

THERMODYNAMICS IN NESTS OF TWO MELIPONA¹ SPECIES IN BRASIL

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SUMMARY

Thermodynamics within 10 nests of *Melipona rufiventris* and *M. seminigra* were recorded during 48 hours with thermocouple probes. Strikingly similar patterns were found for both species. Homeostasis did not occur; temperatures within the brood area, honey and pollen stored in pots and nest cavity space all followed ambient temperature fluctuations. Nest temperatures were consistently higher than ambient in all portions of the nest except the upper extremity of vertically elongate hives. Near the brood, temperature fluctuations were damped and displayed a time lag of one to two hours in following ambient temperature. The thoracic temperature of resting worker bees was near 34°C, and the average brood temperature was 31 – 32°. The involucrum surrounding the brood retained a portion of radiated heat from immatures and workers resting between combs, and brood temperature was two to three degrees higher than internal nest temperature immediately outside the involucrum. The brood chamber, the largest nest structure, contained from 2000 to 6000 immatures, and adult bee populations were less than 1000. The brood nest acts as a heat source at the base of the nest and dissipates heat upwards, creating a thermal gradient. Immature bees appear to supply most of the heat for the nest, and excess heat is shunted

by fanning workers through the nest entrance, usually connected to the brood area. There is no evidence of evaporative cooling from water brought into the nest in these or other species of Meliponinae.

INTRODUCTION

The nests of stingless bees (Apidae: Meliponinae) and their colonies show structural, behavioral and physiological adaptations which protect bees from the external environment. In the tropical forest habitat of these species, particularly near the equator, the change in ambient temperature through a 24 hour period is often as great as that between different seasons of the year (Richards 1952). Thus the degree to which highly social stingless bees regulate nest temperature, and the mechanism by which this is accomplished, should be readily observed. Various workers (Kerr and Liadlaw 1956, Kerr et al. 1967 Zucchi and Sakagami 1972, Wille 1976) have shown that nest temperatures of *Melipona* and *Trigona* are to some extent independent of ambient temperature, although most studies have been made using arti-

(1) running head: *Melipona* thermodynamics

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ficial nests. Regulation of nest temperature by stingless bees has been invoked to explain differences between ambient and nest temperatures, and the comparative ability of species to control the nest environment has been discussed, despite the lack of repeated observations of different colonies of a species (summary by Michener 1974, Wille 1976). More important, no clear definition has been given of "regulation" in this context. Regulation of an intracolony process may involve internal homeostasis, independent of the external environment, or it may involve relative stability, whereby a process varies with external conditions, but fluctuations are relatively slight (Roubik 1982). The regulation of nest temperature also includes both elements of nest architecture and behavioral responses of bees to the nest environment.

Here we provide data on thermodynamics in nestes of *Melipona seminigra* and *M. rufiventris* in Manaus, Brasil. These species are native to Amazonia. We address the questions: (1) To what extent do nidal temperatures vary among different colonies of the same species and between species? (2) To what extent are thermodynamics homeostatic or influenced by external temperature? (3) What features of the nest and bees govern temperature change and control?

MATERIAL AND METHODS

Our study was made using colonies domiciled in hives designed

by Sr. V. de Portugal—Araújo, which closely approximate the size, shape and other properties of natural tree cavities used by *Melipona* (Roubik 1979, 1981, 1983, Portugal—Araújo 1977, Kerr et al. 1967). All observations were made from May 8–28, 1981, on the campus of the Instituto Nacional de Pesquisas da Amazonia in Manaus, Amazonas, Brasil. Five colonies of each species were employed, which had been placed in hives at least three years prior to the study. The hives were of three different designs and provided cavities and brood chambers of differing size (Table 1, see also Portugal—Araújo 1977). All hives were constructed of straight-grained hardwood at least 2.0 cm thick.

Temperatures were recorded with a Bailey BAT-12 thermocouple probe, which measures temperature within 0.1°C accuracy with a constantan/copper tip (Fig. 1). Individual probes with plug attachments were inserted within brood cells near the center of the comb stack, within full honey or pollen storage pots, or in the air space next to the involucre (a thin sheath of wax mixed with resin = cerumen, which surrounds the brood (Fig. 2). Hives were closed for 24 hours prior to recording nest temperatures. Hives which were vertically elongate, like the hollows of trees in which nests are often made, were used to measure temperature also in storage pots and cavity space above the brood area. Tempe-

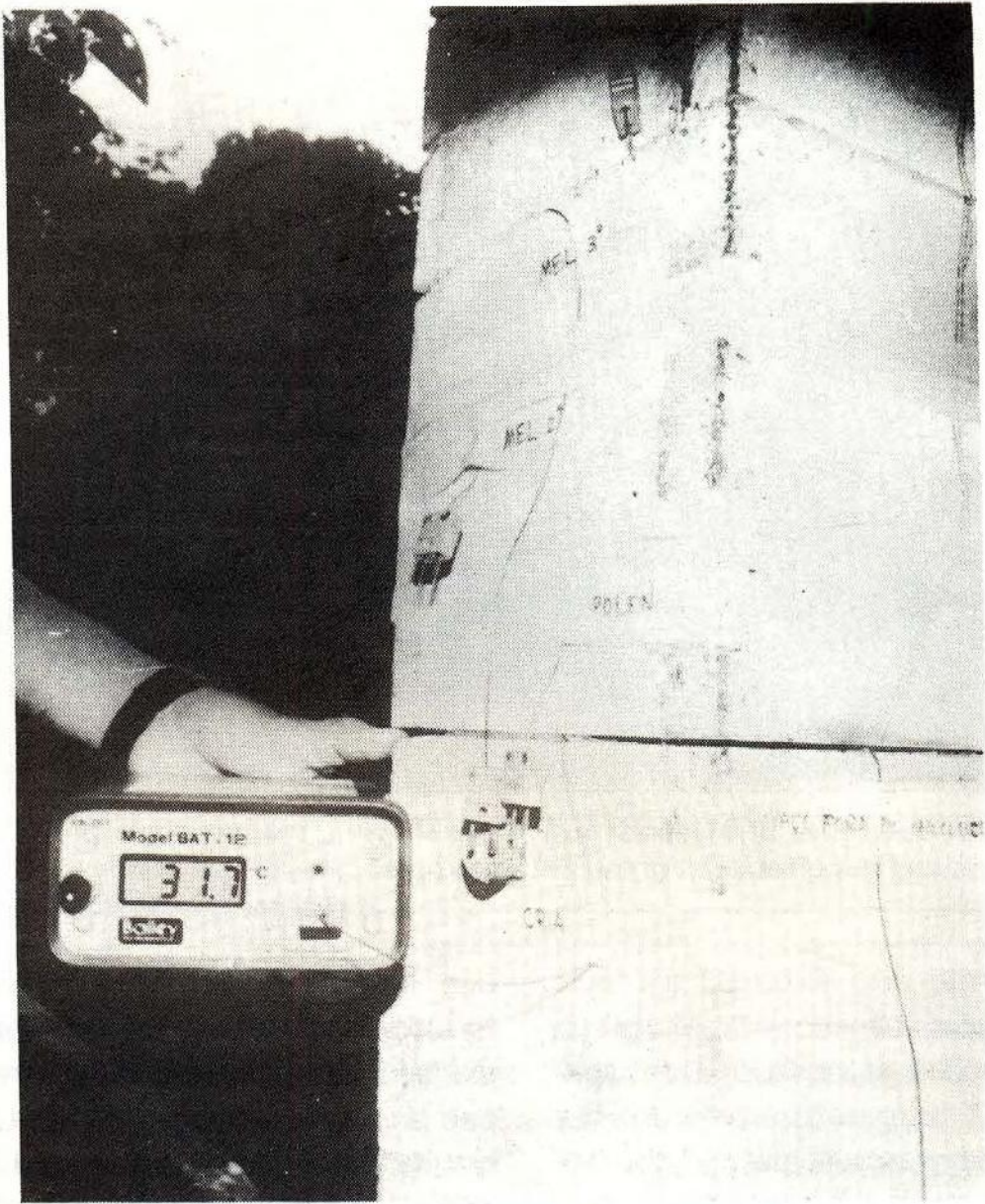


Fig. 1 — A hive of *Melipona seminigra* showing the plugs of thermocouple probes extending from different portions of the nest.

temperature recordings were made hourly from 0600 to 1800 and at 3-hour intervals from 1800 to 0600. Temperature data were taken from each nest during two successive days,

and the external ambient temperature in the shade was measured in a sheltered area less than 10 cm from each hive when nest temperatures were recorded.

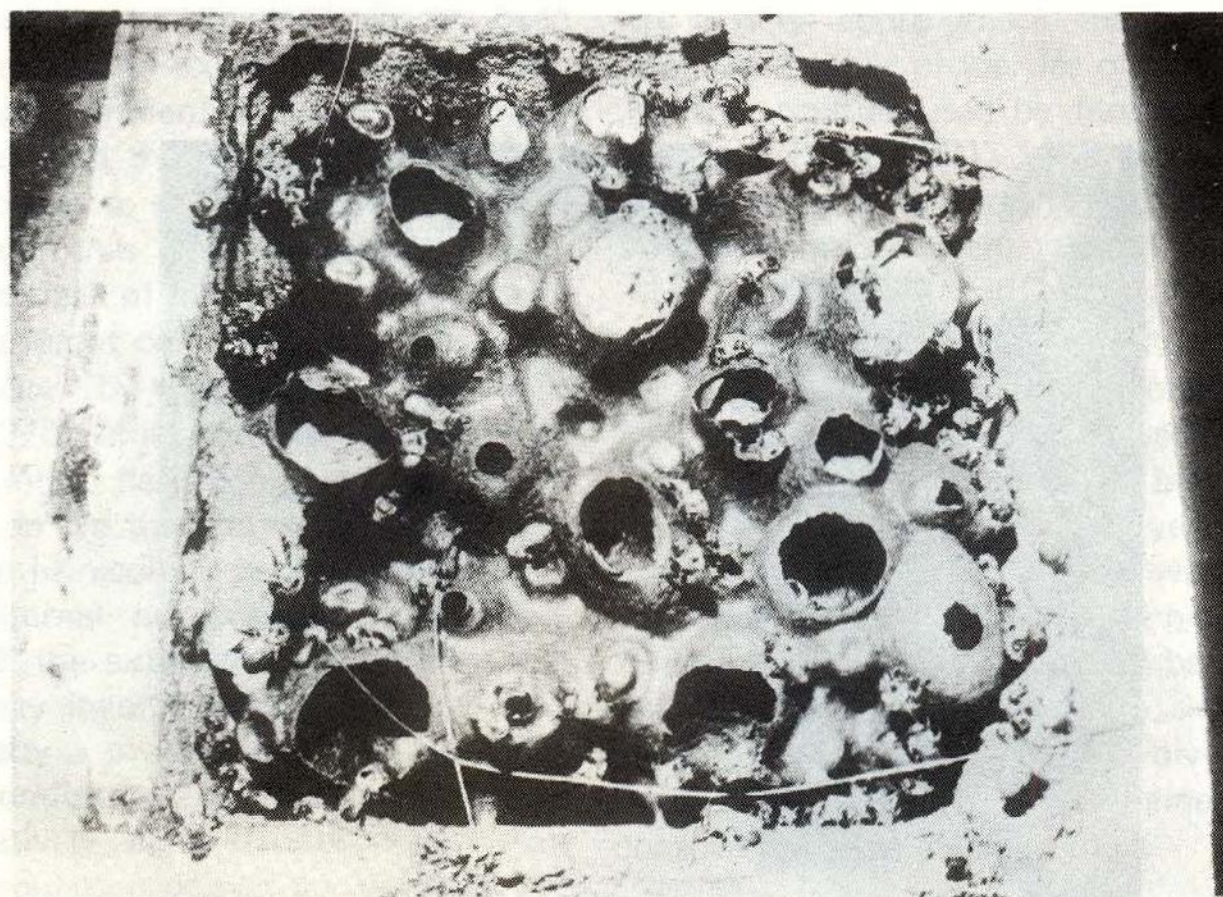


Fig. 2 — An open hive of *Melipona rufiventris* showing thermocouple probe wires leading to pollen and honey in storage pots.

Internal and external thoracic temperatures of worker *Melipona* were recorded at nests of both species. The temperature within the thorax, the warmest part of the body, was measured by inserting a stylet probe in bees resting on the brood comb, and this was done immediately after opening hives to remove thermocouple probes from nests. External thoracic temperatures were recorded both from incoming bees captured with an insect net outside the nest entrance and from bees within the nest. The stylet probe was placed in the middle of the thoracic notum when external

bee temperatures were measured. In order to determine whether the elapsed time between capture of a bee and measurement of its temperature appreciably influenced recorded temperature, we timed the interval between capture and temperature measurement.

Brood temperatures in one nest of each species were recorded for 15 minutes after the hive sections above the brood nest (alças or supers) had been removed. We wanted to show what effect short-term disturbance of the nest had on bee behavior or nest temperature. Therefore we measured temperatu-

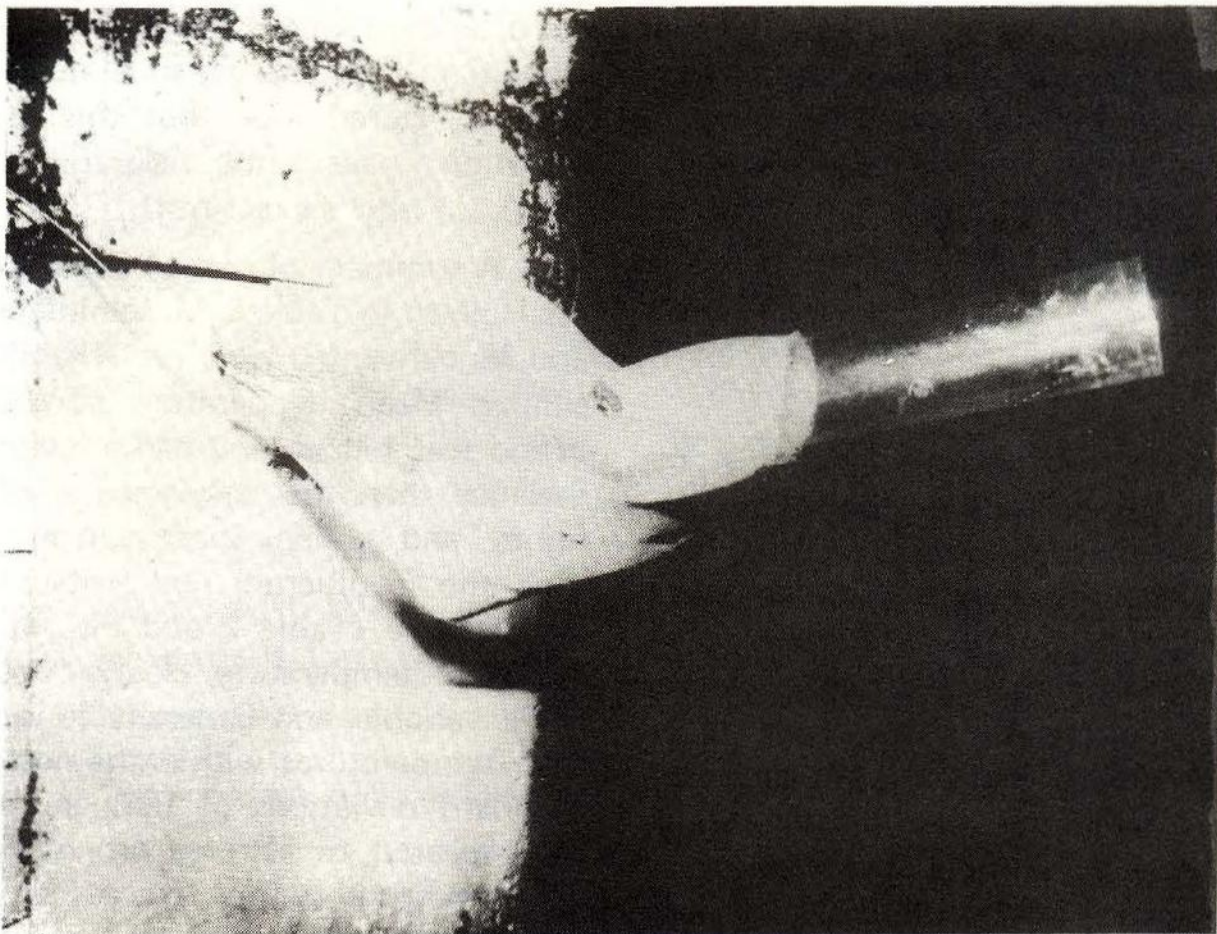


Fig. 3 — A screen and plastic tube funnel for trapping exiting workers from a nest of *Melipona seminigra*.

re only where an abrupt change could conceivably adversely affect the bees.

Total colony food stores and brood were determined by measuring and counting storage pots and brood (Roubik 1979). In addition, worker populations were estimated with inverse sampling, a mark-recapture technique which provides an unbiased estimate of population size and sample variance (Poole 1974). The sampling procedure involved tagging 200 *M. seminigra* or 100 *M. rufiventris* from a colony as they exited from the nest. On the

following day, bees leaving the nest were recaptured until 50 tagged *M. seminigra* or 25 tagged *M. rufiventris* had been sighted, and the total bees exiting the nest in this interval was recorded. Bees were tagged with nontoxic photocopy correction fluid, applied with a brush to the thoracic notum while holding the bees with the fingers. Exiting bees were captured with a wire mesh funnel placed over the entrance, where they were collected in a plastic tube (Fig. 3). The funnels were placed over the nest entrance in such a way that returning bees

entered the nest but exiting bees were trapped. We each tagged foragers at two colonies at the same time, and the tagging procedure for a nest took no more than two hours when performed between 0700 and 1100.

RESULTS

The total brood, food stores and an estimate of the forager population is given in Table 1 for each of the observed colonies. Some colonies could not be sampled effectively to determine worker populations, thus these data are incomplete. Total brood in each nest was far greater than the worker population. Given a low probability of brood mortality that has been observed for other stingless bees, these data imply that, if the colony age structure and population are stable, the egg-adult stage is considerably longer than the average adult life span (Roubik 1979, 1982). The estimates of worker populations are based on workers leaving the nest, which include foragers and bees that carry trash. Calf and young adults presumably do not leave the nest (Michener 1974), so the estimate of worker population is conservative. Total adult populations were probably no more than twice the number of foragers. The forager and trash-carrying bee population consisted of about 300-500 bees for both species (Table 1). It is interesting that colonies with the largest food sto-

res often had smaller brood populations than those having small quantities of stored food, but this association was not significant ($P = 0.3$, Fisher's exact test).

A summary of nest temperatures is given in Table 2. *M. seminigra* and *M. rufiventris* were remarkably similar. Mean temperature of the brood was higher, and had a lower variance than the temperatures of honey and pollen, space surrounding the involucrum and ambient temperature (Table 2 and Fig. 4). Ambient temperature clearly was more variable and generally lower than temperatures within the nest. Standard deviations of temperatures appeared to increase progressively from brood to pots to space surrounding the involucrum.

Despite the differences between ambient and nest temperatures, nidial temperatures tracked those of the external environment (Fig. 4). Colonies in the figure were in the tallest vertical hives (Fig. 1), and their size and shape were identical to natural nests in hollow trees. On the average, colonies of each species maintained the brood nest at 2-3°C above that of air space outside the involucrum. These data show that nest temperature is buffered from ambient temperature change, but there is no indication of homeostasis. Moreover, intranidal temperatures near the top of elongate hives were almost the same as ambient temperatures for each species. In natural nests, the top of

TABLE 1. Colony and nest characteristics of *Melipona rufiventris* and *M. seminigra*

Colony	No. Brood	Volume Pollen and Honey (cm ³)	Total Hive Volume (cm ³)	Brood Nest Volume (cm ³)	Forager Population ^a
<i>seminigra</i> 55	3197	1280	30,020	9500	385 ± 51
<i>seminigra</i> 53	3272	160	12,600	10,000	445 ± 40
<i>seminigra</i> 3	2937	1240	15,744	7424	498 ± 47
<i>seminigra</i> 86	1925	1260	29,078	7812	—
<i>seminigra</i> 28	1970	620	19,747	13,939	—
<i>rufiventris</i> PA1	5974	754	17,280	5632	423 ± 64
<i>rufiventris</i> PA2	6390	130	24,420	7770	336 ± 48
<i>rufiventris</i> IN2	4033	429	25,728	11,520	—
<i>rufiventris</i> 18	3529	260	23,058	9747	478 ± 37
<i>rufiventris</i> A29	3292	850	31,000	10,336	—

^a figures given with standard deviation of estimate (see text)

TABLE 2. Nest temperatures recorded for *Melipona rufiventris* and *M. seminigra*. Thermocouple probes were implanted in the center of the brood, in a full honey or pollen pot above or next to the brood, and in the 1-2 cm open space surrounding the involucre in the brood chamber. All figures are given with their standard deviations.

Colony	Average Temperature					N
	Brood	Storage Pot	Space	Brood - Space	Ambient	
<i>seminigra</i> 55	31.03 ± .68	28.66 ± .94	27.96 ± 1.17	3.07 ± .67	25.86 ± 1.83	43
<i>seminigra</i> 53	31.55 ± .86	29.51 ± 1.21	28.24 ± 1.30	3.30 ± .79	25.97 ± 1.79	43
<i>seminigra</i> 3	32.28 ± .70	29.57 ± 1.46	28.54 ± 1.42	3.12 ± .66	25.95 ± 1.92	43
<i>seminigra</i> 86	32.36 ± .74	26.77 ± 1.26	29.46 ± 1.17	2.77 ± .60	26.59 ± 1.88	46
<i>seminigra</i> 28	31.43 ± .69	29.08 ± 1.02	29.30 ± 1.08	2.85 ± .44	26.52 ± 1.92	46
<i>rufiventris</i> PA1	31.18 ± .49	27.85 ± 1.20	27.14 ± 1.32	3.02 ± .49	25.77 ± 1.63	43
<i>rufiventris</i> A29	31.04 ± .92	—	31.30 ± 1.02	-.92 ± .41	26.88 ± 1.87	46
<i>rufiventris</i> IN2	31.85 ± .84	29.77 ± 1.22	29.41 ± 1.19	2.25 ± .78	26.54 ± 1.91	46
<i>rufiventris</i> PA2	31.16 ± .76	—	27.14 ± 1.34	3.02 ± .76	25.63 ± 1.74	43

the nest either terminates with a perforated batumen plate made of mud and resin, or the nest ends at the upper extremity of the nest cavity. We suppose that the temperature of the tree or hollow space above a nest cavity is similar to ambient temperature. A probe placed in the air space 4 cm below the top cover of a nest of *M. rufiventris*, in which bees had built a batumen plate to cover a screened hole in the cover, showed a temperature range of 24.2 – 29.2°, and that of ambient temperature was 22.7 – 29.6°. A temperature gradient was evident in all vertically elongate hives, with the warmest part of the nest at the base within the brood area, and the coolest temperatures at the top of the cavity. Temperature within full honey or pollen pots was the same at a given distance from the brood, but the average temperature of pollen or honey decreased approximately 1°C for each 6.5 cm (the depth of and alça or super) above the brood. In each of the vertical hives, the upper two or three alças did not contain stores and were largely sealed off with batumen, perhaps indicating that this area could not be maintained at suitable temperatures to support colony activity.

Thoracic temperatures of workers were higher than brood temperatures. In a nest of *M. seminigra*, internal throacic temperatures of bees that had not recently been in flight ranged from 33.0 – 36.4

(\bar{x} = 34.8, n = 22). In a nest of *M. rufiventris*, and among 12 guard bees resting outside the nest entrance, thoracic temperatures ranged from 26.2 – 32.9 (\bar{x} = 34.0, n = 33). The external thoracic temperatures of bees returning to the nest were somewhat higher. External temperatures of *M. seminigra* ranged from 31.9 – 38.0° (\bar{x} = 36.2, n = 20), and those of *M. rufiventris* were from 32.5 – 36.8° (\bar{x} = 34.6, n = 20). The time interval between capture and temperature measurement was, on average, less than six seconds, and temperature changed little in this time. Regression of temperature on time elapsed before measurement (range 3 – 10 seconds) did not produce a line with slope significantly different from zero.

The disturbance caused by opening a hive had no substantial effect during 15 minutes on the temperature of exposed brood. Brood temperature of one colony of *M. seminigra* rose from 32.1 to 32.6°, and that of a *M. rufiventris* colony remained at 31.7°, ambient temperature was near 30.0°. In nests of both species, workers became more active in the brood area and rushed over the exposed surface of the comb after the hive was opened.

DISCUSSION

Melipona did not regulate nest temperature in a homeostatic manner; instead, the environment th-

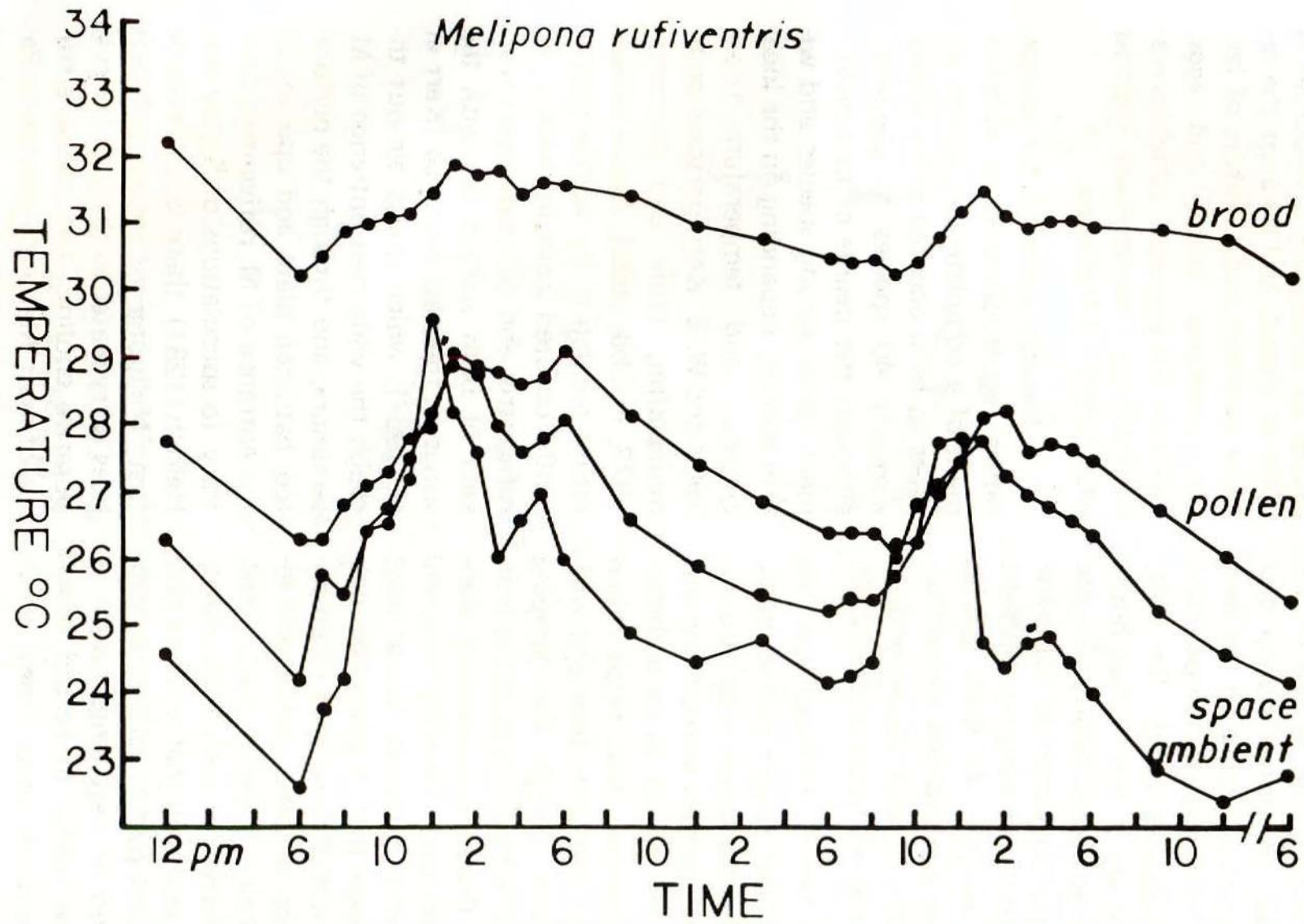


Fig. 4 – Temperatures recorded within brood cells, pollen storage pots, space surrounding the involucrem (immediately outside the brood area) and the external environment of *Melipona* nests.

roughout the nest changed with ambient temperature. Temperature fluctuations outside the nest caused temperatures to rise or fall in the brood area, in stored honey and pollen and in air space in the nest cavity. The change in temperature was less extreme within the nest, and a time lag of one to two hours was observed, particularly in the brood area, for intranidal temperature to follow changing ambient temperature (Fig. 4). Both species maintained a brood nest temperature between 31 and 32.3°, with a range during 48 hours of 1.2–2.9°.

The brood chamber was the principal heat source within nests, and indeed the largest nest structure. The involucrum surrounding this area can be viewed as an architectural adaptation that helps retain heat from immature bees and workers between combs, also damping temperature fluctuation in the entire nest. Brood temperatures were lower than that of worker bees, and the brood population was at least several times that of adult bees between combs. Callow bees often cover the top of lower combs, and some of their body heat must be transferred to the comb. However, it is doubtful that workers play a significant part in actively controlling brood or nest temperature. It would be useful to record brood temperature in nests from which adult bees were removed, but thermoregulatory ability of *Melipona* apparently relies mainly on the pre-

sence of an involucrum, which can trap much of the heat supplied by the metabolism of immatures. The extent to which the involucrum is open or closed, and perhaps the relative position and numbers of larvae, prepupae, pupae and eggs, could be the principal variables responsible for temperature regimes within nests of *Melipona*.

Natural selection for temperature regulation through development of a complete involucrum appears to have occurred in the approximately 40 species of *Melipona*, although the degree of its development varies among species and within species, depending on the local climate and temperature (Kerr 1969 and W. E. Kerr, personal communication, Wille and Michener 1973, Roubik 1983). Active temperature regulation by workers apparently occurred both in nests of *M. rufiventris* and *M. seminigra*; workers of both species fan with the wings at the nest entrance (Kerr et al. 1967), which draws air out through the wide nest entrance of *M. seminigra*, and through the perforated batumen plate and opening at the entrance of *M. rufiventris*. Contrary to speculations of Seeley and Heinrich (1981) there is no evidence that *Melipona* or other stingless bees carry water to the nest for evaporative cooling, and intensive study of four *Melipona* species in Panama, during the hottest part of the year, show that water is not carried by incoming foragers (Roubik

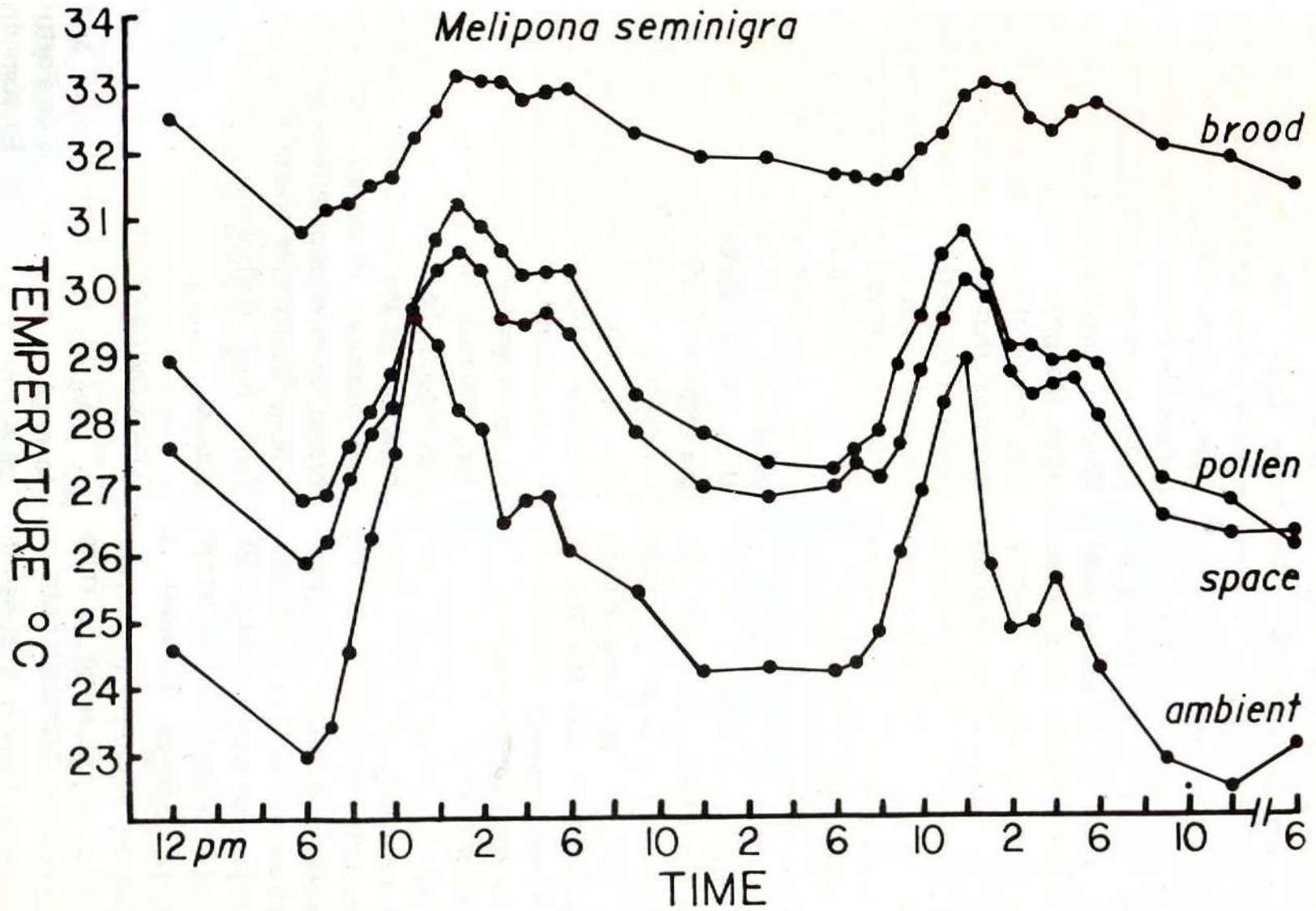


Fig. 4b — Temperatures recorded within brood cells, pollen storage pots, space surrounding the involucrem (immediately outside the brood area) and the external environment of *Melipona* nests.

and Buchmann, unpublished data).

After the involucre is constructed, little additorial investment of time or energy is necessary to maintain it, and the structure makes use of natural colony heat sources. The internal nest entrance of *Melipona* is usually connected to the involucre or the brood area (Roubik 1979, 1983), thus excess heat can be shunted directly through the nest entrance. Worker activity exacts a cost each time it is applied to thermoregulation alone, and colony economy would be likely to give selective advantage to architectural adaptations that minimize additional expenditure by workers. When the observation hives were disturbed and brood was exposed, workers rushed to cover the comb and fanned their wings, but they did not leave the nest to attack. Thus behavior apparently related to thermoregulation is evident, but in normal circumstances the control of temperature within the nest probably is accomplished passively, with occasional fanning by workers. The brood can apparently adjust to the slight temperature changes that occur during a 24 hour cycle. Under more extreme temperature regimes, however, a homeothermic response of worker bees may at times serve to raise nest temperature. Although homeothermy among insects is apparently rare, particularly that involving temperature change without accompanying locomotor activity, the

honey bee *Apis mellifera* and some species of *Bombus* display this phenomenon (Morgan and Bartholomew 1982). These bees are anatomically similar to *Melipona*, and are likewise characterized by having a dense pile on the thorax (Michener 1974, Heinrich 1979, Wille 1958). Thus in environmental extremes, adult *Melipona* may plausibly display homeothermy, although it is doubtful that such controlled temperature regulation is operative for the entire nest or colony in any set of environmental circumstances.

The thermodynamics in nests of both *Melipona* were strikingly similar, despite differences in bee size (five contiguous cells of *M. seminigra* measure 2.85 cm, and five of *M. rufiventris* measure 2.6 cm), and in the size and shape of their hives (Portugal—Araújo 1977 and Table 1). In addition, the involucre of *M. seminigra* was often open on the top, whereas that of *M. rufiventris* was closed. The overall structural similarities of the nests, the quantity and position of stored food and brood, worker populations and metabolic similarities create a comparable nest environment for both species.

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pioneering efforts with an important neotropical bee genus, now disappearing with the forest it inhabits. The first author thanks Dr. S. Dillon Ripley, Smithsonian Institution, for Fluid Research Funding and Smithsonian Institution Scholarly Studies grant 1234S102, which facilitated this study. We also thank Dr. D. W. Inouye for probing comments.

Resumo

A termodinâmica em 10 ninhos de *Melipona rufiventris paraensis* e *Melipona seminigra merrillae* foi registrada através de sensores termoelétricos. Padrões similares foram encontrados para ambas as espécies. Aparentemente não ocorre homeostasis, já que as temperaturas internas registradas da câmara de cría, dos potes de armazenamento de mel e de polen e a do espaço da cavidade do ninho todos acompanhavam as flutuações da temperatura do meio ambiente. No entanto, as temperaturas registradas no ninho foram consideravelmente mais altas que as do meio ambiente em todas as porções do mesmo fazendo exceção a extremidade superior das colmeias verticais. Perto da cría as flutuações de temperatura acompanhavam as flutuações extranidais (do meio ambiente) com uma defasagem de 1 a 2 horas. A temperatura torácica das operárias pousadas nos favos foi perto de 34°C e a média da cría entre 31° e 32°C. O invólucro ao redor da cría retinha uma porção da radiação térmica dos imaturos e das operárias pousadas entre os favos e a temperatura da cría foi de 2 a 3°C mais alta do que a temperatura do ninho imediatamente fora do invólucro. A câmara de cría continha de 2.000 a 6.000 imaturos e a população de adultos foi menor do que 1.000. A cría do ninho atua como uma fonte térmica na base do ninho dissipa-

pando calor para a câmara criando um gradiente térmico. As abelhas imaturas parecem acrescentar mais energia térmica para o ninho.

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