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The Effects of Low Temperature on Metabolism and Survival of Individual Honey Bees (Apis mellifera)

Patricia Ellen De Joy
The College at Brockport

THE EFFECTS OF LOW TEMPERATURE ON METABOLISM AND SURVIVAL OF INDIVIDUAL HONEY BEES (*Apis mellifera*)

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Graduate Coordinator — Date 6/18/98

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Dedication

This work is dedicated to the memory of
Edward E. Southwick
I thank him for his enthusiasm, commitment and compassion;
His joy for life and his fascination with honey bees;
His love for his family and faith in God.

Acknowledgments

I would like to express my gratitude to Dr. Delmont Smith of the Department of Biology at S.U.N.Y. Brockport. Without his guidance, encouragement and thoughtful criticism, this thesis would not have come to completion. Thank you for sticking with me from the beginning to the end. A special thank-you to my committee members, Drs. Christopher Norment and Patricia Harris. I sincerely appreciate your interest in and evaluation of this work. Thanks also to Gary Kazin and Rich Hart who assisted in maintenance of the test equipment.

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Abstract

The honey bee (*Apis mellifera*) is one of the few insects capable of thermoregulation. Heat regulation of an isolated individual under the low temperature conditions normally seen in a northern temperate winter are investigated in this thesis. The factors examined are twofold: the variation in thermal output with ambient temperature and the survival potential from a cold comatose state.

Individual workers attempted to maintain their body temperature through active thermoregulation. Active heating was pronounced and continuous in the range of thoracic temperatures between 19.1-29.3°C. Oxygen consumption, and corresponding heat production, showed a linear increase with a decrease in ambient temperature in the range of $T_{ambient}$ from 35-12°C ($\dot{V}_{O_2} = -2.82 \times T_{ambient} + 96.64$, $r^2 = 0.94$). Large increases in oxygen consumption were seen at temperature differences ($T_{thorax} - T_{ambient}$) ≥ 2.0°C (above the physiologic minimum.) At cabinet temperatures below 12.9°C, the oxygen consumption of individuals did not stabilize, but decreased continuously, representing an abrupt cut-off in metabolic capacity seen at the chill coma point.

The survival potential of a comatose honey bee is high, with a 51% survival rate seen over all tests. In general, the number of survivors decreased with exposure time and exposure temperature. For all bees over all tests, chill coma temperature was dependent on exposure time, but was not dependent on exposure temperature. Revival time was found to be dependent on both exposure temperature and exposure time. An individual was most likely to survive chill coma if it revived in less than 4 min and under 18°C with passive exogenous heating.

Key words: *Apis mellifera*, honey bee, thermoregulation, low temperature, chill-coma, indirect calorimetry, virtual instrumentation, LabVIEW®

Introduction

In general, individual insects adjust to low temperature situations in one of three ways: by passive tracking of environmental temperature, by active thermoregulation to maintain body temperature, or through heat conservation. Few insect taxa expend energy on internal temperature control. As ectotherms, their body temperature tends to follow that of the surrounding environment. Winter survival in north temperate climates usually depends on the ability to produce antifreeze, or diapause in a hardy stage during periods of extended cold.

Thermoregulation is an endothermic response, in which the body temperature of an organism is maintained within set limits by internal heat production under varying external conditions. It is a dynamic, often energy expensive process accomplished through physiological and/or behavioral adjustments of heat production and retention. Some large flying insects, such as bumblebees and sphinx moths, are capable of thermoregulation within limited temperature ranges (Pirsch 1923, Heinrich 1974, Bartholomew 1981, Heinrich 1993). The degree of control over body temperature in these animals is a function of their ability to balance heat production and heat retention capabilities with changing environmental conditions.

Thermogenesis in winged insects is accomplished by low amplitude, high frequency contraction of the thoracic flight muscles (Esch and Bastian 1968, Esch 1988). The conversion efficiency of metabolic energy to mechanical work in this tissue is low, with approximately 80% of the energy expense lost as heat (Weis-Fogh 1972). These thoracic muscles are among the most metabolically active

tissues known and their action can result in body temperatures of flying insects greater than 40°C, approaching and surpassing those of mammals and birds (Bartholomew 1981, Heinrich 1974).

The mechanism of heat production in Hymenoptera takes one of two forms: the asynchronous stretch-activation response used to initiate and maintain flight, or the conventional twitch response used in non-flight conditions (Esch and Goller 1991). Warming in non-flight conditions is termed 'shivering thermogenesis' and is a combination of neurogenic activation of thoracic flight muscles and the mechanical response of the thorax (Esch *et al.* 1991). Neurogenically, it involves tetanus of the dorsolongitudinal wing depressor and dorsoventral wing elevator thoracic muscles. Unequal excitation of these two muscle groups can force one of them against a skeletal stop, physically inhibiting the stretch-activation response that is used in flight (overview in Heinrich and Esch 1994). At low temperatures, most Hymenoptera attempt to continuously heat themselves by this mechanism (Goller and Esch 1990a).

Individual honey bees (*Apis mellifera*) are capable of active thermoregulation through adjustments in their metabolic output in the range of ambient temperatures from 15°C-35°C (Pirsch 1923, Farrar 1931, Allen 1959, Cahill and Lustick 1976, Rothe and Nachtigall 1989, Southwick 1991). The heat generating capacity of the honey bee is broad and dependent on ambient temperature and activity level. Southwick (1991), for example, measured a variation in resting bee metabolic rate of 3.0-29.6 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$ (17.8-175.5 $mW \cdot g^{-1}$) at ambient temperatures ranging from 35°C-15°C. Rothe and Nachtigall (1989) measured a heat output of 240 $mW \cdot g^{-1}$ (40.5 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$) for flying bees. The fuel source for this heat production is the catabolism of carbohydrates (Farrar 1931,

Beenakkers 1969, Rothe and Nachtigall 1989). Heat production has been found to be dependent to some extent on age, caste, time of day, acclimation and air pressure (Woodworth 1936, Allen 1959, Free and Spencer-Booth 1960, Cahill and Lustick 1976, Withers 1981, Blanke and Lensing 1989, Goller and Esch 1990b, Fahrenholz *et al.* 1992).

Under low temperature conditions, body temperature maintenance is possible only when the rate of internal heat production is equal to or greater than the rate of heat loss. A critical factor in heat retention is the size of an animal. The surface area through which internally generated heat is lost to the environment is proportional to two-thirds the power of the body volume ($SA \propto V^{2/3}$). The ratio of surface area-to-volume increases with decreasing animal size, effectively increasing the area available for internal heat loss to the surrounding environment. This results in a greater mass-specific rate of loss for smaller animals (reviewed by Schmidt-Nielsen 1990).

In honey bees, most heat loss from the body is convective (May 1976). Specific passive adaptations have evolved to conserve heat within the thorax. The aorta of the honey bee makes 8-10 hairpin turns in the area of the petiole, as it passes to the thorax from the abdomen (Heinrich 1979a, Dyer and Seeley 1987). Counter-current heat exchange with hemolymph returning to the abdomen is very effective in decreasing thoracic heat loss. By allowing the abdomen to cool, the effective surface area for conductive heat loss is significantly reduced. It has been found that, even when thoracic temperature approaches upper lethal limits, the honey bee does not dump heat to the abdomen (Heinrich 1979a).

Because of low heat retention due to their small size, endothermy in individual insects is heavily dependent on heat production capabilities. With temperature decrease, the difference between thorax and ambient temperature increases, and the rate of heat loss to the environment increases. A point is reached where further increase in heat production cannot offset heat loss and the insect falls into a cold comatose state. Goller and Esch (1990a) have shown the ambient temperature at which this 'chill-coma' occurs (T_{cc}) to be species-specific. For *Apis mellifera*, at ambient temperatures between 8-12°C, metabolic increases cannot maintain internal temperature and a worker bee succumbs to chill-coma (Free and Spencer-Booth 1960, Goller and Esch 1990a). In this state, endogenous heat production capability is lost; a honey bee becomes immobilized and is unable to revive itself.

Free and Spencer-Booth (1960) defined the chill-coma temperature for honey bees as the minimum ambient temperature below which movement was not observed. This is an ambiguous definition because honey bees that are actively producing heat by shivering thermogenesis may appear to be at rest or not moving (Rothe and Nachtigall 1989, Goller and Esch 1990a). Goller and Esch (1990a, 1991) have put a physiological meaning to the term by defining T_{cc} as the temperature at which the amplitude of thoracic muscle junctional potential decays to zero. Esch (1988) relates this decrease in potential amplitude, and a corresponding increase in muscle potential duration, to an interruption of the neuromuscular contraction mechanism in insect muscle that occurs with decreasing temperature.

Agreement is good between values of T_{cc} for *Apis mellifera* workers obtained by Goller & Esch (1990a), 11.2°C averaged over nine individuals, and Free and

Spencer-Booth (1960), 9-12°C for group size between 10-200 bees. This is most likely because all muscles are similarly affected with decrease in temperature, even those not used for thermogenesis (Esch 1988, Goller and Esch 1991). Factors that may influence T_{cc} and survival from chill-coma for a given species include acclimatization, acclimation, food stores (honey crop contents), cooling rate, warming rate and length of time in chill-coma (Mellanby 1939, Colhoun 1954, Free and Spencer-Booth 1958 & 1960, Colhoun 1960, Goller and Esch 1990b).

Honey bees appear to be able to cycle into and out of chill-coma with no adverse effects (Robinson and Visscher 1984). In fact, individuals have been revived after enduring several days in chill-coma (Free and Spencer-Booth 1960, Esch 1988). There are, however, limits to survival in this state. Honeybees have not adapted methods of supercooling as have many nonsocial insects (Southwick 1987, Storey and Storey 1990). Therefore, their tissues are subject to the physical damage inherent in the freezing process (Diamond 1989, Storey and Storey 1990). There is a temperature below which they cannot survive, even when exogenously heated. This temperature, defined as the cold-death temperature, lies between -2 to -6°C (Pirsch 1923, Free and Spencer-Booth 1959 & 1960), but has not been widely investigated.

Honey bees are unique in that they are the only social insects to thermoregulate under continuous cold load, overwintering as adults in north temperate climates (Farrar 1952, Seeley and Visscher 1985, Southwick 1988). Survival potential as a group far exceeds that of the individual, with incorporation of group physiological and behavioral modifications to maintain survival temperatures within the hive (Phillips and Demuth 1914, Free and Spencer-Booth 1959, Southwick 1982). In practice, honey bees always behave as a group to some

extent, thermoregulating over a wide range of ambient temperatures (-80°C to +70°C) (Corkins 1932, Lindauer 1954, Free and Spencer-Booth 1962, Southwick 1987). The honey bee colony, therefore, exhibits thermoregulatory characteristics similar to those of homeotherms (Southwick and Mugaas 1971, Southwick 1983). Collectively, the grouped individuals function together as a single organism: a superorganism (Farrar 1952, Southwick 1983 & 1991, Wilson and Sober 1989, Seeley 1989 & 1997, Moritz and Southwick 1992).

The survival of each individual worker is linked to the survival of the colony (Seeley 1997). A minimum cluster mass is required for winter survival of the group (Jeffree 1955, Harbo 1983, Southwick 1985). Temperature at the periphery of a cluster has been measured to be as low as 7-10°C during the winter months, forcing the bees within that layer to cycle into and out of chill-coma (Phillips and Demuth 1914, Owens 1971, Southwick and Mugaas 1971, Southwick 1985). Individual thermoregulatory behavior at low temperatures has, by necessity, evolved not only to favor honey bee survival when acting individually, but also to maximize group survival.

Many studies have investigated the physiologic response of the *A. mellifera* worker to an induced cold stress (Farrar 1931, Free and Spencer-Booth 1958, Allen 1959, Cahill and Lustick 1976, Rothe and Nachtigall 1989, Southwick 1991, Goller and Esch 1991, Fahrenholz *et al.* 1992). Although the honey bee is one of the most thoroughly studied insects (Heinrich 1993), consistency among measured heat production values found in the literature is low (reviewed in Rothe and Nachtigall 1989, Farenholz *et al.* 1992). There are a number of reasons for these discrepancies:

1. There has been difficulty in establishing a true resting state for the honey bee. Endogenous heating through shivering of the thoracic muscles produces no visible movement of the thorax. Earlier researchers believed that a significant amount of heat was generated only with walking or wing movement (Farrar 1931, Mellanby 1939, Free and Spencer Booth 1958, Rothe and Nachtigall 1989). As a result, most studies do not contain accurate information on the thermoregulatory state of the bees tested. This issue was not completely resolved until Esch and Goller (1991) determined the mechanism of shivering thermogenesis. They proved that the active heating state of a honey bee is most correctly determined with simultaneous detection of thoracic muscle potential and heat production (Esch *et al.* 1991).
2. Measured values for metabolic adjustments with changes in ambient temperature are widely reported in the literature on a per bee basis. Most of these studies, however, were not performed on isolated bees, but on small to relatively large groups (n = 3-1000 bees). The individual energy expense was then calculated by dividing the total output by the number of individuals contained within the test chamber. Honey bees caged in numbers greater than 6 have a tendency to decrease their activity and group close together into a cluster to some degree at ambient temperatures of less than 20°C (Free and Spencer-Booth 1958, Fahrenholz *et al.* 1992). Clustering effectively decreases conductive losses on a per bee basis (Heinrich 1981, Southwick 1985). Even small cluster studies would therefore tend to give artificially low individual metabolic values.
3. Tests performed on isolated workers have typically measured only a small number of individuals, because it is generally not practical to measure large numbers of separated individuals at a time using traditional methods.

Statistically, though, the larger the sample size, the more accurately the data estimate the actual population values (Zar 1984).

4. It historically has been difficult to accurately measure the metabolic rate of a single insect. The metabolic rate of a single bee is low and measurement equipment, until recently, has not contained the inherent sensitivity needed to reliably record such small values (Blanke and Lensing 1989).

The use of sensitive infrared gas analysis recorders to indirectly measure heat output has made it possible to record oxygen consumption values at the low levels seen in insect physiology (Blanke and Lensing 1989, Southwick 1991). A gas analyzer can be used in an open system, comparing the percentage of oxygen and/or carbon dioxide in two streams of air: the air entering and the air exiting through a chamber containing an insect. The flow rate can be adjusted in these systems to accommodate extremely low oxygen consumption levels.

Furthermore, the sensitivity of gas analyzers can be combined with the speed of an on-line computer to record and greatly increase the accuracy of measurements (Farenholz *et al.* 1989, Goller and Esch 1990a, Southwick 1991). Southwick (personal communication) was the first to use a software interface program to control all test equipment and record all data through a virtual instrument panel in insect studies. Such a system has the versatility and input/output capability to monitor the number of variables necessary to produce consistent and accurate data over a wide range of metabolic outputs. In this study, I further refined and analyzed this system for its usefulness in individual honey bee studies.

Using this system, I investigated the characteristics of individual worker honey bees (foragers) during cold stress that may be related to group physiologic and

behavioral responses. The range of test temperatures was chosen to include those measured in previous cluster runs in this lab. In this thesis I, address the following two characteristics:

1. I investigated individual worker responses within the range of temperatures that naturally occurs in an overwintering hive in northern climates.

My working null hypothesis (H_{01}) is: Low exposure temperature does not affect the heat production of *Apis mellifera*.

The questions I will address to evaluate this hypothesis are:

How do isolated individual worker honey bees react to an externally applied cold load?

At what ambient temperatures do individuals attempt to thermoregulate?

Can individual behavior be related to the behavioral response of a group of bees under cold stress?

2. I also investigated the response and survival potential of individuals at maximum cold stress. The differences between bees that survive chill-coma and those that do not are not well defined. I examined two of the factors that may influence the survival rates of worker honey bees: minimum exposure temperature and time at minimum exposure temperature.

My working null hypothesis (H_{O2}) is: Low exposure temperature and time do not affect survival of *Apis mellifera* from chill-coma.

The question I will address to evaluate this hypothesis is:

Do minimum exposure temperature and time of exposure affect the revival and survival rates of comatose honey bees?

Methods

Testing was performed at the Bee Lab in Lennon-Smith Hall on SUNY College at Brockport campus (43° 12'N, 77° 58'W). Honey bees (*Apis mellifera*) were taken from three full-size colonies located near this building and maintained following standard management practices. All metabolic tests were run during May-August 1994 using worker bees. Tests were run under constant dark conditions. Experimental set-up and design were based on previous tests run in this lab (Southwick 1982, 1991). Low-temperature chill-coma tests were run during the months of April and May 1993.

Experimental Design

Equipment

A temperature cabinet (Forma freezer/incubator Model 3770, -20 to +60 ± 0.4°C) was used to set ambient temperature conditions during testing.

Metabolism was measured indirectly as the rate of oxygen consumed ($\dot{V}_{O_2}$) and carbon dioxide given off. Compressed, dried and filtered outside air (20.93% oxygen, 0.03% carbon dioxide) served as the supply to the cabinet. Flow-through air exiting metabolic chambers was sampled, dried (with $CaSO_4$) and analyzed for CO_2 and O_2 content (Beckman Industrial Model 868 and Applied Electrochemistry S-3A, sensitivity 0.001%). The oxygen analyzer, a dual channel instrument, was used as a comparator to measure the relative change in oxygen between the air entering and the air exiting each test chamber. Flow rate was monitored with a digital mass flow meter (Omega Engineering, Inc. FMA5607) to

assure consistency between chambers during multiple chamber tests. Carbon dioxide production was measured for use in calculating RQ, the ratio of oxygen consumed to carbon dioxide produced, and as a check on proper system performance (for carbohydrate metabolism, RQ ± 1). Heat output was determined from $\dot{V}_{O_2}$ with RQ = 1.0. Six channels with bees were monitored simultaneously; a seventh channel was used as a control.

All weights were measured in the lab on a Mettler PH3000 balance.

I. Metabolic Tests

The range of temperatures at which individuals were tested included the range of temperatures known to exist in an overwintering cluster and to be above the chill-coma temperature: 12°C and 15-40°C in 5°C increments.

Collection

Outgoing foragers were taken from the hive entrance during the mid-morning hours (0830-0930). A hand-held Hometrends™ vacuum with a modified opening was used to collect the bees one at a time (Figure 1). Individuals were immediately placed in preweighed 2cc syringes, modified for use as metabolic chambers. The chamber size allowed for limited walking movement, but bees were unable to spread their wings or turn around within these chambers. The needles had previously been removed from the syringes and the plungers modified to allow for airflow through each and, in some cases, to accept a thermocouple lead. A food mixture of powdered sugar and corn syrup was provided *ad libitum* inside the syringes.

Oxygen Consumption

After weighing, the syringes with bees were connected to six Tygon® (3/32" id) manifolds, each having the capacity to hold eight syringes. These manifolds, as well as a control, were connected to separate channels of the metabolic test equipment and monitored with an on-line computer system (Figure 2). Thus, forty-eight individuals were simultaneously tested in parallel circuits. Gas concentration readings for each channel gave an average over the eight bees in parallel with that channel. A small desiccant chamber was placed in line with each of the six channels.

Airflow through the channels and the oxygen analyzer sample pump was adjusted to accommodate for the low rate of oxygen consumption of the individuals. These settings were obtained by using a LabVIEW® real time stripchart that popped up on the computer screen during gas measurements and showed the arrival of the CO_2 peak and corresponding O_2 drop (resulting from the presence of the bees) on each channel. This resulted in a pump flow rate of 80 mL/min, an oxygen sample pump setting of 0.5 and a channel cycle time of two minutes with a 105 second gas delay per channel. Therefore, each channel was sampled for two minutes at fourteen minute intervals.

Two separate runs with forty-eight bees (six channels with eight bees each) were performed at each of the ambient test temperatures. Bees were allowed to accommodate to the cabinet set temperature for 45 minutes and then data were recorded during the following 30 minutes. A preliminary run that sequenced through these temperatures from high to low was used to check the method and as a basis for comparison during the tests that followed.

Temperatures

Thermocouples (40 gauge T-type copper-constantan) were placed inside two syringes per channel during each test to monitor ambient temperature ($T_{ambient}$). Cabinet temperature outside the syringes ($T_{cabinet}$) was monitored using a separate thermocouple. After all metabolic runs were completed, a separate test was performed with thermocouples placed in syringes as noted above and additional thermocouples attached with a dot of beeswax to the thoraxes of randomly selected individuals. This was accomplished by first cooling the bees in a refrigerator until they fell into chill-coma and attaching the thermocouple while they were 'out cold' (as per Esch and Bastian 1968, Esch 1988, Goller and Esch 1990a). This allowed for statistical comparison of thoracic and ambient temperatures.

Mass of Bees

The syringe test chambers were weighed before and after the bees were collected so that the mean weight of individuals could be determined for each of the three supply hives. This information was used to determine the effect, if any, of body mass on results.

Food Consumption

The syringes were weighed at the completion of each test run both before and after the bees were released. The difference between the weights of the empty syringes before and after a run gave the amount of food (in mg) consumed per

bee. The amount of food metabolized was determined by the difference between the food consumed and the weight gain of the individual bee.

Sample equipment and test set-up sheets for the individual metabolic experiments can be found in Appendices 1 and 2.

II. Chill-coma

Collection

Experimental bees were mature workers removed from the periphery of colonies in groups of eight to twelve individuals using the vacuum device shown in Figure 1.

Cage Design

The test cage was a cardboard box with a 7 mm Styrofoam® insert placed in the bottom and over the top. Pieces of cardboard were used to divide the box into six separate chambers to avoid contact between bees and tangling of the fine thermocouple wires. Final cage dimensions were 4.5 x 6.5 x 2.0 cm with chamber dimensions of roughly 3.5 x 3.5 cm. This cage allowed for up to six bees to be tested simultaneously at each test condition. In most cases, five bees were monitored per test, one chamber being used for recording of ambient temperature. The test cage was placed in a foam box (packing foam) open at the top to allow for a more even cooling and warming of the bees under the test conditions.

Test Design

A fine thermocouple was attached to the upper thorax of each test bee. Bees were cold anesthetized for this procedure as was done in the metabolic tests. Extra bees (two to six) were gathered for each test run to assure that this precooling did not affect the survival rates of the bees under test. After preparation, the bees were marked on the abdomen with paint for identification and placed in the test cage.

Once the bees were set up in the cage, it was placed in a freezer (Hotpoint no-frost 14) set at -16.2 ± 0.44°C and allowed to cool to this point. The cage was then transferred to the preset temperature cabinet and left for a specified amount of time. Exposure times and temperatures are given in the test matrix shown in Figure 3. Tests times were determined as an approximately geometric progression. Temperatures were determined by the minimum temperature seen at the periphery of an overwintering cluster (9°C), freezing temperature (0°C) and a midpoint between the two (4.5°C). Due to an error in the temperature cabinet setting, the actual measured low temperature was -2.5°C (not 0°C).

At the end of the test period, the cage was removed from the temperature cabinet, placed on the lab bench at room temperature (ca 23°C), the Styrofoam® cover removed and warming rates monitored. Thermocouple output temperatures were recorded simultaneously and stored to disk throughout the testing period.

Chill Coma Temperature and Time

Temperature at, and time to, first movement of each bee (leg, antenna, abdomen or wing movement) after removal from the temperature cabinet were recorded as the chill-coma temperature (T_{cc}) and chill-coma time (t_{cc}) for these tests. Although this does not necessarily yield exact chill-coma temperatures, as measured by extinction of thoracic muscle potential, Goller and Esch (1990b) have shown this method to give a valid first approximation. Bees were monitored for 15 minutes after removal from the incubator for determination of T_{cc} and t_{cc}. This value was chosen because the bees that I monitored in preliminary tests revived within 15 minutes at room temperature if at all. Bees that survived were tested through two cooling and warming cycles, weighed and returned to the hive (after a 24 hour monitoring period). Food was not provided to the bees during the tests or until after measurement of T_{cc} and t_{cc} was completed.

An individual was considered a survivor only if able to walk or fly 24 hours after end of test. A bee that was unable to walk upright or revived and died within 24 hours was treated as a non survivor. A bee that could not recover from chill-coma beyond this state would most likely not be able to survive in nature (i.e., not be able to fly back to the hive or crawl up the comb to the cluster). All bees were allowed to warm at approximately the same room temperature (ca 23°C). Possible compounding effects of using bees for more than one test were not taken into account in this study. In future tests, bees should be used only once to separate possible cumulative effects.

Computer Interface and Data Acquisition

I. Equipment

Experimental control of the test apparatus, coordination of data acquisition and data storage were automated using National Instruments® LabVIEW®2.2 hardware and software interfaced through Macintosh® microcomputers. Temperature, oxygen consumption, carbon dioxide production, air flow rate and barometric pressure values were recorded and saved to disk as they were measured (Figure 2). Some data processing (e.g. calculation of RQ and correction of oxygen consumption values to STP) was integrated into the program structure and performed on-line.

LabVIEW® is a software package that utilizes a graphical program language to create virtual instruments (VIs). These instruments serve as an interface between signal inputs/outputs and the user. VIs can be obtained from a LabVIEW VI library, customized or created by the user to perform specific control, timing, input, output, data analysis and storage functions. Each virtual instrument consists of two basic parts: a control panel and a corresponding circuit diagram. The control panel is designed to mimic a standard hardware instrument panel and utilizes icons that represent a variety of digital and analog switches, indicators and displays (Figure 4). The circuit diagram shows the order of execution of the instrument, using canned LabVIEW® components or customized subVIs which are 'wired' together by the user (Figure 5). The wiring represents data flow paths between the different software components, subVIs and signal outputs to and inputs from external hardwired breadboards. The subVIs act as subroutines that can be called through the main VI control panel when its input

wires are 'activated.' A large number of subroutines can be nested within a VI in this manner (Figure 6).

The National Instruments® NB-MIO-16L, NB-MIO-16XL and NB-DIO-24 data acquisition boards served to interface between the LabVIEW®2.2 software and the general purpose termination breadboards used for data collection in my experiments. The MIOs, or multifunction input/output boards, contain digital and analog input and output ports as well as counter and timing functions. The 16XL can process an analog input more accurately (using 16-bit analog to digital conversion) than the 16L board (12-bit conversion). The DIO, or digital input/output board, is a 24-bit parallel digital input/output interface. Two general purpose termination breadboards were used to feed externally measured signals to the MIOs: the AMUX-64TR multiplexer and the SC-2070R. These boards can be configured to accept inputs in either a single-ended or differential mode. The differential inputs provided for thermocouple readings that were referenced to a cold junction terminal through an integrated circuit temperature sensor mounted on each board (accurate to ±1°C).

The NB-MIO-16LR board, driven by a Macintosh®IIsi, was used in the low temperature chill coma experiments. Thoracic temperatures of individual bees were monitored using thermocouple (TC) outputs passed through the SC-2070R. This board was set up in the differential mode, simultaneously monitoring seven input signals (TCs) and the on-board temperature sensor.

The NB-MIO-16XLR board, driven by a Macintosh® IIfx, was used in the individual metabolic experiments. Data gathered during these experiments were read through the AMUX-64TR. This board is used as an expansion board for

data acquisition and can sequence through up to 64 single-ended or 32 differential inputs. It was set up to read the differential-mode thermocouple inputs and one single-ended input each from a pressure transducer, mass flow meter, oxygen analyzer and carbon dioxide analyzer. The NB-DIO-24R was used to control channel switching during program execution. Control signals were read by an electromechanical relay digital output board (SC-2062R). This board contains eight single-pole, dual-throw (SPDT) relays that switched on the air pump to one channel at a time. System design allowed for up to seven channels to be monitored sequentially during any test run (six test channels and one control channel).

Care was taken to protect the reference temperature sensors from external temperature fluctuations. Thermocouple measurement accuracy, based on errors associated with the sensors, linearization of the thermocouple outputs, temperature differences across the breadboards and gain errors related to the MIO boards can be as high as ±1.9°C (T-type thermocouple) for the configuration used in these experiments (National Instruments AMUX-64T User Manual 1991, SC-207X Series User Manual 1992).

A flow diagram showing the execution sequence of the LabVIEW® programs used in the chill coma and metabolic experiments is given in Figure 7. A summary of the breadboards used in these experiments in given in Appendix 3.

II. Experimental Setup

Prior to each metabolic test run, the gas analyzers were calibrated with a calibration gas referenced to a 'zero' gas of dried, compressed outside air. Required adjustments were monitored using the LabVIEW® BeePat6 program and made using the front panel controls located on the analyzers. An outline of test initialization procedures is given in Appendix 3.

Data Treatment

Statistical analyses were performed using analysis tools provided by Microsoft Excel® 3.0, MYSTAT®, FASTAT® and LabVIEW®2.2 (Wilkinson 1992). Charts were prepared using Excel® 3.0 and Cricket Graph III® 1.0 was used for graphing of experimental results.

Statistical pooling of data was possible between some of the individual metabolic test runs. In some instances, no significant difference was found between the mean oxygen consumption measured during two different same temperature runs, as determined by Student's t-Test $P \geq 0.05$. For these runs, data were pooled and treated as one data set.

Data taken at 40°C in the metabolic tests was not used in the statistical analyses. The temperature preference of *A. mellifera* has been shown to lie in the range of 31.5-36.5°C (Heron 1952, Heinrich 1993). It is unlikely, then, that the bees would have been actively thermoregulating at 40°C ambient temperature.

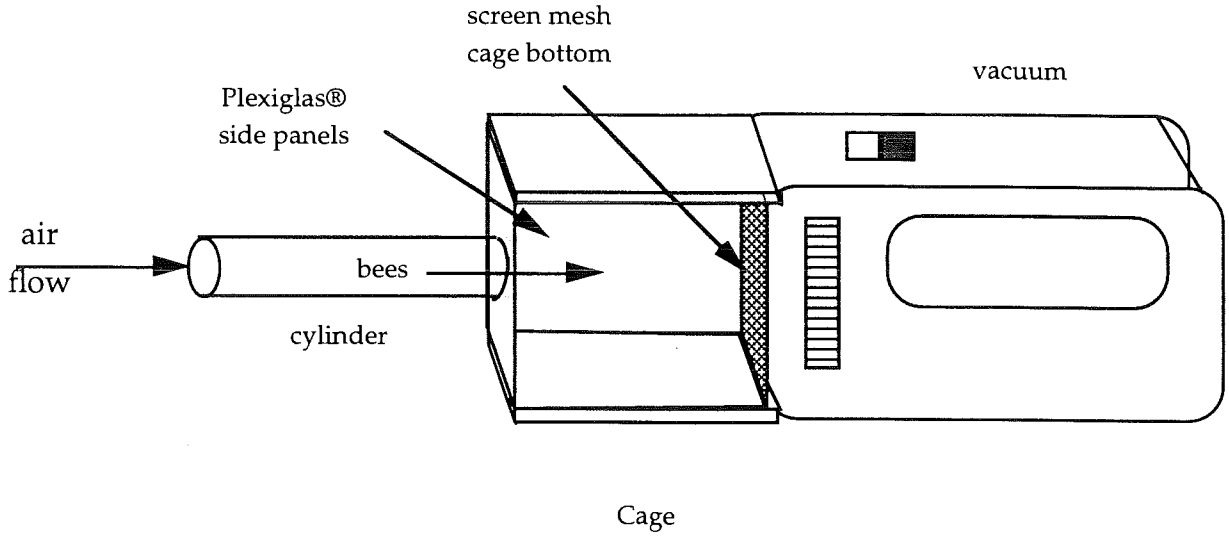

Figure 1. Vacuum apparatus used to collect bees from the hive for chill-coma experiments. Bees were drawn into the cage through the cylinder by the suction of the hand-held vacuum. The whole apparatus is assembled and disassembled quickly and easily. A cork covered the cylinder entrance hole after collection was complete. In metabolic experiments, the cage was replaced with the original vacuum casing. The opening of the casing was modified with a piece of Tygon® tubing so that individuals could be sucked up one at a time at the hive entrance.

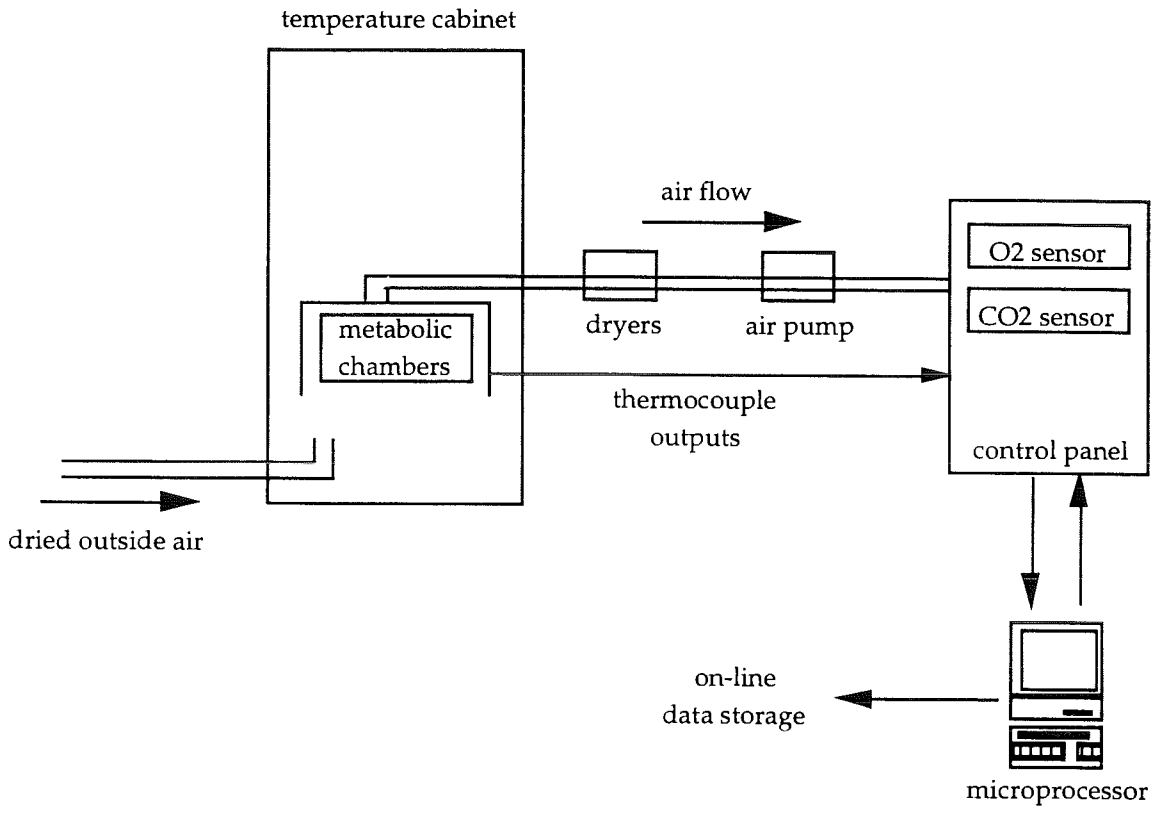

Figure 2. Experimental setup for the measurement of oxygen consumption of individual honey bees at low ambient temperatures. Six separate air flow paths (channels) were connected between the temperature cabinet and gas analyzers at the control panel (only one is shown for clarity). This allowed for up to six metabolic chambers to be monitored independently. Each metabolic chamber consisted of a Tygon® manifold that held eight bees in separate 2cc cages.

	T_{LOW} (°C)			
	9	4.5	-2.5	total n
1 minute	5	5	5	15
10 minutes	3	4	3	10
100 minutes	5	5	5	15
600 minutes	5	5	5	15
total n	18	19	18	

Figure 3. Test matrix for low temperature chill-coma experiments. Numbers within the cells represent the number of individuals tested (n). Time increments were chosen to show the effects of an approximate geometric increase. Test temperatures were chosen to monitor the response of individuals at the minimum temperature seen at a cluster periphery (9°C), at the freezing point (0°C) and midway between the two (4.5°C). The actual measured low temperature (-2.5°C) was below that intended due to an error in the temperature cabinet zero dial setting.

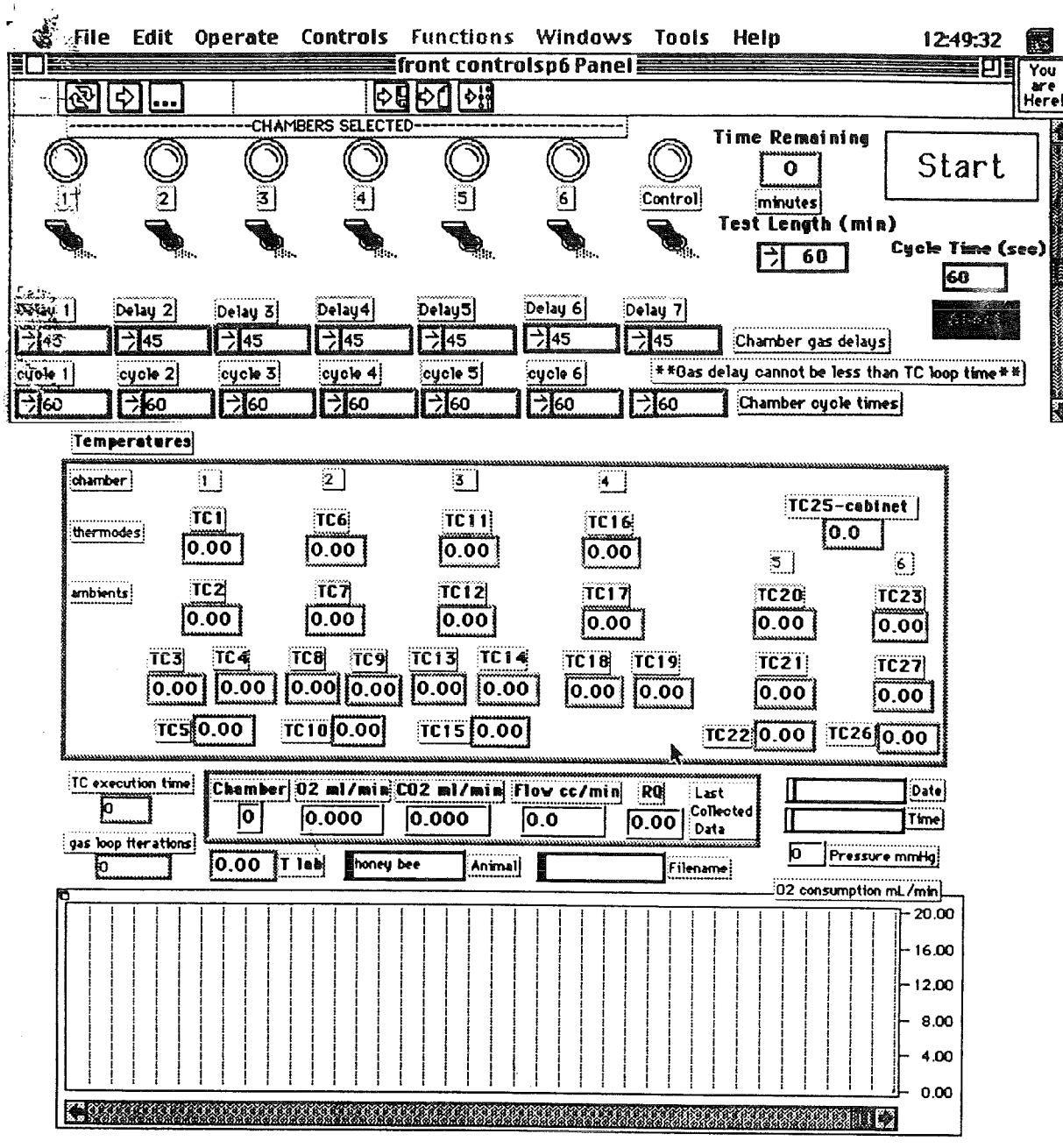

Figure 4. LabVIEW® control panels for individual metabolic experiments. The top panel (front controlsp6 Panel) was used to input test parameters: test channel(s), test length, gas sample cycle time and power switch to begin the test run. The bottom panel (the control VIp6 Panel) was used to display the status of all thermocouple outputs, current gas readings, RQ, system airflow, barometric pressure, time, date and animal id. The stripchart at the bottom of this panel was able to display approximately the last one-hundred oxygen consumption measurements. Measurements previous to these could be accessed using the scroll bar at the base of the chart.

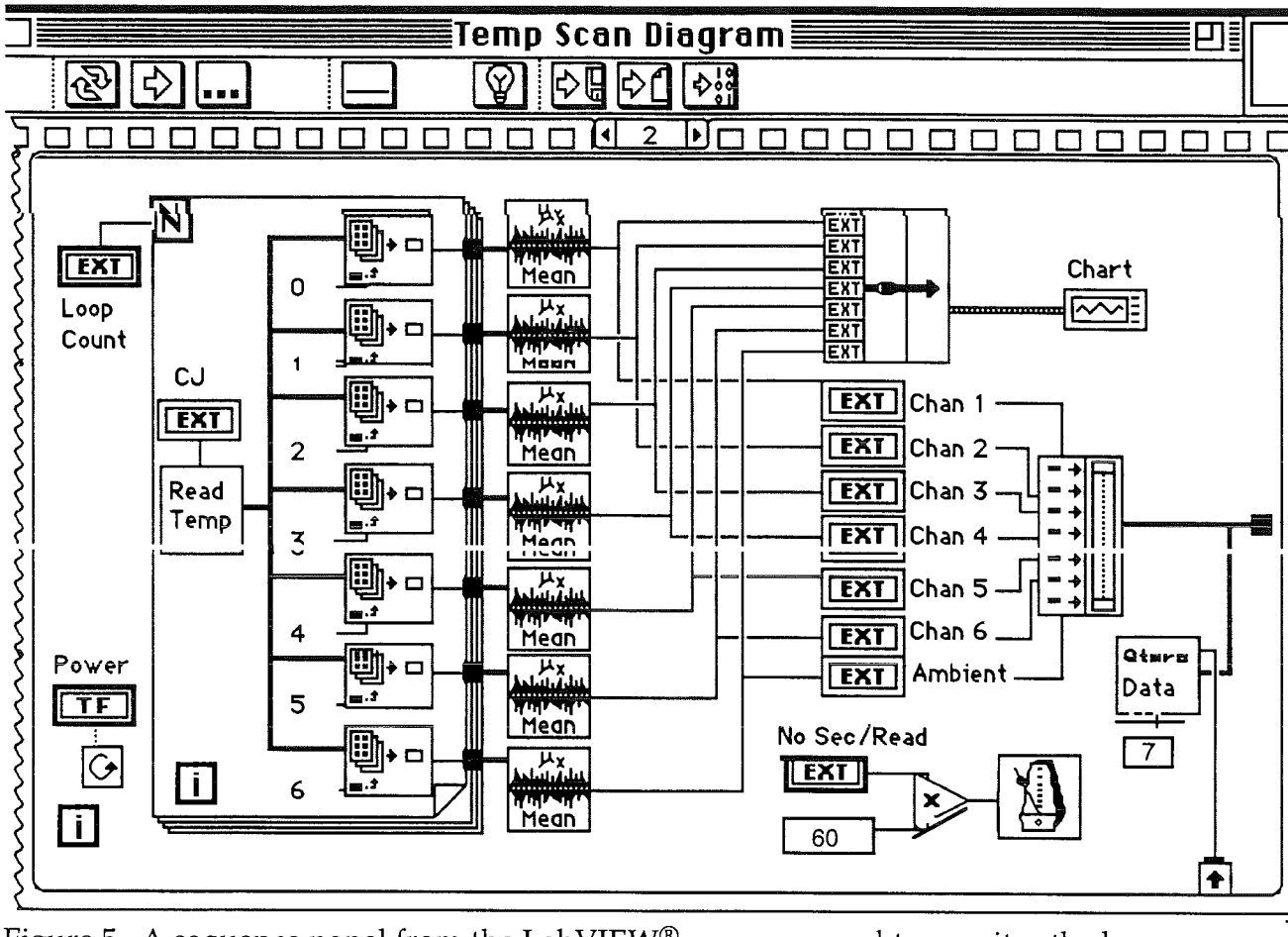

Figure 5. A sequence panel from the LabVIEW® program used to monitor the low temperature chill-coma experiments. Four inputs from the front control panel feed into this sequence: start test (power), number of loop executions (loop count), number of seconds per thermocouple reading (no sec/read) and the cold junction correction temperature (CJ). Two subVIs are called: Read Temp and Store Data. Read Temp reads the output voltage of seven thermocouples simultaneously, converts them to temperatures and feeds them sequentially to seven arrays. Store Data saves the mean temperature (over 'loop count' seconds) per channel to a file on disk. Thermocouple outputs are sent to the computer monitor as a strip chart output similar to the one shown in Figure 4.

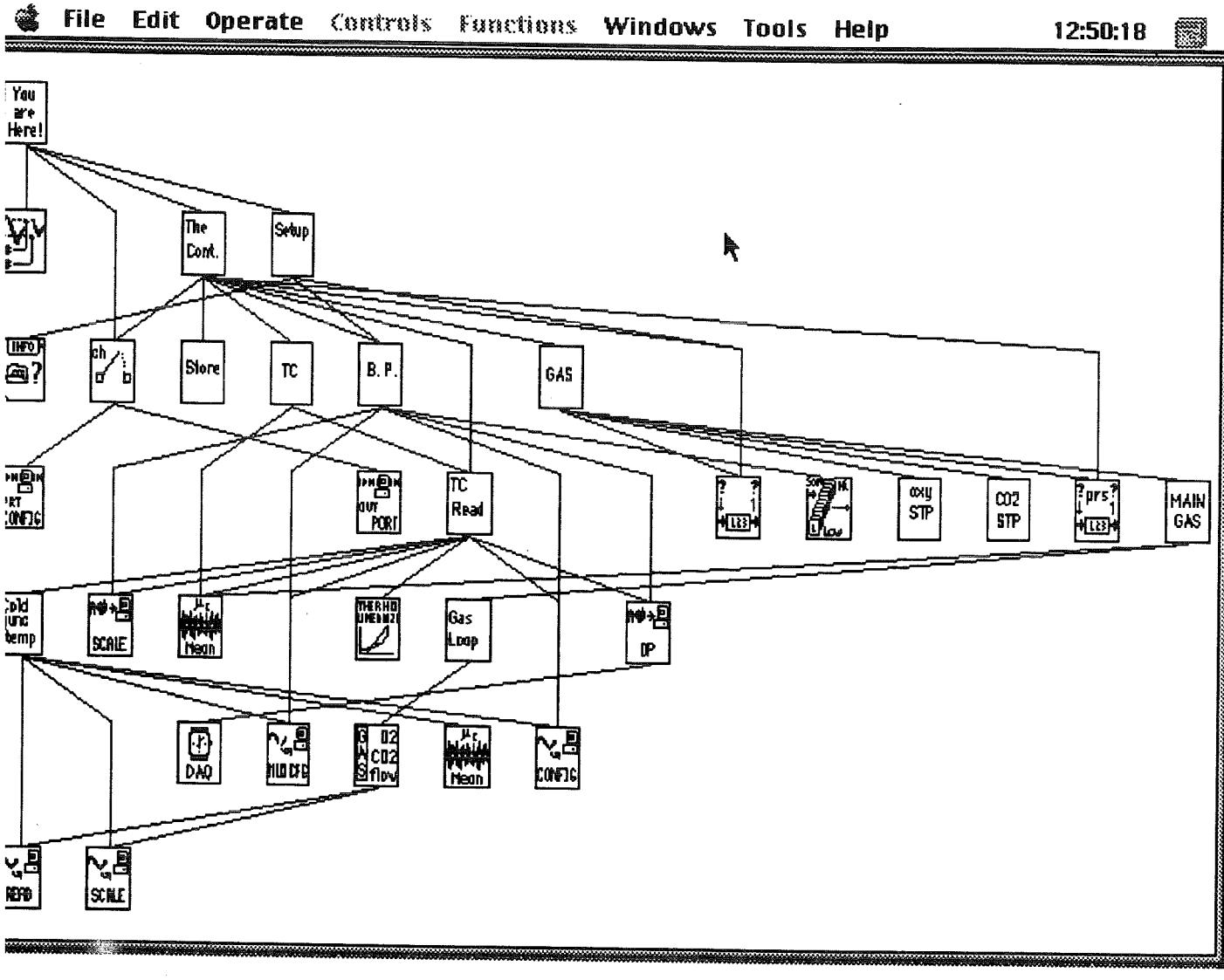

Figure 6. The hierarchy of the BeePat6 program subVIs used to control the individual metabolic experiments. Interactions between, and nesting of, subroutines are indicated by the lines that connect the different panels.

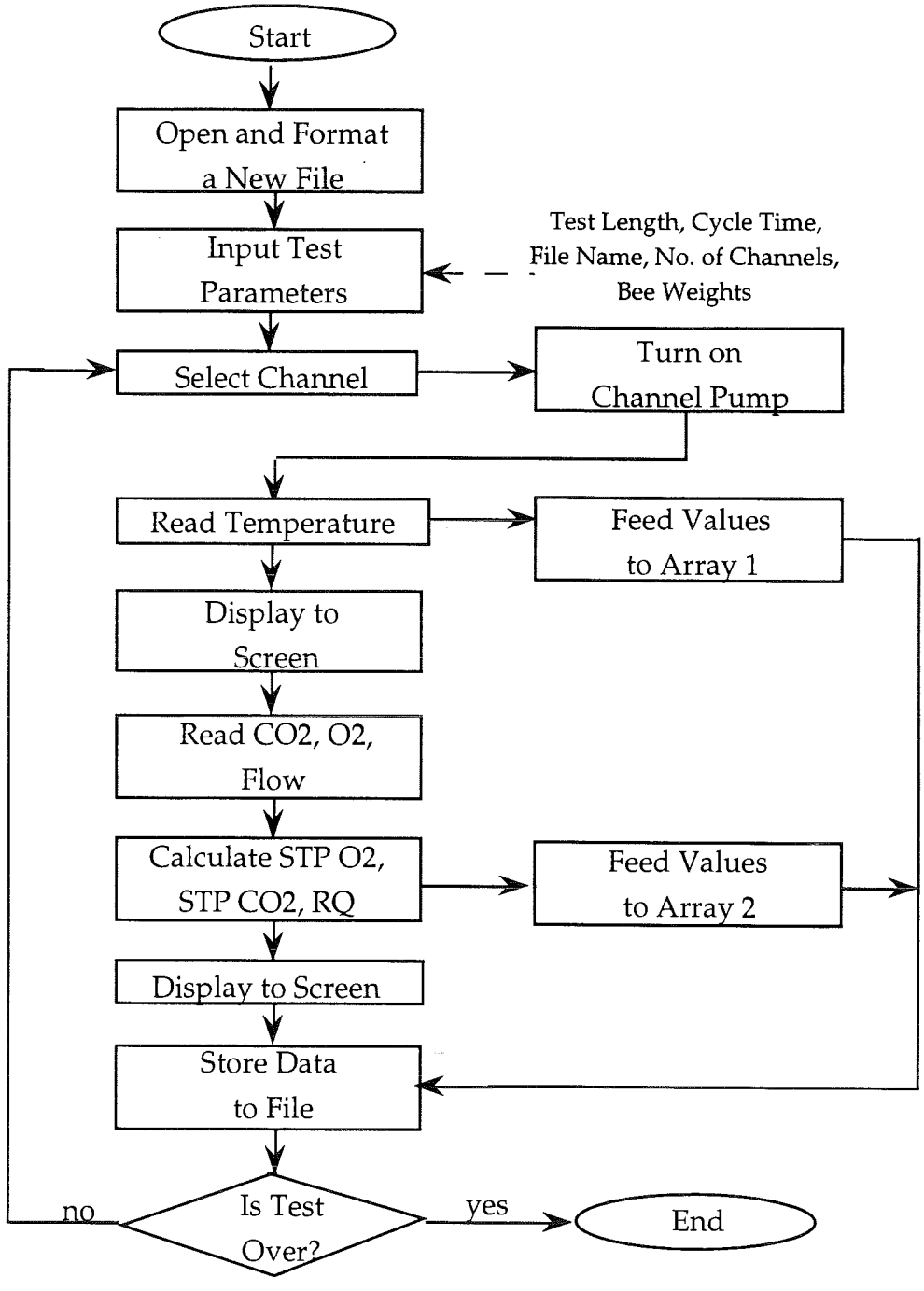

Figure 7. Execution sequence of LabVIEW® program designed to measure and record data in individual metabolic runs. The chill-coma experiments followed the same sequence with the deletion of the gas analysis portion of the program.

Results

I. Metabolic Rate

Mean oxygen consumption, ambient (syringe) air temperature, food consumed and thoracic temperature for all test runs are summarized in Table 1. Cabinet temperature was maintained within 1.1-4.4 percent of the mean cabinet temperature over all test runs. This is equivalent to a maximum deviation of 0.5-1.1°C at ± two standard deviations. Metabolic and food consumption data from any channel were not included if at least one bee or more connected to that channel's manifold ran out of food, succumbed to chill-coma or died during a test run. This occurred to some extent at most test temperatures. In these instances, data could not reliably be averaged over the eight bees connected to that channel. Heat production ($mW \bullet g^{-1}$) was determined from the metabolism of sugar at 5.09 calories burned per mL of oxygen consumed.

Oxygen Consumption

Individual workers attempted to maintain their body temperature through active thermoregulation. Oxygen consumption, and corresponding heat production, of individual honey bees increased with a decrease in ambient temperature (Figure 8). This increase is essentially linear and shows a high dependence of oxygen consumption on ambient temperature:

$$\dot{V}_{O_2} = -2.82 \bullet T_{ambient} + 96.64 \qquad r^2 = 0.94 \qquad n = 58 \qquad P < 0.001.$$

Because of the physiologic limit placed on the ability of *A. mellifera* to thermoregulate below its chill-coma point and above its upper lethal limit, this regression would not be valid outside of the range of test temperatures investigated here (12-35°C). Data at test temperatures 20°C and 35 °C were pooled, according to the criteria outlined in the Methods section of this thesis (Student's t-Test $P \leq 0.05$; values given in Table 2).

Oxygen consumption at 35°C represented a minimum consumption rate of 5.74 ± 0.82 $mL \cdot g^{-1} \cdot hr^{-1}$ for these individual worker bees. This value cannot be considered as a basal or resting rate because observation showed that at least some individuals on each channel were moving throughout the measurement period. This corresponds to the well known tendency of individual honey bees to move constantly when separated from other bees. The maximum observed rate of oxygen consumption was 74.0 $mL \cdot g^{-1} \cdot hr^{-1}$ at 12.9°C.

The variance in oxygen consumption increased with a decrease in cabinet temperature. This is to be expected with heat production increases. Heat production differences due to differences in thoracic muscle mass and food consumption become more pronounced with increased output levels. The increase in variance could also partially be due to the averaging of the data over the eight bees on one channel. The rate of change in thoracic muscle potential may have occurred at slightly different temperatures for the individuals connected to any one channel.

The same trend was seen with eight individuals stepped through a sequential test run from 40°C to 10°C (Figure 9a). This curve has the same characteristics as

that of Figure 8: minimum oxygen consumption between 35-40°C, a steady linear increase in consumption with decreasing ambient temperature in the range of 12-35°C, and an increase in the variability of the data with temperature decrease. At cabinet temperatures below 12°C, the oxygen consumption of individuals did not stabilize, but decreased continuously. The sharp decrease in consumption at ambient temperatures below 12.9°C, in this case, represents an abrupt cut-off in metabolic capacity seen at the chill-coma point. These bees were unable to actively warm themselves. Fifty-eight percent of these individuals were comatose at the completion of this run.

Oxygen consumption varied with thoracic temperature in a manner similar to its variation with ambient temperature (Figure 9b). The thoracic curve, however, is shifted to the right along the temperature axis, indicating maintenance of thoracic temperature over that of ambient. The temperature differential (T_{thorax} - $T_{ambient}$) is a useful indicator of metabolic output. Increases in oxygen consumption were seen at differences greater than 2°C (Figure 9c). Oxygen consumption for a typical individual rises with active heating of the thoracic musculature and falls with its cessation. This bee behaved as an ectotherm, allowing body temperature to follow ambient, at thoracic temperatures above 29.3°C and below 14.6°C. Between these two limits, behavior was endothermic, with maintenance of body temperature higher than ambient (on the average 4.74 ± 1.0°C; minimum = 3.0°C; maximum = 6.2°C; n = 16). The peaks in the thoracic temperature curve represent periods of active heating by the honey bee, the valleys periods of rest or feeding. The peaks occurred at the onset of each downward cycle in cabinet temperature and increased in frequency with decreasing ambient temperature until the onset of chill-coma (Figure 9d). This

was the point at which the junctional potential amplitude of the thoracic musculature decreased to zero as a result of the imposed cold load.

Temperature

On the average, individual bees were able to warm the air in their syringes at test temperatures below 30.1°C. The relationship between ambient and cabinet temperature over all individual runs was described by the linear regression (Figure 10):

$$T_{ambient} = 0.889 \bullet T_{cabinet} + 3.34 \qquad r^2 = 0.99 \qquad n = 1907 \qquad P < 0.001.$$

Comparison of this equation with the equation of equal temperatures ($T_{ambient} = T_{cabinet}$) shows that they are not the same (elevations are significantly different; ANCOVA F=5.345, P=0.038). Simultaneous solution of these two equations shows their intersection point to be 30.1°C. Below this temperature, $T_{ambient}$ is greater than $T_{cabinet}$ and above this temperature, $T_{ambient}$ is less than $T_{cabinet}$. The cooling seen above this temperature is partially an artifact of the test design. Heating seen in the first 24 minutes is passive (Figure 9d) and due to the fact that the cabinet temperature at the start of the test (40°C) was higher than lab ambient (ca 23°C).

The thoracic temperature is seen to always be greater than ambient temperature (Figure 9d). This would not normally be expected during periods of ectothermy. The small size (2cc) of the metabolic chambers and low flow rates may have acted as a buffer for conductive heat loss in this experimental set-up. This is

further substantiated by the fact that the ambient temperature is consistently greater than the cabinet set temperature. The effect increases with an increase in heat production (Figure 11). These three temperatures (cabinet, ambient and thoracic) tend to equilibrate before active thermoregulation begins and after the chill-coma point is reached and heat production ceases.

Mass of Bees

The mean weights of individuals from the three test hives differed significantly (ANOVA $F=12.705$, $P<0.0005$, $df=2, 686$) (Table 3). Multiple comparison using Tukey's Honestly Significant Difference Test showed that the mean weight of individuals from hives B and C were the same, but were different than the mean weight of individuals from hive A ($q=3.058$, $0.05<P<0.10$, $df=686$). The bees from hive A were obtained from a different source than those of hives B and C, and this may account for the weight difference. Practically speaking, this difference is small and no mass-specific differences in metabolic output were seen in individuals from different hives.

Food Consumption

Individual workers consumed more food on the average at cabinet temperatures ranging from 10-25°C than at cabinet temperatures 30-40°C (30.9 ± 9.1mg, $n=319$ vs. 20.6 ± 6.4mg, $n=257$) (Figure 12). This corresponded to the increase in caloric requirements necessary with a shift from passive to active heating. This change in thermoregulatory behavior below 30°C was seen previously. Averages within these two temperature ranges were significantly different from each other

(ANOVA $F=35.45$, $P<0.0001$, $df=586$). Multiple comparison using Tukey's Honestly Significant Difference Test showed that mean food consumption was the same at test temperatures between 30-40°C ($q=0.547$, $P>0.50$, $k=8$) and 11-25°C ($q=4.253$, $0.05<P<0.10$, $k=8$), but that consumption was different between these two temperature ranges.

All bees consumed food during all test runs. This was expected because I gathered outgoing foragers with empty honey crops. Bees tested at higher temperatures (30-40°C), when thoracic temperature followed ambient temperature, did not require large amounts of food. The high cost of thermoregulation below these temperatures required a corresponding increase in food consumption. Since active heating is constant when thoracic temperature falls below 27-30°C (Goller and Esch 1991), a difference in food consumption with decrease in temperature below this ambient is not expected.

A significant contributing factor to the large standard deviations seen in these data is probably the time that it took to weigh the syringes both before and after each test. Collection and weighing of the bees prior to a test run took approximately one hour and weighing after a test run took approximately 35 min. Consequently, there was a large variation in the amount of time that individuals fed on the sugar candy in their separate syringes before weighing. Therefore, food consumption was not used in further analyses. These time spans could also have contributed to the large standard deviations seen in the mean weights of individuals.

II. Chill-coma

Survival

The survival potential of a comatose honey bee is high. A percentage of bees showed at least some temporary movement with heating after all exposure conditions. Fifty-two bees (95%) showed some movement (leg, wing, antenna or abdomen) after initial removal from the temperature cabinet, with thirty-nine bees (71%) showing at least some movement 24 hours after revival. A 51% survival rate was seen across all chill-coma tests (n=55). At least one bee from each test cell showed movement after 24 hours. Walking survivors after 24 hours were seen at all test conditions except two: 100 and 600 minutes at an exposure temperature of -2.5°C.

As expected, the percentage of survivors tended to decrease with a decrease in exposure temperature below 0°C (Table 4). Survival rates of bees exposed to a temperature of 4.5°C tended to be as high, or higher, than those exposed to 9.0 and -2.5 °C, although not significantly so. Over all exposure times, the number of survivors, compared to number of non-survivors, was not different between the 4.5°C and 9.0°C exposure temperatures (chi^2=0.218, df=1, P=0.64, n=37), but was different than bees exposed to -2.5°C (chi^2=4.435, df=1, P=0.035, n=55). This is reflected in the fact that the majority of bees tested above 0°C survived while the majority of bees tested below 0°C did not.

In general, survival rates decreased with an increase in the amount of time a bee remained comatose (Table 4). Survival of bees at 1 and 10 min was high (80% for

each of these exposure times), but fell by a factor of 2 at 100 min and a factor of 4 at 600 min. Over all exposure temperatures, there was a very significant difference between the numbers of survivors and non survivors with exposure time (chi^2=17.654, df=3, P=0.001, n=55). Combining the 1 and 10 minute and the 100 and 600 minute groups, there was a very significant difference between these two groups (chi^2=13.460, df=1, P<0.0005, n=55). This alludes to cumulative physiologic and/or structural changes at the cellular level that are damaging to the honey bee (exposure times at or greater than 100 min in this study) (Storey and Storey 1990).

The interactive effects of exposure time and temperature on survival rate could not be tested statistically due to small and unequal sample sizes. However, exposure time at low temperatures appears to have more of an effect on survival rate than the actual exposure temperature.

Chill-coma Temperature

There was a wide range of thoracic temperatures at which first movement out of chill-coma was observed (7.5 - 25.7°C). Some bees survived even when movement was not seen until their thorax was essentially at room temperature. Thirteen bees, or 23.6% of those sampled, showed movement at T_{thorax} less than 11.2°C. This is below the limit (11.2 ± 0.7°C) observed by Goller and Esch (1990a) for possible heating using thoracic flight muscles. However, their value was obtained during the cooling of bees and what I may have observed is the hysteresis effect known to occur between cooling and warming rates.

For all bees over all tests, chill-coma temperature was not dependent on exposure temperature (ANOVA F=1.67, df=2, 47, P=0.20, n=50), but was dependent on exposure time (ANOVA F=16.26, df=3, 46, P<0.0005, n=50). For survivors, 59% of the variation in T_{cc} was due to the variation in exposure time (Table 5, Figure 13). Since bees cannot feed themselves while comatose, it is possible that some of them may die of starvation at these longer exposure times.

Revival Time

Exposure time is also more predictive of t_{cc} than is exposure temperature. Time to first movement out of chill-coma varied from 0.3 to 14.6 minutes. Revival time was found to be dependent on both exposure temperature (ANOVA F=4.34, df=2, 49, P=0.02, n=52) and exposure time (ANOVA F=10.48, df=3, 48, P<0.0005, n=52). Coefficients of determination were low, especially for non survivors (Table 5, Figure 13).

Chill-coma Temperature and Revival Time

As thoracic temperature and time of warming increase, the likelihood of recovery from a comatose state decreases. It is reasonable to expect that a bee that is capable of shivering will begin doing so at the lowest temperature at which this process is physiologically possible. The best fit regression of revival time and chill-coma temperature is described by a logarithmic function (Figure 14). The logarithmic shape of this curve is expected because, as time at room temperature increases, (T_{room} - T_{thorax}) decreases, with a corresponding decrease in passive warming rate. The correlation between revival time and chill-coma temperature

at warm-up for all bees is 0.92 (survivors: r=0.89, n=27; non survivors: r=0.90, n=23).

The higher the thoracic temperature or longer the time out of the temperature cabinet at first movement, however, the less likely the individual was to survive. All bees that were able to fly 24 hours after testing revived in less than ten minutes and at thoracic temperatures below 25°C, with 89% of these showing movement in less than four minutes and at thoracic temperatures below 18°C. Correlation coefficient between these two variables below these values (4 min, 18°C) was 0.88 for survivors (n=21) and 0.43 for non survivors (n=8). Thus, an individual was twice as likely to survive if it revived in less than 4 min and under 18°C.

These results indicate that exposure time is a more useful indicator of survival potential than exposure temperature. There are no strong predictive indicators of inability to survive after recovery from a comatose state that were examined in these tests.

Table 1. Summary of individual test results

Cabinet Set Point (°C)	$T_{ambient}$ (°C)	n	Oxygen Consumption (mL/g/hr)	n	Food Consumed (mg)	bees	T_{thorax}* (°C)	bees
40	39.1 ± 0.67	10	5.5 ± 0.59	5	16.3 ± 6.5	46	39.6 ± 0.84	1
	39.6 ± 0.55	12	5.0 ± 0.47	6	23.5 ± 5.00	48		
35	34.3 ± 0.26	12	5.2 ± 0.94	6	22.8 ± 5.3	48	36.2 ± 0.44	1
	33.4 ± 0.31	12	4.4 ± 0.84	6	18.2 ± 5.9	48		
30	30.2 ± 2.31	10	14.8 ± 2.31	5	22.6 ± 7.2	40	31.6 ± 0.69	1
	29.4 ± 0.29	12	8.5 ± 3.00	6	19.2 ± 5.3	48		
25	25.1 ± 0.47	10	19.9 ± 2.77	5	NA	0	29.0 ± 0.27	1
	25.0 ± 0.42	12	23.7 ± 3.34	6	30.0 ± 5.8	47		
20	21.4 ± 0.63	10	40.0 ± 4.71	5	29.4 ± 7.5	40	26.3 ± 0.60	1
	21.1 ± 0.71	8	36.8 ± 3.49	4	29.2 ± 9.5	46		
15	16.7 ± 0.70	8	54.0 ± 2.31	4	27.5 ± 8.8	38	22.4 ± 0.18	1
	16.0 ± 0.37	10	44.9 ± 5.71	5	30.8 ± 8.5	40		
12	14.2 ± 0.98	12	62.2 ± 5.23	6	38.8 ± 7.9	48	19.4 ± 0.70	1
	13.3 ± 1.40	12	/	0	27.5 ± 9.4	48		

± error represents ± one standard deviation

/Oxygen consumption of these bees did not stabilize (dropped continuously) during test run

*Average thoracic temperature of one bee that was sequenced through test temperatures (for comparison purposes only)

n is the number of channels with eight individuals per channel

Table 2. Student's t-Test results for individual oxygen consumption tests. Differences between trials at 20°C, 35°C and 40°C were not significant. Trials at these temperatures were pooled in data analyses.

$T_{cabinet}$ (°C)	df	P	t
15	12.4	0.0006	2.179
20	18	0.23	1.261
25	20	0.02	2.481
30	20	0.0003	5.409
35	22	0.08	1.864
40	20	0.05	2.121

Table 3. Summary of weights of individual honey bees collected from test hives for individual metabolic test runs.

Hive	mean weight (grams)	sd	n
A	94.4	12.16	191
B	101.3	13.93	188
C	98.6	14.03	310

Table 4. Percent survival of *Apis mellifera* workers for all chill-coma tests. Table a. gives the percentage of bees that were able to walk and/or fly 24 hrs after removal from the test chamber (survivors). Table b. includes bees that were alive after 24 hr, but unable to walk upright. Number in parentheses is the total number of bees tested per cell.

a. Survivors that were able to walk or fly after 24 hrs:

exposure time (minutes)	exposure temperature (°C)		
	-2.5	4.5	9
1	60 (5)	100 (5)	80 (5)
10	67 (3)	100 (4)	67 (3)
100	0 (5)	60 (5)	60 (5)
600	0 (5)	20 (5)	20 (5)

b. Total bees that showed some movement after 24 hrs:

exposure time (minutes)	exposure temperature (°C)		
	-2.5	4.5	9
1	100 (5)	100 (5)	80 (5)
10	100 (3)	100 (4)	67 (3)
100	40 (5)	80 (5)	80 (5)
600	40 (5)	60 (5)	20 (5)

Table 5. Coefficients of determination (r^2) relating revival time (t_{cc}) and temperature (T_{cc}) from chill-coma to dependent test variables. Survivors (24 hr) are listed separately from non survivors.

	survivors:		non survivors:	
	Texposure (°C)	texposure (minutes)	Texposure (°C)	texposure (minutes)
Chill-coma Temperature	0	0.59	0.03	0.2
Chill-coma Time	0	0.32	0.23	0.08

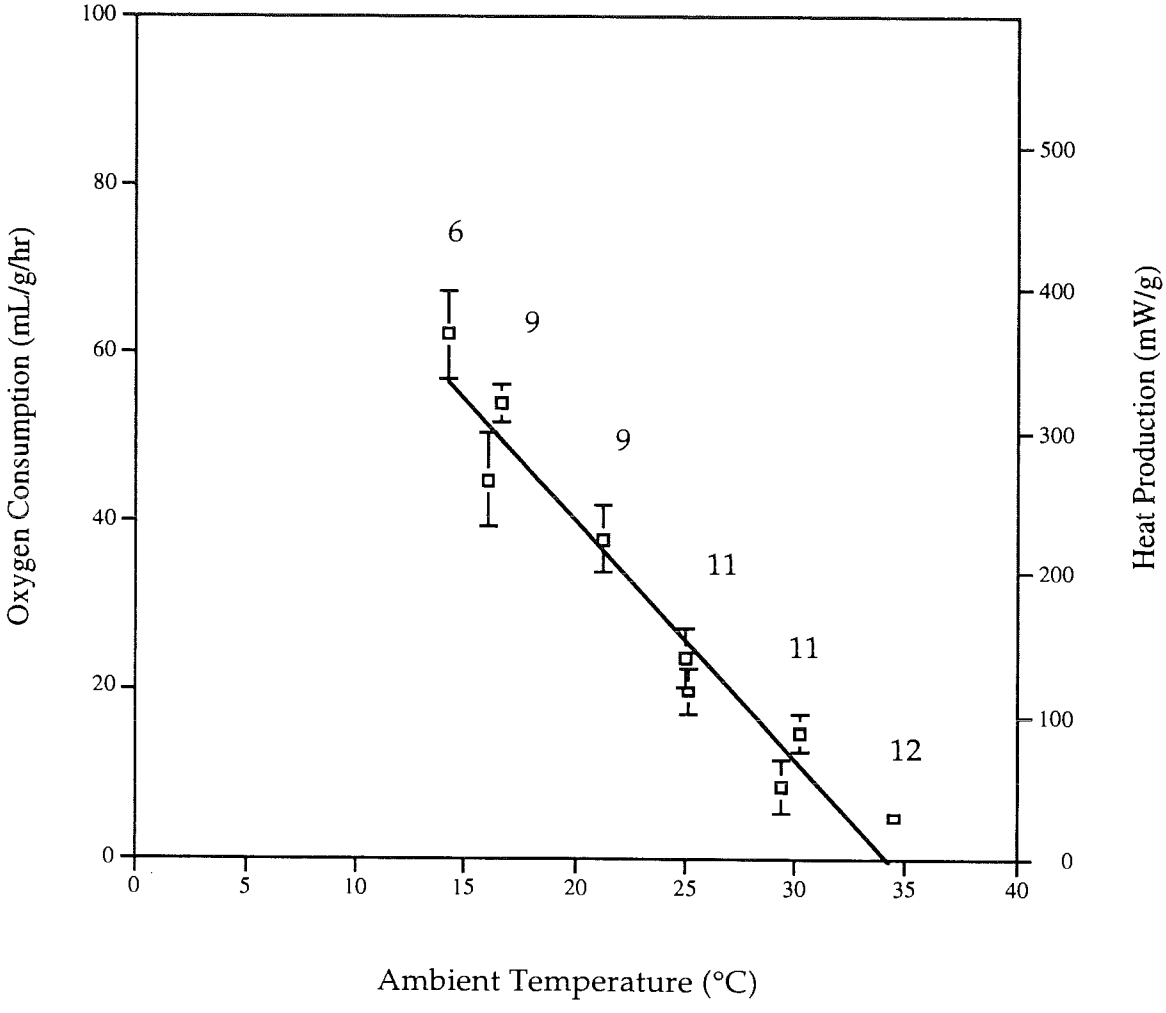

Figure 8. Oxygen consumption and heat production as a function of ambient exposure temperature. Mean consumption and standard deviation of recorded values are shown. Data are pooled where appropriate (refer to Table 2). The linear regression of these data give: $\dot{V}_{O_2} = -2.82 \bullet T_{ambient} + 96.64$ ($r^2 = 0.94$, $P < 0.001$). Number of trials (at eight bees per trial) at each test temperature is shown above symbols.

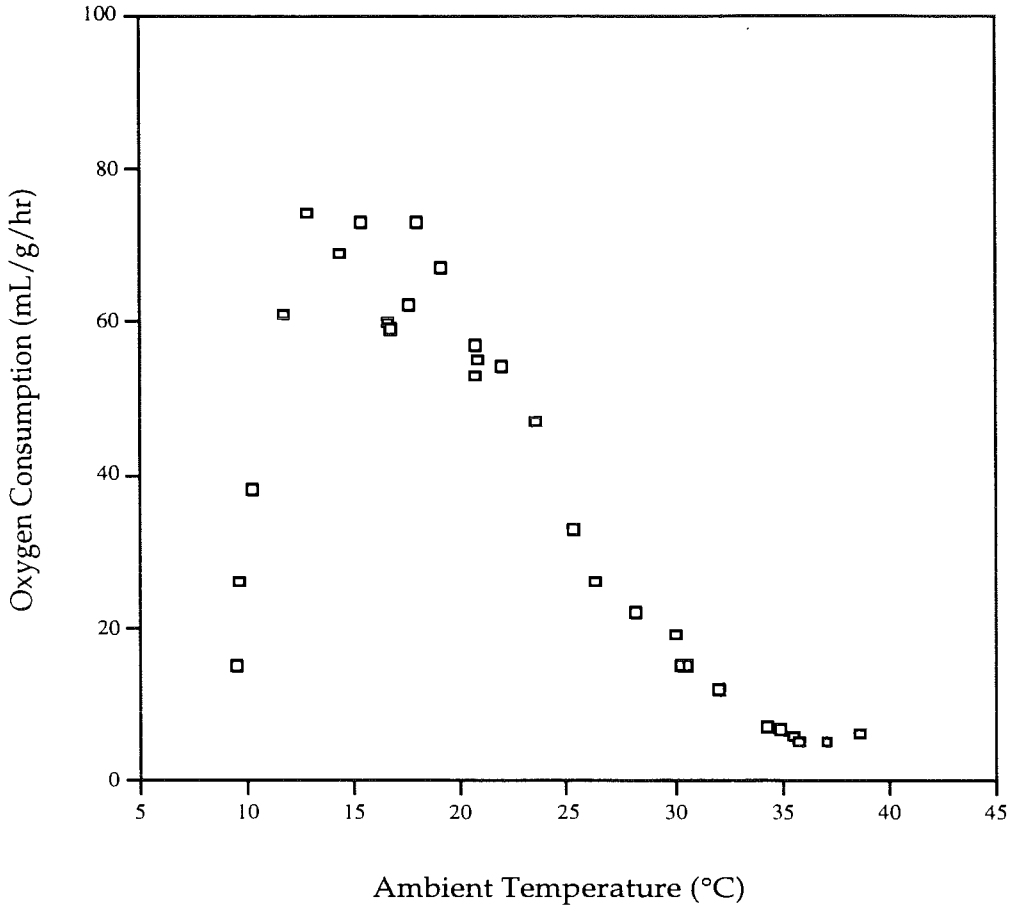

Figure 9a. Oxygen consumption as a function of ambient temperature for a sequential run with cabinet temperature dropped from 40°C to 10°C over a 4 hr period. Oxygen consumption values are averages of eight isolated individuals monitored on one channel.

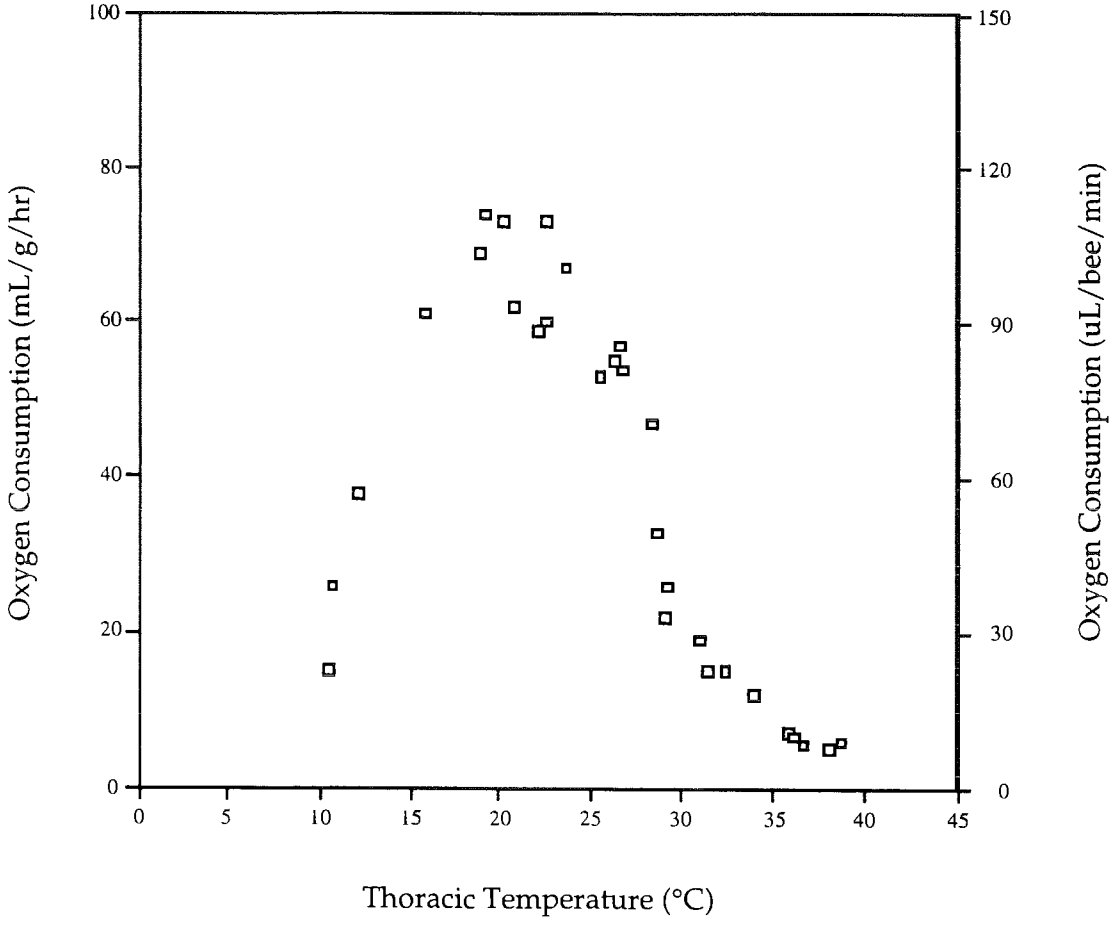

Figure 9b. Oxygen consumption as a function of the thoracic temperature of one individual. Active thermoregulation, defined as (T_{thorax}-$T_{ambient}$) > 2°C, corresponds with a sharp increase in oxygen consumption below thoracic temperature of 29.3°C.

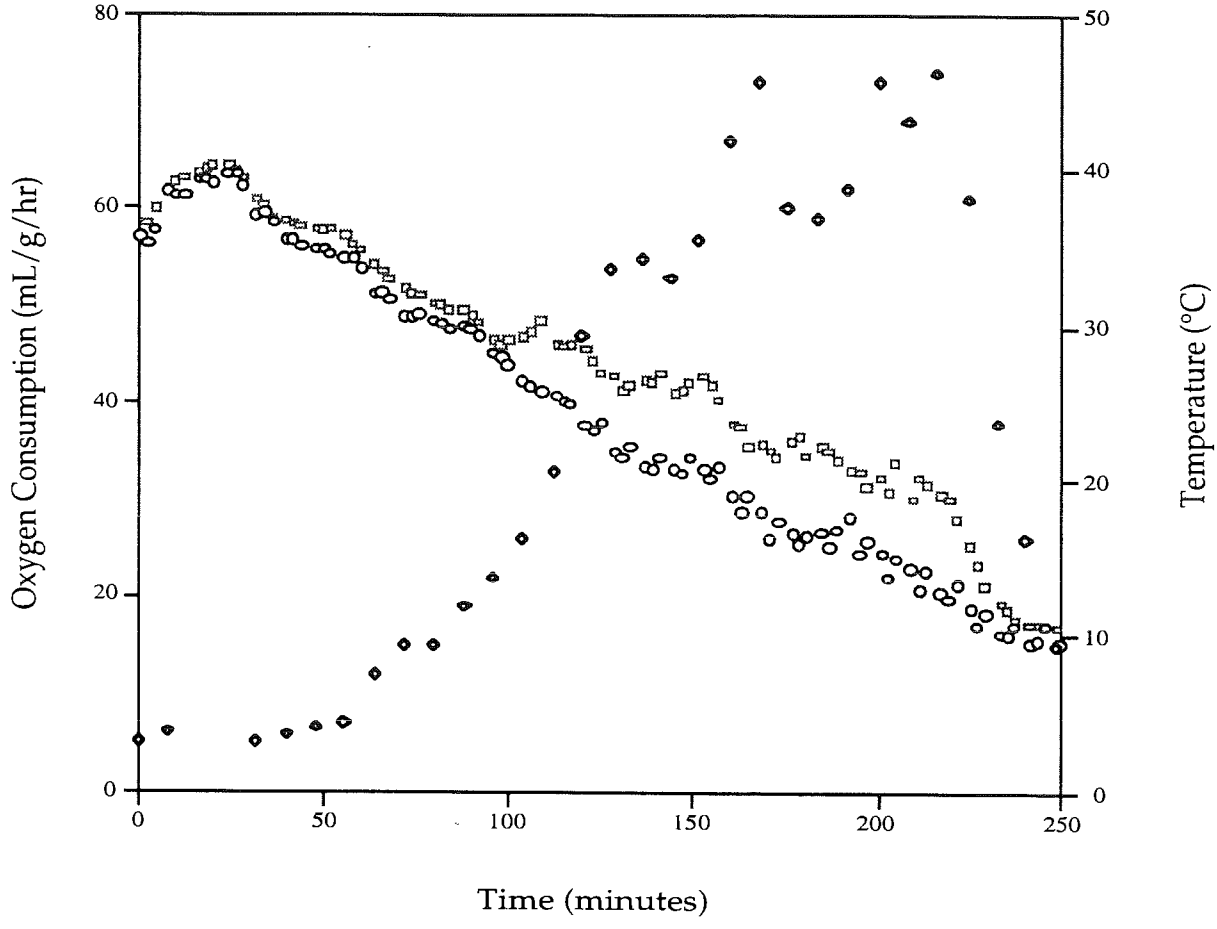

Figure 9c. The variation of oxygen consumption (diamonds) and thoracic (squares) and ambient (circles) temperatures with time for a sequential run with cabinet temperature dropped from 40°C to 10°C over a 4 hr period. Oxygen consumption values are averages of eight isolated individuals monitored on one channel. Thoracic temperature is that of one of these isolated individuals. Active thermoregulation takes place at thoracic temperatures between 29.3-19.1°C. This corresponds with an increase in oxygen consumption in this temperature range.

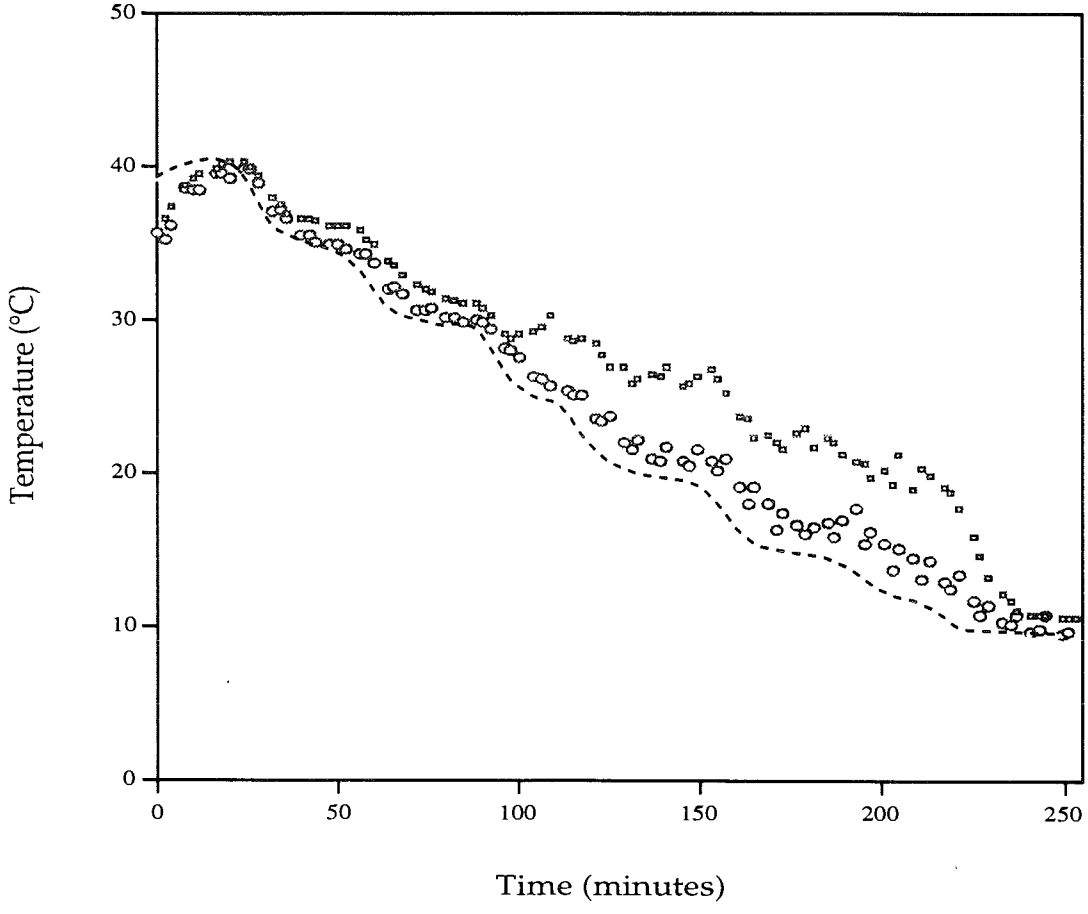

Figure 9d. Thoracic temperature (squares) with decreasing cabinet temperature (dashed line) and ambient (circles) temperatures over a 4 hr period for one individual during a sequential test run. Variation in thoracic temperature represents heating and cooling cycles of thoracic musculature.

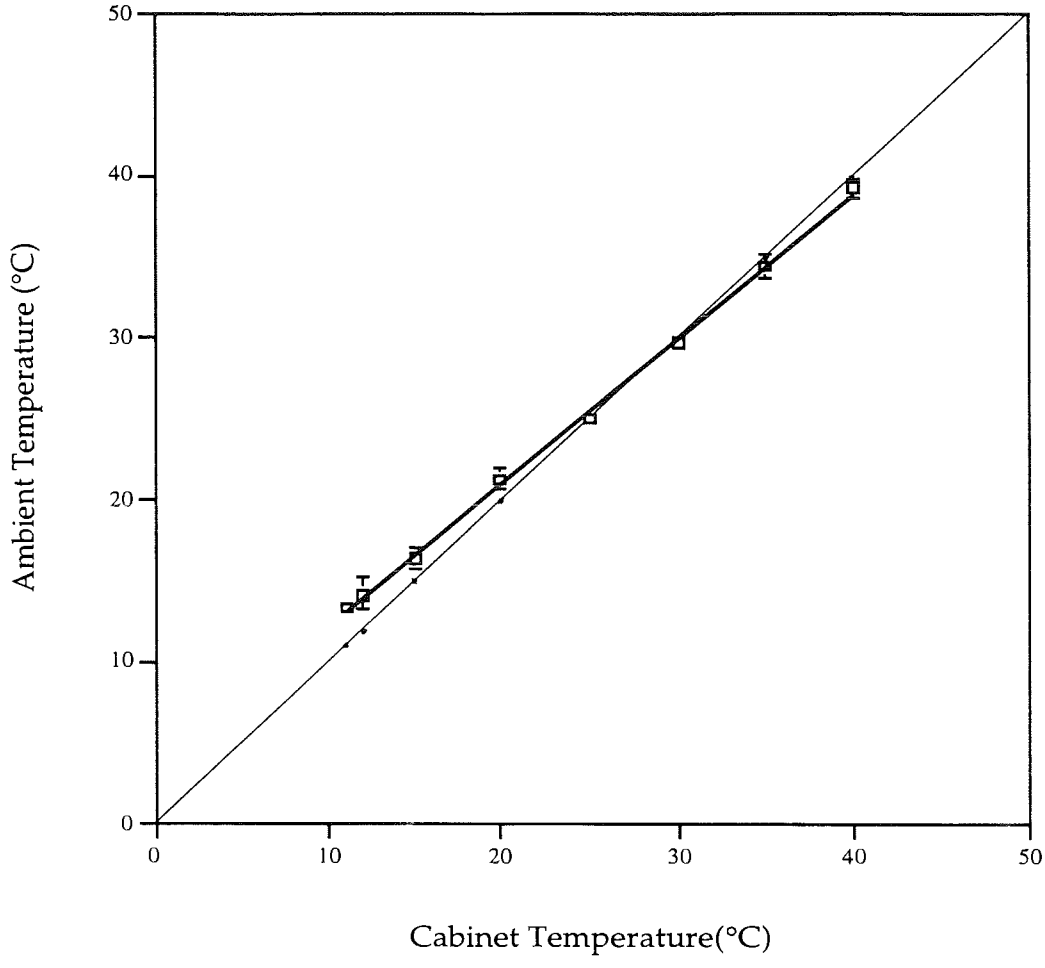

Figure 10. Mean ambient temperature given as a function of cabinet set temperature for all constant temperature individual test runs (squares). Linear regression of these data gives $T_{ambient} = 0.889 \cdot T_{cabinet} + 3.34$ ($r^2 = 0.99$, $P < 0.001$). The line of equal temperatures ($T_{ambient} = T_{cabinet}$) is also shown. Error bars represent the standard error of the mean.

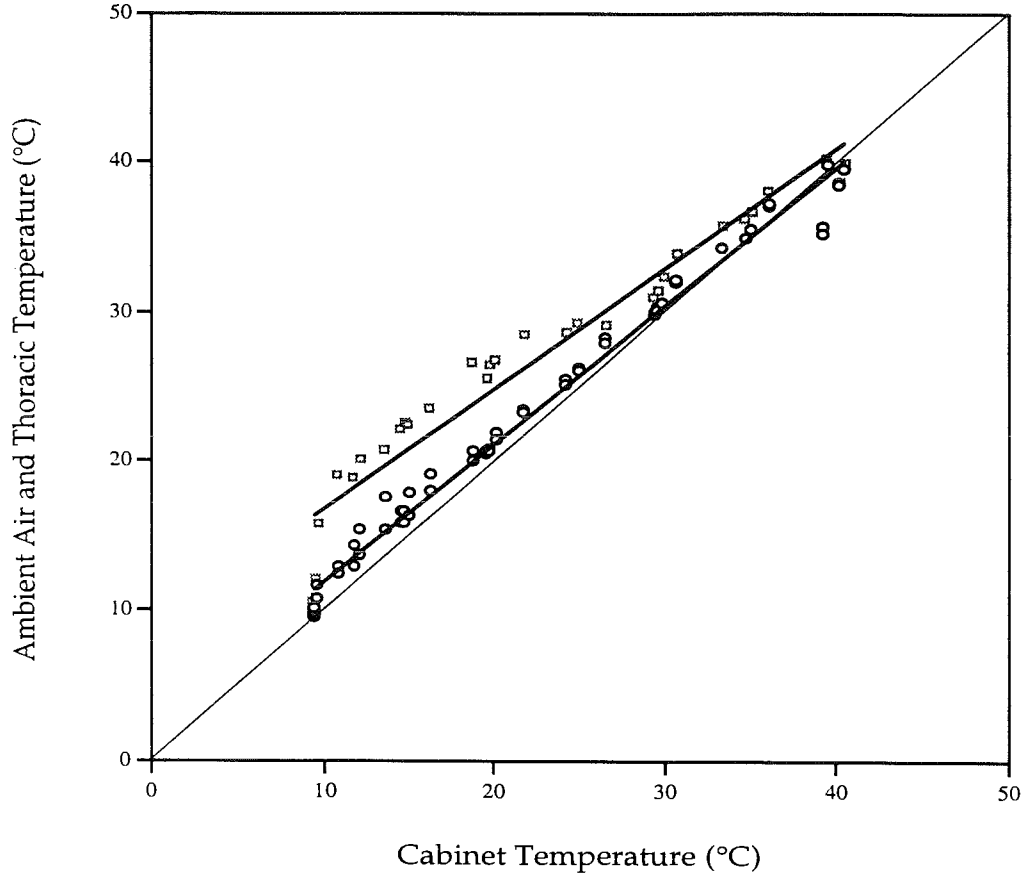

Figure 11. Ambient (circles) and thoracic (squares) temperatures as a function of cabinet temperature for one bee in a sequential test run. Linear regression of air temperature measurements yields $T_{ambient} = 0.93 \cdot T_{cabinet} + 2.68$ ($r^2 = 0.99$, $P < 0.01$) and thorax temperature $T_{thorax} = 0.80 \cdot T_{cabinet} + 8.80$ ($r^2 = 0.94$, $P < 0.01$). The line of equal temperatures ($T_{ambient} = T_{cabinet}$) is also given.

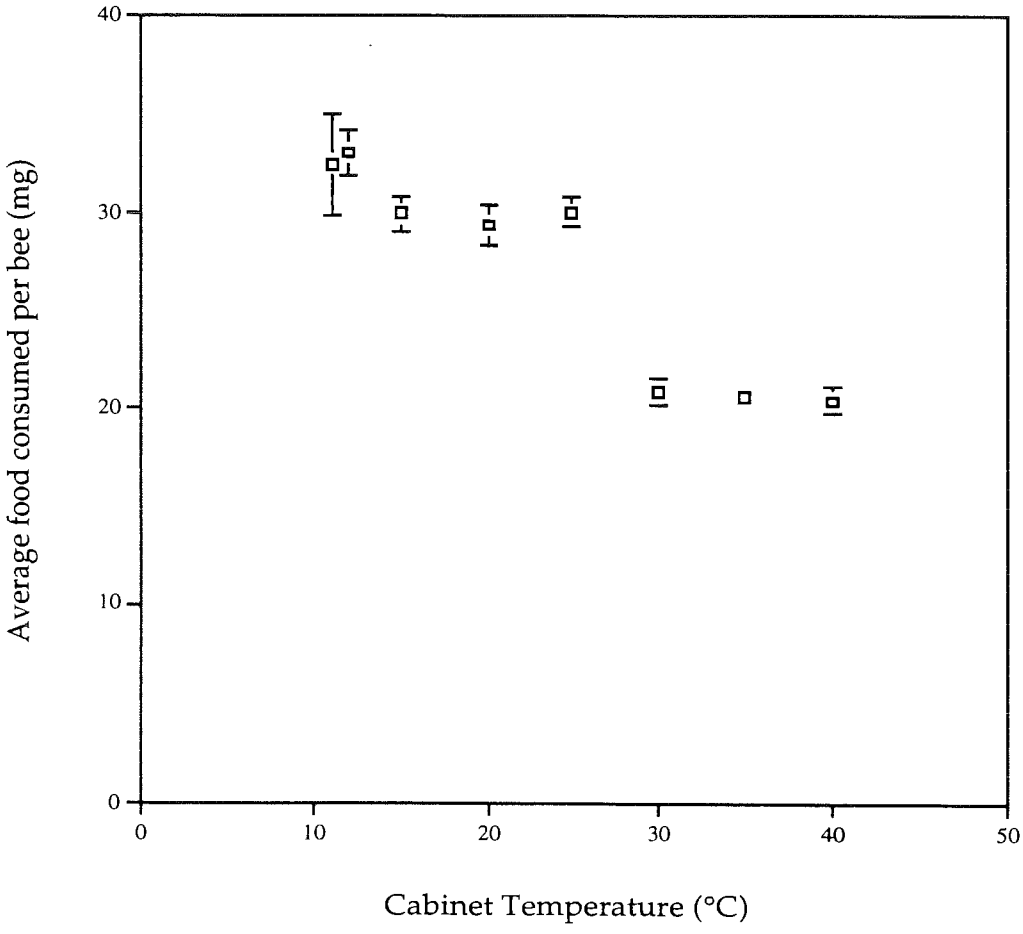

Figure 12. Average food consumed per bee for all test conditions as a function of cabinet temperature (error bars represent the standard error of the mean). ANOVA and Tukey HSD Test show that the food consumed at cabinet temperatures 11-25°C is significantly different than that consumed at cabinet temperatures 30-40°C.

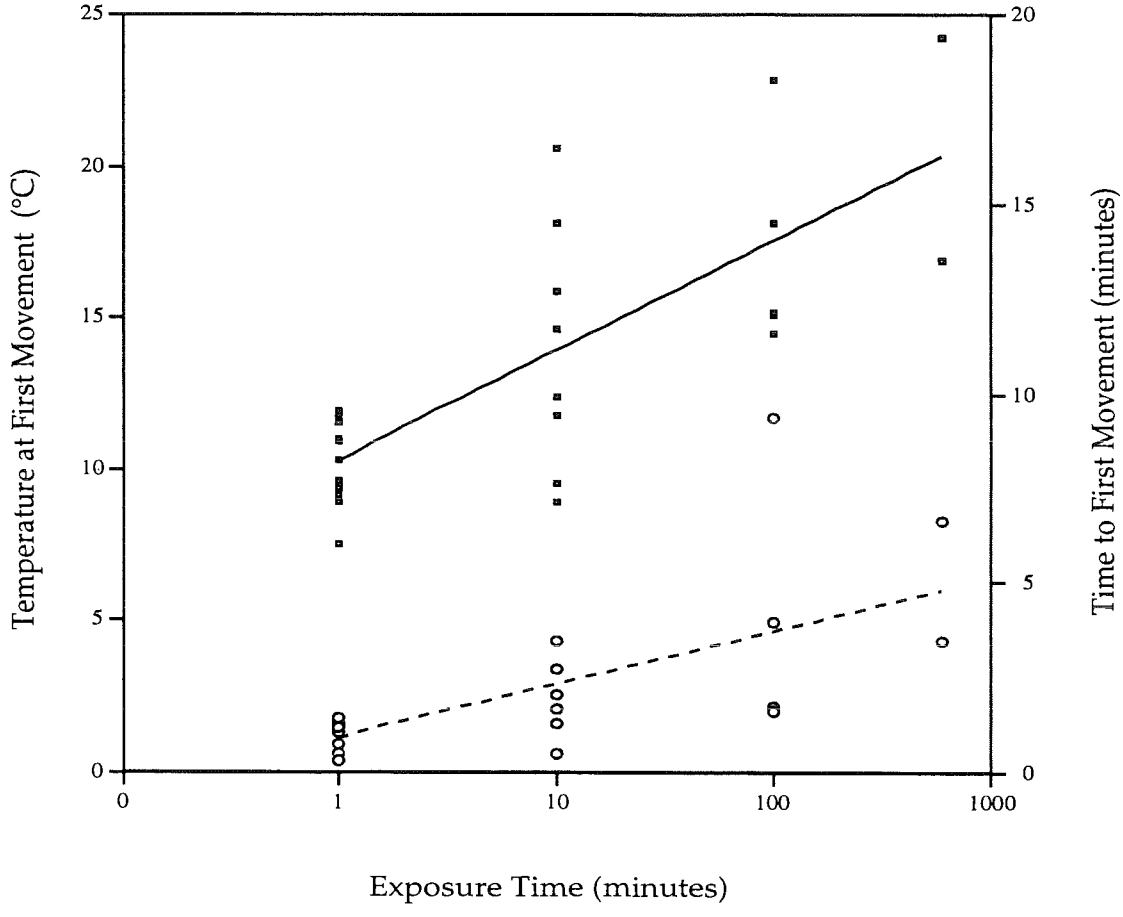

Figure 13. Change in temperature at (T_{cc} squares, solid line) and time to (t_{cc} circles, dashed line) first movement with exposure time in low temperature chill-coma experiments for 24 hr survivors. For these bees, T_{cc} and t_{cc} were both found to be significantly dependent on exposure time at the 5% level (ANCOVA, $P < 0.0005$ in both cases).

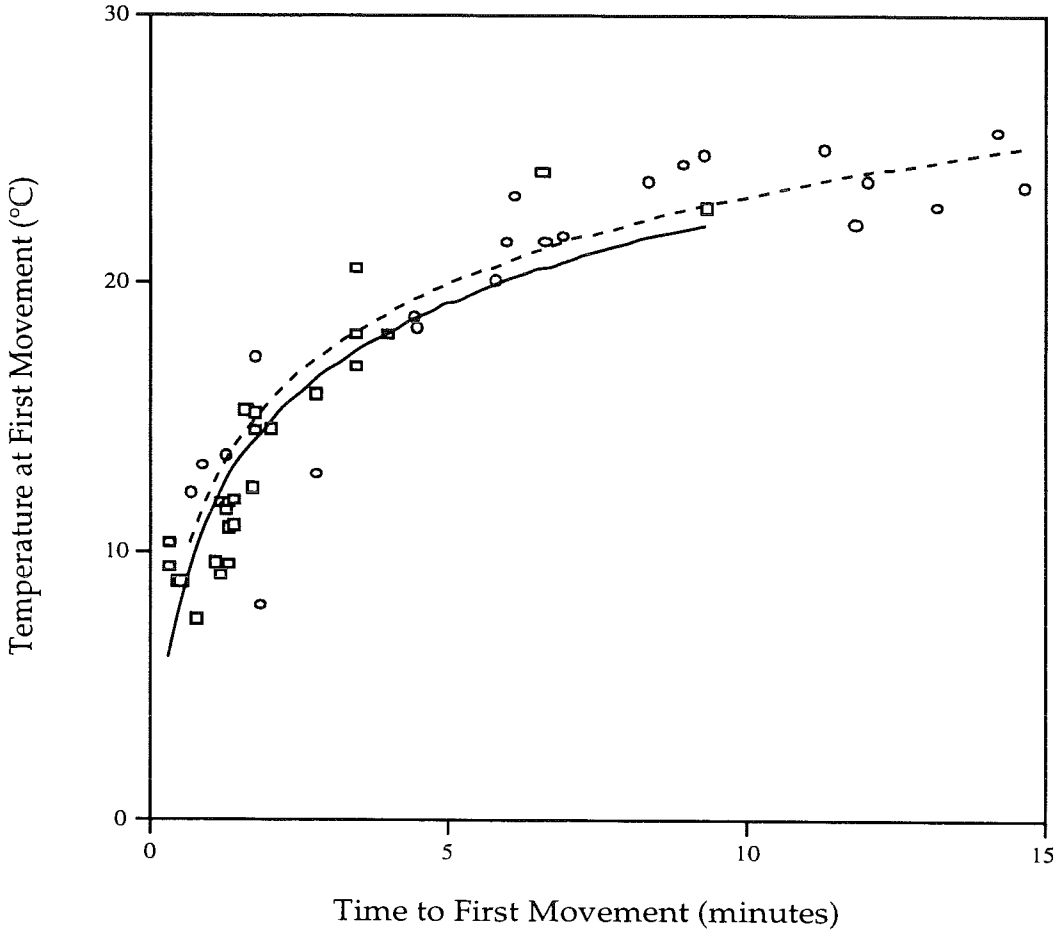

Figure 14. The relationship between chill-coma temperature and revival time for individual workers that revived from test exposure conditions. Survivors (squares, solid line) were defined as bees that were able to walk or fly after 24 hours (r=0.89, n=27). Non survivors (circles, dashed line) indicate bees that either died or could not walk after 24 hours (r=0.90, n=23). Correlation for data below t_{cc}=4 min and Tcc=18°C was 0.88 for survivors and 0.43 for non survivors.

Discussion

Oxygen Consumption

Honey bees have evolved in a high temperature group environment. As social insects, they have not developed adaptive mechanisms to survive singly at low temperatures. Heat retention is not possible for the individual separated from the group. The temperature preference of a worker lies between 31.5-36.5°C (Heran 1952 as noted by Heinrich 1993 and Cahill and Lustick 1976). For individual bees, at temperatures below at least 31.5°C, active temperature regulation is required to maintain body temperature.

Low exposure temperature does affect heat production by *A. mellifera*. The steady increase in oxygen consumption that I measured within an ambient temperature range of 35-12°C is in agreement with similar studies found in the literature for mature workers (Allen 1959, Cahill and Lustick 1976, Southwick 1991). This defines the range of endothermy for these individuals. The high temperature limit is determined by the preferred body temperature of the bee, the low limit by physiologic constraints. The high temperature starting point for increased metabolic output is fairly consistent between studies (32-35°C). In this temperature range, a minimum sustained average rate has been measured within the range of 2.2-6.0 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$. The measured low temperature cut-off for thermoregulation in the literature is more variable (5-17°C) (Allen 1959, Cahill and Lustick 1976, Southwick 1991, Goller and Esch 1991). This large spread is partly a result of the confusion in determining the active state of the thoracic musculature. Some of the discrepancy is due to test design artifact in cases where the ambient temperature points are spaced too far apart near the chill-

coma point. The average maximum sustained rate measured in this experiment (62.2 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$ at 12.9°C) is higher than that found in other studies (50.5 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$ at 5°C Cahill and Lustick 1976; 29.6 $mLO_2 \cdot g^{-1} \cdot hr^{-1}$ at 15°C Southwick 1991).

Variation in individual heat production is much lower at higher ambient temperatures than at lower temperatures. Specifically, standard deviations noticeably increase when ambient temperature approaches 20°C. The increase in oxygen consumption is steady and consistent to 21.9°C ambient; below 21.9°C, the increase in consumption is sporadic and more variable. This is the approximate temperature at which Esch and Goller (1990a) observed interruption of neurological function at the motor end plate of the honey bee's thoracic musculature. The duration of the muscle endplate potential increases exponentially with muscle temperature below 18°C and the amplitude decreases by 50% between 25°C and 15°C. This results in a significant decrease in muscle contractile force and power output (Rothe and Nachtigall 1989). Presumably, the respiratory musculature are similarly affected. This would coincidentally decrease the fuel supply for aerobic conversion and inhibit the muscles used for active heat production.

Increased movement of an individual separated from the group under experimental conditions artificially inflates oxygen consumption values. Fahrenholz *et al.* (1992) showed that groups with as few as three bees had significantly decreased per bee heat production levels compared with isolated individuals. It is well known that a honey bee separated from its sisters will not settle down, even in cases of extreme energy loss (Heinrich 1993). This tendency is contrary to expected survival behavior and implies that a completely natural

test setting is impossible to achieve with individuals. Still, this type of study has value in that the thermoregulatory cues for the individual may be assumed to play a role in group behavioral dynamics.

Thoracic Temperature

The only mechanism available for active heat production in the honey bee is shivering thermogenesis (Heinrich and Esch 1994). Physiologic maintenance of thoracic temperature is the driving force that links ambient temperature and oxygen consumption. The dependence of oxygen consumption on thoracic temperature is similar to its dependence on ambient temperature (see Figures 9a and 9c). As the temperature differential between thoracic musculature and ambient increases ($T_{thorax} - T_{ambient}$), conductive heat loss increases rapidly. This increased loss requires a higher power output and a greater oxygen consumption for aerobic heat conversion in the muscle. However, power output decreases proportionally with temperature due to the disturbance of junctional potential impulses with cold stress. The interplay of these factors determines a low temperature limit for thoracic heating.

This experiment showed maintenance of thoracic temperature over ambient temperature in the thoracic temperature range of 31-12.9°C. This corresponds closely with that found by Esch (1988): 30-11°C. However, thoracic temperature dropped continuously with successive readings below 19°C, implying that the cut off for effective thermoregulation was, more accurately, 19°C (see Figure 9c). Heat production capability was severely limited at thoracic temperatures below 19°C, which is 6°C above the measured chill-coma point in these experiments.

The heat production capability of the honey bee is significantly restricted even before it is immobilized.

Heinrich (1996) proposed that the control set point for shivering thermogenesis is located in the thorax of the honey bee. This would partially explain differences in the literature regarding specific values of heat production related to environmental temperature. Slight differences in experimental conditions could alter the relationship between ambient and thoracic temperature, thus changing the resultant measured relationship between heat production and temperature. This suggests that either T_{thorax} or the differential (T_{thorax} - $T_{ambient}$) would be a better indicator of metabolic output than ambient temperature alone.

Food Consumption

Food consumption has been used qualitatively as an indicator of metabolic activity; Free and Spencer-Booth (1958) saw an increase in feeding with a decrease in environmental temperature. Food consumption, however, is rarely used as the sole indicator of heat production. It is difficult to determine the proportion of metabolized energy that is given off as heat without direct measurement. This is illustrated in Figure 12, which shows only two average values of consumption over the entire test range. The steady increase in metabolism with increased cold load is lost in this analysis.

Bees exposed to ambient temperatures at and above 30°C clearly had a lower total energy expense than those exposed to ambient temperatures below 30°C. This delineation is interesting and indicates the continuous nature of heating below 30°C ambient. Esch and Goller (1991) observed this effect in workers at a

30°C thoracic temperature. A clear division would not have showed up in Free and Spencer-Booth's (1958) study because groups, not individuals, were tested. At any given time, not all bees within a group are necessarily producing heat, even when group output is increasing.

Survival

The ability to survive low temperature exposure has practical applications in the natural environment. The temperature at the outer layer of an overwintering cluster (mantle) may drop to chill-coma levels at low ambient temperatures (Southwick 1985). Bees within this layer must be able to endure cycling into and out of chill-coma in order to move with the cluster to honey stores. Separation from the cluster in cold conditions can be fatal to the individual comatose worker. Honey bees also have been observed foraging at ambient temperatures near 10°C (Heinrich 1979b). A worker foraging at or near this temperature may be unable to maintain the high thoracic temperature necessary for flight (~27°C minimum) and return to the hive. Robinson and Morse (1982) showed that honey bees do get stranded away from the hive at night, returning during the warmer mid-morning hours.

Considering that a honey bee lives through its entire life cycle in a highly socialized and tightly regulated thermal environment, the survival potential of a worker separated from the group is surprising. I saw movement in bees after all exposure conditions (most notably -2.5°C for 10 hours), although not all were alive after the twenty-four hour defined period for survival. Individuals have survived test conditions of 0°C for 80 hours, -2 to -5°C for 0.5-3 hours (Free and Spencer-Booth 1960) and 5°C for several days (Esch 1988).

I found that low exposure temperature and time do affect the survival of *A. mellifera* from chill-coma. Honey bee survival rates were most influenced by the amount of time that an individual was in chill-coma. Colhoun (1960) saw a similar effect of exposure time on survival in the cockroach (*Blattella gemanica* L.).

This effect was compounded by the minimum temperature to which the bee was exposed. Although not statistically significant, more bees survived the exposure temperature of 4.5°C than at either 9°C or -2.5°C (this pattern would have to be further investigated by retesting with a larger number of bees). Free and Spencer-Booth (1960) found survival rates to be highest at 5°C (temperatures tested: 0, 5, 10°C). This effect may simply be related to the Q_{10} effect of temperature on metabolic rate. A bee becomes immobilized when muscle potential amplitude drops to zero, but, to survive, metabolic processes must continue on some level. Given equal food availability, the bee with the lower metabolism (comatose at the lower temperature) will survive the longest. There will be a critical temperature, below which tissue and cell damage will interfere with metabolic processes and survival will not be possible. The critical temperature is most likely related to the freezing point of the bee's tissue, which is at or near the freezing point of water. This is substantiated by the fact that cold-death temperatures quoted in the literature for honey bees have all been below 0°C (-1°C Pirsch 1923, -2 to -5°C Free and Spencer-Booth 1960, -2.5°C this paper). Future studies that take into account body weight and honey crop content are needed to clarify these factors.

Chill-coma temperature at warm up for twenty-four hour survivors was 13.27 ± 4.5°C, with 87.5% seen between 7.5-18°C (11.75 ± 2.7°C). This value is in agreement with chill-coma cool down values found in the literature, ranging

from 7-12°C (Allen 1959, Free and Spencer-Booth 1960, Esch 1988, Southwick 1991). The spread in my data compared with these others is due to at least two factors. One is the known hysteresis effect that occurs between cool down and warm up in the honey bee (Fahrenholz *et al.* 1989). The other was noted by Esch (1988), that most bees require mechanical stimulation to recover if not moving by the time they are warmed up to 16°C. No mechanical stimulation was provided in my experiments. This would not be a factor in the honey bees' natural environment or in group experiments, where stimulation by fellow workers is constant.

LabVIEW® Interface and Data Acquisition

There have historically been problems with consistency between published data on the thermoregulatory characteristics of an individual worker bee. The discrepancies arise mainly from two sources: a lack of knowledge on the mechanism of heat production in the honey bee and the need for highly sensitive measurement equipment to monitor single insect output levels. These problems have begun to be resolved within the last decade (Esch *et al.* 1991, Blanke and Lensing 1989). The LabVIEW® data acquisition and software interface possess the capability and versatility to provide further solutions to these problems.

It is critical that a large number of variables be considered with indirect calorimetry studies: oxygen consumption, carbon dioxide production, thoracic temperature, ambient temperature, air pressure and air flow rate along with system equipment parameters. For statistical comparison, it is best to monitor all parameters simultaneously. The LabVIEW® hardware used in these experiments accepted a minimum of thirty-two output signals from the test chamber.

Sampling of these signals was controlled by a timer on the circuit board and was accomplished virtually simultaneously (i.e. it took a number of milliseconds to sequence through all of the signals). Additional output boards could increase the number of parameters monitored if needed.

It is desirable that the consistency of test conditions between trials be high in any given study. Computer control assures that experimental conditions are essentially the same for each test trial. Flow rates (sample pump and oxygen analyzer) through chamber(s) and cabinet temperature, for example, can be adjusted on-line at the beginning of a test and set to within a few percent from trial to trial. Checks for proper system operation can be built into the software. In my experiments, the RQ was displayed and updated every two minutes as a check on total system performance. Once the test is begun, parameters can be adjusted without disturbing the insects or interrupting the test.

The system used in this study was set up to monitor six channels per test trial with a manifold containing eight individuals per channel. Actually, any number of individuals can be connected in parallel per channel and still be kept separate. Using these manifolds, a relatively large quantity of individuals can be tested simultaneously in a relatively short period of time. In many cases, it took longer to collect and weigh the bees than it did to run my trials.

Data acquisition, evaluation and graphing of test results can be accomplished on-line. These functions can be integrated into the test program or can be performed as the test is running. The LabVIEW® interface does not have to be pulled up on the monitor; data can be stored to a file and analyzed using any spreadsheet or

statistical program contained on the hard drive of the control computer as the test is running.

The versatility of this system is impressive. Once the hardware is set up, variations in test design and parameters usually require adjustment only to the software interface subroutines. For instance, an additional variable, such as thoracic muscle potential, could easily be monitored with the existing equipment. The same equipment rack was used in this laboratory to perform tests on single individuals, small groups of approximately 200 bees and complete supers, with only minor adjustments to the software and system flow rates.

Individual Behavior Related to Group Response

The distribution of an insect species is influenced by external temperature. Minimum and maximum tolerable heat and cold loads (lethal limits) are important determinants of adaptation and dispersal potential. Groups of *Apis* have developed an unusually wide climatic dispersion for an insect species, through adaptation of a typical homeothermic response (Southwick 1991).

The value of assessing an individual honey bees' 'normal' thermoregulatory performance has been questioned because of the social nature of these insects. Isolated bees react more strongly to experimental temperature fluctuations than do groups of bees, with individual measured values much higher than per bee cluster estimates (Free and Spencer-Booth 1958 & 1960, Harrison 1987, Southwick 1991, Fahrenholz *et al.* 1992). Fahrenholz *et al.* (1992) have shown a decrease in metabolism at low temperatures with group sizes as small as 3-6 bees. However, the importance of the response of an individual to shifts in its thermal

environment cannot be discounted. The ability of a bee colony to produce heat is fundamentally dependent on the ability of an individual bee to thermoregulate (Dyer and Seeley 1987). It is the cumulative response of all individuals in a colony reacting to the thermal conditions in their immediate environment that provide for group thermoregulatory characteristics. *A. mellifera* workers thermoregulate in the range of temperatures found within a hive throughout the course of a normal northern winter. It is group behavioral factors that are responsible for the ability to stay warm at low temperatures through the retention of heat generated by individuals.

The driving force for colonial thermoregulation is unclear. It is not known exactly how physiological and behavioral thermoregulatory mechanisms are coordinated in the performance of temperature control. The question then becomes, to what extent do honey bees behave as individuals within the context of the group? This remains an unanswered question in honey bee biology.

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Appendix 1: Equipment Set-up Procedure for Metabolic Experiments

To do before test start:

set temperature cabinet to start temperature

check desiccant on all channels and on input line

turn on outside air to cabinet and oxygen sample pump

calibrate gas analyzers*

turn on appropriate channel pumps

During setup:

place bee manifolds in cabinet

connect manifolds to the channel air (pump) lines

place thermocouples in syringes

To start test:

open, enter start up data and parameters, start LabVIEW® program BeePat6**

BeePat6 is set to display the output from six channels with three to five thermocouples per channel

*gas analyzer calibration

use a calibration gas and a 'zero' gas of dried, compressed outside air

turn on oxygen sample pump and adjust left ball float to 0.5

turn on zero gas and feed it into temperature cabinet

connect calibration gas to channel one of BeeSniffer (leave channel one relay switch turned off)

Appendix 1

adjust control pump gas flow rate to 80 mL/min

open BeePat6 to monitor the flow status and RQ of the calibration gas

(BeePat6 is located on the AppleSkin HD, in the PatThesis folder)

turn on channel switches 1 and 7

run calibration gas until the following readings are obtained on the gas analyzers:

- CO_2 with Range switch on 1: 0.3 with zero gas flowing (adjust with Zero pot) and 23.0 with calibration gas flowing (adjust with Span pot).
- CO_2 and O_2 with Range switch on 3:

	CO_2	O_2	RQ
Zero gas:	0.6-2.0	20.80-20.92	any value
Calibration gas:	93-98	16.08-16.48	0.92-0.99

(use Reference Adjust on the oxygen analyzer front panel to lower O_2 reading, if necessary)

**program (BeePat6) start up:

open the frontcontrols panel in the BeePat6 folder

click on the arrow in the upper left corner of the display

enter bee weights per channel, filename, date; click DONE button

switch on front control channels that correspond to the test

choose test length, gas delay and chamber cycle time per channel; click START button

Appendix 2: Test Set-up Checklist for Metabolic Experiments

Set-up Checklist

_____Date _____Filename

_____Time bees collected _____Animal I.D.

_____Test start time _____$T_{outside}$

_____Test stop time

_____1. Set cabinet starting temperature:_____; time _____.

_____2. Open the outside control gas (ZERO gas) and adjust to about 5 L/min. Calibrate gas analyzers; check all tubing connections; check desiccant for all chambers and the big canister inside the temperature cabinet-change if necessary.

_____3. Weigh syringes (with food)/get bees/weigh syringes with bees.

_____8. Connect two $T_{ambient}$ TCs and one T_{thorax} TC to each channel.

_____9. Enter test data into BeePat6:

Filename_____ Animal I.D._____

bee weights_____

gas delay time_____ cycle time_____

test length_____

turn on chamber switches _____

_____10. Start BeePat6.

_____11. Check flow to all channels:_____. Check flow to oxygen sample pump_____.

_____10. Overflow on all channels? 7 1 2 3 4 5 6

NOTES:

	pre-test			post-test		
yringe #	w/o bee	w/ bee	W_{bee}	w/o bee	w/ bee	W_{bee}
1						
2						
3						
4						
5						
6						
7						
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Appendix 3: Summary of LabVIEW® Circuit Boards

Board Number	Type	Use
NB-MIO-16L	12 bit digital–›analog converter multifunction I/O	data acquisition interface
NB-MIO-16XL	16 bit D–›A converter multifunction I/O	data acquisition interface
NB-MIO-16LR*	multifunction I/O	data acquisition interface
NB-MIO-16XLR#	multifunction I/O	data acquisition interface
NB-DIO-24R	24 bit digital I/O	channel switching control
NB-DIO-24	24 bit digital I/O	data acquisition control
AMUX-64TR#	I/O with single-ended and differential mode options	thermocouple, pressure, flow rate, O_2, CO_2 readings
SC-2070R*	I/O with differential mode	thermocouple reading
SC-2062R	8 SPDT digital output	channel switching relays

*used in chill-coma experiments only
#used in metabolic experiments only