Can commercially imported bumble bees out-compete their native conspecifics?

T. C. INGS,* N. L. WARD†‡ and L. CHITTKA*

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK, and †CABI Bioscience (UK Centre), Bakeham Lane, Egham TW20 9TY, UK

Summary

1. Although invasive species are major topics of research, little consideration has been given to the implications of introducing non-native subspecies or beneficial organisms such as pollinators. However, the extensive trade in bumble bees as pollinators of glasshouse crops involves both. Within regions in Europe, the importation of non-native commercially reared subspecies of *Bombus terrestris* could endanger native bumble bees through competitive displacement and/or hybridization.

2. This study made a direct ecological comparison between commercially imported and native *B. terrestris* colonies growing in the wild in the UK. In particular, using a paired design, we compared the nectar-foraging performance and reproductive outputs of commercial and native colonies growing under identical field conditions.

3. Commercial colonies performed well in the field, with substantially higher nectar-foraging rates than native colonies in four out of five locations. Nectar-foraging performance was positively correlated with forager size, with commercial bees being consistently larger than native bees.

4. All seven commercial colonies studied produced gynes (new queens), with two colonies each producing in excess of 50. In contrast, only two out of seven native colonies produced gynes, and those only produced small numbers (five in total). Males were produced by all colonies but there were no significant differences in numbers between commercial and native subspecies.

5. *Synthesis and applications.* The high reproductive success of commercial colonies indicates that there is an appreciable risk that they will become established and spread within the UK. Furthermore, their superior foraging ability and large colony size could lead them to out-compete native bumble bees. Clearly the invasive potential of non-native subspecies and/or beneficial organisms should not be overlooked. With respect to the current importation of commercial bumble bees, we strongly recommend a precautionary approach: native species and subspecies should be locally reared and the use/disposal of bees should be strictly regulated.

Key-words: *Bombus terrestris dalmatinus*, *Bombus terrestris audax*, bumble bee trade, colony growth, foraging performance, reproductive success, species introductions, subspecies

Correspondence: Thomas Ings, School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK (fax + 44 20 89830973; e-mail t.c.ings@qmul.ac.uk).
‡Present address: School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK.

Introduction

The introduction of non-native species, whether intentionally or accidentally, is a major component of human-induced global change (Vitousek *et al.* 1997). However, little consideration has been given to non-native subspecies or beneficial organisms such as pollinators (Goulson 2003; Moritz, Hartel & Neumann 2005). Furthermore, in the

face of world-wide declines of pollinators (Kearns, Inouye & Waser 1998; Steffan-Dewenter, Potts & Packer 2005), including bumble bees (Sarospataki, Novak & Molnar 2005; Williams 2005), it is more important than ever to pay closer attention to the potential impacts of the establishment of introduced non-native pollinators.

International trade in bumble bees for crop pollination involves the introduction of both non-native species and subspecies. Despite grave concerns over the potential ecological impact of using non-native bumble bees (Dafni & Shmida 1996; Dafni 1998; Widmer *et al*. 1998; Goka *et al*. 2001; Goulson *et al*. 2002a; Ings, Schikora & Chittka 2005), the importation of commercial bees continues on a large scale in many countries (e.g. Chile, South Korea, and throughout Europe). In fact, there is now clear evidence of the establishment and spread of the non-native *Bombus terrestris* L. as a result of its use in glasshouses in several countries (e.g. in Japan; Matsumura *et al*. 2004; Inari *et al*. 2005).

Within Europe, several subspecies of *B. terrestris* occur. Although these subspecies differ in important traits such as colour and foraging performance (Chittka, Ings & Raine 2004; Ings, Schikora & Chittka 2005), some are being imported into countries where they are non-native (e.g. the UK). While we know little about the risks posed by introducing non-native subspecies, negative impacts have been associated with the introduction of non-native honey bee subspecies (Schneider, Hoffman & Smith 2004; Moritz, Hartel & Neumann 2005), geographically isolated plant populations (Keller, Kollmann & Edwards 2000) and farmed fish (McGinnity *et al*. 2003). Therefore, a closer examination of the dangers posed by importation of non-native subspecies of bumble bees is urgently required.

Currently commercially reared colonies of *Bombus terrestris dalmatinus* (Dalla Torre 1882), sourced in south-eastern Europe, are being imported into the UK, where the resident native subspecies is *Bombus terrestris audax* (Harris 1780). While imported bees are typically used in glasshouses (at least in the UK), inevitably many workers (Morandin *et al*. 2001), males and new queens (gynes) are escaping (Goulson *et al*. 2002a) through unobstructed glasshouse vents and from discarded nests. Therefore, there is a clear risk of non-native subspecies of *B. terrestris* establishing in the UK (Ruz & Herrera 2001; Ruz 2002; Inari *et al*. 2005). In addition, there is also a risk of hybridization and introgression between native and non-native subspecies (Widmer *et al*. 1998; Goulson *et al*. 2002a; Ings, Schikora & Chittka 2005; Ings, Raine & Chittka 2005). Ultimately, the native subspecies (*B. t. audax*) could be displaced by the imported one or both could be replaced by hybrids.

Unfortunately, detecting the establishment of non-native *B. terrestris* subspecies is problematic. Worker bees of imported *B. t. dalmatinus* and native *B. t. audax* are visually hard to distinguish, as both have very similar banding patterns. Although genetic techniques can be successfully used to identify members of different populations (Estoup *et al*. 1996; Widmer *et al*. 1998; Jensen *et al*. 2005), and even colonies (Knight *et al*. 2005),

reliable genetic markers are still being developed for the relevant subspecies of *B. terrestris* (M. Knight, personal communication). Therefore, other methods, such as controlled ecological comparisons, are necessary to assess the continued risk of the establishment of non-natives in the UK.

The number and frequency of releases of reproductive individuals, i.e. the propagule pressure, is important for establishment (Lockwood, Cassey & Blackburn 2005; Memmott *et al*. 2005; Von Holle & Simberloff 2005). Although this information is not known for commercial bumble bees in the UK, an estimated 10 000 colonies are being imported each year (Ings, Schikora & Chittka 2005). The frequency of escapes is also likely to be high, as large glasshouses can use several hundred colonies per year (Inari *et al*. 2005) throughout the growing season (February to November in the UK). Hence a few gynes and/or males escaping from each colony would result in high propagule pressure. Escaping gynes and males must breed successfully to become established, and this has clearly occurred elsewhere (e.g. Chile; Ruz & Herrera 2001; and Japan; Inari *et al*. 2005). Consequently, it is important to determine whether commercially imported bees (*B. t. dalmatinus*) will survive in the wild and to assess what affects they are likely to have on native bumble bee populations.

We addressed this by carrying out a direct ecological comparison between commercially imported (*B. t. dalmatinus*) and native (*B. t. audax*) subspecies of *B. terrestris* growing under natural field conditions. Their performance was assessed by a comparison of nectar-foraging rates (Ings, Schikora & Chittka 2005) and reproductive outputs (production of males and gynes). The ability of commercial bees to exploit resources, i.e. their nectar-foraging rates, also provides a good indication of their competitiveness with other species/subspecies occupying a similar niche (Duyck, David & Quilici 2004).

Methods

This study was carried out in southern England at seven sites located close to Egham, Surrey (51:25:44N, 00:32:52E), 30 km to the west of London. These sites were selected to represent a range of habitats where *B. terrestris* is naturally found: grassland, hedgerow and domestic gardens (allotment and garden); grassland, hedgerow, woodland and domestic gardens (CABI, Silwood and Longcross); landscaped gardens and woodland (valley gardens) and riverside meadow, hedgerow, woodland and domestic gardens (Ankerwycke). As *B. terrestris* typically forages up to 600 m from the nest (Osborne *et al*. 1999), extending this to about 1·7 km for super-abundant resources (Walther-Hellwig & Frankl 2000), sites were located at least 1·9 km apart to reduce the possibility of overlap between foraging territories.

COLONY INITIATION

Native British *B. t. audax* colonies (hereafter native) were reared from 79 nest-searching queens captured during

Table 1. Demography of paired commercial and native colonies. The 'placement' date is when paired colonies were first placed in the field and 'workers at start' indicates the approximate number of workers at the date of placement. Reproductive outputs are summarized by the number and size of males and gynes produced. All summary statistics are means ± 1 SEM

Location (placement date)	Subspecies	Foundress size (mm)	Workers at start*	First Male	First Gyne	Last Male	Last Gyne	Number of males	Male size (mm)	Number of gynes	Gyne size (mm)	Foundress death
Allotment	Native	8·0	40	19/6	–	30/7	–	278	5·8 ± 0·0	0	–	13/8
(27/5)	Commercial	8·2	30	19/7	21/7	17/8	17/8	23	5·9 ± 0·0	57	8·5 ± 0·0	15/7
CABI	Native	8·5	50	22/6	–	28/7	–	224	5·7 ± 0·0	0	–	17/7
(23/5)	Commercial	8·6	40	15/6	11/7	3/8	15/7	358	5·9 ± 0·0	5	8·5 ± 0·0	15/6
Garden	Native	8·6	30	22/5	–	24/7	–	154	5·8 ± 0·0	0	–	30/7
(26/5)	Commercial	8·7	25	14/7	17/7	3/8	3/8	56	5·8 ± 0·0	25	8·1 ± 0·0	4/7
Valley gardens	Native	8·4	30	2/6	–	2/7	–	64	5·1 ± 0·1	0	–	20/7
(25/5)	Commercial	7·7‡	30	24/7	6/7	20/7	17/7	165	5·9 ± 0·0	73	8·4 ± 0·0	7/7
Longcross	Native	8·0	45	2/7	13/7	31/7	17/7	351	5·6 ± 0·0	2	8·0 ± 0·3	18/7
(11/6)	Commercial	7·9‡	50	2/7	13/7	22/7	17/7	349	6·0 ± 0·0	5	8·4 ± 0·1	NA
Silwood	Native	8·4	15	19/6	–	20/7	–	63	5·6 ± 0·0	0	–	17/6
(26/5)	Commercial	8·1	20	19/6	13/7	5/8	13/7	196	6·0 ± 0·0	1	8·7	15/6
Ankerwycke	Native	8·6	45	2/7	13/7	NA	15/7	NA	5·7 ± 0·0†	3†	7·1 ± 0·1†	NA
(7/6)	Commercial	8·6‡	50	26/6	8/7	30/7	13/7	230	5·9 ± 0·0	7	8·3 ± 0·2	13/6
Mean	Native							189 ± 47·7		0·3 ± 0·3		
	Commercial							197 ± 49·1		24·7 ± 10·9		

*Numbers to the nearest 5.
†Colony was vandalized but only three empty gyne cells were found after colony death, so all queens emerged prior to vandalism.
‡Size calculated from fresh mass as it was not possible to measure the queen.

the spring of 2005 (16–27 March) from five locations in southern England: Regents Park (London), Ascot (Berkshire), Egham (Surrey), Windsor Great Park (Surrey) and Nailsea (North Somerset). These were kept in a dark room (at 25–28 °C and 60% relative humidity) in bipartite wooden nest boxes (28 × 16 × 11 cm). Two or three male pupae from another nest, or two artificial clay pupae (orientated vertically side by side on a small bed of clay), were added to the nesting chamber to stimulate nest initiation (Velthuis 2002; Kwon, Saeed & Duchateau 2003). All founding queens and subsequent colonies were fed *ad libitum* with pollen and artificial nectar obtained from Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands). Both of these food sources were removed from all colonies before they were placed in the field.

The seven strongest colonies (3–10 workers and healthy brood) were selected from 20 that had produced their first workers. At the same time, seven small colonies of commercially available *B. t. dalmatinus* (hereafter commercial) were obtained from Koppert Biological Systems. These colonies arrived with their first batch of workers, ranging from three to 20, and were matched with the native colonies for worker number and brood size as closely as possible. The matched pairs of colonies were then transferred into identical larger (40 × 28 × 10 cm) bipartite wooden nest boxes for placement in the field. At this stage all colonies were inoculated with B 401®/CERTAN™ (SWARM SA, Bougival, France), a biological Lepidopteran larvicide to prevent attack by wax moths during the early stages of colony growth in the field. After the CERTAN treatment, all colonies were maintained under identical conditions, i.e. in the dark room with *ad libitum* pollen and artificial nectar,

until placement in the field (12–31 days from the arrival of the commercial colonies). Just before placement, commercial and native colonies were rematched for worker number and brood size, with similar sized colonies placed at each location (Table 1). The final matched pairs of colonies were screened for parasites by microscopic (×400) examination of the faeces of five randomly selected workers from each colony. All colonies were deemed to be free from major gut parasites (e.g. *Crithidia bombi* Lipa & Triggiani 1980 and *Nosema bombi* Fantham & Porter 1914).

Prior to placement in the field, the nest entrance of all colonies was restricted to a diameter of 7 mm to prevent the founding queen and any new unfertilized queens (gynes) escaping and establishing (i.e. setting up feral non-native colonies) as a result of our experiment. In addition, to facilitate the foraging observations, a clear plastic (Liteglaze®, Ariel Plastics Ltd, Staveley, UK) tube (20 × 2·5 × 2·5 cm) with four shutters was attached to the entrance of each nest box. The far end of each tube was fitted with a coloured (either blue or yellow) plastic landing platform (7 cm high by 5 cm wide) to aid bees' entry to the nest and allow them to distinguish the correct nest of each pair. To avoid bias, the colour used for each subspecies in a pair of colonies was alternated for the first six pairs and selected at random for the seventh.

As queens were prevented from foraging, only colonies that had produced at least their second batch of workers (mean 35 colony⁻¹) were placed in the field, to avoid premature colony death (Table 1). At each of the seven locations, a pair of colonies, consisting of one commercial and one native colony, was placed in a sheltered position (under trees/shrubs or artificial shelters). Colonies were positioned at least 2 m apart on wooden tables (60 ×

60×35 cm) and were covered by black polythene-coated cardboard boxes to protect them from rain. As the nest boxes were much larger than the colonies at this stage and night-time temperatures were occasionally low (below 10 °C), the nest chambers were insulated with fleece blankets. To prevent the escape of any non-native reproductive bees, all colonies were examined every 48 h (as males tend to leave the nest 2–4 days after emergence; Bourke 1997) and any males and gynes removed.

FORAGING OBSERVATIONS

Foraging performance was measured for five pairs of colonies, and observations were carried out at least 1 week after the colonies were placed in the field to ensure colonies had used up all stored food reserves and that bees were foraging naturally. Observations were carried out between 14 and 23 June 2005 during fine dry weather (this was on average about 3 weeks prior to the production of males and 4 weeks before gynes). At each site, both colonies in a pair were monitored simultaneously between 06:00 and 19:00 h for 2–3 days (depending upon the weather). Bee traffic was controlled by means of shutters in the entrance tube so that all exiting and returning foragers could be captured and weighed. As far as possible, all bees that wished to forage were allowed to do so.

All foraging bees in each colony were uniquely marked with individually numbered tags (Opalith Plättchen, Christian Graze KG, Weinstadt-Endersbach, Germany) prior to foraging observations. During the observations the following were recorded for each bee: (i) departure time, (ii) departure mass, (iii) arrival time and (iv) arrival mass. Bees were captured at the entrance of the Liteglaze tunnel in a plastic vial as they departed and arrived. They were then transferred to an electronic balance (Ohaus Navigator N20330, Ohaus Corporation, Pine Brook, NJ) to measure their change in body mass. Departure time was taken when the bees were released after weighing and the time of arrival was taken when the bees first reappeared at the tunnel. Bees can forage for nectar only, pollen only or nectar and pollen. However, as bees mostly collected nectar only during this study we focused our attention on nectar foragers. The nectar collected by foraging bees was calculated by subtracting the bees' departure mass from its return mass. Once foraging observations finished for the day the shutters were opened to allow bees to forage freely.

REPRODUCTIVE SUCCESS

Reproductive success was measured for all seven pairs of colonies by counting and measuring all males and gynes they produced (Baer & Schmid-Hempel 2003). A size index of males and gynes was taken by measuring the thorax width at its widest point, just below the wings, using analogue Vernier callipers (accurate to 0·1 mm). Unfortunately, the native colony at one of the sites (Ankerwycke) was vandalized 10 days after the first gynes

emerged. Therefore, it was not possible to determine total male production for that colony, but total queen production was estimated by counting empty queen pupae after the colony died (Thomson 2004). Colonies remained in the field until all males and gynes had emerged and the last worker and founding queen had died (Table 1).

ANALYSIS

To avoid the inclusion of bees dividing their time between nectar and pollen collection, we excluded all bees that carried measurable amounts (greater than 0·01 g) of pollen. In addition, trips of bees with negative returns and those of less than 5 min duration were excluded from the analysis to remove bees making exploration and/or defecation flights (Spaethe & Weidenmüller 2002; Ings, Schikora & Chittka 2005). Only $3·2 \pm 0·6\%$ of flights (out of 1551) resulted in zero or negative nectar returns, with no significant differences between commercial and native bees (paired t-test, $t = -0·02$, $P = 0·49$, d.f. = 4). Finally, for all analyses the average performance of individual bees that foraged on three or more occasions (Spaethe & Weidenmüller 2002; Ings, Schikora & Chittka 2005) was used as the unit of replication. For each of these bees we calculated the mean (over all its trips) mass of nectar collected, trip duration and nectar-foraging rates (milligrams of nectar collected per hour). Mean departure weight of individual foragers was used as an index of body size (Ings, Schikora & Chittka 2005).

As the foraging data violated some of the assumptions implicit in standard analysis of variance, i.e. the variance was proportional to the mean, generalized linear models (GLM) with a gamma error distribution and log-link function were used where appropriate (R 2·0·1 statistical language software; R Development Core Team 2004). Body mass was used as a covariate in the GLM, as nectar-foraging rate of *B. terrestris* is known to be positively related to body size (Goulson *et al*. 2002b; Spaethe & Weidenmüller 2002; Ings, Schikora & Chittka 2005). Paired t-tests were carried out to compare the reproductive output of native and commercial colonies: the data for the gynes were $\ln(x + 0·01)$ transformed to correct for unequal variances and non-normality.

Results

NECTAR-FORAGING PERFORMANCE

The nectar-foraging rates (NFR) of 163 bees making an average of $9 \pm 0·5$ (mean $\pm$ 1 SEM) nectar-foraging trips were assessed for five pairs of colonies. Considerable variation in nectar-foraging rates (Fig. 1a), total nectar collected per trip (Fig. 1b) and trip duration (Fig. 1c) occurred among locations and subspecies. Nectar-foraging rates varied significantly between subspecies and were strongly influenced by location (Fig. 1a; location:subspecies, $F_{4,152} = 6·35$, $P << 0·001$). The greatest NFR occurred in the Valley Gardens, where
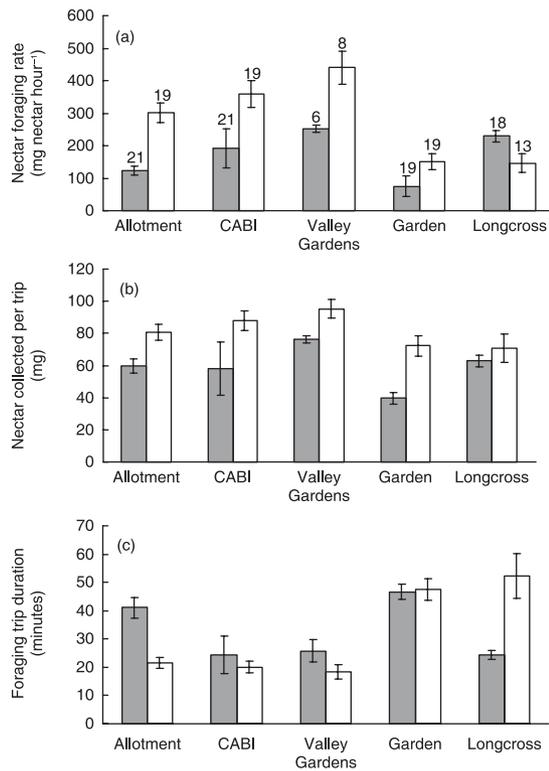
Fig. 1. Comparison of the nectar-foraging parameters between paired native (grey bars) and commercial (white bars) colonies: (a) mean nectar-foraging rates, (b) mean nectar collected per foraging trip, and (c) mean duration of foraging trips. Error bars are ± 1 SEM and the number of foragers per colony are given above bars in (a).

suitable forage, especially *Rhododendron* spp., was abundant. The lowest NFR, less than half those at the Valley Gardens, were observed in the colonies located in the domestic garden (Fig. 1a).
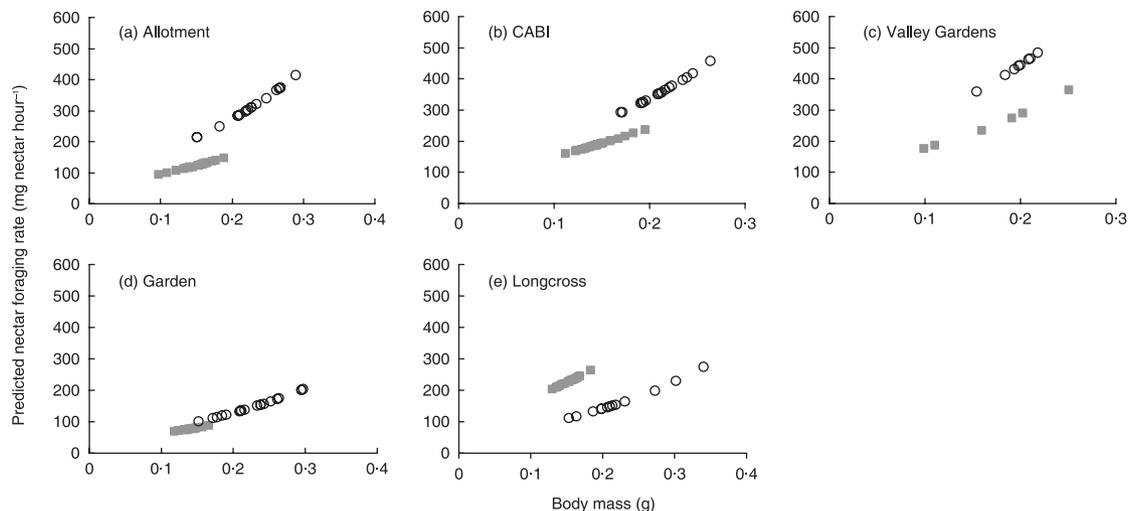
More importantly, commercial bees were substantially better nectar foragers than native bees in four out of five locations (Fig. 1a; Allotment, CABI, Valley Gardens and

Garden; location:subspecies, $F_{4,152} = 6.35$, $P \ll 0.001$). Notably, NFR of commercial bees was more than twice that of native bees in both the poorest (Garden) and richest (Valley Gardens) foraging environments (as reflected by NFR). Furthermore, when viewed at a population level, the average nectar-foraging performance of the commercial bees (279.6 ± 57.8 mg nectar h^{-1}) was much greater than (1.6 times) that of the native bees (174.4 ± 33.1 mg nectar h^{-1}).

Commercial colonies had larger foragers than native colonies, although the relative differences in the body mass of foraging bees varied significantly between pairs of colonies (location:subspecies, $F_{4,153} = 3.3$, $P < 0.05$). The significant interaction reflected the small difference in body mass at the Valley Gardens: foragers from commercial colonies were only 1.2 times heavier on average than native ones compared with 1.4 (CABI), 1.5 (Longcross and Allotment) and 1.7 (Garden) times heavier at the other locations.

After accounting for body mass (GLM; NFR ~ location + subspecies + location:subspecies + startweight), differences in NFR among subspecies varied between locations (location:subspecies, $F_{4,153} = 5.91$, $P < 0.05$). Commercial bees were better nectar foragers than native bees in three out of five locations (Allotment, CABI and Valley Gardens; Fig. 2a–c). Native bees only outperformed commercial bees at Longcross (Fig. 2e), with no differences being evident at the Garden site (Fig. 2d).

The absolute amount of nectar collected per foraging trip was significantly positively related to body mass ($F_{1,156} = 30.68$, $P \ll 0.001$), with the larger commercial bees collecting more nectar than the smaller native ones (Fig. 1b). Conversely, the duration of foraging trips was not affected by body mass but varied significantly between subspecies and pairs of colonies (location:subspecies, $F_{4,153} = 13.86$, $P \ll 0.001$). Commercial bees had shorter foraging trips than native bees at the Allotment and Valley Gardens, and similar length trips at the Garden and CABI (Fig. 1c). Notably, at Longcross commercial

© 2006 The Authors.
Journal compilation
© 2006 British
Ecological Society,
*Journal of Applied
Ecology*, **43**,
940–948

Fig. 2. Summary of predicted nectar-foraging performance plotted against body mass (GLM with gamma error distribution and log link function) for paired commercial (open symbols) and native (closed symbols) colonies.

bees had substantially longer nectar-foraging trips than native bees (Fig. 1c; $52 \cdot 3 \pm 7 \cdot 9$ and $24 \cdot 4 \pm 2 \cdot 7$ min, respectively).

REPRODUCTIVE SUCCESS

Reproductive output varied substantially between colonies both within and between subspecies (Table 1). All colonies produced males and although production varied between colonies (Table 1) no significant differences were evident between subspecies (Paired *t*-test, $t = -0 \cdot 034$, $P = 0 \cdot 974$, d.f. = 5). Males produced by commercial colonies ($5 \cdot 9 \pm 0 \cdot 0$ mm) were slightly, but significantly (paired *t*-test, $t = -2 \cdot 48$, $P < 0 \cdot 05$, d.f. = 6), larger than those produced by native colonies ($5 \cdot 6 \pm 0 \cdot 1$ mm).

Commercial colonies produced significantly more gynes than native colonies (paired *t*-test, $t = 3 \cdot 59$, $P < 0 \cdot 05$, d.f. = 6). In fact, all commercial colonies produced at least one gyne ($24 \cdot 7 \pm 10 \cdot 9$) yet only two native colonies, those at Longcross and Ankerwycke, produced two and three gynes, respectively. Furthermore, two of the commercial colonies, those at Valley Gardens and Allotment, produced 73 and 57 gynes, respectively (Table 1). Although too few native gynes were produced to compare their size statistically with commercial gynes, those from commercial colonies (mean thorax width 8·4 mm) tended to be larger than those from native colonies (7·6 mm) (Table 1).

A significant positive relationship between NFR and gyne production was apparent across the five pairs of colonies tested ($r = 0 \cdot 662$, $P < 0 \cdot 05$, d.f. = 10). Colonies with poor nectar-foraging performance (below 250 mg nectar h^{-1}) produced few or no gynes and only those with high nectar-foraging rates (above 300 mg nectar h^{-1}) produced large numbers (> 30) of gynes.

Discussion

The results presented here clearly show that commercially imported *B. terrestris* colonies are able to survive and grow outside of glasshouses in the UK. In particular, we have shown that not only are commercial bees able to forage for nectar successfully in the wild, in most cases they substantially outperform native bees. Furthermore, we have shown that commercial colonies will frequently produce many more gynes (new queens) than native colonies growing in the same environment. These results are therefore of great importance for policy makers, who should give serious consideration to the continued risks posed by the importation of non-native subspecies of *B. terrestris* to pollinate glasshouse crops. The results highlight the need for conservationists and policy makers to move beyond the invasive 'species' concept and consider non-native 'subspecies' as potentially posing an invasive risk.

FORAGING PERFORMANCE: COMPETITION

In this study, the higher nectar-foraging rate of commercial bees was related to their large size (Goulson *et al.* 2002b; Spaethe & Weidenmüller 2002; Ings, Schikora & Chittka 2005) compared with native bees. There were some differences in nectar-foraging performance between subspecies after the effects of body size had been accounted for, but these were inconsistent. Bearing in mind the small sample size, this suggests that forager size is indeed the most important factor influencing nectar-foraging rates. While it is possible that the size of some foragers was influenced by *ad libitum* feeding prior to placement in the field, both subspecies were given access to the same food supplies. Also, a large part of their development (> 14 days out of an average 22-day development period; Duchateau & Velthuis 1988; Yeninar *et al.* 2000) occurred in the field. Similarly, in laboratory colonies, commercial (*B. t. dalmatinus*) foragers are consistently larger than native (*B. t. audax*) foragers (mean thorax width $5 \cdot 4 \pm 0 \cdot 02$ and $4 \cdot 6 \pm 0 \cdot 03$ mm, respectively, $t = -20 \cdot 4$, $P < 0 \cdot 001$, d.f. = 366, from 15 native and eight commercial colonies; T. C. Ings, N. E. Raine & L. Chittka unpublished data). So what implications do these differences in forager size and foraging performance between subspecies have for the possible establishment and spread of commercial bees?

Our data clearly support the notion that size affects foraging performance and may be important in exploitation competition (Petren & Case 1996; Duyck, David & Quilici 2004). Larger commercial bees are likely to be able to utilize nectar resources more effectively than native bees. Furthermore, although it could be argued that native colonies may be as effective by producing more, smaller foragers, commercial colonies tend to produce more workers than native colonies (e.g. native = $104 \cdot 6 \pm 12 \cdot 7$, *n* = 26 colonies; N. E. Raine & T. C. Ings, unpublished data; commercial = $225 \cdot 3 \pm 30 \cdot 9$, *n* = 11 colonies; Gosterit & Gurel 2005). Hence a commercial colony with a large number of superior nectar foragers will have a greater impact on local nectar resources than a native colony. Although providing evidence of competition between introduced bees and native bees is complex (Goulson 2003), some studies indicate that honey bees can have negative impacts on bumble bees through competition for food (Thomson 2004; Forup & Memmott 2005). Non-native bumble bees can also impact on native bees through resource depletion (Dafni & Shmida 1996; Hingston & McQuillan 1999). Furthermore, foraging *B. ignitus* adjust their foraging ranges when interacting with conspecifics (Makino & Sakai 2005), and bumble bee species with similar foraging niches can displace one another through resource competition (Pyke 1982). Therefore, if large numbers of colonies of commercial *B. t. dalmatinus* become established (as in Japan; Inari *et al.* 2005) they could have deleterious effects on local populations of native *B. t. audax* and other bumble bee species through competition for food.

ESTABLISHMENT: REPRODUCTIVE SUCCESS

Although commercial bees are highly competitive foragers, it is also important to consider whether they

produce reproductive offspring when growing in the field. All commercial colonies produced both males and gynes, and were far superior to native colonies at producing gynes. Interestingly, gyne production (of all colonies) was positively correlated with nectar-foraging rates. However, this must be interpreted carefully as the number of colonies was relatively small and other factors, i.e. the early death of founding queens, can strongly influence gyne production. Yet, although provision of pollen, as the sole protein source, is perhaps more important for sexual production, large amounts of energy in the form of nectar are also required to produce gynes (Sladen 1989; Beekman, van Stratum & Lingeman 1998).

Furthermore, gynes produced by commercial colonies were the largest (thorax width 8·1–8·7 mm), especially where nectar-foraging rates were high. In fact they were at least as big as native queens that survive natural hibernation and produce colonies (7·5–8·8 mm; T. C. Ings, unpublished data). Therefore, unless commercial bees have substantially lower resistance to pathogens or climate during diapause, there is a high risk that commercial gynes after mating will survive to produce new colonies. In fact, mated *B. terrestris* queens escaping from glasshouses have already successfully hibernated and established wild populations in Japan (Inari *et al.* 2005), which has a similar climate to the UK and pathogens potentially novel for the introduced *B. terrestris*. In addition, the reproductive output of feral *B. terrestris* colonies in Japan is more than four times that of native bumble bee species (Matsumura *et al.* 2004). Therefore, the combination of high reproductive outputs and potentially high survival rates could lead to rapid establishment of commercial colonies.

However, before management and policy recommendations can be made, we need to evaluate carefully how representative our results are of the true situation. Although the limited number of colonies used may not capture all the variation that is known to occur between colonies (Schmid-Hempel & Schmid-Hempel 1998; Raine *et al.* 2005), the patterns between subspecies were consistent. Another potential concern is whether the native colonies in this study were truly representative of the natural population. Was their poor foraging performance and low gyne production an experimental artefact? In fact, low gyne production by wild caught queens reared in captivity has been reported elsewhere (Yeninar *et al.* 2000; Gosterit & Gurel 2005). Perhaps native bees do not take to nest boxes well in the first generation and commercial bees have become adapted to artificial nest boxes after several generations of captive rearing. This is possible, but wild-caught queens reared in artificial nest boxes do produce more gynes than those reared in the laboratory for several generations (data from Müller and Schmid-Hempel in Beekman, van Stratum & Lingeman 1998). In addition, our colonies were the strongest ones produced by queens randomly caught at several locations. Therefore, we assume that they were representative of the wild population of *B. t. audax*

in the UK. Furthermore, the ability of commercial colonies to grow rapidly in the wild and produce appreciable numbers of gynes and males is in itself, sufficient to raise concerns about their invasive potential.

A final point that needs to be considered is that the commercial colonies used in this study were produced by queens that had undergone artificial diapause. The high gyne production of these colonies may be a function of a short diapause (Beekman & van Stratum 1998) as breeders are likely to minimize diapause length to increase turnaround. In further generations, queens undergoing longer, natural diapause, may not be so productive. However, Mediterranean populations of *B. terrestris*, the source of commercial bees, typically have at least two generations per year (Rasmont *et al.* 2005), and possibly three in some areas (P. Rasmont, personal communication). In fact, native *B. terrestris* queens are now initiating colonies within the UK during the autumn without any obvious diapause (T. C. Ings, personal observation). This flexibility in phenology shown by *B. terrestris* suggests that diapause length may not be a restrictive factor for gyne production in future generations of commercial bees. Furthermore, it is believed that a few maternal lines are responsible for gyne production in *B. terrestris*, with many colonies producing only males (Gerloff & Schmid-Hempel 2005). Gyne-producing lines were probably selected from the first generation of commercial bees, so gyne production is likely to persist into future generations of commercial strains if they become established in the wild.

SUMMARY AND RECOMMENDATIONS

This study has highlighted the importance of considering the potential risks posed by importation of non-native subspecies of beneficial organisms. We have shown that the non-native commercially imported subspecies of *B. terrestris* are likely to become established in the UK. In fact, considering the large volume of colonies imported over the last two decades, it seems inevitable that feral colonies may already exist. Furthermore, the data on nectar-foraging performance show that commercial bees are highly effective foragers and that this translates into high reproductive outputs. Therefore, commercial bees are likely to be more competitive than native *B. terrestris.* Although difficult to predict, there is a real risk that feral commercial bees, or their hybrids, could displace native *B. terrestris* and, perhaps more importantly, other already threatened species of bumble bee in the UK. It is therefore essential that additional studies using genetic techniques are carried out to determine the extent to which commercial bees have become established, the extent of their spread, and the degree of introgression that may have already occurred (Jensen *et al.* 2005). We would also recommend a precautionary approach to prevent further release of non-native subspecies of *B. terrestris* into the UK and elsewhere in Europe. A number of recommendations are briefly discussed below.

An obvious way of preventing further release of commercial bees into the UK is to ban their use. However, the use of bumble bees by growers is of considerable economic importance. A less effective, but perhaps viable, option would be to tighten up security over the use of bees in glasshouses. For example, queen excluders should be compulsory on all imported nests. Although some breeders have these fitted already, they are too large (more than 10 mm); the diameter should be no more than 7 mm. Unfortunately queen excluders will allow males to escape unless they are regularly removed, which would not be realistic for growers. Use of mesh to cover glasshouse vents could reduce male escapes, but realistically it would not be possible to prevent escape totally as all escape routes cannot be blocked (e.g. opening doors). Disposal of old nests should also be controlled as these often still contain gynes and males that can escape if nests are not disposed of properly, such as killing by freezing for a few days.

While the measures suggested above would reduce the release of non-native subspecies, they would not halt it altogether. Therefore, an obvious alternative solution would be to enforce the rearing of native subspecies. Similar legislation is active in the Canary Islands and Norway and will come into force in Japan in the spring of 2006. However, in Japan breeders have had the opportunity to select and rear an alternative species. Therefore, taking this route may be most effective by initially combining it with stricter regulation on the use and disposal of imported colonies. Furthermore, as the spread of novel parasites is a potential problem (Goka *et al.* 2001), enforcement of quarantined local rearing would be essential.

Acknowledgements

References

Baer, B. & Schmid-Hempel, P. (2003) Effects of selective episodes in the field on life history traits in the bumblebee *Bombus terrestris*. *Oikos*, **101**, 563–568.

Beekman, M. & van Stratum, P. (1998) Bumblebee sex ratios: why do bumblebees produce so many males? *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1535–1543.

Beekman, M., van Stratum, P. & Lingeman, R. (1998) Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). *Entomologia Experimentalis et Applicata*, **89**, 207–214.

Bourke, A.F.G. (1997) Sex ratios in bumble bees. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **352**, 1921–1932.

Chittka, L., Ings, T.C. & Raine, N.E. (2004) Chance and adaptation in the evolution of island bumblebee behaviour. *Population Ecology*, **46**, 243–251.

Dafni, A. (1998) The threat of *Bombus terrestris* spread. *Bee World*, **79**, 113–114.

Dafni, A. & Shmida, A. (1996) The possible ecological implications of the invasion of *Bombus terrestris* (L.) (Apidae) at Mt Carmel, Israel. *The Conservation of Bees* (eds A. Matheson, S.L. Buchmann, C. O'Toole, P. Westrich & I.H. Williams), pp. 183–200. Academic Press, London, UK.

Duchateau, M.J. & Velthuis, H.H.W. (1988) Development and reproductive strategies in *Bombus* colonies. *Behaviour*, **107**, 186–207.

Duyck, P.-F., David, P. & Quilici, S. (2004) A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecological Entomology*, **29**, 511–520.

Estoup, A., Solignac, M., Cornuet, J.-M., Goudet, J. & Scholl, A. (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Molecular Ecology*, **5**, 19–31.

Forup, M.L. & Memmott, J. (2005) The relationship between the abundances of bumblebees and honeybees in a native habitat. *Ecological Entomology*, **30**, 47–57.

Gerloff, C., U. & Schmid-Hempel, P. (2005) Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera: Apidae). *Oikos*, **111**, 67–80.

Goka, K. Okabe, K. Yoneda, M. & Niwa, S. (2001) Bumblebee commercialization will cause worldwide migration of parasitic mites. *Molecular Ecology*, **10**, 2095–2099.

Gosterit, A. & Gurel, F. (2005) Comparison of development patterns of imported and native *Bombus terrestris* L. (Hymenoptera: Apidae) colonies in the Mediterranean coastal region. *Turkish Journal of Veterinary and Animal Sciences*, **29**, 393–398.

Goulson, D. (2003) Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution and Systematics*, **34**, 1–26.

Goulson, D. Hughes, W.O.H. Derwent, L.C. & Stout, J.C. (2002a) Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. *Oecologia*, **130**, 267–273.

Goulson, D. Peat, J. Stout, J.C. Tucker, J. Darvill, B. Derwent, L.C. & Hughes, W.O.H. (2002b) Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Animal Behaviour*, **64**, 123–130.

Hingston, A.B. & McQuillan, P.B. (1999) Displacement of Tasmanian native megachilid bees by the recently introduced bumblebee *Bombus terrestris* (Linnaeus, 1758) (Hymenoptera: Apidae). *Australian Journal of Zoology*, **47**, 59–65.

Inari, N. Nagamitsu, T. Kenta, T. Goka, K. & Hiura, T. (2005) Spatial and temporal pattern of introduced *Bombus terrestris* abundance in Hokkaido, Japan, and its potential impact on native bumblebees. *Population Ecology*, **47**, 77–82.

Ings, T.C. Raine, N.E. & Chittka, L. (2005) Mating preference of commercially imported bumblebees (*Bombus terrestris*) in Britain (Hymenoptera: Apidae). *Entomologia Generalis*, **28**, 233–238.

Ings, T.C. Schikora, J. & Chittka, L. (2005) Bumblebees, humble pollinators or assiduous invaders? A population comparison of foraging performance in *Bombus terrestris*. *Oecologia*, **144**, 508–516.

Jensen, A.B. Palmer, K.A. Boomsma, J.J. & Pedersen, B.V. (2005) Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. *Molecular Ecology*, **14**, 93–106.

Kearns, C.A. Inouye, D.W. & Waser, N.M. (1998) Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology, Evolution and Systematics*, **29**, 83–112.

Keller, M. Kollmann, J. & Edwards, P.J. (2000) Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology*, **37**, 647–659.

Knight, M.E. Martin, A.P. Bishop, S. Osborne, J.L. Hale, R.J. Sanderson, R.A. & Goulson, D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Molecular Ecology*, **14**, 1811–1820.

Kwon, Y.J. Saeed, S. & Duchateau, M.J. (2003) Stimulation of colony initiation and colony development in *Bombus terrestris* by adding a male pupa: the influence of age and orientation. *Apidologie*, **34**, 429–437.

Lockwood, J.L. Cassey, P. & Blackburn, T. (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution*, **20**, 223–228.

McGinnity, P. Prodohl, P. Ferguson, A. Hynes, R.O. Maoileidigh, N. Baker, N. Cotter, D. O'Hea, B. Cooke, D. Rogan, G. Taggart, J. & Cross, T. (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 2443–2450.

Makino, T.T. & Sakai, S. (2005) Does interaction between bumblebees (*Bombus ignitus*) reduce their foraging area? Bee-removal experiments in a net cage. *Behavioral Ecology and Sociobiology*, **57**, 617–622.

Matsumura, C. Nakajima, M. Yokoyama, J. & Washitani, I. (2004) High reproductive ability of an alien bumblebee invader, *Bombus terrestris* L., in the Hidaka region of southern Hokkaido, Japan. *Japanese Journal of Conservation Ecology*, **9**, 93–102.

Memmott, J. Craze, P.G. Harman, H.M. Syrett, P. & Fowler, S.V. (2005) The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, **74**, 50–62.

Morandin, L.A. Laverty, T.M. Kevan, P.G. Khosla, S. & Shipp, L. (2001) Bumble bee (Hymenoptera: Apidae) activity and loss in commercial tomato greenhouses. *Canadian Entomologist*, **133**, 883–893.

Moritz, R.F.A. Hartel, S. & Neumann, P. (2005) Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience*, **12**, 289–301.

Osborne, J.L. Clark, S.J. Morris, R.J. Williams, I.H. Riley, J.R. Smith, A.D. Reynolds, D.R. & Edwards, A.S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology*, **36**, 519–533.

Petren, K. & Case, T.J. (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology*, **77**, 118–132.

Pyke, G.H. (1982) Local geographic distributions of bumblebees near Crested Butte, Colorado: competition and community structure. *Ecology*, **63**, 555–573.

Raine, N.E. Ings, T.C. Ramos-Rodriguez, O. & Chittka, L. (2005) Intercolony variation in learning performance of a wild British bumblebee population (Hymenoptera: Apidae: *Bombus terrestris audax*). *Entomologia Generalis*, **28**(4).

Rasmont, P. Regali, A. Ings, T.C. Lognay, G. Baudart, E. Marlier, M. Delcarte, E. Viville, P. Marot, C. Falmagne, P. Verhaeghe, J.C. & Chittka, L. (2005) Analysis of pollen and nectar of *Arbutus unedo* as a food source for *Bombus terrestris*

(Hymenoptera: Apidae). *Journal of Economic Entomology*, **98**, 656–663.

R Development Core Team (2004) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

Ruz, L. (2002) Bee pollinators introduced to Chile: a review. *Pollinating Bees. The Conservation Link Between Agriculture and Nature* (eds P.G. Kevan & V.L. Imperatriz-Fonesca), pp. 155–167. Ministry of Environment, Brasila, Brazil.

Ruz, L. & Herrera, R. (2001) Preliminary observations on foraging activities of *Bombus dahlbomii* and *Bombus terrestris* (Hym: Apidae) on native and non-native vegetation in Chile. *Acta Horticulturae*, **561**, 165–169.

Sarospataki, M., Novak, J. & Molnar, V. (2005) Assessing the threatened status of bumble bee species (Hymenoptera: Apidae) in Hungary, central Europe. *Biodiversity and Conservation*, **14**, 2437–2446.

Schmid-Hempel, R. & Schmid-Hempel, P. (1998) Colony performance and immunocompetence of a social insect, *Bombus terrestris*, in poor and variable environments. *Functional Ecology*, **12**, 22–30.

Schneider, S.S., Hoffman, G.D. & Smith, D.R. (2004) The African honey bee: factors contributing to a successful biological invasion. *Annual Review of Entomology*, **49**, 351–376.

Sladen, F.W.L. (1989) *The Humble-Bee.* Logaston Press, Little Logaston, UK.

Spaethe, J. & Weidenmüller, A. (2002) Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Sociaux*, **49**, 142–146.

Steffan-Dewenter, I., Potts, S.G. & Packer, L. (2005) Pollinator diversity and crop pollination services are at risk. *Trends in Ecology and Evolution*, **20**, 651.

Thomson, D. (2004) Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology*, **85**, 458–470.

Velthuis, H.H.W. (2002) The historical background of the domestication of the bumble-bee, *Bombus terrestris*, and its introduction in agriculture. *Pollinating Bees. The Conservation Link Between Agriculture and Nature* (eds P. Kevan & V.L. Imperatriz Fonseca), pp. 177–184. Ministry of Environment, Brasila, Brazil.

Vitousek, P.M., Dantonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.

Von Holle, B. & Simberloff, D. (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology*, **86**, 3212–3218.

Walther-Hellwig, K. & Frankl, R. (2000) Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. *Journal of Applied Entomology*, **124**, 299–306.

Widmer, A., Schmid-Hempel, P., Estoup, A. & Scholl, A. (1998) Population genetic structure and colonization history of *Bombus terrestris* s. I. (Hymenoptera: Apidae) from the Canary Islands and Madeira. *Heredity*, **81**, 563–572.

Williams, P. (2005) Does specialization explain rarity and decline British bumblebees? A response to Goulson *et al.* *Biological Conservation*, **122**, 33–43.

Yeninar, H., Duchateau, M.J., Kaftanoglu, O. & Velthuis, H. (2000) Colony developmental patterns in different local populations of the Turkish bumble bee, *Bombus terrestris dalmatinus*. *Journal of Apicultural Research*, **39**, 107–116.