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Original Article

Males from multiple colonies improve queen mating success in the bumblebee *Bombus lantschouensis* (Hymenoptera: Apidae)

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Abstract – Mating is an important process in bumblebees that could affect queen diapause survival and offspring reproduction. Both queens and males could influence mating failure. Here, we used the indigenous bumblebee *Bombus lantschouensis* to evaluate the mating interactions of males and gynes. The effects of kin recognition and males and gynes from multiple colonies on mating latency, mating duration, and the mating success rate were investigated. The results showed that gynes mated with related males had a longer mating latency than those mated with unrelated males (42.88 ± 3.8 min and 24.15 ± 2.5 min, respectively, $P < 0.05$) and that the mating success rate was significantly higher in unrelated groups than in related groups (49.29 ± 4.1% and 36.74 ± 2.6%, respectively, $P < 0.05$). However, no preference for related or unrelated males was observed in the mixed mating groups (mating success rate 30.63 ± 3.1% and 30.73 ± 2.4%, respectively, $P > 0.05$). Interestingly, the occurrence of males from multiple sources significantly increased the mating success rate (one colony 39.1 ± 4.2% to four colonies 60.05 ± 5.7%, $P < 0.05$). Nonetheless, an increase in the number of gyne sources had no effect on the mating success rate (one colony 39.50 ± 4.9% to four colonies 43.52 ± 5.7%, $P > 0.05$). Mating latency was significantly more influenced by males and gynes from multiple colonies than by kin relationship, male number, and gyne number ($P < 0.05$). In conclusion, there is no evidence that the bumblebee *B. lantschouensis* can recognize kin relationships before mating. The presence of males and gynes from multiple colonies can influence mating latency. Moreover, males from multiple colonies can significantly enhance mating success, which has implications for bumblebee ecological conservation and artificial mass rearing.

Bumblebee / *Bombus lantschouensis* / Mating / Kin recognition / Artificial rearing

1. INTRODUCTION

Bumblebees, as pollinators of many greenhouse crops, are used as a strategy in agriculture that has become increasingly popular worldwide since the 1980s (Reade et al. 2014; Velthuis and Doorn 2006). The artificial mass rearing of bumblebees to meet the requirements of crop pollination has become an urgent need (Lecocq et al. 2016). The year-round rearing of bumblebees involves three critical processes: the rearing of colonies, the mating of queens, and the breaking of diapause (Velthuis and Doorn 2006). Mating is an important period in the artificial rearing of bumblebees. The physical and chemical attributes of males often influence their mating success (Amin et al. 2012; Zácek et al. 2009). As haplodiploid Hymenoptera, bumblebee individuals are males if they are homozygous at one or more sex-determining loci, and heterozygotes

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at this locus are females (Paxton et al. 2000). Therefore, bumblebees are particularly susceptible to the costs of inbreeding due to their single-locus complementary sex determination (SL-SCD) system (van Wilgenburg et al. 2006; Zayed and Packer 2005). Similar to the system in honeybees, haplodiploidy determines the sex of bumblebees, and fertilized eggs laid by mated gynes develop into adult diploid males if they are homozygous at a single sex locus (Paxton et al. 2000). The production of diploid males gradually increases when the gyne undergoes inbreeding. In a controlled environment, determining how to avoid inbreeding and promote the mating success of bumblebees is critical to obtain quality colonies over successive generations (Beekman and Veerman 1999). Therefore, it is important to understand the mechanism preventing in breeding between males and gynes.

Diploid males represent significant fitness costs, primarily through their inviability and sterility (El Agoze et al. 1994; Petters and Mettus 1980). In addition, diploid males deplete the worker force and reduce the number of reproductive females (Duchateau et al. 1994). Moreover, inbreeding has an effect on each stage of bumblebee year-round rearing. Whitehorn et al. (2009b) observed that sibling mating is associated with longer mating latency than nonsibling mating in gynes of *Bombus terrestris* and interpreted this observation as evidence for kin recognition for inbreeding avoidance. Relatively low survival in gynes mated with related males during diapause was also observed (U. Gerloff and Schmid-Hempel 2005). However, it was found that diapause survival did not differ between gynes mated with siblings and those mated with nonsibling males (Bortolotti et al. 2020). Inbreeding was also demonstrated to affect colony initiation, colony development, and queen fecundity (Gosterit 2016; Whitehorn et al. 2009a).

The ratio of gynes to males in the mating cage has important effects on successful mating and subsequent colony foundation activity. The mating propensity of *B. terrestris* was found to be significantly different depending on the ratio of gynes and males, and the highest mating propensities occurred at a gyne-to-male ratio of 1:2 under artificial conditions with different photoperiodic regimes (Kwon and Suh 2006). A higher mating percentage was found at a gyne-to-male ratio of 1:1.5 then at a ratio of 1:2 (Amin et al. 2010). Amin et al. (2012) found that mating latency gradually increased with the number of males via interference among them, while the mating duration was unaffected. Mating duration, which normally lasts 20–40 min, was found to be determined by males even though most sperm are transferred within the first 2 min (Amin et al. 2007, 2009; Baer et al. 2003; Duvoisin et al. 1999). Within experimental mating cages, the number of bumblebees gynes influences mating success by impacting the available space in the cage and number of males. The most suitable gyne number in a mating cage was found to be 30 for two bumblebee species, *Bombus lucorum* (cage size: 100 × 100 × 100 cm) and *Bombus ignitus* (cage size: 55 × 45 × 65 cm) (Wu et al. 2005; Yoon et al. 2007). Therefore, bumblebee species differ in their responses to mating cage size.

The Asian bumblebee *Bombus lantschouensis* is a polylectic and primitively eusocial bee that is widely distributed across medium- to high-elevation mountains and grasslands in northern China (An et al. 2014). It was found to have potential for artificial rearing because of its efficient pollination ability, colony size, and capacity for domestication (Zhang et al. 2020; Zhou et al. 2015). The effects of males and gynes on the mating success of this bumblebee have not been previously investigated.

In this study, we explored the mating behavior of the bumblebee *B. lantschouensis* in artificial mating cages. We aimed to answer two major questions: (1) Do gynes have the ability to recognize related and unrelated males? (2) Can males and gynes from multiple colonies affect mating behavior? Our aim was to verify whether a mating preference exists as a mechanism for inbreeding avoidance and whether males and gynes from multiple colonies can improve the mating success rate. The results from this study will provide guidance for bumblebee ecological conservation and artificial mass rearing.

2. MATERIALS AND METHODS

2.1. Bumblebees

Queens of the bumblebee *B. lantschouensis* were captured from Liancheng Natural Reserve of Gansu Province, China (E 102° 83′, N 36° 59′), in April 2019 and 2020. The queens were taken to the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, for captive breeding. One hundred and twenty colonies were established in an artificially regulated climate-controlled room at 27 ± 2 °C and 60 ± 5% relative humidity in continuous darkness. The bumblebees were provided with fresh frozen pollen and sugar solutions (1:1, w/v) ad libitum as a diet. Within 24 h after the emergence of sexual individuals in the colonies, these individuals were transferred to transparent plastic boxes (27 × 16 × 15 cm). We marked the gynes and males with the date of emergence and origin of the colony. A total of 984 gynes and 2500 males were obtained from 20 colonies. The boxes of gynes and males were placed in separate dark rooms and fed fresh pollen and sugar solutions until use.

Gynes of 7 days of age and males of 10–15 days of age were used to determine the mating latency, mating duration, and the mating success rate in flight cages (95 × 95 × 95 cm). Medium- and large-sized gynes and males were used to avoid the influence of small individuals (Amin et al. 2012; Bogo et al. 2017). In addition, we collected males from the colony before competition began to ensure that all the males were from the queen and not from workers. We marked gynes and males with different colors according to the origin of the colony. The ratio of gynes to males was 1:2 in all experiments. The mating tests were performed under natural light during 08:00 to 11:00 am mating periods on sunny days.

2.2. Mating experiments

The mating observations began when the gynes and males were released into the mating cages (Figure 1). The mating observation period was limited to 2 h from the release of gynes. Mating latency was calculated by measuring the time between the introduction of males and gynes into the mating cage and the initiation of copulation (Amin et al. 2012). Each mating pair was removed from their respective mating cages when mating began. The mating duration was determined by observing a mating pair in a small transparent plastic box (11 × 7 × 6.5 cm). We calculated the mating success rate as the number of mating pairs divided by the number of released gynes.

Figure 1. Bumblebee (*Bombus lantschouensis*) mating experiment on a sunny day. **A** Two mating cages (95×95×95 cm). **B** Copulation of a gyne and male.

2.2.1. *Experiment 1: effects of related and unrelated males on mating*

To evaluate the effects of related and unrelated males on queen mating, mature gynes were placed in separate cages with their brothers or unrelated males from randomly selected colonies. In addition, males from the same colony and different colonies were placed into the same mating cages as mixed mating groups (gynes were selected from same colony) (Figure S1: Experiment 1). To better distinguish the related and unrelated males, they were marked with different colors on the thorax. Mating latency, mating duration, and the mating success rate were recorded in the different mating groups. The numbers of gynes in each mating cage ranged from 6 to 20, which was determined by the numbers of emerging gynes and males. A total of 71 experimental mating groups (26 related cages (210 gynes), 22 unrelated cages (154 gynes), and 23 mixed cages (172 gynes)) were established.

2.2.2. *Experiment 2: effect of males from multiple colonies on mating*

To explore the effect of males from multiple colonies on mating success, the gynes in each mating cage were derived from the same colony, with their number ranging between 8 and 20. Males from one, two, three, and four colonies were released into the mating cages (Figure S1: Experiment 2). To effectively distinguish the origin of the males, they were marked with different colors on the thorax. The proportions of males in each treatment group were equal. Mating latency, mating duration, and the mating success rate were recorded across the four treatments. Each treatment was replicated five times, for a total of 20 treatments (225 gynes in total).

2.2.3. *Experiment 3: effect of gynes from multiple colonies on mating*

To observe the effect of gynes from multiple colonies on mating success, males from the same colony were mated with gynes from multiple colonies (Figure S1: Experiment 3). To distinguish the gynes on the basis of their origin, they were marked on the thorax with different colors. The proportions of gynes were consistent between treatment groups. Mating latency, mating duration, and the mating success rate were recorded. Each treatment was replicated five times, for a total of 20 treatments (223 gynes in total).

2.2.4. *Statistical analysis*

Data were first checked for normality (Kolmogorov–Smirnov and Shapiro–Wilk tests). When the data did not fit a normal distribution, we used nonparametric statistical tests. Mating percentages were transformed using the arcsine-square-root transformation. Differences in mating latency, mating duration, and the mating success rate among the three mating types (related, unrelated, and mixed) were analyzed with the nonparametric Kruskal–Wallis H test. Related and unrelated males mated with gynes in mixed groups were compared with the Mann–Whitney U test. The effects of males and gynes from multiple colonies on mating success were tested with one-way ANOVA and Duncan's multiple range test. Statistical analyses were performed with R project software version 4.0.4 (https://cran.r-project.org).

3. RESULTS

3.1. Effects of related and unrelated males on mating

A total of 71 mating groups were evaluated, and the results showed that mating latency significantly differed among the related, unrelated, and mixed groups ($H = 40.486$, $P < 0.05$) (Figure 2A). The average mating latency was 42.9 ± 3.77 min for related groups, 24.2 ± 2.53 min for unrelated groups, and 16.8 ± 1.8 min for mixed groups. Mating duration did not significantly differ among the three groups ($H = 4.457$, $P > 0.05$) (Figure 2B). In terms of mating success, the

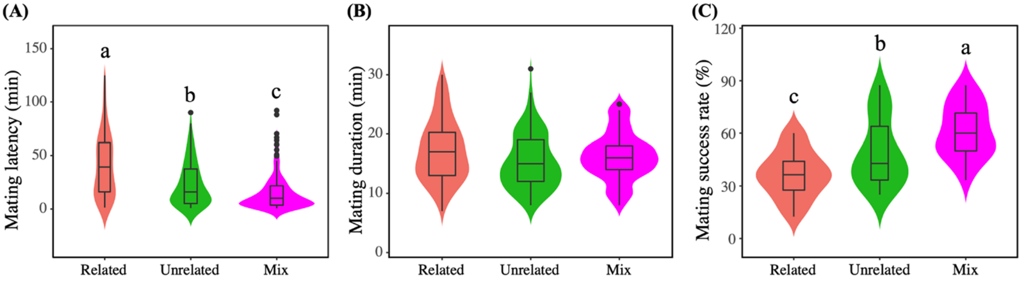

Figure 2. Effects of related, unrelated, and mixed related and unrelated males on the mating behavior of the bumblebee *B. lantschouensis*. **A** Difference in mating latency among the different mating groups. **B** Difference in mating duration among the different mating groups. **C** Difference in the mating success rate among the different mating groups. Violin plots show these results; the violin shape presents the distribution of the values, with a box plot inside. The black line in the center indicates the median, the top of the black box represents the upper quartile, and the bottom of the black box represents the lower quartile. Different letters denote significant differences at $\alpha = 0.05$.

mating groups significantly differed ($H = 20.892$, $P < 0.05$) (Figure 2C). However, no difference in mating success was found between related and unrelated males mated with gynes in the mixed groups (Table I, mating latency ($U = 1332$, $P > 0.05$), mating duration ($U = 1348$, $P > 0.05$), and mating success ($U = 250$, $P > 0.05$)).

3.2. Effect of males from multiple colonies on mating success

Males from multiple colonies had no significant effect on mating latency or mating duration when the four different-source male groups were evaluated (ANOVA, $P > 0.05$) (Figure 3A, B). However, males from multiple colonies had a significant effect on mating success (ANOVA, $P < 0.05$) (Figure 3C). Males originating from three and four colonies had a higher mating success rate (57.6 ± 4.76% and 60.05 ± 5.73%) than males originating from one or two colonies (39.1 ± 4.30% and 39.5 ± 3.72%). The mating success rate did not significantly differ between males originating from three and four colonies ($P > 0.05$).

3.3. Effect of gynes from multiple colonies on mating success

There was no significant difference in mating latency or mating success among the four treatment groups (ANOVA, $P > 0.05$) (Figure 4A,

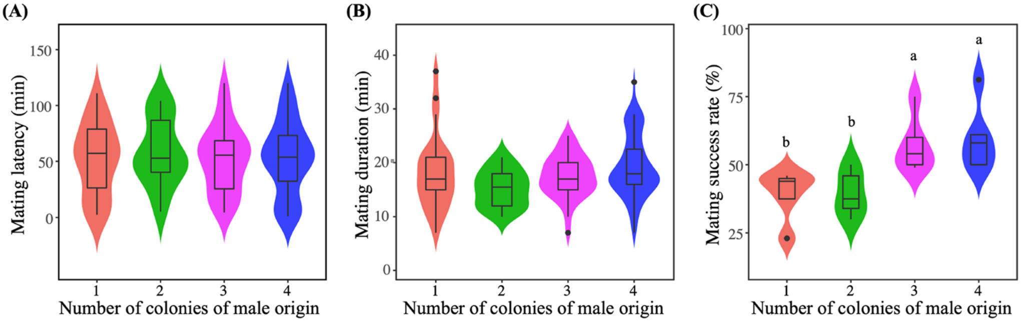

Figure 3. Effects of males from different numbers of colonies on the mating behavior of the bumblebee *B. lantschouensis*. **A** Effect of the number of colonies of male origin on mating latency. **B** Effect of the number of colonies of male origin on mating duration. **C** Effect of the number of colonies of male origin on the mating success rate. Violin plots show these results; the violin shape presents the distribution of the values, with a box plot inside. The black line in the center indicates the median, the top of the black box represents the upper quartile, and the bottom of the black box represents the lower quartile. Different letters denote significant differences at $\alpha = 0.05$.

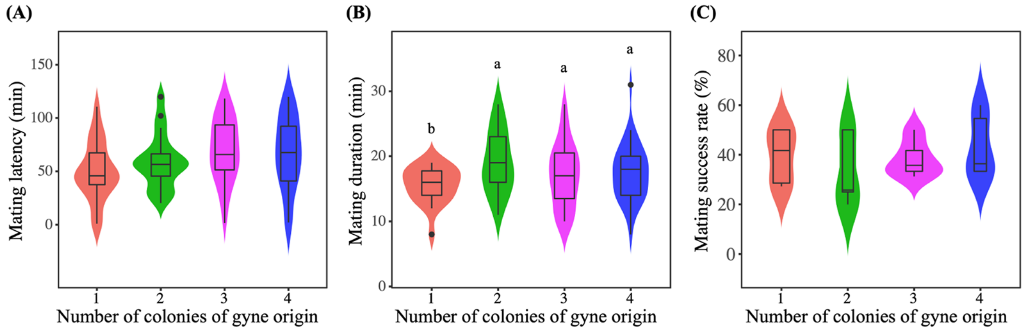

Figure 4. Effect of gynes from different numbers of colonies on the mating behavior of the bumblebee *B. lantschouensis*. **A** Effect of the number of colonies of gyne origin on mating latency. **B** Effect of the number of colonies of gyne origin on mating duration. **C** Effect of the number of colonies of gyne origin on the mating success rate. Violin plots are shown; the violin shape presents the distribution of the values, with a box plot inside. The black line in the center indicates the median, the top of the black box represents the upper quartile, and the bottom of the black box represents the lower quartile. Different letters denote significant differences at $\alpha = 0.05$.

C). However, gynes from one colony had a shorter mating duration (14.77 ± 0.71 min) than gynes from multiple colonies (the duration for two to four colonies was 19.1 ± 1.19 min, 17.1 ± 0.97 min and 17.6 ± 0.97 min, respectively) (Figure 4B).

3.4. Effects of different factors on the response variables

A nonparametric Kruskal–Wallis H test showed the effects of different factors (kin relationship, multiple sources of males and gynes,

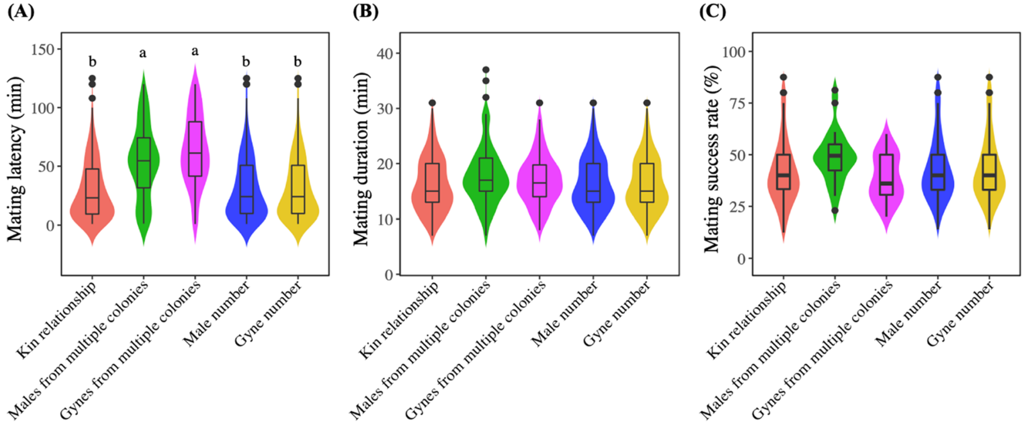

Figure 5. Effect of different factors (kin relationship, males from multiple colonies, gynes from multiple colonies, male number, and gyne number) on the mating behavior of the bumblebee *B. lantschouensis*. **A** Effect of kin relationship, males from multiple colonies, gynes from multiple colonies, male number, and gyne number on mating latency. **B** Effect of kin relationship, males from multiple colonies, gynes from multiple colonies, male number, and gyne number on mating duration. **C** Effect of kin relationship, males from multiple colonies, gynes from multiple colonies, male number, and gyne number on the mating success rate. Violin plots are shown; the violin shape presents the distribution of the values, with a box plot inside. The black line in the center indicates the median, the top of the black box represents the upper quartile, and the bottom of the black box represents the lower quartile. Different letters denote significant differences at $\alpha = 0.05$.

and number of males and gynes) on the response variables (mating latency, mating duration, and mating success) ($H = 82.343$, $P < 0.05$). Males and gynes from multiple colonies had a more significant influence on mating latency than kin relationship, male number, and gyne number ($P < 0.05$) (Figure 5A). However, there were no significant effects of in kin relationship, males and gynes from multiple colonies, male number, and gyne number on mating duration and mating success ($P > 0.05$) (Figure 5B, C).

4. DISCUSSION

Mating is an important process affecting the sustainability of bumblebee factory rearing. The prevention of inbreeding and increasing mating success are crucial for the establishment and development of bumblebee colonies. In this study, we used the indigenous bumblebee *B. lantschouensis* to test the mating interaction of males and gynes. The results indicate that the gynes of this species have no kin-recognition ability, at least of individuals (males) from the same locality under controlled conditions. Furthermore, the presence of males from multiple colonies is an important mechanism influencing mating success in this species. To the best of our knowledge, this is the first systematic study of mating between gynes and males of *B. lantschouensis*. The findings will help to guide the artificial rearing of this native bumblebee.

Mating latency is an index used to reflect mating propensity. A previous study showed a shorter mating latency in outbred groups than in inbred groups of the bumblebee *B. terrestris* (Whitehorn et al. 2009b). Our study further demonstrated that related groups of the native bumblebee *B. lantschouensis* have a longer mating latency (42.88 ± 3.8 min) than unrelated groups (24.15 ± 2.5 min) (Figure 2A). Furthermore, the mating latency of the mixed groups was shorter than that of the related and unrelated groups. This result may have been caused by competition between related and unrelated males. We found no significant difference in mating latency between related and unrelated males (Table I). This result was consistent with the observation of Bogo et al. (2018b). Therefore, mating latency is not directly correlated with mating preference in this bumblebee, and gynes from the same colonies apparently cannot distinguish between related and unrelated males. However, mating latency may be affected by interference among males, in which multiple males attempted to mate with the gynes simultaneously (Amin et al. 2012). However, the effect of male-male competition on the mating latency of the bumblebee *B. lantschouensis* needs to be tested in the future.

Mating latency may differ among bumblebee species. Our results show that mating latency in the bumblebee *B. lantschouensis* in three different mating groups (related, unrelated, and mixed) was longer than that in the bumblebee *B. terrestris* (mating latency time is less than 20 min) (Amin et al. 2012; Bogo et al. 2018b). The most likely explanations for this result are differences in the sex pheromone contents of different species, the localities of the experiments, temperature, the ages of individuals, and luminosity (Amin et al. 2007, 2010; Bogo et al. 2018b).

There was no evidence for the existence of an inbreeding avoidance system in the bumblebee *B. lantschouensis* in the artificial mating cages. However, different systems used to prevent inbreeding have been found in bumblebees under natural conditions. Gynes and males of the

Table I Mating preferences of related and unrelated males when mating in mixed groups

Index	Related	Unrelated	*U*	*P* value
Mating latency (min)	18.98 ± 2.8	14.59 ± 2.3	1332	0.17
Mating duration (min)	16.73 ± 0.6	15.77 ± 0.5	1348	0.199
Mating success rate (%)	30.63 ± 3.1	30.73 ± 2.4	250	0.749

bumblebees *B. frigidus* and *B. bifarius* have been found to have the ability to recognize nestmates, while the bumblebees *B. californicus* and *B. rufocinctus* do not have an inbreeding avoidance system (Foster 1992). This difference could be due to variation in precopulatory behavior: *B. californicus* and *B. rufocinctus* show nest surveillance behavior to find females and do not survey their own nest, while the bumblebee species *B. frigidus* and *B. bifarius* exhibit patrolling behavior. The latter two species may therefore frequently encounter young queens in their own nest, and an inbreeding-avoidance system is thus advantageous. Under artificial rearing, an inbreeding mating avoidance mechanism may exist in bumblebee through changes in the timing of gyne and male emergence and regulation of the sex ratio (Bogo et al. 2018a).

Mating duration can be used by insects to adjust their mating investment in relation to their relatedness (Tabadkani et al. 2012). In bumblebees, a long copulation duration is used by males to manipulate paternity: it promotes effective sperm transfer and produces a so-called “mating plug” secretion, which is applied to the female genital tract to prevent young queens from remating (Baer et al. 2001, 2000; Brown and Baer 2005). Previous research suggests that there is no significant difference in copulation time between related and unrelated bumblebees (Bogo et al. 2018b). Our results also showed no difference between the related and unrelated groups in the bumblebee *B. lantschouensis*. The average mating duration of the bumblebee *B. lantschouensis* was less than 20 min. Research has indicated that the mean mating duration of the bumblebee *B. terrestris* is more than 30 min (Amin et al. 2009; Brown et al. 2002). The reasons for these observations may be related to the speed of sperm and mating plug migration in the different species. Additionally, the number of male individuals did not affect the mating duration (Amin et al. 2012). This was also reflected in our observation that the number of males did not affect the mating duration ($P > 0.05$). However, the source of gynes influenced mating duration. Gynes from one colony mated with unrelated males had a shorter mating duration than those from multiple colonies. The reasons for these observations may be that gynes from multiple colonies favor sperm filling of the queen’s spermatheca, which is affected by a longer mating duration (Brown and Baer 2005). Further evidence is needed to validate the hypothesis.

The successful mating of bumblebees is influenced by many factors, including male choice, female choice, male courtship behavior, female response to this courtship, and female reproductive status (Patrick 1983). In our experiments, we found that the mating success rate of unrelated groups was significantly higher than that of related groups. The mating success rate of the mixed groups was 60%, which could be some sort of female strategy dependent on environmental conditions (Figure 1C). On the basis of the mixed groups, we further demonstrated no mating preference between related and unrelated groups. No mating preference for related or unrelated partners was also found in the bumblebee *B. terrestris* (Bogo et al. 2018b).

Previous research has shown that the number of male and gyne individuals influences mating success (Amin et al. 2010; Kwon and Suh 2006; Wu et al. 2005; Yoon et al. 2007). However, few studies have focused on the effects of different sources of males and gynes on mating success. Our results suggest that males from multiple colonies, in terms of the number of colonies of origin, can significantly enhance the mating success rate. However, gynes from multiple colonies has no influence on the mating success rate. These results provide some technical guidance and a theoretical basis for indigenous bumblebee factory rearing.

5. CONCLUSIONS

We conclude that males originating from different colonies have an overall effect on the mating success of the studied species under artificial and controlled conditions. Our observations demonstrate that gynes cannot recognize related males, which may be an important consideration in the artificial rearing of bumblebees (Bogo et al. 2018b). As these are primitively eusocial bees, no avoidance mechanism would significantly reduce fitness in the offspring. In artificial mating cages, males should originate from more than three colonies to increase mating success and improve the fitness of offspring. Furthermore, we observed longer mating durations in cages in which gynes were from multiple colonies. Interestingly, gynes may benefit from prolonged mating with a greater quantity of transferred sperm (Brown and Baer 2005). Therefore, we suggest that the gynes in a mating cage be from more than two colonies. Despite our assessment of the interactions between males and queens, future work should include an evaluation of the effects of environmental factors, cage size, and male maturity on queen mating and the effectiveness of queen insemination under the conditions investigated in our study. Our understanding of mating aspects in males and gynes has the potential contribute to bumblebee ecological conservation and artificial mass rearing.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

QZ, YJ, and JH conceived and designed the experiments; QZ, JL, and JW performed the experiments; QZ, GD, and JH contributed analytical tools and performed data analysis; and QZ and JH wrote and edited the paper. All authors participated in the revisions of the paper and approved the final manuscript.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interest.

REFERENCES

Amin M., Bussiere L. & Goulson D. (2012). Effects of male age and size on mating success in the bumblebee *Bombus terrestris*. *Journal of Insect Behavior*, *25*(4), 362-374. https://doi.org/10.1007/s10905-011-9306-4

Amin M.R., Kwon Y.J. & Suh S.J. (2007). Photoperiodic influence on the body mass of bumblebee, *Bombus terrestris* and its copulation duration. *Journal of Applied Entomology*, *131*(8), 537-541. https://doi.org/10.1111/j.1439-0418.2007.01162.x

Amin M.R., Than K.K. & Kwon Y.J. (2010). Mating status of bumblebees, *Bombus terrestris* (Hymenoptera: Apidae) with notes on ambient temperature, age and virginity. *Applied Entomology and Zoology*, *45*(3), 363-367. https://doi.org/10.1303/aez.2010.363

Amin M.R., Than K.K. & Kwon Y.J. (2009). Copulation duration of bumblebee *Bombus terrestris* (Hymenoptera: Apidae): Impacts on polyandry and colony parameters. *Journal of Asia-Pacific Entomology*, *12*(3), 141-144. https://doi.org/10.1016/j.aspen.2009.02.010

An J., Huang J., Shao Y., Zhang S., Wang B., Liu X., Wu J. & Williams P.H. (2014). The bumblebees of North China (Apidae, *Bombus* Latreille). *Zootaxa*, *3830*(1), 1–89. https://doi.org/10.11646/zootaxa.3830.1.1

Baer B., Inwood M. & Schmid-Hempel P. (2001). A nonspecific fatty acid within the bumblebee mating plug prevents females from remating. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(7), 3926-3928. https://doi.org/10.1073/pnas.061027998

Baer B., Maile R., Schmid-Hempel P., Morgan E.D. & Jones G.R. (2000). Chemistry of a mating plug in bumblebees. *Journal of Chemical Ecology*, *26*(8), 1869-1875. https://doi.org/10.1023/a:1005596707591

Baer B., Schmid-Hempel P., Høeg J.T. & Boomsma J.J. (2003). Sperm length, sperm storage and mating system characteristics in bumblebees. *Insectes Sociaux*, *50*(2), 101-108. https://doi.org/10.1007/s00040-003-0641-0

Beekman M., van P. & Veerman A. (1999). Selection for non-diapause in the bumblebee *Bombus terrestris*, with notes on the effect of inbreeding. *Entomologia Experimentalis et Applicata*, *93*, 69-75. https://doi.org/10.1046/j.1570-7458.1999.00563.x

Bogo G., de Manincor N., Fisogni A., Galloni M., Bortolotti L (2017) Effects of queen mating status, pre-diapause weight and pupae's sex on colony initiation in small-scale rearing of *Bombus terrestris*. *Apidologie, 48,*845-854. https://doi.org/10.1007/s13592-017-0529-z

Bogo G., de Manincor N., de Fisogni A., Galloni M., Zavatta L., Bortolotti L (2018a) Different reproductive strategies and their possible relation to inbreeding risk in the bumble bee *Bombus terrestris*. *Insectes Sociaux, 65*(2), 289-295. https://doi.org/10.1007/s00040-018-0611-1

Bogo G., Manincor N., de Fisogni A., Galloni M., Zavatta L., Bortolotti L (2018b) No evidence for an inbreeding avoidance system in the bumble bee *Bombus terrestris*. *Apidologie, 49*(4), 1-11. https://doi.org/10.1007/s13592-018-0575-1

Bortolotti L., Fiorillo F., Zavatta L., De Rogatis A. & Bogo G. (2020). Influence of inbreeding in the early stages of artificially reared colonies of *Bombus terrestris*. *Journal of Applied Entomology*, *144*(7), 637-646. https://doi.org/10.1111/jen.12765

Brown M. & Baer B. (2005). The evolutionary significance of long copulation duration in bumble bees. *Apidologie*, *36*(2), 157-167. https://doi.org/10.1051/apido:2005008

Brown M.J.F., Baer B., Schmid-Hempel R. & Schmid-Hempel P. (2002). Dynamics of multiple-mating in the bumble bee *Bombus hypnorum*. *Insectes Sociaux*, *49*(4), 315-319. https://doi.org/10.1007/pl00012654

Duchateau M.J., Hoshiba H. & Velthuis H.H.W. (1994). Diploid males in the bumble bee *Bombus terrestris*. *Entomologia Experimentalis et Applicata*, *71*(3), 263-269. https://doi.org/10.1111/j.1570-7458.1994.tb01793.x

Duvoisin N., Baer B. & Schmid-Hempel P. (1999). Sperm transfer and male competition in a bumblebee. *Animal Behaviour*, *58*(4), 743-749. https://doi.org/10.1006/anbe.1999.1196

El Agoze M., Drezen J.M., Renault S. & Periquet G. (1994). Analysis of the reproductive potential of diploid males in the wasp *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Bulletin of Entomological Research*, *84*(2), 213-218. https://doi.org/10.1017/S0007485300039717

Foster R.L. (1992). Nestmate recognition as an inbreeding avoidance mechanism in bumble bees (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, *65*(3), 238-243.

Gosterit A. (2016). Adverse effects of inbreeding on colony foundation success in bumblebees, *Bombus terrestris* (Hymenoptera: Apidae). *Applied Entomology and Zoology*, *51*(4), 521-526. https://doi.org/10.1007/s13355-016-0427-2

Kwon Y. & Suh S. (2006). Mating propensity of *Bombus terrestris* reared in different photoperiodic regimes. *Apidologie*, *37*(10), 679-686. https://doi.org/10.1051/apido:2006047

Lecocq T., Rasmont P., Harpke A. & Schweiger O. (2016). Improving international trade regulation by considering intraspecific variation for invasion risk assessment of commercially traded species: the *Bombus terrestris* case. *Conservation Letters*, *9*(4), 281 - 289. https://doi.org/10.1111/conl.12215

Patrick B. (1983). *Mate choice*. New York: Cambridge University Press.

Paxton R., Thorén P., Gyllenstrand N. & TengÖ J. (2000). Microsatellite DNA analysis reveals low diploid male production in a communal bee with inbreeding. *Biological Journal of the Linnean Society*, *69*, 483-502. https://doi.org/10.1111/j.1095-8312.2000.tb01220.x

Petters R.M. & Mettus R.V. (1980). Decreased diploid male viability in the parasitic wasp, *Bracon hebetor*. *Journal of Heredity*, *71*(5), 353-356. https://doi.org/10.1093/oxfordjournals.jhered.a109385

Reade C., Goka K., Thorp R., Mitsuhata M. & Wasbauer M. (2014). CSR, Biodiversity and Japan's stakeholder approach to the global bumble bee trade. *Journal of Corporate Citizenship*, *2014*(56), 53-66. https://doi.org/10.9774/gleaf.4700.2014.de.00006

Tabadkani S., Nowzari J. & Lihoreau M. (2012). Inbreeding and the evolution of sociality in arthropods. *Die Naturwissenschaften*, *99*, 779-788. https://doi.org/10.1007/s00114-012-0961-x

U. Gerloff C. & Schmid-Hempel P. (2005). Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera: Apidae). *Oikos*, *111*(1), 67-80. https://doi.org/10.1111/j.0030-1299.2005.13980.x

van Wilgenburg E., Driessen G. & Beukeboom L. (2006). Single locus complementary sex determination in Hymenoptera: An "unintelligent" design? *Frontiers in Zoology*, *3*, 1. https://doi.org/10.1186/1742-9994-3-1

Velthuis H.H.W. & Doorn A.v. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*, *37*(4), 421-451. https://doi.org/10.1051/apido:2006019

Whitehorn PR., Tinsley MC., Brown MJ., Darvill B., Goulson D (2009a) Impacts of inbreeding on bumblebee colony fitness under field conditions. *BMC Evolutionary Biology, 9*,152. https://doi.org/10.1186/1471-2148-9-152

Whitehorn PR., Tinsley MC., Goulson D (2009b) Kin recognition and inbreeding reluctance in bumblebees. *Apidologie*, 40(6), 627-633. https://doi.org/10.1051/apido/2009050

Wu J., Peng W., An J., Guo Z., Tong Y. & Li J. (2005). Rearing techniques of *Bombus lucorum*. *Chinese Bulletin of Entomology*, *42*(6), 717-720.

Yoon H.J., Kim S.E., Lee K.Y., Lee S.B. & Park I.G. (2007). Mating conditions favorable for improving mating rate of the bumblebee, *Bombus ignitus*. *International Journal of Industrial Entomology*, *15*(2), 107-114.

Zácek P., Kalinová B., Sobotník J., Hovorka O., Ptácek V., Coppée A., Verheggen F. & Valterová I. (2009). Comparison of age-dependent quantitative changes in the male labial gland secretion of *Bombus terrestris* and *Bombus lucorum*. *Journal of Chemical Ecology*, *35*(6), 698-705. https://doi.org/10.1007/s10886-009-9650-4

Zayed A. & Packer L. (2005). Complementary sex determination substantially increases extinction proneness of haplodiploid populations. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(30), 10742-10746. https://doi.org/10.1073/pnas.0502271102

Zhang H., Shan S., Gu S., Huang X., Li Z., Khashaveh A. & Zhang Y. (2020). Prior experience with food reward influences the behavioral responses of the honeybee *Apis mellifera* and the bumblebee *Bombus lantschouensis* to tomato floral scent. *Insects*, *11*(12), 11120884. https://doi.org/10/gh2z89

Zhou Z., Zhang H., Liang C., Zou Y., Dong J., Yuan X., Huang J. & An J. (2015). Foraging preference of the honeybee *Apis mellifera* and the bumblebee *Bombus lantschouensis* (Hymenoptera: Apidae) in peach greenhouse. *Acta Entomologica Sinica*, *58*(12), 1315–1321. https://doi.org/10.16380/j.kcxb.2015.12.007