Causes of colony mortality in bumblebees

Dave Goulson[1,*], Steph O'Connor[2], Kirsty Park[2],

[1]*School of Life Sciences, University of Sussex, BN1 9QG, UK*
[2]*Biological and Environmental Sciences, School of Natural Sciences, University of Stirling, FK9 4LA, UK*

**Author for correspondence.*

Emails: D. Goulson, d.goulson@sussex.ac.uk
S. O'Connor, steph.oconnor@yahoo.co.uk
K.J. Park, k.j.park@stir.ac.uk

Running header: Causes of colony mortality in bumblebees
Keywords: *Bombus; nest; predation; survival; reproduction;* Aphomia sociella, Meles meles

Abstract

Despite considerable interest in bumblebees and their conservation, few data are available on basic life history parameters such as rates of nest predation and the proportion of wild nests that survive to reproduction. Here we use a combination of data collected by volunteers and our own direct observations which together describe the fate of 908 bumblebee nests in the UK between 2008 and 2013. Overall, 75% of nests produced gynes, with marked differences between species; the recently arrived species, *B. hypnorum*, had the highest proportion of colonies surviving to gyne production (96%), with the long-tongued *B. hortorum* having the lowest success in reaching gyne production (41%). There were also large differences between bumblebee species in the timing of nesting, gyne production and nest mortality, with *B. hypnorum* and *B. pratorum* nests starting early, producing most gynes before mid-summer, and then dying off in June, while at the other end of the spectrum *B. pascuorum* nests started late and produced gynes mainly in August. There was evidence for the partial or complete destruction of 100 nests. The main reported causes were excavation by a large mammal, probably primarily *Meles meles* (50%). Human disturbance was the second greatest cause of nest mortality (26%), followed by flooding (7%). Wax moth infestations were common (55% of nests), with *Bombus hypnorum* nests most frequently infested. However, infestation did not results in reduced likelihood of gyne production, perhaps because infestations often do not become severe until after some gynes have been produced. Our study provides novel insights into the little-studied biology of wild bumblebee nests and factors affecting their survival; collecting similar data sets in the future would enable fascinating comparisons as to how parameters such as nest survival and reproduction are changing over time, and are affected by management interventions for bees.

Introduction

Interest in bumblebee conservation has grown greatly in the last two decades, driven in part by realization that some species are in decline (Goulson *et al.,* 2011, 2015). However, bumblebee nests are notoriously difficult to find, and hence we still have a poor understanding of bumblebee nesting and population biology (Osborne et al, 2008; Goulson *et al.,* 2010; Lye *et al.,* 2012). Much of our understanding of the ecology of bumblebee nests is based upon observations made decades ago (for example, Sladen, 1912; Cumber, 1953) and since then there have been extensive land use change in the UK (Robinson and Sutherland, 2002), which has acquired a new species of bumblebee, *Bombus hypnorum* (Goulson & Williams, 2001), lost *Bombus subterraneus,* and experienced notable range reductions in the majority of other species (Williams, 1982; Goulson, 2010).

As with many eusocial hymenopterans, each nest represents a single breeding female, and hence the population trajectory of a species will depend on the frequency of success or failure of nests (Chapman & Bourke, 2001). What proportion of bumblebee nests survive to reproduce? What are the major causes of nest mortality? How does this vary between species and with location? It would be of great value to conservationists if we had answers to these questions, for it would enable us to interpret effects of altered land use, conservation schemes or climate change (Suzuki *et al.,* 2009; Williams & Osborne, 2009; Goulson, 2010). However, at present we have few recent data on the fate of real, wild bumblebee colonies in any setting.

In a study of 80 *Bombus pascuorum* nests at a site in southern England, Cumber (1953) reported that 23 produced queens, (i.e. 28.8%) and this is the only direct estimate of fecundity in natural bumblebee nests. The failure of most nests to produce reproductives is thought most often to be due to predators and parasites (Edwards & Williams, 2004). Nest survival has been estimated by calculating numbers of nests at the start and end of the summer using microsatellites to identify sister clusters (e.g. Goulson *et al.,* 2010). However, such genetic estimates are crude and subject to bias if average foraging range changes through the season (as is highly likely).

A more common approach to studying the nesting ecology of bumblebees has entailed monitoring and manipulation of artificially reared nests which have been either maintained in the laboratory or placed in the field and allowed to forage. Rates of nest survival and fecundity, effects of internal parasites, *Psithyrus* invasions and usurpation attempts have been studied in this way (for example, Müller & Schmid-Hempel, 1992; Frehn & Schwammberger, 2001; Goulson *et al.,* 2002; Carvell *et al.,* 2008; Otti & Schmid-Hempel, 2008). These studies have provided valuable information, but such colonies are unlikely to be accurately representative of wild nests. For example, invasion by wax moths, *Psithyrus* or foreign queens or workers may be more likely in reared colonies as such colonies are not concealed as natural bumblebee nests are.

The ecology of interactions between bumblebee nests and vertebrate species is an area that has also been largely neglected. Small mammals are thought to attack bumblebee nests, consuming the brood and pollen stores, particularly before the first brood of workers have emerged (Sladen, 1912; Free & Butler, 1959; Pouvreau, 1973; Alford, 1975). In New Zealand, mice were suspected of destroying 11 nests (in a study of 84 nests in artificial domiciles) (Donovan & Wier, 1978). Sladen (1912) attributed mice or shrews to the demise of several nests but he did not directly observe depredation events.

The destruction of nests caused by larger predators such as *M. meles* is usually obvious and this species is a well-known predator of bumblebee nests (Pease, 1898; Sladen, 1912; Pouvreau, 1973; Alford, 1975; Benton, 2006). *Meles meles* seek out nests, excavate them and consume the entire comb (Pease, 1898). They have been blamed for depredating commercially reared bumblebee colonies during experiments investigating colony growth (Goulson *et al.*, 2002). Other mammals such as foxes (*Vulpes vulpes*), stoats (*Mustela ermine*), moles (*Talpa europaea*) and hedgehogs (*Erinaceus europaeus*) are thought to depredate bumblebee nests, but the evidence is less clear (Sladen, 1912; Pouvreau, 1973; Alford 1975; Benton, 2006, Goulson, 2010).

Bumblebee nests may also be invaded by a range of invertebrates including cuckoo bumblebees (*Psithyrus*) and wax moths. Cuckoo females typically attack strong, early nests prior to the emergence of the second brood of workers (Muller & Schmid-Hempel, 1992). *Psithyrus* females lay their eggs in the nest and the *Bombus* workers of the host nest will rear a new generation of *Psithyrus* gynes and males. The wax moth *Aphomia sociella* is said to cause the demise of many nests each year (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Goulson *et al.,* 2002), yet we have few data on the actual rates of infestations by wax moths or the damage they cause to colonies (in terms of preventing reproduction).

Here, we aim to gather data on the duration of survival, rates of gyne production and (where possible) on the causes of nest mortality of a large sample of natural bumblebee nests in Britain, based on direct observation of nests and data gathered by the public. These data are intended to form a baseline so that in future we may examine how nest survival rates change over time, or have been affected by specific conservation strategies. Additionally, identifying significant sources of colony mortality may help us to devise appropriate management recommendations to reduce mortality.

Methods

Nests were located between 2006 and 2013 using a trained bumblebee nest detection dog and deliberate human searches (methods provided in Waters *et al.*, 2011; O'Connor *et al.*, 2012). The majority of these nests were located in rural locations around Stirling, in central Scotland. Once located, these nests were visited a minimum of once every fortnight and observed for 20-30 minutes on each occasion to ascertain if each nest was still active, if gynes or males were present, or if it had succumbed to a predator. The entrances to a subset of 32 nests were filmed to provide more detailed information on the predators that might visit these nests (details of the cameras can be found in O'Connor 2013). It was sometimes possible to collect or excavate nests once activity ceased. In this case, they were stored at -18ºC and later inspected to reveal invasion by wax moths and presence of gyne cells.

Using social media, members of the Bumblebee Conservation Trust and the wider public were asked to contact us if they had found a bumblebee nest. Additionally, we contacted local bee keepers and pest control agencies between 2010 and 2012 since these organisations are often contacted by people who have unwanted bumblebee nests. Members of the public reporting a nest were asked to fill in a brief online questionnaire describing the location of the nest, and those that were willing were asked to observe nests weekly for fifteen minutes and record worker activity, production of gynes and males and report any interesting activity with a photograph where possible. Some people were unable to participate in the weekly observations but were willing to submit occasional reports, or report if they noticed something unusual. In eight cases, bumblebees nested in bird boxes fitted with purpose made camera recorders.

Volunteers were asked to email photographs of bees so that the species could be verified. Occasionally volunteers preferred to post dead samples or record videos, and other nests were identified by experts (often survey coordinators of the Bumblebee Conservation Trust). In some cases, species were verified through detailed description alone. If volunteers were unsure how to identify gynes, they were asked to send photographs for confirmation. Where spurious results were received (for example, reports of many new gynes or males but no workers during their fifteen minute survey) these records were not included in analysis but were used to establish longevity of the nest.

Gyneless nests were so determined if no gynes had been observed during regular observations, there were no gyne cells at nest dissection and/or if nests were known to fail prematurely (i.e. April-May). An additional method of assessing gyne production was available for *B. hypnorum*, where a 'swarm' of males can be seen at entrances to nests producing new gynes.

The remains of 113 nests were inspected. This allowed the presence or absence of wax moth caterpillars and their silk to be determined, and in some cases presence or absence of gyne pupae cells could inform gyne production (some volunteers were unable to identify cells, but photographs or posted nest remains revealed this information).

Statistical Analysis

All analyses were performed in IBM SPSS Statistic 21. A χ^2 test of association was used to compare how the proportion of nests that went on to produce gynes differed among bumblebee species, and also to compare the proportions of nests found in each location (above ground, below ground, or on the ground surface) across bumblebee species. Date of first detection of nests, of gyne production, and of nest death were each compared across species using Kruskal-Wallis tests. Kaplan-Meier survival analysis was used to compare survivorship curves across species, with differences between species tested using a log rank (Mantel-Cox) test. Binary logistic regressions were used to examine whether infestation by wax moth, *Aphomia sociella*, affected the likelihood of nests producing gynes.

Results

In total data for 908 nests were collated (135 nests were located by the authors, 773 by members of the public), from across the UK but clustered in areas of high human population density (Figure S1). Species were identified for 821 of these nests (244 *B. hypnorum*, 208 *B. terrestris*, 118 *B. lapidarius*, 98 *B. lucorum*, 61 *B. pratorum*, 50 *B. pascuorum* and 42 *B. hortorum*). There were marked differences in the locations of nests of the different species, with nests of *Bombus hypnorum* almost entirely above ground (Figure 1), while the other species all occupied a range of sites but with a majority of nests below ground.

Dates of first detection of nests differed between species (Kruskal-Wallis test statistic = 142.3, d.f. = 7, $p<0.001$, Figure 2), with *B. hypnorum* and *B. pratorum* nests being detected earliest (mean Julian dates 136 and 138, respectively, equating to mid May). The remaining species were all found on average between Julian dates 150 and 160 (early June) except for *B. pascuorum* which was detected latest (mean Julian date 182, early July).

Date of first gyne production also varied markedly between species, exhibiting a similar pattern to date of first nest detection (Kruskal-Wallis test statistic = 192.5, d.f. = 7, p<0.001, Figure 3). *Bombus hypnorum* gynes tended to be observed first (mean Julian date 159, early June) followed on average 6 days later by *B. pratorum*. *Bombus pascuorum* were by far the latest nests to produce gynes (average Julian date 217, early August, approximately two months later than *B. hypnorum*).

Dates on which nests expired (the first date on which no activity was detected) also varied significantly between species, although the data were more variable (Kruskal-Wallis test statistic = 160.8, d.f. = 7, p<0.001, Figure 4). *Bombus pratorum* nests expired first (mean Julian date 181, end of June), followed by *B. hypnorum* (mean Julian date 188, early July). Once again, *B. pascuorum* nests expired on average later than the other species (mean Julian date 215, early August). Kaplan-Meier survival analysis reveals these same patterns in more detail (Figure S2). Survival curves differed significantly between species (Log Rank (Mantel-Cox) test, $\chi^2_7 = 141$, p<0.001).

Across records for all species, 76.2% of nests which were monitored went on to produce new gynes (399 of 489). Excluding unverified/unknown species, 76.4% nests produced gynes (356 of 466 nests). This proportion varied between species, (χ^2_6=74.51; P < 0.001) with a larger proportion of *B. hypnorum* nests producing gynes than any other species (Figure 5). Survival to gyne production was lowest in the two longer tongued species, *B. pascuorum* and *B. hortorum* (48 and 41%, respectively).

Of 24 nests which were discovered when only the queen was present, only 54.2% produced gynes, compared to 76.1% of nests detected after emergence of workers (n = 465). However, there was no significant difference between these proportions (χ^2_1 =0.64, P = 0.422).

Evidence of partial or complete destruction of nests was noted for 100 nests (excluding wax moths which are considered separately) (Table 1). Large animals, probably badgers, were responsible for the greatest number of nest failures (50). Human disturbance (for example, gardening and construction projects) resulted in 26 nest failures. Other causes of nest loss include flooding (7) and attack by ants (4).

Nests predation by large animals was recorded from May to September (Figure S3), with most events occurring in June and July. Only nine of the 50 nests destroyed by large mammals were found before the predation event; the large majority (41) were only discovered after they had been excavated. Nests discovered after destruction were not included in survival estimates.

Nineteen percent (117 nests) were in bird nest boxes. Thirty one incidences where bumblebees interacted with nesting birds were reported. In one case, a great tit was filmed using its bill to remove a queen *B. hortorum* which had entered the box three days previously. Birds had at least inspected (n = 8), started to build (n = 17) or laid eggs (n = 1) in nests which they then abandoned and immediately or soon after were inhabited by bumblebees. It is impossible to know the proportion of bird nests which were usurped by bumblebees versus those abandoned for other reasons shortly before bumblebees took up residence. Bird species apparently ousted by bumblebees include 14 *Parus caeruleus,* 2 *Passer domesticus,* 1 *Parus major* and 1 *Parus ater*. There was a single record of *Picus viridis* predation of a nest of *B. pascuorum* (Table 1).

It was possible to inspect 133 of the bumblebee nests for infestation by wax moth, *Aphomia sociella*, and 55% of nests were infested. These nests were disproportionately over-represented by *B. hypnorum* as this species tends to nest in bird nest boxes which are readily inspected. The proportion of infested nests differed significantly between species (χ^2_4 = 541, p<0.001; calculation excludes *B. lucorum* and *B. hortorum* for which too few records were available). *B. hypnorum* were most frequently infested, followed by *B. lapidarius* (Figure S4). *Bombus hortorum* and *B. pascuorum* were least frequently infested. Interestingly, wax moth infestation did not seem to affect the likelihood of a nest going on to produce gynes (binary logistic regression, χ^2 = 3.04, p=0.22); the weak trend was towards infested nests being more likely to produce gynes (40/52, 77%) compared to uninfested nests (26/40, 65% produced gynes).

Discussion

Rates and causes of bumblebee colony mortality, and the frequency with which colonies survive to reproduce, has very rarely been recorded for wild bumblebee nests. We present a unique data set quantifying the fate of 908 bumblebee nests encompassing all seven of the common UK species. Nests of *B. hypnorum*, a species that did not arrive in the UK until 2001 (Goulson & Williams 2001), are probably over-represented in our sample as this species frequently nests in bird boxes and in the eaves of houses where it is readily observed.

The phenology of the seven bumblebee species closely followed known differences (Goulson 2010). Nests of *B. hypnorum* and *B. pratorum* were, on average, detected earlier in the year than the other species, and nests of these two species also produced gynes earlier and died off earlier in the year. These patterns are unlikely to be due to differences in the geographic distributions of six of the seven species since they are found throughout the UK, but *B. hypnorum* was not found in Scotland at the time of our study and this might exaggerate differences in timing of emergence. However, previous studies suggest that *B. hypnorum* and *B. pratorum* do have a strategy of emerging and breeding early, and their life cycle is usually complete before midsummer (Goulson 2010). No evidence was found of a second generation. In contrast, *B. pascuorum* seems to adopt a more leisurely strategy, founding nests later and producing gynes in late summer. It is interesting to note that *B. hypnorum* suffered particularly badly from infestation by wax moths, while *B. pascuorum* nests were infested least often. It may be that *B. hypnorum*'s short life cycle is a strategy that has evolved to minimise harm from wax moths or other parasites by completing the life cycle before the moths can do much harm. Equally, it may be that species with a short colony cycle do not need to invest so much in nest defence. Whatever the explanation, it would appear that *B. hypnorum's* strategy is currently successful, for nests of *B. hypnorum* produced gynes more frequently (96%) than those of any other species in our study. In 15 years since colonisation this species has become one of the most abundant of UK bumblebees, particularly in gardens, bucking the generally negative trend in bee populations. Its success may hinge on the ready availability of artificial bird boxes for it to nest in, aided by its apparent ability to oust nesting birds such as *P. caeruleus*. Bird boxes are plentiful in UK gardens, and are beyond the reach of *M. meles.*

Competition over nests between birds and bees has been reported elsewhere. *Bombus niveatus* oust common redstart (*Phoenicurus phoenicurus*) from bird boxes at all stages of nesting, even after brood have hatched, however, nests of *P. major* using nest boxes in this study were never invaded (Rasmont *et al.*, 2008) and *Bombus polaris* queens may utilise the nests of snow buntings (*Plectrophenax nivalis*) in the Arctic (Heinrich, 1993), sometimes causing the birds to abandon their clutch of eggs (Kukal & Pattie, 1988). In a Finnish study of 1219 broods of *P. major*, four were abandoned after *Bombus* spp. invaded their nests (Orell, & Ojanen, 1983) and in South Korea *Bombus ardens* ousted oriental tits (*Parus minor*) and varied tits (*Poecile varius*) from nest boxes (Jablonski *et al.*, 2013). From our study, it seems bumblebee encounters with nesting *P. caeruleus* typically result in bumblebees ousting birds, whereas in at least one instance, a *P. major* was seen to remove a queen *B. hortorum*.

The most frequently confirmed cause of bumblebee nest destruction was by large animals, presumed to be *M. meles*, which destroyed 50 nests (5.5%), mainly in June and July when nests tend to be large. Although a badger was only directly observed in one of these 50 cases, dietary evidence confirms that badgers regularly consume bees. For example, examination of the stomach contents of 686 badgers (Cleary *et al.*, 2009) from March 2005 –

September 2006 in Ireland found that bees and wasps occurred in 3% of all samples and made up an estimated 1% of the total ingested bulk of badgers' diets. In June-August, bees and wasps remains occurred in 12% of samples, accounting for an estimated 6.5 % ingested bulk of the badgers' summer diets (Cleary *et al.*, 2009, see also Kruuk & Parish, 1981). It seems plausible that badgers have a significant negative impact on bumblebee populations, and it would be interesting to investigate whether the controversial badger culls that are currently ongoing in parts of the UK are benefitting bumblebees.

The only other large mammal that might plausibly excavate and eat bumblebee nests in the UK is the fox, *Vulpes vulpes.* Insects are common in the diet of *V. vulpes* (Lever 1959; Leckie *et al.*, 1998; Baker *et al.*, 2006). In particular, several studies note coleopterans as frequently occurring prey (Lever, 1959; Baker *et al.*, 2006). However, no hymenopterans were found in any of these studies (1,868 scat samples where insect remains were identified as far as possible), suggesting that foxes do not regularly depredate bumblebee nests.

Humans were the second most frequent cause of bumblebee nest destruction (26 nests, 2.9%). It is difficult to evaluate how representative these data are, for these nests were sometimes discovered by the very act of destruction; this might lead to us overestimating how often this happens. On the other hand, nests might be destroyed frequently by agricultural operations such as silage or hay cutting, but these events would not ordinarily be noticed or recorded.

Other causes of colony mortality were few. Seven nests were flooded during heavy rain, and we might speculate that this could become more frequent under climate change as extreme weather events become more common. Ants and social wasps (*Vespula* spp.) were found infesting four and three nests, respectively, but we cannot be certain that this was the cause of nest decline or opportunistic invasion of a nest that has declined for other reasons.

Previous authors have suggested that small mammals are significant predators of bumblebee nests, particularly in the early stages of nest development (Sladen, 1912; Free & Butler, 1959; Pouvreau, 1973; Alford, 1975), but we found no evidence for this. Traces of chitin have been found amongst the stomach and gut contents of wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) throughout the year, indicating that insects and other invertebrates are routinely eaten in small quantities (Watts, 1968; Flowerdew & Gardner 1978). However, no hymenopteran remains have been reported. This does not mean that small mammals may not depredate brood (for bee larvae have few recognisable chitinous structures), or steal food stores (as suggested by Sladen 1912). Such events would not have been detected by our methods.

Wax moths are widely believed to be amongst the most harmful predators of bumblebee colonies (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Goulson *et al.*, 2002), and our data confirm that the majority of nests are attacked (55%). Wax moths have been reported to pupate in June, with the adults on the wing and invading nests in August (Alford, 1975; Free and Butler, 1959), but our data suggest that this is incorrect. Infestations were detected in early to mid June, and since larvae are only likely to be spotted when at least part-grown it seems likely that adult moths can be on the wing in May. Despite their high frequency, and the obvious damage that wax moths do in late stages of infestation (the larvae can entirely consume the bumblebee brood, wax and food stores), our data suggest that most infested nests successfully reach gyne production. However, we are unable to discern if the ravages of the moth reduce the number of gynes produced.

It should be noted that our data on the proportion of nests that go on to produce gynes are undoubtedly overestimates (overall 76%). Nest discovery is inevitably biased towards large nests which are presumably likely to go on to reproduce. Only 24 (3.4%) of our nests were discovered before the first workers appeared, and this early stage is likely to be far more vulnerable. These nests did show a lower survival rate to gyne production (54%), although the small sample size precludes any confidence in this estimate.

In summary, we provide some novel insights into the nesting ecology, survival and reproductive rates of bumblebee nests, using a data set largely collected by volunteers. Overall, 76% of nests survived to produce at least some new gynes, with some differences between individual bumblebee species. Studying wild bumblebee nests is difficult, but is necessary if we wish to understand the population biology of these important pollinators. Our data provides a useful baseline against which future studies of nest survival and reproduction could be compared, for example to determine whether survival has changed over time, and how it is influenced by management interventions.

Acknowledgements

We would like to thank all of the people who volunteered their time to observe bumblebee nests. This work was partly funded by BBSRC grants BB/K014579/1 and BB/J014753/1, and the Leverhulme Trust.

References

Alford, D.V. (1975) *Bumblebees*. Davis-Poynter Ltd., London.

Baker, P., Furlong, M., Southern, S. & Harris, S. (2006) The potential impact of red fox *Vulpes vulpes* predation in agricultural landscapes in lowland Britain. *Wildlife Biol.* **12**, 39-50.

Benton, T. (2006) *Bumblebees*. HarperColins Publishers, London.

Carvell, C., Rothery, P., Pywell, R.F. & Heard, M.S. (2008) Effects of resource availability and social parasite invasion on field colonies of *Bombus terrestris*. *Ecol. Entomol.* **33**, 321-327.

Chapman, R.E. & Bourke, A.F.G. (2001) The influence of sociality on the conservation biology of social insects. *Ecology Letters* **4**, 650-662.

Cleary, G.P., Corner, L.A.L., O'Keeffe, J. & Marples, N.M. (2009) The diet of the badger *Meles meles* in the Republic of Ireland. *Mammalian Biology* **74**, 438-447.

Cumber, R.A. (1953) Some aspects of the biology and ecology of the humble-bees bearing upon the yields of red clover seed in New Zealand. *New Zealand Journal of Science and Technology* **34**, 227-240.

Donovan, B.J. & Wier, S.S. (1978) Development of hives for field population increase, and studies on the life cycle of the four species of introduced bumblebees in New Zealand. *New Zealand Journal of Agricultural Research* **21**, 733-756.

Edwards, M. & Williams, P.H. (2004) Where have all the bumblebees gone and could they ever return? *British Wildlife* **June**, 305-312.

Flowerdew, J.R. & Gardner, G. (1978). Small rodent populations and food supply in a Derbyshire ashwood. *Journal of Animal Ecology* 47, 725-740.

Free, J.B. & Butler, R.F. (1959) *Bumblebees*. Collins Press; London, UK.

Frehn, E. & Schwammberger, K.H. (2001) Social *parasitism* of *Psithyrus vestalis* in Free-ranging Colonies of *Bombus terrestris* (Hymenopters: Apidae). *Entomologia Generalis* **25**, 103-105.

Goulson, D. (2010) *Bumblebees; their behaviour, ecology and conservation*. Oxford University Press; Oxford, UK.

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

Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L & Darvill B. 2010. Effects of land use at a landscape scale on bumblebee nest density and survival. *Journal of Applied Ecology* 46: 1207-1215

Goulson, D., Nicholls E., Botías C., & Rotheray, E.L. 2015. Combined stress from parasites, pesticides and lack of flowers drives bee declines. SCIENCE 347: 1435-+.

Goulson, D., Rayner, P., Dawson, B. & Darvill, B. (2011) Translating research into action; bumblebee conservation as a case study. *Journal of Applied Ecology* **48**, 3–8.

Goulson, D. & Williams, P. (2001) *Bombus hypnorum* (Hymenoptera: Apidae), a new British bumblebee? *British Journal of Entomology and Natural History* **14**, 129-131.

Heinrich, B. (1993) *The Hot-blooded insects: Strategies and Mechanisms of Thermoregulation*. Harvard University Press, Cambridge.

Jablonski, P.G., Cho, J.C., Song, S.R., Kang, C.K & Lee, S. (2013) Warning signals confer advantage to prey in competition with predators: bumblebees steal nests from insectivorous birds. *Behavioural Ecology and Sociobiology* **67**, 1259-1267.

Kruuk, H. & Parish, T. (1981) Feeding Specialisation of the European Badger *Meles meles* in Scotland. *The Journal of Animal Ecology*. **50**, 773-788.

Kukal, O. & Pattie, D.L. (1988) Colonization of snow bunting, *Plectrophenax-nivalis, nivalis*, nests by bumblebees, *Bombus polaris*, in the high arctic. *Canadian Field Naturalist*. **102**, 544-544.

Leckie, F.M., Thirgood, S.J., May, R. & Redpath, S.M. (1998) Variation in the diet of red foxes on Scottish moorland in relation to prey abundance. *Ecography* **21**, 599-604.

Lever, R.J.A.W. (1959) The Diet of the Fox Since Myxomatosis. *Journal of Animal Ecology*, **28**, 359-375.

Lye, G.C., Osborne, J.L., Park, K.J. & Goulson, D. (2012) Using citizen science to monitor *Bombus* populations in the UK: nesting ecology and relative abundance in the urban environment. *Journal of Insect Conservation* **16**, 697-707.

Müller, C.B. & Schmid-Hempel, P. (1992) Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecological Entomology* **17**, 343-353.

O'Connor, S. (2013) *The nesting ecology of bumblebees*. PhD Thesis, University of Stirling.

O'Connor, S., Park, K.J. & Goulson, D. (2012) Humans versus dogs; a comparison of methods for the detection of bumblebee nests. *Apidologie* **51**, 204-211.

Orell, M. & Ojanen, M. (1983) Breeding success and population dynamics in a northern Great tit Parus major population. Ann Zool Fennici 20, 77-98.

Osborne, J.L., Martin, A.P., Shortall, C.R., Todd, A.D., Goulson, D., Knight, M.E., Hale, R.J. & Sanderson, R.A. (2008) Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. *Journal of Applied Ecology* **45**, 784-792.

Otti, O. & Schmid-Hempel, P. (2008) A field experiment on the effect of *Nosema bombi* in colonies of the bumblebee *Bombus terrestris*. *Ecological Entomology* **33**, 577-582.

Pease, A.E. (1898) The badger; a monograph. Lawrence and Bullen Ltd., London.

Pouvreau, A. (1973) The Enemies of Bumblebees. *Apidologie* **4**, 103-148.

Rasmont, P., Aytekin, A.M. & Kaccar, M.S. (2008) Ousting of the common Redstart (Aves: Turdidae: *Phoenicurus phoenicurus*) from its nests by the bumblebee *Bombus niveatus vorticosus* (Hymenoptera: Apidae). *Annales de la Société Entomologique de France* **44,** 251-255.

Robinson, R.A. & Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology* **39**, 157-176.

Sladen, F.W.L. (1912) *The Humble-bee*. Macmillan and Co. Ltd., Hereford, UK.

Suzuki, Y., Kawaguchi, L.G., Munidasa, D.T. & Toquenaga, Y. (2009) Do bumblebee queens choose nest sites to maximize foraging rate? Testing models of nest site selection. *Behavioural Ecology and Sociobiology* **63**, 1353-1362.

Waters, J., O'Connor, S., Park, K.J. & Goulson, D. 2011. Testing a Detection Dog to Locate Bumblebee Colonies and Estimate Nest Density. *Apidologie* 42: 200-205

Watts C.H.S. (1968) The foods Eaten by Wood Mice (*Apodemus sylvaticus*) and Bank Voles (*Clethrionomys glareolus*) in Wytham Woods Berkshire. *The Journal of Animal Ecology* **37**, 25-41.

Williams, P.H. (1982) The distribution and decline of British bumblebees (*Bombus* Latr.) *Journal of Apicultural Research* **21**, 236-245.

Williams, P.H. & Osborne, J.L, (2009) Bumblebee vulnerability and conservation world-wide. *Apidologie* **40**, 367-387.

Table 1. Possible causes and available evidence for mortality of 100 nests.

Nests (*n*)	**Cause**	**Evidence for cause. Number (*n*) given where relevant.**
50	*Meles meles* (badger)	Nests excavated by large animal, probably *M. meles*. Soil or vegetation removed, tooth and claw marks in soil, tree roots, etc.
26	People	Nests disturbed through gardening or building work
7	Flood	Nest flooded from heavy rain.
4	Ants	Many ants found in nest post death.
3	*Psithyrus* spp.	*B. sylvestris* filmed entering nest. (1)
		Psithyrus sp. photographed in nest (2)
2	*Apodemus sylvaticus*	Filmed covering/blocking entrance with leaves. (1)
		Droppings/mice found within nest remains. (1)
3	*Vespula* spp.	Nest contained *Vespula* spp. during decline. (2)
		Observed *Vespula* spp. attack and kill a worker at nest entrance. (1)
2	Usurpation by bumblebee	*B. terrestris* queen filmed repeatedly entering *B. pratorum* nest which failed shortly afterwards. (1)
		B. terrestris workers filmed repeatedly entering *B. lapidarius* nest which ceased shortly afterwards. (1)
2	Birds	*Parus major* filmed ousting queen *B. hypnorum*. (1)
		Picus viridis bill marks in destroyed *B. pascuorum* nest. (1)
1	Spider	Spider and queen filmed fighting repeatedly. Several days later, queen was dead.
100	Total	

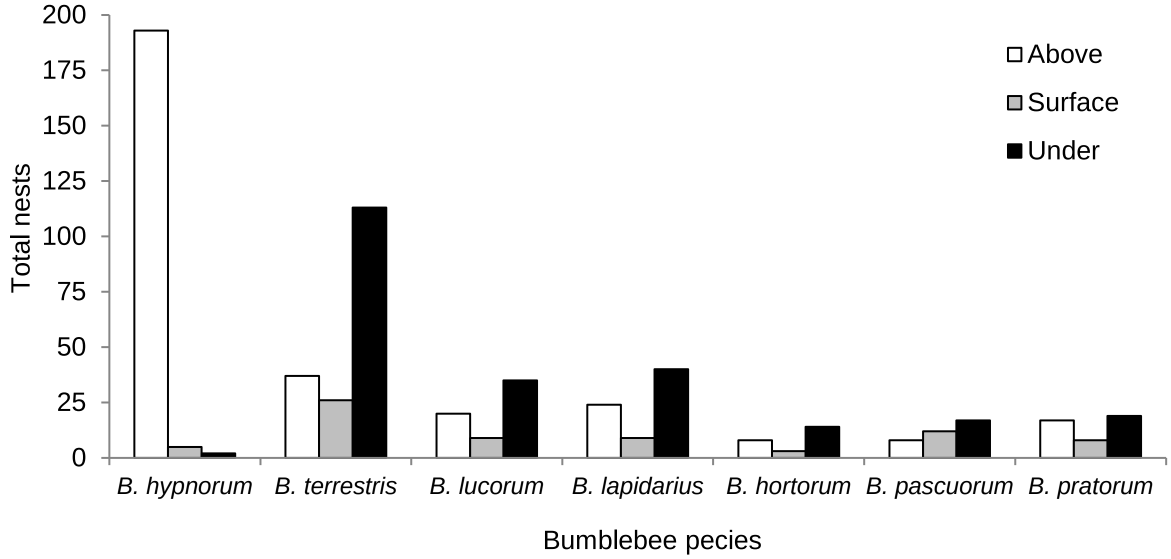


Figure 1. Locations of nests (above the ground, on the surface or subterranean) by species for 619 nests of verified species for which locations were obtained.

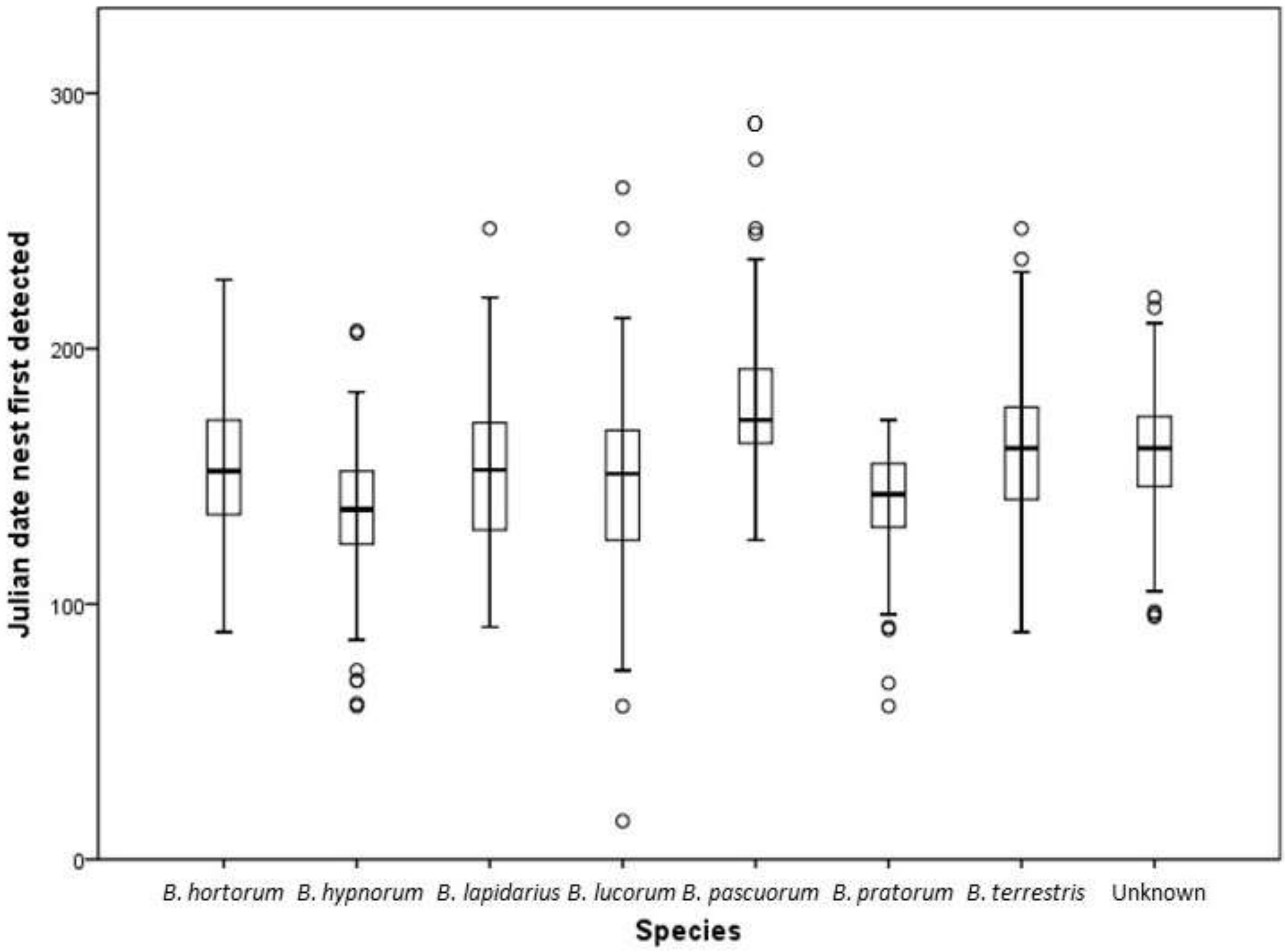


Figure 2. Dates of first detection of bumblebee nests according to species (median, quartiles, 95% confidence limits and outliers).

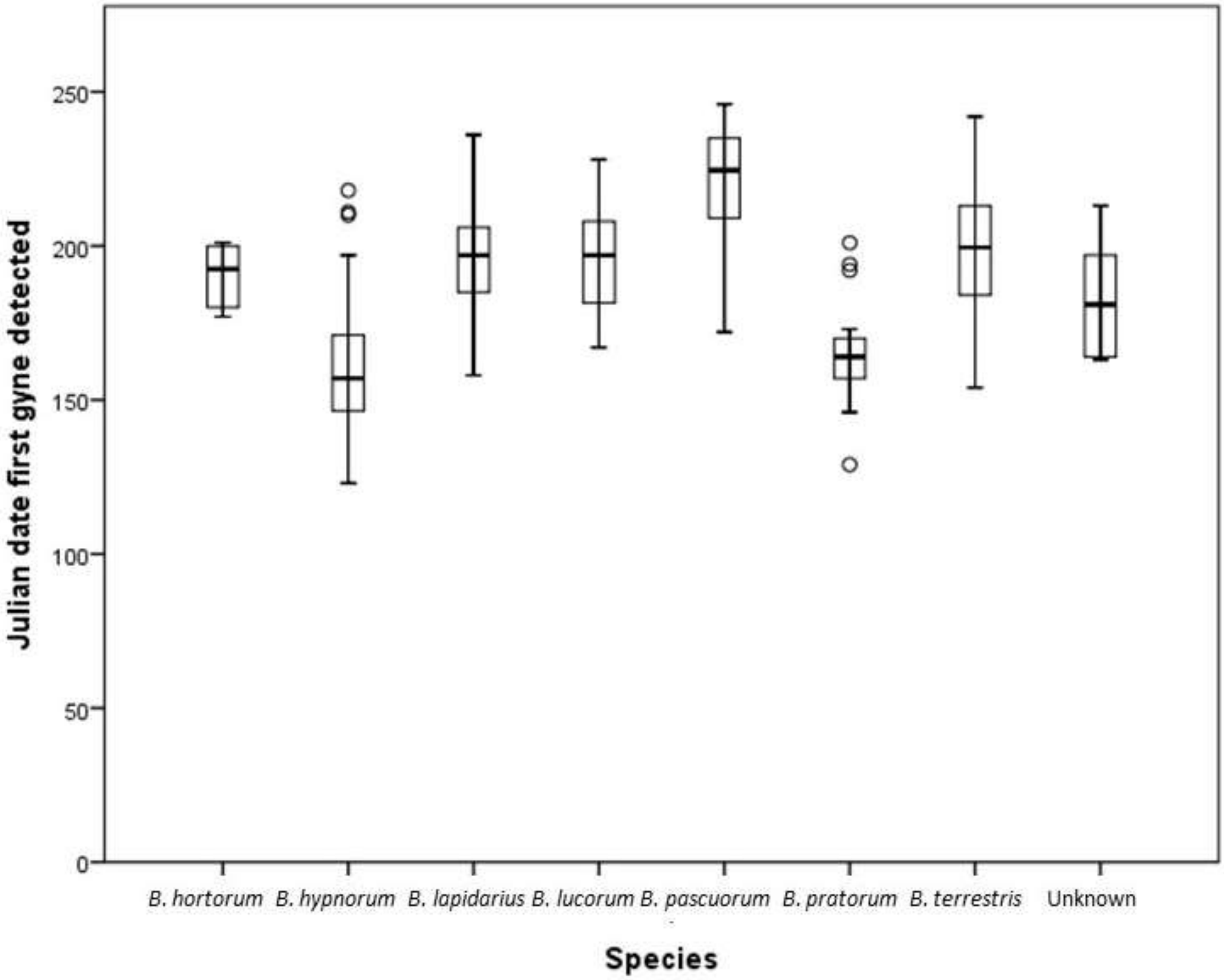


Figure 3. Dates of first detection of gyne production of bumblebee nests according to species (median, quartiles, 95% confidence limits and outliers).

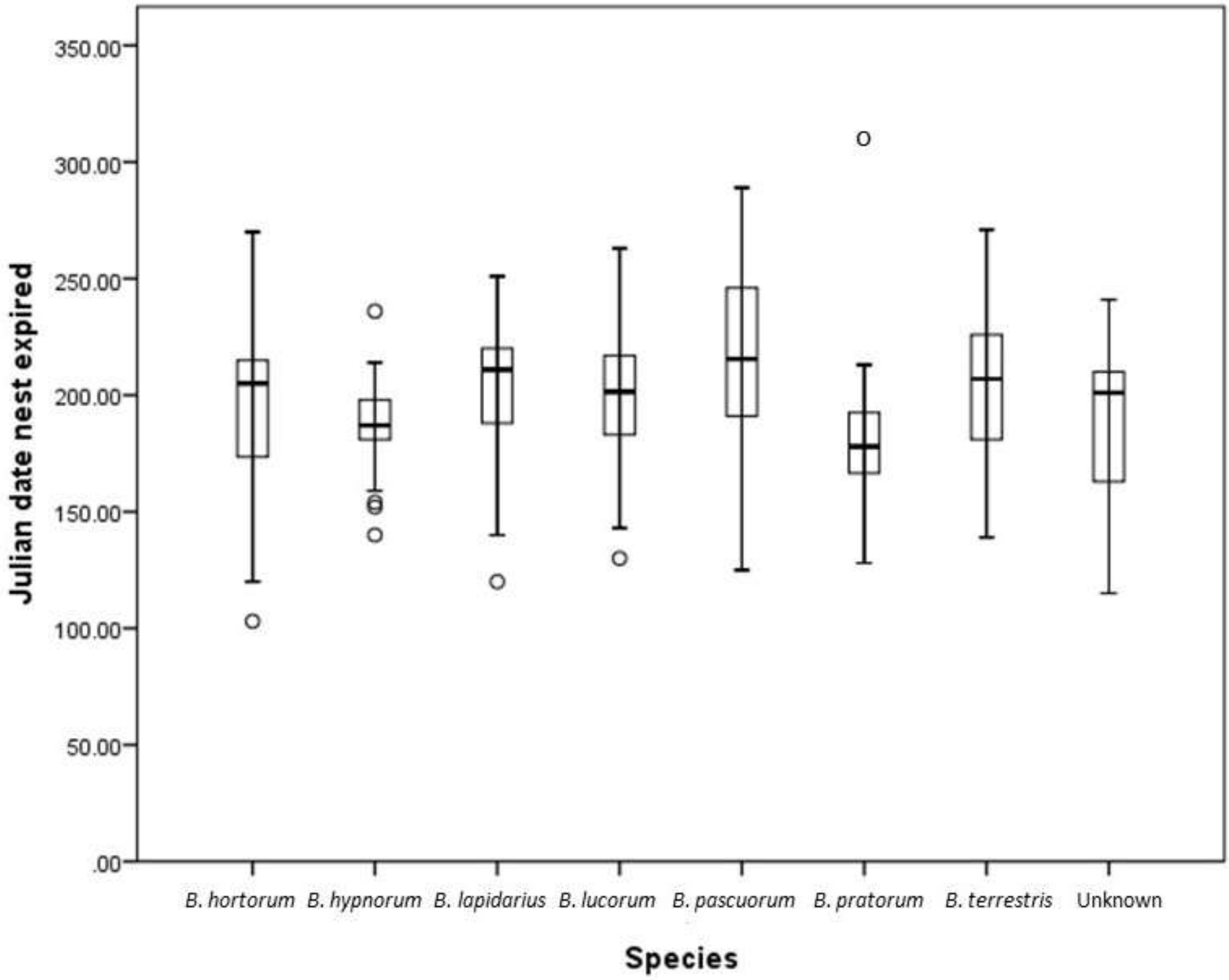


Figure 4. Dates of cessation of nest activity according to bumblebee species (median, quartiles, 95% confidence limits and outliers).

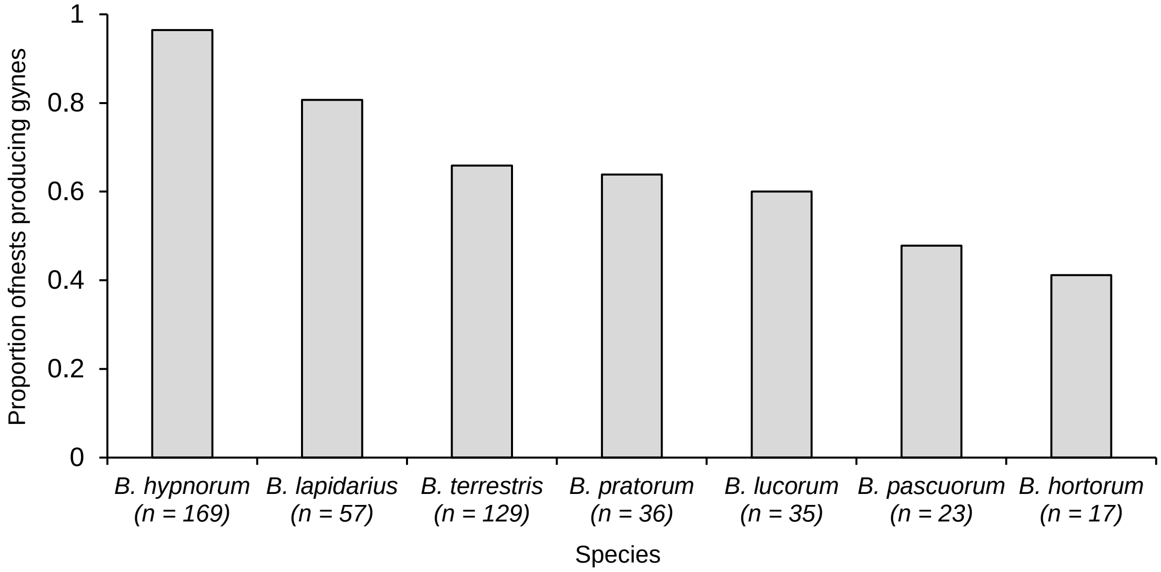


Figure 5. Proportions of nests producing gynes (using data where species was verified, n =466).

Supplementary Materials

Figure S1. Locations of the 908 bumblebee nests. Some sites have multiple nests. Site A in Scotland contained 33 nests found by the researchers.

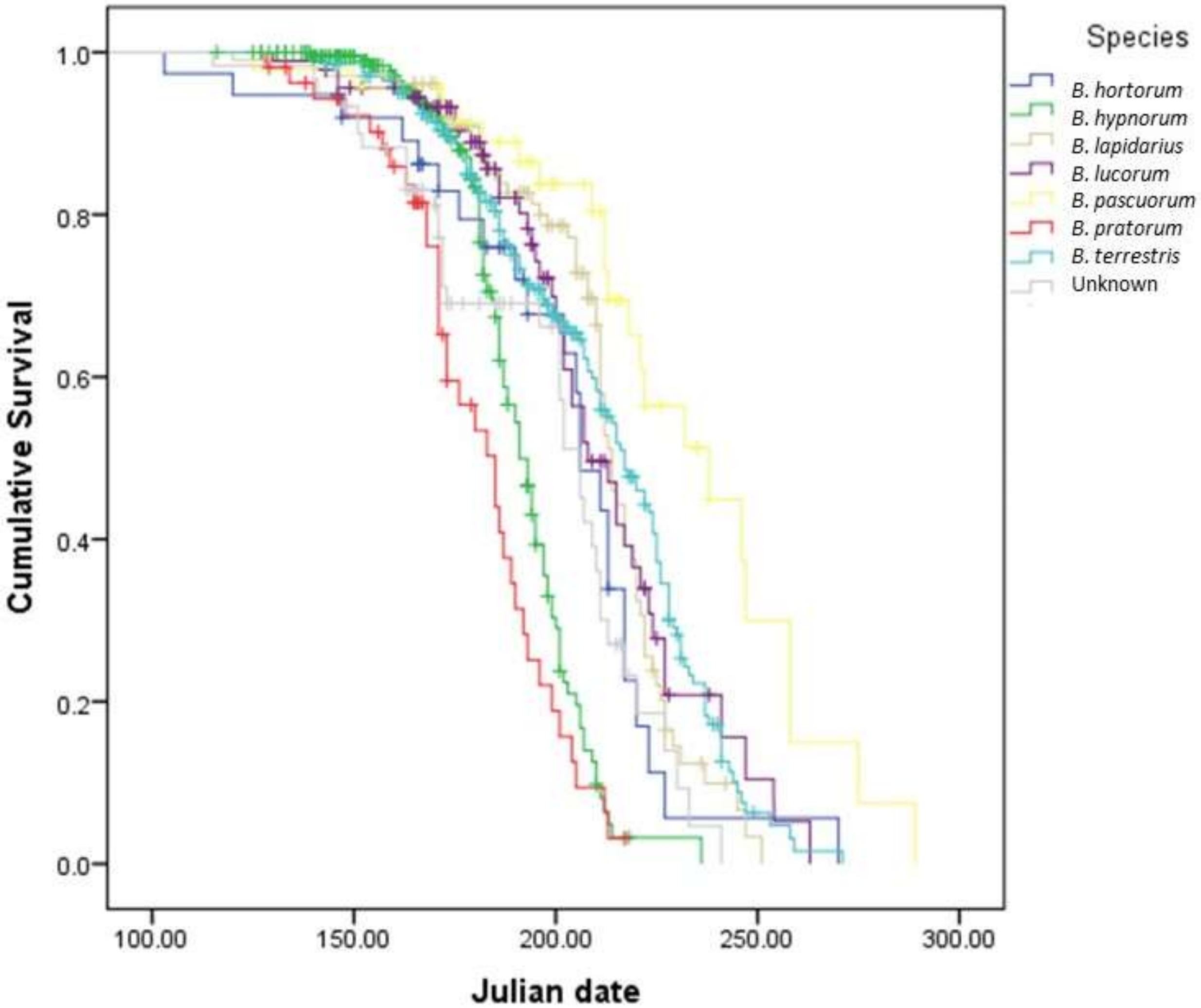
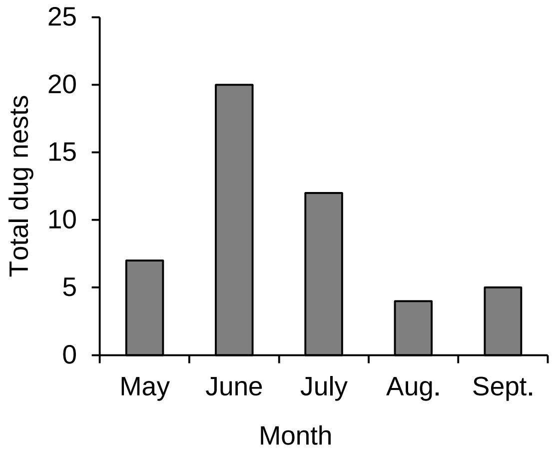
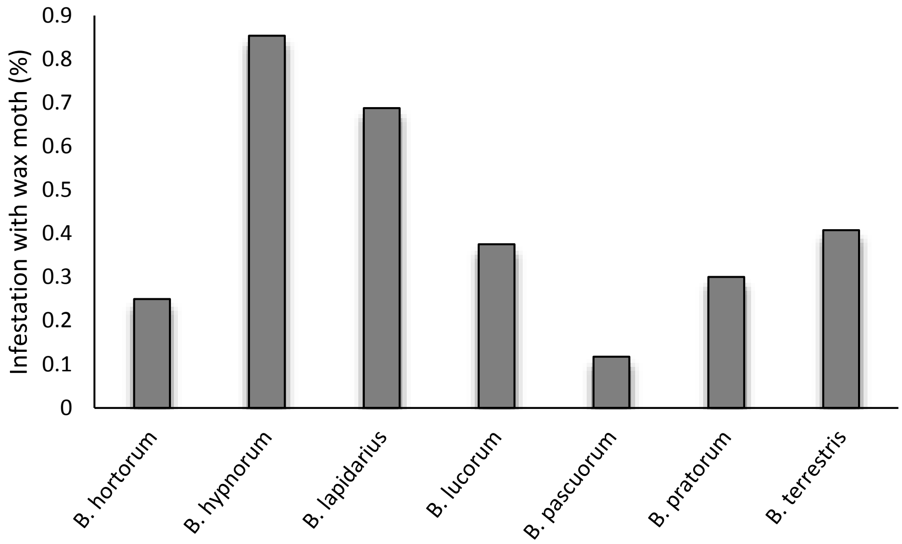


Figure S2. Survival curves for nests of seven bumblebee species according to Julian date. + indicated censored data. Based on 818 nests.



Supplementary Figure S3. Month in which nests excavated by large animals (probably *M. meles*) were discovered (n=48; no date was given for two reported dug nests).



Supplementary Figure S4. Proportion of nests infested by the wax moth, *Aphomia sociella*