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Effects of brood temperature on honey bee *Apis mellifera* wing morphology*

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西方蜜蜂幼虫发育温度对成体翅膀形态的影响*

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摘　要　为探讨不同恒定温度条件对培育蜜蜂蛹翅膀形态特征的影响，作者将进入蛹期的西方蜜蜂（*Apis mellifera*）放入人工气候箱里，分别在32、35和36℃的恒定温度条件培育，直到蜜蜂蛹羽化出房为止。测量了蜜蜂翅膀的标准形态特征，包括翅的大小、肘脉长和11个翅肘脉角，所得到的数据进行多变量比较分析、相关性分析、主成分分析和区别分析。研究结果表明，不同温度条件对蜜蜂翅膀形态特征有明显的影响［动物学报 51（4）：768－771，2005］。

关键词　西方蜜蜂　幼虫发育温度　翅膀形态

Key words　Honey bee, *Apis mellifera*, Brood temperature, Wing morphology

Brood care in honey bees involves a remarkable ability to keep the brood temperature within a narrow range of 33 － 36℃ (Corkins, 1930; Merrill, 1924; Seeley, 1981). Particularly during pupae development, brood is sensitive to temperature variation and morphological malformations occur if the brood temperature is lower than 32℃ or higher than 37℃ (Himmer, 1927, 1932) over prolonged periods. Even apparently normally developed individuals may be affected by suboptimal temperatures. Pigmentation patterns appear to be temperature-sensitive. Pupae kept at 25 － 38℃ in the laboratory emerged into yellow-type adults at high temperature of 34℃ but were black-type at temperatures below 30℃ adult (Tsuruta et al., 1989). As other morphological characters might also be affected, this study investigates whether brood raising temperature would influence the morphology

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of the wings as wing size, cubital index and wing venation characterized by wing angles.

In this study, we explored the effect of raising pupae at different constant temperature on wing morphology. Pupal stages of honeybee *Apis mellifera* workers were raised within their cells until emerged in an incubator at three different temperatures of 32, 35 and 36℃, respectively. Standard morphometric characters including wing size, length of cubital veins and 11 wing venation angles were measured. Data were analysed by ANOVA analysis and correlation analysis. Variables were reduced by principal component analysis, and temperature groups were confirmed by discriminant analysis. The results showed a significant influence of temperature on wing morphology.

1 Materials and methods

Three brood combs from closely related and evenly kept colonies of *Apis mellifera* were placed directly after cell sealing into incubators set to temperatures of 32℃, 35℃ and 36℃ respectively. After hatching, the worker bees were stored in a freezer. The experiments were repeated three times (July 2003, July 2004 and August 2004) in the Bee Station of Wuerzburg University, Germany. For morphometric analysis, 20 worker bees were taken from each of the 9 samples, dissected and measured according to the methods described by Ruttner et al. (1978) and Ruttner (1988). Measurements were performed using a stereo microscope and a computer-aided measuring system based on a video system and measuring program (Meixner, 1994) in the Bee Institute of Frankfurt University of Germany. Of the 41 morphometric characters listed in Ruttner et al. (1978), 17 wing characters were determined, including length and width of the forewing, forewing index, length of cubital vein 1 and 2, cubital index, and 11 wing venation angles.

Data were analysed by multifactorial analysis of variance, and by correlation analysis (Pearson correlation coefficients) Variables were reduced by principal component analysis, and temperature groups were confirmed by discriminant analysis. All calculations were performed using the SPSS for Windows 10.00 statistical package.

2 Results

Data were analyzed by multivariate ANOVA with trial and temperature as fixed factors, showing significant differences and interactions for both variables ($P < 0.0005$, Wilks-Lambda). Means for temperatures are given as estimated marginal values in Table 1 for each variable. All 17 morphometric characters showed significant differences ($P < 0.05$). In multiple comparisons, the 32℃ and 35℃

Table 1 Estimated marginal values for morphometric measurements at three brood raising temperatures

	32℃ Mean SD	35℃ Mean SD	36℃ Mean SD	P
Length forewing	921.03 ± 1.11^{ab}	921.98 ± 0.96^{a}	922.95 ± 1.08^{b}	0.006
Width forewing	318.90 ± 0.89^{a}	315.84 ± 0.67	318.27 ± 0.99^{a}	0.001
Cubital 1	64.48 ± 0.83^{a}	64.94 ± 0.79^{a}	64.24 ± 1.07^{a}	0.001
Cubital 2	22.19 ± 0.43^{a}	22.40 ± 0.61^{a}	19.71 ± 0.45	0.005
Angle A4	27.32 ± 0.26^{a}	27.26 ± 0.42^{a}	29.24 ± 0.38	0.001
Angle b4	111.33 ± 0.86^{a}	110.42 ± 1.17^{ab}	108.19 ± 1.08^{b}	0.001
Angle D7	94.89 ± 0.67^{ab}	93.70 ± 0.59^{a}	96.15 ± 0.45^{b}	0.004
Angle E9	23.55 ± 0.34	24.39 ± 0.33^{a}	24.56 ± 0.28^{a}	0.001
Angle G18	91.72 ± 1.11^{a}	89.12 ± 0.55^{b}	90.45 ± 0.45^{ab}	0.014
Angle J10	52.24 ± 0.58^{a}	51.80 ± 0.65^{a}	54.17 ± 0.8	0.001
Angle J16	92.39 ± 0.63^{a}	92.50 ± 0.62^{a}	92.27 ± 0.60^{a}	0.001
Angle K19	80.57 ± 0.55	77.81 ± 0.42^{a}	76.48 ± 0.54^{a}	0.001
Angle L13	13.02 ± 0.24^{a}	12.64 ± 0.22^{ab}	12.06 ± 0.21^{b}	0.001
Angle N23	93.02 ± 0.62^{a}	93.45 ± 0.58^{a}	93.30 ± 0.66^{a}	0.001
Angle O26	36.04 ± 0.72^{a}	37.52 ± 0.67^{a}	33.81 ± 0.58	0.001
Cubital index	2.94 ± 0.07^{a}	2.98 ± 0.10^{a}	3.33 ± 0.11^{a}	0.004
Forewing index	34.63 ± 0.10^{a}	34.26 ± 0.06	34.48 ± 0.09^{a}	0.001
PC1	-0.23^{a}	-0.28^{a}	0.51	0.001
PC2	0.08^{a}	0.33^{a}	-0.41	0.001
PC3	-0.33^{a}	-0.16^{a}	-0.49	0.001

Length measurements are given in 0.01 mm, angles in °. For each variable the significance for temperature effects is indicated by P, post hoc comparisons (LSD) are given by superscript letters where same letters indicate no difference ($P > 0.05$).

group differed significantly in only 5 characters (width of forewing, Angle E9, G18, K19 and forewing index), while the 36℃ group differed from the 35℃ group in 9 (length and width of forewing, length of cubital vein 2, angles A4, D7, J10, O26, cubital index and wing index) and from the 32℃ group in 9 characters (length of cubital vein 2, angles A4, B4, E9, J10, K19, L13, O26 and cubital index). Significantly positive correlations to temperature existed in 3 of the characters (E9, A4 and cubital index) and negative in 5 characters (cubital vein 2, B4, K19, L13 and 026), while the relation was irregular or absent in the others. To reduce information, data were submitted to factor analysis, resulting in three principal components which represented 40.7% of the variation. PC1 correlated strongly ($|r| > 0.5$) with A4, B4 and J10, PC2 mainly with cubital vein 1, D7 and L13 and PC 3 mainly with cubital vein 2, E9 and J10. Sample scores of the 36℃ group differed significantly from the other groups in all three principal components while the other two groups were indistinct (Table 1); PC1 and PC3 correlated positively with temperature.

Whether temperature groups differ was further investigated by discriminant analysis, in which the first function, correlating predominantly with A4 and length of cubital vein 2, accounted for 36.4% and the second function, correlating predominantly with width of forewing and G18, accounted for 19.2% of the total variation in the data (eigenvalue 2.784, $r = 0.856$ and 1.450, $r = 0.769$, respectively). A graph of sample values on the two discriminate axes show that centroids of the three temperature groups are separate. Each group differed from the others ($P < 0.0005$, Wilks Lambda) (Fig.1). Samples show some degree of overlap, and discriminant analysis classified most of the samples (74.4%) correctly into their temperature groups (66.7%, 80.0% and 76.7% for the 32℃, 35℃ and 36℃ group, respectively).

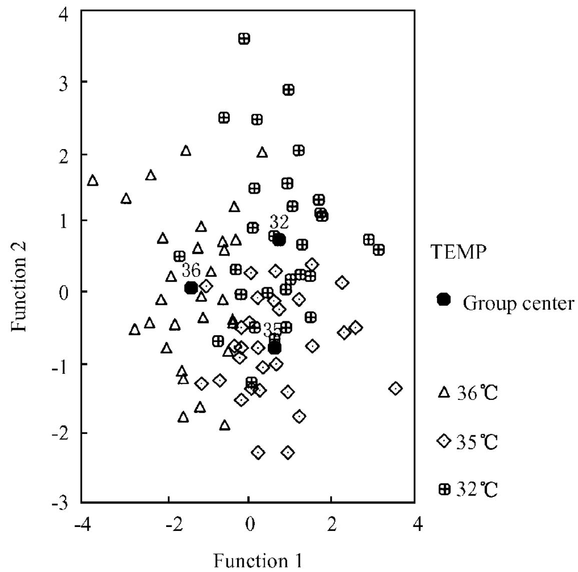

Fig.1 Plot of the bees in three temperature groups (32℃, 35℃ and 36℃) on the two discriminant functions

3 Discussion

Our analysis clearly showed that raising the bee pupae at different temperatures affected wing morphology. These results may help to explain the existence of seasonal influences found in morphometric measures of honeybees (Mattu and Verma, 1984; Gromisz, 1962; Michailoff, 1927a). Although only few parameters were in common, some degree of correspondence of the results is apparent. The forewing appears to be longer during the warmer periods in all three studies. This is also consistent with a study by Michailoff (1927b) who found an increase in forewing length in bees raised at 35℃ in comparison to bees raised at 30℃. Mattu and Verma (1984) reported higher values in the wing angles A4 and E9 during summer and these two wing angles also showed a distinct response to increased temperature in our study. Though the relationship of ambient temperature and brood temperature is unclear, the brood area is known to be constant in the centre, but might show some fluctuation in the peripheral area (Free, 1977) which might link seasonal morphological changes to brood temperature.

In the morphometric study of the honey bee, wing size and wing venation angles are characteristics traditionally used in morphometry-based taxonomy of *Apis* species (Ruttner, 1988; Engel, 2001). Though seasonal influences on brood temperature might to some degree affect the measurements (Mattu and Verma, 1984), the direct phenotypic influence of ambient temperature is unlikely to be a major factor in the overall geographical distribution of morphological characters. Locally adapted biotypes will be strongly selected to regulate brood temperature close to the optimum even in extreme climatic regions. An independent relation between bee morphology and environmental temperature is evident from the tendency of bees to be larger in high altitude or northern latitudes (Ruttner, 1988), and from the negative correlation of mean ambient temperature with wing size (Tan et al., 2003; Tsuruta et al., 1989) which is contrary to the effect of brood-temperature on wing morphology reported in this study.

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