

HONEYBEE QUEEN DOCILITY NOTICED DURING QUEEN DUELS

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S u m m a r y

Most animals compete for mates and most competition includes some type of fighting. But if losses are too high, one of the opponents withdraws. *Apis mellifera* is a rare example of a species where this competition most frequently results in deadly fights. However, “weak” queens sometimes refrain/retreat from fighting. This study is to confirm the occurrence of such behavior and to investigate how bee breed and prior insemination influence the queen contests. A pair of queens (10 or 14 days old), were introduced into an arena together with 3-day old workers. The comparison concerned the behaviour of 2 x 50 pairs of the crossbreed, 100 pairs of native and 100 pairs of Caucasian queens, as well as the behaviour of 17 pairs of inseminated and 19 pairs of virgin queens. Three types of fight behaviour were described. Super-domination (SD), one queen attacked, whereas the other tried to withdraw. SD results not from the extraordinary aggression of the dominating queen but rather from the backing out of the fight by her opponent. The “weak” queen either refrained from fighting, without any physical contact with her opponent (RF) or she tried to retreat after the first clash (RT was observed in the SD and the DD pairs). Domination (DD), one queen dominated and attacked, but the other fought back and even counterattacked. Occasionally, the opponent of the dominating queen began to retreat from fighting after several clashes (RT). No-domination, both queens attacked but neither of them was able to dominate or, in two cases, both of the dueling queens refrained from fighting (RF). Prior insemination and breed (genotype) significantly influenced the duration, pattern and violence of the queens. The percentage of DD was 33% - 42%, ND was 41% - 52% and SD was 9% - 26% and it was influenced only by breed not by the prior insemination. The percentage of RT+RF was 17% and 22% in crossbreeds and natives, but as much as 47% in Caucasian queens (RF was respectively 7%, 5% and 12%). RT and RF behavior seem to be important, genetically determined, mechanisms of bee queen contest/selection. RF queens were also able to assess their own strength before engaging into a conflict, and just therefore they withdraw from fighting.

Keywords: *Apis mellifera*, queen, behaviour, conflict, duel.

INTRODUCTION

Most animals compete for mates and most competition includes different types of fighting (Archer, 1988). An important feature of evolution is minimizing the costs of such contests (Krzanowska and

Łomnicki, 2002). Therefore, if losses connected with fighting are assessed to be too high, a conflict participant decides to withdraw. This is particularly true in aggressors and when the combat losses are relatively high, such a strategy could be effective for the future reproduction

success (Maynard-Smith and Price, 1973). To apply this strategy successfully it is necessary to be able to assess one's own strength as well as the fighting abilities of an opponent and next, to intimidate the opponent. That is why animals, including insects, have developed many different cue systems, which enable them to make such assessments (Archer, 1988; Tibbets and Dale, 2004; Slessor et al., 2005). It is believed, however, that in *Apis mellifera* the queen-reproductive-competition mostly results in deadly fights (Gilley, 2001; Tarpy and Mayer, 2009). On the other hand, a diversity of defense strategies employed by worker-bees was observed during a colony defense response. Kastberger et al. (2004, 2009) revealed that in some of the colonies most of the workers were attackers (out-flying, ready to sting) whereas in others most of the workers were retreaters (retreating to the nest). Dietemann et al. (2008) revealed that queens deprived of their weapon also tried to avoid a lethal fight and that "weak" queens could refrain from fighting since they probably were able to assess their low chances of winning. My former studies (Paleolog, 1993) also showed that a few of the duelling queens tried to avoid their opponents after the first physical contact. Since workers and queens have the same genes (HGSC, 2006) the following questions arise: are bee queens really only attackers or could docility versus aggressiveness be also taken into account during their reproductive conflicts, and another, could a queen's docility influence the selection of the queens within a honeybee colony?

The aim of this study was to tackle these questions and to investigate how breed and prior insemination could influence the strategies/pattern of the bee queen contest.

MATERIAL AND METHODS

Just emerged queens were individually marked (different colours and numbers) and then kept in queen banks. The behavioural assays were performed within wooden, square arenas (9.0 x 9.0 x 2.5 cm) equipped with glass covers, bottoms lined with the wax foundation, openings (1 cm²), internal feeders and ventilation slots. The arenas were placed in an air-conditioned chamber (temp. = 29°C -30°C; H = 65% - 75%). The queen stings were blunted (less than 1 mm) under CO₂ anaesthesia using micro-surgery scissors and an insemination device, when the queens were 7 days old. At the same time the queens were weighed. When the queens were 10 days old a pair of queens was placed in an arena. Queens of similar weight were paired. Three-day-old worker bees were also introduced into each arena (n=15) under CO₂ anaesthesia 1 minute before queen introduction. The course of the queen fights was monitored.

The following experimental design was applied. In Experiment 1, the fight behaviour of 100 crossbred queen pairs of unknown origin was examined in two repetitions, 50 pairs per repetition. Then, the fight behaviour of the native European (*Apis mellifera mellifera* x black-bee drones) and the Caucasian (*Apis mellifera caucasica* - purebred) queens, 100 pairs per group, was compared.

In Experiment 2, the stings were blunted in 80 *Apis mellifera carnica* (F₁) crossbred queens and 40 of them were instrumentally inseminated at the age of 10 days. Fight behaviour of 19 pairs of virgin and 17 pairs of inseminated queens (queen losses) was compared 4 days after the insemination.

An observed versus expected χ^2 test (1:1; binary variables, df=1) was used to detect significant differences between the trait distributions Fig. 1 (24 independent

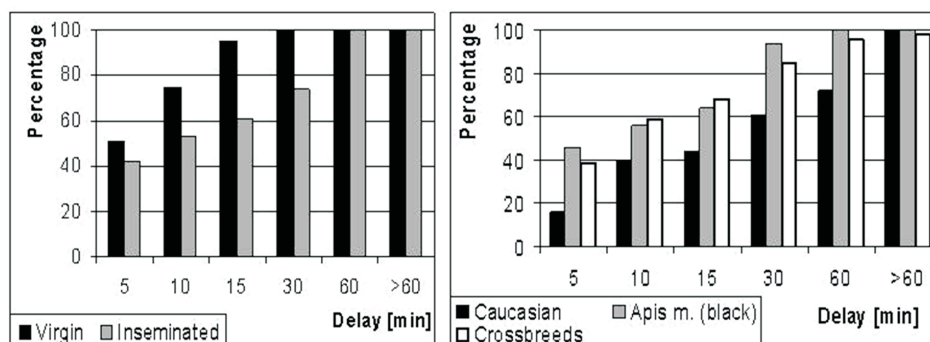


Fig. 1. Percentages of queens which started to fight 5, 10, 15, 30, 60 and >60 minute after the introduction of the double-queens into arenas.

Statistical verification (χ^2 test, $df=1$) of all the differences between expected (1:1) and obtained values for all the distributions compared on this figure are shown in Table 1. The comparison Crossbreeds:Caucasian, Crossbreeds: *A. mellifera* (black) and Caucasian: *A. mellifera* (black) were considered separately

comparisons) and in Tab. 3 (20 independent comparisons). One-way ANOVA plus the Duncan multiple range test were used for statistical verification of an influence of the queens' breed on their fighting duration and pattern, and also to detect significant differences between the

means in Tab. 2, Exp. 1. The only ANOVA was used to verify an influence of the prior insemination on values of these traits in Tab. 2, Exp. 2.

Table 1

The "auxiliary" table showing the χ^2 values in the tests performed separately within all the comparisons presented at Fig. 1 and also in Tab. 3.

Phase 1 of the queen combat; - Fig. 1					Behavior of the dueling queens; -Tab. 3				
Time interval	Comparison				Behav. type	Comparison			
	Vg:In	Cu:Na	Cu:Cb	Cb:Na		Vg:In	Cu:Na	Cu:Cb	Cb:Na
5 min	1.087	14.516	9.619	0.576	DD	0.077	1.080	0.671	0.049
10 min	3.881	2.867	3.910	0.138	ND	0.222	1.301	0.186	0.505
15 min	7.410	3.854	4.400	0.032	SD	0.200	8.257	3.870	1.087
30 min	3.885	7.410	3.945	0.556	RT+RF	0.200	9.058	14.063	0.641
60 min	-	4.558	3.699	0.046	RF	0.333	4.263	1.316	0.333
>60 min	-	-	-	-					

For $p = 0.05$ $\chi^2 = 3.841$; for $p = 0.01$ $\chi^2 = 6.635$; $df=1$. Shaded cells - a difference between expected (1:1) and obtained value of a given distribution is significant; **Vg** - virgin queens; **In** - inseminated queens; **Cu** - Caucasian queens; **Na** - Native European (*Apis m. mellifera* - black); **Cb** - crossbred queens; **ND** - No domination; **DD** - Domination; **SD** - Super-domination; **RT** - one of the opponents is retreating from fighting; **RF** - one of the dueling queens is refraining from fighting without any physical contact with her opponents. Phase 1 is the phase of mutual examination (assessment), the first physical contact, and finally (not always), a very short preliminary clash of the opponents.

Table 2

Duration of the phase [h] of the most intensive queen fighting (Phase 2) and also of the gradual calming and final cessation of their aggression (Phase 3)

Experiment 1								
PP	Phase 2				Phase 3			
	100%		80%		100%		80%	
Bees	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Caucasian	0:1 - 9:1	8:5 ^A	0:5 - 8:3	(7:8) ^A	9:4 - 18:1	(8:3) ^A	14:2 - 17:2	(2:5) ^A
Native	0:1 - 12:3	12:2 ^B	0:6 - 9:8	(9:2) ^B	13:2 - 24:1	(10:4) ^B	18:1 - 23:3	(5:3) ^B
Crossbreeds*	0:0 - 11:8	11:5 ^B	0:4 - 10:1	(9:7) ^B	12:1 - 23:3	(11:2) ^B	16:1 - 22:3	(6:2) ^B
Experiment 2								
Inseminated	0:5 - 7:5	(7:1) ^A	1:1 - 6:2	(5:2) ^A	8:0 - 23:5	(15:3) ^A	15:1 - 21:3	(6:2) ^A
Virgin	0:1 - 10:5	(10:4) ^B	0:3 - 7:1	(6:5) ^B	11:2 - 0:5	(9:4) ^B	17:1 - 19:5	(2:4) ^B

A phase duration indicates its beginning and terminating after the queens were introduced into an arena. The average duration is given in brackets. “*” - data from two repetitions were pooled. Different capital letters - averages differ significantly $p < 0.01$. The averages were compared within each of the rows and within each of the experiments, separately.

PP - Percentage of the queen pairs showing a given behaviour. “Native” means Native European bees (*Apis m. mellifera* - black). **Phase 2** - the most intensive queen fighting. **Phase 3** - gradual calming and final cessation of queen aggression.

RESULTS

Three consecutive phases of the queens' duel were differentiated: Phase 1: mutual examination (assessment), the first physical contact, and finally (not always), a very short preliminary clash of the opponents, Phase 2: the intensive/violent combat which consists of consecutive clashes and breaks and Phase 3: gradual calming and final cessation of aggression.

Significantly less Caucasian than native queens were already engaged in fights 5, 15, 30 and 60 minutes after the double-queens had been introduced into the arenas whereas no significant differences between the crossbreeds and the native queens were found (Fig. 1 versus Tab. 1). More virgin than inseminated queens were already fighting, but only 10, 15 and 30 minutes after the double-queen introduction. Therefore, both the queen's breed/genotype and the prior insemination significantly influenced the duration and pattern of Phase 1. The fights were

significantly shorter (Tab. 2 - experiment 2 - average fight duration) and aggression decreased significantly faster in Caucasian than in native and crossbreed queens. In the virgin queens, the phase of the most intensive fighting lasted significantly longer but the phase of gradual calming and cessation of aggression lasted significantly shorter than in the inseminated queens.

Three different types of queen dueling behavior, and therefore, three different strategies of the queen conflicts were observed: No-domination (ND), both queens attacked alternately but neither of them was able to dominate. Super-domination (SD), one queen dominated and attacked, whereas the other tried to withdraw/run away. Different types of the docile queen's behaviour were observed within the SD pairs. Either the “weak” queen retreated and even tried to run away after the first clash or first few clashes (RT) or, sometimes, the “weak”

Table 3

Number of the queen pairs showing each of the three dueling behaviour types (Part I), as well as the docile behaviour which was observed within SD and DD (Part II)

A queen type	No. of queen pairs	Part I			Part II	
		ND	DD	SD	RT+RF	RF
Crossbreeds total *	100	45	40	14	17	5+2#=7
Caucasian	100	41	33	26	47	12
Native100	100	52	42	9	22	5
Inseminated	17	8	7	2	2	1
Virgin	19	10	6	3	3	2

* data from two repetitions were pooled. (#) - both contesting queens avoid fighting.

ND - No domination; **DD** - Domination; **SD** - Super-domination; **RT** - one of the opponents is retreating from fighting; **RF** - one of the dueling queens is refraining from fighting without any physical contact with her opponents.

Statistical verification (χ^2 test, $df=1$) of all the differences between expected (1:1) and obtained values for all the distributions compared on this figure are shown in Table 1. The comparison Crossbreeds: Caucasian, Crossbreeds: Native and Caucasian: Crossbreeds were considered separately.

queen refrained from fighting without any physical contact with the dominating one (RF). Such RF queens even tried to escape from the arenas when one of the entrances was opened. Moreover, it has been observed (only in two cases) that both of the dueling queens refrained from fighting and tried to avoid each other (both queens were showing RF). Such a situation is hereafter referred to as ND behaviour. Domination (DD), one queen dominated and most frequently attacked, but the other fought back (did not run away) and sometimes even counterattacked. Occasionally, however, the opponent of the dominating queen begun to refrain from fighting and to run away but only after several consecutive clashes, i.e. DD changed into SD (RT). Thus, RF or RT of one of the dueling queens was mostly connected with the SD of her opponent but sometimes RF or RT also occurred in DD queen pairs.

The queen's breed significantly influenced but prior insemination did not, the frequency of the types of dueling behavior (Tab. 3 versus Tab. 1). However,

frequency of the ND behaviour was 41% - 52% and did not depend on the queen's breed. Frequency of DD was slightly lower in Caucasian than in native and crossbreed queens, but this tendency was also insignificant (Tab. 3 - Part I versus Tab. 1). The most interesting is that frequencies of SD, as well as RF+RT or RF, were about twice as high in the Caucasian than in the native/crossbreed queens, and the distributions were significantly different (Tab. 3 - Part II versus Tab. 1).

DISCUSSION

Bee queens are eliminated by a multi-level selection within the colony. The first level is the queen-queen competition which includes queen duels (Gilley and Tarpy, 2005). The second is the elimination of the queen by workers (Szabo, 1977; Paleolog, 2001; Schneider et al., 2001). Queen duels are believed to be the most important (Tarpy and Mayer, 2009). Physical contact between queens is essential for the beginning of the duel (Pflugfelder and

Koeniger, 2003), but dueling queens sometimes avoided contact with each other or they tried to hide within the comb cells (Paleolog, 1993; Gilley, 2001). Could such behaviour be profitable for competing queens and how frequently does it occur during the queen duels? My results revealed that queen docility can even occur in more than 40% of the queen conflicts. Gilley and Tarpy (2005) found that, instead of fighting and killing her rival queens (the 1st possibility), a newly emerged honey bee queen could also surrender the queenship to her sister queens and be killed (the 2nd possibility), or she could depart with an after-swarm (the 3rd possibility). In my experiment, one of the duelling opponents retreated from fighting in about 20% of all the native and crossbreed queen pairs, but in 47% in the Caucasian. So, the 4th possibility is that one of the duelling queens withdraws from fighting and tries to wait until the danger decreases (such queens often try to hide in the hive - Paleolog - unpublished). Such docile behaviour could increase the chances of the retreaters, since some of the remaining queens could be wounded during the conflict (Gilley, 2001). The considerably higher frequency of the retreaters, as well as the significantly different pattern, duration and violence of the queen combats observed more in Caucasian than in the other queens in my experiment, suggests the genetic background of the docile/fighting behaviour. If the worker bees defence behaviour/strategies differ between races/ecotypes and there are aggressive and docile worker colonies (Kastberger et al., 2009), why should not the queen conflict strategies, patterns, or violence not be divergent in different bee races/ecotypes and why should some of the queens not be docile. Consequently, retreaters/attacker ratio in bee queens could also be

genetically determined and therefore be under evolutionary pressure.

The queens are also selected by workers and many different factors influence their decisions (Szabo, 1977; Tarpy and Fletcher, 1998; Tarpy et al., 2000; Paleolog, 2001; Tarpy and Mayer, 2009). My studies revealed one more such factor, i.e. prior queen insemination. The docile behavior of some queens is conducive to maintaining the multiple-queen colony status and therefore conducive to the queen being selected by workers. That behavior could also protect a colony from queen-lessness since as a result of it, there are often a few spare (docile) queens within the colony. My studies confirmed that such queens could occur in a bee colony just because they had abilities to retreat/refrain from fighting.

Dieteman et al. (2008) found that weak queens refrain from fighting as a result of a self assessment showing that their chances of winning are low. Super domination (SD) observed in my studies results not from the extraordinary aggression of a dominating queen but rather from the retreating of her opponent from fighting. The most efficient form of the docile behavior is refraining from fighting without any physical contact with the opponent (Krzanowska and Łomnicki, 2002). Such behavior was observed in 5% to 12 % of all dueling queen pairs in my studies (RF). Gradual calming and final cessation of the fighting queens' aggression observed in my experiment, confirmed that when the opponents were not able to win and the effort and possible fight losses increased, they decided to retreat from fighting. All these observations suggest that queens are able to assess their own strength before engaging in combat (or at the initial phase of the combat) and, consequently, decided to choose docility as their strategy in queen duels.

CONCLUSIONS

Docile behaviour of some of the opponents during queen duels was connected with retreating or refraining from fighting and appeared not to be so rare in reproductive conflicts between queens. This behaviour seems to have a genetic background and be under evolutionary pressure. Therefore, this study confirmed the occurrence of another, less-known mechanism, of the multi-level queen selection process in *Apis mellifera*.

Dueling queens were probably able to assess their own strength or chances of winning and consequently withdraw from the combat. They withdrew either before or at the initial phase of the fight or even after a long period of fighting.

Prior insemination is one of the factors which might influence the patterns, strategies and results of queen combats.

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UNIKANIE WALKI PRZEZ MATKI PSZCZOŁY MIODNEJ PODCZAS ICH RYWALIZACJI

Paleolog J.

S t r e s z c z e n i e

Zwierzęta rywalizują/walczą aby uzyskać sukces reprodukcyjny. Jednak gdy straty/koszty są zbyt duże jedna ze stron wycofuje się. Ważna jest tu ocena własnych możliwości przed rozpoczęciem konfliktu. U nielicznych gatunków, w tym *Apis mellifera*, konkurencja reprodukcyjna przyjmuje postać walki matek ze śmiertelnym skutkiem. U robotnic poza atakiem zaobserwowano też strategię ucieczki do gniazda. Takie strategię uciekania lub unikania walki czasami obserwowano też wśród matek. W tej pracy badano jak często rywalizujące matki stosują strategię ucieczki (wycofywania) i jak rasa oraz uprzednia inseminacja wpływają na częstość występowania tych strategii. W klatce (9,0 x 9,0 x 2,5 cm) z 15 młodymi robotnicami (3 dniowe) umieszczano dwie młode matki (10 dniowe) z przepięcionymi żąłkami i obserwowano ich zachowania. Dwukrotnie porównano zachowania 50 par matek nieznanymi mieszańców (2 x 50 = 100 par), jak też zachowania 100 par matek kaukaskich i 100 par europejskich matek rodzimych. Dodatkowo porównano rywalizację 17 par matek uprzednio unasienionych i 19 par matek nie unasienionych (kraińskich - F₁ - wiek 14 dni). Superdominacja (SD), pierwsza matka miała przewagę, atakowała, a druga uciekała. SD wynikała raczej z unikania walki przez jedną z przeciwniczek niż z nadmiernej agresji drugiej i przejawiała się na dwa sposoby. Albo pierwsza matka bez kontaktu fizycznego powodowała ucieczkę drugiej (RF) albo po pierwszym starciu (lub kilku) jedna z matek unikała dalszej walki (RT). Dominacja (DD), jedna matka uzyskiwała przewagę i najczęściej atakowała ale druga walczyła, a nawet atakowała. Niekiedy, po pewnym okresie kontynuowania walki, jedna z matek przyjmowała typ zachowania RT, czyli zaprzestawała walki i uciekała. Bez dominacji (ND), obie matki atakowały i żadna nie uzyskiwała przewagi. W dwu przypadkach dwie matki unikały walki (RF). Uprzednie unasienienie oraz rasa (genotyp) wpływały istotnie na przebieg, długość i gwałtowność. Odsetek par wykazujących DD wynosił 33% - 42%, ND 41% - 52% a SD 9% - 26% i nie zależał od uprzedniego unasienienia ale zależał od rasy. Udział procentowy RT+RF wynosił 17% i 22% dla matek mieszańców i matek miejscowych ale aż 47% dla matek kaukaskich (RF odpowiednio 7% i 5% oraz 12%). Zachowania typu RT i RF mogą być jednym ze znaczących mechanizmów w rywalizacji i selekcji matek. Zachowanie te mają genetyczne podłoże i mogą podlegać oddziaływaniu procesów ewolucji. Matki (np. RF) były w stanie ocenić własne siły przed zaangażowaniem się w konflikt i ewentualnie się z niego wycofać.

Słowa kluczowe: *Apis mellifera*, matki, zachowanie, konflikt, walka.