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Sexual selection against inbred mates and inbreeding effects on some mating properties in *Bombus terrestris*

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Abstract

Bumblebees are phylogenetically related to honey bees and have ecological and economic importance as pollinators of both natural flora and many crops. The mating stage is one of the most important stages in *Bombus terrestris* (L.) life cycle since it affects diapause success and offspring reproduction. Unlike honey bees, *B. terrestris* colonies are at higher risk of homozygosity because queens are monandrous. Accordingly, the quality of queens and males, as well as the kinship relationships of individuals, affect mating success much more compared to polyandrous species. In this study, we aimed to investigate sexual selection against inbred mates and inbreeding effects on some mating properties in *B. terrestris*. The results showed that inbreeding did not cause a significant difference in sexual selection for both queens and males of *B. terrestris* reared under laboratory conditions. Moreover, there were no significant differences in mating latency and mating duration between related and unrelated couples. Additionally, the weights of queens and males were measured. The results were evaluated, and *B. terrestris* queens and males were not affected by kinship in the selection of mates during the mating phase.

Key words: bumblebee, kinship, mating choice, sexual selection.

Introduction

Bee species in the order Hymenoptera exhibit complexity and great diversity in terms of mating behaviour (Ayasse *et al.*, 2001). This specific mating behaviour contributes to their survival throughout generations. In species that especially exhibit parthenogenesis or haplodiploidy, mating strategies play a critical role in avoiding inbreeding depression, which is characterized by related offspring being less fit than unrelated offspring. For example, in honey bees, which are polyandrous, queens mate with about 15 males in flight far from their nest site (Tarpy *et al.*, 2004; Cobey, 2007). This mating pattern protects honey bee populations against the destructive effects of homozygosity at the single-sex locus that lead to diploid male production (Bienefeld *et al.*, 1989; Gempe *et al.*, 2009).

Bumblebees, which are phylogenetically related to honey bees, have ecological and economic importance as pollinators of both natural flora and many commercial crops (Wilfert *et al.*, 2006). This genus consists of many species that differ in their ecological, behavioural, and physiological properties (Williams, 1998; Pedersen, 2002). *Bombus terrestris* (L.) is most widely reared commercially and used as a pollinator agent in conventional agriculture (Uzun Yigit *et al.*, 2023). Scientific findings about mating behaviour reported by different studies have been a guide for both researchers and breeders. In *B. terrestris*, queens mate only once, but males can remate (Gosterit and Gurel, 2016), mating lasts about 30 minutes and can be observed in the cage (Amin *et al.*, 2009), male body size directly affects their mating success (Amin *et al.*, 2012), optimal mating age is 12 days for males and 6 days for queens (Djegham *et al.*, 1994), and optimal ambient temperature for a high mating rate is 23 degrees centigrade (Amin *et al.*, 2010). Because *B. terrestris* is an obligatorily eusocial species with colonies that are always headed by a single, once-mated queen, the dramatic effects of inbreeding depression can be clearly visible in the next generation in their mass rearing process. Therefore, one of the most important requirements to improve the rate of colony foundation success is to prevent inbreeding (Schmid-Hempel and Schmid-Hempel, 2000; Gosterit, 2016; Bortolotti *et al.*, 2020).

Studies on the effects of inbreeding on colony life and colony fitness in *B. terrestris* in native and laboratory conditions were reported by different researchers (Gerloff and Schmid-Hempel, 2005; Schmid-Hempel *et al.*, 2007; Whitehorn *et al.*, 2009; Gosterit, 2016; Bortolotti *et al.*, 2020). In populations with reduced genetic diversity, inbreeding may take place, resulting in inbreeding depression and decreased fitness. This can manifest as diminished reproductive rates, slower growth rates, and limited adaptability to diverse environmental conditions (Zayed and Packer, 2005). At the initial stages of its life cycle, a *B. terrestris* colony is led by a solitary monandrous queen who holds exclusive responsibility for egg-laying (Schmid-Hempel and Schmid-Hempel, 2000). Following the competition point (Duchateau and Velthuis, 1988; Bloch and Hefetz, 1999), workers have the ability to lay eggs that give rise to male offspring (Alaux *et al.*, 2004). Early in the colony's life, the detrimental consequences of inbreeding became evident. The inbred group experienced an earlier emergence of the first males compared to the outbred group, resulting in a reduced worker force within the colonies of the inbred group. Additionally, Gerloff and Schmid-Hempel (2005) found that inbreeding has a substantial impact on the queen's chances of surviving hibernation, leading to a significant reduction in survival probability. In another study, Bogo *et al.* (2022) found that diploid male wings resulted in a narrower and slightly longer configuration than haploid male wings. *B. terrestris* is a remarkably invasive species, capable of thriving despite experiencing a significant genetic bottleneck. This invasiveness could be attributed to its haplo-diploid sex determination system, which subjects recessive, harmful mutations to natural selection. As a result of this purging process, the descendants of these lines might develop increased resilience to inbreeding (Schmid-Hempel *et al.*, 2007). It is clear that mating with relatives is common in *B. terrestris*. It has even been observed that males and queens mate inside the nest (personal observation). However, there is limited or contradictory knowledge about whether males and young queens have sexual selection against related mates. According to a previous study on this topic by Whitehorn *et al.* (2009), *B. terrestris* reproductive can discriminate between related mates and related couples take longer to mate than unrelated couples. On the other hand, it is reported that there is no sexual preference to avoid inbreeding, but related couples have shorter mating latency than unrelated couples in a more recent study by Bogo *et al.* (2018). Here, we conducted an experimental study to contribute to the available scientific data on this topic. In addition to mate preference by both males and queens against related opposite sexual, inbreeding effects on some mating properties such as mating latency and mating duration were also determined in *B. terrestris.*

Materials and methods

Materials

This study was carried out in the Apicultural Research and Application Laboratory at Isparta University of Applied Sciences, in Turkey. We used *B. terrestris* colonies, which are reared in our laboratory, and known for their pedigree information. Eight colonies producing both sexes (males and females) were selected. Four of them were used to test the mate preference of queens against related males, whereas others were used to test the mate preference of males against related queens. Colonies with similar growth characteristics were selected for standardisation of colony effects. The preference test for each sexual was repeated twice.

Methods

The ages of males and queens play crucial roles in mating success. As reported in a previous study, queens and males have to be 12-14 days old and 6-8 days old, respectively, for the best mating success rate (Djegham *et al.*, 1996; Amin *et al.*, 2010). Therefore, all males and queens of unknown age were removed from the colonies. Afterwards, two colonies from each group were chosen for the first and second mating trials, where sibling mating was conducted to create a related status within those colonies. The remaining colonies in each group were reserved for unrelated mating purposes. All newly emerged males and queens were collected and isolated from the colonies. They were put into another empty special-designed rearing box (16 × 19 × 5 cm) separately according to sex and colony code until the mating day of the experiment. These males and queens of known age were also fed sugar syrup (50 Brix) and fresh thawed pollen ad libitum in the laboratory rearing conditions (27-28 °C, 50-55% RH).

The mating room was prepared at 23 °C and RH 70%, as described by Amin *et al.* (2010). Two experiments were conducted. In the first experiment, mate preferences by queens against related and unrelated males were investigated in two mating trials. Individuals were marked on their thorax in a different colour with queen marker pens according to the colony code before placing the mating cages. For this purpose, 30 queens (marked with white colour) from the colony (a), 15 related males (marked with white colour) from the same colony (a), and 15 unrelated males (marked with red colour) from a different colony (b) were placed in a mating cage at the same time in the first trial. This process was applied for the second trial using another mating cage with different two colonies (x, y) in first experiment. In the second experiment, mate preference by males against related and unrelated queens was observed in two mating trials. Like in the first experiment, two mating trials were carried out in this experiment, each in a different mating cage. For this, 30 males (marked with white colour) from the colony (k), 15 related queens (marked with white colour) from the same colony (k), and 15 unrelated queens (marked with red colour) from a different colony (l) were placed in the mating cage at the same time in the first trial. This process was applied for the second trial of the second experiment with two different colonies (w, z). Experimental settings are given figure 1.

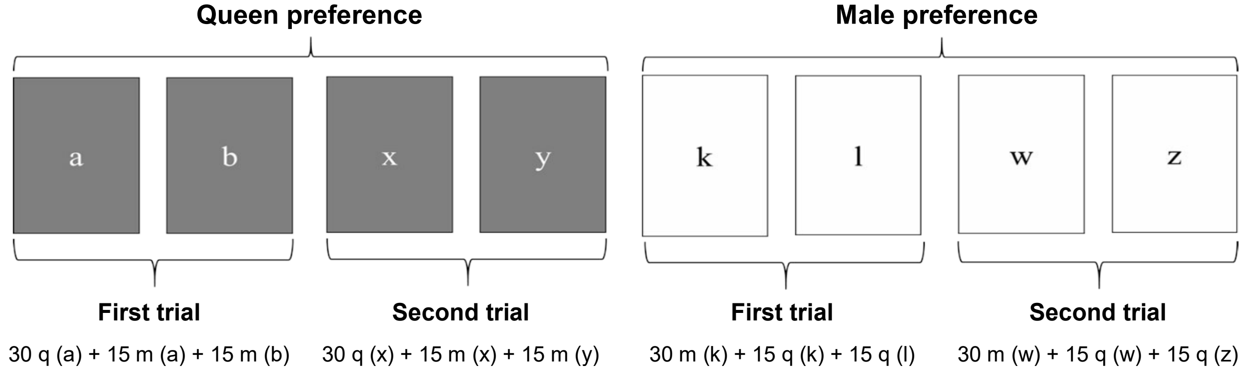

Figure 1. Experimental design.

For all mating trials in the two experiments, some traits were determined. For the determination of mating latency (time elapsed from the transfer in the mating cage to the start of copulation) (minutes) and mating duration (time elapsed between the start and the end of copulation) (minutes), we first recorded the transfer time of individuals to the mating cage. As soon as the copulation began, the time was noted again, and couples were transferred to transparent boxes separately. These boxes were observed, and the termination time for each mating was noted. Since it is known that an individual's body weight affects mating performance (Amin *et al.*, 2012), males and queens were weighed separately after mating. The mate preference ratio of both males and queens against related opposite sexual species was also calculated.

Data analysis

Data were analysed in Minitab statistical software (version 16.2.4). Descriptive statistics for traits were calculated. According to the Shapiro-Wilk normality test, it was determined that mating latency and mating duration were not normally distributed, while other properties showed normal distribution. Square root transformation was used for data that was not normally distributed. Proportional data related to mating preferences were compared using two proportional Z tests.

Results

Data related to mate choices of queens against related and unrelated males obtained are given in table 1. The general mating ratio was determined to be 56.67% and 63.33% in the first and second mating trials, respectively ($P_{\text{mating trail 1}} = 0.732$, $P_{\text{mating trail 2}} = 0.746$; $Z_{\text{mating trail 1}} = 0.34$, $Z_{\text{mating trail 2}} = -0.32$). There was no statistically significant difference between the two mating trials with regard to the mating ratio (52.95, 47.05, 47.37, and 52.63%, respectively). According to the findings, whereas 9 of 17 mated queens preferred to mate with related males, the others mated with unrelated males in the first mating trial. In the second mating trial, 9 of the 19 mated queens mated with related males, and the remaining queens chose to mate with unrelated males. Differences in the proportion of related and unrelated couples in the same mating trial were not also significant (table 1).

Mate preference by males against related and unrelated queens was also determined. The result showed that 8 of 19 successfully mated males chose to mate with related queens, while others preferred the unrelated queens in mating trial 1. On the other hand, in mating trial 2, whereas 12 males chose to mate with related queens, 9 males mated with unrelated queens (table 2). Like in the experiment of mate preference by the queen, differences both in terms of general mating ratio among the first (63.33%), and second mating trials (70%), and proportion of related and unrelated couples in the same mating trial were not significant in the experiment of mate preference by the males ($P_{\text{mating trail 1}} = 0.330$, $P_{\text{mating trail 2}} = 0.355$; $Z_{\text{mating trail 1}} = -0.97$, $Z_{\text{mating trail 2}} = 0.93$). The findings of the study indicate that there is no significant impact of inbreeding on the mate choosing behaviour of *B. terrestris* sexuales, namely males and queens, as seen by the results presented in table 2.

In this study, mating latency and mating duration for related and unrelated couples were also determined (table 3). The mating latency of related and unrelated couples was found to be 16.34 ± 15.24 minutes and 15.34 ± 12.33 minutes, respectively. The mating duration was found to be 29.45 ± 10.26 minutes for related couples and

Table 1. Mate preference by *B. terrestris* queens against related and unrelated males.

	Queens Colony code	N	Males Colony code	N	Number of couples	Ratio in all couples (%)	P value	Z value
Mating trial 1	a	30	a	15	9	52.95	0.732	0.34
			b	15	8	47.05		
Mating trial 2	x	30	x	15	9	47.37	0.746	−0.32
			y	15	10	52.63		

Table 2. Mate preference by *B. terrestris* males against related and unrelated queens.

	Males Colony code	N	Queens Colony code	N	Number of couples	Ratio in all couples (%)	P value	Z value
Mating trial 1	k	30	k	15	8	42.10	0.330	−0.97
			l	15	11	57.90		
Mating trial 2	w	30	w	15	12	57.15	0.355	0.93
			z	15	9	42.85		

Table 3. Mating latency and mating duration of related and unrelated couples.

Property	N	Related couples Mean ± SD	Unrelated couples Mean ± SD	P value
Mating latency (minutes)	38	16.34 ± 15.24	15.34 ± 12.33	0.885
Mating duration (minutes)	38	29.45 ± 10.26	29.71 ± 8.71	0.760

Table 4. Correlation between mating weights of males and queens (grams).

Property	N	Queen weight Mean ± SD	Male weight Mean ± SD	The ratio of queen weight / male weight
Related couples	38	0.914 ± 0.170	0.274 ± 0.047	3.453 ± 0.938
Unrelated couples	38	0.881 ± 0.132	0.268 ± 0.051	3.405 ± 0.836
Total	76	0.897 ± 0.152	0.271 ± 0.049	3.429 ± 0.882

29.71 ± 8.71 minutes for unrelated couples. According to our results, there were no significant differences in terms of both mating latency and mating duration between the related and unrelated couples (table 3).

We also determined the weights of queens and males in each couple to evaluate the role of weight on sexual selection in *B. terrestris* (table 4). The queens' and males' weights were found to be 0.914 ± 0.170 gram and 0.881 ± 0.132 gram in inbreeding couples, whereas these values were 0.274 ± 0.047 gram and 0.268 ± 0.051 gram for non inbreeding couples, respectively. The groups with related and unrelated couples were determined as queen weight, male weight and the ratio of queen weight: male weight. As a result of the statistical analysis of each feature, the features examined are not statistically significant. The weight of the opposite sexes had no effect on the mate selection decision, according to the queen weight to male weight ratio (table 4).

Discussion and conclusion

Like the other haplodiploid social insects, homozygosity at the single-sex locus causes diploid male production in *B. terrestris* (Bienefeld *et al*., 1989; Gempe *et al*., 2009). To avoid this situation, some species have adapted to alternative solutions such as flying long distances from the nest area to mate (Michener, 1974) and being able to mate with more than one, to reduce homozygosity (Page, 1980). Compared to polyandrous species like honeybees, *B. terrestris* colonies are at higher risk of homozygosity because queens are monandrous (Estoup *et al*., 1995). It is known that homozygosity due to inbreeding leads to devastating effects on *B. terrestris* (Whitehorn *et al*., 2009; Gosterit, 2016; Bortolotti *et al*., 2020) and it negatively affects the sustainability of mass rearing. Whitehorn *et al*. (2007) reported that *B. terrestris* was a remarkably invasive species, demonstrating the ability to establish its presence even after a significant genetic bottleneck. One potential reason for its invasive nature could be attributed to the haplo-diploid sex determination system, which makes recessive, harmful mutations susceptible to natural selection. Consequently, the offspring from these purged lineages might possess increased tolerance to inbreeding, enabling their successful adaptation and proliferation. Therefore, mating stages are critical for the mass rearing of *B. terrestris*. On the other hand, inbreeding is an obstacle to biodiversity when considered in terms of natural populations.

There are limited studies on the mechanism of incest avoidance through the kin recognition of *Bombus* species. While there is the mechanism of incest avoidance through kin recognition in *Bombus frigidus* Smith and *Bombus bifarius* Cresson, it is not present in *Bombus californicus* Smith and *Bombus rufocinctus* Cresson (Foster, 1992). In addition, there is also no evidence for this topic in *Bombus lantschouensis* Vogt (Zhang *et al*., 2021). Whitehorn *et al*. (2009) found that related mating duration was longer than unrelated mating duration in *B. terrestris*. Additionally, they reported that *B. terrestris* sexuales have kin recognition and this information might be useful in mating behaviour studies. However, Bogo *et al*., (2018) reported that there is no incest avoidance in *B. terrestris*, and for this reason, both queens and males can mate with nestmates.

In this study, we investigated whether kinship influences mate selection in the mating stages of queens and males of *B. terrestris* and their mating preferences. According to our findings, there are no significant differences in terms of mating choice between related and unrelated couples. Moreover, we recorded some mating traits for related and unrelated couples. In the experiment with couples, no significant differences in mating latency or mating duration were found. Additionally, we measured the queens' and males' weights after mating. Our results have some similarities with the observations of Bogo *et al*. (2018) such as mating success, mating latency. However, when the results were compared to the results of Whitehorn *et al*. (2009), there were some differences in terms of mating duration, proportion of queens and males mated with unrelated, compared to related.

Consequently, we tested sexual selection against inbred mates and inbreeding effects on some mating preferences in *B. terrestris*. According to the results we have obtained, during the mating trials performed in laboratory conditions, kinship is not a determinative factor in mate preference behaviour for both queens and males. Besides, similarity was determined in terms of mating latency and mating duration among related and unrelated couples. As mentioned above, there are a few studies about mate selection and kin recognition in bumblebees. For researchers, the situation in natural populations is particularly interesting and thrilling. So, more research needs to be done on these subjects to figure out how to avoid incest through both kin recognition and mate selection.

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