Temperature as a causative factor in the seasonal colour dimorphism of Apis cerana japonica workers

T. Tsuruta, M. Matsuka, M. Sasaki

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Temperature as a causative factor in the seasonal colour dimorphism of *Apis cerana japonica* workers

T. Tsuruta, M. Matsuka and M. Sasaki

Faculty of Agriculture, Tamagawa University, Machida-shi, Tokyo 194 Japan

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Summary — The pigmentation of the scutellum, sclerites of the 3rd and 4th tergites, and the abdominal ventral side of Japanese honey bee workers was examined. A predominantly yellow (summer) type of worker appeared from August to October, whereas a black (winter) type appeared from late October to May. The pigmentation grades differed among colonies collected from different regions of Japan.

When pupae were kept at 25—38°C in the laboratory, the resultant adult pigmentation was affected. The yellow type appeared at temperatures higher than 34°C, and the black type appeared at temperatures lower than 30°C. The temperature at several places in a colony was measured using microthermistors. At the central brood area, it was consistently 33—34°C and the peripheral area fluctuated from 30° to 34°C. The seasonal colour variation in the Japanese honey bee in relation to the accuracy of thermoregulation in the brood area is discussed.

***Apis cerana japonica* — seasonal dimorphism — body colour — temperature**

Résumé — La température, cause du dimorphisme saisonnier de couleur chez les ouvrières d'*Apis cerana japonica*. *On a étudié les degrés de pigmentation du scutellum, des sclérites des 3e et 4e tergites et de la face ventrale abdominale chez les ouvrières de l'abeille japonaise. Deux cents abeilles ont été prélevées à plusieurs reprises durant l'année. Le type jaune (été) apparaît d'août à octobre, et le noir (hiver) de la fin d'octobre à mai. Les degrés de pigmentation varient en fonction de l'origine géographique des colonies.*

La pigmentation de l'adulte est influencée par la température à laquelle les nymphes ont été conservées au laboratoire (25, 28, 30, 34 et 38°C). Le type jaune apparaît aux températures supérieures à 34°C et le noir aux températures inférieures à 30°C. Les expériences faites aux températures intermédiaires montrent que la période sensible est le début du stade nymphal (nymphe avec les yeux blancs ou roses). Dans la colonie, la température a été contrôlée par des microthermistors dans le nid à couvain (parties centrale et périphérique) et dans les provisions de miel. La température au centre du nid à couvain a été régulièrement de 33—34°C, alors qu'à la périphérie elle a varié de 30 à 34°C. La variation saisonnière de la couleur chez l'abeille japonaise est discutée par rapport à la précision de la thermorégulation à l'intérieur du nid à couvain.

***Apis cerana japonica* — dimorphisme sexuel — couleur du corps — température**

Zusammenfassung — Temperatur als causaler Faktor für den säsonalen Farbdimorphismus bei der Arbeitsbiene von *Apis cerana japonica*. *Der Pigmentierungsgrad des Scutellums, der Sklerite des 3. and 4. Tergits und der 4. Bauchschuppe des Abdomens wurden an Arbeiterinnen der japanischen Honigbiene untersucht. Jeweils 200 Bienen wurden zu verschiedenen Zeiten über* *das ganze Jahr hinweg gesammelt und ausgewertet. Der gelbe (Sommer-) Typ erschien in der Zeit von August bis Oktober, der dunkle (Winter-) Typ von Ende Oktober bis Mai. Der Pigmentierungsgrad unterschied sich zwischen den Völkern, die aus verschiedenen Regionen von Japan stammten.*

Wenn Puppen bei 25, 28, 30, 34 und 38°C im Labor gehalten wurden, konnten Einflüsse der verschiedenen Temperaturen auf die Pigmentierung dargestellt werden. Der gelbe Typ erschien bei höheren Temperaturen als 34°C, der dunkle Typ bei Temperaturen unter 30°C. Temperaturwechsel während des Experiments zeigte, daß die sensitive Phase in der frühen Puppenzeit mit weißen oder rosa Augen liegt. Die Temperatur in den Völkern wurde zentral und peripher zum Brutnest und bei den Honigvorräten mit Hilfe von Mikrothermistoren gemessen. Die Temperatur im zentralen Brutbereich war konstant (33-34°C), in den peripheren Bereichen schwankte sie zwischen 30 und 34°C. Das säsonale Auftreten von Farbvariationen bei der japanischen Honigbiene wird daher im Zusammenhang mit der Genauigkeit der Thermoregulation im Brutnest diskutiert.

Apis cerana japonica — *säsonaler Farbdimorphismus — Pigmentierung — Temperatur*

Introduction

Body colour is one of the characteristics used in the traditional taxonomy of the *Apis* species (Ruttner, 1988) and the variation in body colour of *Apis cerana* has been described as a characteristic of local strains or varieties (Tokuda, 1924; Muttoo, 1956). Mattu and Verma (1984) measured the width of light and dark bands on the tergites to characterize Indian regional bees. Yano (1955) and Shiraki (1973) distinguished three subspecies or varieties of Japanese honey bees : *A. c. japonica* (black abdomen), *A. c. nigrocincta* (yellow bands among each tergite), and *A. c. peroni* (2—3 yellow tergites and black posterior abdomen).

However, Okada (1986) found that workers of the Japanese honey bee *(Apis cerana japonica)* are dimorphic within a colony; the yellow type appears in colonies from July to October, whereas the black type emerges from October to May. In the present study, we examined the body colour of several colonies during 1 yr and laboratory-tested the effect of temperature as a causative factor affecting body colour.

Materials and Methods

Japanese honey bee colonies were obtained from several different regions in 1985 and 1986 and kept in the apiary of the Institute of Honeybee Science, Tamagawa University.

To determine the seasonal change of the dimorphs in the colonies, 200 worker bees were collected at intervals from each colony and the colour of the body parts was graded as shown in Fig. 1.

As a preliminary test to examine the effect of temperature on body colour, parts of combs containing pupae were removed and put in an incubator in which compartments were set to 25—38 °C. The colour of the body parts was examined after emergence of the bees. Pigmentation developed during tanning but the colour pattern itself did not change after emergence.

Pupae were collected, divided into separate developmental stages according to the grade of eye pigmentation, and subjected to temperatures of 30 or 34 °C in filter paper dishes to determine the temperature sensitive stage(s). Relative humidity was kept at 76% with supersaturated NaCl solution during the pupal incubation.

The temperature in various parts of a colony was automatically recorded from 15 to 18 November 1985 by using microthermistors (Takara Ind. Co., TZL-64) in which sensory pinpoints were inserted into cells or left free.

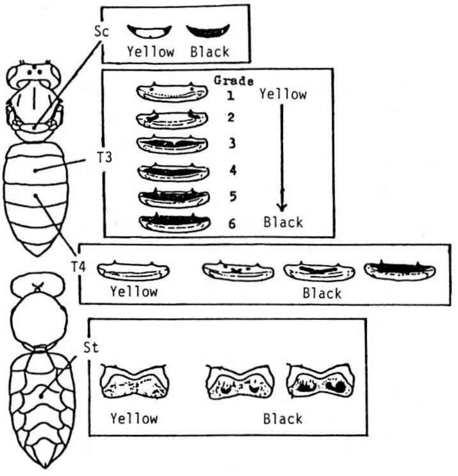


Fig. 1. Colour grading of four body parts of *Apis cerana japonica* (Sc : scutellum, T3 : 3rd tergite, T4 : 4th tergite, and St : abdominal sternite).

Records were collected at hourly intervals to calculate the average temperature ± SD. During this period, the outside ambient temperature was low enough to easily detect temperature differences, if any, within a colony.

Results and Discussion

Colour change in colonies

Body colour was partially genetically determined. Dark strains, which came from Ehime, Shikoku Island, showed a predominance of dark bees with colour grades for T3 of more than 4, except during August to October (E1 and E2 in Fig. 2).

In contrast, strains from Kumamoto, Kyushu island (K1 and K2) were lighter in colour. The colour grade of winter-type bees in this strain was 3 for T3. These genetic variations correspond to the colour varieties reported previously.

The data represented in Fig. 2 also agree with the seasonal colour change reported by Okada (1986). Yellow bees (T3 less than 2) appeared from July until October. The number of dark bees (T3 more than 5) decreased strikingly during this period but increased in October.

Colour grades of other body parts examined are shown in Table I. These parts also showed seasonal colour changes. The sternite, the most sensitive

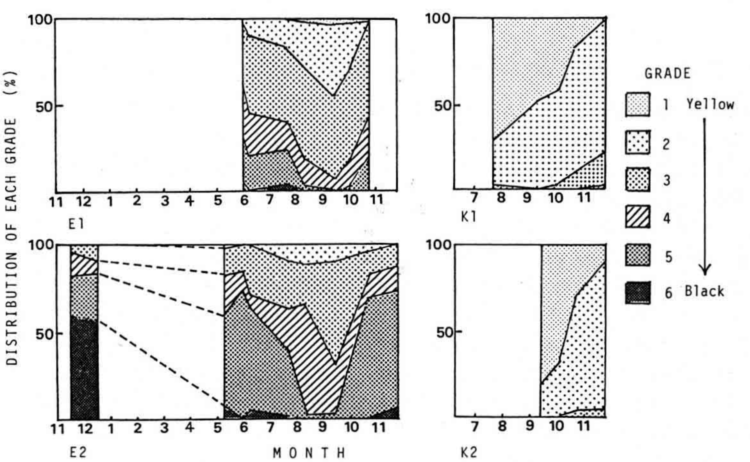


Fig. 2. Seasonal colour variation in 3rd abdominal tergite of *Apis cerana japonica.*

Table I. Seasonal variation in pigmentation of scutellum (Sc), fourth tergite (T4) and sternites (St) of *Apis cerana japonica*.

	Colony [1]	*Dec 19*	*May 9*	*May 25*	*Jun 7*	*Jul 18*	*Aug 10*	*Sep 13*	*Oct 2*	*Oct 22*	*Nov 19*
					(% of dark types)						
Sc	E1	–	–	89.0	88.0	85.3	84.6	77.3	77.3	91.3	–
	E2	90.8	83.6	84.7	86.1	80.0	82.6	76.0	79.1	88.3	87.0
	K1	–	–	–	–	0	–	0	0	0.6	5.9
	K2	–	–	–	–	–	–	0	0.4	0.5	9.8
T4	E1	–	–	72.4	56.4	40.8	19.5	10.3	22.3	60.3	–
	E2	100	87.8	88.5	74.8	40.8	35.6	39.6	54.5	73.5	100
	K1	–	–	–	–	0	–	0	0	0	5.3
	K2	–	–	–	–	–	–	0	0	0	1.2
St	E1	–	–	20.2	16.5	11.4	1.3	0	16.0	93.8	–
	E2	98.3	46.0	21.3	23.5	0	0	0	30.0	67.8	100
	K1	–	–	–	–	0	–	0	0	12.0	47.9
	K2	–	–	–	–	–	–	0	0	4.7	10.8

[1] E and K represent bees originating from Ehime and Kumamoto respectively.

part, was almost always yellow during summer and black during winter.

Temperature treatment in an incubator

Since the seasonal colour change may reflect the temperature at which bees developed, the effect of temperature during the pupal stage was examined. Sealed brood were removed from colonies and put in a temperature-programmed incubator. Emerged bees were examined for colour (Table II). The pigmentation in the Kumamoto strain was lighter than the Ehime strain as described above. The lower the temperature, the more pigmented bees that emerged. The colour of the abdominal sternites in the Kumamoto strain was more sensitive to temperature. No dark spot appeared in bees incubated at 38 °C; only 6.7% of bees were dark at 34 °C and a 100% black abdomen occurred after incubation at 30 °C or lower. Mortality was high at incubation temperatures lower than 28 °C.

In another experiment, pupae at known developmental stages were subjected to high (34°C) and low (30°C) temperatures. The results are shown in Fig. 3. The colour grade of T3 for the high-temperature treated individuals (throughout pupal stage, row G in the figure) was 3.0, and 0—8% of bees were dark at other parts (Sc, T4, and St). However, the same pigmentations were 3.7 and 67—100%, respectively, for bees kept at the low temperature (row A). From intermediate experiments (shown in the

Table II. Effect of temperature during pupal period on pigmentation in *Apis cerana japonica.*

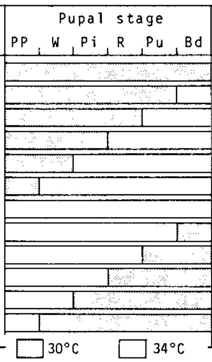
Colony [2]	*Temp. (°C)*	*N*	*Grade of T3*							*Pigmentation (%)* [1]		
			1	*2*	*3*	*4*	*5*	*6*	*Av.*	*Sc*	*T4*	*St*
E 2	38	43		7	28	8			3.0	48.8	18.6	0
	34	30			7	16	7		4.0	83.3	66.7	6.7
	30	10			2	2	5	1	4.5	80.0	100	100
	28	1			1				3	0	100	100
	25	1					1		5	100	100	100
K 3	38	39	35	4					1.1	0	0	0
	34	71	26	41	4				1.7	8.5	2.8	0
	30	86	3	34	50	1			2.6	4.7	41.9	33.7
	28	65	1	30	31	3			2.6	6.2	43.1	35.4
	25	18		2	6	4	3	3	5.8	55.6	83.3	83.3

[1] % pigmentation of scutellum (Sc), 4th tergite (T4) and sternite (St).
[2] E and K represent bees originating from Ehime and Kumamoto respectively.

figure), it is evident that the earlier or longer the treatment with high temperature, the lighter the bees appeared, and *vice versa.* The results indicate that the temperature-sensitive period is the early pupal stage with white or pink eyes.

Temperature fluctuation in a colony

Brood area temperature in a honey bee colony is known to be constant (Free, 1977). However, if this is always true, the colour variation described above is difficult to explain on the basis of temperature change alone, and the phenomenon has to be explained by other factor(s). Consequently, the temperatures at the central and peripheral brood area, and honey stock area in a Japanese honey bee hive were monitored with microthermistors. The outside ambient temperature was also measured (Fig. 4).



Group	N	Mortality (%)	T3 Grade	Pigmentation (%) Sc	T4	St
A	10	70	3.7	67	100	100
B	8	25	3.5	67	83	83
C	5	0	3.6	60	80	80
D	6	67	3.0	0	100	100
E	7	0	3.3	29	100	57
F	8	13	3.0	0	43	43
G	13	0	3.0	0	0	8
H	5	40	3.0	0	67	0
I	7	0	3.0	0	71	71
J	7	43	3.0	0	100	100
K	6	33	2.8	25	100	100
L	7	14	3.3	67	100	100

Fig. 3. Effect of temperature changes during the pupal period on adult pigmentation in *Apis cerana japonica.* Pupal stages are classified as prepupa (PP), pupa with white (W), pink (Pi), red (R) and purple (Pu) eyes, and with darkened body colour (Bd).

The ambient temperature during the experimental period showed a diurnal fluctuation between 5 and 18 °C (10.0 ± 3.1 °C, average). The temperature at the central brood area was very stable at 34 °C (33.9 ± 0.3 °C). However, the peripheral brood area temperature was lower than at the centre with some diurnal fluctuation (31.3 ± 1.7 °C). The honey storage area was also kept much warmer than the outside air, but was cooler than the brood area and was affected by diurnal change (25.9 ± 3.4 °C).

Verma (1970) has also reported seasonal temperature differences in a hive of *Apis indica*.

Temperature as a causative factor of colour dimorphism

Body colour and pattern of the Japanese honey bee are largely determined genetically. Kumamoto (Kyushu island) colonies produce light coloured bees while Ehime (Shikoku island) bees are dark. This agrees with the results of Tokuda (1924). However, Ono and Tsuruta (1987) have reported yellow morphs from Iwate (Northern Honshu island). Experimental colonies in the present study showed a distinct colour dimorphism as reported by Okada (1986) which were irrespective of geographical origin. Laboratory experiments proved that a 4° temperature difference during pupal development caused the dimorphism. The temperature difference between the central and peripheral brood area in a colony is about 2.5 °C, which could be a major cause of the colour dimorphism in adult bees. The variation in pupal period in *Apis cerana* (10—12 d, Kapil, 1959) may be caused by the temperature differences in the brood. Average pupal durations in the present study at 38°, 34°, and 30 °C were 10.5, 11.6, and 17.0 d, respectively.

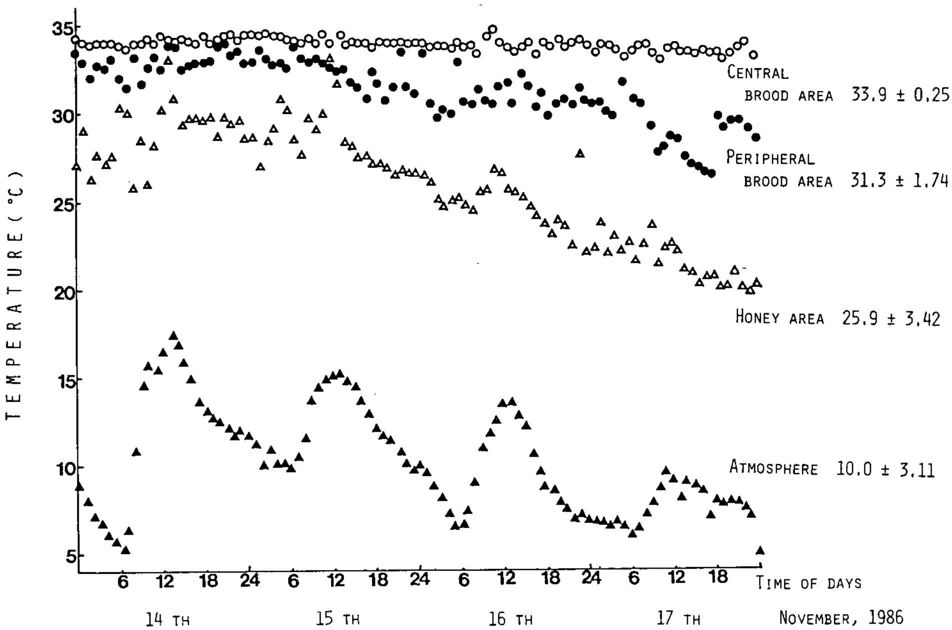


Fig. 4. Outside air temperature and temperature of central and peripheral brood area, and honey storage area within an *Apis cerana japonica* colony during 4 days in November, 1986.

Factors determining seasonal dimorphism among insects, especially Lepidoptera, have been investigated. Photoperiodism and humidity, as well as temperature, are known to be major factors affecting the variation (Endo *et al.*, 1985). Although the influence of these other factors should be examined carefully in the honey bee, the present experiments were carried out under controlled conditions where only temperature was varied. Seasonal variation has been found in the morphometric characteristics of *A. c. indica* including the length of tergites and sternites (Mattu and Verma, 1984). The authors inferred that colony strength and surplus food caused maximal development of these and other characteristics. Combining their inference with our results, colony strength could have an effect by raising the hive temperature; however, nutritional factors need to be investigated.

Since body colour in workers of *A. c. japonica* is affected by temperature, description of varieties should not be based only on body colour (Ruttner, 1988).

References

Endo K., Maruyama Y. & Sasaki K. (1985) Environmental factors controlling seasonal morph determination in the small copper butterfly *Lycaena phlaeas daimio. J. Insect Physiol.* 31, 525-532

Free J.B. (1977) *The Social Organization of Honey Bees.* E. Arnold, London, p. 68

Kapil R.P. (1959) Variation in the developmental period of the Indian bee. *Indian Bee J.* 21, 3-6 and 26

Mattu V.K. & Verma L.R. (1984) Morphometric studies on the Indian honey bee, *Apis cerana indica* F. Effect of seasonal variations. *Apidologie* 15, 63-74

Muttoo R.N. (1956) Facts about beekeeping in India. *Bee World* 37, 125-133

Okada I. (1986) Biological characteristics of the Japanese honey bee, *Apis cerana japonica.* Proc. 30th Int. Apic. Congr., Apimondia. pp. 119-122

Ono M. & Tsuruta T. (1987) Variation of body colour in the Japanese honey bee, *Apis cerana japonica. Honeybee Sci.* 8, 113-114 (in Japanese)

Ruttner F. (1988) *Biogeography and Taxonomy of Honeybees.* Springer-Verlag, Berlin, Heidelberg, RFA, p. 284

Shiraki T. (1973) *Classification of Insects.* Hokuryukan, Tokyo, p. 625 (in Japanese)

Tokuda Y. (1924) Studies on the honeybee, with special reference to the Japanese honeybee. *Trans. Sapporo Natl. Hist. Soc.* 9, 1-27 (in Japanese)

Verma L.R. (1970) A comparative study of temperature regulation in *Apis mellifera* L. and *Apis indica* F. *Am. Bee J.* 110, 390-391

Yano S. (1950) *Iconographia Insectorum Japonicorum.* 2nd ed., Hokuryukan, Tokyo, p. 1494 (in Japanese)