Trialling techniques for rearing long-tongued bumblebees under laboratory conditions

Joanne D. CARNELL¹, Sam PAGE², Dave GOULSON¹, William O. H. HUGHES¹

¹School of Life Sciences, University of Sussex, Falmer, Brighton, UK

²Bumblebee Conservation Trust, Stirling University Innovation Park, Stirling, UK

Received 28 February 2019 – Revised 4 September 2019 – Accepted 18 October 2019

Abstract – Bumblebees are important pollinating insects, but many species have suffered declines over the last century. Long-tongued bumblebees have been identified as particularly at risk, partly due to their more selective diet. Attempts to study these species in captivity have been impeded by stress-induced behaviours which cause queens to kill or abandon their brood. Here, we attempt to further develop techniques, using queen pairing and *Bombus terrestris* cocoons, to successfully rear two common long-tongued bumblebee species (*B. pascuorum* and *B. hortorum*) in captivity. Approximately half of queens laid eggs and 29% produced workers. Although challenges remain, there is a great deal to be gained from optimising the captive rearing of these species.

pocket makers / pollination / *Bombus pascuorum* / *Bombus hortorum* / *Bombus terrestris* / rearing / cocoon / queen pairing

1. INTRODUCTION

Bumblebees (*Bombus* spp.) are ecologically and economically important pollinating insects, but many species have suffered severe declines in recent decades across Europe and North America (Williams and Osborne 2009; Goulson et al. 2015). Nutritional stress, pathogen infection and exposure to pesticides are key drivers in their decline, but these pressures do not act independently of one another and so their effects on bees are not straightforward (Vanbergen and Initiative 2013). Techniques to rear bumblebees in captivity have been developed over the last century (Van

den Eijnde et al. 1990; Pouvreau 2004; Velthuis and Van Doorn 2006). However, these experiments have been almost exclusively conducted on short-tongued species (such as *B. terrestris* and *B. impatiens*). In the wild, these species are generally common, characterised by long colony life-cycles and the utilisation of resources from multiple plant groups and habitats (Fussell and Corbet 1992; Goulson et al. 2005, 2006). While these species are suitable as models for studying some aspects of social insect biology and behaviour, they are not representative of all bumblebee species and differ significantly in their ecological sensitivity and response to stressors (Goulson et al. 2005).

A small number of studies have succeeded in rearing bumblebee species other than *B. terrestris* or *B. impatiens* in captivity. Lhomme et al. (2013) provided the first precise protocol for rearing two cuckoo species, *B. vestalis* and *B. sylvestris* and Moerman et al. (2016) showed differential responses to pollen diet between *B. terrestris*,

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13592-019-00707-7>) contains supplementary material, which is available to authorized users.

Corresponding author: J. Carnell,
j.carnell@sussex.ac.uk
Manuscript editor: Cedric Alaux

B. pratorum and *B. hypnorum* micro-colonies reared in captivity. Like *B. terrestris* and *B. impatiens*, these other species also have short-medium tongue lengths (Falk 2015). However, the bumblebees that have declined most severely tend to be the long-tongued species that rely heavily on flowers from the Fabaceae plant family (Goulson et al. 2005; Biesmeijer et al. 2006). Unlike short-tongued species, many of which have thrived despite land use intensification, long-tongued bumblebees have a restricted range of foodplants and are from smaller colonies with a smaller foraging range (Goulson et al. 2008). As a result, they are more sensitive to local and global pressures (Goulson 2003; Goulson et al. 2005). Unfortunately, these species have remained relatively understudied in laboratory conditions due to difficulties of rearing the colonies in captivity. During laboratory rearing trials, queens generally perform stress-induced behaviours (Pomeroy and Plowright 1979; Weidenmüller et al. 2002), including failure to settle in nest boxes provided, failure to utilise pollen, neglecting eggs and larvae and direct ovicide or infanticide, resulting in early colony failure. Rearing conditions, such as nest box design and pollen type, have a substantial effect on the health and reproductive success of bumblebees reared in captivity. There is some evidence to show that giving young, long-tongued colonies access to flowers allows natural foraging and improves colony development (Lhomme et al. 2013; Ptáček et al. 2015; Moerman et al. 2016). Many of the long-tongued bumblebees can be characterised as pocket makers, feeding their larvae a solid rather than liquid diet (Den Boer and Duchateau 2006). How this might affect the physiological and behavioural responses of queens, workers and larvae to environmental stressors remains unclear.

The first attempts at long-tongued bee rearing were carried out by Lindhard (1912). At the same time, Sladen (1912) was testing stimuli to encourage oviposition in short-tongued queens. These methods have been combined and developed since although their effects can be highly variable. Stimuli for egg-laying include various interspecies pairings at different life stages, including the provision of cocoons and callow workers.

Providing brood has a stimulatory effect on queens of several bumblebee species (Yoneda 2008; Bučánková and Ptáček 2010), and cocoons of *B. terrestris* have commonly been used in rearing trials of long-tongued species (Kwon et al. 2003; Bučánková and Ptáček 2012; Ptáček et al. 2015). Allowing cocoons to hatch and callows to remain in the colony, or adding *B. terrestris* or *Apis mellifera* callows, can also have a stimulatory effect on queen egg-laying and *B. pascuorum* and *B. ruderarius* are at least somewhat encouraged to oviposition by honey bee workers (Ptáček 1983, 1985; Ptáček et al. 2015). Callows have also been observed caring for the queens own brood; Ptáček et al. (2015) observed *B. terrestris* workers feeding *B. pascuorum* and *B. sylvarum* larvae. However, in another trial, *B. hortorum* queens were aggressive towards multiple stimuli and have been observed destroying *B. terrestris* cocoons and killing honey bee workers (Bučánková and Ptáček 2012). The pairing of queens, first tested by Sladen (1912), can also encourage oviposition and brood care later on (Plowright and Jay 1966; Alford 1975; Duchateau 1985; Ptáček et al. 2000, 2015). This sometimes involves an excluder, which keeps queens physically separated in a shared nest box. Once they are placed together, one female usually becomes dominant and begins egg-laying (Ptáček et al. 2015). Ptáček et al. (2000) and Ptáček et al. (2015) found queen pairing effective for *B. pascuorum* but not for *B. humilis* or *B. ruderarius*, who displayed 'resentful' and aggressive behaviours. Of the three *B. humilis* females they trialled, one broke through the excluder to reach and kill her neighbour. In another study, queens of *B. hortorum* were more likely to establish an egg cell when kept alone (100%, $n = 9$), compared with those who were paired (66%, $n = 6$) or who had been given a honey bee worker (71%, $n = 7$) (Bučánková and Ptáček 2012). These various interspecific combinations have therefore yielded contrasting results, from cohabitation to killing, but many of the observations are based on a very small number of individuals, making species-level assessments and progress difficult.

To support the conservation of these long-tongued bumblebee species, a more detailed

understanding of their biological and behavioural response to natural threats is needed. Developing methods to rear them in captivity would be of great benefit to research in this area. In this study, we attempt to further develop techniques in long-tongued bumblebee rearing with two common long-tongued species, *B. pascuorum* and *B. hortorum*, under laboratory conditions.

2. MATERIALS AND METHODS

Rearing trials took place at the University of Sussex (UK) in 2017 (Figure 1). Queens of *B. pascuorum* and *B. hortorum* were collected from surrounding chalk grasslands in the spring (March to mid-April). Pollen foraging occurs post-nest establishment (Evans et al. 2007), and so only queens without pollen in their pollen baskets were collected. Bees were stored in ventilated 5 ml Eppendorf tubes cooled beside ice packs for transport back to the lab. Queens were then placed in a dark room (30 ± 1 °C, 20% RH) in ventilated $15 \times 15 \times 15$ -cm plastic boxes. We observed previously that queens failed to thermoregulate brood and so we used the highest temperature described in similar long-tongued rearing trials (Ptáček et al. 2015). We did not observe any nest fanning behaviour by queens or workers during the experiment that would have indicated overheating. Queens were provided with a piece of cotton wool to simulate nesting material and 50% (v/v) sugar water (10% fructose, 90% sucrose). For the first feeding, sugar water was placed onto the floor of the box or pipetted directly to queens if they were lethargic. Thereafter, sugar water was provided in an external feeder *ad libitum* (Figure 1c). Queens were given a fresh 1 g pollen ball every 2 days. Pollen balls were made by grinding dry pollen granules to a powder and combining with 50% (v/v) sugar water (10% fructose, 90% sucrose) until a sticky dough was formed. We found that this wet pollen with a high sugar water content was consumed readily by both species. All bees started on mixed *Erica*, *Salix* and *Prunus* pollen (Pollenergie, France) and from April a pre-made polyfloral wildflower mix (mille fleurs, Pollenergie, France) was added, which became 100% of their food by May to simulate natural conditions for workers. All pollen had

been freeze-stored prior to use. Pollen balls were generally placed in the box for bees to collect but attempts were made to feed the first larvae by scattering or gently pressing ground pollen onto the brood casing.

Following Ptáček et al. (2015), all *B. pascuorum* queens were paired to prompt one to become dominant and begin egg-laying (Figure 1c). We identified dominant individuals as those which spent the most amount of time in the centre of the box and standing on the pollen provided, since this is naturally where they would lay their eggs (Den Boer and Duchateau 2006). Some of these queens also exhibited a lengthening of the abdomen, also observed by Ptáček et al. (2015). Submissive queens (which tended to be the smaller of the two) were more active and tended to roam the edges of the box. Once identified, submissive females were removed and paired together, prompting another to establish dominance. Paired queens that appeared unsettled or not engaged in egg-laying or brood care were also separated and either re-paired or given their own nest box. The action taken for each queen were based on each queen's dominant/submissive characteristics and the availability of other single queens available for pairing and nest boxes (see Supplementary Tables I, II and III for all pairings and actions). When testing the response of two *B. hortorum* queens being paired, they were highly aggressive, so were separated and no further *B. hortorum* queens were paired together.

For the first 33 days, we recorded the progress of queens without additional stimuli. From day 34, to further stimulate egg-laying and encourage brood care behaviours, 2–5-day-old laboratory-reared *B. terrestris* pupae were introduced to the *B. pascuorum* and *B. hortorum* queens. Three times a week throughout the experiment, we recorded (i) the day of first egg-laying, (ii) observable ovicide, larvicide or neglect, (iii) brood pupation, (iv) worker emergence, (v) gyne and male production, (vi) aggression between paired queens, or queens and *B. terrestris* cocoons/workers and (vii) use of cotton wool as nesting material. All efforts were made to minimise disturbance throughout the experiment.

To check for differences in behaviour and reproductive success between species, we used

Figure 1 a Flowchart illustrating the methodology and stimuli used in this study to encourage wild-caught *B. pascuorum* (n = 57) and *B. hortorum* (n = 11) bumblebee queens kept in captivity to produce colonies. *B. pascuorum* queens were all initially paired (79 pairings in total). **b** Flowchart showing the response of *B. pascuorum* queens to pairing (see Supplementary Tables I, II and III) and the resulting action taken, which was based on each queen's dominant/submissive characteristics and the availability of single queens and nest boxes. **c** Top left to right: Nesting boxes used to rear single or paired long-tongued bumblebee queens; a *B. pascuorum* worker and her queen. Bottom left and right: two colonies of *B. hortorum* utilising cotton wool as nesting and food storage material.

Kruskal-Wallis tests for queen survival, the day they first produced eggs or workers, and the number of workers produced. Any pairs of queens that produced workers together were counted as a single queen. Chi-square tests were used to determine if there was a difference between species in whether or not queens produced eggs or workers, and ejected or neglected eggs or larvae.

3. RESULTS

A total of sixty-eight queens (11 *B. hortorum* and 57 *B. pascuorum*) were used in the experiment, of which forty queens laid eggs (59%) (Figure 1). Six additional pairs of queens showed close co-operative brood care behaviour with their partner, and as a result, it was not possible to tell which queen, if not both, were egg-laying (Table I). There were significant differences between species in whether or not they ejected eggs or larvae, and whether or not they produced workers ($\chi^2 = 10.6$, $df = 1$, $p = 0.001$ and $\chi^2 = 9.9$, $df = 1$, $p = 0.0002$, respectively). *B. pascuorum* queens were more likely to eject their eggs or larvae (79% of the 34 queens that laid eggs) compared with *B. hortorum* (16% of the 6 queens that laid eggs), and *B. hortorum* queens were nearly three and a half times as likely to produce workers (Table I). There were no significant differences between species in their survival, whether or not they laid eggs, the number of days it took to produce their first egg or worker, the number of workers they produced and whether or not they neglected their eggs or brood (respectively: $\chi^2 = 0.04$, $df = 1$, $p = 0.85$; $\chi^2 = 0.09$, $p = 0.75$; $\chi^2 = 0.21$, $df = 1$, $p = 0.64$; $\chi^2 = 0.71$, $df = 1$, $p = 0.40$; $\chi^2 = 3.10$, $df = 1$, $p = 0.08$; $\chi^2 = 0.95$, $df = 1$, $p = 0.32$).

Survival of queens ranged from 2 to 178 days (Figure 2), with a 12% mortality rate observed in the first 7 days. Of the 40 queens that laid eggs, two paired *B. pascuorum* queens (BpW and BpM) and one individual *B. hortorum* queen (BhF) did so before the addition of any *B. terrestris* cocoons (Table II). Across both species, 70% of queens ejected eggs and larvae and 33% neglected them. These behaviours were repeatedly observed throughout the experiment. Queens would lay eggs and then either remove

them or the developing larvae, or would fail to feed the larvae so they died in the brood case. This cycle continued even after the first workers were produced. Scattering and pressing additional pollen over the larvae was unsuccessful; the larvae were not observed to feed and the pollen dried around them. Nine single queens (*B. hortorum* queens BhJ, BhK, BhE, BhD and BhF, and *B. pascuorum* queens BpB and BpAQ) and three pairs of queens (*B. pascuorum* queens BpAL-BpAM, BpBC-BpBK and BpAD-BpAZ) produced pupae, and all pupae successfully eclosed and emerged as workers.

3.1. Rearing of *B. pascuorum* queens

In the first 10 days, 91% of *B. pascuorum* queens performed nest-associated behaviours, including carding with cotton wool, making nectar cups and sitting on pollen (Figure 1c). All 57 queens were paired and a total of 79 pairings (up to three per queen, $\bar{x} \pm \text{s.e. } 1.4 \pm 0.08$) were made over the course of the experiment (Tables SI, SII and SIII). Only in one case did a *B. pascuorum* queen exhibit aggression, where a previously submissive queen killed her new partner. The duration of the other 78 pairings varied between 1 and 129 days (32 ± 4.7). The behaviour of paired queens could be categorised as aggressive ($n = 1$), unsettled (i.e. avoidance, no egg-laying or interruption of egg-laying) ($n = 21$), not engaged with brood care ($n = 29$), settled (i.e. not aggressive or avoidant) ($n = 12$) or co-operative (jointly caring for brood) ($n = 16$) (Table SI, SII and SIII). Of the 34 queens that were observed to lay eggs, 32 (94%) produced their first eggs before the addition of cocoons, and two (6%) 2–5 days after.

At the point *B. terrestris* cocoons were given to queens (which was determined by the availability in the source colony), 16 of the *B. pascuorum* queens were paired and 20 were single. The cocoons were always ignored, and workers emerged 1–4 days later. Thirty-one queens actively avoided their *B. terrestris* worker until the worker was removed. In eight nests, the *B. terrestris* worker took over and laid its own eggs. When this happened, the

Table I. A summary of colony development, behaviour and longevity of *B. hortorum* and *B. pascuorum* bumblebee queens kept under laboratory conditions. *B. hortorum* queens were kept individuals ($n = 11$). *B. pascuorum* queens were kept in pairs or singly after initial pairing ($n = 57$ queens). Queens were provided with food and sugar water *ad libitum* and stimuli to encourage oviposition and brood-rearing were provided at various points in colony development (Table II)

	<i>B. hortorum</i> queens ($n = 11$)	<i>B. pascuorum</i> queens ($n = 57$)
Mean $\pm$ SE days survived (queens)	74 $\pm$ 20	74 $\pm$ 7
Use of cotton wool	91% (10/11)	100% (57/57)
Aggression between queens	-	2% (1/57)
Egg-laying	54% (6/11)	60% (34/54)
Unconfirmed egg-laying	-	11% (6/57)
Mean $\pm$ SE days to egg-laying	16 $\pm$ 2.3	20.6 $\pm$ 1.9
Egg/larval ejection	16% (1/6)	79% (27/34)
Larvae neglect	50% (3/6)	29% (10/34)
Pupation	83% (5/6)	24% (8/34)
Production of workers	83% (5/6)	24% (8/34)
Mean $\pm$ SE days to first worker	81 $\pm$ 9.2	66.4 $\pm$ 11.9
Mean $\pm$ SE number of workers	4 $\pm$ 1.4	3 $\pm$ 0.4
Gyne production	50% (3/6)	0% (0/34)

B. pascuorum queens ceased their cyclic egg-laying and egg or larval ejection and, even after the *B. terrestris* worker was removed, did not resume egg-laying again for some time. Two queens killed their donor *B. terrestris* after emergence, including one that had successfully produced a worker before the addition of the cocoon (BpAQ; 25 days post-capture; Table II). Only one pair of queens accepted their worker and exhibited positive physical contact (BpAD-BpAZ). In the remaining nests, the *B. terrestris* workers stayed around the perimeter of the boxes and we observed very few interactions between them and the *B. pascuorum* queens. We saw no evidence that *B. terrestris* workers assisted the *B. pascuorum* queens in brood care.

For five queens (pairs BpAZ-BpAD and BpAL-BpAM and single queen BpB), the addition of a *B. terrestris* cocoon was followed by the successful production of *B. pascuorum* workers, 11–15 days later. One additional pairing produced their first worker 41 days later (BpK-BpBC).

Successful queens (i.e. those which produced workers) produced up to three small *B. pascuorum* workers each (Figure 1c). No

males or gynes were produced. One queen (BpAQ), which had produced a worker before the addition of cocoons, produced another two after the *B. terrestris* worker was removed. One *B. pascuorum* worker laid eggs after the death of the queen. These were neglected and died at the ~ L2 larval stage.

3.2. Rearing of *B. hortorum* queens

Due to their aggression, *B. hortorum* queens were not paired. All but one of the 11 queens carried out nest building using the cotton wool (Figure 1c). Fifty-four per cent of queens laid eggs, and of these, 83% went on to produce 4 ± 1.4 workers; three of these also produced males (1, 4 and 38 individuals respectively; Table II).

Three queens survived for less than 17 days in captivity and did not lay any eggs in that time (BhG, BhB and BhI; Table II). Of the three queens which survived between 25 and 36 days (BhC, BhA and BhH), only one laid eggs. These were reared up to the L3 larval stage before she ceased brood care and remained relatively still in a corner for 10 days

Figure 2 Survival of wild-caught **a** *B. hortorum* ($n = 11$) and **b** *B. pascuorum* ($n = 57$) bumblebee queens reared in captivity to produce colonies. Fourteen per cent of queens died in the first 10 days and 76% of queens that survived this period went on to lay eggs.

before dying. Five queens survived between 97 and 178 days (BhJ, BhK, BhE, BhD and BhF), all successfully producing workers. These queens were first observed with eggs or L1 stage larvae on days 8–23, but did not produce their first workers until, at the earliest, day 58 and at the latest, day 109.

The donor cocoons used to prompt oviposition and brood care in *B. hortorum* were given to all queens that survived to this period (from day 41 post-capture) and produced mixed behavioural responses. Queens were not observed interacting with the cocoons and generally continued their own egg-laying and larval feeding. BhF was given a cocoon on the day her first daughter emerged. Within a week, BhF had (for the first time) ejected her remaining larvae. She did not kill or show any aggression to the *B. terrestris* worker but laid new eggs 4 days later, which later emerged as males. Three other queens responded

negatively to the *B. terrestris* worker when it emerged. BhD killed it within a day of emergence and BhK and BhE actively avoided theirs, ceasing all nest-associated behaviours until the workers were removed. BhJ was the only queen whose interactions with her *B. terrestris* worker appeared consistently natural (settled physical contact). No evidence of *B. terrestris* workers engaging in brood care behaviour when paired with *B. hortorum* queens was observed.

4. DISCUSSION

Queens of both species readily laid eggs in the study, with more than three-quarters laying eggs if they survived past day 10. Even *B. hortorum* queens, who were given no initial stimulus beyond nesting material, had generally laid their first eggs approximately 2 weeks after capture. Despite not being a natural

Table II. Survival and reproductive success (day of first egg, day of first worker and number of offspring produced), of *B. hortorum* and *B. pascuorum* queens kept in laboratory conditions. Egg-laying shown in italics denotes co-operation between queens, where it was not possible to tell if one or both queens contributed to egg-laying. *B. pascuorum* queens were all initially paired and based on their behaviour, reproductive success and survival were either paired (P) or single (S) at different stages of colony production (see Supplementary Tables I, II and III for all pairings and justifications for keeping some queens paired and giving some their own nest box). If a *B. pascuorum* queen was paired, the number of pairings she had is shown (P1-3). All queens were initially given cotton wool, pollen and sugar water *ad libitum* at the start of the experiment, then later, a *B. terrestris* cocoon to further encourage oviposition and care

	Queen	Days survived	Day of first egg	Day of first worker	Day <i>B. terrestris</i> cocoon was added	Long-tongued offspring produced		
						Workers	Males	Gynes
<i>Bombus hortorum</i>	BhG	2	-	-	-	-	-	-
	BhB	13	-	-	-	-	-	-
	BhI	17	-	-	-	-	-	-
	BhC	25	13	-	-	-	-	-
	BhA	29	-	-	-	-	-	-
	BhH	36	-	-	-	-	-	-
	BhJ	97	16	95	42●	3	-	-
	BhK	105	8	109	41□	1	-	-
	BhE	152	14	72	47□	8	1	-
	BhD	158	22	71	41◆	6	38	4
	BhF	178	23	58	58□	1	4	-
<i>Bombus pascuorum</i>	BpAB, BpC	3	-	-	-	-	-	-
	BpD	5	-	-	-	-	-	-
	BpAW	6	-	-	-	-	-	-
	BpG, BpR	7	-	-	-	-	-	-
	BpBD, BpE	8	-	-	-	-	-	-
	BpT	9	-	-	-	-	-	-
	BpF	12	(S) 11	-	-	-	-	-
	BpS	16	-	-	-	-	-	-
	BpQ	17	-	-	-	-	-	-
	BpAJ	20	-	-	-	-	-	-
	BpAA, BpAV	22	-	-	-	-	-	-
	BpAT	23	(P1) 20○	-	(S) 48□	-	-	-
	BpU	23	-	-	-	-	-	-
	BpAN	24	-	-	-	-	-	-
	BpAK	25	-	-	-	-	-	-
	BpAU	28	(P2) 21○	-	-	-	-	-
	BpAX	47	(P1) 9	-	-	-	-	-
	BpBF	50	(P1) 14	-	(P1) 48□	-	-	-
	BpBE	52	(P1) 14	-	(P1) 48□	-	-	-
	BpAY	60	(P1) 46○	-	(P1) 41□	-	-	-

Table II (continued)

Queen	Days survived	Day of first egg	Day of first worker	Day <i>B. terrestris</i> cocoon was added	Long-tongued offspring produced		
					Workers	Males	Gynes
BpAZ	61	(P1) 13○	(P1) 64	(P1) 53●	3	-	-
BpBB	62	(P1) 20○	-	(P1) 56□	-	-	-
BpAL	65	(P1) 16○	(P1) 64	(P1) 51□	3	-	-
BpAM	65	(P1) 16○	(P1) 64	(P1) 51□	3	-	-
BpAG	69	(P1) 13	-	(S) 48□	-	-	-
BpAS	71	(S) 30	-	(P2) 50□	-	-	-
BpAE	76	(P1) 13	-	(P1) 59□	-	-	-
BpM	77	(P1) 36	-	(P1) 34□	-	-	-
BpAC	80	(P2) 17	-	(S) 59□	-	-	-
BpN	82	(P1) 46○	-	(P1) 41□	-	-	-
BpW	92	(P1) 36	-	(P1) 34□	-	-	-
BpX	103	(S) 22	-	(S) 57□	-	-	-
BpB	114	(S) 23	(S) 84	(S) 69□	3	-	-
BpAO	117	(P2) 12	-	(S) 35□	-	-	-
BpV	119	(P2) 28	-	(S) 45□	-	-	-
BpAD	123	(P1) 13○	(P1) 64	(P1) 53●	3	-	-
BpBC	123	(P1) 9○	(P1) 95	(P1) 54□	1	-	-
BpK	124	(P1) 9○	(P1) 95	(P1) 54□	1	-	-
BpO	126	(S) 16	-	(S) 49□	-	-	-
BpAR	129	(P1) 19○	-	(P1) 55□	-	-	-
BpL	130	(S) 20	-	(S) 48□	-	-	-
BpBA	132	(S) 20○	-	(P1) 56□	-	-	-
BpH	132	(P2) 4○	-	(P3) 58□	-	-	-
BpP	132	(P1) 5	-	-	-	-	-
BpJ	133	(P2) 13	-	(P2) 59□	-	-	-
BpAQ	136	(P1) 16	(S) 25	(S) 59◆	3	-	-
BpHI	136	(P1) 30	-	(S) 50□	-	-	-
BpAF	140	(S) 13	-	(S) 48□	-	-	-
BpI	141	(S) 38	-	(S) 58◆	-	-	-
BpY	142	(P2) 21	-	(S) 35□	-	-	-
BpAP	149	(P1) 19○	-	(P1) 55□	-	-	-
BpAH	153	(P2) 28	-	(S) 50□	-	-	-
BpZ	160	(S) 55	-	(S) 62□	-	-	-

Response of queens to the resulting *B. terrestris* workers: Bullet represents *B. terrestris* worker accepted by queen (i.e. not killed and not avoided); square represents queen actively avoided *B. terrestris* worker until it was removed; diamond represents queen killed her *B. terrestris* worker or showed aggression

pollen choice for long-tongued bumblebees, the diet was sufficiently good for the queens to produce workers ($n = 7$ single queens and 3 pairs) and reproductives ($n = 3$). Very few workers were needed for the colony to produce males, demonstrated by a *B. hortorum* colony

(queen BhD) which only produced six workers before producing 38 males. Problems arose for both species during larval development, when queens neglected or ejected their young. Although we cannot exclude eggs or larvae being abandoned due to poor health, we suggest this behaviour was more likely a response to stress or a perceived lack of resources (Smith 1985; Parmigiani and Vom Saal 1994). However, we did not observe any queen consuming their abandoned larvae, or failure of the larvae to feed pollen provided by the queen. Queens of both species were found to commit ovicide and larvicide, and this behaviour did delay worker production noticeably in both species, which has also been observed in trials with *B. lapidarius* (Bučánková and Ptáček 2012). The cyclic egg-laying-ovicide behaviour presents multiple opportunities to encourage larval feeding and care. It may also suggest that, while queens may be ready to lay eggs and start a colony, they may also be particularly sensitive to external conditions, such as temperature, humidity and disturbance, which interfere with rearing. Behaviourally, *B. pascuorum* seemed much less aggravated by the artificial conditions and stimuli provided, while *B. hortorum* queens responded more sensitively and more aggressively if disturbed. This included pollen feeding, during which the lid of the nest box had to be removed. However, given the frequency of ovicide and larvicide in both species, reducing stress post-egg-laying should be a continuing priority in rearing trials.

The proportion of *B. hortorum* queens used in the experiment that went on to produce workers (45%) is very promising. A much lower success rate was achieved for *B. pascuorum*, with only eight queens (14%) being involved in worker production. This may be improved by giving younger cocoons to queens as soon as they have acclimatised to their nest boxes. This could not be done in our experiment due to availability of cocoons, but other studies have demonstrated that younger cocoons and earlier exposure can prompt queens to settle quicker in their boxes—and ultimately produce workers sooner

(Bučánková and Ptáček 2012; Ptáček et al. 2015). Previous rearing trials have generally assumed that the presence of cocoons prompts queens to engage in natural brood care activities. This could be as a result of the cocoon's scent (Heinrich 1974; Gamboa et al. 1987), or slightly higher temperature (Barrow and Pickard 1985). While it is possible the queen might assume the brood are her own, nest intrusions occur naturally in the wild (Lopez-Vaamonde et al. 2004; Goulson et al. 2018), and so the cocoons may instead be perceived as evidence of a female competitor being present. This would explain why queens have also been documented destroying cocoons and may stimulate females to assert dominance over the apparent rival via their own egg-laying, essentially stressed into developing a colony. Assuming that this is the case, there must be factors which discourage queens from performing natural rearing behaviours before the addition of cocoons, but which can be at least somewhat overridden when cocoons are added. Given that queens naturally would self-select nesting sites, it is possible that they do not recognise their captive surroundings and that the addition of cocoons functions at the very least as an indicator that the surrounding environment is a suitable nest.

As a result of intermittent feeding by the queen, the long-tongued workers (mainly *B. pascuorum*) that did emerge were small and may have made little contribution to colony development, suggesting queens may need continued support even after their first workers emerge. We did not screen queens for pathogens and it is possible that parasite infections could have affected their propensity to lay eggs, and the size and health of their larvae. Providing *B. terrestris* cocoons did encourage *B. pascuorum* queens to engage in more larval care (Table II) but had less effect of *B. hortorum* queens. After emergence, most *B. terrestris* workers made little to no physical contact with the queen or her brood, which is in contrast to previous studies in which *B. terrestris* workers actively engage in brood care and larval feeding for *B. pascuorum* and *B. sylvarum* queens (Ptáček et al. 2015).

We did observe co-operative brood care between paired *B. pascuorum* queens and up to 17 queens appeared to contribute equally to egg-laying and brood care. For *B. pascuorum*, the value of intra- and inter-specific pairings clearly needs further investigation.

B. hortorum and *B. pascuorum* are amongst the most generalist of the long-tongued bumblebees. They are common in the UK and share the same association with Fabaceae as those species most in decline, making them suitable models for rearing trials and laboratory experiments. In our study, we found that queens of *B. pascuorum* and *B. hortorum* could rear small colonies through to the reproductive stage even when fed pollen they may not naturally collect in the wild. We found that queens lay eggs readily under artificial conditions even without the use of cocoons as stimuli; this might be further improved using CO₂ exposure (Röseler 1985; Tasei 1994). It is also clear that species responded differently to captive conditions, as previously shown in other bumblebee species (Bučánková and Ptáček 2012; Ptáček et al. 2015; Moerman et al. 2016). Repeated egg/larvae abandonment remains a problem. Future trials should test methods to manage queen stress from the initial collection through to colony initiation. Trialling various nest box types and pollen diets will further clarify their nesting preferences (Lhomme et al. 2013; Moerman et al. 2016). Since queens may respond positively to stress-inducing stimuli due to a perceived competitive pressure, techniques to restrict stressful conditions to the first egg-laying phase might elucidate this. Future research should examine how pollen type and preparation (e.g. water content) affects larval development. Pollen prepared with a higher liquid content than we used here may be more suitable for manual larval feeding. Although rearing long-tongued bees may require more sensitive, species-specific maintenance in captivity, our results show that it is possible. Given the ecological implications and conservation status of many long-tongued species, further work to refine the protocols is clearly worthwhile.

ACKNOWLEDGEMENTS

We thank Rosaline A. Hulse for help on this manuscript and two anonymous reviewers for their many constructive comments.

AUTHOR CONTRIBUTION

All authors conceived the research; WH and JC designed the experiment; JC performed experiments and analysis; WH and JC wrote the paper and WH and DG participated in the revisions of it. All authors approved the final manuscript.

FUNDING INFORMATION

This work received funding from the Bumblebee Conservation Trust and Natural Environment Research Council (NE/M00949/1)

OPEN ACCESS

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Essai de méthodes d'élevage de bourdons à longue langue en condition de laboratoire.

***Bombus pascuorum* / *Bombus hortorum* / *Bombus terrestris* / élevage / cocon / accouplement de reine.**

Test von Methoden zur Aufzucht langzüngiger Hummeln unter Laborbedingungen.

***Bombus terrestris* / Aufzucht / Kokon / Paarung.**

REFERENCES

- Alford, D. V. (1975). Bumblebees. London, Davis-Poynter.
- Barrow, D. and R. Pickard (1985). Larval temperature in brood clumps of *Bombus pascuorum* (Scop.). J. Apic. Res.. **24**(2): 69-75.

- Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele and W. E. Kunin (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **313** (5785): 351-354.
- Bučánková, A. and V. Ptáček (2010). Experiences in rearing of *Bombus lucorum* L. (Hymenoptera: Apoidea) in captivity. *Úroda* **12**: 621-624.
- Bučánková, A. and V. Ptáček (2012). A test of *Bombus terrestris* cocoon and other common methods for nest initiation in *B. lapidarius* and *B. hortorum*. *J. Apic. Sci.* **56** (2): 37-47.
- Den Boer, S. P. A. and M. Duchateau (2006). A larval hunger signal in the bumblebee *Bombus terrestris*. *Insect. Soc.* **53** (3): 369-373.
- Duchateau, M. (1985). Analysis of some methods for rearing bumblebee colonies. *Apidologie* **16**: 225-227.
- Evans, E., I. Burns and M. Spivak (2007). Befriending bumble bees: a practical guide to raising local bumble bees. St. Paul: University of Minnesota Extension Service.
- Falk, S. J. (2015). Field guide to the bees of Great Britain and Ireland, British Wildlife Publishing, Totnes.
- Fussell, M. and S. A. Corbet (1992). Flower usage by bumblebees: a basis for forage plant management. *J. Appl. Ecol.* **29**: 451-465.
- Gamboa, G. J., R. L. Foster and K. W. Richards (1987). Intraspecific nest and brood recognition by queens of the bumble bee, *Bombus occidentalis* (Hymenoptera: Apidae). *Can. J. Zool.* **65** (12): 2893-2897.
- Goulson, D. (2003). Bumblebees. Behaviour and Ecology, Oxford University Press, Oxford.
- Goulson, D., M. E. Hanley, B. Darvill, J. S. Ellis and M. E. Knight (2005). Causes of rarity in bumblebees. *Biol. Conserv.* **122** (1): 1-8.
- Goulson, D., M. E. Hanley, B. Darvill and J. S. Ellis (2006). Biotope associations and the decline of bumblebees (*Bombus* spp.). *J. Insect Conserv.* **10** (2): 95-103.
- Goulson, D., G. C. Lye and B. Darvill (2008). Diet breadth, coexistence and rarity in bumblebees. *Biodivers. Conserv.* **17** (13): 3269-3288.
- Goulson, D., E. Nicholls, C. Botías and E. L. Rotheray (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347** (6229): 1435.
- Goulson, D., S. O'Connor and K. J. Park (2018). The impacts of predators and parasites on wild bumblebee colonies. *Ecol. Entomol.* **43** (2): 168-181.
- Heinrich, B. (1974). Pheromone induced brooding behavior in *Bombus vosnesenskii* and *B. edwardsii* (Hymenoptera: Bombidae). *J. Kansas Entomol. Soc.* **47**: 396-404.
- Kwon, Y. J., S. Saeed and M. J. Duchateau (2003). Stimulation of colony initiation and colony development in *Bombus terrestris* by adding a male pupa: the influence of age and orientation. *Apidologie* **34** (5): 429-437.
- Lhomme, P., A. Sramkova, K. Kreuter, T. Lecocq, P. Rasmont and M. Ayasse (2013). A method for year-round rearing of cuckoo bumblebees (Hymenoptera: Apoidea: *Bombus* subgenus *Psithyrus*). *Ann Soc Entomol Fr, Taylor & Francis, Didcot*.
- Lindhard, E. (1912). Humlebien som Husdyr: spredte Traek af nogle danske Humlebiartes biologi. *Tidsskr PIAvI* **19**: 319-326.
- Lopez-Vaamonde, C., J. W. Koning, R. M. Brown, W. C. Jordan and A. F. Bourke (2004). Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature* **430** (6999): 557.
- Moerman, R., N. Roger, R. De Jonghe, D. Michez and M. Vanderplanck (2016). Interspecific variation in bumblebee performance on pollen diet: new insights for mitigation strategies. *PLoS One* **11** (12): e0168462.
- Parmigiani, S. and F. S. Vom Saal (1994). Infanticide and parental care, Taylor & Francis, Didcot.
- Plowright, R. C. and S. C. Jay (1966). Rearing bumble bee colonies in captivity. *J. Apic. Res.* **5** (3): 155-165.
- Pomeroy, N. and R. Plowright (1979). Larval ejection following narcosis of bumble bees (Hymenoptera: Apidae). *J. Kansas Entomol. Soc.* **52**: 215-217.
- Pouvreau, A. (2004). Les Insectes Pollinisateurs. Lausanne, Delachaux et Niestle.
- Ptáček, V. (1983). Stimulation of brood rearing of bumblebee queens (Hymenoptera, Bombidae) by contacting honey bees (*Apis mellifera* L.) (preliminary report)). *Sb. Ved. Pr. Vyzk. Slechtitelskeho Ustavu Pčininorskeho*, Troubsko Brna **8**: 157-161.
- Ptáček, V. (1985). Testing three methods of rearing bumble bees (Hymenoptera, Bombinae). *Sbornik ved. Praci VSUP Troubsko* **9**: 56-57.
- Ptáček, V., R. Borovec and E. Pernova (2000). The two-queen cascade method as an alternative technique for starting bumble bee [*Bombus*, Hymenoptera, Apidae] colonies in laboratory conditions: a preliminary study. *Pszczelnicze Zesz Nauk* **44** (2): 305-309.
- Ptáček, V., A. Votavová and O. Komzáková (2015). Experience in rearing common carder bees (*Bombus pascuorum* Scop.), with some notes on three similar species: shrill carder bee (*B. sylvarum* L.), red-shanked carder bee (*B. ruderarius* Müll.), and brown-banded carder bee (*B. humilis* Ill.) (Hymenoptera: Apidae). *Acta Univ. Agric. Silv. Mendel Brun* **63** (5): 1535-1542.
- Röseler, P.-F. (1985). A technique for year-round rearing of *Bombus terrestris* (Apidae, Bombini) colonies in captivity. *Apidologie* **16** (2): 165-170.
- Sladen, F. (1912). The humble-bee, its life-history and how to domesticate it. London, Macmillan & Co.
- Smith, R. (1985). Oviposition, ovicide and larval competition in granivorous insects. *Behav. Ecol.* **25**: 423-488.
- Tasei, J. (1994). Effect of different narcosis procedures on initiating oviposition of prediapausing *Bombus terrestris* queens. *Entomol. Exp. Appl.* **72** (3): 273-279.

- Van den Eijnde, J., A. De Ruijter and J. Van der Steen (1990). Method for rearing *Bombus terrestris* continuously and the production of bumblebee colonies for pollination purposes. VI International Symposium on Pollination 288.
- Vanbergen, A. J. and I. P. Initiative (2013). Threats to an ecosystem service: pressures on pollinators. *Front. Ecol. Environ.* **11** (5): 251-259.
- Velthuis, H. H. and A. Van Doorn (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37** (4): 421-451.
- Weidenmüller, A., C. Kleineidam and J. Tautz (2002). Collective control of nest climate parameters in bumblebee colonies. *Anim. Behav.* **63** (6): 1065-1071.
- Williams, P. H. and J. L. Osborne (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie* **40** (3): 367-387.
- Yoneda, M. (2008). Induction of colony initiation by Japanese native bumble bees using cocoons of the exotic bumblebee *Bombus terrestris*. *Entomol. Sci.* **11** (1): 123-126.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.