

1
2
3
4
AQ1
6
AQ2
AQ3
9
AQ4
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
AQ5
58

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

Evidence for hilltopping in bumblebees?

DAVE GOULSON, E.L. SANGSTER and J.C. YOUNG School of Biological and Environmental Sciences, University of Stirling, Stirling, U.K.

Abstract. Male bumblebees are known to exhibit a range of mate-location behaviours, including perching on prominent objects and darting at passing queens, patrolling of scent-marked flight routes, and waiting outside nest entrances for virgin queens to emerge. Here we provide evidence for a fourth strategy, known as hilltopping. This behaviour is widely known from a range of invertebrates, but has not previously been described in bumblebees. We studied the distribution of bumblebees along transects ascending four hills in Scotland and demonstrate that, relative to workers, males of four bumblebee species or species groups (*Bombus lapidarius*, *B. monticola*, *B. pascuorum*, and *B. lucorum/magnus/cryptarum/terrestris*) tend to congregate at or near the tops of hills. This is, to our knowledge, the first evidence for hilltopping in bumblebees and the first record of any putative mate-locating behaviour for male *B. pascuorum*, a very common species in Europe. However, we note that, in common with most previous studies of mate-locating behaviour in bumblebees, attraction of virgin queens and mating were not observed.

Key words. Apidae, *Bombus*, Hymenoptera, male aggregation, mate location, vertical stratification.

Introduction

The genus *Bombus* comprises approximately 250 species worldwide which exhibit a varied and somewhat enigmatic range of mate location activities (reviewed in Goulson, 2010). Males of a small number of species are highly territorial and aggressive, pursuing females from scent-marked look-out posts (e.g. *Bombus confusus*, *B. mendax*, *B. nevadensis*, *B. griseocollis*, and *B. rufocinctus*) (Saunders, 1909; Krüger, 1951; Schremmer, 1972; Haas, 1976; Alcock & Alcock, 1983; O'Neill *et al.*, 1991). Other species, including *B. subterraneus*, *B. californicus*, *B. sonorus*, *B. fervidus*, *B. muscorum*, and *B. ruderarius*, have been seen to stake out the entrance to nests from which young queens are about to emerge (Smith, 1858; Tuck, 1897; Krüger, 1951; Postner, 1951; Lloyd, 1981; Free, 1987; Villalobos & Shelly, 1987; Foster, 1992; Darvill & Goulson, 2007). A swarm of males may be observed outside each nest, and when a queen emerges they fight furiously, and the males may also pursue her back into the nest. The most common mate-location strategy involves males patrolling a regular circuit of a few hundred metres in length, along which they have marked a number of objects such as twigs or leaves with a species-specific pheromone secreted from

their labial gland. This was described in detail by Darwin (1886) in *B. hortorum*, and has since been observed in many others including *B. sylvestris*, *B. bohemicus*, *B. pascuorum*, *B. lapidarius*, *B. terrestris*, and *B. lucorum* (Haas, 1949; Krüger, 1951; Awram, 1970; Bringer, 1973; Svensson, 1979; Bergström *et al.*, 1981; Prys-Jones & Corbet, 1991; Bergman & Bergström, 1997). An interesting feature of this behaviour is that many males of the same species may follow the same route, so that streams of them pass by any particular point. However, virgin queens have not been observed to be attracted to these patrolling routes (discussed in Goulson, 2010).

An as yet unexplained phenomenon is that mate location behaviour is very rarely seen in some common bumblebee species. For example *B. pascuorum* is abundant in much of Europe, but its mate-locating mechanism is poorly known (Awram, 1970; Fussell & Corbet, 1992). It may be that there are other mating systems used by bumblebees that are not easily observed (Williams, 1991).

Based purely on anecdotal evidence, Goulson (2010) suggests that *B. lucorum* and *B. sylvestris* may exhibit hilltopping, a phenomenon well known from other insects such as butterflies, flies, and wasps whereby any insect seeking a mate heads to the top of the nearest hill, a behaviour that serves to concentrate sexually active individuals (e.g. Alcock, 1994, 2007; Alcock & Kemp, 2006). Here, we examine the relative distribution of worker versus male bumblebees along transects that

Correspondence: Dave Goulson, School of Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, U.K. E-mail: dave.goulson@stir.ac.uk

ascend hills in Scotland, with the aim of testing whether male bumblebees do indeed aggregate at the tops of these hills.

Methods

Transects were set up from the bottom to the top of four small but prominent hills in Central Scotland, UK, each of which rises sharply from a substantial lowland area and is visible from a distance of many kilometres. These were Stirling Castle (altitude 89 m, 56°07'26"N, 3°56'53"W), Wallace Monument (altitude 99 m, 56°08'19"N, 3°55'04"W), Dumyat Hill (altitude 389 m, 56°09'27"N, 3°52'34"W), and Loudon Hill (altitude 279 m, 55°36'53"N, 4°12'43"W). The habitats encompassed by the transects were varied; Stirling Castle was mostly grassland and scrub close to an urban area; Wallace Monument was along woodland edge; Dumyat Hill was through acidic grassland and heath; Loudon Hill was rough grassland, scrub, and woodland edge. The transects were divided into four sections of equal length, hereafter described as lower, middle, upper, and top. Each transect was visited eight times during August and September 2010, and walked in a random direction at a steady pace, recording and counting the species and caste of all bumblebees within 2 m either side. When in doubt, bees were captured for closer inspection and then released. Workers of the closely related species *B. terrestris*, *B. lucorum*, *B. magnus*, and *B. cryptarum* cannot be reliably separated in the field, so these species were pooled for data analysis and are henceforth described as the *B. terrestris* group.

The counts of bees within each section of the four transects were analysed using a generalised linear model with Poisson errors with a log link function in PASW Statistics version 17.0, using position on the hill, sex, and species as explanatory factors, with all two-way and the three-way interactions.

Results

In total 507 bumblebees (254 workers and 275 males) of seven species or species groups were recorded: *B. terrestris* group ($n = 196$), *B. lapidarius* ($n = 132$), *B. pascuorum* ($n = 114$), *B. monticola* ($n = 41$), *B. pratorum* ($n = 10$), *B. sylvestris* ($n = 8$), and *B. hortorum* ($n = 4$). For all species and hills pooled, the proportion of males to workers differed according to height, with the lowest proportion of males at the bottom of the hills (0.289) and the greatest in the two highest sections (0.634 for the upper section and 0.614 for the hilltop) (χ^2 test of association, $\chi^2_2 = 36.0$, $P < 0.001$, Fig. 1). For species-level analysis using the generalised linear model, *B. pratorum*, *B. sylvestris*, and *B. hortorum* were excluded as sample sizes were small. In accordance with the simple χ^2 test, there was a significant two-way interaction between sex and position on the hill, with more males and fewer workers in the higher sections of the transects ($\chi^2_2 = 23.2$, $P > 0.001$, Fig. 2). However, there was no significant three-way interaction, suggesting that all four species included in the model exhibit the same trend of increasing proportions of males at higher altitude on the hills ($\chi^2_2 = 9.09$, $P = 0.429$). Although the trend for *B. lapidarius* appears to be less convincing than for the other three species,

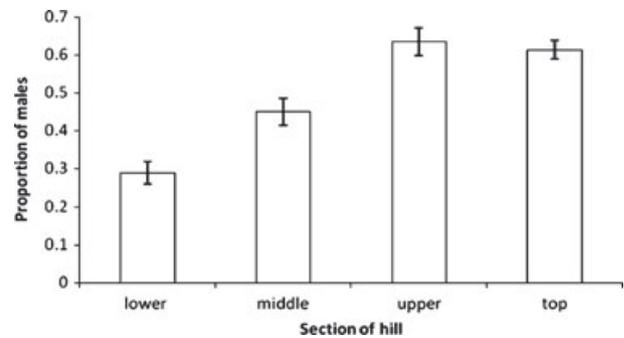


Fig. 1. The proportion of males of all bumblebees recorded according to position along a linear transect running from the bottom to the top of four hills (hills and bee species pooled) (\pm SE).

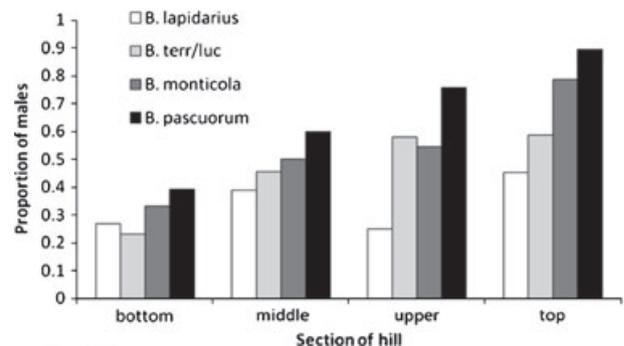


Fig. 2. The proportion of males of four bumblebee species recorded according to position along a linear transect running from the bottom to the top of four hills (hills pooled).

for all four species the largest proportion of males was recorded at the tops of the hills (Fig. 2). There was also a significant two-way interaction between sex and species, indicating that the ratio of males to workers differed between the four species ($\chi^2_3 = 24.1$, $P > 0.001$, proportion of males 0.36, 0.48, 0.59, and 0.67 for *B. lapidarius*, *B. terrestris/lucorum*, *B. monticola*, and *B. pascuorum*, respectively).

Discussion

This study shows that male bumblebees become proportionately more abundant, relative to workers, at or near the tops of hills compared with the bottom. This pattern held true for four species or species groups, *B. lapidarius*, *B. monticola*, *B. pascuorum*, and the *B. terrestris* group. This pattern could conceivably be explained if the hilltops tended to provide flowers that males prefer, while the lower areas tended to provide flowers that workers prefer. As a broad generalisation, males and workers do differ in their foraging preferences, with males generally favouring Asteraceae such as *Centaurea* and *Cirsium* spp., and workers tending to favour Fabaceae, although there is considerable overlap and flexibility (e.g. Goulson *et al.*, 2005, 2008). Although the vegetation was not quantified in detail, this possibility seems unlikely because Asteraceae are not generally associated with hilltops and vegetation did not

change markedly along the transects. For example, the transect at Dumyat Hill was dominated by acidic grasslands and heather moorland, with *Calluna* and *Erica* providing the only significant sources of forage for bumblebees. The Wallace Monument transect was entirely through woodland, following a broad track, with *Epilobium* spp. the main source of forage. An alternative explanation might be that bumblebees have an innate tendency to forage uphill. For montane species it might make sense for workers to travel uphill from their nest so that their fully laden return journey is downhill, and it is conceivable that this might have non-adaptive consequences for male behaviour which, since males do not have to return to their nest, could result in them accumulating at hill tops. However, we suggest that the most likely explanation for the predominance of males at hilltops is that bumblebees use hilltopping as a mating strategy, as many other insects are known to do, with males and virgin females gravitating towards the highest local point.

As with almost all studies of mate location strategies in bumblebees, an obvious weakness of our study is that no mating was observed, or indeed any queens. However, the sex ratio of bumblebees is heavily male biased (reviewed in Bourke, 1997; see also Beekman & Van Stratum, 1998) and virgin queens are thought to find a mate swiftly and rest while mating in dense vegetation, so observing the encounters between males and virgin queen is inevitably rare even while the efforts of males to find females may be conspicuous (for example when males patrol scent marks). It might be informative in this respect to use harmonic radar tags (Riley *et al.*, 1999) to follow virgin queens, although estimates of queen movement suggest that they may rapidly travel beyond the ~700 m detection range of this technology (Lepais *et al.*, 2010). It would also be valuable to study the behaviour of males directly, using mark-recapture to examine how long they remain on hill tops, and also to collect data on their behaviour at hill tops. The males recorded in this study were not actively involved in any obvious mate-locating activity, but were mostly feeding or resting on flowers. Further studies are required before we can conclude beyond doubt that the accumulation of male bumblebees on hilltops is a behaviour related to mate location.

The distribution of different mate-location behaviours in bumblebees does not appear to correspond to phylogeny. For example, when compared against the phylogeny of Cameron *et al.* (2007), bumblebees that are territorial fall into five separate subgenera, while patrolling species fall within eight subgenera (O'Neill *et al.*, 1991). Some of the species that we suggest are hilltopping are also known to patrol scent-marked routes (e.g. *B. lucorum*, *B. lapidarius*, Haas, 1949; Awram, 1970), and it seems likely that the strategy used may be flexible and vary according to circumstance (for example, there are not always prominent hills present). As noted in the Introduction, prior to this study we had very little information on the mating behaviour of *B. pascuorum*, one of the most abundant species in Europe. It exhibits the most marked bias towards males being found on hilltops of the four species found in sufficient abundance to analyse, so it may be that hilltopping is the primary mate location mechanism in this species. In lowland areas it might use prominent landmarks such as the tops of tall trees, which could explain the paucity of observations.

For many bumblebee species, including some common ones, there remain no records of their mate-location strategy, and this remains an intractable but fascinating subject that challenges the ingenuity of future researchers.

Acknowledgements

We would like to thank two anonymous reviewers for their comments.

References

- Alcock, J. (1994) Alternative mate-locating tactics in *Chlosyne californica* (Lepidoptera, Nymphalidae). *Ethology*, **97**, 103–118.
- Alcock, J. (2007) Hilltopping behavior by three species of *Tachytes* wasps (Hymenoptera: Crabronidae). *Journal of the Kansas Entomological Society*, **80**, 361–368.
- Alcock, J. & Alcock, J.P. (1983) Male behaviour in two bumblebees, *Bombus nevadensis auricomus* and *B. griseicollis* (Hymenoptera: Apidae). *Journal of Zoology*, **200**, 561–570.
- Alcock, J. & Kemp, D.J. (2006) The hilltopping mating system of *Leschenaultia adusta* (Loew) (Diptera: Tachinidae). *Journal of Insect Behavior*, **19**, 645–656.
- Awram, W.J. (1970) *Flight route behaviour of bumblebees*. PhD thesis, University of London, London, U.K.
- Beekman, M. & Van Stratum, P. (1998) Bumblebee sex ratios: why do bumblebees produce so many males? *Proceedings of the Royal Society of London B*, **265**, 1535–1543.
- Bergman, P. & Bergström, G. (1997) Scent marking, scent origin, and species specificity in male pre-mating behaviour of two Scandinavian bumblebees. *Journal of Chemical Ecology*, **23**, 1235–1251.
- Bergström, G., Svensson, B.G., Appelgren, M. & Groth, I. (1981) Complexity of bumblebee marking pheromones: biochemical, ecological and systematical interpretations. *Biosystematics of Social Insects* (ed. by P. E. Howse and J. L. Clement), pp. 175–183. Academic Press, London, U.K.
- Bourke, A.F.G. (1997) Sex ratios in bumble bees. *Philosophical Transactions of the Royal Society of London B*, **352**, 1921–1933.
- Bringer, B. (1973) Territorial flight of bumble-bee males in coniferous forest on the northernmost part of the island of Öland. *Zoon* (Suppl.), **1**, 15–22.
- Cameron, S.A., Hines, H.M. & Williams, P.H. (2007) A comprehensive phylogeny of the bumble bees (*Bombus*). *Biological Journal of the Linnean Society*, **91**, 161–188.
- Darvill, B. & Goulson, D. (2007) Aggregations of male *Bombus muscorum* (Hymenoptera: Apidae) at mature nests. Incestuous brothers or amorous suitors? *Apidologie*, **38**, 518–524.
- Darwin, C. (1886) Über die wege der hummelmännchen. *Gesammelte kleinere Schriften von Charles Darwin. Ein supplement zu seinen grösseren werken*, Vol. 2 (ed. by E. Krause). Leipzig, Germany.
- Foster, R.L. (1992) Nestmate recognition as an inbreeding avoidance mechanism in bumble bees (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, **65**, 238–243.
- Free, J.B. (1987) *Pheromones of Social Bees*. Chapman & Hall, London, U.K.
- Fussell, M. & Corbet, S.A. (1992) Observations on the patrolling behaviour of male bumblebees (Hym.). *Entomologist's Monthly Magazine*, **128**, 229–235.
- Goulson, D. (2010) *Bumblebees; their Behaviour, Ecology and Conservation*. Oxford University Press, Oxford, U.K.

- Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. & Knight, M.E. (2005) Causes of rarity in bumblebees. *Biological Conservation*, **122**, 1–8.
- Goulson, D., Lye, G.C. & Darvill, B. (2008) Diet breadth, coexistence and rarity in bumblebees. *Biodiversity and Conservation*, **17**, 3269–3288.
- Haas, A. (1949) Arttpische flugbahnen von hummelmännchen. *Zeitschrift für Vergleichende Physiologie*, **31**, 281–307.
- Haas, A. (1976) Paarungsverhalten und nestbau der alpinen Hummelart *Bombus mendax* (Hymenoptera: Apidae). *Entomologica Germanica*, **3**, 248–259.
- Krüger, E. (1951) Über die bahnlüge der männchen der gattungen *Bombus* und *Psithyrus*. *Zeitschrift für Tierpsychologie*, **8**, 61–75.
- Lepais, O., Darvill, B., O'Connor, S., Osborne, J.L., Sanderson, R.A., Cussans, J. *et al.* (2010) Estimation of bumblebee queen dispersal distances and a comparison of sibship reconstruction methods for haplodiploid organisms. *Molecular Ecology*, **19**, 819–831.
- Lloyd, J.E. (1981) Sexual selection: individuality, identification, and recognition in a bumblebee and other insects. *Florida Entomologist*, **64**, 89–111.
- O'Neill, K.M., Evans, H.E. & Bjostad, L.B. (1991) Territorial behaviour in males of three North American species of bumblebees (Hymenoptera: Apidae, *Bombus*). *Canadian Journal of Zoology*, **69**, 604–613.
- Postner, M. (1951) Biologisch-ökologische untersuchungen an hummeln und ihren nestern. *Veröffentlichungen des Museum Bremen, Reihe A*, **1952**, 45–86.
- Prys-Jones, O.E. & Corbet, S.A. (1991) *Bumblebees*. Richmond Publishing, Slough, U.K.
- Riley, J.R., Reynolds, D.R., Smith, A.D., Edwards, A.S., Osborne, J.L. & Williams, I.H. (1999) Compensation for wind drift by bumblebees. *Nature*, **400**, 126.
- Saunders, E. (1909) Bombi and other aculeates collected in 1908 in the Berner Oberland by the Rev. A.E. Easton, M.A. *Entomologist's Monthly Magazine*, **45**, 83–84.
- Schremmer, F. (1972) Beobachtungen zum paarungsverhalten der männchen von *Bombus confusus* Schenk. *Zeitschrift für Tierpsychologie*, **31**, 503–512.
- Smith, F. (1858) Notes on aculeate Hymenoptera, with some observations on their economy. *Entomologist's Annual*, **1858**, 34–46.
- Svensson, B.G. (1979) Patrolling behaviour of bumble bee males in a subalpine/alpine area, Swedish Lapland. *Zoon*, **7**, 67–94.
- Tuck, W.H. (1897) Note on the habits of *Bombus latreillellus*. *Entomologist's Monthly Magazine*, **33**, 234–235.
- Villalobos, E.M. & Shelly, T.E. (1987) Observations on the behavior of male *Bombus sonorus* (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, **60**, 541–548.
- Williams, P.H. (1991) The bumble bees of the Kashmir Himalaya (Hymenoptera: Apidae, *Bombini*). *Bulletin of the British Museum (Natural History) (Entomology)*, **60**, 1–204.

Accepted 1 June 2011

Uncorrected Proofs

QUERIES TO BE ANSWERED BY AUTHOR

IMPORTANT NOTE: Please mark your corrections and answers to these queries directly onto the proof at the relevant place. DO NOT mark your corrections on this query sheet.

Queries from the Copyeditor:

- AQ1.** We have typeset this article as an Original article. Please confirm if this article should be an 'Original article' or 'Short Communication'.
- AQ2.** Please spell out the authors' forenames (E.L. Sangster and J.C. Young).
- AQ3.** Please provide the department name for affiliation, if appropriate.
- AQ4.** Please add numbers so that the abstract is in list format (1., 2. . .) – see previous papers.
- AQ5.** Please provide the department name for the corresponding author, if appropriate.
- AQ6.** Please check the capitalization of other language title in the Reference list.
-