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Decision Making Plasticity of Swarming Honeybees (*Apis cerana cerana*)

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Abstract

Honeybee which is a very important and useful model insect of social behavior language and pheromone study has been widely studied around the world from many aspects. With the development of the colony, honeybees make many decisions to flourish the colony and breed it stronger. During the swarming season, honeybees will decide when to swarm, who joins the swarming, who stays in the colony, and where the new home is. In this paper, we focused on whether workers can consciously decide to leave or to stay in the colony during the swarming, and if they can, what can be the underline impetus. The experiment was conducted with three colony Apis cerana cerana under natural swarming conditions. Genetic relatedness and subfamily composition were analyzed by using four Microsatellites. The results showed that subfamily distribution in swarms and the workers staying in the colony were significantly different from random decision making. Workers prefer to stay with their super sister virgin queens. Our data first indicates genetic relatedness may affect workers making non random decisions on whether to leave or to stay in the colony during swarming. We presume non random decision making set the evolutionary success for honeybees.

Keywords: *Apis cerana cerana*, genetic relatedness, decision, swarming.

Introduction

Swarming is the most important type of reproduction at colony level.During swarming,the mother queen will leave the colony with about half of the workers and some drones looking for a new home[1]. One necessary preparation for the swarming is the queen would lay eggs in the pre-constructed queen cells. Different hypotheses have been proposed to explain what triggers swarming. These include a surplus of young bees resulting in too much brood food, crowding of adult workers and limited space for brood, and reduced transmission of queen mandibular pheromone among workers[2]. These factors, as well as colony size and worker age distribution, all play roles in stimulating swarming preparation; however, none of them alone consistently induces swarming[3-4].

Honeybee queens are highly polyandrous and the colony is composed of super-sister and half-sister workers. Workers all have the same genetic relatedness with the mother queen G=0.5. Immature queens in the queen cells relate super-sister workers by G = 0.75 and relate half-sister workers by G = 0.25, because of the haplodiploid [5]. The theory of kin selection suggests that individuals should show less aggression, and more altruism, towards closer kin [6-7], according to which workers should preferentially invest in the super-sister immature queens and selectively choose to stay in the colony during swarming.

Even though many decision making behaviors[8-13] and swarming tendency[14-15] have been reported, what triggers honeybee to do so and why some subfamilies tend to join the swarming while others tend to stay in the colony are still unclear. We try to explain this from the effects of the genetic relatedness among swarms, immature queens and workers staying in the colony during the swarming, and provide data as evidence of super-sister workers of the immature queens selectively stayed in the colony during the swarming. When the eggs are laid in the queen cells, the discrimination and the decision making process may have been under way.

Material and Methods

Sampling: Three 4-frame colonies (colony #39, #43 and #58) *Apis cerana cerana* were kept in the apiary at the Honeybee Research Institute, Jiangxi Agricultural University of China. Before the first queen cell was sealed, combs were checked every two days to calculate when the swarming would emerge. When swarms have clustered in the nearby trees, 50 workers were randomly sampled from the swarms to reflect the genotypic composition of the swarms and preserved in 95% alcohol for further DNA analysis. Then all the queen cells were controlled in individual wooden cages and transferred into the incubator to emerge. After that, another 50 workers were sampled randomly from the mother colony as the genotypic composition of the workers who chose to stay in the colony during the swarming. Finally, queen cells were observed every 30 minutes to record the emerging order of immature queens to see whether the super sister workers of the first emerged immature queen have stronger staying tendency than super sister workers of other immature queens. All of the virgin queens emerged successfully in the incubator. We obtained four queen cells from colony 39 (no virgin queen emerged when sampled), one virgin queen from colony 43 (other queen cells have been completely destroyed and there are no larvae or pupae left in the destroyed queen cells when sampled), and six queen cells from colony 58 (no virgin queen emerged when sampled).

Subfamily analysis: DNA was extracted from individual using phenol-chloroform protocol[16].Genetic relatedness and subfamily composition were analyzed using four microsatellites (A76; B124; A14; A107)[17].

Statistical analysis: To determine if there were subfamily differences in tendency to stay in the colony during the swarming, we used Fisher's exact test, 2*n contingency table (n represents the subfamily number).

Do workers with a super-sister immature queen have a greater tendency to stay in the colony was tested by Fisher's exact test, 2*2 contingency table. Do super-sister workers of the first emerged immature queen have stronger tendency to stay in the colony than super-sister workers of other immature queens was tested by Fisher's exacttest,2*2contingencytable.Are there within patriline difference in tendency to leave or to stay in the colony was tested by a binomial distribution. All the analysis is conducted with SPSS 16 analysis package.

Results and Discussion

The subfamily analysis data (table 1) showed that the three colonies were composed of 13 subfamilies, 9 subfamilies and 12 subfamilies respectively. The statistical results showed that there are subfamily differences in tendency to stay in colony 39 and colony 43 ($P<0.01$, Fisher's exact test), but not in colony 58 ($P>0.05$, Fisher's exact test). Our test did not reject colony 58 has subfamily staying tendency. In order to further evaluate the staying tendency, we used the following model to further test the swarming behavior.

According to the theory of kin selection, workers with a related queen should selectively stay in the colony during the swarming. Our test results supported the theory of kin selection and showed workers with a related queen have a greater staying tendency than workers without a related queen (in all three colonies $P<0.001$, Fisher's exact test) which suggests that workers may use kin selection to make swarming decisions. Workers with the super sister virgin queen no matter whether it is the first emerged one have the same staying tendency (in colony 39 and colony 58 $P>0.05$, Fisher's exact test. colony 43 can not be calculated for there is only one virgin queen left).

We can assume that if swarming is a random behavior, subfamily distribution in swarms and workers staying in the colony should be approximate to 1:1 and the there would be no subfamily differences in the tendency to stay in the colony. But if the swarming is not a random behavior, the distribution should deviate statistically from 1:1 and the subfamilies would have different staying tendency.If the workers can not only discriminate the immature queens but also foresee which immature queen would be the first emerged one, super-sister workers of the first emerged queen would have stronger tendency to stay in the colony than super-sister workers of other immature queens. But the test results did not support this hypothesis.

In colony 58, even though subfamily frequency was not different, swarming tendency is significantly different between workers with and without an immature queen which still can demonstrate that honeybees can selectively decide to stay with super sister virgin queens.Some subfamilies made a very high contribution to the swarms whereas others were less active[15]. One explanation to the swarming tendency is that some genotypes are inclined to swarm, but we explain this question from the relationship between workers and immature queens. Workers have been proved to have the ability to discriminate the kin from the non kin[18-22], and the related queen from the unrelated queen[23-25].

Kryger and Moritz successfully proved that subfamilies have swarming tendency under natural conditions, but the evidences to support the theory of kin selection hasn't been found in the after swarm between after swarms and the virgin queen[15]. One problem is that primary swarms were transferred back to the colony. Mostly, swarms won't come back to the colony, except when the queen doesn't join the swarming or the queen suddenly died in the swarming. The swarming passion of the primary swarms may not calm down so quickly, and those swarms became the organizer and important composition of the after swarms.

Our data support the theory of kin selection and the observed behaviors suggest that honeybees can make non random decisions both at general level and individual level. We assume that some subfamilies that are inclined to stay in the colony may trigger other subfamilies deciding to join the swarming. Honeybees know exactly what they are doing which may be constructed during the long term evolution of the species.

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Table 1
Paternal distribution and genetic alleles of honeybees in three colonies

		Virgin queens						Drones mating with the mother queen												
		N.1	N.2	N.3	N.4	N.5	N.6	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
Colony 39	A14	216/212	229/210	229/210	224/212			210	218	218	217	214	212	226	224	221	221	229	216	216
	A76	225/204	213/204	213/214	230/214			201	204	200	209	217	214	227	230	210	215	213	228	225
	A107	166/169	164/158	164/158	158/158			147	149	155	164	151	166	162	158	169	151	164	176	166
	B124	224/236	220/216	220/236	235/236			225	235	216	220	213	235	216	235	220	216	220	222	224
	ws							3	0^{A}	5	1	0	2^{A}	2	7^{a}	1	3	8^{A}	4	14^{a}
	wl							0	13^{B}	13	1	5	14^{B}	1	0^{b}	0	0	0^{B}	0	3^{b}
	qd	D13	D11	D11	D8															
Colony 43	A14	245/219						220	209	220	219	224	224	219	245	245				
	A76	220/226						210	228	218	228	210	222	210	228	220				
	A107	154/156						144	150	144	156	154	167	156	167	154				
	B124	240/234						212	206	226	234	222	242	212	258	240				
	ws							0	0	5	0	11	5	0^{a}	1	28^{A}				
	wl							5	1	2	2	22	7	7^{b}	1	3^{B}				
	qd	D9																		
Colony 58	A14	208/208	208/208	248/231	208/208	248/231	208/208	224	208	229	256	248	208	236	208	229	208	256	224	
	A76	220/224	220/268	236/268	220/224	236/224	208/268	208	220	224	232	236	208	248	244	252	264	254	202	
	A107	145/159	145/159	140/182	145/182	140/159	172/182	160	145	140	182	140	172	174	176	140	174	182	158	
	B124	214/214	214/214	220212/	214/212	220/212	232/214	232	214	212	232	220	232	238	212	229	214	238	220	
	Ws							1	16^{a}	1	13	3	10^{A}	0	2	0	3	0	1	
	Wl							1	9^{b}	0	21	1	4^{B}	1	4	1	3	2	3	
	qd	D2	D2	D5	D2	D5	D6													

Notice: N. representing the emerging order of virgin queens in the incubator (in colony 43, there is only one virgin queen available, other queen cells were destroyed when sampled), *qd* representing queen distribution, *ws* representing workers staying in the colony in the swarming, *wl* representing workers leaving the colony in the swarming. Workers within patrilines were analyzed by binominal distribution, different letters represent a significant difference with a, b ($P<0.05$), and with A, B ($P<0.01$).

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