The Effect of Temperature on Hind wing Vein of *Apis cerana cerana* during Sealed Brood's Development[1)]

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Abstract The sealed broods of *Apis cerana cerana* were incubated at constant temperature 30℃ or treated at 24℃ for 24 to 72 h, and then incubated at 35℃ (the optimum temperature for brood development) until emergence. Significant three types of mutations including appearance of new vein, protrusion and absence of virgin vein were observed in hind wing vein. A new radiomedial crossvein appeared between radial sector and mediae exterior margin of basal cell. Mediae and radial sector protruded towards basal cell. Cubito-anal crossvein and mediae protruding towards discal cell disappeared. This discovery will have a significant influence on the study of germplasm resources evaluation, evolutional biology and phylogenetics of honeybee.

Key words honeybee; temperature; development; vein

1 INTRODUCTION

Honeybee is a kind of social insect. Nest thermoregulation could come true by individual worker producing heat (Kleinhenz M et al., 2003) and by wing fanning and water intake for evaporative cooling (Norman, 1993), and colonies maintain stable brood nest temperatures of about 35℃ (Winston, 1987). Broods (including egg, larva and pupa) naturally complete development at relatively constant brood nest temperature.

Honeybee's temperature sensitivity is a product of evolutionary process. Brood's developmental period, survival rate, original quality, ovariole number (Zhou bingfeng et al., 2002), ultrastructure of the mucus glands of drones (Lien M et al., 2005) and behavioural performance of adults (Tautz Jet al., 2003) could be influenced by deviations from an optimal temperature during development.

Very little is known about the effect of environmental factor on vein morphology of insect. The ozone could cause filial generation of housefly vein variations including the 4th longitudinal vein defect or malformation, medial cross-vein variation, disappearance of radiomedial crossvein, deficient wing and vestigial wing etc. (Kou yu et al., 2003), while there is no literatures on honeybee's vein. In the study of the effect of temperature on development of honeybee, however, we accidentally discovered significant variations in honeybee vein (Zhou bingfeng et al., 2007).

Vein morphology of hymenoptera is important for classification and phylogenesis (Chen jiahua et al., 1994). If changes of vein morphology are because of environmental factor, it would provoke a challenge to the traditional method of insect taxonomy. A further study on effect of temperature on vein morphology may reveal evolution pattern of honeybee.

1 MATERIALS AND METHODS

1.1 Samples

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Honeybees (*Apis cerana cerana*) were obtained from hives in Fuzhou Fujian province (China).

Queens were placed on an empty comb to laid eggs, thus providing us brood combs in which all the larvae would pupate almost simultaneously. The queens were transferred to a new, empty comb the nest day. The combs with the eggs were left in the colony for about 8 days until most of the brood cells had been capped. Samples were those which capped within 2 hours.

1.2 Methods

The capped brood samples of *Apis cerana cerana* were treated at 24□ for 24 to 72 h in incubator (HWS-250, Shanghai Jing Hong Laboratory Instrument Co., Ltd., China; precision, ±0.5℃), and then incubated at 35□ (the optimum temperature for brood development) until emergence using a thermoprobe (95-A, Shanghai Jinghua Meter Factory, China; precision, ±0.5℃) and a precise dry-humidity thermometer (WQG-11, Shanghai Medical Instrument Co., China; allowance, ±0.0℃) to monitor the brood temperature continuously during the entire incubation periods to guarantee the brood developing under the expected temperatures. Deviations from the preset temperatures were minimal, ranging within ±0.5℃.

1.3 Vein morphology observation

Hind wings of samples were mounted on a microscope slide for morphometric analysis. Microscope slides of wings were projected onto a TV screen, and vein morphology were photographed in details using a stereomicroscope (GL-99TI, Guilin Optical Instrument Factory, China) and a computer-aided measuring system based on a video system (IK-CP230Ⅱ and MV-2000).

Hind wings were described according to Gauld（1988）（Fig. 1）.

3 RESULTS

Significant 3 types of mutations including appearance of new vein, protrusion and absence of virgin vein were observed in hind wing vein of *Apis cerana cerana*.

3.1 Appearance of new vein in hind wing vein

There was a new radiomedial crossvein (rs-m) appeared between radial sector (Rs) and mediae (M) (Fig.1, 2). However there is only one short rs-m between Rs and M in normal hind wing vein.

The worker's and drone's sealed broods of *Apis cerana cerana* were treated at 24℃ for 24 to

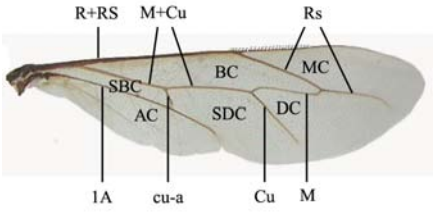


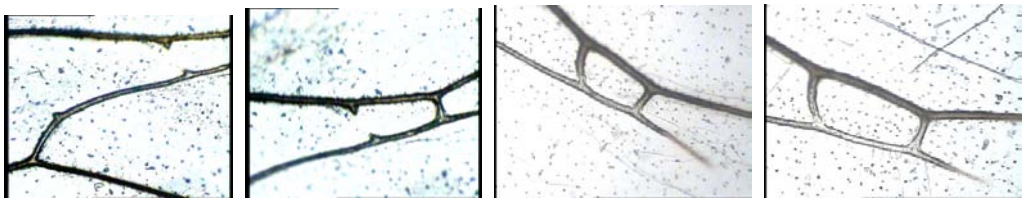
Figure 1 Name of vein and wing cell of *Apis cerana cerana*

1A：first anal vein；Cu：cubitus；M：mediae；R：radial vein；Rs：radial sector；cu-a: cu-a crossvein

AC：anal cell；BC：basal cell；DC：discal cell；MC：marginal cell；SBC：sub basal cell；SDC：sub discal cell

Figure 2 The new rs-m's position of hind wing

72 h, and then incubated at 35℃ (the optimum temperature for brood development) until emergence or incubated at constant temperature 30℃. A new rs-m was observed between Rs and M of the adults' hind wing. There were different degrees of the new rs-m. Some only had two protuberances protruding towards each other (Fig.3AB); some were rounded new vein with different distance position, close to the original rs-m (Fig.3C) or distant to the originalrs-m relatively (Fig.3D). As the original rs-m, the new rs-m had a "feeble point" at the back end (Fig.3CD).



A B C D

Figure 3 The new rs-m between Rs and M of hind wing of *Apis cerana cerana*

3.2 Protrusion mutations in hind wing vein

There were protrusion mutations in M and Rs (Fig.4).

3.2.1 The protrusion mutation in M

The normal M of hind wing vein had a "turning point", but had no protrusion (Fig.4).

The drone's sealed broods of *Apis cerana cerana* were treated at 24℃ for 24 h, and then incubated at 35℃ until emergence or incubated at constant temperature 30℃. The M of the temperature-treated adults' hind wing vein protruded towards BC. Some protruded slightly (Fig.5A), some protruded visibly (Fig.5B) and some protruded significantly forming a long vein (Fig.5C). All of the mutations were observed at the "turning point" of M. In addition, in some samples Rs and M had two protuberances protruding towards each other (Fig.3A).

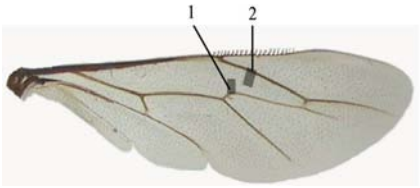
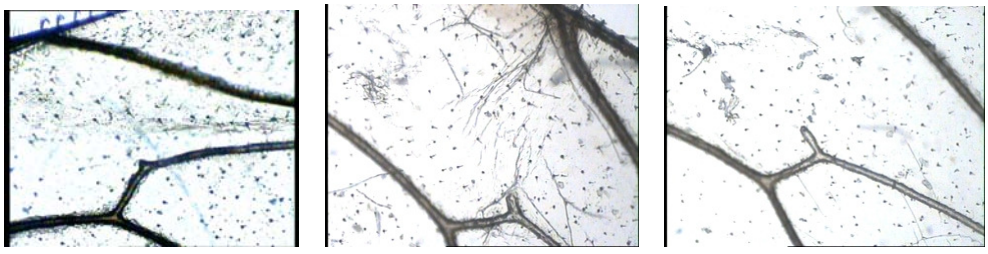


Figure 4 The protrusion mutations' position
1. the protrusion mutation in M; 2.the protrusion mutation in Rs

This kind of mutation was only observed in drones, but not in workers.



A B C

Figure 5 The protrusion mutations in M towards BC

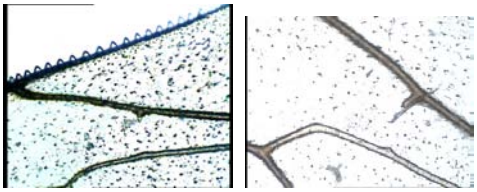
3.2.2 The protrusion mutation in Rs

This kind of mutation was observed at the foreside of Rs (Fig.4). The normal Rs had no protrusion.

The worker's sealed broods of *Apis cerana cerana* were treated at 24℃ for 72 h, and then incubated at 35℃ until emergence or the drone's sealed broods were incubated at constant temperature 30℃. The Rs protruded towards BC of temperature-treated adults' hind wing vein. Some protruded slightly (Fig.6A), some protruded visibly (Fig.6B).

3.3 Absence of virgin vein in hind wing vein

The virgin vein observed absent were the cu-a of SBC and the M in the DC (Fig.7).



A B

Figure 6 The Rs protruding towards BC

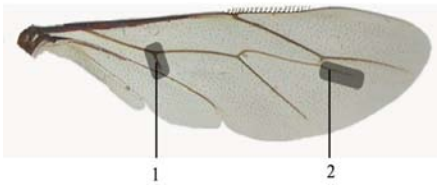


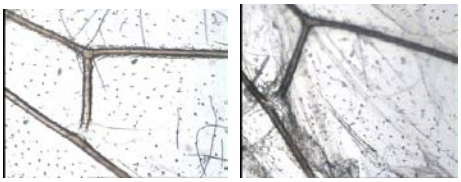
Figure 7 The positions of the virgin vein with absence 1. cu-a; 2. M

3.3.1 The absence of cu-a

The normal cu-a joints cu and A, and at the back end of cu-a is the "feeble point", but not broken (Fig.8).

The drone's sealed broods were incubated at constant temperature 30℃ until emergence. Two of the temperature-treated drones were observed with absence cu-a at the "feeble point" (Fig.9).

Figure 8 the normal cu-a



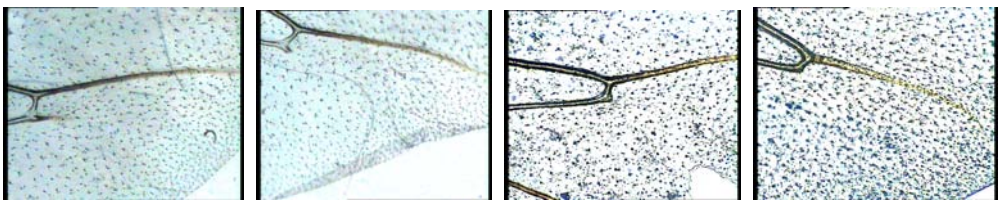
A B

Figure 9 the cu-a with absence

3.3.2 The absence of M

The normal M is stretching towards DC in hind wing of *Apis cerana*, which is one of the important morphological characters to distinguish *Apis cerana* and *Apis mellefera*.

The worker's sealed broods of *Apis cerana cerana* were treated at 24℃ for 24-72 h, and then



A B C D

Figure 10 the absence of the tail end of M of the hind wing of *Apis cerana cerana*

incubated at 35℃ until emergence. The mediaes stretching towards DC of large numbers of temperature-treated worker adults were absent at different degrees. Some was absolutely shortened (Fig.10A); some only had vestige left (Fig.10BC); some were absent completely (Fig.10D), which were same with *Apis mellefera* entirely (Fig.11).

At the same time, so many samples were observed with shortened rs-m (Fig.12A) or without rs-m (Fig.12B).

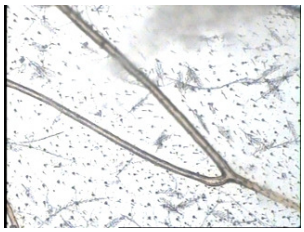
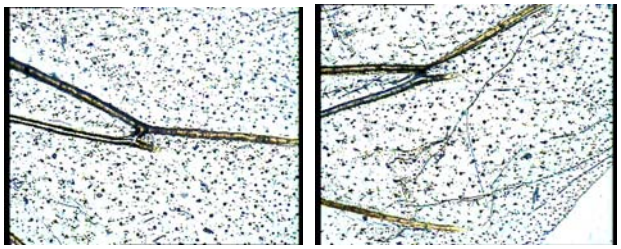


Figure 11 the outside of BC in hind wing of *Apis mellefera ligustica*



A B

Figure 12 Shortened rs-a and disappeared rs-m of the hind wing of *Apis cerana cerana*

4 DISCUSSIONS

（1）The new veins produced by decreased developmental temperature were likely to be the ones had disappeared during evolution. That was to say the genes of these veins still existed, but could not be expressed under normal conditions. If this hypothesis can be confirmed by molecular biology, the relationship between honeybee and other hymenoptera, the evolutionary process of honeybee and phylogenesis of Apidae can be inferred by these veins.

（2）Vein tends to decrease during evolutionary history of hymenoptera (Gauld et al., 1988), so vein of honeybee may keep on decreasing in the future. The absent veins in this study may disappear in the future. Whether this happens would be determined by whether the disappearing of the vein influences the fitness of honeybee. The results of this study have a good potential to predict the trend of vein's evolution in the future and also to study the phylogenesis of hymenoptera.

（3）Vein's distribution pattern provides an important reference for insect taxonomy, however, developmental temperature could cause vein variation, which provoke a challenge to the traditional method of insect taxonomy.

REFERENCES

Chen J.H., Wu Z.S. 1994. The alysiini of China: Hymenoptera Braconidae Alysiinae. Beijing: agriculture publishing house of China, 10-11

Kou Y., Ni X.P. 2003. Study on Biological Effect of Housefly in Ozone. Chin J Vector Bio&Control, Vo1. 14, No. 2: 108-109

Zhou B.F., Lin S.H., Su J. et al. 2002. the effect of temperature on fertilized eggs and sealed queen pupa of honeybee. Journal of Fujian Agricultural and Forestry university (natural Science Edition), 31(4): 511-513

Zhou B.F., Li Y., Zhu X.J. et al. 2007. The effect of temperature on forewing vein of honeybee. Apicalture of China, 58(5): 5-8

Gauld I, Bolton B. 1988. The Hymenoptera. Oxford: Oxford University Press. 66-75

Kleinhenz M, Bujok B, Fuchs S, et al. 2003 Hot bees in empty broodnest cells: heating from within. J Exp Biol. 206(23): 4217-4231

Lien M, Olivier S, Gudrun K, et al. 2005 Morphological and ultrastructural changes in the mucus glands of *Apis mellifera* drones during pupal development and sexual maturation. Apidologie 36 (2005) 245-254

Norman E G. 1993. Activities and behavior of honey bees. In: Joe M. The hive and the honey bee. Hamilton: Dadant & Sons, 312

Tautz J, Maier S, Groh C, et al. 2003. Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc Natl Acad Sci U S A.100(12):7343-7347

Winston M L. 1987, The biology of the honey bee. London: Harvard University Press.