

## Non-destructive genotyping and genetic variation of fanning in a honey bee colony

Songkun Su<sup>a,\*</sup>, Stefan Albert<sup>b</sup>, Shaowu Zhang<sup>c</sup>, Sven Maier<sup>d</sup>, Shenglu Chen<sup>e</sup>,  
Honghu Du<sup>a</sup>, Jürgen Tautz<sup>d,\*</sup>

<sup>a</sup>College of Animal Sciences, Zhejiang University, Hangzhou, 310029, China

<sup>b</sup>Institute of Medical Radiation and Cell Research, University of Würzburg, Versbacher Strasse 5, 97078 Würzburg, Germany

<sup>c</sup>Centre of Excellence in Vision Science, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, A.C.T. 2601, Australia

<sup>d</sup>Beegroup, Biocenter, University of Würzburg, Am Hubland, D-97074, Würzburg, Germany

<sup>e</sup>College of Agriculture and Bio-technology, Zhejiang University, Hangzhou, 310029, China

Received 1 November 2006; received in revised form 10 January 2007; accepted 11 January 2007

### Abstract

The relationship between workers from different patriline in a naturally mated queen honey bee colony is very complex due to queen polyandry, and still poorly characterized. Here, we report a means of determining the genotype of living workers in a natural honey bee colony by a new non-destructive method, which makes it possible to observe the relationship between behaviours and genotypes. DNA was extracted from the exuvia, found at the bottom of each brood cell, and confirmed to be identical to the DNA extracted from the thorax muscle of the bee emerging from that particular cell. The genotypes were thus determined using DNA from the exuviae without having to hurt or kill the organisms. The emerging workers were marked with coloured, numbered tags to enable behavioural observations over their entire life. Using this new method, we determined 20 patriline in a naturally mated queen colony, and discovered that the patriline composition of bees exhibiting fanning behaviour was significantly different from the patriline composition of the whole colony. Our results confirm that the genetic structure of a natural insect society plays a fundamental role in the division of labour. The new non-destructive method reveals a novel avenue for the determination of relationships between the behaviours and genes of social insects.

© 2007 Elsevier Ltd. All rights reserved.

**Keywords:** *Apis mellifera*; Genotyping; Fanning behaviour; Genetic variation; Microsatellites; PCR

### 1. Introduction

The honey bee (*Apis mellifera*) is a highly eusocial insect (Linksvayer and Wade, 2005) with complex patriline structure (Michener, 1974), making it an ideal model organism for research on social behaviours (Amdam et al., 2006; Honeybee Genome Sequencing Consortium, 2006; Robinson et al., 2005). In recent years, there has been a surge of interest in the genetic underpinnings of honey bee

behaviour (Robinson, 2002; Ruppell et al., 2004). Robinson and Page reported the genetic variation in guarding and undertaking behaviour in artificially inseminated honey bee colonies containing three patriline (Robinson and Page, 1988). By artificially inseminating a queen with semen from two unrelated drones, and using genetic lines that express distinguishable colour morphs in worker bees, Getz and Smith found that honey bees can discriminate between full and half sisters (Getz and Smith, 1983). Page and colleagues, using body colours or allozyme markers to identify two or three subfamilies in experimental colonies, reported that honey bee workers discriminate nepotistically among nest mate patriline under experimental conditions (Frumhoff and Schneider, 1987; Getz et al., 1982; Page

\*Corresponding authors. Tel.: +86 571 86971095; fax: +86 571 86971095.

E-mail addresses: [susongkun@zju.edu.cn](mailto:susongkun@zju.edu.cn) (S. Su), [tautz@biozentrum.uni-wuerzburg.de](mailto:tautz@biozentrum.uni-wuerzburg.de) (J. Tautz).

et al., 1989). However, the relationship between different patriline in a naturally mated queenright colony is very complicated because the queen mates with multiple drones (polyandry). Therefore, the results of the above studies may not accurately reflect the situation in natural colonies, in which queens may mate with 7–17 males (Winston, 1987). The phenomena of kin discrimination and nepotism in the honey bee colony have been subjects of controversy since the 1990s (Carlin and Frumhoff, 1990; Page et al., 1990). Mainly because of the complex composition of patriline, there is still insufficient evidence to conclusively support the existence of kin recognition and nepotistic phenomena in a honey bee colony with a naturally mated queen.

The progress in DNA fingerprinting (Blanchetot, 1991; Moritz et al., 1991), RAPD (Hunt and Page, 1992), and the availability of microsatellite genetic markers (Estoup et al., 1993) in honey bee research have made it possible to determine the precise number of patriline in naturally mated honey bee colonies (Estoup et al., 1994). Using these tools, different aspects of honey bee behaviour have been shown to be driven by the genetic background of individuals (Breed et al., 2004; Kryger et al., 2000; Moritz et al., 1996; Rüppele et al., 2004). Unfortunately, the broader temporal interactions between honey bees of different patriline in naturally mated colonies are generally less understood. The major stumbling block is that it is difficult to remove tissue from a honey bee for genotype determination, without affecting its normal functioning.

Here, we tested whether larval exuvia may serve as a source of DNA for further genetic studies. Motivated by the recent extraction of DNA from human hair (Suenaga and Nakamura, 2005), we tried to extract DNA from the larval exuvia remaining in the brood cell after eclosion, before it is eaten by cleaner bees. We thereby established a method to determine the genotypes of living honey bees in a naturally mated colony, which would enable the easy and accurate determination of patriline. We then attempted to correlate an important and easily observable behaviour in worker honey bees, namely fanning behaviour. Fanning, where worker honey bees stand in or at the entrance of the hive, and rapidly fan their wings to produce an air current, is an important, but infrequently studied part of the bee behavioural repertoire, and is essential for the cooling of the hive and for honey maturation. In a first proof of concept, we discovered that the patriline composition of honey bee workers exhibiting fanning behaviour was significantly different from the whole colony patriline composition.

## 2. Materials and methods

### 2.1. DNA extraction from exuvia and muscle of honey bees

We took five workers' exuviae from their respective brood cells (see Fig. 1), and transferred them to

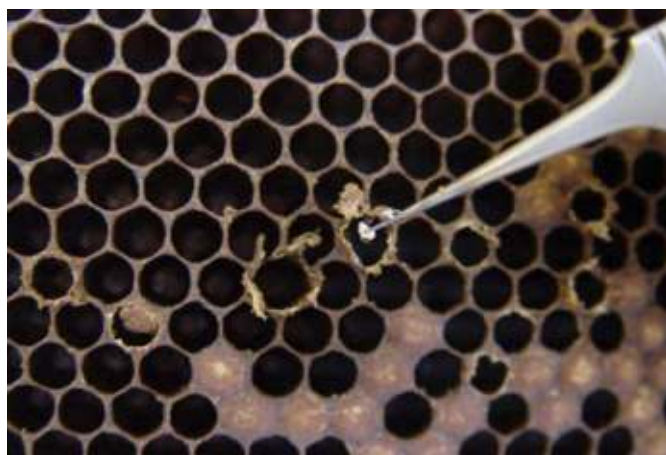


Fig. 1. Exuvia collection from the newly emerged worker cell. The material on the tip of the forceps is the exuvia, which was just collected from the floor of the newly emerged worker cell.

Eppendorf tubes to extract DNA. We used three protocols, which included the phenol/chloroform method (Kocher et al., 1989), the PUREGENE DNA Purification Kit (Biocompare, San Francisco, CA) and the Chelex 100 (Bio-Rad Laboratories, CA), to extract DNA, and carried out polymerase chain reaction (PCR) on the DNA with microsatellite primers. The Chelex 100 method was found to be the most suitable for extraction of genomic DNA from the exuviae. In order to confirm that the DNA isolated from exuvia was the same as the body DNA, we removed exuviae from five worker brood cells, five queen cells, and three drone cells from three different honey bees colonies (*Apis mellifera ligustica*) (ZND No.1), from the experimental apiary of Zhejiang University. We also extracted DNA from the thoracic muscles of the individuals, and compared the PCR products.

### 2.2. Microsatellite PCR amplification and electrophoretic analysis

DNA was extracted using the Chelex method (Suenaga and Nakamura, 2005) with a few modifications. Two hundred microliters of 5% Chelex 100 and 10  $\mu$ L of 10 mg/mL Proteinase K and 7  $\mu$ L DTT were added to each piece of exuvia placed in a 1.5-mL eppendorf tube. The solution was incubated at 55  $^{\circ}$ C for at least 10 h. Afterwards, the mixture was vortexed and incubated at 100  $^{\circ}$ C for 8 min to inactivate the Proteinase K. After centrifugation at 13,000 g for 3 min, the supernatant was transferred to another 1.5-mL microcentrifuge tube, and used as a template for PCR amplifications.

PCR was carried out with five microsatellite loci primers (Estoup et al., 1994, see Table 1) labelled with the IRD 700/800 fluorescent dye (METABION, Germany). PCR was conducted under the following

Table 1  
Primer sequences and PCR conditions for the 5 primers used

Locus	Sequence of primers	[Mg <sup>2+</sup> ] (mM)	Annealing temperature (°C)	Number of cycles
A107	5'-CCGTGGGAGGTTTATTGTCG-3' 5'-GGTTCGTAACGGATGACACC-3'	2.0	60	35
A14	5'-GTGTCGCAATCGACGTAACC-3' 5'-GTCGATTACCGATCGTGACG-3'	2.5	58	40
A29	5'-AAACAGTACATTTGTGACCC-3' 5'-CAACTTCAACTGAAATCCG-3'	2.5	54	35
A43	5'-CACCGAAACAAGATGCAAG-3' 5'-CCGCTCATTAAGATATCCG-3'	2.0	55	35
B124	5'-GCAACAGGTCGGGTTAGAG-3' 5'-CAGGATAGGGTAGGTAAGCAG-3'	2.0	55	40

We used five microsatellite loci (Estoup et al., 1994) to determine the genotypes of marked workers.

conditions (μL):

H <sub>2</sub> O	8.4-X
Buffer	1.25
MgCl <sub>2</sub> (25 mM)	1.25
DNTPs (2 mM each)	1.00
Primer1 (10 μM)	0.25
Primer2 (10 μM)	0.25
Taq polymerase (5U/μL)	0.10
DNA Template	X
Total	12.5

Between 1 and 6 μL of DNA template was added to the reaction mixture, depending on the primers. The microsatellite PCR products were separated on a gel to determine the worker genotype; this was done with the LI-COR 4300 DNA analyser. Individual genotypes were analysed and confirmed with the SAGA software. We used the MATE-SOFT software (Moilanen et al., 2004), which has been used in several previous studies (Kronauer et al., 2004; Sumner et al., 2004), to analyse the patriline present in the colony.

### 2.3. Preparation of the experimental bee colony and collection of exuviae

From a honey bee (*Apis mellifera carnica*) colony with a naturally mated queen, one brood frame with capped brood cells was transferred into an incubator on July 29, 2005, where the pupae were kept at a constant temperature of 35 °C until they hatched as adult bees. Thus the influence of pupal temperature on bee behaviour (Tautz et al., 2003) was eliminated, and it was hypothesized that any observed behavioural differences would be due to genetic differences. Workers emerging from this brood comb were marked with plastic number tags, and the same time, the exuviae were collected from the cells from which they hatched. The identity of individual bees and their corresponding exuvia

Table 2

Comparison of PCR products obtained with DNA extracted from exuvia and thoracic muscle

Sample ID	Primers			
	A14	A29	A43	B124
wc1	214/214	148/176	123/139	212/214
wc2	214/214	148/176	123/123	212/214
wc3	214/224	148/162	123/139	214/222
wc4	214/214	148/178	125/125	212/214
wc5	214/224	150/150	125/125	214/216
wm1	214/214	148/176	123/139	212/214
wm2	214/214	148/176	123/123	212/214
wm3	214/224	148/162	123/139	214/222
wm4	214/214	148/178	125/125	212/214
wm5	214/224	150/150	125/125	214/216
qc1	214/224	148/150	125/139	214/216
qc2	214/230	148/148	139/139	214/214
qc3	214/224	148/150	123/123	214/216
qc4	214/224	150/162	123/123	214/222
qc5	214/230	148/148	123/139	214/214
qm1	214/224	148/150	125/139	214/216
qm2	214/230	148/148	139/139	214/214
qm3	214/224	148/150	123/123	214/216
qm4	214/224	150/162	123/123	214/222
qm5	214/230	148/148	123/139	214/214
dc1	216	156	123	216
dc2	214	148	123	214
dc3	216	156	123	214
dm1	216	156	123	216
dm2	214	148	123	214
dm3	216	156	123	214

Note: wc1–5, worker exuvia; wm1–5, worker muscle; qc1–5, queen exuvia; qm1–5, queen muscle; dc1–3, drone exuvia; dm1–3, drone muscle. Thus, wc1 and wm1 represent exuvia and muscle tissue from the same worker, qc1 and qm1 signify exuvia and muscle tissue from the same queen, and so on. These samples were taken from three different colonies. The exuvia and muscle pairs from all individuals produce DNA fragments of identical sizes.

The numbers represent the sizes of the DNA fragments (in bp) generated by primers A14, A29, A43 and B124. Worker and queen tissue produce one or two bands following PCR (depending on homo- or heterozygosity at given locus), while drone tissue (haploid) produces only one.

was noted. This way, 237 emerging workers and their exuviae were marked and collected within 11 h.

A single-cohort colony (Whitfield et al., 2003) was prepared from 1500 newly emerged workers (<40 h old) and all 237 marked workers in a two-frame observation hive, together with one honey and pollen-bearing frame and one queen.

#### 2.4. Behavioural observations

We spent 4 hours every day (from 9:00 to 10:00 and 10:30 to 11:30 a.m., from 14:30 to 15:30 and 16:00 to 17:00 p.m.) observing the fanning behaviour of marked bees in the experimental observation hive. We carried out observations over 23 days from August 11 to September 9. We only recorded fanning behaviour, which lasted more than 5 s at a time.

Table 3  
Fanning behaviour in marked workers during the experimental period

Date	Number of fanning workers
2005-8-11	Y11, Y33, Y73, B6, B43, B6, B82, Y4, Y23, B2, W65, B16, Y68, W50, Y66, B9, B1, B31, Y18, B70, Y16, Y46, B65, Y20, B50, Y44, Y44, B89, B2
2005-8-12	W13, Y68, B29, B54, B48, W2, Y42, Y56, B6, Y69, Y21, Y20, B3, Y70, Y1, B59, W29, Y69, Y31, Y31, B1
2005-8-13	W1, W58, B27, B9, W54, Y43, B9, W43, W37, Y58, B31
2005-8-14	Y53, B16, W16, B14, W63, Y56, Y56, B23, Y56, Y34, B9, Y85, W63, Y34, B30, B43, Y44, Y70, Y44, B27, Y70, B14, B41, Y40, B8, Y47, B87, B51
2005-8-15	Y3, Y34, W58, B90, B91, B77, B2, B33, Y81, W27
2005-8-16	B33, B47, B8, Y34
2005-8-17	B59, W1, W8, W3, B27, W27, B50, B16, B9, W53, B68
2005-8-18	W63, B79, Y48, B44, B20, B1, B85, B68
2005-8-19	W37, Y53, B62, B6, Y54, B37, B8, Y71v
2005-8-20	B6, W64, Y53, B51, W43, B79, W63, B23, Y75, B62, Y20, Y33, W32
2005-8-21	Y44, W8, B8, Y60, Y72, W29, B31, B7, W37, W45
2005-8-22	B81, B50, B8, Y1, W46, W9, Y42, W64, W4, W45, W63, B16, W45, W65, Y60, W9, B39v
2005-8-23	W41, W63, Y20, B45, B29, W9, Y46, W36, B51, B20, B96, B27Y44, B8, B29, Y62, Y44, Y45, Y15, B87, B3, B30, B62, W29, W65, B58, W65, B58, W31, Y60, W18, W34
2005-8-25	B29, W16, B87, W45, B6, W37, Y53, Y1, Y60, B69, Y75, B17, Y32
2005-8-26	W53, Y83, B64, Y53, B39, B1, Y73, B51, W45, B96, B64, Y68
2005-8-27	B76, Y75, B30, B44, B71, Y8, Y83, W29, Y45, W29, B3, B31, Y46, B4, Y54, Y56
2005-8-29	B82, B75, Y44, B29, Y44, W39, B10, Y68, B47, B77, B97, B20, Y62, Y88
2005-8-30	W52, B47, W41
2005-9-1	Y60, B79, B71, W63, Y20, B16, W37, W59
2005-9-6	Y4, B87, W22, B57, B3, W9, W23, W48, W21, Y56, W61, W26, Y71, W9, W18, W62, B75
2005-9-7	Y8, W36, B97, W9, W22, W4, B39, Y61, W43, W27, W9, B47, W23, W62, W65, B50, B29, Y15, B4, W9, Y15, W23, W13
2005-9-8	Y56, Y21, Y88, W62, W35, W13, Y45, Y8, B64, W36, W35, W57, B32, B17, Y73, Y45, W26, W58, B58, W8, Y15, B71, W67, B58, W62, B64, Y2, W28, W21, B71, Y88, W53, B27, B64, W28, B59, W59, W22, Y71, W64, Y45, Y75, W9, W13, W21, B59, B20
2005-9-9	W59, W9, W13, W43, Y15, B47, W37, B77, W62, Y73, W28, W37, B32, B96, Y45, W36, Y56, W27, B58, W62, Y2, W9, W65, B69, W21, W59, B30, B20, W67, W23

Fanning behavior was observed over 23 days from August 11th to September 9th, 2005. 385 individuals, including Y11 and Y73, were recorded exhibiting fanning behaviour during these 24 days. Some of the marked workers performed fanning behaviour several times during the experimental period.

#### 2.5. Statistical analyses

The frequency of each patriline among all marked workers (235 individuals except 2 workers) was calculated by dividing the number of individuals of a certain patriline with the total number of all tested workers.

The frequencies of fanning workers of a particular patriline for a particular day were calculated by dividing the number of marked fanning workers of a particular patriline with the number of marked fanning workers in one day.

We analysed the differences in frequency of fanning behaviour among workers of different patrilines and on different days, using the *G*-test of DPS software (Tang and Feng, 1997). We also used the *G*-test (Sokal and Rohlf, 1995; Tang and Feng, 1997) (DPS software) to test the significance of differences in patriline frequencies between the fanning workers and the whole colony.

### 3. Results

#### 3.1. Comparison of PCR with DNA isolated from exuvia and muscle

We used the Chelex method to extract genomic DNA from the exuvia and muscle of five emerging workers, five emerging virgin queens and three emerging drones. We then amplified these DNA samples with four microsatellite primers, separated the PCR products on a gel, and analysed them using the SAGA software. The results are shown in Table 2. It can be seen that the banding profiles with the DNA from workers', virgin queens' and drones' exuviae are the same as those from the muscle of the corresponding individuals. Thus, the DNA from the exuvia found in brood cells can be used to determine the genotypes of corresponding individual honey bees.

#### 3.2. Fanning behaviour

We observed the fanning behaviour of the marked bees, and noted the tag number of workers exhibiting fanning behaviour from August 11 to September 9, 2005. We recorded 385 individuals exhibiting fanning behaviour during those 23 days; some of the marked workers performed fanning behaviour several times during the experimental period. The results are shown in Table 3. We found that the frequency of fanning varied under different conditions.

#### 3.3. Genotype determination and patriline analysis

Genotypes of worker honey bees were determined on the basis of the polymorphism of microsatellite loci from the collected exuviae. The genotypes of 237 marked workers,

produced by the LI-COR 4300 DNA Analyzer, were confirmed using the SAGA program (Fig. 2). Twenty patriline were found and confirmed among 235 marked workers using the MateSoft program. The patriline of some individuals (e.g. Y11 and Y73) could not be unequivocally determined, possibly due to low DNA concentration. Therefore, these individuals were removed from the dataset. All confirmed patriline are shown in Table S1, and the frequency of each patriline is shown in Fig. 3.

#### 3.4. Genetic component of fanning

Based on the available patriline information, we calculated and analysed the distribution of fanning behaviour for each day of observation, as well as for the whole observation period (24 days). *G*-test results showed that there was no significant difference in fanning behaviour between days according to patriline ( $P = 0.1787$ ). However, as shown in Fig. 4, when considering fanning behaviour over the whole experimental period, we found a significant difference in patriline composition (*G*-test,  $P = 0.0001$ ). This suggests that honey bee workers of some patriline are more likely to perform fanning. Thus, genetic composition seems to at least co-determine the division of labour in a naturally mated colony, consistent with the results obtained by other researchers (Giray et al., 2000; Jones et al., 2004; Kryger et al., 2000; Robinson and Page, 1988).

### 4. Discussion

#### 4.1. Potential applications of the non-destructive genotyping method

So far, it has been difficult to determine the genotype of living honey bee workers without damaging or killing

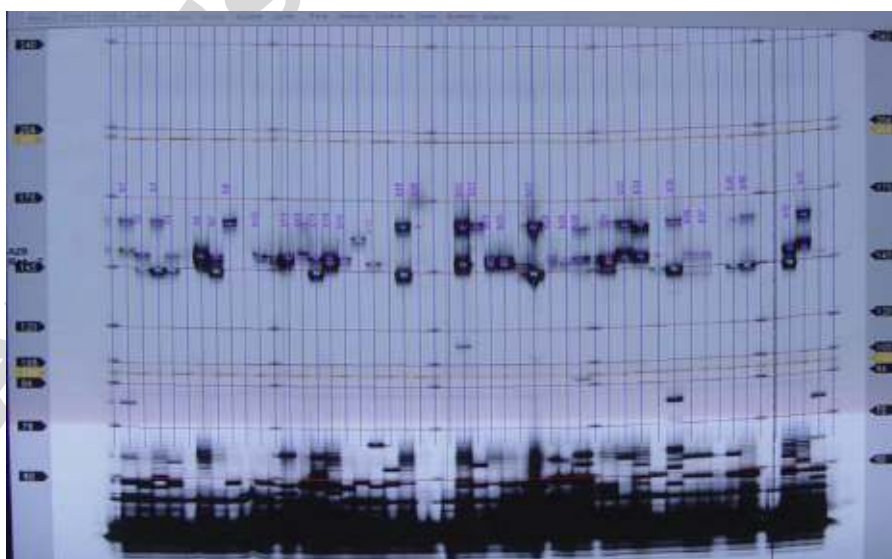


Fig. 2. The profile of PCR products of worker individuals determined with A29 microsatellite primers. There were 50 lanes on this gel, including 6 lanes of DNA markers and 44 lanes of samples. The size of PCR products, amplified with A29 primers, was between 100 and 200 bp, as determined with SAGA software.

them. Genotype determination of living honey bees has important implications in the fields of genetics, breeding and behaviour, and could contribute significantly to our understanding of the genetic structure of bee colonies, interactions among workers from different subfamilies, and the effects of genes on individual behaviour. This method could also find many applications in insect research beyond honey bee biology.

Our method of genotyping allows the convenient study of the interactions between individuals belonging to different subfamilies, in spite of the complicated patriline compositions of natural colonies. In this study, we investigated the genetic variation in fanning behaviour in a colony with 20 patrilines, an ideal situation to study the relationship between behaviour and genes with this new method. Another advantage of our method is that it allows the observation of the marked workers' behaviour without having to later catch them for DNA extraction.

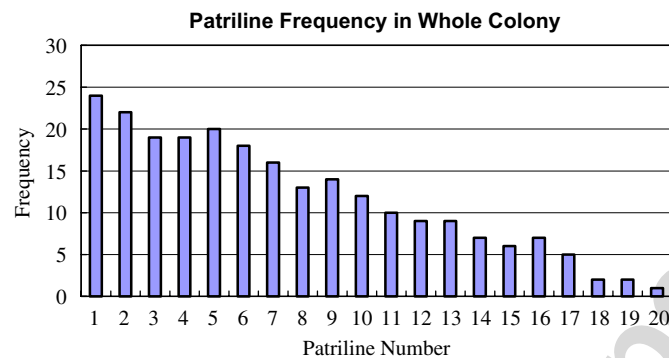


Fig. 3. Frequency of patrilines among all the marked workers. Twenty patrilines were identified among newly emerging and marked workers ( $n = 235$ ) based on genotype determination with five microsatellite primers. The frequencies of different patrilines range from 10.21% to 0.43% in this colony.

Most of the current genotyping methods require the killing or injuring of individual bees for DNA extraction, after behavioural observations. Unfortunately, this loss of individuals can affect the structure of the observation colony. Moreover, marked bees might be lost for a variety of reasons during the experimental period, if their collection were to be scheduled for the end of that period.

As shown in the Results, DNA could also be extracted from the exuviae of workers, drones and virgin queens. We believe that the genetic characterization of virgin queens using DNA extracted from exuviae can find many applications, particularly in bee breeding.

PCR with primers relating to special biological or economic characters (such as disease resistance, high production of royal jelly, low defense behaviour, etc.) could be performed to select for queens bearing a given character. With the sequencing of the honey bee genome, it is inevitable that more and more genes will be discovered, to such desirable characteristics.

#### 4.2. Genetic variation of fanning in the honey bee colony

Fanning within the nest may be performed for a number of purposes, i.e. cooling the colony, concentrating honey by evaporation, and decreasing the humidity and carbon dioxide levels inside the colony (Seeley, 1995; Winston, 1987). Fanning behaviour is one of the most prominent factors modulating the climate within the hive. Jones et al. found that brood nest temperatures in genetically diverse colonies tend to be more stable than in genetically uniform ones (Jones et al., 2004). This reconciles well with our results showing that workers of different patrilines performed fanning behaviour with different frequencies. Genetically determined diversity in workers' response thresholds to different stimuli may thus modulate the hive-ventilating behaviour of individual workers and contribute to a fine-tuning of the hive climate.

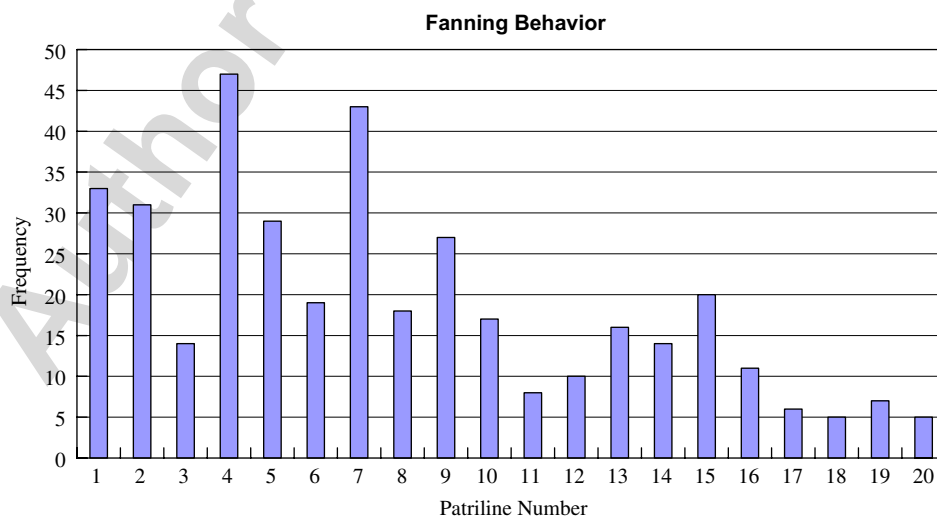


Fig. 4. Patriline frequency of workers exhibiting fanning behaviour. A total of 380 marked individuals were observed and recorded fanning during the 24-day experimental period. The fanning performance data were pooled together because there were no significant differences ( $G$ -test,  $P = 0.1787$ ) between days. A significant difference ( $G$ -test,  $P = 0.0001$ ) of patriline composition was found between fanning workers and the whole colony.

Natural selection theory tells us that whenever there is genetic heterogeneity within a group, there is great potential for conflicts among the group's members. Our new non-destructive method combined with relatively long-term behavioural observations reveals a novel avenue for the study of the complex relationships between different patriline in social insects.

### Acknowledgements

We thank Dirk Ahrens for help with beekeeping, H. Feldhaar and K. Möller for help with genotyping, G.E. Robinson for helpful discussions and suggestions that improved the manuscript, Aung Si for reading the manuscript and valuable comments, Q.Y. Tang for help with the statistical analysis. This study was supported by the National Natural Science Foundation of China (Grant no. 30571409) and the Zhejiang Provincial Natural Science Foundation of China to SKS, grants from the Bavarian Ministry of Agriculture to JT and the ARC COE Vision Science (CE0561903) and ARC DP-0450535 to SWZ.

*Author information:* Reprints and permissions information is available at <http://www.elsevier.com/locate/jinsphys>.

### Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jinsphys.2007.01.002](https://doi.org/10.1016/j.jinsphys.2007.01.002).

### References

- Amdam, G.V., Csondes, A., Fondrk, M.K., Page Jr., R.E., 2006. Complex social behaviour derived from maternal reproductive traits. *Nature* 439, 76–78.
- Blanchetot, A., 1991. Genetic relatedness in honeybees as established by DNA fingerprinting. *Journal of Heredity* 82, 391–396.
- Breed, M.D., Guzman-Novoa, E., Hunt, G.J., 2004. Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annual Review of Entomology* 49, 271–298.
- Carlin, N.F., Frumhoff, P.C., 1990. Nepotism in the honey bee. *Nature* 346, 706.
- Estoup, A., Solignac, M., Harry, M., Cornuet, J.M., 1993. Characterization of (GT)<sub>n</sub> and (CT)<sub>n</sub> microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nucleic Acids Research* 21, 1427–1431.
- Estoup, A., Solignac, M., Cornuet, J.-M., 1994. Precise assessment of the number of patriline and of genetic relatedness in honeybee colonies. *Proceedings of the Royal Society of London B* 258, 1–7.
- Frumhoff, P.C., Schneider, S., 1987. The social consequences of honey bee polyandry: the effects of kinship on worker interactions within colonies. *Animal Behaviour* 35, 255–262.
- Getz, W.M., Brückner, D., Parisian, T.R., 1982. Kin structure and the swarming behavior of the honey bee *Apis mellifera*. *Behavioral Ecology and Sociobiology* 10, 265–270.
- Getz, W.M., Smith, K.B., 1983. Genetic kin recognition: honey bees discriminate between full and half sisters. *Nature* 302, 147–148.
- Giray, T., Guzman-Novoa, E., Aron, C.W., Zelinsky, B., Fahrbach, S.E., Robinson, G.E., 2000. Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. *Behavioral Ecology* 11, 44–55.
- Honeybee Genome Sequencing Consortium, 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443, 931–949.
- Hunt, G.J., Page, R.E., 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theoretical and Applied Genetics* 85, 15–20.
- Jones, J.C., Myerscough, M.R., Graham, S., Oldroyd, B.P., 2004. Honey bee nest thermoregulation: diversity promotes stability. *Science* 305, 402–404.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86, 6196–6200.
- Kronauer, D.J., Schöning, C., Pedersen, J.S., Boomsma, J.J., Gadau, J., 2004. Extreme queen-mating frequency and colony fission in African army ants. *Molecular Ecology* 13, 2381–2388.
- Kryger, P., Kryger, U., Moritz, R.F.A., 2000. Genotypical variability for the tasks of water collecting and scenting in a honey bee colony. *Ethology* 106, 769–779.
- Linksvayer, T.A., Wade, M.J., 2005. The evolutionary origin and elaboration of sociality in the aculeate Hymenoptera: maternal effects, sib-social effects, and heterochrony. *The Quarterly Review of Biology* 80, 317–336.
- Michener, C.D., 1974. *The Social Behavior of the Bees*. Harvard University Press, Cambridge.
- Moilanen, A., Sundström, L., Pedersen, J.S., 2004. Matesoft: a program for deducing parental genotypes and estimating mating system statistics in haplodiploid species. *Molecular Ecology Notes* 4, 795–797.
- Moritz, R.F., Meusel, M.S., Haberl, M., 1991. Oligonucleotide DNA fingerprinting discriminates super- and half-sisters in honeybee colonies (*Apis mellifera* L.). *Naturwissenschaften* 78, 422–424.
- Moritz, R.F.A., Kryger, P., Allsopp, M.H., 1996. Competition for royalty in bees. *Nature* 384, 31.
- Page, R.E., Robinson, G.E., Fondrk, M.K., 1989. Genetic specialists, kin recognition and nepotism in honey-bee colonies. *Nature* 338, 576–579.
- Page, R.E., Breed, M.D., Getz, W.M., 1990. Nepotism in the honey bee. *Nature* 346, 707.
- Robinson, G.E., 2002. Development. Sociogenomics takes flight. *Science* 297, 204–205.
- Robinson, G.E., Page, R.E., 1988. Genetic determination of guarding and undertaking in honey-bee colonies. *Nature* 333, 356–358.
- Robinson, G.E., Grozinger, C.M., Whitfield, C.W., 2005. Sociogenomics: social life in molecular terms. *Nature Reviews Genetics* 6, 257–270.
- Rüppell, O., Pankiw, T., Page Jr., R.E., 2004. Pleiotropy, epistasis and new QTL: the genetic architecture of honey bee foraging behavior. *Journal of Heredity* 95, 481–491.
- Seeley, T.D., 1995. *The Wisdom of the Hive*. Harvard University Press, Cambridge.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, New York.
- Suenaga, E., Nakamura, H., 2005. Evaluation of three methods for effective extraction of DNA from human hair. *Journal of Chromatography B* 820, 137–141.
- Sumner, S., Hughes, W.O., Pedersen, J.S., Boomsma, J.J., 2004. Ant parasite queens revert to mating singly. *Nature* 428, 35–36.
- Tang, Q.Y., Feng, M.G., 1997. *Practical Statistics and DPS Data Processing System*. China Agricultural Press, Beijing.
- Tautz, J., Maier, S., Groh, C., Rössler, W., Brockmann, A., 2003. Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proceedings of the National Academy of Sciences of the United States of America* 100, 7343–7347.
- Whitfield, C.W., Cziko, A.M., Robinson, G.E., 2003. Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296–299.
- Winston, M.L., 1987. *The Biology of Honey Bee*. Harvard University Press, Cambridge.