure. The peak warm-up rate (PWR) shown by a species is the highest rate maintained over a 5°C temperature interval for the species.

3. Thoracic temperatures in flight.

Data for T_{th} of flying bees at ambient temperatures of 20-22°C have been used. These were obtained using the 'grab-and-stab' techniques described in Chapter 2. In species for which 20-22°C lies at the lower limit of the ambient temperature range in which they were active, emphasis was put on sampling as many bees as possible within this temperature range. Where 20-22°C lies in the middle of the T_a range over which a species was active, an average T_{th} was estimated either from data taken in the required T_a range or by extrapolation from a plot of T_{th} on T_a. In each case the number of values in the required ambient temperature range is given in parentheses in Table 6.2. Results from other studies used in section 6.4 are either T_{th} quoted for the temperature range 20-22 °C, or mean values estimated from figures of T_{th} as a function of T_a, or values calculated from best-fit regression equations to the same figure. There will be some variation in precisely what an ambient temperature of 22°C means - whether this is in the presence or absence of direct sunlight, for example, or of moving air. It is assumed, however, that such variation merely constitutes noise, and is unlikely to bias any statistical tests systematically. The errors involved in comparing 'grab-and-stab' data between species where T_{es} data are not available will be to some extent parallelled by errors in the MTA data. Thus, when examining the relationship between body temperature and body mass, MTA can be used as an approximate control for the errors in 'grab-and-stab' data. The errors associated with 'grab-and-stab' measurement of T_{th} are assumed to be small (Stone and Willmer 1989a) and independent of investigator, body mass and taxonomy.

6.3. Comparative analysis within the genus *Anthophora*.

Data have been used for 20 species within the genus, of which all but *Anthophora quadrimaculata* and *A. plumipes* were studied in Israel. *A. plumipes* was studied both in Israel and in Britain. The taxonomic distribution of the species investigated (with the exception of two species yet to be identified) is shown in Fig.6.1, based on the work of Brooks (1988). Data summaries for each species in the following analyses are presented in Table 6.1.

Table 6.1 Summary of data on warm-up rates and body temperatures within the genus Anthophora.

Species	Where studied	sex	Mean body mass ± 1 S.E. (n) (mg)	MWR ± 1 S.E. (n) (°C)	PWR (°C)	VFT ± 1 S.E. (n) (°C)	SFT ± 1 S.E. (n) (°C)	MTA (°C)
Subgenus *Anthophora*								
A. plumipes (Pallas)	U.K.	m	160	8.85	15.1	33.1	31.7	3.0
A. plumipes (Pallas)	U.K.	f	185	12.3	19.6	35.1	35.1	0.0
A. plumipes (Pallas)	Israel	m	141±20 (5)	9.7±0.6 (17)	14.8	34.3±0.3 (14)	32.6±0.4 (4)	9.0
A. plumipes (Pallas)	Israel	f	177±5 (6)	11.05±0.4 (40)	16.3	35.7±0.4 (33)	34.1±0.3 (7)	6.0
A. senescens Lepeletier	Israel	m	100±10 (3)	10.4±1 (6)	14.4	38.7±0.6 (3)	34.8±0.1 (3)	10.5
A. senescens Lepeletier	Israel	f	140±5 (2)	11.0±0.6 (21)	15.75	35.1±0.3 (15)	32.1±0.1 (5)	10.5
A. fulvitarsis Brullé	Israel	m	237±5 (2)	11.9±0.7 (8)	15.65	37.1±1.0 (6)	37.4±0.2 (6)	8
A. fulvitarsis Brullé	Israel	f	242±7 (2)	13.6±0.9 (8)	16.75	38.3±0.3 (6)	37.4±0.2 (6)	8
Subgenus *Pyganthophora*								
A. erschowi Fedtschenko	Israel	f	132±5 (3)	8.75±0.5 (24)	12.45	37.1±0.7 (4)	33.7±0.8 (4)	8
A. libyphaenica Gribodo	Israel	m	145±14 (3)	8.9±0.3 (28)	11.6	35.1±0.7 (24)	33.65±0.7 (7)	6
A. libyphaenica Gribodo	Israel	f	247.5±6 (4)	11.9±0.6 (22)	16.65	37.6±0.6 (17)	37.0±0.1 (13)	6
A. nigriceps Morawitz	Israel	m	85.4±1.4 (5)	6.5±0.4 (25)	10.0	29.5±0.6 (13)	27.7±0.5 (12)	9.5
A. nigriceps Morawitz	Israel	f	114±5 (4)	7.4±0.6 (8)	10.5	34.3±0.6 (7)	31.6±0.3 (4)	9.5
A. rubricus Dours	Israel	m	93±3 (10)	6.85±0.3 (67)	12.5	30.85±0.35 (40)	29.95±0.65 (18)	9.0
A. rubricus Dours	Israel	f	120±6 (4)	7.85±0.3 (53)	13.5	34.25±0.6 (26)	32.2±0.4 (13)	9.0
A. sergius (Nurse)	Israel	m	80.0±13 (3)	6.2±0.5 (19)	12.0	34±0.8 (4)	31.7±0.5 (4)	11
A. sergius (Nurse)	Israel	f	85±8 (4)	7.6±0.4 (36)	12.65	33.6±0.6 (16)	30.0±0.6 (8)	11
A. sp.aff.*sergius*	Israel	f	160 (1)	8.55±0.8 (8)	10.8	39.8±0.1 (3)	35.0 (1)	8
A. vernalis Morawitz	Israel	f	361±3 (3)	9.5±0.4 (27)	14.4	37.95±0.6 (8)	37.2±1 (2)	10

Table 6.1 (continued)

Species	Where studied	sex	Mean body mass ± 1 S.E. (n) (mg)	MWR ± 1 S.E. (n) (°C)	PWR (°C)	VFT ± 1 S.E. (n) (°C)	SFT ± 1 S.E. (n) (°C)	MTA (°C)
Subgenus Lophanthophora								
A. biciliata Lepeletier	Israel	m	87±4 (2)	11.1±0.8 (18)	19.5	35.5±0.8 (8)	32.5±0.2 (3)	9.5
A. caelebs Gribodo	Israel	m	316±13 (2)	8.8±0.5 (4)	12.35	36.8±0.3 (3)	*	10
A. caelebs Gribodo	Israel	f	460±10 (2)	7.7±0.8 (8)	11.7	35.0±0.7 (6)	35.2±0.5 (6)	10
A. dispar Lepeletier	Israel	m	181±7 (8)	9.3±0.4 (56)	17.85	35.3±0.5 (27)	32.7±0.5 (15)	6
A. dispar Lepeletier	Israel	f	231±4 (6)	11.9±0.6 (22)	16.65	37.6±0.6 (17)	37.0±0.1 (9)	5
A. disparilis Friese	Israel	m	121±0.5 (4)	10.2±0.4 (35)	14.4	35.5±0.35 (25)	33.9±0.3 (11)	8
A. disparilis Friese	Israel	f	196.5±11.0 (2)	10.7±0.8 (19)	18.75	34.8±0.3 (13)	33.3±0.3 (4)	8
A. hispanica (Fabricius)	Israel	m	321±34 (3)	9.6±0.8 (15)	16.65	37.9±0.7 (9)	35.95±0.40 (3)	8
A. hispanica (Fabricius)	Israel	f	442±24 (3)	10.74±0.7 (20)	14.8	37.5±0.50 (11)	37.35±0.20 (9)	8
A. rutilans Dours	Israel	f	244±7 (3)	12.25±0.60 (33)	19.3	38.25±0.45 (18)	38.1±0.4 (7)	6
Subgenus *Petalosternon*								
A. priesneri Alfken	Israel	f	101±5 (2)	7.6±0.5 (9)	9.7	33.2±0.2 (2)	*	11
A. wegelini Friese	Israel	m	62.5±8 (2)	5.35±0.6 (8)	7.2	32.2±0.2 (2)	29.9±0.1 (3)	13
Subgenus *Dasymegilla*								
A. quadrimaculata (Panzer)	U.K.	f	90±4.5 (3)	5.1±0.5 (10)	7.0	30.8±0.35 (3)	*	10

A. Mean warm-up rate. Mean warm-up rates within the sample of *Anthophora* studied ranged from 5.1°C min^{-1} in female *A. quadrimaculata* (mean mass 90mg) and 5.3°C min^{-1} in male *A. wegelini* (mean mass 62.5mg) to 13.6°C min^{-1} in female *A. fulvitarsis* (mean body mass 242mg) (Fig.6.2, Table 6.1), the highest MWR value obtained for any bee. Fig.6.3a shows the relationship between log (body mass) and log (MWR). Within the species with a body mass less than 250mg (Fig.6.3a) mean warm-up rate increases linearly with body mass, but three large species in 2 different subgenera (*Anthophora vernalis*, *A. caelebs* and *A. hispanica*) have relatively low mean warm-up rates (Table 6.1). As the figure shows, there is no difference in the form of the relationship between males and females.

Fig.6.3b shows the relationship between log (body mass) and minimum air temperatures for activity (MTA). Because males and females of each species have different MTA and MWR values, data for both males and for females are incorporated in the figure. Bees able to fly at a lower air temperature are capable of higher rates of warm-up at 22°C. Multiple regression reveals that there is no significant effect of sex, and that both the negative correlation between MTA and log (MWR) and the positive correlation between log (body mass) and log (MWR) remain significant when the other relationship has been controlled for (n=32, R^2=0.47. MTA p=0.010; log (mass) p=0.011; sex p=0.88). To show that mass remains positively correlated with warm-up rate once the effect of MTA has been controlled for, the relationship between the residuals in log (MWR) after regression on MTA and log (body mass) is shown in Fig.6.3c, divided between the three main subgenera into which the species in my sample are divided by Brooks (1988).

Some variation in log (MWR) remains when these factors are controlled for. Species in the subgenus *Anthophora* tend to lie above the across-genus regression (and thus have positive residuals), while species in the genus *Pyganthophora* tend to lie below the line (and thus have negative residuals) (Fig.6.3d). Fig.6.4 shows residuals in log (MWR) after regression on MTA and log (body mass) for all of the species in the analysis, divided by subgenus. It is apparent from the figure that the residuals in the subgenera *Anthophora* and *Lophanthophora* are higher, on average, than those in the subgenus *Pyganthophora*. One way analysis of variance shows that there is a significant difference in residuals between subgenera (n=32, $F_{4,32}$ = 3.57, p=0.018). It is tempting to suggest that this result indicates some fundamental difference between the subgenera, shared by the members of each subgenus as a result of their common ancestry. However, the species studied form a small proportion of the total species in each subgenus (3 of 11 in *Anthophora*, 6 of 61 in *Pyganthophora* and 6 of 32 in *Lophanthophora* for example) (Brooks 1988), and any such conclusion can therefore only be tentative.

B. Voluntary flight temperature. VFT within the genus varied from near 30°C in the males of *A. nigriceps* and *A. rubricus* to near 38°C in *A. rutilans, A. fulvitarsis* and *A. hispanica.* The relationship between log (VFT) and log (body mass) is shown in Fig.6.5a. There is a strong positive correlation between the two when the effects of sex and MTA have been controlled for ($n=30$, $R^2=0.4$. log (body mass) $p=0.002$; MTA $p=0.680$; sex $p=0.62$). Again the form of the relationship is the same for males and females. The relationship is curvilinear, and the same large species mentioned in section A above have lower VFT values than some of the smaller species, such as *A. fulvitarsis.*

C. Stable flight temperature. Stable thoracic temperatures during tethered flight varied from less than 30°C in the males of *A. rubricus* and *A. nigriceps* to over 37°C in several of the larger species (Table 6.2). There is a clear positive correlation between log (SFT) and log (body mass) (Fig.6.5b), and the relationship is of the same form for males and females. This relationship remains significant when the effects of MTA and sex have been controlled for ($n=29$, $R^2=0.65$, log (body mass) $p<0.001$; MTA $p=0.65$; sex $p=0.56$).

6.4. Comparative analysis across the Apoidea.

Because of the wide variety in sexual dimophism within the Apoidea, from small, relatively short-lived males (e.g. *Osmia rufa*, Megachilidae) to relatively large, long-lived, robust males (e.g. *A. plumipes*, Anthophoridae), only data for females have been used in this analysis. The females share the common need to provision cells, and are all relatively long-lived and feed on similar nectar diets. In the cases of *Bombus edwardsii* separate data for workers and for queens have been used. The data for females in the *Anthophora* data set have been incorporated in the following analysis. So that differences between the *Anthophora* data set and data for the rest of the Apoidea can be readily seen, data for the genus *Anthophora* are represented by a different symbol in the figures. In all cases, the lines fitted on the figures represent best fit regressions to the whole data set. In addition to the *Anthophora* data, new data for 26 species have been collected, and published data are used for a further 27 species (Table 6.2); Apidae (23 species), Anthophoridae (14 species), Megachilidae (10 species), Andrenidae (3 species) Colletidae (2 species) and Halictidae (3 species). Analyses of this data set without data for the *Anthophora* species studied in Israel, and incorporating only warm-up rate and 'grab-and-stab' data, are presented in Stone and Willmer (1989b).

A. Mean warm-up rate. Fig.6.6a shows the relationship between untransformed warm-up rate and body mass data. 'Naïve' cross-species analysis reveals no significant correlation between log

Table 6.2 Summaries of warm-up rates, body temperatures and body masses for bees not in the genus *Anthophora* used in comparative analyses.

Species	Mean body mass (mg) (n)	MWR ($°Cmin^{-1}$)	PWR ($°Cmin^{-1}$)	VFT (°C)	SFT (°C)	'Grab and stab' T_{th} at T_a=22°C(°C)	MTA (°C)	Source
APIDAE								
Apis m.mellifera L.	89(7)	4.8(34)	9.3	33.6	28.9	*	4.8	Stone & Willmer (1989)b
Apis.m.mellifera L.	92.7	*	*	*	*	32.5	7	Heinrich (1979)
Apis m.mellifera L	100.8	*	*	*	*	38	12	Cooper *et al.* (1985)
Apis.m.adansonii Latreille	60.8	*	*	*	*	36	7	Heinrich (1979)
Bombus lapidarius (L.)	136(12)	6.1(83)	10.7	30.95	31.0	*	4	Stone & Willmer (1989)b
B. pascuorum (Scop.)	123(8)	5.45(64)	8.33	*	31.6±0.2 (3)	*	*	Stone & Willmer (1989)b
B. terrestris (L.)	250(8)	8.8(47)	12.5	*	35.2	*	3	Stone & Willmer (1989)b
B. vosnesenskii Rad.(Queen)	550	12.3	*	*	*	40	2	Heinrich, (1972c,d; 1975), Heinrich& Heinrich,(1983)
B. edwardsii Cresson (Queen)	400	*	*	*	*	42	2.5	as above
B. edwardsii Cresson .	120	*	*	*	*	37.5	2.5	as above
B. vagans Smith	120	*	*	*	*	33	5	Heinrich (1972a), Heinrich&Heinrich (1983)
B. terricola Kirby	150	*	*	*	*	36.5	5	Heinrich (1972b), Heinrich&Heinrich (1983)
Psithyrus vestalis (Geoffroy)	195(4)	6.3(28)	9.6	32.7±0.2	*	*	*	Stone & Willmer (1989)b
Euglossa variabilis Friese	95	5.7	*	*	*	*	22.5	May (1976a)
E. igneiventris Friese	102	4.8	*	*	*	*	*	May (1976a)
E. imperialis Cockerell	160	7	*	*	*	34.4	22.5	May (1976a), May & Casey (1983).
E. saphirina Moure	70	*	*	*	*	32.3	*	May & Casey (1983)
E. tridentata Moure	110	*	*	*	*	35.5	*	May & Casey (1983)
Exaerete smaragdina (Guerin)	383	4.8	*	*	*	*	*	May (1976a)
Exaerete frontalis (Guerin)	680	6.2	*	*	*	33.4	19	May (1976a), May& Casey (1983)
Eulaema nigrita Lep.	420	5.8	*	*	*	36.6	19	May (1976a), May& Casey (1983)

Species	Mean body mass (mg) (n)	MWR ($°Cmin^{-1}$)	PWR ($°Cmin^{-1}$)	VFT (°C)	SFT (°C)	'Grab and stab' T_{th} at T_a=22°C(°C)	MTA (°C)	Source
Eulaema meriana (Olivier)	880	7	*	*	*	35.4	19	May (1976a), May& Casey (1983)
Eufriesia pulchra (Smith)	460	7	*	*	*	36.4	19	May (1976a), May& Casey (1983)
Euplusia schmidtiana (Friese)	460	6.9	*	*	*	*	19	May (1976a)
ANTHOPHORIDAE								
Xylocopa capitata Smith	1300	4.5	*	38.0	38.0	37	23	Louw & Nicolson (1983), Nicolson& Louw (1982)
X. californica Cresson	590	*	*	*	*	40	12.5	Chappell (1982)
X. virginica L.	510	*	*	*	*	41	15	Baird (1986)
X. varipuncta Patton	673	2.25	*	*	*	37	12	Heinrich & Buchmann (1986)
X. pubescens (Spinola)	640	4.2	*	*	*	36	14	Willmer (1988)
X. sulcatipes Maa	420	3.2	*	*	*	34	19	Willmer (1988)
Xylocopa (*Koptortosoma*) spp.	260(10)	*	*	*	*	38.6(11)	16	Stone & Willmer (1989)b
Centris pallida Fox	197	*	*	*	*	39	25	Chappell (1984)
Amegilla sapiens (Cockerell)	117(38)	2.15(1)	6.5	30.4	30.4	33(11)	18	Stone & Willmer (1989)b, Stone *et al.* (1988)
Thyreus quadrimaculatus (Rad.)	100(14)	1.75(6)	2.1	27.5	27.5	28(7)	26	Stone & Willmer (1989)b
Melecta albifrons (Fab.)	110(6)	5.35(55)	10.7	28.9±0.3	28.9	*	12	Stone & Willmer (1989)b
Eucera spp.nov. (teste Baker)	75(6)	3.5(6)	*	*	*	27.6(8)	18	Stone & Willmer (1989)b
MEGACHILIDAE								
Creightonella frontalis (Fab.)	305(59)	5.4(7)	9	33.4	33.4	33(10)	20	Stone & Willmer (1989)b
Megachile spp.	87(14)	*	*	*	*	32.5(9)	*	Stone & Willmer (1989)b
Megachile spp.	125(8)	*	*	*	*	34(8)	*	Stone & Willmer (1989)b
Megachile willoughbiella (Kirby)	121(8)	6.3(16)	10.4	30.25	30.3	*	16	Stone & Willmer (1989)b
Megachile centuncularis (L.)	64(4)	3.7(15)	*	29.3±0.4	24.5±0.2 (3)	*	*	Stone & Willmer (1989)b

Species	Mean body mass (mg) (n)	MWR ($°Cmin^{-1}$)	PWR ($°Cmin^{-1}$)	VFT (°C)	SFT (°C)	'Grab and stab' T_{th} at T_a=22°C(°C)	MTA (°C)	Source
Coelioxys spp.	90(4)	1(9)	1.4	27.3	27.3	*	26	Stone & Willmer (1989)b
Osmia rufa (L.)	85(5)	10.5(20)	12.2	35.5±0.2	35.5	33.5(8)	5	Stone & Willmer (1989)b
Osmia leaiana (Kirby)	55(6)	4.1(10)	*	27.2±0.1	*	*	*	Stone & Willmer (1989)b
Chalicodoma sicula (Rossi)	190	4.2	*	*	*	32	17	Willmer (1986)
C. montenigrense (Dours)	128(4)	*	*	*	*	28.9(4)	*	Stone & Willmer (1989)b
ANDRENIDAE								
Andrena clarkella (Kirby)	85(4)	6.2(38)	7.7	27.0±0.3	26.1	*	8	Stone & Willmer (1989)b
Andrena fulva (Mulleer in Allioni)	81(6)	4(39)	6	29.6±0.6	29.3	*	12	Stone & Willmer (1989)b
Andrena nigroaenea (Kirby)	108(4)	6.95(21)	10.5	30.3±0.7	30.2	*	9	Stone & Willmer (1989)b
COLLETIDAE								
Colletes cunicularius (L.)	112(8)	7.35(121)	13.5	31.2±0.5	30.9	*	10	Stone & Willmer (1989)b
Colletes daviesanus Smith	36(4)	3.8(16)	6.0	27.1±0.3	25.1±0.1 (3)	*	*	Stone & Willmer (1989)b
HALICTIDAE								
Nomia spp.	43(10)	*	*	*	*	28.5(7)	20	Stone & Willmer (1989)b
Nomia spp.	45(10)	*	*	*	*	30(9)	20	Stone & Willmer (1989)b
Lasioglossum smeathmanellum Kirby	10(4)	1.25(12)	2.33	*	23.8±0.1 (7)	*	*	Stone & Willmer (1989)b

(MWR) and log (body mass) (Fig.6.6b). A major contributing factor to the absence of any overall correlation is the fact that most of the larger bees active in warmer thermal regimes (e.g. *Xylocopa* sp.) have much lower warm-up rates than smaller species (e.g. *Osmia rufa*, see Table 6.2) adapted to cooler climates. Across all species there is a highly significant negative correlation between log (MWR) and MTA (minimum ambient temperature for activity) ($n=45$, $R^2= 0.480$, $p<0.0001$; Fig.6.6c). 'Naïve' multiple regression shows MTA to correlate negatively and significantly with log (MWR) after controlling for log (body mass) ($n=45$, $R^2=0.52$ $p<0.0001$), while mass gives a significant positive correlation ($p=0.06$) having controlled for the effect of MTA (Figs.6.5d,e). Phylogenetic regression reveals that each of log (body mass) ($F_{1,11}= 9.4$, $p<0.025$, positive correlation) and MTA ($F_{1,11}= 43.42$, $p<0.001$, negative correlation) correlate significantly with log (MWR) when the effect of the other is controlled for. The greater significance of the relationship between body mass and warm-up rate obtained using phylogenetic regression suggests that the absence of an overall significant correlation between body mass and warm-up rate is due at least in part to differences in the form of the relationship between taxa. Examination of Figs.6.6c-e shows that data for the genus *Anthophora* are almost always above the best-fit regression line for the whole data set. The best-fit regression for the genus *Anthophora* alone would therefore lie above the regression for all bees. The large *Anthophora* data set skews the line for all Apoidea towards high warm-up rate values, away from the lower warm-up rates for bees of similar mass in other families (such as the three *Andrena* species, Table 6.2). This clearly shows the necessity of controlling for taxonomic effects in the overall interspecific analysis.

While warm-up rates within the genus *Anthophora* appear to be generally high, some of the warm-up rates for other species able to fly in cool conditions are also very high. For example, *Osmia rufa* (Fig.6.7) has a high MWR of 10.5°C min^{-1}, and a peak rate of 12.2°C min^{-1} (Table 6.2). However, no bee in any other family matches the rates shown by the females of several of the *Anthophora* species, with mean rates of over 13°C min^{-1} and a peak rates of 18-19°C min^{-1}, the highest warm-up rates reported to date by a considerable margin. The smallest bee examined in this study was *Lasioglossum smeathmanellum* (Halictidae) (Fig.6.8), with a body mass of about 10mg. Although the errors involved in accurately determining warm-up rates in a species this small are assumed to prohibit the use of data for it in the analyses in this paper (Chapter 2), despite having no apparent ability to maintain an elevated temperature during tethered flight, this species nevertheless elevated T_{th} 2-3°C above T_a before flight.

Females are forced to forage during less than optimal conditions if they are to minimize the time for which their cells are open to parasitism (Willmer 1985a,b). Kleptoparasitic species, however, are freed from the need to forage and provision cells during less than optimal climatic

conditions, and it might be predicted that these species should have lower warm-up rates for their mass and a higher MTA than females of provisioning species. Certainly the kleptoparasitic species in this data set (*Psithyrus*, Apidae; *Melecta* and *Thyreus*, Anthophoridae; *Coelioxys*, Megachilidae, Table 6.2) all have low warm-up rates for their body mass. Though some preliminary data on *Thyreus quadrimaculatus* were discussed in Chapter 5, I have insufficient data to allow a conclusion on this point. A similar point could be made about males, although here the complication of the wide diversity of mating systems shown by bees (c.f. Chapter 5) precludes general predictions. There are very few data available concerning intraspecific male-female differences in endothermic physiology.

B. Thoracic temperatures in flight at an air temperature of 22°C. Fig.6.9a shows the relationship between untransformed data for T_{th} in flight and body mass. 'Naïve' regression reveals a significant positive correlation between log (T_{th} at 22°C) and log (body mass) over the whole data set (n=34, R^2=0.348, p<0.001 Fig.6.9b), and a negative correlation with MTA (n=28, R^2= 0.185, p=0.02 Fig.6.9c). 'Naïve' multiple regression shows that each correlates significantly with log T_{th} when the other is controlled for (n=28, R^2=0.621; MTA: p=0.02; log body mass: p=0.001 Figs 6.9d,e). Controlling for taxonomic effects using phylogenetic regression confirms these results [MTA: $F_{1,7}$=13.8, p<0.05; log (body mass): $F_{1,7}$=7.19 p<0.01]. There is no significant interaction. Thus for bees with a given minimum ambient temperature for activity, T_{th} in flight increases with mass. More tentatively (bearing in mind possible errors in MTA) for bees of a given body mass, those able to forage at lower T_a have higher T_{th} at 22°C.

C. Voluntary flight temperature (VFT). Figure 6.10a shows the significant positive correlation between log (VFT at T_a=22°C) and log (body mass) (n=37, R^2=0.5 p<0.001). There is also a significant negative correlation between log (VFT) and MTA (n=32, R^2=0.198, p=0.01) (Fig.6.10b). In the latter case this 'naïve' regression is heavily biassed by the *Anthophora* data set (Fig.6.10b), and so this result should not be trusted. Once again, bees in this genus have high values relative to other Apoidea. 'Naïve' multiple regression shows that both log (body mass) and MTA are significantly correlated with log (VFT) when the other is controlled for (n=33, R^2=0.63, MTA p=0.0001; log (body mass) p=0.0001) (Figs. 6.10c,d). Phylogenetic regression removes the biassing effect of the *Anthophora* data set, and shows that both MTA and log (body mass) remain significantly correlated with log VFT (MTA: $F_{1,7}$=23.45, p<0.005. log (body mass): $F_{1,7}$=9.36, p<0.025). For bees active at a given MTA, VFT increases with body mass, and for bees of a given body mass, VFT increases with decreasing MTA.

D. Stable flight temperature (SFT). Fig.6.11a shows the significant positive correlation between log (body mass) and log (SFT) (n=31, R^2=0.65, p<0.001). 'Naïve' multiple regression reveals that both log (body mass) and MTA correlate significantly with log (SFT) once the other has been controlled for (n=30, R^2=0.755, log (body mass) p=0.0001; MTA p=0.0001) (Fig.6.11b,c). Phylogenetic regression shows that both MTA and log (body mass) remain significantly correlated with log (SFT) when the other and phylogenetic effects have been controlled for (MTA: $F_{1,7}$=15.9, p<0.01. log (body mass) $F_{1,7}$=27.5, p<0.005).

E. Conductance. Conductance was calculated from cooling curves as described in Chapter 2 (Table 6.3). 'Naïve' regression yields a strong negative correlation between log (conductance) and log (body mass) (n=17, R^2=0.793, p=0.0001) (Fig.6.9). The conductance values obtained for my bees are compatible with those obtained for euglossine bees by May and Casey (1983).

Discussion.

In the discussion which follows I shall take the genus *Anthophora* as an example of what the patterns between the variables discussed are when phylogenetic problems are minimised. This does not mean that problems associated with phylogeny do not exist within this data set. Whenever an ancestral group gives rise to daughter taxa, evolutionary patterns can arise anew, and this is as true when species evolve within a subgenus as when families diverge within an order. However, the longer the divergences have been happening over evolutionary time, the greater the differences between taxa will be, and I am assuming that the differences within the *Anthophora* data set, among species which have diverged relatively recently, are smaller than those across the Apoidea in general.

A. Mean warm-up rate.

Fig.6.3a shows that within a group of closely related species (the genus *Anthophora*) there can be a significant correlation between body mass and mean warm-up rate. The same positive correlation was found within the species *A.plumipes* in Chapter 4. However, Fig.6.6a shows that there is no obvious simple relationship between these variables across the Apoidea. Even if the relationship between body mass and warm-up rate were the same within each genus, analysis of a data set comprising all the members of all genera might well not yield an overall relationship.

It is only when the variation in mean warm-up rates due to thermal regime and phylogeny is controlled for, using phylogenetic regression, that the relationship between body mass and MWR becomes clear. Within the genus *Anthophora* MTA is significantly correlated with mean warm-up rate, and this pattern persists across the whole data set. The relationship between body size and warm-up rate thus appears to be real over a wide range of comparative levels, from members of a

Table 6.3 Summary of Conductance data for British bees obtained during work on this thesis. The number of individuals of each species tested is shown. Errors are ±1 standard error.

Species	Mean body mass (mg)	n	Cooling constant ($°Cmin^{-1}°C^{-1}$)	Conductance ($mWg^{-1}°C^{-1}$)
Apidae				
Apis mellifera L.	93	2	1.13±0.05	63±3
Bombus pascuorum (Scop.)	126	23	1.00±0.10	56±6
Bombus terrestris (L.)	345	8	0.68±0.10	38±6
Bombus lucorum	370	2	0.36±0.10	20±6
Psithyrus vestalis (Geoffroy)	195	2	0.88±0.20	49±11
Anthophoridae				
Anthophora plumipes (Pallas) m	159	38	0.94±0.03	53±2
Anthophora plumipes (Pallas) f	186	21	0.99±0.05	55±3
Anthophora quadrimaculata (Panz)	90	2	1.14±0.04	64±2
Melecta albifrons (Fab.)	124	8	0.97±0.05	54±3
Megachilidae				
Megachile willoughbiella (Kirby)	94	6	1.14±0.2	64±11
Osmia rufa (L.)	85	5	1.08±0.13	60±7
Osmia leaiana (Kirby)	52	2	1.37±0.05	77±3
Andrenidae				
Andrena clarkella (Kirby)	80	6	1.18±0.10	66±6
Andrena fulva (Mulleer in Allioni)	78	4	0.97±0.10	54±6
Andrena nigroaenea (Kirby)	124	2	1.00±0.20	56±11
Colletidae				
Colletes cunicularius (L.)	112	10	1.07±0.10	60±6
Colletes daviesanus Smith	36	2	1.60±0.20	90±11

single sex of a single species (*A.plumipes*, Chapter 4) to members of different families. The form of the relationship between body mass and warm-up rate within the Apoidea thus supports the predictions of May (1976a) and Bartholomew (1981). This result is compatible with the suggestion in the Introduction of this chapter that at the body mass range examined in this investigation, heat loss and surface area to volume ratios are important predictors (and probably major determinants) of warm-up rate. Thus although warm-up rates in endothermic insects lie generally within the range predicted by a continuation to lower body masses of the relationship within vertebrate endotherms, the negative correlation between warm-up rate and body mass found for vertebrate heterotherms does not exist within the Apoidea.

The gradient of the best fit 'naïve' regression of log (MWR) on log (body mass) is less than 1 both within the genus *Anthophora* and over all species (Figs. 6.3a, 6.6b). This implies that overall smaller bees have a relatively higher warm-up rate per unit mass than larger bees. This is in accordance with studies by Bartholomew & Casey (1978) and Bartholomew *et al.* (1981) on hovering moths and Chappell (1982) on hovering *Xylocopa californica*, which report that mass specific oxygen consumption per unit time decreases with increasing body mass, and the statement by May (1976a) that heat production per gramme body mass is higher in smaller euglossine bees than in larger species. However, it is clear from the scatter in Fig.6.6b that mass is not a fundamental constraint across species. The gradient of the relationship between log (MWR) and log (body mass) for female *A. plumipes* (0.88 ± 0.26: $n=28$, $R^2=0.314$, $p=0.002$)) approaches unity, implying that within a sex of a species endothermic abilities per unit mass may be more or less constant and further suggesting that warm-up rates are a property of the flight machinery of each particular species, and are not determined solely or simply by mass and surface area to volume ratios.

Thermal regime, represented in this analysis by the minimum T_a at which the species flies in the field, is also of major importance in determining warm-up rates. The negative correlation between MWR and MTA within the genus *Anthophora* is highly significant (Fig.6.3b). How closely warm-up rates correlate with thermal regime across all species should depend on how much each species relies on exogenous heat to maintain body temperatures. Differences in reliance on exothermic regulation may cause some of the variation in MWR between species with a similar MTA seen in Fig.6.6b; for example, a bee species with a low metabolic warm-up rate could remain active under conditions favouring species with higher levels of endothermy by virtue of increased levels of basking. Such variation in the role of behavioural thermoregulation will cause noise in the relationship between warm-up rate and MTA which is not considered in this analysis.

Why should there be such a strong correlation between MWR and MTA? In

thermoregulating endotherms, the T_{ex} which must be generated, and thus the total energy expenditure in warm-up, is maximal at the lowest T_a at which warm-up occurs (e.g. Heinrich 1975a, and Chapter 4 for *A. plumipes*). The total time required for warm-up will also be maximal under these conditions (Bartholomew & Heinrich 1973; Heinrich 1975a; Casey *et al.* 1981; Chapter 4). Several authors have suggested that the time taken to warm up should be minimized for a number of reasons, including minimizing the time during which the insect is exposed to predators without the ability to escape (Bartholomew & Heinrich 1973) or the time for which foraging is impossible, and minimising the total energy expenditure associated with the warm-up. Insects which use endothermic flight musculature should therefore have thermogenic ability sufficient to achieve a minimum tolerable warm-up rate at the minimum T_a at which they fly. The time taken to complete warm-up depends not only on warm-up rate but also on the T_{ex} which must be established. If it is assumed for the moment that T_{ex} at take-off is relatively constant across species, then part of the relationship between MWR and MTA shown in this study is explained. Bees adapted to a relatively warm thermal regime have a lower warm-up rate at 22°C than bees adapted to lower temperatures, because 22°C is relatively closer to the minimum T_a to which their thermogenic systems are adapted. This implies that were we to examine warm-up rates across species at the minimum T_a at which each species flies we would find similar warm-up rates (complicated by taxonomic considerations).

It seems probable that were we to control for thermal regime most bees would have similar relationships between warm-up rate and ambient temperature. Certainly it is true that warm-up rates at T_a=22°C in most species examined to date are dependent on ambient temperature (e.g. Heinrich & Bartholomew 1971; Bartholomew & Casey 1973; Heinrich & Casey 1973; Heinrich 1975a; Casey *et al.* 1981; Heinrich & Buchmann 1986; Chapter 4). Warm-up rates in bees whose MTA values are near 22°C are approximately 4-5°C min^{-1} (Table 6.2). My reasoning predicts that different species will have similar warm-up rates at their MTA. For example, at T_a=8°C the MWR for female *Anthophora plumipes* is 5-6°C min^{-1} rather than the 12.3°C min^{-1} shown at 22°C. It is probable that warm-up rates would approach 4°C min^{-1} at T_a= MTA for this species. Similarly, at T_a as far above their MTA's as 22°C is for some cold-adapted species, some warm-adapted species show far higher warm-up rates. For example, at an ambient temperature of 32°C (12-14°C above their MTA's) the tropical bees *Amegilla sapiens* (Anthophoridae) and *Creightonella frontalis* (Megachilidae) show mean warm-up rates compatible with those shown by cold-adapted species in this analysis (*A. sapiens*: 6.7±1.4°C min^{-1}, n=6; *C. frontalis*: 8.7±0.3°C min^{-1}, n=7; Tables 5.3, 5.5). At 22°C their warm-up rates are much lower (*Amegilla sapiens* 2.15°C min^{-1}; *C. frontalis* 5.4°C min^{-1}) (Table 6.2).

My assumptions concerning temperature excesses generated at MTA values across species

should be modified. In general among endothermic insects it appears that species capable of flight at very low T_a generate temperature excesses at their MTA which are larger than those generated by species with higher MTA values (e.g. Heinrich 1987). For this reason we would predict greater endothermic abilities in cold-adapted species, even near their MTA, than in warm-adapted species, simply because they have further to warm up and can less afford the great inefficiency of slow warm-up for prolonged periods of time (Heinrich 1987). In bees there is an indication that the temperature excesses which warm-regime bees sustain at their MTAs are relatively low compared with those generated by bees flying at very low T_a. For example, the 26°C T_{ex} maintained at T_a=12°C by 1-2 g *Xylocopa varipuncta* (Anthophoridae) from Arizona (Heinrich & Buchmann 1986) is somewhat less than the 32°C excess maintained at a T_a=4°C by the queens of *Bombus vosnesenskii* (Apidae) weighing only 0.25-0.6 g (Heinrich 1975a).

It is easy to accept that at the minimum air temperatures at which they are active all bees will have lower warm-up rates than they do at higher T_a. It is also predictable that bees generating high T_{ex} from low T_a should have low initial warm-up rates, particularly if they are small (Chapter 4). It is harder, however, to see why large tropical bees should show such low warm-up rates at the relatively high ambient temperature minima which they experience. It seems certain that large tropical bees such as *Xylocopa* species could have evolved a higher warm-up rate if required, given that close relatives such as *Anthophora plumipes* have extremely high warm-up rates. Some indication that xylocopines is capable of higher warm-up rates is shown by data for 2 female *Xylocopa iris* studied in Israel. Although rather smaller than the *Xylocopa* species listed in Table 6.2 (body mass 180-190mg), this species, able to fly at T_a down to 13-14°C, has a mean warm-up rate of 4.8°C min^{-1}, and a peak rate of 9.1°C min^{-1}. It may be that efficiency in warm-up, in terms of maximizing warm-up rate at low T_a, has been less of a selective pressure for warm-regime bees. Because of the high T_a at which some of these species fly, and the very high T_{th} which are generated in some of the larger *Xylocopa* spp. (e.g. Chappell 1982; Heinrich & Buchmann 1986), an important selective pressure for warm regime bees must have been tolerance of high ambient and body temperatures. Species capable of warm-up at very low T_a may become heat stressed at relatively low T_a, and thus cannot remain active at high T_a (e.g. Heinrich 1987): warm-up at very low T_a and activity at high T_a would require excellent thermoregulatory abilities. It is possible that a flight system tolerant of extremely high working temperatures in warm-regime bees [up to 48°C in female *Creightonella frontalis* at an ambient temperature of 35-38°C; a similar upper limit has been reported by Chappell (1982) for the desert-living *Xylocopa californica*, and for hovering male *Centris pallida* (Chappell 1984)] is only capable of warm-up at low rates, even at relatively high T_a.

The generally higher warm-up rates in cold-regime bees could be due either to a reduction

in conductance or to an increase in metabolic heat generation. The species in my analysis with the lowest MTA values have extremely dense insulating pile (particularly *Bombus* spp., *Anthophora plumipes* and *Osmia rufa*). Pile has been shown to be an effective insulator of the thorax in moving air, but in still air May and Casey (1983) were unable to show any significant difference in the conductance of glabrous and pubescent species of euglossine bees. This suggests that the relationship between pubescence and warm-up rate during warm-up in still air is not a simple one. It is also possible that even in the absence of forced convection a bee may generate air currents during vigourous abdominal pumping strong enough to disrupt the boundary layer around its body, and thus contribute to enhanced convective cooling (Dr. Peter Miller, personal communication). Furthermore, since the form and distribution of pubescence tends to be similar in closely related species, an analysis of changes in conductance over evolutionary time in response to thermal regime would have to control for taxonomic effects. Other studies have supported the suggestion that it is mainly variation in metabolic heat production, rather than differences in conductance, that causes differences in warm-up rates between different endothermic insects (e.g. Casey *et al.* 1981). It is probable, therefore, that within the Apoidea variation in metabolic rate during warm-up is a major response to differing thermal regimes.

B. Thoracic temperature in flight.

Because maintenance of a given T_{ex} during flight at a given ambient temperature becomes relatively more expensive per unit of body mass the smaller you are, small endotherms might be expected to make concessions to the cost of endothermy by reducing the T_{ex} they maintain at a given T_a. This appears to be the case among mammals (McNab, 1970), where sustained body temperatures are a function of body mass up to a 'critical mass' above which body temperature is independent of body mass. Bartholomew & Heinrich (1973) demonstrated a similar non-linear relationship between body mass and T_{th} when moths of several families were considered together. However, analysis of this relationship within families gave significant positive correlations for only two of the six families considered. T_{th} in flight was found to correlate positively with wing loading in all six families. Thus, the positive correlation between body mass and T_{th} found overall and in the two families may be due to inter-familiy and within-family variation in wing loading. The importance of controlling for taxonomic effects is shown by their comment that although T_{th} correlates with wing loading within a family, species in different families with the same wing loading can have very different T_{th}. Heinrich & Casey (1973) found no significant correlation between body mass and T_{th} for sphingid moths of 13 species over the mass range 0.3-3.5 g. Heinrich & Heinrich (1983) report that for workers and queens of a variety of *Bombus* species over the mass

range 100-750 mg there was no overall correlation between body mass and T_{th} while foraging. My data reveal a clear positive correlation between body mass and T_{th} in flight once the effects of thermal regime and taxonomic effects have been controlled for. The same qualitative relationship between these variables was obtained within *A. plumipes* (Chapter 4). As Bartholomew & Heinrich (1973) suggested, within the Apoidea the relationship is non-linear (Fig.6.9a,b), and most pronounced at low body masses. The data presented by Heinrich & Heinrich (1983) showing no correlation between mass and T_{th} were gathered over a wide range of T_a (2.5-22°C). Their data concerned both large queens and smaller workers, and they mention that queens were able to forage at somewhat lower T_a than workers. This suggests to me that, although at higher T_a there was indeed no relationship between body mass and T_{th} for the species they examined, at T_a near the lower limit tolerated by workers there may well have been a relationship between the two.

Thoracic temperatures in flight, like warm-up rates, appear to be affected by thermal regime. Having controlled for the effects of body mass and taxonomy, there is a significant negative correlation between T_{th} in flight at 22°C and MTA. Bees adapted to cooler regimes fly hotter at a given T_a. Although the nature of the MTA statistic describing thermal regime necessitates caution in making conclusions, clearly the thermal regime to which a species is adapted is a factor that must be considered in comparative analyses. It appears to be true that, as McNab (1970) states for mammals, the levels of regulated body temperatures are capable of some variation independent of body mass in a manner adaptive to climate.

C. Voluntary flight temperatures and stable flight temperatures.

Both VFT and SFT increased significantly with increasing body mass within the genus *Anthophora* and across the Apoidea. These results are qualitatively similar to those obtained for *A. plumipes* in Chapter 4. Having controlled for the effect of body mass, there is a significant negative correlation between MTA and each of SFT and VFT across the Apoidea, but not within the genus *Anthophora*.

The thermogenic properties of the flight musculature in these bees is shown in all of the measures of endothermy used here. If it is accepted that heat generation is brought about by 'shivering' rather than by a system independent of muscular contraction (Chapter 1), heat generation during warm-up will be unavoidably correlated with heat generation during flight. If this is true, then the similarity in the relationships between each of MWR, SFT and VFT, and MTA and body mass are to be predicted. In the Introduction to this chapter I suggested that the relationship between SFT and body mass might be stronger than that between VFT and body mass because of the stronger convective cooling, and thus a greater importance of surface area to volume ratios, encountered

during flight. The strengths of these relationships can be quantified by comparing the values of R^2 calculated for the least squares linear regressions fitted to the data, or by comparing the F values obtained using phylogenetic regression. Values are given for both simple regressions, and regressions having controlled for the effect of MTA, in Table 6.4 (below). In every case the correlation with SFT is stronger than that with VFT, suggesting that the prediction may be correct.

One result still to be explained is the finding that having controlled for body mass, MTA correlates significantly with both VFT and SFT over the Apoidea, but with neither within the genus *Anthophora*. If body mass and MTA are strongly correlated, then controlling for the effect of body mass in these analyses may leave no significant relationship with MTA. Chapters 3 and 4 both show, using independent methods, that ability to warm-up and fly at low temperatures increase with body mass within a single species. Fig.6.13a shows that MTA and body mass are indeed strongly correlated within the genus *Anthophora* (n=32, R^2=0.129, p=0.043) and this may explain the absence of any significant relationship between MTA and SFT or VFT when the effects of body size have been controlled for. There is no significant correlation across the Apoidea (Fig.6.13b: n=45, R^2=0.021, p=0.34), and it is probable that over these less closely related species factors other than body size which effect both MTA and SFT or VFT become more significant, and significant correlations between them, independent of mass, emerge.

Table 6.4 Values of R2 for regressions of log (VFT) and log (SFT) on log (body mass).

Naïve regression:	Values of R^2 (sample size)	
Data set:	Simple regression	Controlling for MTA
Genus *Anthophora*		
log (body mass) v log (SFT)	0.625 (30)	0.519 (28)
log (body mass) v log (VFT)	0.384 (32)	0.279 (31)
All Apoidea		
log (body mass) v log (SFT)	0.656 (31)	0.703 (31)
log (body mass) v log (VFT)	0.500 (37)	0.533 (36)
Phylogenetic regression, all Apoidea.		
log (body mass) v log (SFT)		$F_{1,7}$=27.5
log (body mass) v log (VFT)		$F_{1,7}$=15.9

D. Thermoregulatory Strategies.

Measurements of warm-up rate and SFT indicate that body size does affect thermoregulatory ability in bees. *Lasioglossum smeathmanellum*, weighing only 10mg (Fig.6.8), did demonstrate detectable preflight warm-up (MWR 1.25 °Cmin^{-1}, Table 6.2), but the mean thoracic

temperature excess maintained in flight at T_a=21°C was only 1.8°C (Table 6.2). Although the gradient of T_{th} on T_a is required to establish thermoregulatory ability, the maintenance of such a small T_{ex} at so moderate a T_a suggests that while *Lasioglossum smeathmanellum* is undoubtedly endothermic, it is unlikely that it is able to regulate T_{th} significantly over a wide range of T_a. While it is true that all the Apoidea examined in this thesis are endothermic, the extent of significant endothermic thermoregulation remains to be established. A comparative analysis of the gradients of T_{th} on T_a as a function of body size and phylogeny would therefore be of considerable interest. As yet, most of the data on thermoregulatory ability have been obtained for bees in the families Apidae and Anthophoridae (Table 4.6), and the smallest of the species tested (*Apis mellifera*) is still relatively large. The assessment of the effect of body size on thermoregulatory ability is complicated by the effect of body size on the errors associated with insertion of thermocouples, which makes accurate measurement of the body temperatures of very small bees difficult (Chapter 2). It is probable that small bees depend largely on behavioural thermoregulation.

There are several compatible solutions to thermoregulation at low T_a, including an increase in thermogenic ability, a decrease in thermal conductance, and a reduction in the minimum tolerated body temperature necessary for the activity concerned. My analyses suggest that the first has occurred within the Apoidea, and that there is considerable ability to adjust the setting of the 'thermogenic thermostat' over evolutionary time in response to environmental conditions. It should be noted that even for species of similar mass and MTA there is variation in warm-up rate. For example, *Osmia rufa* (Megachilidae: mean mass 85mg, MTA 5°C) at 22°C warms up at a mean rate of 10.5°C min^{-1} whereas *Andrena clarkella* (Fig.6.14) (Andrenidae: mean mass 85mg, MTA 8°C) has a much lower mean warm-up rate of 6.2°Cmin^{-1}.

Data for these same two species suggests that the third option may also have occurred. *Osmia rufa* generates an excess of 13.6°C before takeoff at T_a = 22°C, whereas *Andrena clarkella* generates a much lower mean T_{ex} of 4.2°C at take-off. However, because there are good reasons for warm-up rates and VFT to be correlated, it is not yet possible to say whether bees such as *A. clarkella* have evolved low warm up rates *and* low VFT's, or whether they have low VFT's *because* of their low warm-up rates. Indeed, all the *Andrena* species examined here have relatively low warm-up rates, and this not only prompts questions about the evolution of different thermal strategies in bees living under similar conditions but also emphasizes the need for an awareness of phylogeny.

There are as yet insufficient data to test whether, having controlled for the effect of body mass, conductance increases with MTA. Most bees active at low temperatures are relatively pubescent, and those active at high temperatures generally less so, and given that pubescence does

affect conductance, I would predict that a relationship between conductance and MTA will be demonstrated.

It is now possible to make two generalisations:-

(1) Body mass correlates positively with (and is probably an important determinant of) warm-up rates, VFT, SFT and T_{th} during flight in the Apoidea, confirming the predictions of May (1976a) and Bartholomew (1981). This is true within a species, within a genus, and across families. Thus, within the Apoidea, although smaller bees show higher warm-up rates (and thus metabolic rates) per unit mass, it is probably the strong negative correlation between conductance and body mass which negates this effect and leads to slower overall warm-up rates.

(2) It is clear that correlations with body mass alone are insufficient to explain all of the observed variation in warm-up rate, SFT, VFT and T_{th} in flight between species. Indeed, across the Apoidea the relationship between body mass and warm-up rate only becomes apparent when the considerable effects of thermal regime and phylogeny have been controlled for. Species active at lower temperatures have both higher warm-up rates and higher T_{th} in flight.

Dyer & Seeley (1987) stated that "general scaling relationships based on body mass alone may fail to predict qualitative physiological differences even within a closely related group of species". The analyses above show that it is essential to take into account both phylogenetic and ecological differences between the species involved. Warm-up rates appear to have been very susceptible to selective change within the absolute constraints imposed by size. There can be no doubt that warm-up rates have evolved to match physiologically average conditions in which the insect is active. I predict that warm-up rates in kleptoparasitic bees, which are freed from the need to forage during sub-optimal conditions, will be lower than those in related bees of similar mass. When physiological and activity pattern data become available for more male Apoidea an interesting comparative analysis linking endothermic abilities, body size, thermal regime and mating system will be possible. Differences in mating systems among species and higher taxa can be expected to have exerted different selective pressures on the endothermic machinery of males of different species, although we should bear in mind that just as female warm-up rates may be phylogenetically related, so male warm-up rates may be phylogenetically linked to female warm-up rates. Answers to these questions must await more extensive and detailed data.

This chapter demonstrates that endothermy is widespread in the Apoidea. The importance of body mass and thermal regime in predicting warm-up rates and body temperatures is shown to be considerable, both within the genus *Anthophora* and across the Apoidea, thus repeating the patterns observed within *A. plumipes*. Differences in warm-up rates and body

temperatures between subtaxa within the Apoidea mean that the effects of phylogeny must be controlled for in the comparative analysis.

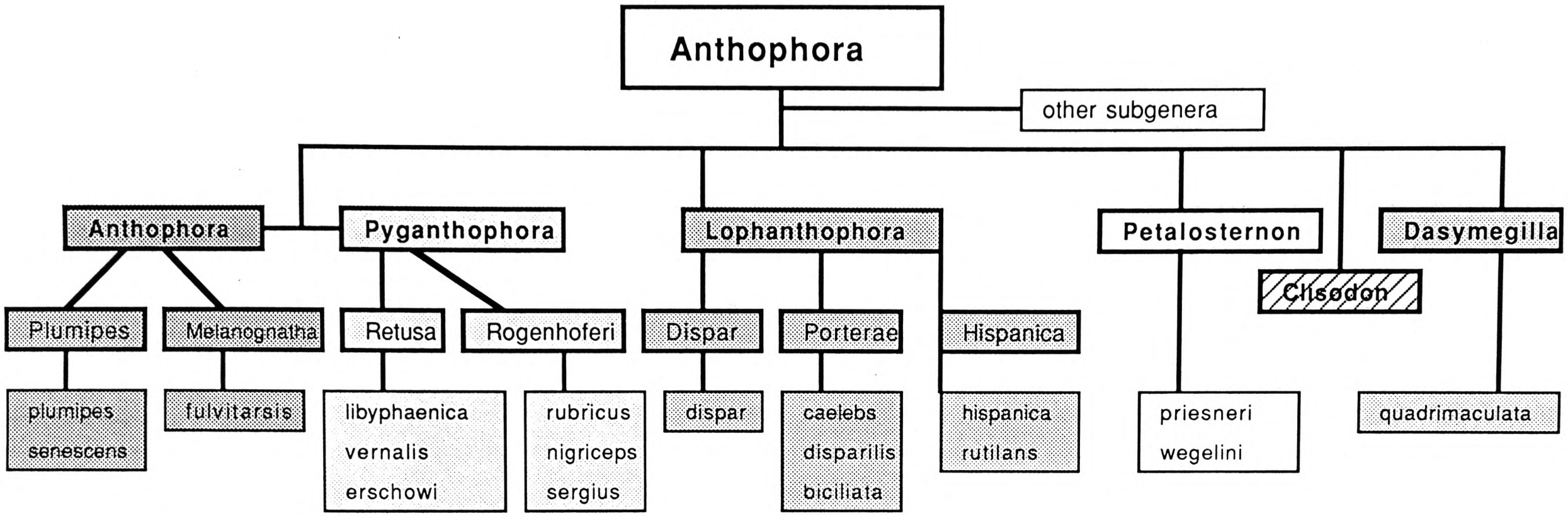


Fig.6.1 Partial taxonomy and phylogeny of the genus *Anthophora*, after Brooks (1988).



Fig.6.2 A female *Anthophora fulvitarsis* from Avdat, Israel. Females of this species illustrated the highest mean warm-up rates of any species investigated in this thesis (Table 6.2).

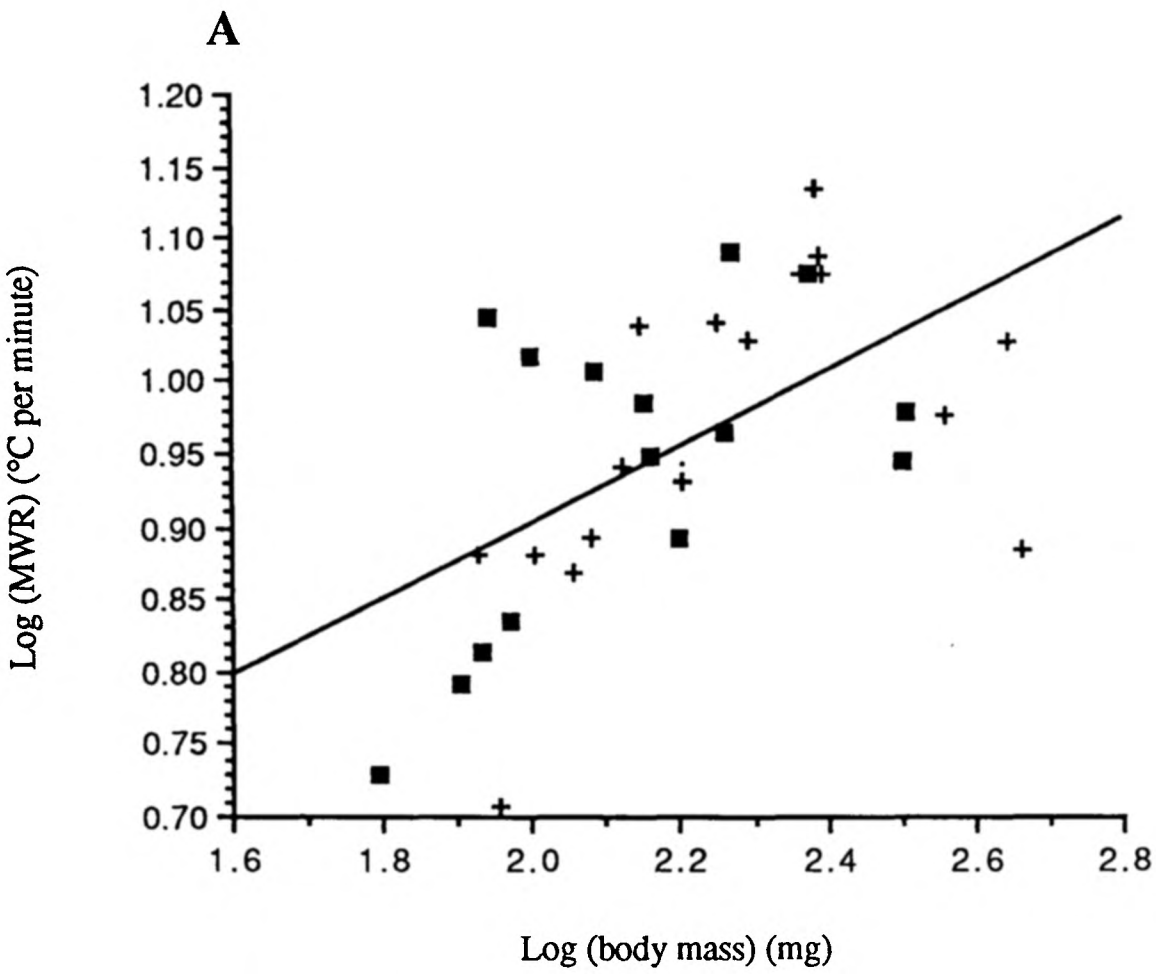


Fig.6.3 (a) The relationship between log (body mass) and log (MWR) for males (■) and females (❐) in the genus *Anthophora* (y=0.37+0.27x, R^2=0.319). **(b)** Log (MWR) as a function of MTA for males and females in the genus *Anthophora* (y=1.15-0.023x, R^2 =0.313).

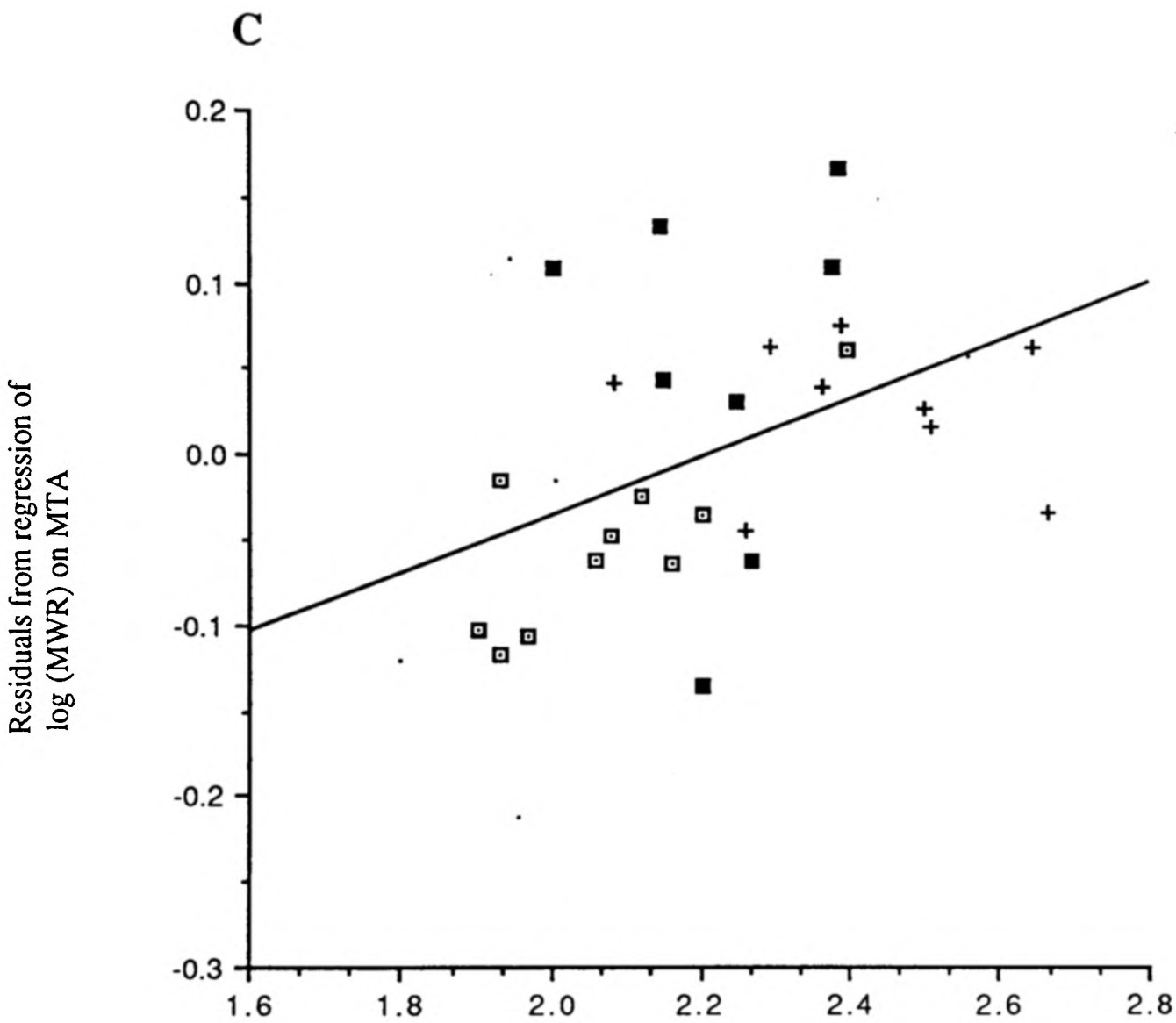


Fig.6.3 (cont.) (c) Residuals from regression of log (MWR) on MTA as a function of log (body mass) for males and females in the genus *Anthophora* divided by subgenus: *Pyganthophora* (■), *Anthophora* (❐), *Lophanthophora* (✛) and others (·) (all bees: $y=-0.38+0.18x$, $R^2=0.194$).

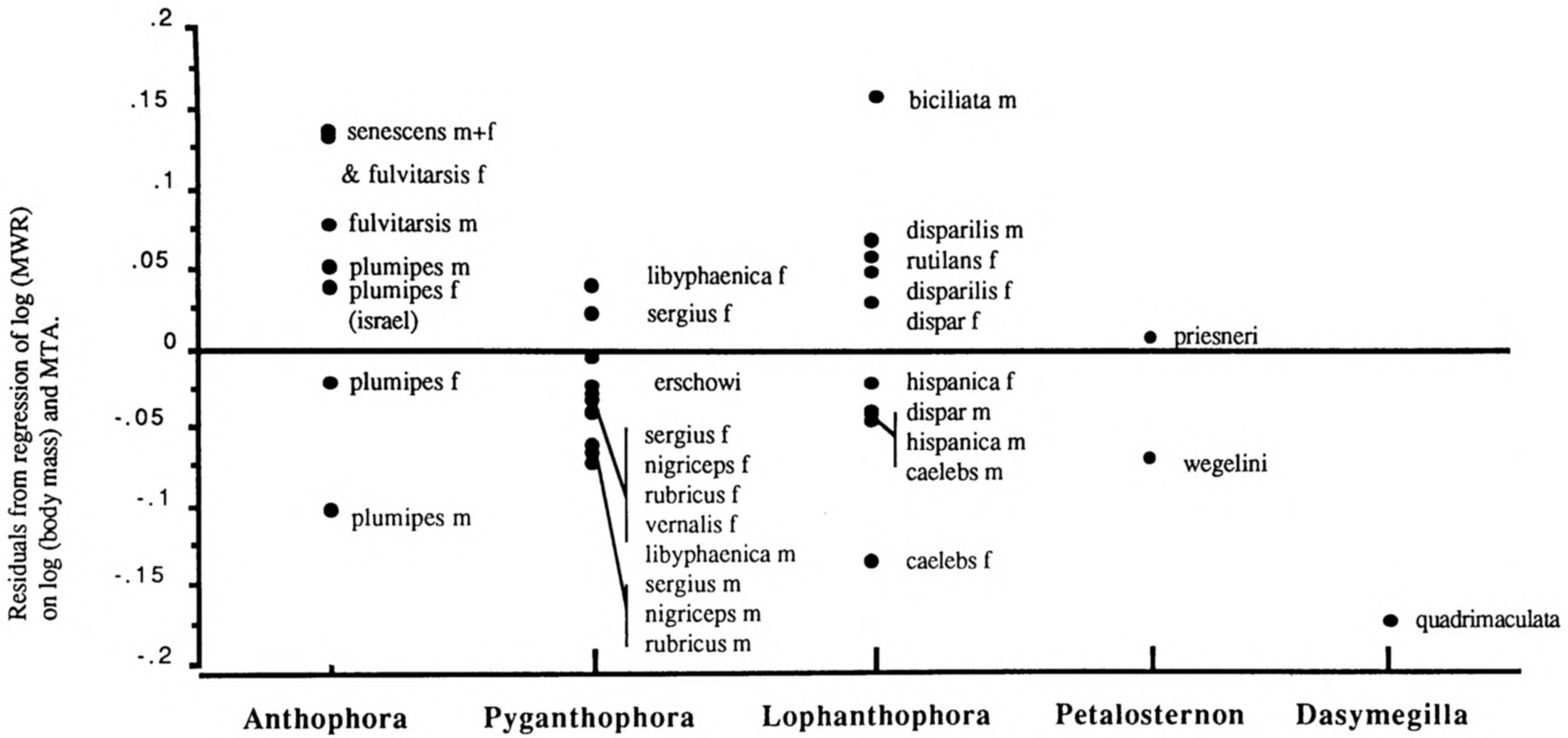
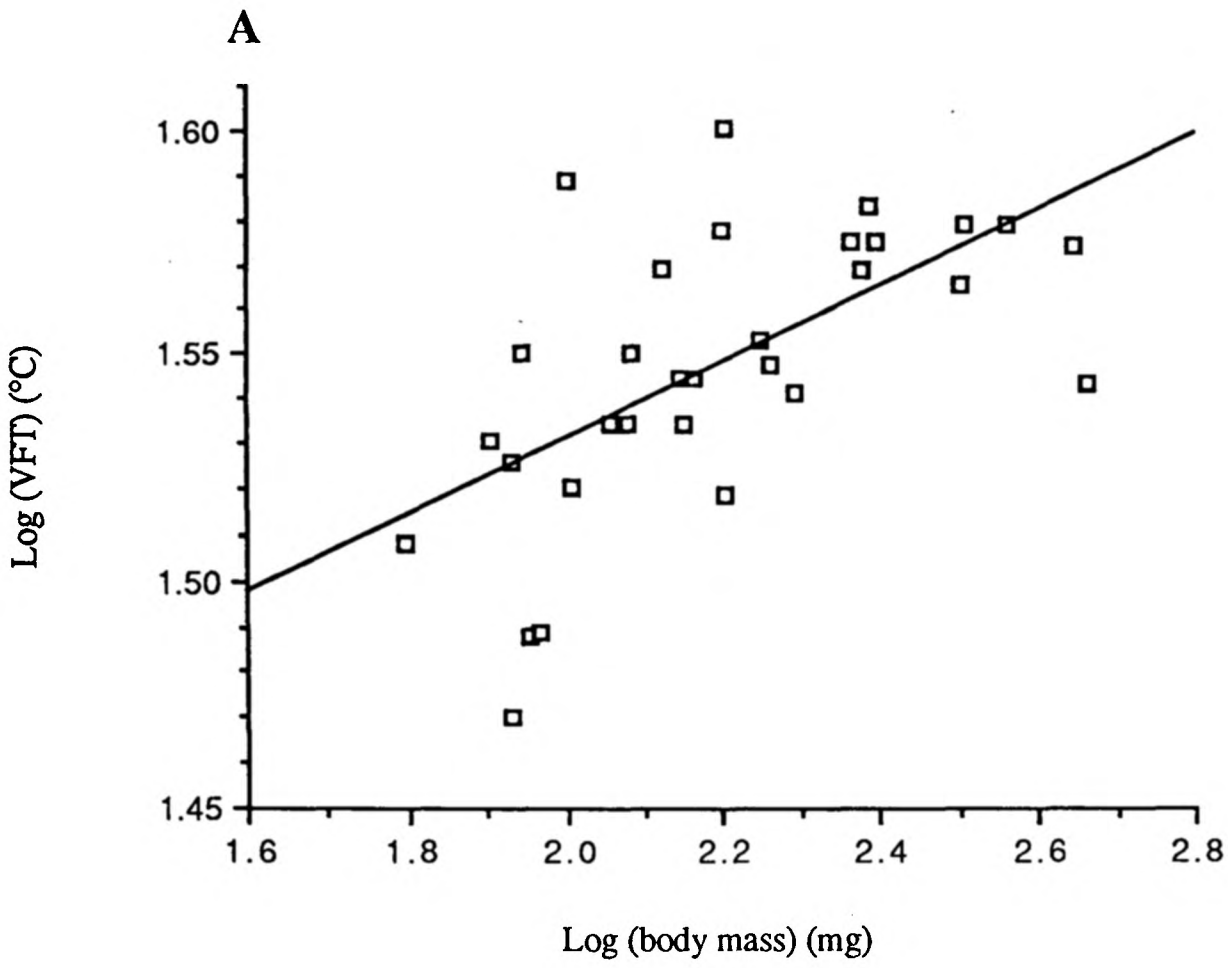


Fig.6.4 Differences between the residuals after regression of log (MWR) on log (body mass) and MTA between subgeneral in the genus *Anthophora*.



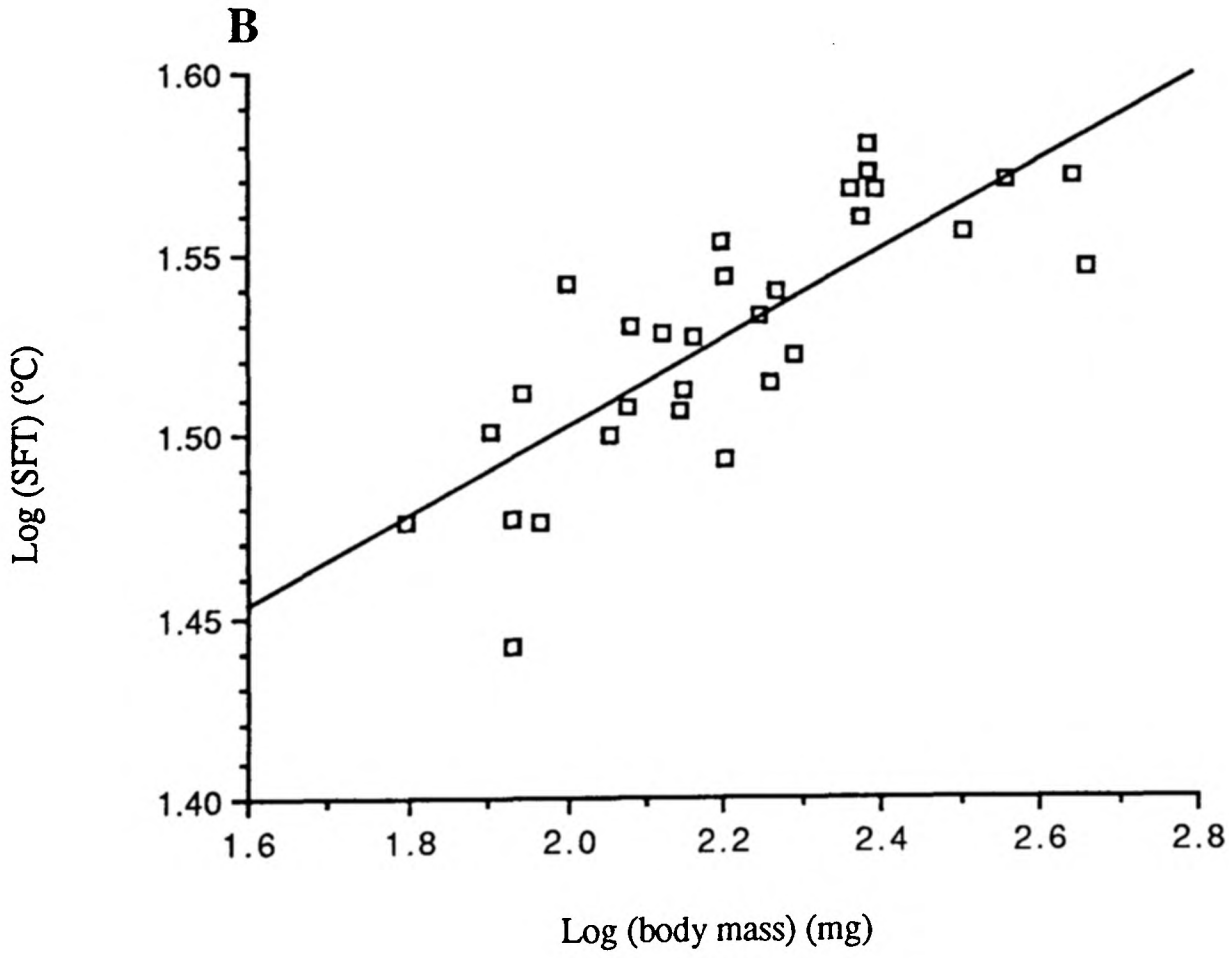


Fig.6.5 **(a)** The relationship between log (VFT) and log (body mass) for males and females in the genus *Anthophora* ($y=1.36+0.085x$, $R^2=0.384$). **(b)** The relationship between log (SFT) and log (body mass) for males and females in the genus *Anthophora* ($y=1.25+0.12x$, $R^2=0.624$).

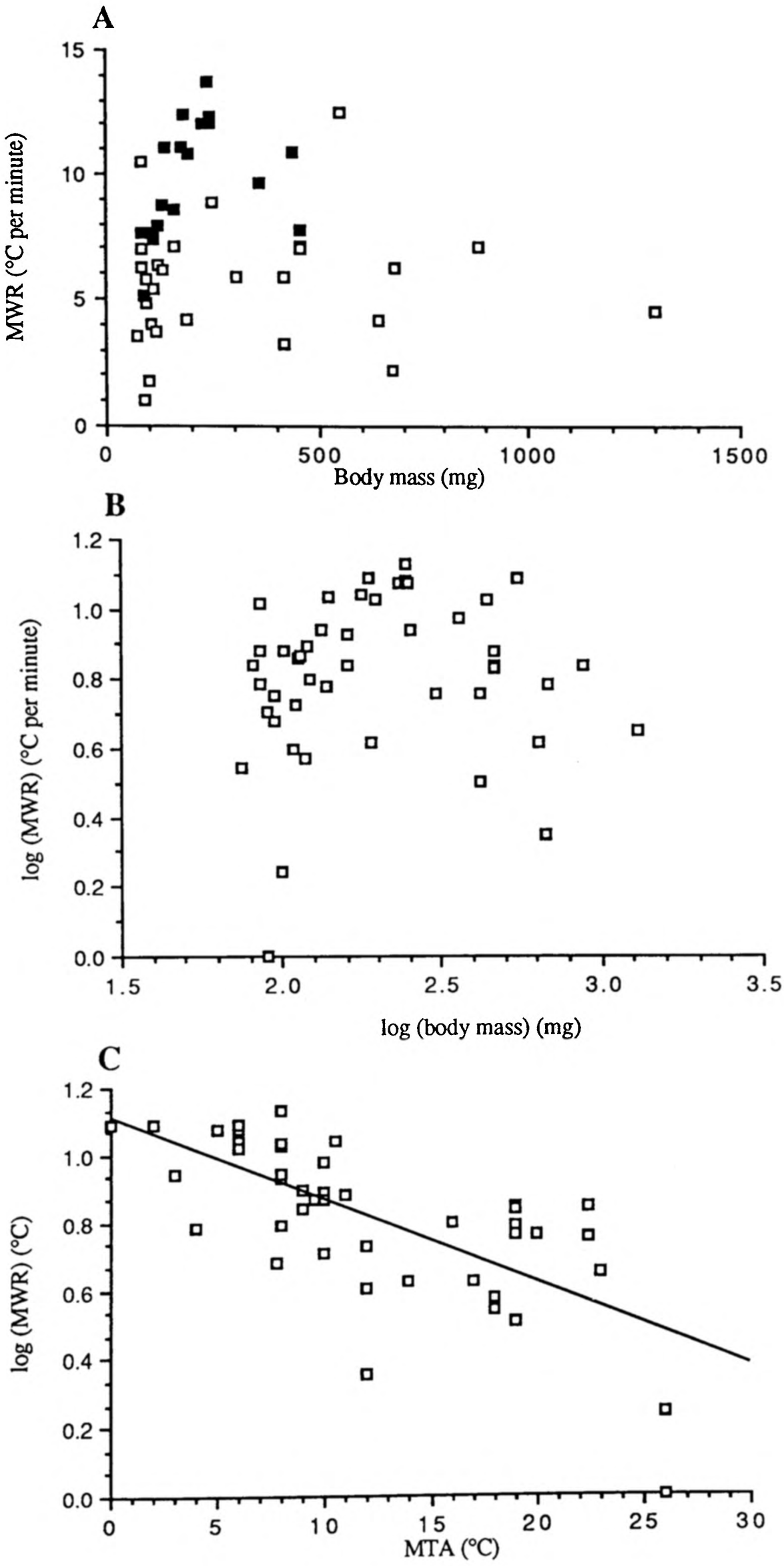
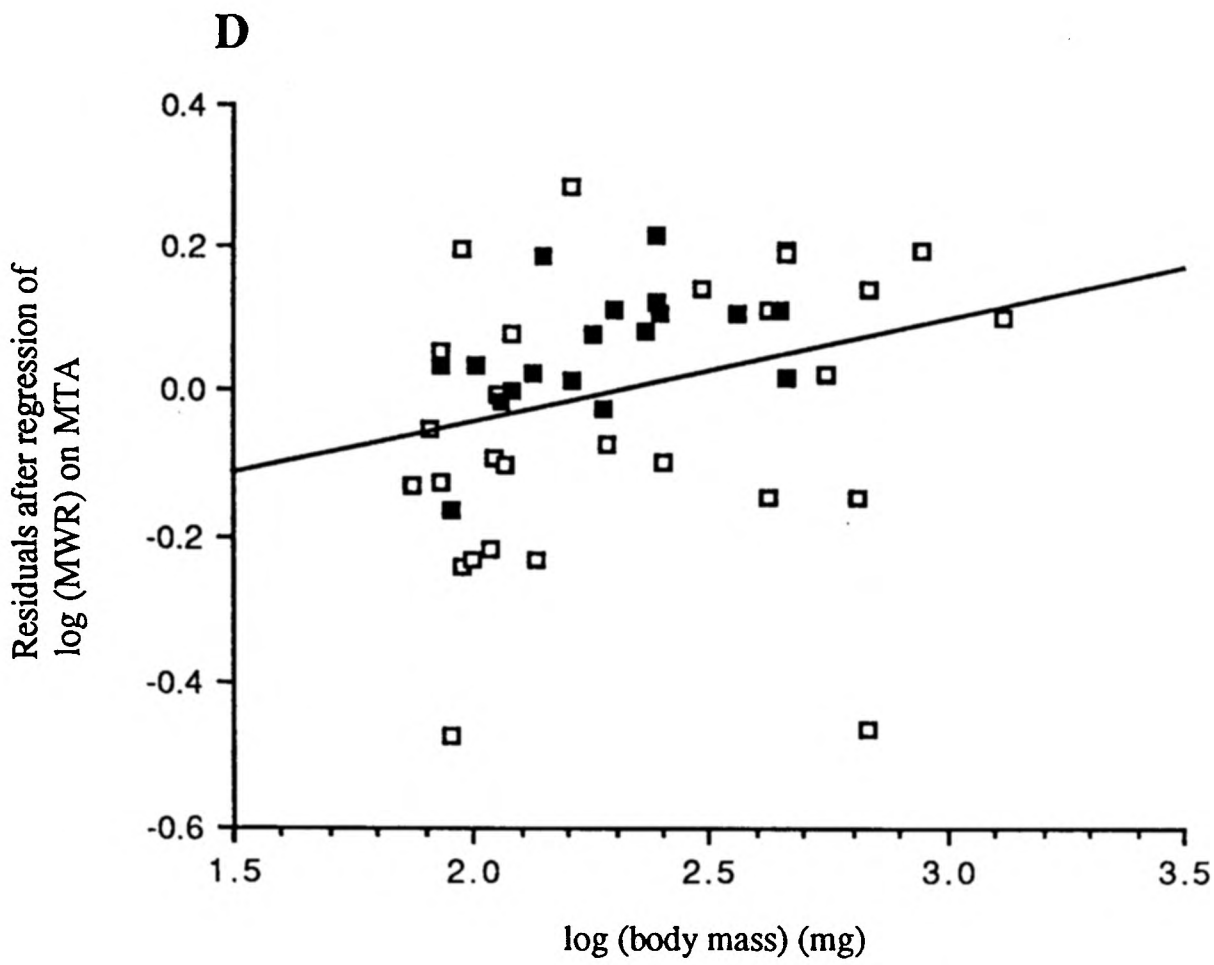


Fig.6.6 (a) MWR as a function of body mass for members of the genus *Anthophora* (■) and for other bees (❐). **(b)** log (MWR) as a function of log (body mass) for all bees. **(c)** Log (MWR) as a function of MTA for all bees (y=1.1-0.024x, R^2 =0.476).



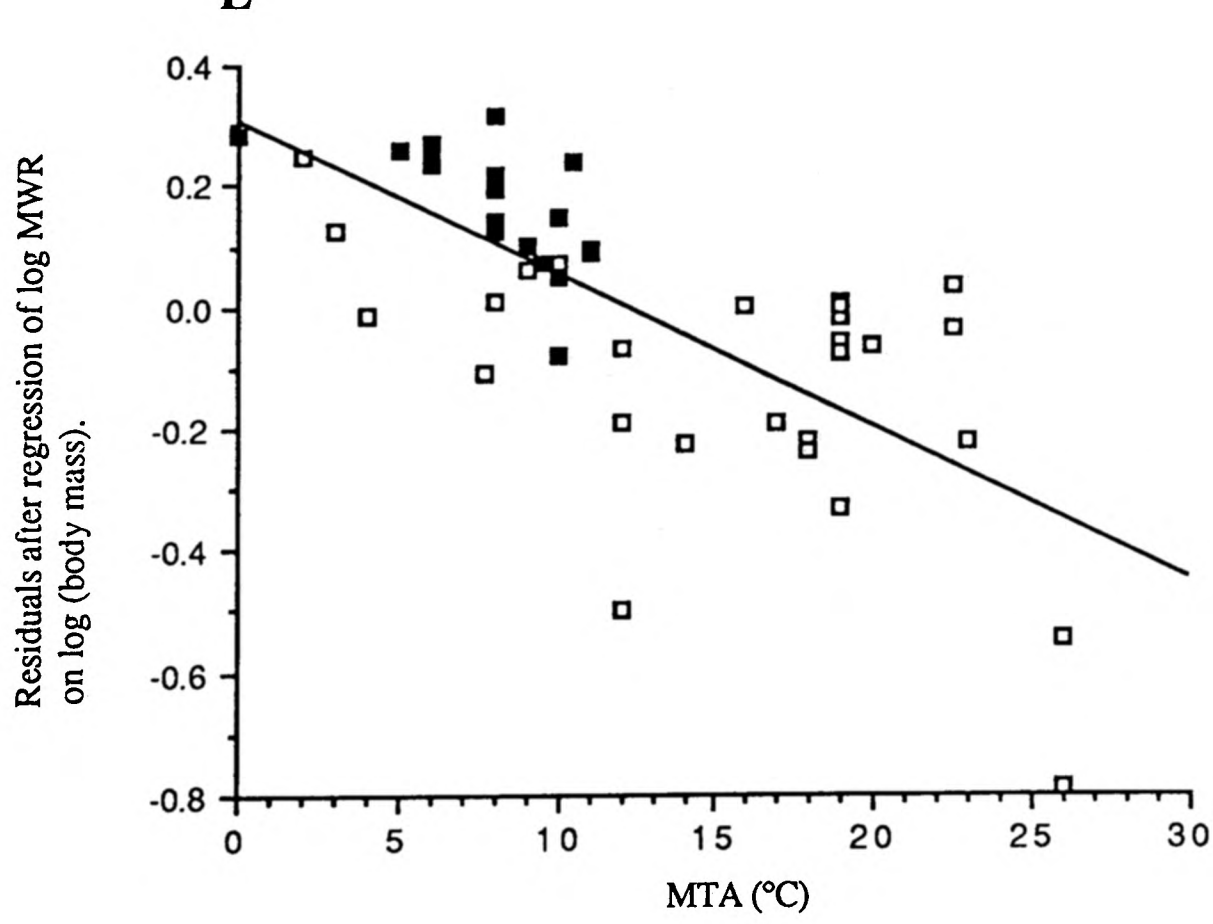


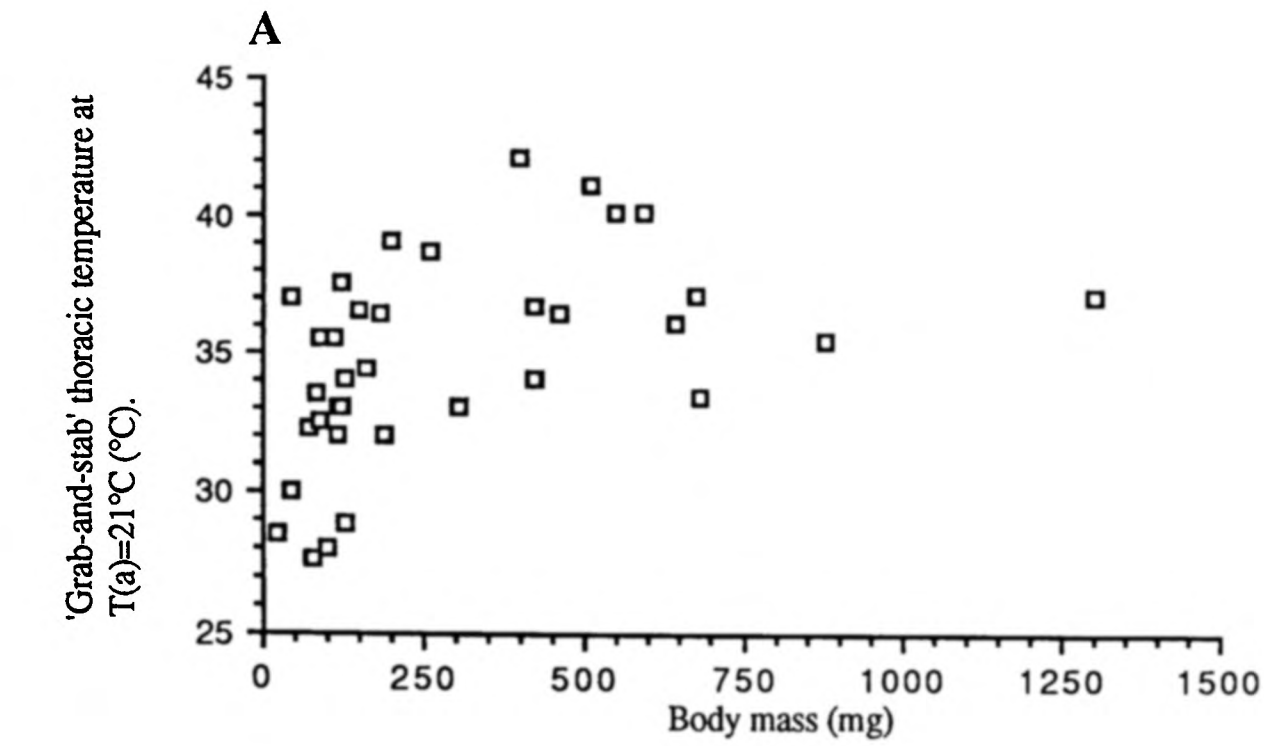
Fig.6.6 (cont.) (d) Residuals after regression of log (MWR) on MTA as a function of log (body mass) for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: $y=-0.33+0.14x$, $R^2=0.078$). **(e)** Residuals after regression of log (MWR) on log (body mass) as a function of MTA for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: $y=0.31-0.025x$, $R^2=0.504$).

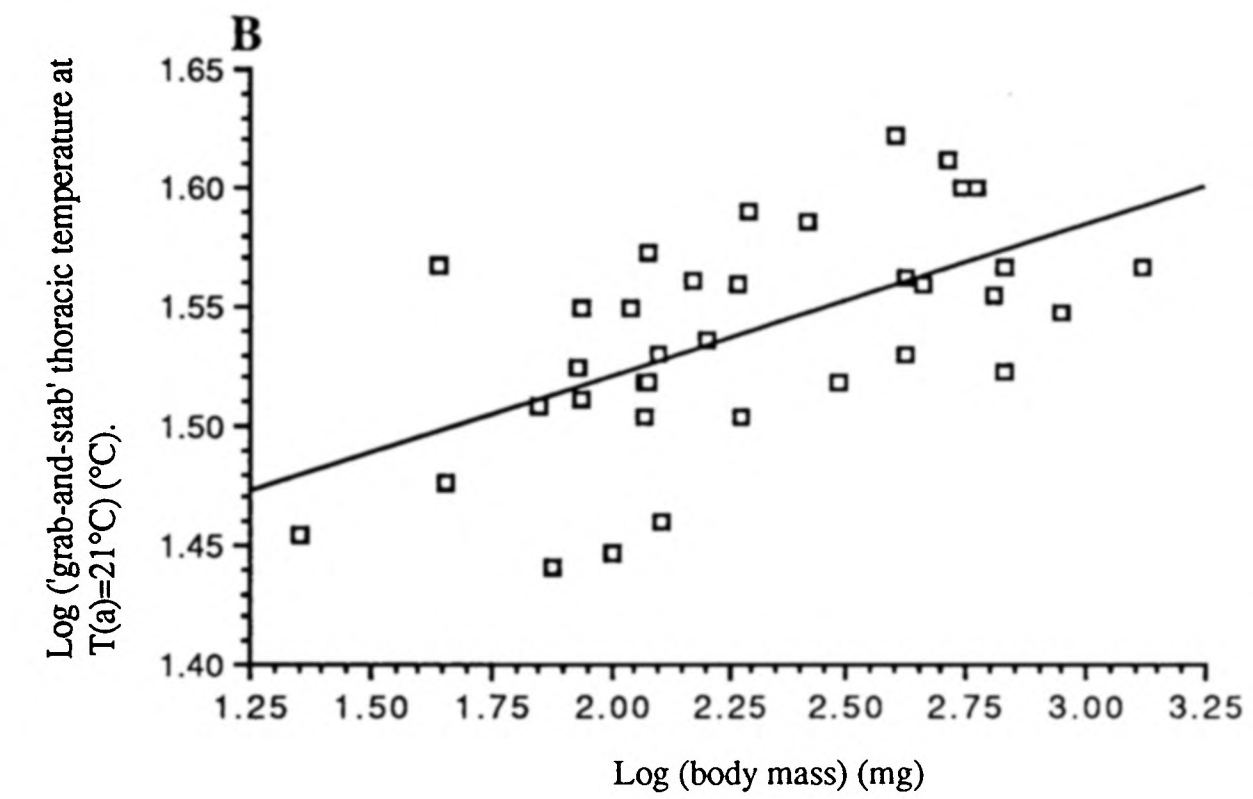


Fig.6.8 A female *Lasioglossum smeathmanellum* (Halictidae). This spcies, with a body mass of about 10mg, was the smallest bee investigated in this thesis.



Fig.6.7 A female *Osmia rufa* (Megachilidae) foraging from *Pulmonaria saccharata* at the Botanical Gardens, Oxford. This species has a very high warm-up rate for its body mass.





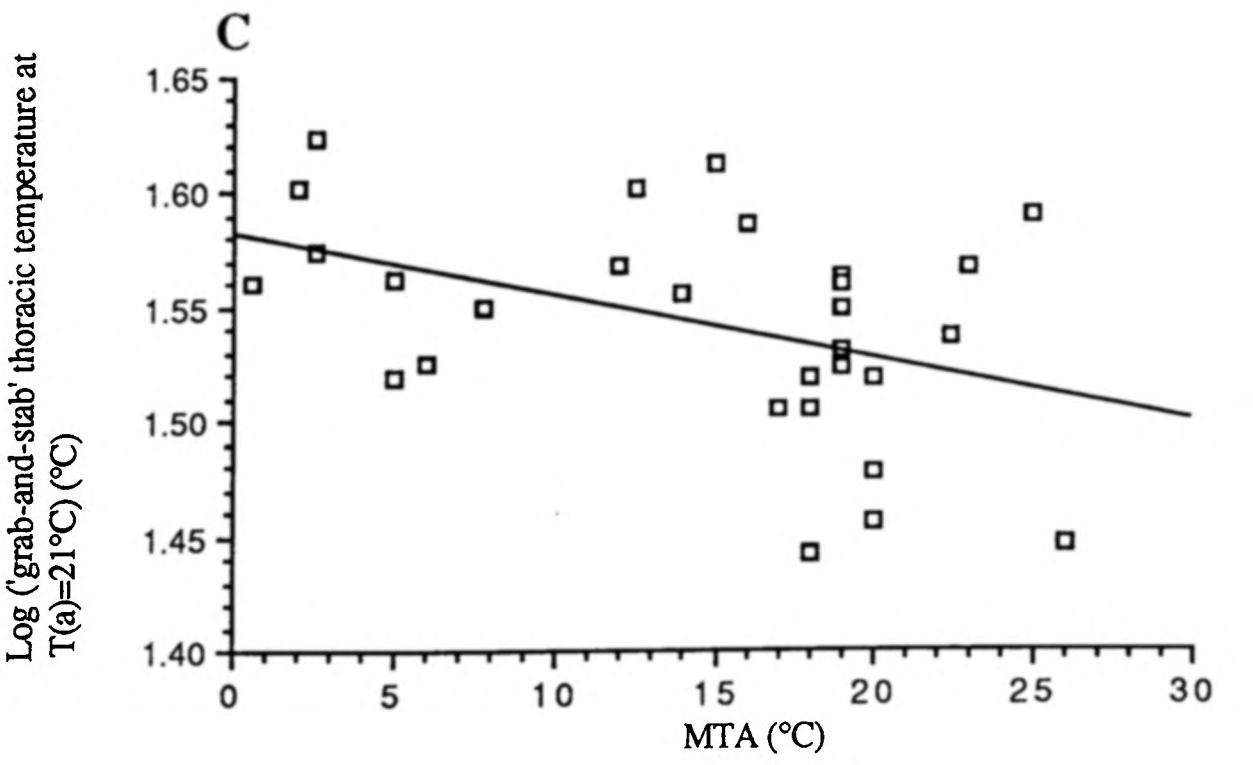
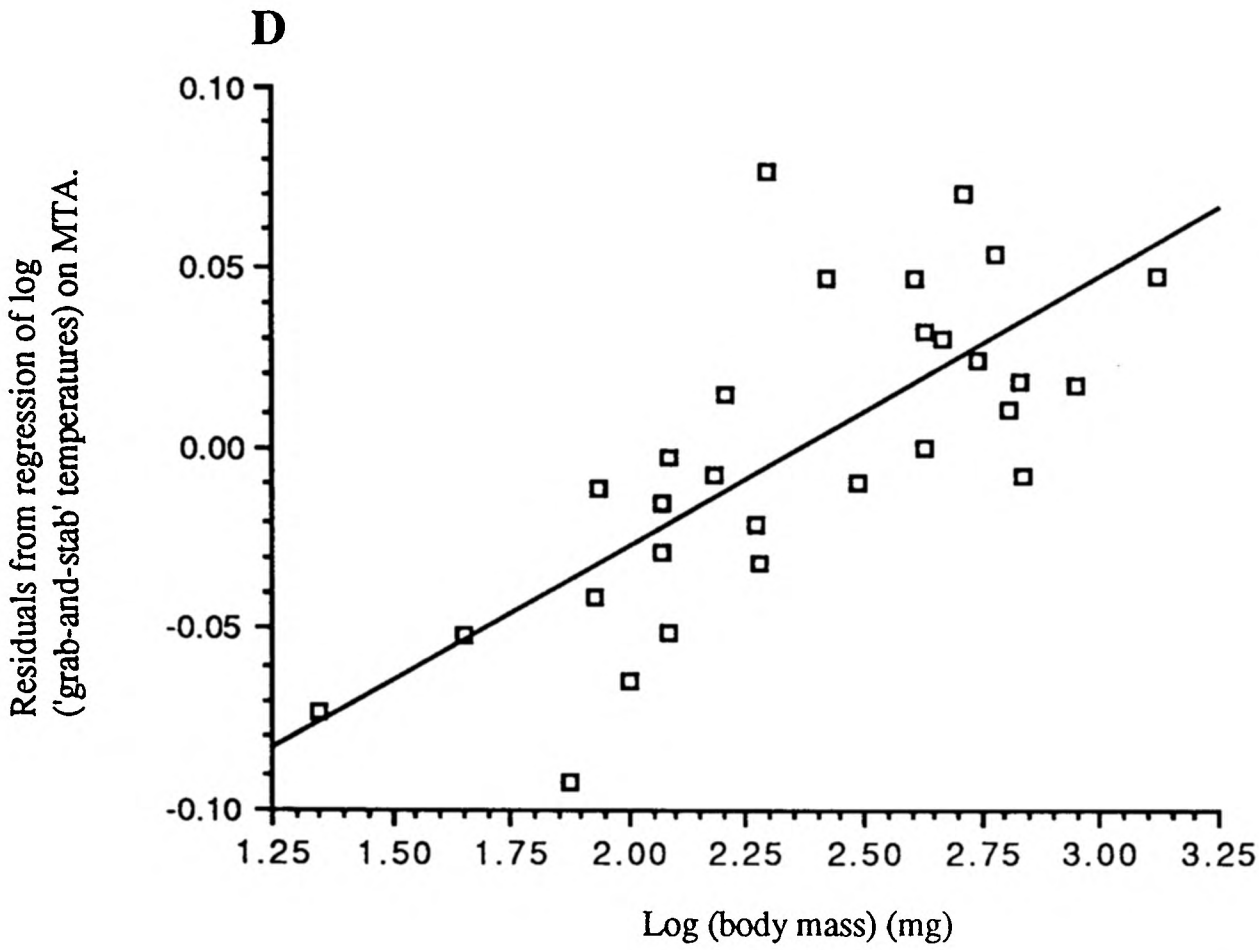


Fig.6.9 (a) 'Grab-and-stab' thoracic temperatures at an ambient temperature of 21°C as a function of body mass across the Apoidea. **(b)** Log ('grab-and-stab' thoracic temperatures) as a function of log (body mass) across the Apoidea ($y=1.39+0.065x$, $R^2=0.348$). **(c)** Log ('grab-and-stab' thoracic temperatures) as a function of MTA across the Apoidea ($y=1.58-0.003x$, $R^2=0.185$).



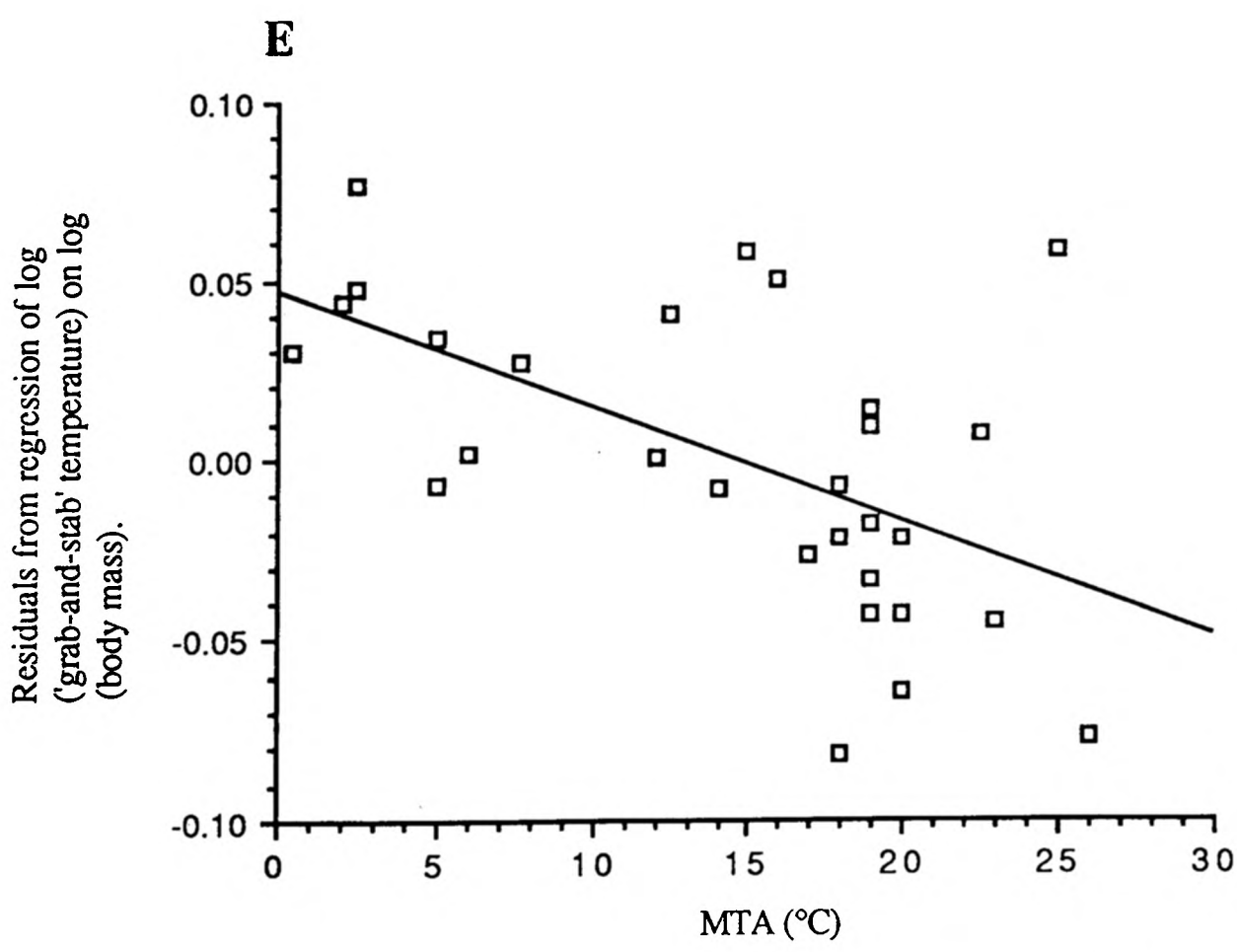


Fig.6.9 (cont.) (d) Residuals from regression of log ('grab-and-stab' thoracic temperatures) on MTA as a function of log (body mass) across the Apoidea ($y=0.18+0.075x$, $R^2=0.533$). **(e)** Residuals from regression of log ('grab-and-stab' thoracic temperatures) on log (body mass) as a function of MTA across the Apoidea ($y=0.05-0.0035x$, $R^2=0.314$).

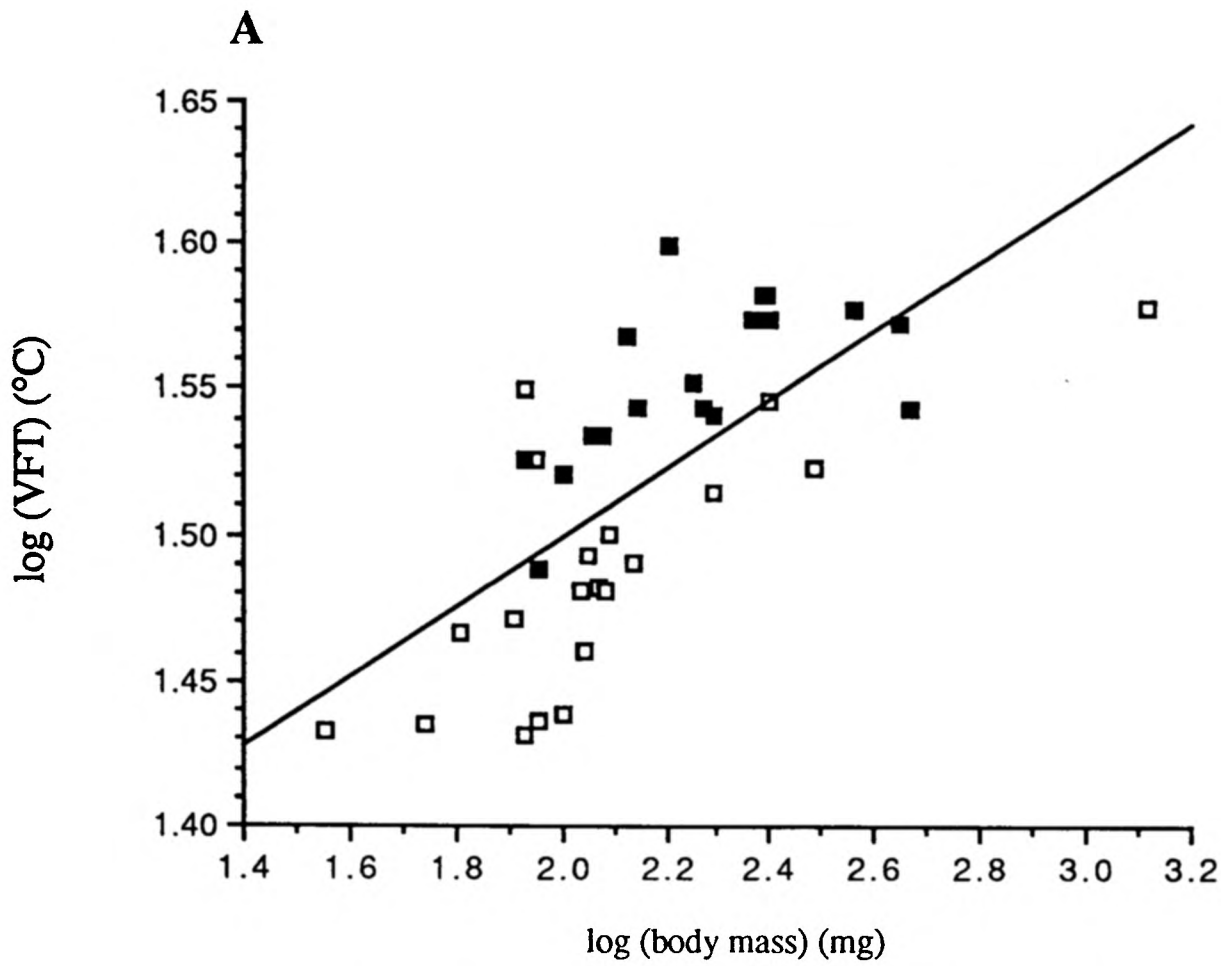
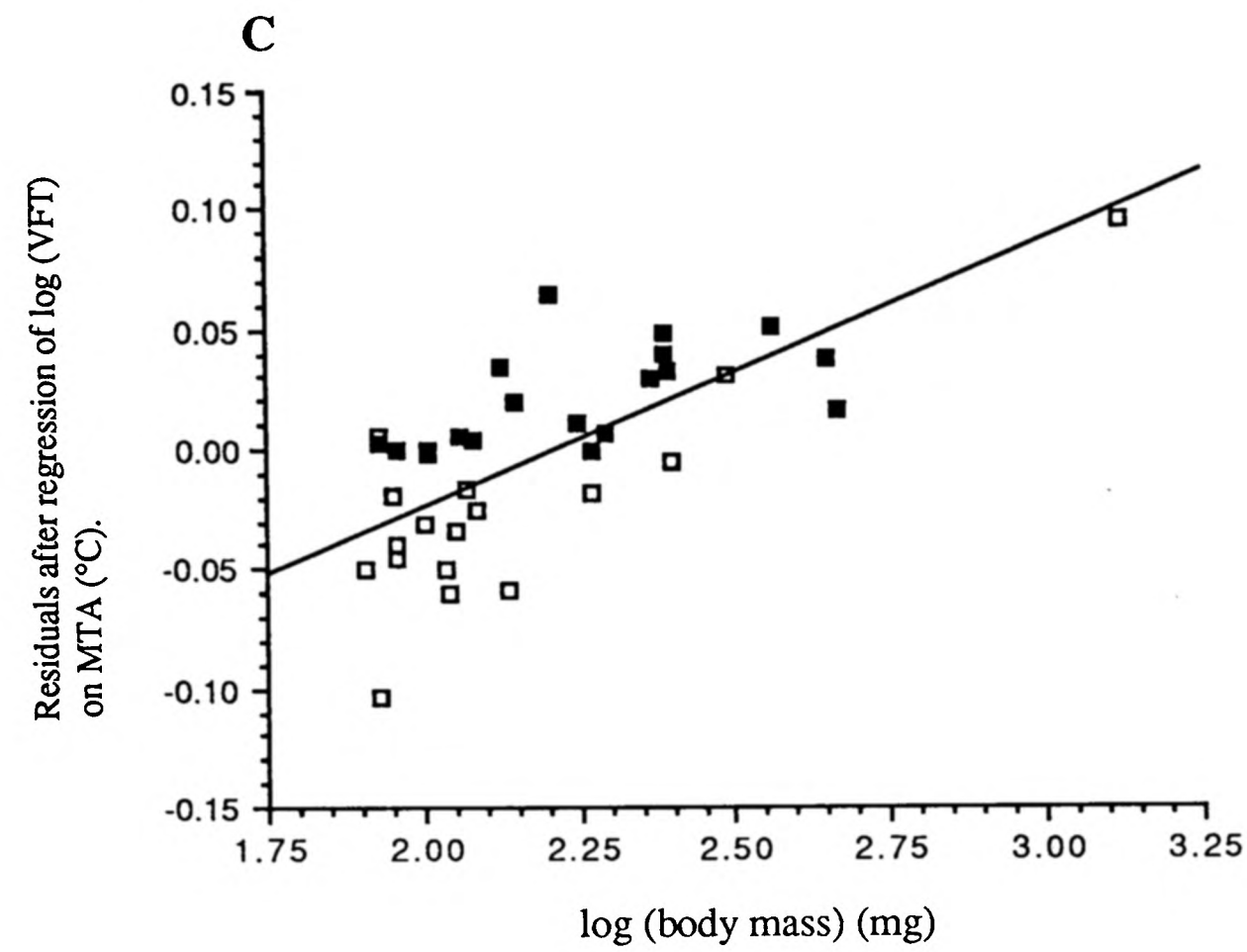


Fig.6.10 (**a**) Log (VFT) as a function of log (body mass) for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: y=1.25+0.12x, R^2 =0.504). (**b**) Log (VFT) as a function of MTA for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: y=1.6-0.003x, R^2 =0.04).



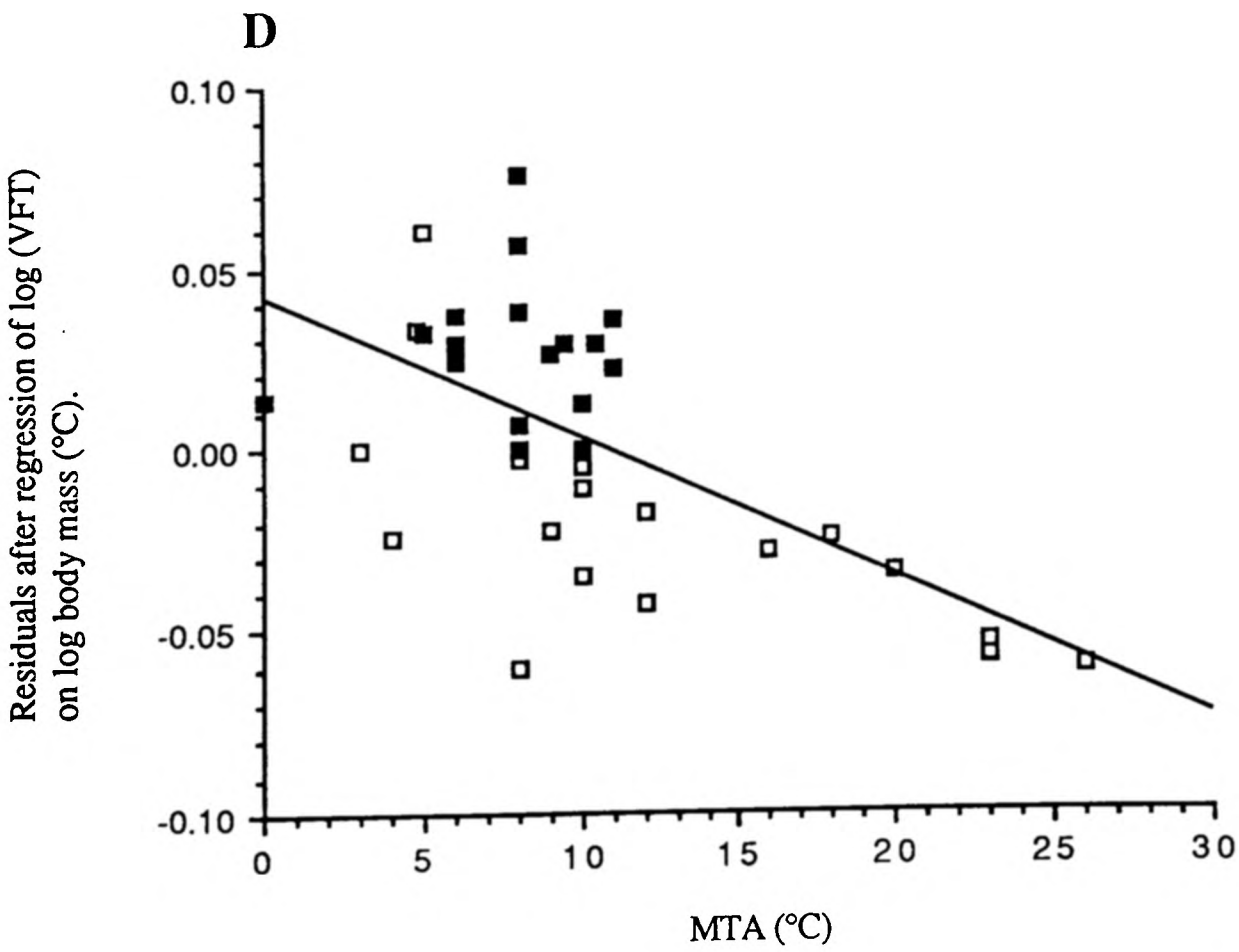


Fig.6.10 (cont.) (c) Residuals after regression of log (VFT) on MTA as a function of log (body mass) for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: $y=-0.25+0.11x$, $R^2=0.533$). **(d)** Residuals after regression of log (VFT) on log (body mass) as a function of MTA for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: $y=0.04-0.004x$, $R^2=0.384$).

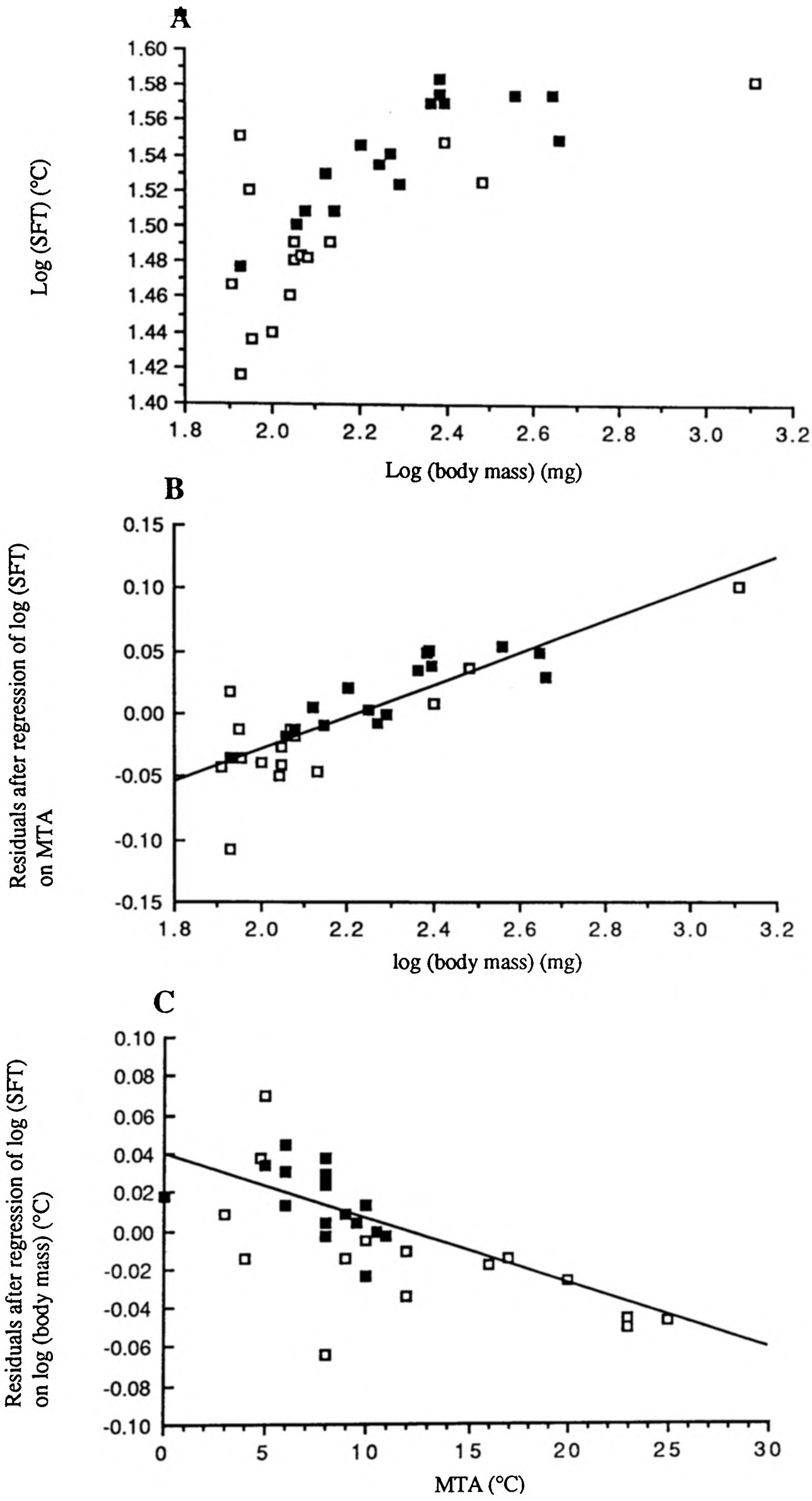


Fig.6.11 (a) Log (SFT) as a function of log (body mass) for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: y=1.3+0.12x, R^2 =0.656). **(b)** Residuals from regression of log (SFT) on MTA as a function of log (body mass) for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: y=-0.28+0.13x, R^2 =0.706). **(c)** Residuals from regression of log (SFT) on log (body mass) as a function of MTA for members of the genus *Anthophora* (■) and for other bees (❐) (regression for all bees: y=0.04-0.003x, R^2=0.250).

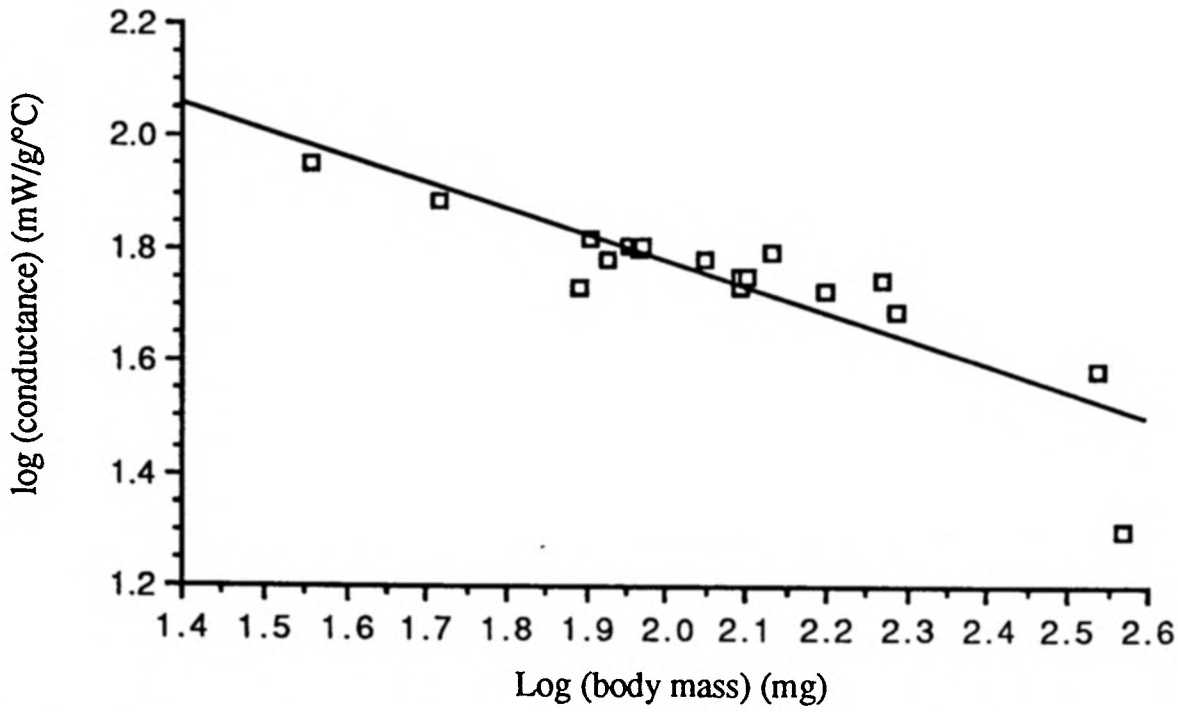


Fig.6.12 Log (conductance) as a function of log (body mass) for British bees (y=2.7-0.46x, R^2=0.750).

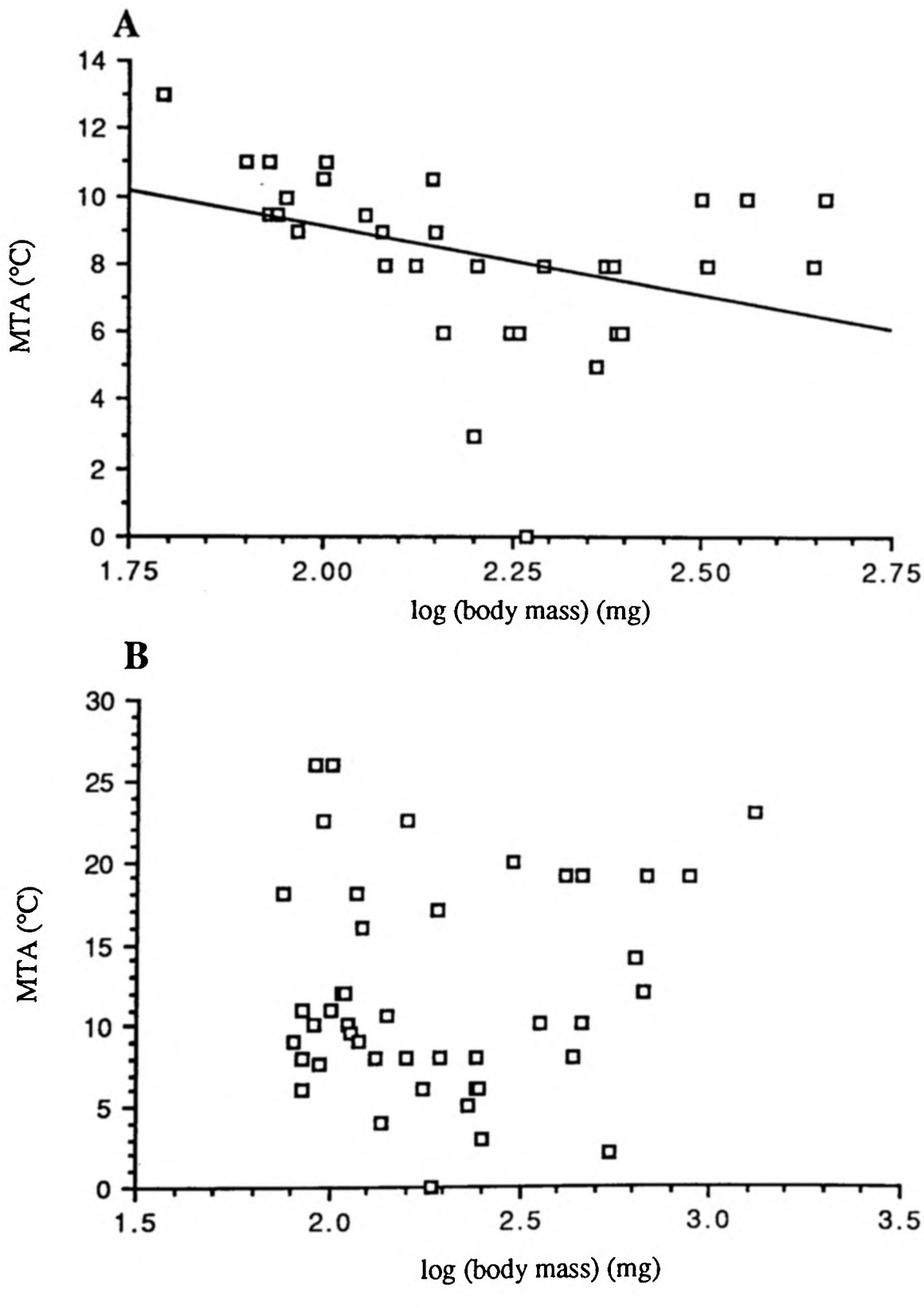


Fig.6.13 (a) MTA as a function of log (body mass) for males and females within the genus *Anthophora* (y=17.2-4.0x, R^2 =0.130). **(b)** MTA as a function of log (body mass) across the Apoidea.



Fig.6.14 A female *Andrena clarkella* (Andrenidae). This species is one of the first solitary bees to emerge in the spring in Britain, flying before the end of February in many years (Dr. Rob Paxton, personal communication). Although endothermic, this species showed relatively low warm-up rates and body temperatures.

Chapter 7. Conclusions and new directions.

7.1 Introduction; a summary of the findings of this thesis.

This last chapter summarises the main results of this thesis and then develops several ideas which I consider to be interesting avenues for future research in insect endothermy.

My findings may be divided into three sections. The first concerns the activity patterns and behaviour of bees, and the second, body temperatures and warm-up rates. The third examines errors associated with methodology.

A. Behaviour and activity patterns.

Chapter 3 showed that T_a placed considerable limits on the activities of *A. plumipes*. Ambient conditions had a significant effect on which females were able to forage, what time they initiated foraging in the morning, and the type and mass of provisions collected. The behaviour of males was also strongly dependent on T_a, which affected not only when they emerged from their nest tunnels, but also how long they spent basking, when and where they fed, and whether they showed courtship behaviour.

The activity patterns and behaviour of males and females over time were shown to correlate with a complex array of factors. Activity patterns of females depended on the site at which they were observed, body mass, T_a, the position of the female in her nest-provisioning cycle, and levels of male interference at foraging sites. Male behaviour not only depended on body size and T_a, but also on which other bees (particularly male and female conspecifics) were encountered while patrolling food sources and at the nest site.

Chapter 5 extended some of these findings by showing that the importance of factors affecting activity patterns, such as T_a and the temporal availability of floral resources, may vary markedly and in predictable fashions for different bees experiencing different climates.

B. Body temperatures and warm-up rates.

Chapters 4 and 6 showed that endothermy in bees is much more widespread than previously thought. Endothermic warm-up before flight is present to some degree in all the species examined. Levels of thermoregulation achieved, however, vary considerably between species. Warm-up rates in bees, and thoracic temperatures in free and tethered flight, have been shown to depend on T_a and body mass within a species (for temperate and tropical examples), across members of a genus and across the Apoidea as a whole. The persistence of these relationships over a range of comparative levels suggests that they are of fundamental importance, even though phylogeny does affect their

form. Furthermore, body temperatures may also depend, in at least some cases, on sex and there may be differences within a group of related species between provisioning and parasitic forms. The interaction of all these factors is complex, and the predictive value of a variable such as body mass does not always emerge unless sophisticated techniques are used to control for other variables.

C. Errors and methodology.

The errors associated with two common methods in the measurement of insect body temperatures have often been loosely discussed but rarely quantified. Chapter 2 contains the first published examinations of (a) the magnitude and possible effects of errors in 'grab-and-stab' measurement of body temperature, and (b) the approximate errors in measurement of body temperature using fixed sensors linked by thermally conducting leads to measuring devices. In neither case do the demonstrated errors preclude use of the technique, but care with interpretation is required. In both cases, measurement of T_{th} in small bees involves the largest errors, and this is the most serious obstacle to comparisons of endothermic and thermoregulatory abilities over the full range of body sizes found in the Apoidea.

7.2. Activity patterns in bees: a brief review and some speculations.

Many authors have described differences in the level of activity of bee species over time. The daily activity of some species is reported as bimodal, and of others as unimodal. Some examples are given in Table 7.1. Can general conclusions be made about the factors which determine activity patterns in bees? Factors which must be important include limitations imposed by floral food sources, limitations of climate, the effects of competion from other nectar-feeding animals, and aspects of the biology of the species, such as the nesting cycle or mating system, that make demands on its time.

Times of nectar and pollen release by flowers impose ultimate limits on the times at which both males and female bees can forage for food. Because most female bees need to provision cells and thus collect nectar and pollen far beyond their own needs, and given that nectar and pollen are usually limited resources, we would predict females to arrive at the flowers as soon as possible after the resource they are collecting becomes available. When nectar secretion and/or anther dehiscence occurs at low ambient temperatures, we would predict competition for limited resources to lead to the evolution of considerable powers of endothermy in bees large enough to maintain elevated thoracic temperatures (e.g. Willmer [1983] for *Bombus*). Departure of such bees from foraging sites may occur due to relative exhaustion of food supplies before climatic changes over the day, such as rising air temperatures, become important, even if departure from the foraging site does correlate with

climatic change. This type of activity pattern is seen in some matinal and vernal bees in deserts (e.g. *Ptiloglossa arizonensis* (Colletidae) and *P. jonesi* foraging from Creosote bush, *Larrea tridentata* in the deserts of the southern United States (Hurd and Linsley 1975). When the resource becomes available in the late evening, and evening foraging does not deplete the resource to some minimum level, bees may return in the morning. Examples include *Anthophora neglecta* and *A. affabilis* foraging from *Oenothera pallida* (Linsley *et al.* 1963) and *Andrena raveni*, also feeding from an evening opening *Oenothera* spp. (Linsley and MacSwain 1959). Resource availability may thus generate a bimodal activity pattern (Table 7.1).

The strength of the correlation between times of bee activity and the time at which floral resources become available would be expected to depend on the size of the bee. Smaller bees, which require smaller pollen and nectar loads, can forage profitably from flowers in which the resource levels have been more greatly reduced than can large bees, as long as they can reach and collect the resource and the resource is of suitable quality (for example, nectar concentration; Corbet 1978). Such small species would be expected to continue to forage after the larger species have departed. Because maintenace of elevated thoracic temperatures in very small bees is not feasible, the arrival of such bees at forage sites may be dependent on increasing ambient temperatures throught the day. This change in forager size through the day is well-documented (e.g. Willmer 1983).

When nectar and pollen are available at different sources and differ in their availablity over time the activity patterns shown by provisioning females will depend on the site at which they are observed. If changes in activity at a nectar or pollen site correlate with changes in temperature it may be tempting to assume that activity levels are dependent on this variable. But the possibility that the source visited by the population may have changed must always be considered. For example, Linsley, *et al.* (1963) described the mass movement of a population of *Andrena omninigra clarkiae* (Andrenidae) from their *Clarkia* pollen source from mid-morning to mid-afternoon to their *Brassica* nectar source in the evening. A similar shift from one forage source to another by a population of females is described in Chapter 3. It should be stressed that nectar and pollen availability and quantity may themselves be related to microclimate. The full range of nectar and pollen sources visited by a species must be observed before limitations of climate can be inferred.

When nectar and/or pollen supplies vary less absolutely, and ambient temperature varies over a wide diurnal range, a dependence of activity on temperature is more probable, particularly in small species where the presence of substantial physiological thermoregulatory ability is unlikely. For such small bees, activity may be limited to periods of the day when levels of solar radiation and T_a allow behavioural thermoregulation alone to achieve the necessary T_{th} for flight. Many of the unimodal activity patterns in Table 7.1 are produced by small or very small bees (*Andrena, Perdita,*

Table 7.1 Examples of unimodal and bimodal patterns of activity at foraging sites over time in bees, with possible causes of the observed activity pattern. Cases in which activity correlates strongly with changes in climate, and where temporal availability of floral resources is not restricted to a narrow period, are referred to as temperature-limited. Cases in which activity is limited to short periods for which nectar and pollen are available, despite long periods of tolerable temperatures, are referred to as flower-limited. This is a very simple classification, and does not imply that combinations of both effects do not occur under conditions not recorded in the cited studies (see text). In many cases there is insufficient information to implicate a causal factor.

Species	Source	Possible cause of observed pattern
Unimodal activity patterns		
Melissodes pallidisignata (Anthophoridae)	Thorp and Chemsak (1964)	temperature-limited
Exomalopsis chionura (Anthophoridae)	Rozen and MacNeil (1957)	temperature-limited
Diadasia angusticeps (Anthophoridae)	MacSwain *et al.* (1973)	temperature-limited, flower-limited
Xenoglossa patricia (Anthophoridae)	Linsley (1960)	flower-limited
Apis mellifera (Apidae)	Butler and Finney (1942)	temperature-limited
Megachile gravita (Megachilidae)	MacSwain *et al.* (1973)	temperature-limited
Megachile pascoensis (Megachilidae)	MacSwain *et al.* (1973)	flower-limited
Hoplitis anthocopoides (Megachilidae)	Eickwort (1977)	temperature-limited
Halictus umbripennis (Halictidae)	Eickwort and Eickwort (1971)	temperature-limited
Andrena placida (Andrenidae)	Thorp and Stage (1968)	temperature-limited
Andrena erythronii (Andrenidae)	Michener and Rettenmeyer (1956)	temperature-limited
Andrena rozeni (Andrenidae)	Linsley and MacSwain (1959)	flower-limited
Andrena raveni (Andrenidae)	Linsley and MacSwain (1959)	flower-limited
Andrena omninigra clarkiae (Andrenidae)	Linsley *et al.* (1963)	flower-limited
Andrena complexa, A. suavis (Andrenidae)	Linsley and MacSwain (1959)	temperature-limited
Perdita nuda (Andrenidae)	Torchio (1975)	temperature-limited
Perdita texana (Andrenidae)	Barrows *et al.* (1976)	temperature-limited
Perdita opuntiae (Andrenidae)	Bennett and Bread (1985)	temperature-limited
Perdita maculigera maculipennis (Andrenidae)	Michener and Ordway (1963)	temperature-limited
Bimodal activity patterns		
Melissodes pallidisignata (Anthophor.)♂	Thorp and Chemsak (1964)	
Xylocopa tabaniformis (Anthophoridae)	Janzen (1964)	
Xylocopa tabaniformis (Anthophoridae)	Hurd (1958)	
Xylocopa virginica (Anthophoridae)	Balduf (1962)	
Xylocopa spp. (Anthophoridae)	Kapil and Dhaliwal (1969)	
Xylocopa sulcatipes (Anthophoridae)	Gerling *et al.* (1983)	temperature-limited
Anthophora affabilis (Anthophoridae)	Linsley *et al.* (1963)	flower-limited
Anthophora neglecta (Anthophoridae)	Linsley *et al.* (1963)	flower-limited
Bombus pennsylvanicus (Apidae)	Posey, Katayama and	temperature- and
Bombus nevadensis (Apidae)	Burleigh (1986)	flower-limited.
Anthidium palmarum (Megachilidae) ♂	Wainwright (1978)	
Ptiloglossa eximia (Colletidae)	Schrottky (1907)	
Ptiloglossa arizonensis (Colletidae)	Linsley (1962b); Linsley and Cazier (1970)	
Caupolicana yarrowi (Colletidae)	Linsley and Cazier (1970)	
Caupolicana electa (Colletidae)	Mitchell (1960)	
Lasioglossum galpinsiae (Halictidae)	Linsley *et al.* (1963)	temperature- and flower-limited

Halictus). In highly endothermic species which generate a high T_{th}, high T_a during the middle of the day may lead to the generation of intolerable heat loads in flight, and abandoning of the foraging site. If food supplies persist into the evening, such species may show a bimodal activity pattern. Most of the species showing bimodal activity patterns in Table 7.1 are either known to be capable of substantial endothermy (*Bombus, Xylocopa, Anthophora*) or good potential candidates in which the presence of this phenomenon has yet to be investigated (*Ptiloglossa, Caupolicana*). The same species (e.g. *A. plumipes*, Chapter 3) may show a single activity peak on a cool day and a bimodal activity pattern on a hot day. A similar change in activity has been described for *Xylocopa pubescens* in Israel by Mordechai *et al.* (1978) and Willmer (1988). Analysis of visitation levels over a range of climatic conditions and levels of resource availability is necessary before the factors which most strongly influence activity patterns can be identified.

As well as provisioning their cells with pollen and nectar, female bees must construct and seal their cells. These activities preclude foraging for at least some of the period over which they are active, and periods of activity inside the nest may constitute a considerable proportion of the total activity period (e.g. the 3 female *A. plumipes* illustrated in Fig.3.6 spent a mean of 42.6±3.4% of their period of activity, or 5.2±0.6 hours, in the nest; see also Willmer 1986; Willmer and Stone 1988). If activity inside the nest characteristically occurs at a particular part of the day, such as the evening periods of nest digging in female *A. plumipes* in Fig.3.6, it will affect the activity periods observed at forage sites. Where possible increased time spent by females in the nest should correlate with decreasing availablity of resources, as in foraging *C. frontalis* (Willmer and Stone 1988). The factors which affect female activity may also change if there are phases of different behaviour over the females' life. Although there was no detectable difference in the endothermic abilities of provisioning and searching female *A. plumipes*, these two behavioural phases showed quite different responses to ambient temperature (Chapter 3). It may therefore not be possible to generalise about the effects that changes in various factors will have on the behaviour of a female unless her position in the nesting cycle is known.

The purpose of male activity is to locate and mate with receptive females. In the case of species in which females mate only once, males must locate that fraction of the female population that is unmated. Males must also forage for nectar, and will respond to climatic change. Male activity patterns must therefore be the result of complex interactions between, on the one hand, climate, nectar availability, and any other factors which affect the activity patterns of unmated females; and on the other, of the direct affects of nectar availability and climate on the males themselves. Many studies document changes in the behaviour of male bees with changes in the activity of females at foraging sites (e.g. Cazier and Linsley 1963; Thorp and Chemsak 1964; Linsley and Cazier 1970;

Roberts 1971) and, in species with territorial males, the necessity to divide time between foraging and territoriality (e.g. Cazier and Linsley 1963; Raw 1975; Gerling and Hermann 1978). Chapter 3 showed that male *A. plumipes* may also have strong effects on the behaviour of conspecific females.

This brief discussion demonstrates that inference of the factors determining activity in bees merely by counting the numbers flying at a particular place at a particular time can only produce credible results if a great deal of the biology of the species is known. Illustration of the complex interactions which result in the activity patterns shown by male and female *A. plumipes* is a justification for detailed study of a single species.

7.3 Body temperatures in bees: some more speculations.

A. Genotypic adaptation and phenotypic adjustment.

The heat produced by a given mass of endothermic tissue will depend on the conditions under which it is examined. Thoracic and ambient temperature have strong effects on the heat produced, and the resulting warm-up rate (Chapter 4). At a cellular level, heat production depends on the rates of endothermic reactions, and such a dependence of endothermy on temperature is thus to be expected. More interesting are the possible results of qualitative intra- and inter-specific differences in the metabolic systems used.

Prevailing environmental temperatures when and where endothermic insects are active vary widely, and species can adapt to them in the long term through evolution (genotypic adaptation), and more immediately through acclimation (modification within a single individual of metabolic processes in response to changes in the external environment) (phenotypic adjustment). If there has been selective pressure to increase the intensity and duration of physical performance - and flying insects exhibit some of the highest sustained metabolic rates known - then it is probable that the enzymes of aerobic metabolism have evolved to function best in the relatively narrow range of body temperatures that can be maintained most easily, given the prevailing thermal environment (Chapter 6).

Although it has been little studied, acclimation apparently occurs in at least one endothermic insect. Kammer (1971) showed that adults of the Monarch butterfly, *Danaus plexippus*, kept at 4-5C for 'a few days' more readily showed warm-up behaviour at an ambient temperature of 15-16°C than those kept at 23-4°C. The mechanism of this apparent acclimation is unknown. In the cockroach *Periplaneta americana*, cold-acclimated adults showed higher metabolic rates than warm-acclimated individuals at the same T_a (Singh and Das 1977). A contributing factor may have been an increase in mitochondrial density in the muscles of cold acclimated animals; cold-acclimated

individuals had a 25% higher density of mitochondria in their coxal muscles than warm-acclimated animals (Singh and Das 1977). Variation in the metabolic rate probably exists between closely related species of endothermic insects. In the honeybee *Apis mellifera*, the African variety *Apis mellifera adansonii* maintains almost the same thoracic temperatures at a given T_a as the European form *Apis mellifera mellifera*, even though it is only 66% of the body mass of the European subspecies. The African bees are also more rapid fliers at a given Ta, suggesting that the comparable temperature excess they maintain during flight is due to a greater metabolic rate, counteracting their higher rates of heat loss due to their smaller size (Heinrich 1981).

Either acclimation or long term evolution could explain the importance of MTA in predicting warm-up rates in the bees compared in Chapter 6. The role of each phenomenon could be investigated easily in a species such as *Apis mellifera*, providing an interesting avenue for further research.

Chapter 6 showed that there are differences in the warm-up rates of bees that are characteristic of taxa within the Apoidea. For example, for bees of a given body mass members of the genus *Anthophora* have higher warm-up rates than those in the genus *Andrena*. It is improbable that differences in rates of heat loss could account for all the differences observed; *Anthophora quadrimaculata* and *Andrena clarkella* have similar masses and almost identical thermal conductance (Chapter 6). These differences suggest that there are phylogenetic differences in the thermogenic properties of the flight muscles. Given that any level of thermogenic ability has associated with it costs and benefits, it is likely that these systematic differences constitute different energetic strategies. At the moment, too little about these differences is known, both in terms of the thermogenic machinery of the taxa concerned, and of the energetics of their life history strategies for any differences in strategy to be assessed.

A potential source of interspecific and intraspecific variation in warm-up rates may be differences in the levels of activity of a substrate cycling heat source (see Chapter 1). Newsholme *et al.* (1972) showed that levels of activity of both FDPase and PFKase (Chapter 1) differed between six species of *Bombus*, correlating negatively with body mass both within a species and across species. This result could account in part for the higher warm-up rates per unit mass found in smaller bees (Chapter 6), although the range of bees demonstrating the potential for endothermy using substrate cycling remains to be established. Levels of FDPase and PFKase activity have also been shown to correlate with the ability of different bumblebee species to forage for periods of time without flight on large inflorescences of small flowers: the more important foraging from such inflorescences is for a species, the higher the activities of both substrate cycle enzymes (Prys-Jones 1986).

As well as differences between bumblebee species, Newsholme *et al.* (1972) found large differences in the activities of these enzymes between bumblebees and other bees: *Apis mellifera* and two species of cuckoo bee (*Psithyrus vestalis* [Geoffroy in Fourcroy] and *P. campestris* [Panz.]) had rates of FDPase activity far lower than those of the bumblebees investigated. It is interesting that *Anthophora plumipes*, a member of the most endothermic insect genus known, does not show high levels of FDPase activity (Surholt and Newsholme 1981), and thus apparently does not utilise this particular substrate cycle as a heat source.

B. Ageing.

The endothermic ability of an individual insect could also change in the same thermal regime, not only in the short term in response to feeding, as discussed in Chapter 4, but also in response to changes in the flight musculature through its life. Although no study of the effect of ageing on endothermic ability in insects has been published, the phenomenon of ageing of the flight muscles of insects in general is well known (Sohal 1985, reviewed by Collatz and Sohal 1986). In those endothermic insects in which heat is generated by the thoracic flight muscles, it is reasonable to expect that degenerative processes affecting flight ability may also affect endothermic ability. Perhaps the best known example of the ageing of insect flight systems is in an endothermic insect - the honeybee. Neukirch (1982) showed that, regardless of the period over which she completes them, a worker honeybee dies due to senescence after flying approximately 800km. Collatz and Wilps (1986) showed that senescence in the scorpionfly *Panorpa vulgaris* is accompanied by clear ultrastructural changes in the muscle cells, including deformation and destruction of mitochondria, swelling of the sarcoplasmic reticulum and dissolution of the myofibrils. Degenerative processes in the flight muscles with age are thought to be widespread in insects; Johnson (1980) reports ageing of the flight systems of 20 families in 8 insect orders. Degeneration of the flight muscles is not always permanent: Nair and Prabhu (1985) showed that the indirect flight muscles of *Dysdecus cingulatus* (Heteroptera: Pyrrhocoridae) degenerate during winter torpor, and regenerate in the spring prior to migration.

The mechanisms affecting ageing of the flight systems are thought to affect the energy provision systems required for flight. In the locust, which uses fats as the fuel for flight, the system thought to be affected by ageing is the lipid transport system involving lipoprotein A^+ (Wheeler and Goldsworthy 1983, Collatz and Sohal 1986). In the blowfly *Phormia*, which utilises carbohydrate as its flight fuel, Collatz and Wilps (1986) implicated decreases in the activity of the enzymes arginine phosphate kinase (APK), glycogen phosphorylase (GP) and phosphofructokinase (PFK) as

causal factors in the deterioration of flight performance. APK and GP catalyse early stages in the energy yielding metabolic chain, and are apparently responsible for cutting off the energy flow needed to drive the flight muscles. A role for PFK is of particular interest because of its proposed importance in the substrate cycling hypotheses discussed in Chapter 1.

Since endothermy also depends on supply of fuel to rapidly metabolising thermogenic tissues, ageing may therefore not only result in a decrease in the physical efficiency of the flight muscles, but also in a reduction in the ability of the muscle to maintain itself at the temperatures to which it is adapted, leading to further reduction in performance. It must be borne in mind that loss of flight activity need not depend on disturbance to the ultrastructural integrity of the muscle cells. There may be changes in the neural information reaching the muscles. Although it is known that both in myogenic and neurogenic fliers the rate of motoneuron firing and wingbeat frequency both increase for a short while after eclosion (Kutsch and Hug 1981; Kutsch and Stevenson 1981), it is not known whether disturbance or desynchronisation of neuromuscular interaction occurs in senescence.

No study of changes in endothermic ability with age in insects have been attempted, although methods allowing repeated examination of individuals now exist. Examination of the endothermic abilities of different age classes of *Apis mellifera* would seem to be an ideal way to investigate this further. Further investigation of *Apis mellifera* would be of particular value in the light of an increasing body of knowledge on the trade-offs between foraging effort and longevity (Schmid Hempel and Wolf 1988; Wolf and Schmid Hempel 1989). There will soon be an oportunity to assess the energetics of foraging in this species not only as a physiological phenomenon but also as a life history strategy.

C. Body mass.

Factors other than the metabolic output per unit mass of muscle tissue also affect warm-up rates and body temperatures, and therefore thermoregulatory strategies as well. The importance of body mass has been shown to be considerable (Chapter 6). Size has a strong effect on whether thermoregulation is possible. Although the flight muscles of all insects obligatorily produce heat during flight, the smallest fliers, which rapidly lose heat by convection, are not necessarily endothermic regulators, despite significant heat production. Mosquitos weighing 1-2mg generate thoracic temperature excesses of less than 1°C in flight, and *Musca domestica* (body mass 14mg) only 2°C (Heinrich 1981). This figure is compatible with that obtained in Chapter 6 for *Lasioglossum smeathmanellum*, which has a similar body mass.

While low body mass may make maintenance of high T_{th} at low T_a impossible, high body

mass and high levels of endothermic activity may result in body temperatures that exceed thermoregulatory abilities, resulting in unregulated hyperthermia (Heinrich 1981), leading to damage or death if flight is continued. The limit to endurance in flight is apparently related to body size, and the metabolic heat generated. Mosquitos and other small flying insects cannot overheat, no matter how vigorously they fly. The endurance of bumblebees or sphinx moths 100-1000 times their body mass is limited to about 2 minutes of continuous flight at T_a =35°C as a result of hyperthermia, despite their impressive ability to thermoregulate at lower T_a.

These effects of mass may explain (at least partially) some more general patterns. As well as the changes in the masses of bees visiting a floral source over time mentioned in section 7.1, there are changes in body size within a group of closely related species as temperatures increase through the season. Linsley *et al.* (1955), Linsley and MacSwain (1959) and Linsley *et al.* (1963) showed that at two sites in the western United States larger species in the genus *Andrena* emerged earlier in the season, and foraged at lower T_a, than smaller species. The dense pubescence on the thoraces of some of the early season species (such as *A. perimelas*; Linsley and MacSwain 1959) in this genus, which is known to contain endothermic representatives (Chapter 6), suggests that a thermoregulatory role of body mass may be important. A similar pattern, across all bee species, and at a variety of floral sources, has been studied by Shmida and Dukas (1990) for Israeli desert bee communities. However, the probable effect of flower size and nectar availability on bee size must be considered before such a broad spectrum pattern can be explained in terms of thermoregulatory strategies.

7.4 How widespread is endothermy in the Hymenoptera?

How widespread is endothermic regulation in the Hymenoptera as a whole? I know of no studies examining body temperatures in the most 'primitive' Hymenoptera - the sawflies (sub order Symphyta). Many sawflies are small insects, and as such unlikely to be endothermic regulators, but some species, such as the Giant Woodwasp (*Urocerus gigas*; Siricidae), are certainly large enough to be so. Other moderate sized sawflies, such as *Trichiosoma tibiale* (Cimbicidae) are strong, active fliers, as are many endothermic insects. Although none of these species are obviously hairy, unlike many well-known endothermic insects, lack of pubescence is not a safe indicator that the insect is not endothermic (see below).

The sub-order Apocrita ('waisted' Hymenoptera) is divided into the Parasitica and the Aculeata. The former are nearly all parasites, and some of the superfamilies in this assemblage consist of insects that are too small for thermoregulation to be feasible - in particular the Proctotrupoidea, Chalcidoidea and Cynipoidea. Brothers (1975) divides the Aculeata into three superfamilies; the Bethyloidea, Vespoidea and Sphecoidea (Fig.7.1). The superfamily Bethyloidea

includes the brilliantly-coloured metallic wasps of the family Chrysididae. Although relatively small, and completely lacking pubescence, at least one species is endothermic. Fig.7.2 shows a warm-up curve for a female *Stilbum cyanurum* (Foerster) from Papua New Guinea (body mass 90mg). At a T_a of 24°C the wasp warmed to a T_{th} of 30.2°C at a mean warm-up rate of 1.7°C min^{-1}, maintaining a thoracic temperature excess of 3°C in stable tethered flight. This species appears phylogenetically far removed from the other Hymenoptera in which endothermy is known. While elevated T_{th} and observations of warm-up indicate the presence of endothermy in these wasps, thermoregulation has yet to be demonstrated.

Brothers (1975) groups the ants (Formicidae) within the superfamily Vespoidea, which includes the social wasps. Although metabolic heat production is thought to be important in the maintenance of nest temperatures in some ant species (Seeley and Heinrich 1981), the extent to which the thoracic flight muscles are used in heat generation is unknown, and the metabolic heat output per individual is very small. Kneitz (1964) estimated that the oxygen consumption of a *Formica polyctena* worker at an ambient temperature of 20°C is 700$\mu l g^{-1} hr^{-1}$, or approximately one 400,000th of the maximum oxygen consumption of an *A. plumipes* during warm-up (4.6$ml g^{-1} min^{-1}$; Chapter 4). It is the huge number of individuals per nest (estimated by Kneitz to be 1 million) and the insulation of the nest which leads to the generation of high nest temperatures.

Endothermy is also found in the family Scoliidae, which includes some of the largest Hymenoptera. Females of a *Scolia* spp. (also studied in P.N.G.) caught in flight at a T_a of 24.5-26°C and with body masses ranging from 315-360 mg (n=4) had T_{th} ranging between 29.7 and 32.5°C. A warm-up curve for a 490mg female is shown in Fig.7.1.

I know of no studies of endothermy in solitary wasps of the familys Pompilidae. The 'true' wasps of the family Vespidae include many endothermic forms, although to date there have been no studies of solitary families such as the Eumenidae. Among the social species, although there is no evidence of endothermy in paper wasps (genus *Polistes*), common wasps (*Vespula* spp.) and hornets (*Vespa* spp.) are both known to be endothermic (Ishay 1972). Warm-up rates in large hornets can be high (Fig.7.3). Queens of the hornet *Vespa orientalis* L., studied in Israel, reached warm-up rates of 6.65 °Cmin^{-1} and a T_{th} of over 38°C at a T_a of 21°C.

The bees are grouped by Brothers (1975) within the superfamily Sphecoidea, which includes several families of wasps. Body temperature and body size are known to be important in the foraging and reproductive success of some sphecids (*Cerceris arenaria*, Willmer 1985a,b; *Bembix rostrata* Larrson 1989a,b). Some sphecids are large, and have a dense pile of pubescence on the thorax, and it is quite possible that these are endothermic regulators.

Endothermy is clearly widespread in the bees, among both social and solitary species. It is

my feeling that all bees have some endothermic ability, and those above a minimum body mass of perhaps 30-40mg will prove to have varying endothermic thermoregulatory abilities. The activity of some large bees at low air temperatures (in the absence of solar warming) leads me to believe that these species may be as endothermic as *Anthophora*. This is particularly so for bees in the genera *Caupolicana* (Colletidae; Table 7.1), *Ptiloglossa* (Colletidae; Table 7.1), *Crawfordapis* (Colletidae) (Roubik and Michener 1984), *Protoxaea* (Oxaeidae) (Cazier and Linsley 1963), *Xenoglossa* (Anthophoridae) (Linsley 1962b) and *Epicharis* (Anthophoridae) (Linsley 1962b). Even very small bees can regulate nest temperatures if they are social. For example, *Trigona spinipes*, a small stingless bee, can regulate its nest temperature between 34 and 36°C over a T_a range from 15.5-28°C (Seeley and Heinrich 1981). However, since colonies of *T. spinipes* may number 100,000 individuals (Seeley and Heinrich 1981), it is difficult to know whether the heat generated is general metabolic heat, as in ants, or endothermic activity involving the flight muscles alone. No physiological regulation of nest temperature is known for another group of small bees with varying levels of sociality - the sweat bees (such as *Lasioglossum* spp.) of the subfamily Halictinae (Halictidae), and it is probable that both groups lie below the minimum body mass for individual endothermic regulation.

The taxonomic extent of endothermy outside the Apoidea is thus little known. Demonstration of endothermy in so unlikely a candidate as a glabrous, metallic chrysidid wasp suggests that endothermy may be more widespread than is currently accepted. This is exciting because it may mean that we can ask comparative questions about the evolutionary and ecological significance of endothermy over a wide phylogenetic group. Unlike some other insect orders containing endothermic representatives, such as the Odonata or Lepidoptera, the Hymenoptera includes species showing a very wide range of life history strategies, from parasitism to pollen foraging, from solitary insects to complex sociality, with a wide range of mating systems over a wide range of body sizes and in a wide variety of habitats. Investigation of the true extent of endothermy within the Hymenoptera, and comparative analysis of correlations between endothermic abilities and ecology and phylogeny is an exciting prospect and may lead to a shift from the prevailing view that the only 'real' endothermic insects in the world are bumblebees, hawkmoths and the odd dungbeetle!

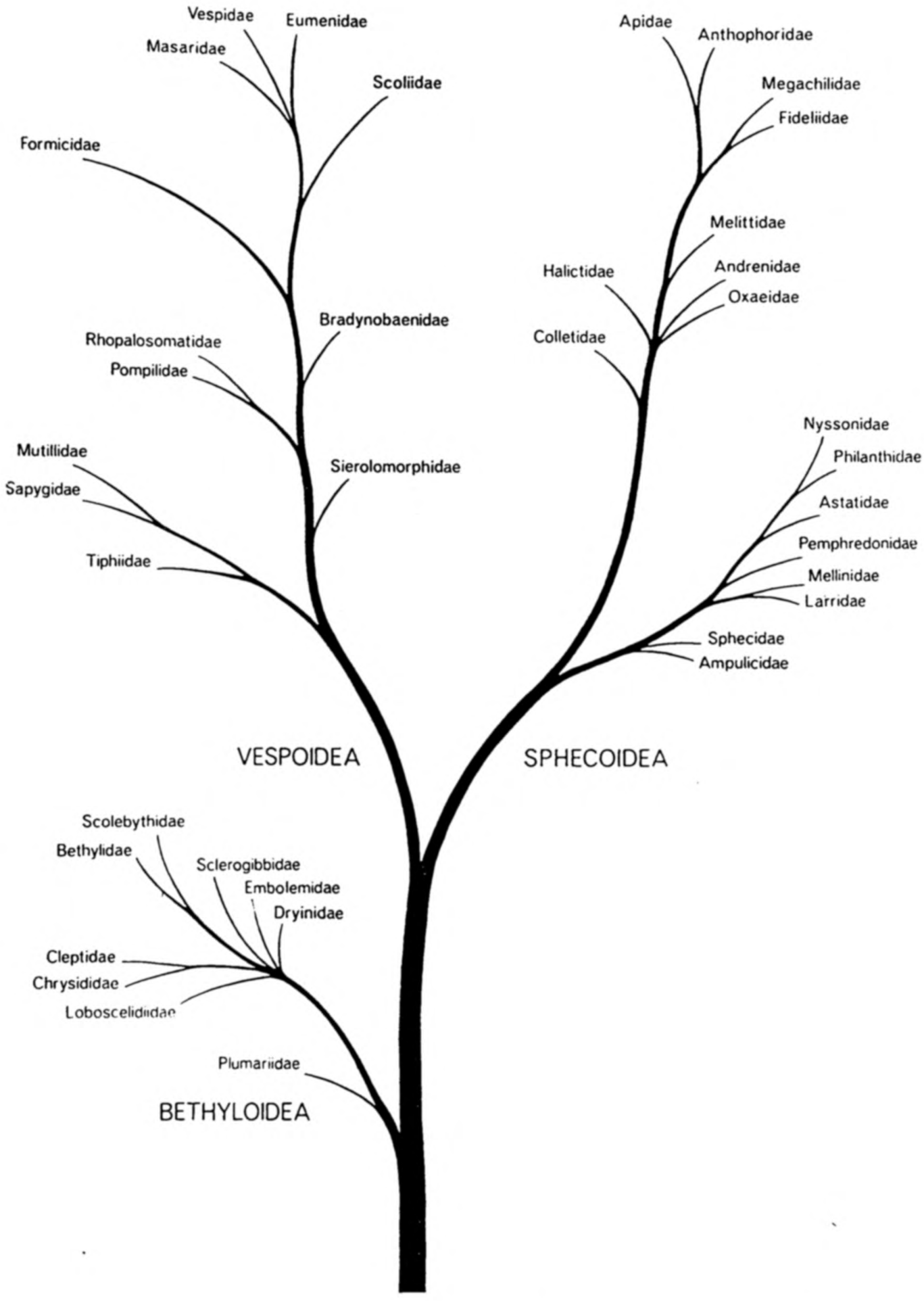


Fig.7.1 Phylogeny of the families of Hymeoptera Aculeata, as proposed by Brothers (1975).

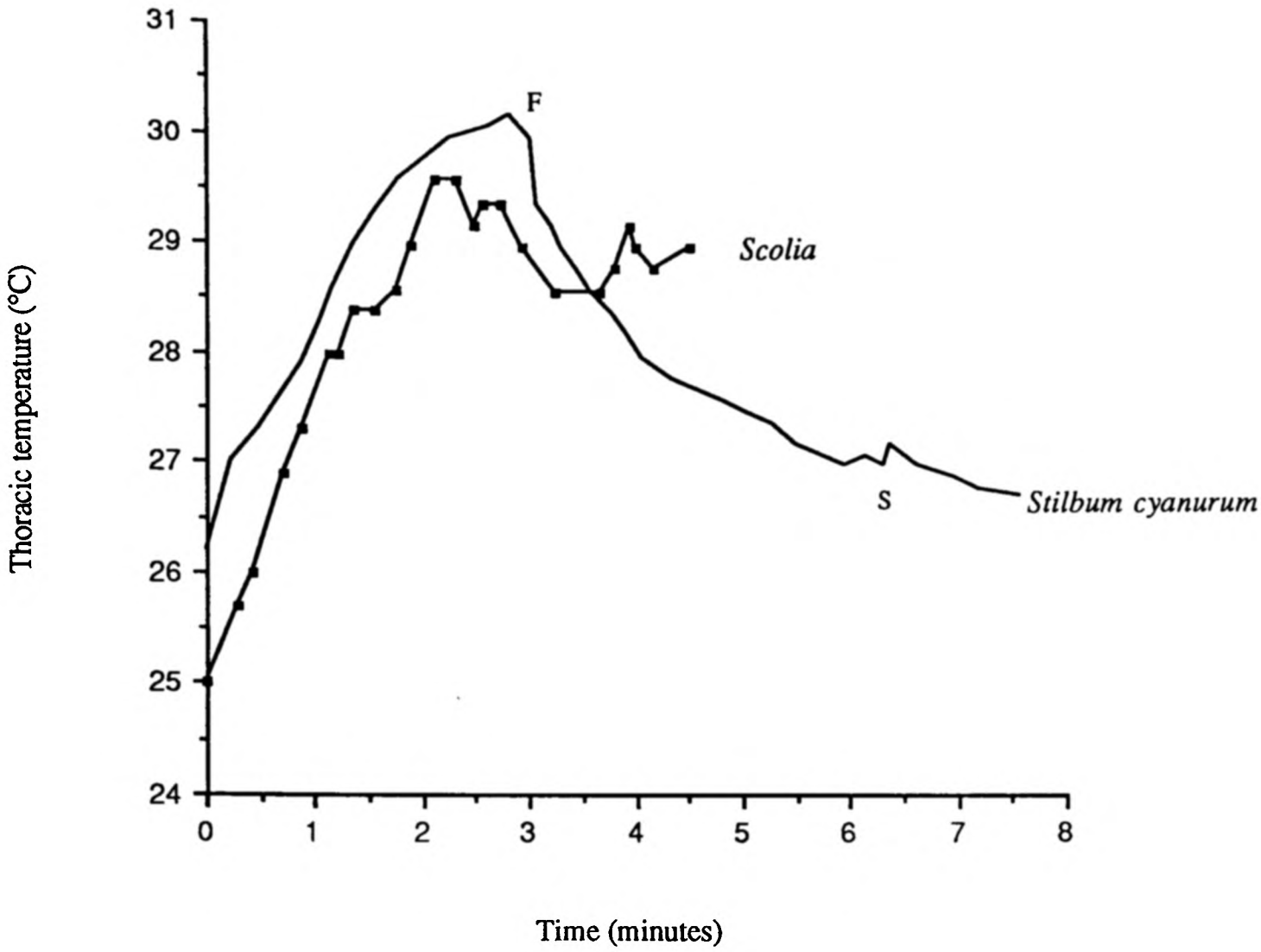


Fig.7.2 Body temperatures over time for a female *Stilbum cyanurum* (Chrysididae) (body mass 90mg) and a female *Scolia* spp. (Scoliidae) (body mass 490mg) at an ambient temperature of 24°C.

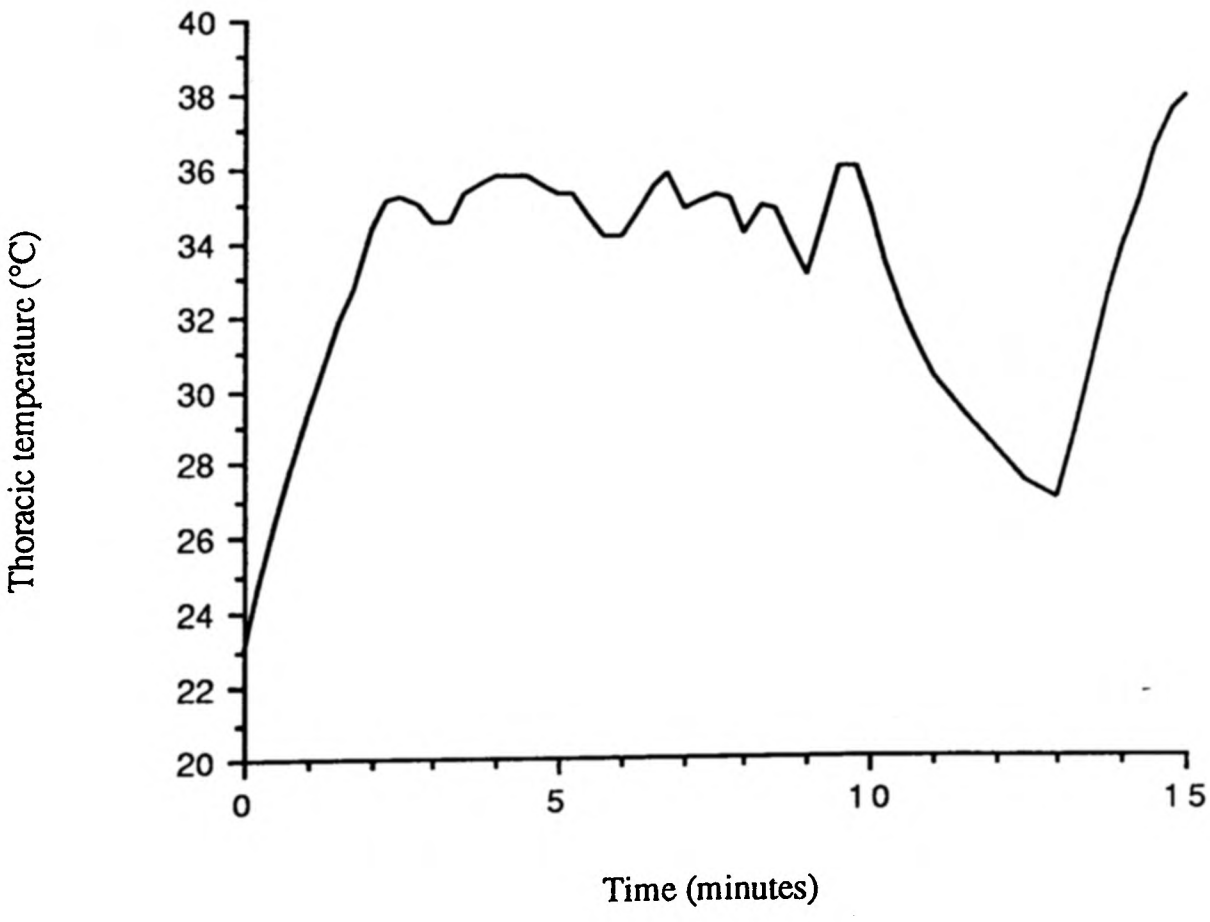


Fig.7.3 Body temperatures over time for a queen hornet, *Vespa orientalis* (Vespidae) (body mass 940mg), at an ambient temperature of 21°C.

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