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Comparative behavior and physiology of feral and domestic honeybees, *Apis mellifera* L.

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The University of Arizona, 1992

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COMPARATIVE BEHAVIOR AND PHYSIOLOGY OF FERAL AND DOMESTIC HONEYBEES, *APIS MELLIFERA* L.

by

Anita Hanna Atmowidjojo

A Thesis Submitted to the Faculty of the

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In Partial Fulfillment of the Requirements
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STATEMENT BY AUTHOR

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ABSTRACT

To determine if temperature and water conservation/ collection play a significant role in the success of feral honeybees, experiments in foraging behavior, temperature tolerance, water balance, hemolymph osmolality regulation and uric acid concentrations of both feral and domestic bees were conducted.

This study shows that feral honeybees collect larger volumes of liquid and have higher critical thermal maxima (CTMs) than domestic honeybees. Feral bees may need more liquid for thermoregulation than do domestic bees. The rate of water loss did not differ significantly between the two types of honeybees. Hemolymph osmolality was slightly significant lower in feral bees. In response to desiccation, both feral and domestic bees showed reduced concentrations of proteins and amino acids in the blood. Therefore both types of honeybees maintained hemolymph osmolality during desiccation. There were no differences in mean concentrations of uric acid in feral vs domestic bees and no difference as a result of desiccation. This indicates that uric acid is regulated but is not an osmoeffector.

INTRODUCTION

RACES OF HONEYBEES

The races (sub species) of honeybees, *Apis mellifera L.*, vary in morphological, behavioral, physiological and colony characteristics. These different characteristics have evolved in response to variability in ecological conditions that correspond to the geographical origin of the races (Winston et al., 1983).

Variations between races can be seen in defensive behavior, swarming, robbing tendencies, amount of drifting (Ruttner, 1988), and preferences in pollen collecting (Mackensen and Nye, 1966). There are differences in flower constancy among four races *mellifera, carnica, caucasia* and *ligustica* and in foraging preferences and dispersal distances (Ruttner, 1988). When nectar is abundant, European honeybees store more nectar than Africanized honeybees (Rinderer et al., 1985; Danka et al., 1986, 1987).

Feral honeybees, although not a distinct race, show differences in biology and behavior in comparison to domestic honeybees. Feral honeybees have a longer life span, and a lower rate of reproduction as measured by swarming than do domestic honeybees (Seeley, 1978; Taber, 1979). Feral nests differ in amount of honey stored in the nest and feral bees often build

exposed nests (Winston et al., 1983). It is suggested feral honeybees posses characteristics essential for survival in natural environments that are unfavorable for domestic bees.

FORAGING BEHAVIOR OF HONEYBEE COLONIES

Colony requirements

Since honeybees need a balanced diet and large amounts of energy, the supply of available pollen and nectar can limit development, productivity, growth, reproduction and winter survival (Seeley and Visscher, 1985; Erickson, 1990). Nectar, pollen, water and resin are needed by honeybees to support life in the colony (Winston, 1987). Pollen is used as source of protein and nectar as source of carbohydrate. Farrar (1968) estimated that to rear brood and feed the adults, the productive colony needs pollen 40 - 60 pounds and several hundred pounds honey annually. Stored honey is also used by the honeybee colonies to thermoregulate in the hive during fall and winter (Rinderer, 1982).

Responses of honeybees to environmental conditions

Diet selection is probably influenced strongly by the climatological constraints which regulate the survival and reproductive capacities of the

honeybee ecotypes (Danka et al., 1987). Many physical factors, such as mean daily temperature, solar radiation, relative humidity, monthly rainfall, wind, photoperiod (day-length), and plant physiological processes, such as photosynthesis, respiration, translocation, transpiration, and flower characteristics interact with each other and with the colony to determine honeybee foraging patterns (Winston, 1987; Seeley, 1985).

The availability of nectar and pollen in plants limits forager activity. Nectar secretions vary among flowers even within species depending on soil conditions and shaded areas (Gould and Gould, 1988). Sugar concentration of nectar varies in different regions and years, depending on many factors such as air temperature, humidity, soil fertility, ground water, precipitation and global solar radiation (Shuel, 1975; Corbet et al., 1979).

Shuel (1955) found a positive correlation between nectar production and the amount of solar energy or radiation received by the plants in the preceding 24 hours. When day-length was 20 hours in Canada, the nectar of legumes was secreted in large volume. In another experiment, Shuel (1975) reported a correlation between nectar secretion and temperature or sunshine in temperate climates. The nectar of white clover (*Trifolium repens*) increases when ambient temperatures during the day are high and low at night. He concluded that the activity of enzymes which are involved in nectar secretion are influenced by ambient temperatures. He further showed that nectar

secretion is limited by water supply and minerals in the soils.

Workers collect nectar and pollen at certain times of the day (Michener, 1974; Crane, 1975). These foraging activities are influenced by light intensity, temperature, nectar secretion and pollen dehiscence. Nectar and pollen foraging behaviors are affected by genetic and environmental factors (Ribbands, 1953; Winston, 1987). Crane (1975) reported that honeybees collecting nectar (or pollen) follow the fluctuating of availability nectar secretion annually and the plant flowering cycle (Mattu and Verma, 1985). Nectar or pollen gatherers usually forage above temperatures of 12-14°C. Wind and rain will decrease foraging activities. Flight patterns can be related to the time of day, usually by early to mid afternoon forager numbers will decrease. Winston (1987) suggested that nectar availability in flowers decreases in the afternoon.

Foraging choice can be affected by the degree of insolation of flowers (Roubik, 1989). Flowers can be used to trap solar heat so body temperatures of foragers in cool ambient temperatures will increase (Kevan, 1975). Percival (1965) explained that nectar secretion of flowers in direct sunlight is earlier and greater than in flowers in shaded areas.

The composition and quantity of sugars in nectar collected by honeybees per flower are varied (Winston, 1987; Roubik, 1989). Spring nectars harvested by honeybees in New York have 10 to 68% sugars (Southwick et al., 1981). In Panama, honeybees visited flowers with sugar concentration in nectar 10 to

65%.

EFFECTS OF TEMPERATURE ON INDIVIDUAL BEES

The large amounts of heat generated by bees may place additional stress on them in hot arid environments. Stress, illness and the death of organisms can be caused by fluctuations of the physical environment through time and space (Huey and Bennett, 1990). To minimize the worst effects of those fluctuations, organisms respond with immediate adjustments in behavior and physiology. Temperature is one important environmental variable that can produce changes in behavior and physiology as in control of body temperature (temperature tolerance) and the longer term process of acclimatization.

Several factors affect lethal temperature thresholds such as genetic background, acclimation temperature, size, age, hormonal state, diet and environmental factors such as oxygen and photoperiod. Increasing body temperature in terrestrial insects decreases oxygen transport and increases water loss through evaporation, and causes insects to become desiccated. Enzymes become inactivated, and cuticular permeability increases (Prosser, 1973).

A parameter frequently used in studies of the tolerance of organisms to temperature is the critical thermal maximum or CTM. In vertebrates, CTM is

defined as the temperature that induces torpor at 100% humidity (Brattstrom, 1965). In insects, CTM is the temperature at which the insects undergo 'knock down' or the inability to right themselves immediately when on their backs (Jackson and Cohen, 1984).

Morphological responses to high temperatures

The body hairs of bees function to reduce heat. Abdominal hair on 6 races of *Apis mellifera* showed a gradation in length from short (0.22 mm for bees which live in hot environments) to long (0.51 mm for bees living in cool environments) (Southwick, 1985). Increased hairiness reduces loss of heat from flight muscles. The hairy races of honey bees have a good tolerance to cool and freezing weather (Southwick, 1985; Seeley and Heinrich, 1981).

Behavioral responses to high temperatures

Honeybee colonies respond to increased temperature by increasing evaporative heat loss from the hive. One behavioral response of honeybees to increasing temperature is the fanning of their wings at the hive entrance for air circulation to cool the hive (Kronenberg & Heller, 1982). The increase of hive temperature will also stimulate bees to collect water that is evaporated in cells

(Heinrich, 1979; 1981). When the hive becomes overheated many bees leave the hive to cluster outside the entrance. As a result, less heat is produced by the bees remaining inside hive and the temperature inside decreases (Dunham, 1931: Lensky, 1958).

Physiological responses to high temperatures

Acclimatization

Heinrich (1980) observed that at 45-50°C, honey bees survived only four hours, but at 10-15°C, the bees could survive for several days (Free and Spencer-Booth, 1960). Free and Spencer-Booth (1962) reported that more honeybees which were acclimated to normal hive temperatures (35°C) survived at 45 - 47°C than honeybees which were acclimated to ambient temperature (20°C).

Heat shock proteins (HSPs)

The majority of organisms will die if they are exposed to severe heat shock. If they survive, they will respond to heat shock at the cellular level, by using alternative metabolic pathways of biological processes (Morimoto et al., 1990) or the induced synthesis of some proteins named "heat shock proteins" (HSPs) (Ashburner and Bonner, 1979; Lindquist, 1986). Petersen and Mitchell

(1985) define heat shock proteins as a group of proteins synthesized in response to stimulation by temperature and chemical or environmental changes.

The range of temperature tolerance of organisms in their natural environment is influenced by the production of HSPs (Lindquist, 1986; Riabowol et al., 1988). Different habitats affect these resistance adaptations. For example, insects from hot and dry deserts will adjust their bodies to higher optimal temperatures and critical thermal limits (Prosser, 1973).

EFFECT OF WATER LOSS ON INDIVIDUAL BEES

Water requirements of insects

Water is essential for insect life because it has important roles in biochemical reactions, diffusion of solutes, heat distribution, temperature control and processes of basic metabolism (Prosser, 1973; Edney, 1977). If insects fail to minimize water loss or dehydration, it can cause death.

Insects, because of their small size, have a large ratio of surface area to volume, so water loss is a constant problem (Price, 1985). To maintain water balance under xeric conditions, terrestrial insects have physiological, structural and behavioral adaptations that maximize water gain and minimize water loss under variable environmental conditions (Edney, 1957, 1974; Beament, 1958, 1960; Arlian and Eckstrand, 1975; Appel and Rust, 1985).

Honey bees will suffer heat stress, if water is unavailable, followed by death of all adults and brood in harsh conditions (Crane, 1990). Bees which gather nectar containing 5% or less of sugar are categorized as water carriers (Sylvester et al., 1983). Honeybees use water to cool the hive and to dilute honey for larval food (Gary et al., 1979).

Pathways of water loss

In insects, water can be lost via transpiration, excretion (including defecation, quinone secretion and spinning, and egg laying) (Flemister, 1964; Edney, 1977), and the oral and anal openings (Cooper, 1983). Water loss through transpiration consists of both respiratory water loss and transcuticular water loss (Edney, 1977; Zachariassen et al., 1987). In resting insects, the rate of water loss via spiracles is lower than via the cuticle. In flying insects, the rate of water loss via spiracles is higher than the cuticle because insects actively ventilate their tracheal system (Chapman, 1982). During desiccation, most water loss is by transpiration, and faecal water loss represents only a small part of the total (Wall, 1970). The survival of terrestrial insects completely depends on a low level of water loss through the excretory and respiratory systems (Rafaeli and Applebaum, 1982).

Resistance to desiccation

The term *resistance to desiccation* has two meanings. The first meaning relates to a tolerance of large volumes of water loss and low body water content. The second meaning pertains to a limitation of water loss from living tissue (Edney, 1977). For arthropods living in arid climates, restriction of water loss is the most important means of resistance to desiccation (Edney, 1974).

Different insect species can tolerate water loss ranging from 17% to 89% of normal body water content (Arlian and Staiger, 1979; Edney, 1974, 1977; Bursell, 1974). The loss of more than 15-25% of body water in most species of insects can cause death (Toolson, 1987).

MECHANISMS AGAINST WATER LOSS

Morphological adaptations against water loss

Spiracle structure may also affect rates of water loss. Insects living in dry environments tend to have small spiracles (Richer, 1969). In addition some insect spiracles are sunken or hidden (Cloudsley-Thompson, 1975).

Most insects killed in dry air die from desiccation rather than by over heating (Prosser, 1973). The structure of the cuticle, as well as the chemical composition of cuticular lipids, restricts water loss (Edney, 1977). The outer

layers of the cuticle consist of a thin layer of lipid (usually wax) which plays a very important role in the physiology of insects, protecting them from dehydration (Beament, 1976; Wigglesworth, 1976). Degree of waterproofing is correlated with composition of epicuticular hydrocarbons. Xeric adapted species are characterized by long-chain hydrocarbons which serve to restrict water loss (Hadley, 1978).

The major barrier to cuticular water loss is the epicuticular lipid layer. Rates of water loss and the permeability of the cuticle can be affected by environmental factors, such as temperature and humidity (Beament, 1958; Edney, 1977). At a constant temperature, the rate of water loss via evaporation through the cuticle is proportional to humidity and to wind speed (Chapman, 1982).

Lockey (1985; 1988) noted the main functions of cuticular lipids is to limit desiccation due to transpiration. Desert insects are adapted to high saturation deficits by decreasing permeability of the cuticle which affects the rapid rate of evaporative water loss and involves physiological and biochemical mechanisms (Cloudsley-Thompson, 1975; Toolson and Hadley, 1977; Hadley, 1980; Blomquist et al., 1987; Toolson, 1987). Usually, cuticle permeability changes until a certain temperature, known as the transition temperature, is reached. Edney (1977) suggests that cuticular permeability is low at low humidity, promoting water conservation and retention.

Blomquist et al. (1980) studied the cuticular wax of honeybees. Hydrocarbons are identified as the major components of cuticular wax of the honeybees. Hydrocarbons (58%) composed the largest portion followed by monoester (23%) and lower amounts of diester, triester, free fatty acids and more polar material. Among hydrocarbons, alkanes are the major components (48%), and 52% of hydrocarbons are unsaturated. Methyl branched alkanes contribute to the waterproofing of the cuticle (Jackson and Blomquist, 1976; Lockey, 1988; Espelie and Brown, 1990).

Cuticular lipid is synthesized by oenocytes (Wigglesworth, 1976), and is probably transported to the cuticle by lipophorin, a lipid-transporting lipoprotein in insect hemolymph (Chino and Nelson, 1978).

Behavioral adaptations against water loss

Desert cockroaches live in habitats that are dry and hot, but they use a behavioral mechanism to avoid extreme conditions. During the day, the desert cockroach *Arenivaga* spp. avoids water loss by burrowing at depths of 20 - 60 cm in dune sand. At night, the males can be found at the surface and females and nymphs 1 - 3 cm bellow the surface (Hawke and Farley, 1973).

Physiological adaptations against water loss

Closure of the spiracular opening is a physiological response to desiccation by restricting tracheal water loss (Mellanby, 1934; Wigglesworth, 1945; Miller, 1964; Edney, 1977). Changes in temperature and humidity can rapidly effect the spiracular closure so that respiratory water loss will be limited (Bursell, 1957; Loveridge, 1968; Ahearn, 1970; Cooper, 1983). Rates of spiracular openings and water loss are affected by age in some insects (Bursell, 1974; Edney, 1977).

Maintenance of hemolymph osmolality

Insect hemolymph is defined as "the extracellular circulating fluid that fills the body cavity or hemocoel" (Mullins, 1985). One important function of the hemolymph is to ensure insect survival under severe conditions such as low temperature and dehydration (Patterson and Duman, 1978). Hemolymph is composed of water and a fluid plasma volume with nucleated cells (Chapman, 1982). Water makes up the largest part of the hemolymph and plasma volume varies depending on life stages and physiological state (Mullins, 1985). Insect hemolymph contains both inorganic and organic solutes (Florkin and Jeuniaux, 1974). Organic solutes include carbohydrates, nitrogenous constituents (such

as proteins, amino acids), lipids, free fatty acids, organic acids, organic phosphates and polyols. Inorganic ions include sodium, potassium, calcium, magnesium, phosphate and chloride (Mullins, 1985).

Osmolality or *hemolymph osmotic pressure* "is a measure of the total number of moles of solute per liter of hemolymph" (Mullins, 1985). Osmotic pressures among insects vary between 250 mOs and 700 mOs (Buck, 1953; Florkin and Jeuniaux, 1974; Wharton and Richards, 1978). Insects have the ability to regulate osmolality of the hemolymph under conditions of severe stress (Edney, 1977).

The hemolymph and body tissues are the two major water reservoirs in insects, and water can be transported between the hemolymph and the tissues (Nicholson, 1980). Ionic concentrations are maintained by moving solutes from one water compartment to another : i.e., by moving Na^+, K^+ and urate from the hemolymph to the fat body or by moving Cl^- and urate from the hemolymph to the hind gut (Arlian and Veselica, 1979).

In insects, physiology and behavior can be influenced by the changes in hemolymph concentration (Chapman, 1982). Most of the water lost from insects during desiccation comes from the hemolymph. In many insects, osmolality remains relatively constant during dehydration even though hemolymph volume decreases (Edney, 1977).

Many arthropods actively regulate hemolymph volume and osmolality

(Edney, 1977). Regulation of osmolality is important because chemical and osmotic composition which affect the activities within cells, work most efficiently within a very narrow range (Schin and Moore, 1977; Chapman, 1982). Besides, homeostasis can be influenced by physiological parameters such as osmotic pressure and hemolymph volume, diet, development, molting, and environmental stress (Punzo, 1990). Osmolality tends to rise during dehydration because of decreasing hemolymph volume. In order to maintain osmolality, osmotically active substances must be removed from the hemolymph during dehydration and returned to it in rehydration (Nicholson, 1980).

Hemolymph proteins and free amino acids

Hemolymph proteins help to maintain osmotic balance in many insect species (Collet, 1976; Evans and Crossley, 1974; Junnikkula, 1977). Hemolymph proteins have roles in development (Chen, 1966; Wyatt, 1980), storage (Wyatt and Pan, 1978; Mullins, 1985), transport (Chino, 1981; 1985), immunity (Wyatt and Pan, 1978; Wyatt, 1980) and reproduction (Wyatt and Pan, 1978). Usually the hemolymph contains all of the amino acids that make up proteins (Chen, 1985; Mullins, 1985).

In Endopterygotes, the concentration of free amino acids in hemolymph

is high compared to other animals and is variable depending on family and insect species (Sutcliffe, 1963; Florkin and Jeanniaux, 1974; Chen, 1985). Disease, diet, insect activities, stage of development, molting (Florkin and Jeanniaux, 1974), starvation (Cohen and Patana, 1982), and stress (Collett, 1979) influence the concentration of amino acids (Mullins, 1985).

One interpretation of the high organic content of hemolymph is that amino acids are involved in osmoregulation (Lazar and Mohamed, 1988). The free amino acids in the hemolymph appear to play an important role in the maintenance of osmotic pressure in Hymenoptera, Lepidoptera and some Coleoptera (Buck, 1953; Wyatt, 1961; Sutcliffe, 1963; Florkin and Jeanniaux, 1974). Naidu and Hattingh (1986) suggested that removal of amino acids from the hemolymph occurs during dehydration.

Uric acid regulation

An important function of the excretory system is to void excess nitrogen ingested from food. Primary urine, a watery substance containing ions, amino acids and nitrogenous waste products, is produced in the Malpighian tubules and then is transported to the anterior hind-gut (Wigglesworth, 1972). Other materials, including uric acid, are transported by the hemolymph, and pass into the Malpighian tubules during the waste-secretory phase (Cochran, 1975;

Chapman, 1982). In a number of insect species, uric acid is actively transported from the hemolymph to the Malpighian tubule lumen (Maddrell, 1971, 1981; Wessing and Eichelberg, 1978).

Uric acid is the major form of nitrogenous excretory product for a number of insect species. It is insoluble and relatively non-toxic. Uric acid can be excreted as dry powder, requiring minimal water for excretion so the insect can conserve water (Cochran, 1985).

OBJECTIVES OF THE STUDIES

Feral honeybees (*A. mellifera L.*) have lived in southern Arizona for at least one and probably four hundred years (Sheppard, 1989). Population size of feral honeybees is restricted by lack of suitable nesting sites (Ruttner, 1988). In the forested temperate environments, the nests of feral bees are cavities of trees (Seeley and Morse, 1976). In dry and arid areas, cavities in the ground are utilized most extensively (Erickson, unpublished). Since feral bees are generally believed to be adapted to local conditions (Erickson, 1990), desert-dwelling feral bees may be specially adapted to environments subjected to water and temperature stress. It is possible that temperature and water conservation / collection responses may play a significant role in the adaptive biology of these bees. Therefore, I examined the foraging activities of feral and

domestic honeybees relative to nectar and water collection to determine if there were differences in volumes collected or in preferences for concentrations of nectar over time. In addition, I examined indicators of adaptive mechanisms favoring the survival of honeybees in the arid Southwest. These included thermoregulation, water balance, and the ability to regulate hemolymph osmolality and uric acid concentrations in individual feral and domestic honeybees.

MATERIALS AND METHODS

ANIMALS

The honeybees used in this study were of two types, feral and domestic. The feral honeybees were derived from a single colony which was caught in a swarm trap at Picacho Peak, Phoenix, Arizona, in June 1990. This colony, with the queen marked, was maintained at the Carl Hayden Bee Laboratory, Agriculture Research Service, USDA, Tucson, Arizona.

The foraging behavior studies utilized bees derived from the feral colony. These were compared to bees from control colony that were derived from a single domestic colony obtained from the USDA closed population.

To limit the differences in environmental factors such as temperature and humidity, all colonies were maintained in a single apiary at the Carl Hayden Bee Research Laboratory, ARS, USDA, Tucson, Arizona. The data for temperature and humidity in the vicinity of the experimental site were obtained from the National Climatic Data Center (NCDC), Asheville, North Carolina 28801.

All specimens for the studies on temperature tolerance, water loss rates, and hemolymph composition were taken directly from either the feral colony or the domestic colony. To obtain specimens for study, a flight screen-wire cone was placed against the entrance of the hive to capture foragers (Gary, 1967).

After 15 minutes, the flight cone was removed from the hive and covered with black cloth. The bees were transferred to small cups, which were then transferred to a cooler containing ice, for transportation into the laboratory. Prior to being used, bees were placed in a refrigerator for 30 minutes to immobilize them, in order to avoid regurgitation problems and to minimize the possibility of stinging and resultant harm to the bees (Pesante et al., 1987).

FORAGING BEHAVIOR

One colony each of feral and domestic bees were used in this experiment. Twenty returning foragers from each feral or domestic colony were captured randomly by using a modified pollen trap and vacuum aspirator (Erickson et al, 1973). To block the outgoing flight of honeybees, a metal gate was inserted in the pollen trap during sampling. Plastic sample bags were used to collect and store samples by fitting them to the aspirator head. The samples were placed in a cooler with ice (as described above) before bees were carried to the laboratory. In the laboratory, samples were stored in the refrigerator before analysis.

The experiments were conducted in November 1991 and the samples were collected three times daily at 10:00 a.m, 12:00 noon and 2:00 p.m for three days (Erickson et al.,1973).

Bees with pollen loads were discarded prior to analysis. The abdomen from each of 20 randomly selected bees was pulled apart to draw out the honey stomach. The honey stomach contents of each bee was then extracted by squeezing with the finger tips. Care was taken to avoid contamination of the nectar. The liquid was drawn into a 10, 5 or 1 μl microcapillary pipette (Drummond Scientific Co, Broomall, PA) (Erickson et al., 1973; Sylvester et al., 1983). The sugar concentration (percent, wt/wt) was measured by placing a drop of the liquid into the refractometer (Bausch and Lomb, Rochester, N.Y) (Bolten et al, 1979).

TEMPERATURE TOLERANCE (CTM)

The Critical Temperature Maximum (CTM) is the temperature at which the bees fail to undergo of an immediate righting response (Cohen and Pinto, 1977; Jackson and Cohen, 1984). In order to compare the CTM of feral and domestic bees, the modified oven of a gas chromatograph (Model 1400, Varian Ins®, Sunnyvale, CA) was used to produce the necessary temperature regimes. A sheet of clear plexiglass was installed in the oven door, so that the test subjects could be observed (Appel et al., 1983). Plastic vials, 25 mm in diameter, were cemented to the bottom side of a sheet of clear plexiglass. These were used as observation cages for the individual test subjects. The lids

of the vials were perforated and covered with organdy cloth to allow air to flow over the bees. The oven was temperature programmed to start at 30°C and increase to 60°C at the rate of 0.5°C /minute. The temperature was monitored by a BAT 6® microprobe (Bailey Instruments Saddle Brook, N.J) placed in one of the cages.

In order to maintain relative humidity at 100% in the cage , saturated sponges were put in Petri dishes inside the oven. Ten bees from the feral colony and ten bees from the domestic colony were then removed from the refrigerator and allowed to warm up to room temperature before the oven was turned on. The experiment was conducted every month from November 1990 until January 1992.

The dorsal turning reflex (Cohen and Pinto, 1977; Jackson and Cohen, 1984) was used to assess temperature tolerance. Bees unable to right themselves immediately were classed as intolerant to the given temperature. It was assumed that insect body temperatures were equivalent to the ambient temperature. It has been demonstrated in other insects, similar in body mass to honey bees, that core temperatures reached ambient temperatures within 30-45 seconds (Cohen and Pinto, 1977).

WATER LOSS

Rates of water loss were estimated gravimetrically as described by Cohen (1982). The test chamber consisted of a plastic box 27 x 40 x 15.5 cm deep with a lid of clear plexiglass. The space between the box and the lid was sealed with a vinyl foam gasket.

Individual bees were weighed to the nearest 1 mg using a Cahn Model 25 automatic electrobalance (Cahn Instruments, Cerritos, CA). For weighing, individual bees were placed in small, light tubes (<600 mg), made from nylon organdy, with the ends stapled shut.

Cages were placed in desiccation jars that contained appropriate solutions of sulphuric acid, water, or drierite (Table 1). The experimental groups were maintained at 25, 30 or 35°C and at 0, 25, 50, 75 or 100% relative humidity using three incubators (Whirpool, RCA 15). Thus, there were 15 experimental treatments. Three replicates of each experiment were performed. For each replicate, the test subject was exposed to experimental conditions for two hours and then reweighed . Rates of water loss were expressed as $mg.g^{-1}.h^{-1}$, (Edney, 1977). Treatments were randomized within each treatment and overall temperatures. Nine hundred bees from both feral and domestic population were used in this experiment.

Table 1. Composition of substances added to a desiccation jars to maintain relative humidity.

% humidity	Composition
0	drierite
25	56% H_2SO_4 solution
50	44% H_2SO_4 solution
75	31% H_2SO_4 solution
100	distilled water

Note: all solutions are expressed as v/v percentages (Solomon, 1957).

HEMOLYMPH OSMOLALITY, PROTEINS AND FREE AMINO ACIDS

Twenty bees each from the feral colony and twenty bees from the domestic colony were selected for study of hemolymph osmolality. Half of the bees from each group were desiccated by placing the bees in an incubator at 30°C and 0% humidity for two hours. Honeybees were then immobilized on a wax plate with pins. The right forewing was removed at the distal end with forceps. One μl of hemolymph was collected from each honeybee using a 1 μl microcapillary pipette (Drummond Scientific Co, Broomall, PA). Samples of approximately 1-2 nanoliters were placed in platform wells (of a Direct Reading Nanoliter Osmometer-Clifton Technical Physics, Hartford, New York) that were filled with high viscosity immersion oil. Standards of NaCl (500 or 1000 mOsmol/kg) solution and distilled water were simultaneously analyzed with samples. The melting point of each sample was measured by direct observation of disappearance of the last crystal and simultaneous observation of the digital reading which was calibrated to osmolality.

Twenty bees from the feral colony and twenty bees from the domestic colony were selected for hemolymph protein study. Half of the bees from each group were desiccated using the procedures described above. The hemolymph was stored in a -20°C freezer until analysis.

Total hemolymph protein concentrations were determined

Spectrophotometer at a wavelength 595 NM, Response I Spectrophotometer (Guilford Instruments, Oberlin, Ohio). Bovine serum albumin (Sigma Chemical, St. Louis, MO) was used as a standard at concentrations of 0, 10, 15, 20 and 25 μl/ml.

In the study of amino acids, twenty bees each from feral and domestic colonies were used. Ten bees from each group were dehydrated using procedures described above. Hemolymph was collected as described above. Total hemolymph amino acids were measured spectrophotometrically as ninhydrin positive substances according to the methods of Moore and Stein (1954) and Keleti and Lederer (1974). Leucine was used as the standard in concentrations of 0, 5, 10, 15 and 20 μg/μl. The samples were assayed at 570 NM.

URIC ACID

Twenty bees from both feral and domestic colonies were used in this study. Half of the bees in each group were desiccated using the procedures described above. Each bee was dissected and the Malpighian tubules and hind gut were removed, placed in capped plastic tubes, then held at -20°C before analysis. The Malpighian tubules and hind gut were weighed to the nearest 10 μg using a Sartorius MC1 balance.

μg using a Sartorius MC1 balance.

The instrument used for analysis of uric acid was a Beckman Model 332 HPLC operated isocratically. Separation of samples was achieved with a 4.6 x 250 mm column packed with Ultrasil ODS (C-18)(10μm) and a 10 μm ODS pre-column (Cohen, 1983).

For determination of uric acid in the hemolymph, one hundred μl of 0.5% Li_2CO_3 solution were added to the hemolymph sample. For determination of uric acid concentrations in excretory tissue, samples were mixed with 1 ml of Li_2CO_3 solution and were ground and mixed thoroughly in a micro tissue grinder. Samples were then placed in a microfiltration apparatus and filtered through a 0.45 μ filter (Bioanalytical Systems Inc., West Lafayette, IN) at 5000 r.p.m for 5 minutes at 4°C in a refrigerated centrifuge from Sorvall RC-2 (Ivan Sorvall Inc., Norwalk, CN). A 20 μl sample of this solution was injected into the HPLC (Cohen and Jackson, 1986) and detected at 254 nm. A Beckman CRI - A recording integrator was used to quantify the area under peaks (Cohen, 1983).

The solvent was pumped at 1.0 ml/min 900 : 100 : 25 of H_2O : MeOH : Pic-A (Waters Associates, Milford, MA). To compare retention times and peak areas, authentic standards of hypoxanthine, xanthine and uric acid (Sigma Chemicals, St.Louis, MO) were used with quantification by a Shimadzu CR-1A Recording Integrator (Cohen, 1983).

STATISTICS

Means and standard errors for foraging behavior, temperature tolerance, water loss, hemolymph proteins, amino acids, uric acid, and osmolality were calculated. The data were tested by one way of ANOVA (Analysis of Variance) and Duncan's Multiple Range Test with SAS (Zar, 1984; SAS Institute Inc., 1989). All tests in the study were evaluated at the 5% level of significance.

RESULTS

FORAGING BEHAVIOR

Foraging for liquid by feral and domestic workers was quantified in 3 ways : percent of foragers returning with nectar and water, liquid volume and liquid concentration. The profile analysis of nectar and water foraging is shown in Tables 2 and 3. In both feral and domestic honeybee colonies, the majority were nectar foragers during three days of observations. On the first day of observation more than 80% of total foragers were nectar foragers, but the third day the percentage decreased to 60%. There were no significant differences between feral and domestic honeybees in the allocation of foragers to nectar and water collection.

The mean volumes of honey stomach contents for feral and domestic honeybees are shown in Figure 1. In feral bees, the highest volumes were recorded at midday on November 26. In domestic honeybees, the highest volume recorded was in the morning, and the lowest was at 12:00 noon on all three days. Analysis of variance showed significant differences in the volumes of liquid collected (Table 4, $P<0.0001$). And feral honey bees collected significantly larger mean volumes ($10.06 \pm 0.42\ \mu l$) than domestic honeybees ($8.4 \pm 0.36\ \mu l$) ($P<0.0236$). There was significant difference in effect of time

Table 2. Foraging profile analysis of feral bees

Tucson, AZ. Nov, 1991.

(Total # = 30).

Date or time	% collectors	
	water	nectar
	Between days - across all times	
Nov. 26	16.67	83.33
Nov. 27	16.67	83.33
Nov. 29	40.00	60.00
	Between times - across all days	
10:00 a.m	13.33	86.67
12:00 noon	20.00	80.00
2:00 p.m	40.00	60.00

Table 3 . Foraging profile analysis of domestic bees.

Tucson, AZ. Nov. 1991.

(total # = 30).

Date or time	% collectors	
	water	nectar
	Between days - across all times	
Nov. 26	13.33	86.67
Nov. 27	36.67	63.33
Nov. 29	36.67	63.33
	Between times - across all days	
10:00 a.m	30.00	70.00
12:00 noon	16.67	83.33
2:00 p.m	40.00	60.00

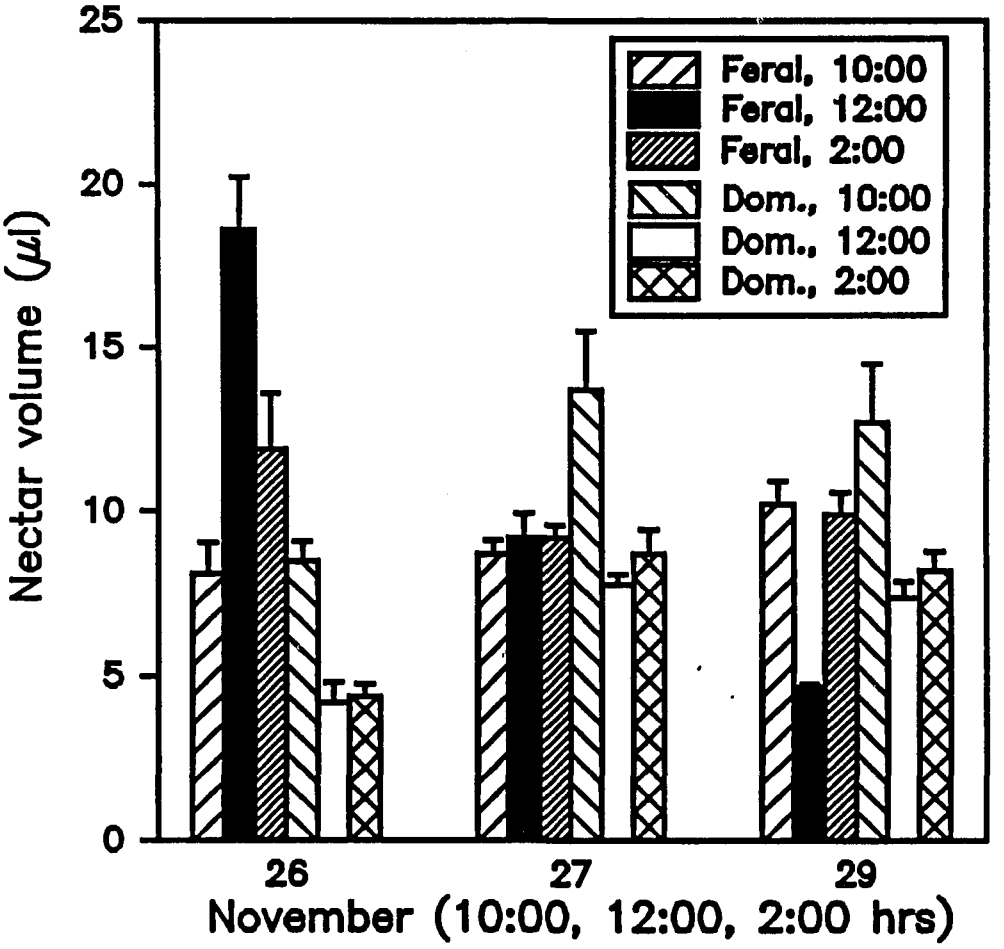

Figure 1. Nectar volumes collected by feral and domestic honeybees.

Table 4. Analysis of variance of volumes of nectar and water collected by feral and domestic honeybees.

Source	DF	SS	Mean square	F value	Pr > F
Colony	1	179.1249	179.1249	5.22	0.0236
Time	2	331.2664	165.6332	4.83	0.0092
Day	2	31.6025	15.8012	0.46	0.6318
Source**	1	694.9555	694.9555	20.25	0.0001
Colony* Time	2	679.9023	339.9512	9.91	0.0001
Colony* Day	2	296.5022	148.2511	4.32	0.0149
Colony* Source	1	12.1209	12.1209	0.35	0.5531
Time* Day	4	233.6799	58.4200	1.70	0.1520
Time* Source	2	300.1049	150.0525	4.37	0.0142
Day* Source	2	198.2321	99.1161	2.89	0.0586

Note : Source** = liquid (water and nectar).

of day on volume of nectar and water collected by both colonies ($P<0.009$) and there no significant difference among the three days ($P=0.632$).

The means of nectar concentration collected by feral and domestic honeybees are shown in Figure 2. There was a significant difference overall in concentrations ($P<0.0001$)(Table 5) and a significant difference in concentration of nectar collected at different times of day ($P<0.04$). In feral bees, the higher nectar concentrations were recorded in the afternoon on three days observations, and no consistent pattern for domestic honeybees. However, there were no significant differences in nectar concentration between feral and domestic honeybee colonies.

From this experiment, I concluded there are no significant differences between feral and domestic honeybees in proportion of nectar foragers and nectar concentration collected by feral and domestic honeybees, but there is a difference in nectar volume.

TEMPERATURE TOLERANCE (CTM)

The CTMs (Critical Thermal Maxima) of feral and domestic honeybees are shown in Figure 3, along with the average monthly temperatures at the experimental site. In general, the temperature tolerance of feral bees was higher than that of domestic bees. Analysis of variance showed a highly significant

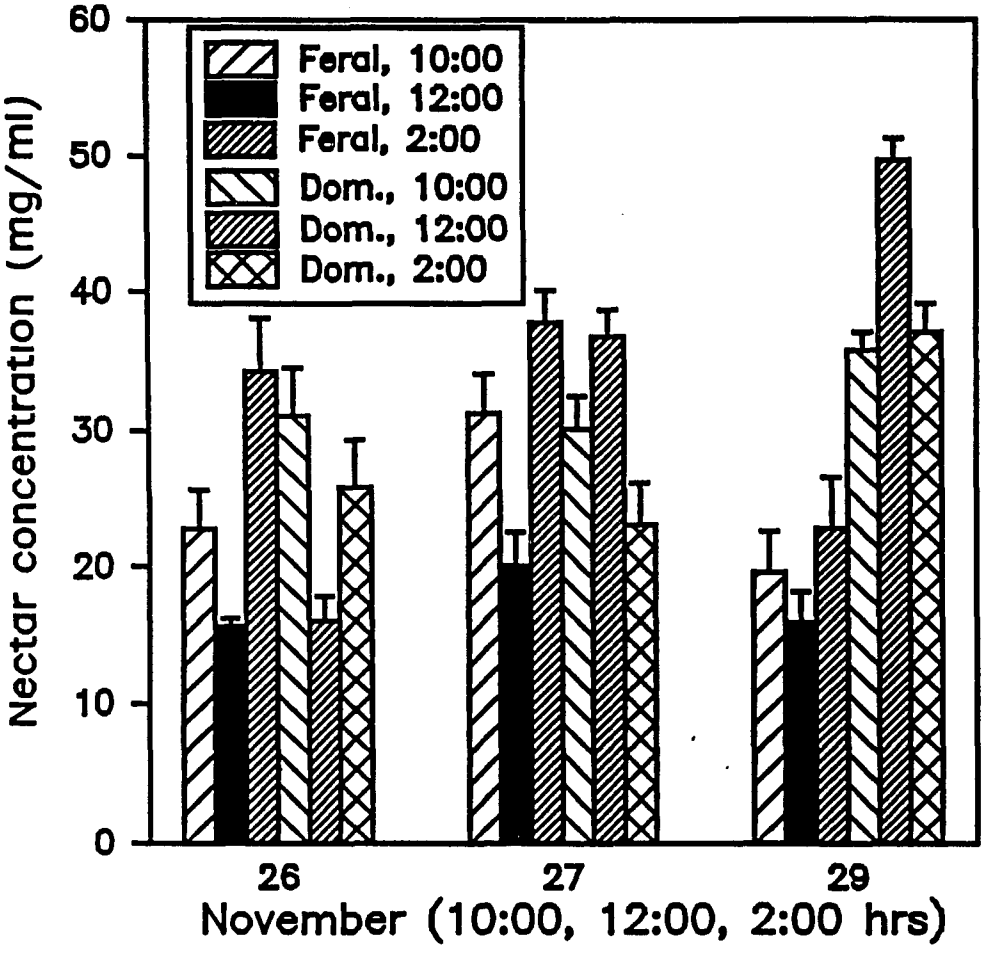

Figure 2. Nectar concentration collected by feral and domestic honeybees.

Table 5. Analysis of variance of concentration of nectar collected by feral and domestic honeybees,

Source	DF	SS	Mean square	F value	Pr > P
Colony type	1	128.9538	128.9538	0.84	0.3600
Time	2	979.2595	489.6298	3.20	0.0434
Day	2	64.2599	32.1300	0.21	0.8109
Source**	1	18512.9379	18512.9379	120.97	0.0001
Colony* Time	2	1049.1205	524.5602	3.43	0.0349
Colony* Day	2	703.5712	351.7856	2.30	0.1037
Colony* Source	1	237.5713	237.5713	1.55	0.2146
Time* Day	4	2940.2061	735.0516	4.80	0.0011
Time* Source	2	128.5531	64.2765	0.42	0.6578
Day* Source	2	46.4571	23.2286	0.15	0.8593

Note : Source** = liquid (water and nectar).

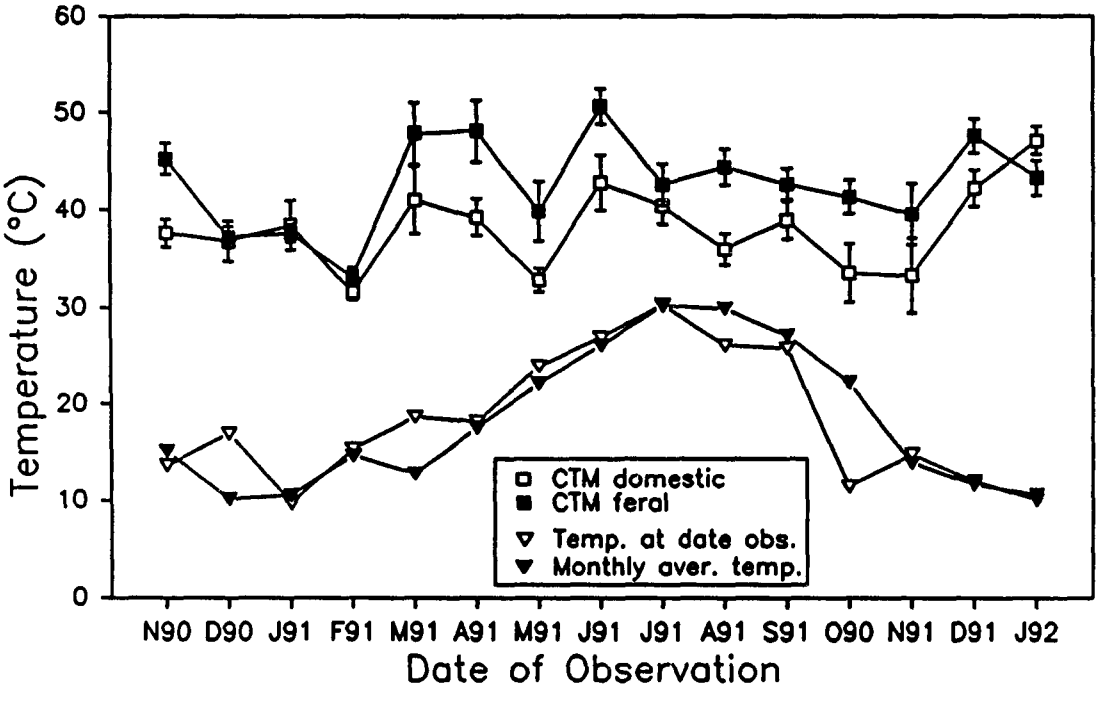

Figure 3. Temperature tolerance of feral and domestic honeybees.

Table 6. Analysis of variance of temperature tolerance of feral and domestic honeybees

Source	DF	SS	Mean square	F value	Pr > P
Colony type	1	1429.2101	1429.2101	29.31	0.0001
Date	14	4763.7059	340.2647	6.98	0.0001
Colony * Date	14	1002.1599	71.5828	1.47	0.1227

difference between CTMs of domestic and feral bees ($P<0.001$). Each value shows the means ± SE calculated from 10 bees. There was also a highly significant effect of sampling date on CTMs ($P<0.0001$) (Table 6).

The highest mean CTM for the feral bees was 50.7 ± 1.9°C recorded in June 1991. The highest mean CTM for domestic honeybees (42.8 ± 2.8°C) was also recorded in June 1991. These maxima were not correlated with the highest of mean ambient temperature (30.2°C) which was recorded in July 1991. Regression analysis, showed no correlation between CTM and the ambient temperatures (Figure 4). The lowest CTM for feral honeybees (33.2 ± 0.9°C) and the lowest CTM for domestic honeybees (31.6 ± 0.8°C) were both recorded in February 1991. The lowest mean ambient temperatures were recorded in January of 1991 (9.5°C) and 1992 (9.4°C).

The results of a Duncan's Multiple Range test for CTMs are shown in Table 7. Mean CTM values with the same Duncan grouping letter were not significantly different at the $P < 0.05$ level. The highest mean CTM for feral and domestic honeybees (combined) was 46.8 ± 1.9°C in June 1991. This value is significantly different from values found at other times. The lowest mean CTM (32.4 ± 0.6°C) occurred in February 1991. This value was significantly lower than all other mean CTM values.

Figure 4. Regression of CTMs on mean ambient temperature

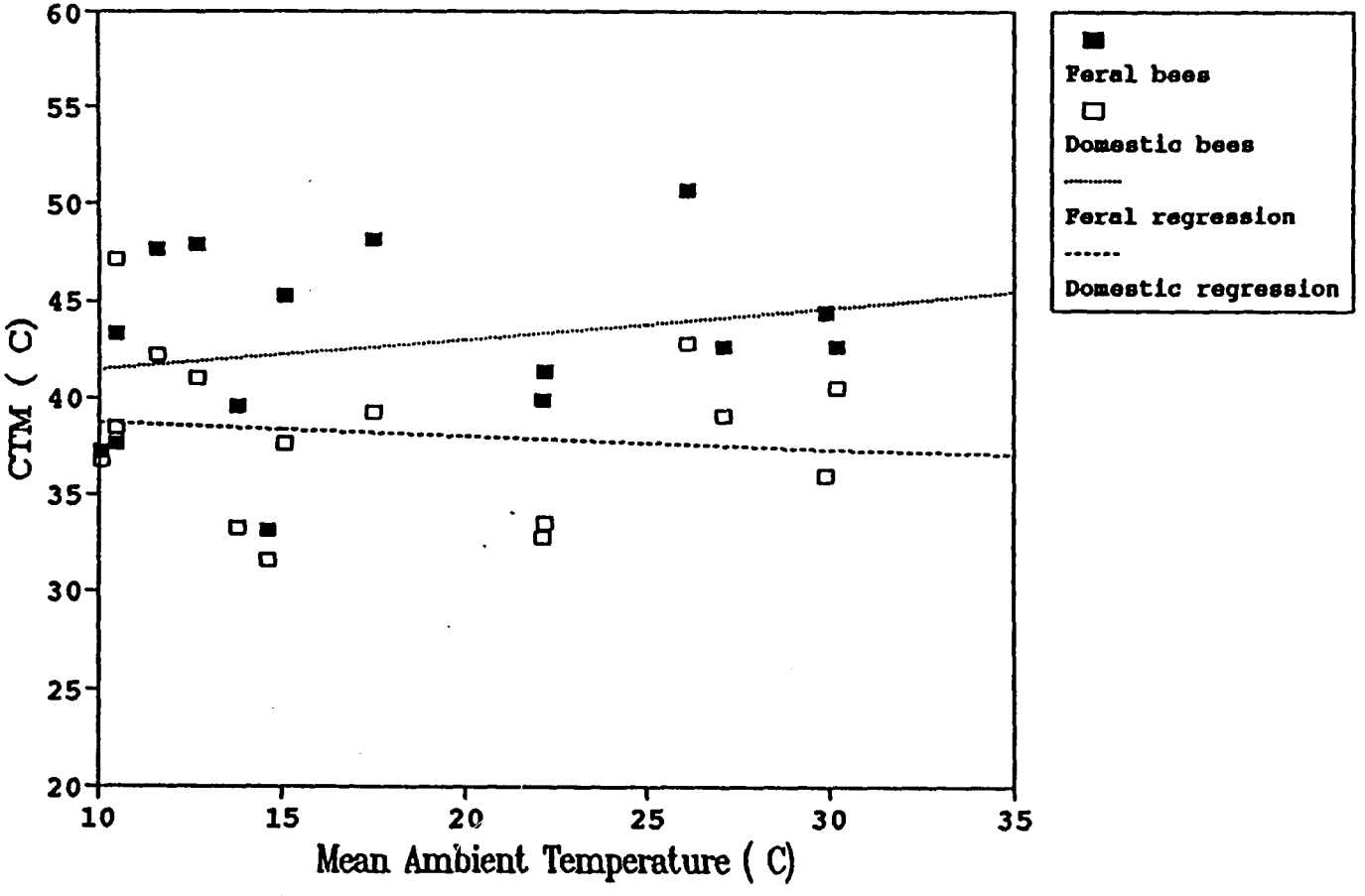

Table 7. Duncan's Multiple Range Test of Critical Thermal Maxima.

Means with the same letter are not significantly different.

Duncan Grouping	Mean of CTM	Number of bees (F & D)	Date
A	46.75	20	Jun 91
A B	45.19	20	Jan 92
A B	44.91	20	Dec 91
A B	44.45	20	Mar 91
A B C	43.68	20	Apr 91
B C D	41.52	20	Jul 91
B C D E	40.55	20	Sep 91
B C D E	41.18	20	Aug 91
C D E	39.39	20	Nov 91
D E	38.02	20	Jan 91
D E	37.41	20	Oct 91
D E F	36.73	20	Dec 90
E F	36.39	20	Nov 91
E F	36.33	20	May 91
F	32.36	20	Feb 91

Note: F = Feral honeybees
D = Domestic Honeybees.

WATER LOSS

Rates of water loss from my experiments with 900 bees at different temperatures (25, 30 and 35°C) and humidities (0, 25, 50, 75 and 100%) are shown in Figure 5. Each value represents the mean ± SE calculated from 30 bees (10 bees per treatment and three replicates).

The rates of water loss were highest in dry air (0%) at 35°C, with an average value of 6.82 ± 0.33 $mg.g^{-1}.hr^{-1}$ for feral bees and 6.90 ± 0.26 $mg.g^{-1}.hr^{-1}$ for domestic bees. The lowest rates of water loss were at 75% RH and 25°C with an average value of 1.83 ± 0.18 $mg.g^{-1}.hr^{-1}$ for feral and at 100% RH and 30°C with value 2.12 ± 0.22 $mg.g^{-1}.hr^{-1}$ for domestic bees. There were highly significant differences in the rate of water loss between relative humidities 0, 25, 50, 75 and 100% ($P < 0.001$) (Table 8).

There were highly significant differences in the rates of water loss at temperatures of 25, 30 and 35°C ($P<0.001$). At 35°C and 100% relative humidity the rate of water loss was more than twice that at water loss at 25°C (4.94 compared to 2.37 $mg.g^{-1}.Hr^{-1}$.) (Figure 5). The results of analysis of variance of water loss in feral and domestic bees are shown in Table 8. Rates of water loss did not differ significantly between feral and domestic honeybees.

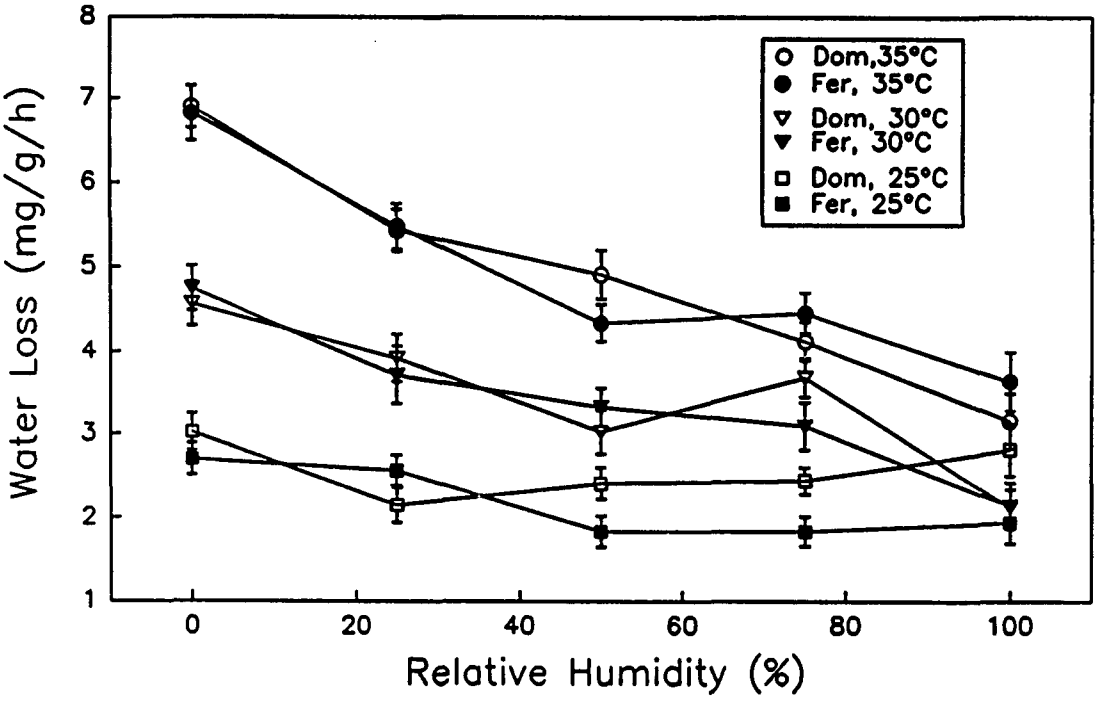

Figure 5. Water loss in feral and domestic honeybees.

Table 8. Analysis of variance of water loss.

Source	DF	SS	Mean square	F value	Pr > P
Colony type	1	0.3829	0.3829	0.44	0.5076
RH	4	48.4370	12.1097	14.04	0.0001
Temperature	2	100.0562	50.0281	57.99	0.0001
Colony * RH	4	0.4663	0.1166	0.14	0.9689
Colony * Temp	2	0.8694	0.4347	0.50	0.6065
RH * Temp	8	17.1081	2.1385	2.48	0.0202

HEMOLYMPH OSMOLALITY , PROTEINS AND FREE AMINO ACIDS

Hemolymph osmotic pressures from desiccated and undesiccated feral and domestic honeybees are presented in Figure 6. For undesiccated and desiccated feral honeybees, the means ± SE of osmotic pressures were 530 ± 29 and 493 ± 19 mOsm/kg, respectively and for undesiccated and desiccated domestic honeybees were 596 ± 27 and 619 ± 15 mOsm/kg, respectively. There were no significant differences for feral and domestic bees between desiccated and undesiccated treatments. There was, however, a significant difference between feral and domestic honeybees ($P < 0.004$) (Table 9), with feral honey bees having a lower osmolality overall than the domestic ones.

The effect of desiccation on the concentration of hemolymph proteins in feral and domestic honeybees is shown in Figure 7. For feral bees, the mean ± SE of concentration of hemolymph protein in undesiccated bees was 9.89 ± 1.32 $\mu g/\mu l$ hemolymph compared to 1.35 ± 0.45 $\mu g/\mu l$ in desiccated bees. For domestic honeybees, the mean concentration in hemolymph protein of undesiccated bees was 6.39 ± 1.69 $\mu g/\mu l$ hemolymph compared to 1.01 ± 0.28 $\mu g/\mu l$ hemolymph in desiccated bees. Analysis of variance shows that there was a highly significant difference in hemolymph protein concentrations between undesiccated and desiccated honeybees ($P<0.001$) (Table 10), but

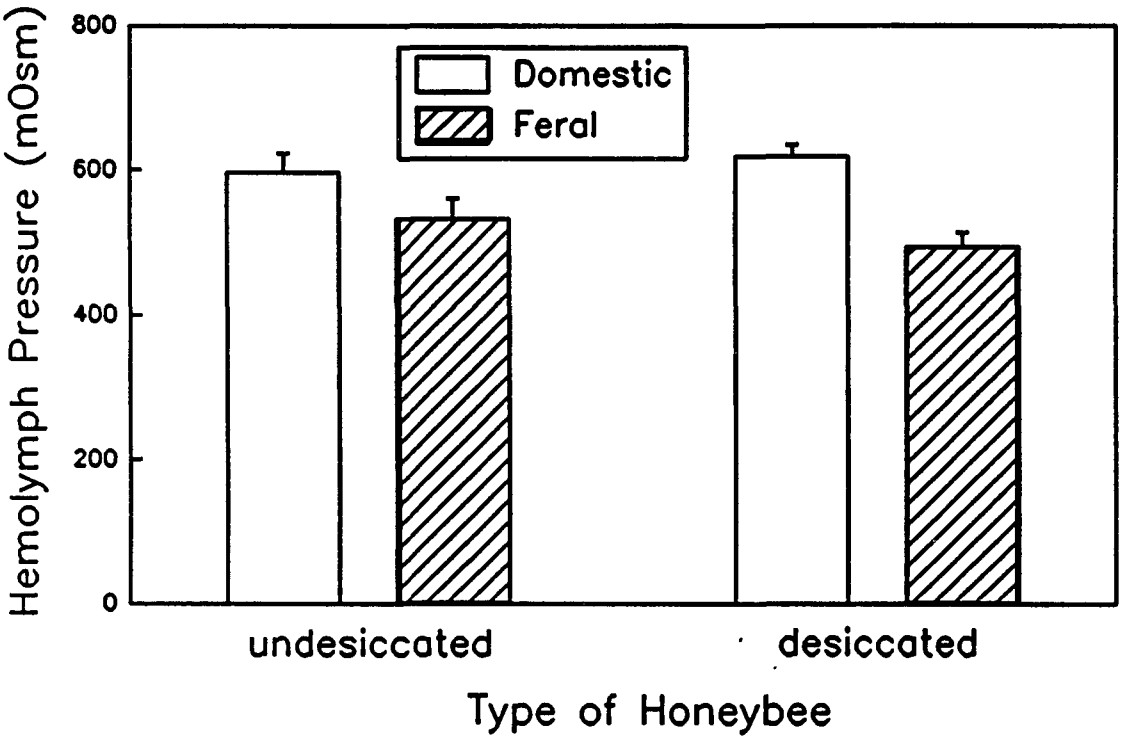

Figure 6. Hemolymph osmolality of feral and domestic honeybees.

Table 9. Analysis of variance of osmolality

Source	DF	SS	Mean square	F value	Pr > F
Colony type	1	54023.1598	54023.1598	10.47	0.0040
Treatment	1	290.3177	290.3177	0.06	0.8148
Colony * Treatment	1	5349.5658	5349.5658	1.04	0.3201

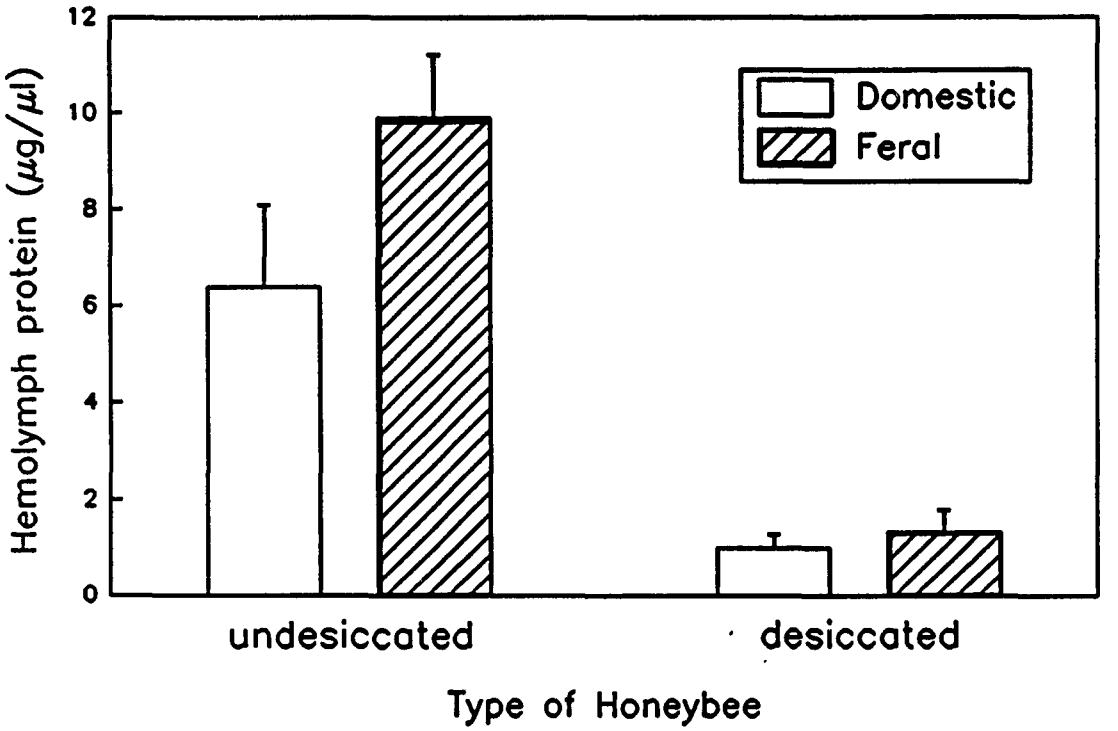

Figure 7. Hemolymph protein concentrations of feral and domestic honeybees.

Table 10. Analysis of variance of hemolymph proteins

Source	DF	SS	Mean square	F value	Pr > F
Colony type	1	36.6560	36.6560	3.00	0.0920
Treatment	1	484.6018	484.6018	39.61	0.0001
Colony * Treatm	1	25.0511	25.0511	2.05	0.1611

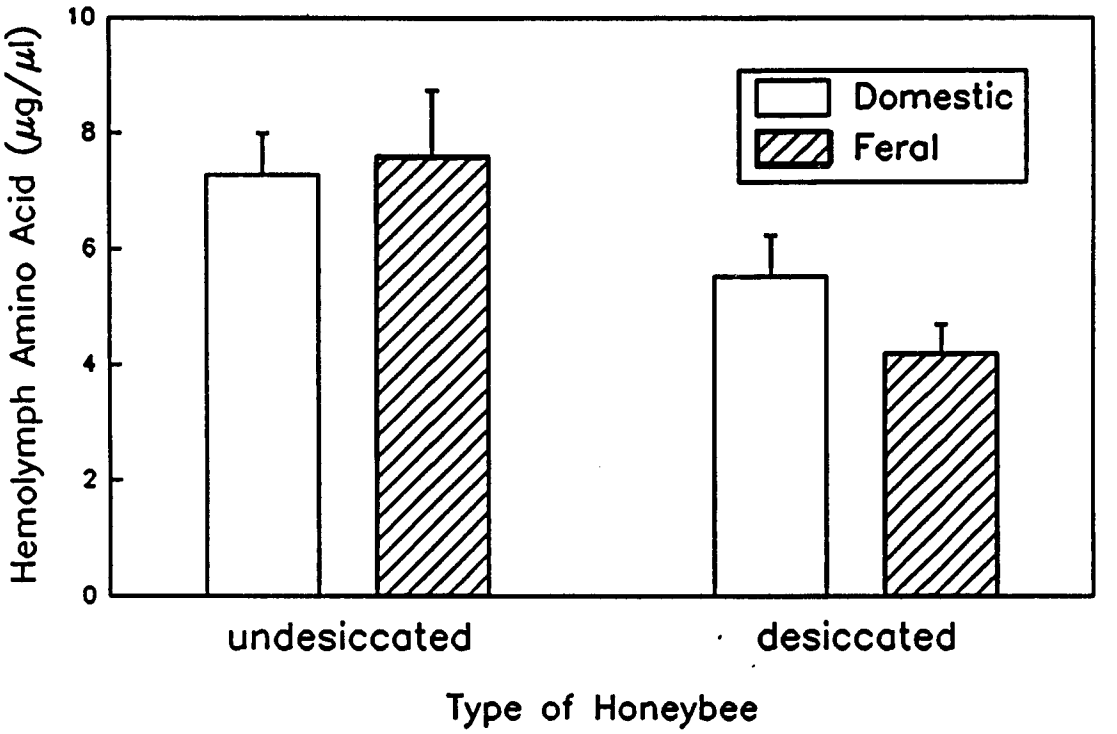

Figure 8. Hemolymph amino acid concentrations of feral and domestic honeybees.

Table 11. Analysis of variance of hemolymph amino acids

Source	DF	SS	Mean square	F value	Pr > F
Colony type	1	2.6855	2.6855	0.41	0.5254
Treatment	1	65.8674	65.8674	10.09	0.0031
Colony * Treatm	1	6.6599	6.6599	1.02	0.3193

there was no significant difference between bees from feral and domestic colonies.

The effect of desiccation on the concentration of hemolymph amino acids in feral and domestic honeybees is shown in Figure 8. For feral bees, the mean and SE of concentration of amino acids in undesiccated was 7.57 ± 1.16 $\mu g/\mu l$ hemolymph compared to 4.18 ± 0.51 $\mu g/\mu l$ of hemolymph in desiccated bees. For domestic honeybees, the mean concentration of amino acids from undesiccated bees was 7.27 ± 0.72 $\mu g/\mu l$ hemolymph compared to 5.52 ± 0.71 $\mu g/\mu l$ of hemolymph in desiccated bees. The results of analysis of variance of amino acids concentration are shown in Table 11. There was a highly significant difference in the concentration of amino acids between undesiccated and desiccated honeybees ($P<0.0031$), but no significant differences in concentrations between bees from feral and domestic colonies.

URIC ACID

Uric acid concentrations from desiccated and undesiccated of feral and domestic honeybees are presented in Figure 9. The means and SE of uric acid concentrations in undesiccated and desiccated feral bees were 121.5 ± 21.1 ng uric acid (U.A)/mg of hindgut (HG) and 151.3 ± 45.5 ng U.A/mg of HG, respectively. For domestic honeybees, the mean of uric acid concentrations in

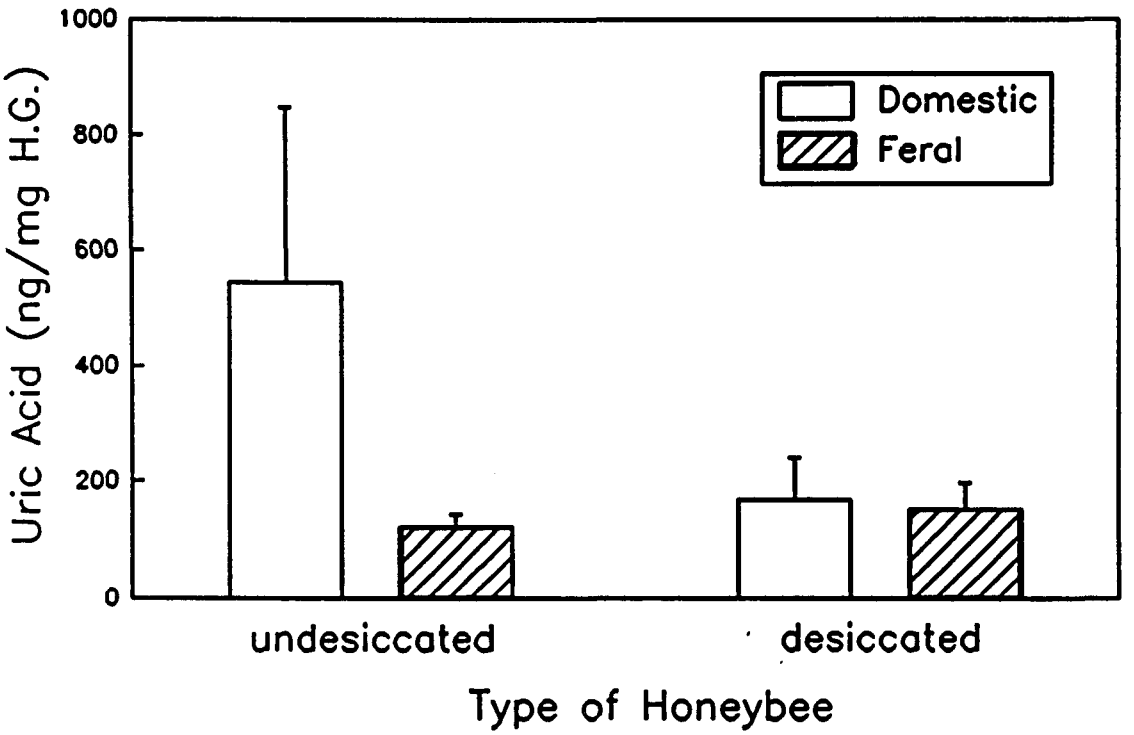

Figure 9. Uric acid concentrations of feral and domestic honeybees.

Table 12. Analysis of variance of uric acid

Source	DF	SS	Mean square	F value	Pr > F
Colony type	1	481256.100	481256.100	1.92	0.1745
Treatment	1	298900.8766	298900.8766	1.19	0.2822
Colony * Treatment	1	411024.9391	411024.9391	1.64	0.2087

undesiccated and desiccated were 543.6 ± 304.6 ng U.A/mg of HG and 168 ± 71.2 ng U.A/mg of HG, respectively. There were no differences between treatments and no differences between feral and domestic honey bees (Table 12).

DISCUSSION

NECTAR AND WATER FORAGERS

The honey-sac content of honeybees depends on nectar flows, nectar temperature and concentration, and the condition of workers (age and experience of the bees) (Free, 1957; Nunez, 1970). Free (1960) and Ribbands (1953) explained that foragers prefer to collect nectar. Erickson et al, (1973) reported that in *A.mellifera*, percentage of nectar collectors was greater than other foragers.

The volume and concentration of nectar collected by feral and domestic honeybees fluctuates during the day (Figure 1). Rinderer et al. (1984) reported that the volume of nectar collected by European honeybees and Africanized honeybees fluctuates daily, but they did not see similar fluctuations in nectar concentrations.

Soehngen and Jay (1973), in their study in Canada, in summer, found that the weight of the honey bee sac was positively correlated with the intensity of the nectar flows. In addition, nectar sugar concentrations were generally highest in the morning. My data showed that the sugar concentrations tended to be higher starting midday. Differences in the daily pattern of nectar concentration may be due to differences in climate between

Arizona and Canada, and season; my experiment was conducted in the fall.

From this study of foraging behavior, I concluded that there are differences in liquid volume collected by feral and domestic honeybees. Feral bees collected more nectar than domestic ones, even though feral bees are smaller than domestic bees. Possibly extra liquid is needed by feral bees for thermoregulation. Foragers with experience in overheating in the desert at high ambient temperature show specific behaviors such as expelled liquid from their tongues (proboscis) when exposed to higher temperatures (Heinrich, 1980; Willmer and Corbet, 1981; Cooper et al., 1985). However, there is no difference between time of days collecting.

TEMPERATURE TOLERANCE (CTM)

Feral honeybees were more tolerant to high temperatures than domestic honeybees (Figure 3). Insects adapted to xeric habitats have been shown to have higher CTM than those living in more moderate environments (Cohen and Cohen, 1981). Cohen and Pinto (1977) reported their experiments with meloid beetles that there was positive correlation between CTM and the temperature of the beetle habitat. Cohen (1982) studied of 2 Hemiptera *Geocoris punctipes* and *Lygus hesperus* and attributed difference in CTMs as selective pressure associated with *G.punctipes* spending most of its time in a hotter microclimate.

Appel (1991) reported the CTM data for several cockroaches species (*Diploptera punctata, Pycnoscelus surinamensis, Blatta lateralis*) and concluded that CTM may be related to habitat temperatures.

Feral bees tolerate higher temperatures than domestic bees. Feral bees are probably adapted to low and high temperatures because they confront the high ambient temperature all the time unlike domestic bees which are frequently imported from other areas very well protected by beekeepers.

One possible basis of thermal tolerance is heat shock protein production. Severson et al. (1990) exposed worker honeybees to 42°C and induced the appearance of HSPs. It is thought that HSPs have important roles for cell survival because they can prevent damage and repair the tissues of the organisms subjected to high temperatures (Lindquist, 1986). Therefore, they buffer environmental stress and regulate cellular homeostasis (Severson et al., 1990). It is possible that heat shock proteins of feral bees are different in their quantity and quality from heat shock proteins of domestic honeybees, so feral honeybees are more tolerant to high temperature than domestic honeybees.

WATER LOSS

In both feral and domestic bees, water loss (total transpiration) increased with increasing temperature and with decreasing humidity (Fig. 5) and there

were no significant differences between the two types of bees (Table 8).

Increased water loss with increasing temperature and decreasing humidity is a common pattern in insects (Cohen et al., 1984; Jackson and Cohen, 1984; Lighton and Feener, 1989), e.g. parasitic wasps *Anaphes ovijentatus* (Cohen and Jackson, 1986), *Lygus* (Cohen et al., 1984), the desert ant *Pogonomyrmex rugosus* (Lighton and Feener, 1989). Rates of water loss can differ significantly among species, with desert adapted species showing lower rates (Ahearn and Hadley, 1969).

One factor important in determining rates of water loss is surface hydrocarbons. The composition and the quantity of the surface hydrocarbons support the theory that they limit water loss (Hadley, 1978, 1980). Therefore, cuticular hydrocarbons can be expected to affect rates of water loss in honey bees. But feral bees showed the same rates of water loss as domestic honeybees which live in less harsh conditions. Feral honeybees have lived wild in the United States for more than one hundred years (Sheppard, 1989). Therefore, feral bees in the Sonoran desert do not to appear to have evolved mechanisms for reducing cuticular water loss. It may be that the hundred year time frame of living in the wild under harsh conditions is not sufficient time to evolve differences in the cuticle layer and its permeability. The presence and the amount of hydrocarbons in cuticular lipids is under the influence of genetic control of the insects, and is not influenced by short term environmental

changes (Smith, 1990). Evolution of different cuticular hydrocarbon profiles requires genetic variation,on which we have no data, and time, of which 100 years may have been too little. In addition, it may be that the social behavior of bees and their creation of a protected microclimate in the hive may reduce the environmental and selective pressure to evolve advanced water-proofing mechanisms.

HEMOLYMPH OSMOLALITY, PROTEINS AND FREE AMINO ACIDS

Insects from hot and arid regions have been reported to maintain osmolality during high temperatures and dehydration by regulating hemolymph protein and free amino acids (Cohen and Pinto, 1977; Cohen, 1978, Cohen and Patana, 1984). Also, many xeric-adapted insects can maintain fairly stable hemolymph osmolalities during periods of severe dehydration (Crawford, 1981; Riddle, 1986).

In insects, hemolymph osmolality tends to increase during desiccation (Edney, 1977; Cohen, 1984). During dehydration loss of water from hemolymph can concentrate solutes. The hemolymph acts as a source of water for stressed tissue (Edney, 1977). If osmotic pressure of hemolymph is to be kept constant, osmotically active substances must be removed from the hemolymph during desiccation (Nicholson, 1980). Osmolality can be

controlled by regulation of hemolymph free amino acid concentrations (Cohen, 1978; Cohen et al., 1986; Djajakusumah and Miles, 1966; Broza et al., 1976). For both feral and domestic honeybees, osmolalities were similar for desiccated and undesiccated treatments, suggesting some sort of regulation. In both types of bees, protein and amino acid concentrations fell during desiccation. The decrease in hemolymph protein concentration does not affect significantly in osmolality, since proteins have such large molecules, they do not directly contribute very much to regulation of osmotic homeostasis. They may have some other functions.

These changes represent a rapid physiological adjustment for both types of honeybees to high temperature and shows that amino acids and proteins are involved in honeybee osmoregulatory mechanisms.

From my experiments, I concluded that in both feral and domestic honeybees, in order to maintain stability of osmolality, protein and amino acids of hemolymph were decreased. Other hemolymph components, such as inorganic ions and other organic solutes are (or may be) involved in osmotic regulation.

URIC ACID

Uric acid can have an important role as osmoeffector in the regulation of ion and water balance in hemolymph (Schwantz and Schwantz, 1990). The use of uric acid as an excretory product could be important in conserving water in dry and hot environments. Uric acid contains less hydrogen per atom of nitrogen than other nitrogenous waste products of animals, so its production needs less water. Further more, uric acid is relatively non toxic and thus requires little dilution (Chapman, 1982). However, I found no significant differences between treatments or between feral and domestic honey bees. The basis for the high variability in uric acid content of domestic honeybees is not understood.

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