

## COMPARISON OF SOME PROPERTIES OF DIFFERENT SUPER SISTER QUEEN GROUPS IN BUMBLEBEE

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### Abstract

Commercial bumblebee colonies have been used for the pollination of a number of crops, mainly of tomatoes (95%). In comparison with other bumblebee species, *Bombus terrestris* has the most year round rearing. The year round rearing procedure of *B. terrestris* involves some main stages: colony foundation, obtaining of young queens and males, mating and diapause control. Success of these stage are affected by environmental conditions, physiological properties and genetic structure of the queens. Knowledge of the effects of these factors will increase the rearing success. Because of the *B. terrestris* queens mate once, female individuals in their colonies are supersister. There is a 75% genetic relatedness between the supersister young queens. This study was conducted to compare the different super sister queen groups and to determine the effect of genotype on weight, mating performance and diapause performance of queens in *B. terrestris*. Results showed that genetic differences affected the copulation duration and pre and post diapause weight of queens, but survival ratio during the diapause duration did not affected by genotypic differences.

**Key words:** bumblebee, *Bombus terrestris*, genotype, supersister queen.

### INTRODUCTION

The majority of food consumed by humans are provided from some plant species, and 75% of these plant species are pollinated by bees (Klein et al., 2007). Honeybees (*Apis mellifera* L.) are most effective pollinators for both natural and cultivated plants. In addition to honeybees, bumblebees have incontrovertible importance among the pollinator insects because of their some features such as long tongue, large and hairy bodies, and high pollinator capacity. Currently, about 250 species of bumblebees have been determined and five of these (*Bombus terrestris*, *B. lucorum*, *B. ignitus*, *B. occidentalis* and *B. impatiens*) are commercially reared. *B. terrestris* is most commonly reared bumblebee species commercially and used for pollination agent (Velthuis and van Doorn, 2016). This species is indispensable pollinator, especially for greenhouse tomato production, because of its pollination efficiency, which reduces pollination labor costs and improves the quality and quantity of crops (Ahmad et al., 2015).

In bumblebees, the expression of artificial rearing is called in different names such as the mass rearing, the rearing under controlled condition, the laboratory rearing or the year

round rearing. This rearing procedure of bumblebee takes place in four main stages: colony foundation from queens, obtaining of young queens and males from colonies, mating of queens and males, and controlling of diapause (Beekman et al., 2000; Velthuis and van Doorn, 2006). These stages should be realized in controlled conditions for sustainability of production. Some losses occur in each of these stages in mass rearing procedure of *B. terrestris*. Especially, losses in colony initiation stage are critical for success of mass rearing. One of the main criteria is how many of 100 queens can establish colonies suitable for pollination in mass rearing. Pollinator colony production ratio is usually ranges from 30% to 40%. This low ratio leads to increasing of the production cost and colony price (Gosterit and Gurel, 2016).

Like many other organisms, two main element affect the phenotypic value of bumblebee in terms of any characteristic: genotype and environment (Vogt et al., 2008). Traits related with colony foundation success and colony development are phenotypic characteristics in *B. terrestris*. There are significant variations in the egg laying performances and colony foundation success of queens, in the total number of workers, males and young queens produced by

colonies, and in the time of production of sexuals (male and young queens) in *B. terrestris* (Duchateau et al., 1988; Gosterit, 2011). While some queens egg lay, some queens do not egg lay after diapause. On the other hand, male production occurs at variable times of colony development, either early in colony development before the start of young queen production or toward the end of the colony life cycle after the start of young queen production. Under controlled conditions, the part of the colonies produce only males or only queens, other colonies produce both males and queens, while other colonies produce neither males nor queens (Duchateau and Velthuis, 1988; Cnaani et al., 2000; Alaux et al., 2005; Lopez-Vaamonde et al., 2009; Gosterit, 2011). The effects of many factors such as mating success of founder queen, environmental condition, availability and quality of food, diapause duration, parasites and diseases, and nest area on these variations related with colony development were studied by different researchers (Amin et al., 2010; Saglam and Gosterit, 2015; Imran et al., 2017). Colony development traits of different native populations were compared to determine the effects of genetic differences (Gosterit and Gurel, 2005; Gosterit, 2017). A honeybee colony comprises a superfamily of three worker groups: supersisters, full sisters, and half sisters. The relationship is 75%, between the super sisters, 50% between full sisters and 25% between half sisters (Harbo and Rinderer, 1980). In contrast to honeybees, *B. terrestris* queens mate once, and their colonies only include supersisters (Schmid-Hempel and Schmid-Hempel, 2000; Gosterit, 2016). As a result, female individuals produced by *B. terrestris* queens are super sisters, and relationship between these individuals is 75%. This present study was conducted to compare the different super sister queen groups and finally determine the effect of genetic differences on weight, mating performance and diapause performance of queens were aimed.

## MATERIALS AND METHODS

### Experimental groups

A total of 8 *B. terrestris* colonies that produced males and young queens were used. To prevent the adverse effect of inbreeding on survival rate in diapause (Gerloff and Schmid-Hempel,

2005; Gosterit, 2016), males were obtained from 4 colonies (M: male colony group) which taken from a bumblebee breeder, while young queens were obtained from other 4 colonies (Q: queen colony group) which provided from our laboratory stocks. Super sister queens produced by same colony (Q1) were mated with males produced by other same colony (M1). Similarly, queens produced in Q2, Q3 and Q4 colonies were mated with males produced in M2, M3 and M4 colonies in time of their sexual maturity (7 days for queens and 12 days for males), respectively (Amin et al., 2010). At the end of the mating process, four different super sister queen groups were designed.

### Mating and diapause control

Queens and males were mated in mating cage in an illuminated room ( $23 \pm 1^\circ\text{C}$  and  $50 \pm 5\%$  RH). Copulation duration of queens were determined following the method reported by Gosterit and Gurel (2016). Immediately after the initiation of copulation, the time was noted and each mating pair was separately transferred to a transparent plastic box. As soon as copulation was terminated, the time was noted again and copulation duration was calculated. All mated queens were weighted on an electronic balance (0.001 g sensitivity) to obtain pre diapause weight of queens. Then, all mated queens were put into artificial diapause at  $2.5^\circ\text{C}$  for 2 months. At the end of the diapause duration, survival ratio of queens was determined and surviving queens were weighted to obtain their post diapause weight.

### Statistical analysis

In the experiment, 160 queens (40 queens for each group) were used. Data were square-root transformed and tested for normality before analysis. Survival ratio of queens in diapause were compared two-proportion z-tests. One-way analyses of variance were run to determine the effects of genetic differences on weight and copulation duration of queens (Minitab Statistical Software, Version 16.2.4).

## RESULTS AND DISCUSSIONS

In *B. terrestris*, copulation duration of queens which lasts 20-40 minutes does not affect colony foundation period, but it has significant effects on the number of workers produced in first

brood (Amin et al., 2009; Gosterit and Gurel, 2016). On the other hand, worker number produced in first brood could have directly effect on other colony development patterns, especially for number of individuals produced during the colony life (Gosterit, 2011; 2016). Copulation durations of 160 *B. terrestris* queens (40 queens for each genotype group) were determined in this study (Table 1). According to results, genotypic differences affected the copulation duration. One group was significantly different from other three genotype groups in terms of copulation duration ( $P<0.01$ ).

Table 1. Copulation duration of queens in different super sister group (a, b:  $P<0.01$ )

Groups	N	$\bar{x} \pm s.e$	Min	Max
Q1 x M1	40	$38.40 \pm 2.46^a$	13.00	77.00
Q2 x M2	40	$25.82 \pm 1.37^b$	13.00	49.00
Q3 x M3	40	$33.75 \pm 1.41^a$	19.00	57.00
Q4 x M4	40	$33.60 \pm 1.67^a$	15.00	62.00

It is clear that quality of the queens directly affects the colony quality in *B. terrestris*. Studies relating to the weight of bumblebee queens have been focused mainly on the effect of the weight on survival during diapause and their colony foundation success. According to Gosterit and Gurel (2007), the weight of the queen after diapause has no effect on variation between the colony development traits.

However, the weight of the queens before diapause affects their survival ratio during diapause. Queens weighing less than 0.6 g before diapause may not survive during diapause according to long duration (Beekman et al., 1998). Therefore, known of factors affect the weight of young queens is valuable to diapause success. Some factors such as food quality, diseases and social structure of colonies may affect the queen weight. Our results also showed that variation of queen weight was significantly affected by genetic differences (Table 2 and Table 3). Significant differences were determined between the pre diapause weights of different super sister queens groups ( $P<0.01$ ). Pre diapause weight of queens changed from 0.520 to 1.118 gram, while post diapause weight of queens changed from 0.571 to 1.069 for all queens used in the experiment.

Table 2. Pre diapause weight of queens in different super sister group (a, b:  $P<0.01$ )

Groups	N	$\bar{x} \pm s.e$	Min	Max
Q1 x M1	40	$0.984 \pm 0.012^a$	0.705	1.118
Q2 x M2	40	$0.852 \pm 0.013^b$	0.607	1.010
Q3 x M3	40	$0.770 \pm 0.018^{bc}$	0.601	1.019
Q4 x M4	40	$0.801 \pm 0.019^c$	0.520	1.111

Table 3. Post diapause weight of queens in different super sister group (a, b:  $P<0.01$ )

Groups	N	$\bar{x} \pm s.e$	Min	Max
Q1 x M1	39	$0.865 \pm 0.013^a$	0.648	1.069
Q2 x M2	37	$0.799 \pm 0.013^b$	0.657	0.943
Q3 x M3	37	$0.713 \pm 0.018^c$	0.574	0.962
Q4 x M4	35	$0.751 \pm 0.018^{bc}$	0.571	1.056

Inbred mating reduces the likelihood that the queen will survive in diapause (Gerloff and Schmid-Hempel, 2005). Survival ratios of queens in different super sister groups were given in Figure 1. In groups, survival ratios were determined as 97.5%, 92.5%, 92.5% and 87.5%, respectively. However, no significant differences were found between the different super sister queen groups in terms of survival ratio during the diapause duration.

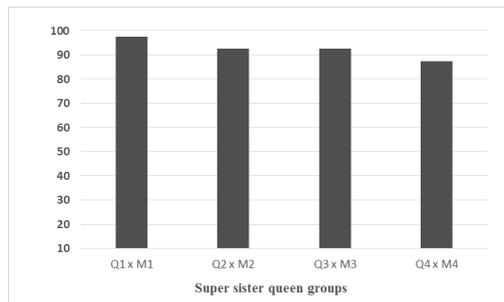


Figure 1. Survival ratio of queens in different super sister group

## CONCLUSIONS

Knowledge related to rearing process are needed to sustainability of bumblebee mass rearing. Therefore, it is vital to determine the factors affect the colony development. It is estimated that mating success of queens, environmental conditions, food quality and availability, diseases and parasites, diapause conditions, and genotype of queens and males affect the rearing success. In this study, we investigated the effects of genotype on weight,

mating performance and diapause performance of queens. According to results, significant differences were determined between the different super sister queen groups in terms of mating duration and pre and post diapause weight of queens in *B. terrestris*. However, genetic differences had no effect on survival ratio during the diapause duration.

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