Original Article

ABSENCE OF NEPOTISM IN WAGGLE COMMUNICATION OF HONEYBEES (*APIS MELLIFERA*)

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A b s t r a c t

The polyandrous mating behavior of the honeybee queen increases the genetic variability among her worker offspring and the workers of particular subfamilies tend to have a genetic predisposition for tasks preference. In this study, we intended to understand whether there is nepotism in dance communication of honeybees during natural conditions. Microsatellite DNA analyses revealed a total of fourteen and twelve subfamilies in two colonies. The subfamily composition of the dancer and the followers did not deviate from random. The majority of the subfamilies did not show kin recognition in dance-recruit communication in honeybee colonies, but some subfamilies showed significant nepotism for workers to follow their super-sister dancer. Because it seems unlikely that honeybee would change the tendency to follow dancers due to the degree of relatedness, we conclude that honeybees randomly follow a dancer in order to e benefit colony gain and development.

Keywords: microsatellite DNA, nepotism, recruits, subfamily, waggle dance

INTRODUCTION

Honeybees are a model organism to study the genetic basis of task specialization in social insects due to the polyandrous queen (Robinson et al., 2005). Generally, from six to twenty subfamilies (paternity) co-exit within a honeybee colony that are genetically different, and these genetic differences have also translated into different behavior preference (Page & Robinson, 1991). Genetic variability has been described for such behavioral traits as nectar and pollen foraging (Page & Robinson, 1991), nest site scouting (Robinson & Page, 1989), plant choice for pollen collection (Oldroyd et al., 1992), foraging distance (Oldroyd et al., 1993), water collecting and scenting (Kryger et al., 2000), fanning (Su et al., 2007), emergency queen-cell building (Xie et al., 2008), mite (*Varroa destructor*) parasitism rate (Liu et al., 2009), swarming (Huang & Zeng, 2009), survival differences (Wang et al., 2012) and feeding preference (Yi et al., 2018).

Honeybees have evolved numerous mechanisms to increase colony-level foraging efficiency, mainly the combined system of scout-recruit division of labor and recruitment communication (von Frisch, 1967; Seeley, 1998). This recruitment process incorporates information about the food reward, the colony food stores and the environmental food availability (George & Brockmann, 2019). At the individual level, the efficiency of a scout depends on its ability to find and inform the nest mates of the food source. A successful forager performs waggle dances on the surface of the comb where it interacts with nectar receivers and dance followers (Seeley,

1998). The responses to the waggle dance signal reflect the contextual information possessed by various honeybees on the dance floor (Seeley & Towne, 1992). Honeybee workers might be able to behave nepotistically and favor the reproductive success of their subfamily (Getz et al., 1983; Page et al., 1989, 1990).

Oldroyd (1991) showed that a strong tendency for recruits to follow their super-sister dancers. This may increase honeybee colony efficiency in dance communication. Arnold (2002) suggested that sub-familial variance for propensity may vary both for waggle and tremble dances. However, neither in a colony consisting of only two subfamilies nor in a colony consisting of seventeen subfamilies was there any evidence of subfamily discrimination among dancers and their followers foraging from artificial feeding site that provided sucrose solution (Kirchner & Arnold, 2001). However, the result might be biased by the subfamilies' preferences of nectar and pollen foraging (Page & Robinson, 1991). In order to avoid the foraging preference, we used colonies during natural foraging rather than using an artificial feeder. The aim of the present study is to determine any nepotism in dancing recruitment under natural conditions. We therefore determined the subfamily frequencies of natural mated colonies, as well as the subfamily of the dancers and followers through microsatellite genetic markers.

MATERIAL AND METHODS

Samples Collection

The experiments were conducted with two honeybee colonies (colony A and colony B) with naturally mated queens during natural foraging from food resources rather than a artificial feeding site providing sucrose solution. Each colony was composed of four frames of honeybees and housed in observation hives. One side of the observation hives was open during the experiments to pick honeybees from the combs for genetically analysis. The dancing floor area of the observation hive was observed. The foragers which had been observed ten times in a row to perform a waggle dance after returning from the food source were classified as waggle dancers. The dancing followers were defined as the honeybees attending a waggle dance persistently for several circuits. When the foragers were dancing, the recruits followed her to dance, forming a circle. The outer followers were captured first in a box and the center dancer was captured later in an EP tube. A dancer and dance followers were defined as a group (on average eight followers per dancer). The dancer and dance followers were immediately frozen on dry ice and kept for later determination of their subfamily membership. Thirteen groups of honey bees were collected from colony A, including thirteen dancers and 117 followers. Twelve groups of honey bees were collected from colony B, including twelve dancers and ninety-three followers. For each dancer, a minimal of five followers was collected. Additionally, sixty-four (colony A) and forty-eight (colony B) workers were randomly picked out to represent the overall subfamily composition of the colonies.

DNA Amplification and Genetic Analysis

After the sampling, a genomic DNA extraction kit (StarSpin Animal DNA Kit) was used to extract DNA of each sample individually. Three microsatellite loci (A14, A24 and A113) were selected to determine the subfamilies according to previous reports (Tian et al., 2013; Zhang et al., 2017; Yi et al., 2018) (Tab. 1). These sequences have a high degree of polymorphism. The PCR reaction mixture (25 µL) was as follows: 1 µL of DNA (100 ng/µL), 1 µL each of forward and reverse primer (10 µM), 12.5 µL of SinoBio 2×Master Mix (Taq DNA Polymerase, dNTP, Mg^{2+}, reaction buffer, Vazyme Biotech CO., Ltd) and 9.5 µL of ultrapure sterile water (ddH_2O). The loci were amplified using Bio-RAD T100™ thermal cyclers through thirty cycles consisting of denaturation for 30 s at 94°C, annealing for 45 s at 55-60°C (depending on the locus) and elongation for 60 s at 72°C (Tab. 1). The allele of each microsatellite marker was determined with capillary electrophoresis (QIAxcel Advanced system). Then, the subfamily was analyzed using Mate-soft (Moilanen, et al., 2004).

Table 1.

Description of primer sequences and reaction condition used for microsatellites

Locus	Sequence of primers	Allele size (bp)	Annealing temperature (℃)	No. cycles
A14	F5'-GTGTCGCAATCGACGTAACC-3' R 5'-GTCGATTACCGATCGTGACG-3'	215/220/225/235245	58	30
A24	F5'-CACAAGTTCCAACAATGC-3' R 5'-CACATTGAGGATGAGCG-3'	110/120	55	30
A113	F5'-CTCGAATCGTGGCGTCC-3' R 5'-CCTGTATTTTGCAACCT CGC-3'	210/215/225/235	60	30

Statistic Analysis

All statistic analysis was conducted using SPSS Statistics 26.0. The distribution of workers in each subfamily between dancing followers and randomly collected workers was analyzed using Fisher's exact test. The distribution of recruits in all super-sister of against all other half-sisters was analyzed using Fisher's exact test (Kryger et al., 2000).

RESULTS

Genotypes of paternity were calculated in each colony respectively (Tab. 2). As the subfamily frequencies were unequal in our samples, we extended the statistical analysis by considering the subfamilies separately. Colony A consisted of fourteen subfamilies, and twenty-two of

Table 2.

Genotypes of paternity were in each colony

Colony A			Colony B		
Subfamilies	Genotypes of paternity A014/A024/A113	Random samples	Subfamilies	Genotypes of paternity A014/A024/A113	Random samples
1	225/120/215	18	1	215/120/215	6
2	225/120/235	12	2	215/120/210	4
3	225/120/225	9	3	235/120/215	8
4	215/120/235	8	4	220/120/215	7
5	215/120/215	6	5	225/120/215	5
6	215/120/225	2	6	215/120/235	5
7	245/120/215	2	7	235/110/225	5
8	235/120/225	2	8	235/120/235	3
9	225/110/215	1	9	245/120/215	0
10	235/120/235	2	10	235/120/210	1
11	245/110/225	0	11	220/120/235	3
12	245/120/235	2	12	220/120/210	1
13	235/110/235	0			
14	235/110/215	0			

Note: Colony A consisted of 14 paternity, colony B of 12.

Table 3.

Distribution of super-sisters and half-sisters in colony A

Dancer		Recruits				
No.	Subfamilies	Super-sister Observed	Half-sisters Observed	Super-sister Expected	Half-sisters Expected	P values
A1	5	1	11	1.1	10.9	1.000
A2	7	0	11	0.3	10.7	1.000
A3	7	1	8	0.3	8.7	1.000
A4	6	0	8	0.2	7.8	1.000
A5	3	3	4	1.0	6.0	0.559
A6	1	1	6	2.0	5.0	1.000
A7	2	2	4	1.1	4.9	1.000
A8	1	3	6	2.5	6.5	1.000
A9	6	5	2	0.2	6.8	0.021
A10	10	1	6	0.2	6.8	1.000
A11	3	1	10	1.6	9.4	1.000
A12	6	1	12	0.4	12.6	1.000
A13	5	3	7	0.9	9.1	0.582

Note: The last column gives the P- values for Fisher exact tests for the distribution of the number of super-sister against all other half-sisters in each group. Fisher's exact test (two-tailed) for the distribution of the number of super-sister against all other half-sisters on the whole ($\chi^2 = 3.760$, df = 6, P = 0.768).

Table 4.

Distribution of super-sisters and half-sisters in colony B

Dancer		Recruits				
No.	Subfamilies	Super-sister Observed	Half-sisters Observed	Super-sister Expected	Half-sisters Expected	P values
B1	1	1	6	0.9	6.1	1.000
B2	4	1	7	1.2	6.8	1.000
B3	1	2	7	1.1	7.9	1.000
B4	6	0	8	0.8	7.2	1.000
B5	1	4	1	0.6	4.4	0.206
B6	2	1	6	0.6	6.4	1.000
B7	9	1	6	0	7.0	1.000
B8	5	2	5	0.7	6.3	1.000
B9	3	1	8	1.5	7.5	1.000
B10	1	6	4	1.3	8.7	0.057
B11	1	3	4	0.9	6.1	0.559
B12	8	1	8	0.6	8.4	1.000

Note: The last column gives the P- values for Fisher exact tests for the distribution of the number of super-sister against all other half-sisters in each group. Fisher's exact test (two-tailed) for the distribution of the number of super-sister against all other half-sisters on the whole ($\chi^2 = 5.516$, df = 7, P = 0.498).

117 dance followers were super-sisters of the dancers and ninety-five were half-sisters (Tab. 2, 3). Colony B consisted of twelve subfamilies, where twenty-three of ninety-three analyzed followers were super-sisters of the dancers and seventy were half-sisters (Tab. 2, 4). In colony A, a single group showed significant nepotism for workers to follow super-sister dancer (chi-square test: χ^2 = 7.778, df = 1, P = 0.021). However, significant preference to nepotism was not observed between the distribution of the waggle dancers and that of the dance followers overall in colony A (chi-square test: c^2 = 3.760, df = 6, P = 0.768) (Tab. 3). For colony B, neither individual group nor whole colony showed a significant preference to nepotism between the waggle dancers and that of the dance followers on the whole (chi-square test: c^2 = 5.516, df = 7, P = 0.498) (Tab. 4).

DISCUSSION

For our study, the data show that there was no subfamily nepotism among dancers and their followers. Oldroyd (1991) reported that honeybees dance preferentially with their super-sisters, which suggests a strong tendency to recruit super-sisters and indicates subfamily discrimination. The tendency of a recruit to follow a dancer was affected by the subfamily of the recruit, the subfamily of the dancer, foraging preferences of dancer and what she was carrying (Waddington, 1989; Page & Robinson, 1991). Here we show that recognition between super-sisters and half-sisters is at least not a general feature of the dance communication system.

Honeybees are eusocial insects, specialized in information sharing, division and cooperation. Even though the location and quality of the food resource, as well as the season shapes the foraging behavior, any significant impacts on nepotism have not been identified (Oldroyd et al., 1992, 1993, 1994). Kirchner and Arnold (2001) found no evidence for subfamily discrimination among dancers and their followers in a colony. However, honeybees just foraged from an artificial feeding site providing sucrose solution. So, the result might be due to different foraging preferences of the subfamilies. Gilley (2014) also suggest that the waggle-dance hydrocarbons play an important role in honeybee foraging recruitment by stimulating foragers to perform waggle dances. In addition, dance activity for food sources was regulated by an inter-play between individual response thresholds and the social context obtained through interaction with nest mates (George & Brockmann, 2019). Thus, honeybee would unlikely change the tendency to follow dancers due to the degree of relatedness. The single significant nepotism group found in our data might be due to the difficulty of sampling and the low number of followers in that group. This helps the colony to efficiently utilize its foraging force to effectively exploit food sources in its environment . Thus, we conclude that honeybees do not show kin recognition in dance-recruit associations under natural conditions.

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