

ORIGINAL RESEARCH ARTICLE

Comparison of the colony development of two native bumblebee species *Bombus ignitus* and *Bombus lucorum* as candidates for commercial pollination in China.

Jilian Li[1], Jie Wu[2*], Wanzhi Cai[1], Wenjun Peng[2], Jiandong An[2], Jiaxing Huang[2].
[1]Department of Entomology, China Agricultural University, Yuanmingyuan West Road, Beijing 100094, China
[2]Institute of Apicultural Research, Chinese Academy of Agricultural Science, Xiangshan, Beijing 100093, China.

Received 4 August 2007, accepted subject to revision 9 October 2007, accepted for publication 24 October 2007.

*Corresponding author. Email: bumblebeeljl@hotmail.com

Summary

Studies of the colony development of the Chinese bumble bees *Bombus ignitus* and B*ombus lucorum f*ound that there were four oviposition phases. The average number of workers produced per colony of *B. ignitus* and *B. lucorum* was 107 and 104 respectively, there being no significant difference between them ($p>0.05$). Colonies produced daughter queens in Phase 4 and the average number of new queens produced per colony of *B. ignitus* was smaller than that of *B. lucorum* ($p<0.05$). The proportion of *B. ignitus* and *B. lucorum* queens which established nests was on average 89% and 84% respectively ($p>0.05$). The average time to the emergence of the first workers in *B. lucorum* was longer than in *B. ignitus*, and this was significantly different ($p<0.05$), but the percentage of successful colony production of *B. lucorum* was lower than that of *B. ignitus*, and the number of workers which had emerged at above 30 days after first the oviposition in *B. lucorum* was more than in *B. ignitus*.

Comparación del desarrollo de la colonia de dos especies nativas de abejorros *Bombus ignitus* y *Bombus lucorum* como candidatos para la polinización comercial en China.

Resumen
Los estudios del desarrollo de la colonia en los abejorros chinos *Bombus ignitus* y *Bombus lucorum* comprobaron que hay cuatro fases de oviposición. El número medio de obreras producidas por colonia de *B. ignitus* y *B. lucorum* fue de 107 y 104 respectivamente, sin existir diferencias significativas entre ellos (p> 0,05). Las colonias produjeron reinas hijas en la fase 4 y el número medio de nuevas reinas producidas por colonia de *B. ignitus* fue menor que el de *B. lucorum* (p <0,05). La proporción de reinas que establecieron colmenas en *B. ignitus* y *B. lucorum* fue, en promedio, del 89% y 84%, respectivamente (p> 0,05). El tiempo medio para la aparición de las primeras obreras fue mayor en *B. lucorum* que en *B. ignitus*, y éste fue significativamente diferente (p <0,05), pero el porcentaje de éxito en la producción de la colonia fue menor en *B. lucorum* que en *B. ignitus*, y el número de obreras que surgieron después de los 30 días tras la primera oviposición fue mayor en *B. lucorum* que en *B. ignitus*.

Keywords: : Chinese bumble bees, *Bombus ignitus, Bombus lucorum*, colony development, oviposition, queen, worker, reproduction, commercial pollination, nesting rate.

Introduction

Bumble bees are well known as important pollinators in their natural habitat, and their potential economic value has been recognized for a long time. Their tongues are generally longer than those of honey bees, so they are better at pollinating flowers with deep corollas. Furthermore, the dense hairs on the bodies of bumble bees allow efficient pollen transfer from flower to flower, and they can also sonicate ("buzz pollinate") wild flowers and crops (including tomato) whose flowers shed pollen through apical pores. Because of this, their use to pollinate various glasshouse crops has been developed widely and industrial rearing techniques have been developed extensively in many countries (Velthuis *et al*., 2006). Techniques have been developed to rear colonies in captivity (Sladen, 1912; Pouvreau, 1976; Van Honk and Hogewek, 1981; Röseler, 1985; Duchateau and Velthuis, 1988; Van Doorn and Chrambach, 1989; Pfiidal and Hofbauer, 1998).

There are many species of bumble bees which have been reared and used for commercial crop pollination in the world, such as *Bombus terrestris, Bombus lucorum, Bombus occidentalis*, *Bombus ignitus*, and *Bombus impatiens*. Of these, *B. terrestris* has the most prominent role and has already been imported into many countries such as New Zealand, Japan, U.S.A., Canada and China, for commercial crop pollination. Due to its wide distribution, large colony production, and adaptability to artificial conditions, populations of *B. terrestris* have become naturalized and have expanded their ranges, exploiting floral resources and potentially competing with other pollinators, including native bees (Velthuis *et al*., 2006). Concern continues to grow about the effects of invasive *B. terrestris* on native pollinators and their established relationships with local plants in native ecosystems. In new environments, *B. terrestris* may threaten populations of native pollinators by introducing new diseases, displacing natives through competition for resources, or disrupting genetic adaptations by hybridizing with native species. Thus both the U.S.A. and Canada prohibit the importation of *B. terrestris*, and American growers use commercialized native bumble bees (*B. impatiens* and *B. occidentalis*) for crop pollination. In Japan, two native bumble bees, *Bombus hypocrita* and *B. ignitus* are promising candidates for crop pollination, and laboratory colonies have been successfully reared from post-hibernating queens. Native bumble bees have been reared and then for tomato pollination, no significant different in pollination efficiency between them and *B. terrestris* having been found (Asada and Ono, 1996; 1997).

The Chinese native bumble bees, *B. ignitus, B. lucorum* and *Bombus patagiatus* are promising candidates for crop pollination, and have been reared in the laboratory from post-hibernating queens for scientific studies of social behaviour, biology, and pollination since 1996, and remarkable progress has been made (Liang *et al*., 1999; Peng *et al*., 2003; Guo *et al*., 2003; Wu *et al*., 2005; Li *et al*., 2006). *Bombus eximius* and *Bombus sonani* have also been collected in the An-Mar mountain areas of central Taiwan and reared successfully under laboratory condition (Chiang *et al*., 2006). Chinese farmers now use several hundred native bumble bee colonies per farm for the pollination of glasshouse tomato, pimiento, and Chinese watermelon. For the above reasons, we have examined the differences in colony development of *B. lucorum* and *B. ignitus* in China, in order to select the better species for crop pollination.

Materials and Methods

Laboratory rearing

Colonies of *B. ignitus* and *B. lucorum* were reared in the laboratory from field collected queens in early May (2004-2006) in Beijing and identified by Yao Jian from the Institute of Zoology of the Chinese Academy Sciences. A dark (red lamp) rearing room was climate controlled to 28 – 29°C and 60-65% RH. Colonies were fed with 50% sucrose solution and pollen collected from honey bees using pollen traps and then frozen at -20°C. The newly emerged queens and males were collected and transferred to new boxes. The ages of these queens and males after emergence were recorded by color codes on the thorax in order to determine their ages for mating. Queens aged 5–10 days after emergence and males aged 6-11days were transferred to a flight cage (80 × 80 × 80 cm) for mating. Caged queens and males were kept at 24–25°C and fed on 50% sucrose solution. During the course of the experiments, pollen from rape (*Brassica campestris L*.) and apricot (*Prunus armenica L*.) was used. The pollen was replaced every other day. Sucrose solution was prepared in 1:1 proportion by weight, provided in vertical feeders and replaced every other day.

Monitoring Colony Development

During 2004, 2005 and 2006, 55, 45 and 50 *B. ignitus* and 65, 55 and 80 *B. lucorum* queens were used, respectively. Of these, 40 colonies of each species were selected at random. Duchateau (1991) noted that *B. terrestris* queens had a fixed egg laying pattern, consisting of four phases: Phase 1: first brood from a few egg cells; Phase 2: second brood cells constructed on top of first brood cells at pupation of latter; Phase 3: continuous production of brood cells (queen switches abruptly from laying diploid eggs to laying haploid eggs and phase ends with onset of competition); Phase 4: phase after onset of competition. We observed and recorded the number of egg laying queens, the start date of the first brood from a few egg cells and second brood cells constructed on top of first brood cells at pupation of the latter, the start date of first brood from a few egg cells each phase, and then counted the duration of each of these phases. We also recorded the number of workers and new queens per colony, the rate of nesting (The ratio of number of egg laying queens to the number of queens), the successful copulation rate of the new queens (the ratio of number of successful copulation queens to the number of new queens).

In order to explore the colony development, we monitored colonies for signs of competition and for adult gyne and male emergence. The onset of the competition was identified when one or more of the following events were observed: 1. worker oviposition; 2. egg eating (oophagy); 3. clear signs of egg cup destruction; and 4. two or more open egg cups. Queens typically construct one egg cup at a time, so the occurrence of two open egg cups is a sign that workers are also constructing egg cups or that an already sealed cup has been destroyed (Duchateau *et al*., 1988; Bloch *et al*., 1999).

Results were verified by one-way analysis of variance (ANOVA). If not indicated otherwise, values were given as means and S.D. All analyses were performed with SAS 6.12.

Table 1. The duration of each phase in *B. ignitus* and *B. lucorum* given as means and S.D. from 2004 to 2006, and verified by one-way analysis of variance (ANOVA). Dates followed by the same letters indicate no difference at p>0.05 level; the different letters indicate significant difference at p<0.05 level.

Species	**n**	**Phase 1 (days)**	**Phase 2 (days)**	**Phase 3 (days)**	**Phase 4 (days)**
B. ignitus	40	11.68 ± 2.03 a	14.50 ±1.84 a	40.78 ±2.44 a	46.78 ±1.40 a
B. lucorum	40	11.40 ± 2.25 a	13.83 ±1.57 a	30.35 ±3.53 b	49.10 ±1.15 b

Table 2. Comparison of worker reproduction and new queen production in colonies of *B. ignitus* and *B. lucorum* initiated by field collected queens reared in the laboratory. The number of workers produced per colony and the number of new queens produced per colony are given as means and S.D. from 2004 to 2006, and verified by one-way analysis of variance (ANOVA). Dates followed by the same letters indicate no difference at p>0.05 level; the different letters indicate significant difference at p<0.05 level.

Species	**Number of colonies**	**Number of workers per colony**			**Number of new queens per colony**		
		mean ±SD	Min.	Max.	mean ±SD	Min.	Max.
B. ignitus	40	107.03 ± 46.99a	19	240	55.10 ±39.86a	3	155
B. lucorum	40	104.55 ± 48.33a	19	202	121.48 ± 86.03b	10	431

Table 3. Nesting rate of field collected queens reared in the laboratory. Dates followed by the same letters indicate no difference at p>0.05 level; the different letters indicate significant difference at p<0.05 level.

Species	**Year**	**Number of queens (a)**	**Number of egg-laying queens(b)**	**Nesting rate (%)(b/a×100)**
B. ignitus	2004	55	46	83.64
	2005	45	40	88.89
	2006	50	40	80.00
	Total	**150**	**126**	**84.00a**
B. lucorum	2004	65	60	92.31
	2005	55	48	87.27
	2006	80	70	87.50
	Total	**200**	**178**	**89.00a**

Table 4. The time to the emergence of the first workers in *B. ignitus* and *B. lucorum* reared in the laboratory given as means and S.D. from 2004 to 2006, and verified by one-way analysis of variance (ANOVA). Dates followed by the same letters indicate no difference at p>0.05 level; the different letters indicate significant difference at p<0.05 level.

		The developmental time of the first workers (days)				
Species	**Number of colonies**	mean ±SD	Min.	Max.	≥30d[1]	Successful colony production (%)[2]
B. ignitus	93	23.97±3.44b	17	33	3	80
B. lucorum	67	28.43±5.94a	16	39	29	60

Note: 1= the number of workers that had emerged at above 30 days after the first oviposition
2= successful colony production means it can be used for pollination of glasshouse crops.

Results

Table 1 shows that the colony development of *B. ignitus* and *B. lucorum* consisted of four phases. In the laboratory colonies, the average number of workers reproduction per colony of *B. ignitus* and *B. lucorum* was 107.03 and 104.55 respectively, there being no difference between them ($p>0.05$). The average number of new queens produced per colony of *B. ignitus* was significantly ($p<0.05$) lower than that of *B. lucorum* (Table 2), this difference probably being due to a longer fertilized laying in Phase 3 and a shorter fertilized laying in Phase 4 in *B. ignitus*, with new queen production occurring in Phase 4. We confirmed that competition, such as egg robbing had an effect on the number of workers produced by both species. The average nesting rate of *B. lucorum* and *B. ignitus* was 84% and 89% respectively (Table 3), there being no significant difference between them ($p>0.05$). The average time to the production of the first workers in *B. lucorum* was longer than in *B. ignitus* ($p<0.05$), but the percentage of successful colony production was lower than that of *B. ignitus*. The number of workers which had emerged at above 30 days after first oviposition in *B. lucorum* was more than in *B. ignitus* (Table 4). The successful copulation rate of *B. lucorum* was higher than *B. ignitus* at 75% and 35% respectively.

Discussion

Worker production is an important factor in evaluating the potential pollination efficiency of an insect. Asada and Ono (2000) reported that *B. ignitus* tends to produce more workers (107.06) than other important native pollinators and Hannan *et al*. (1997) reported similar results. Our experiments showed that worker production (107.03) of *B. ignitus* colonies in China was similar to that in Japan, but lower than in Korea (180) (Yoon *et al*., 2002). The reason for this may be genetic differences that exist between populations of *B. ignitus* in different locations (Shao *et al*., 2004), so this should be investigated through further work. The worker productivity of *B. ignitus* was slight higher than that of *B. lucorum*, but not significantly so.

Duchateau (1991) reported that failure of the foundress queen in first brood rearing seemed to influence the development of subsequent colonies. Our results showed that the number of workers which had emerged at above 30 days after first oviposition seemed to influence the development of further colonies. For example the number of workers which had emerged at above 30 days after first oviposition in *B. lucorum* was more than in *B. ignitus*, but the rate of successful colony production in *B. lucorum* was lower than in *B. ignitus*. *Bombus lucorum* produced more queens per colony than *B. ignitus*, and the successful copulation rate in *B. lucorum* was higher. *Bombus lucorum* was therefore more suitable for producing daughter queens than *B. ignitus*. If taking into account commercial use and a year round rearing, *B. lucorum* was a more promising candidates than *B. ignitus* for use in the commercial pollination of glasshouse crops.

Velthuis *et al* (2006) stated that there were over 30 commercial producers rearing bumble bees worldwide. Different producers reared different *Bombus* species, although Pfiidal and Hofbauer (1998) reported that the queens of *B. terrestris* formed colonies four times as large as those of *B. lucorum*. The colonies of *B. lucorum* soon reached the "switch point", developed only into weak colonies, and were therefore not suitable for laboratory rearing and for pollination purposes. *Bombus ignitus* had some clear disadvantages compared to *B. terrestris*; for example, its rearing success rate was much lower, and fully grown *B. ignitus* colonies were much smaller. Currently in China *B. lucorum* and *B. ignitus* are used predominantly in glasshouses for the pollination of various crops.

Acknowledgements

We thank Dr. Wei Shi, Zhan-Bao Guo, Yue-Ming Tong and Hai-Yan Shi for help and advice in the lab, and comments on original versions of the manuscript. This work was financially supported by a grant from the China National Project of the Tenth Five-Year Plan.

References

ASADA, S; ONO, M (1996) Crop pollination by Japanese bumble bees, *Bombus* spp. (Hymenoptera: Apidae): tomato foraging behaviour and pollination efficiency. *Applied Entomology Zoology* 31: 581–586.

ASADA, S; ONO, M (1997) Tomato pollination with Japanese native bumble bees (*Bombus* spp.). *Applied Entomology Zoology* 437: 289–292.

ASADA, S; ONO, M (2000) Difference in colony development of two Japanese bumble bees *Bombus hypocrita* and *Bombus ignitus* (Hymenoptera: Apidae). *Applied Entomology Zoology* 35: 597–603.

BLOCH, G; HEFETZ, A (1999) Regulation of reproduction by dominant workers in bumble bee (*Bombus terrestris*) queenright colonies. *Behavioral Ecology and Sociobiology* 45:125–135.

CHIANG, C H; SUNG, I H; CHEN, Y W; KUANG, H K; YANG, P S (2006) Rearing Taiwanese native bumble bees (Hymenoptera: *Bombus* spp.) in the laboratory and its application in the glasshouse. *Chinese Journal of Entomology* 8: 111–117

DUCHATEAU, M J; VELTHUIS, H H W (1988) Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour* 107: 186–207.

DUCHATEAU, M J (1991) Regulation of colony development in bumble bees. Proc.6th Int. Symp. Pollination. *Acta Horticulturae* 288: 139–143.

HANNAN, M D; MAETA, A Y; HOSIKAWA, K (1997) Colony development of two species of Japanese bumble bees *Bombus (Bombus) ignitus* and *Bombus (Bombus) hypocrita* reared under artificial conditions (Hymenoptera, Apidae). *Japanese Journal of Entomology* 65: 343–354.

LIANG, S K; WU, J; PENG, W J; ZHANG, G L; WANG, J C; AN, J D (1999) Observation on the biology of bumble bees and their breeding in their laboratory. *Apiculture of China* 50(5): 17–18 (in Chinese).

LI, J L; PENG, W J; WU, J; AN, J D; GUO, Z B; TONG, Y M; HUANG, J X (2006) Strawberry pollination by *Bombus lucorum* and *Apis mellifera* in glasshouses. *Acta Entomologica Sinica* 49(2): 342–348 (in Chinese).

PENG, W J; AN, J D (2003) A study of the bumble bees for pollinating white gourd in glasshouses. *Journal of Bee* 6: 3–4 (in Chinese).

PENG, W J; WU, J; GUO, Z B; AN, J D (2003) A study on mating of *Bombus lucorum* under artificial conditions and the influential factors. *Journal of Bee* 54(2): 3–4 (in Chinese).

POUVREAU, A (1976) Contribution à la biologie des Bourdons (Hymenoptera, Apoidae, Bombinae). Étude de quelques paramètres écologiques et physiologiques en relation avec l'hibernation des reines, Thèse, Univ. Paris-Sud, 273 p.

PŘIDAL, A; HOFBAUER, J (1998) Activation of laboratory-reared bumble bee queens. *Acta.Univ.Agric. Silvic.mendel. brun*. (Brno) 46: 79–83.

RÃSELER, P F (1985) A technique for year-round rearing of *Bombus terrestris* (Apidae: Bombini) colonies in captivity. *Apidologie* 16: 165–170.

SHAO, Z Y; MAO, H X; FU, W J; ONO, M; WANG, D S; ZHANG Y P (2004) Genetic Structure of Asian Populations of *Bombus ignitus* (Hymenoptera: Apidae). *Journal of Heredity* 95(1): 46–52.

SLADEN, F W L (1912) *The humble bee, its life history and how to domesticate it.* Macmillan, London, 514pp.

VAN DOORN, A; CHRAMBACH, A. (1989) Retinue behaviour in bumble bee workers (*Bombus terrestris* L.). *Journal of Apicultural Research* 28: 66–70.

VAN HONK, C; HOGEWEG, P (1981) The ontogeny of the social structure in a captive *Bombus terrestris* colony. *Behavioral Ecology Sociobiology* 9: 111–119.

VELTHUIS, H H W; VAN DOORN, A.(2006) A century of advances in bumble bee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37: 421–451.

WU, J; PENG, W J; AN, J D; GUO, Z B; TONG, Y M; LI, J L (2005) Rearing techniques of *Bombus lucorum*. *Entomological Knowledge* 42(6): 717–720 (in Chinese).

YOON, H J; KIM S E; KIM Y S (2002) Temperature and humidity favorable for colony development of the indoor-reared bumble bee, *Bombus ignitus*. *Applied Entomology Zoology* 37(3): 419–423.