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Are there risks to wild European bumble bees from using commercial stocks of domesticated *Bombus terrestris* for crop pollination?

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Are there risks to wild European bumble bees from using commercial stocks of domesticated *Bombus terrestris* for crop pollination?

Mass produced colonies of the Eurasian bumble bee, *Bombus terrestris* L., have been used in Europe for over 30 years for the pollination of horticultural crops. In recent years, the practice has been identified as a risk factor for wild bumble bees in Europe and some researchers have claimed that it is causing environmental harm. The specific risks include competitive displacement of wild bumble bees, gene introgression, and the spread of disease. We have reviewed the scientific evidence on risk factors associated with managed colonies of *B. terrestris* in Europe, and we highlight the strengths, weaknesses and gaps in the current knowledge base. In contrast to other reports, we conclude that there is currently not enough reliable, consistent evidence to support claims that the current use of managed *B. terrestris* in Europe is harmful to wild populations of *B. terrestris* and other bumble bees, and therefore the issue remains unresolved. In the case of disease risks in particular, there is conflicting evidence published by some researchers on the prevalence of pathogens in managed colonies versus audited data published by one of the main bumble bee producers. The current lack of consistent evidence makes it difficult for government regulators to make informed risk assessments, and we argue that more work is needed to demonstrate both the reproducibility of published findings and to understand the mechanisms of action of risk effects. Recommendations are made for future work to better understand if the proposed risks are occurring in practice and to put in place preventative measures and mitigations if required.

Keywords: *Bombus terrestris*, managed, pollination, environment, risk

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Introduction

Bumble bees (*Bombus* spp., Hymenoptera, Apidae) pollinate a wide range of flowering plants and are often considered to be keystone species, but there are widespread concerns about their conservation (Goulson, 2010; Goulson, Lye & Darvill, 2008; Plowright & Laverty, 1984). Approximately 250 different *Bombus* species are recognized, of which roughly 10% have been reported to be at risk of extinction, while others, although not under extinction risk, have been exhibiting long term reductions in abundance and range extent (Goulson et al., 2008; Kosior et al., 2007; Potts et al., 2010; Williams & Osborne, 2009) (see Supplemental Material S1 for details). Changes in land use, agricultural intensification and habitat loss are recognized as important drivers for these reductions (Carvell et al., 2006; Fitzpatrick et al., 2007; Goulson, Hanley, Darvill, Ellis & Knight, 2005; Grixti, Wong, Cameron & Favret, 2009; Kremen, Williams & Thorp, 2002; Williams, 2005; Williams & Osborne, 2009). In addition, the use of mass-produced, managed bumble bee colonies for crop pollination has been highlighted as an additional risk factor for wild bumble bees, and this issue has received increasing attention in recent years (Cameron, Lim, Lozier, Duennes & Thorp, 2016; Cameron et al., 2011; Colla & Packer, 2008; Goka, 2010; Goka, Okabe, Niwa & Yoneda, 2000; Kondo et al., 2009; Morales, Arbetman, Cameron & Aizen, 2013; Tsuchida, Kondo, Inoue & Goka, 2010; Williams & Osborne, 2009). This practice has grown widely since the late 1980s and is used for the pollination of fresh produce such as tomato and strawberry, mainly in glasshouses and polytunnels (Department for Environment, Food and Rural Affairs [DEFRA], 2014a). In this system, bumble bee hives produced by a specialist supplier are placed temporarily in the crop, with each hive lasting typically for 6 – 8 weeks after delivery. New hives are added through the season as the pollination demand increases and / or to replace older hives that have gone past the peak of worker production. The use of domesticated bumble bees in this way is now an important part of the horticultural industry. However, it is not without controversy, and it has been criticized by some researchers, who have proposed that it is either a causal factor in wild bumble bee declines or presents a high-risk potential that is not being managed correctly. Specific concerns have been raised about competitive effects of managed bumble bees on wild bees, hybridization between domesticated and wild bees, and elevating infectious disease levels in wild bee populations (Cameron et al., 2016; Cameron et al., 2011; Goulson et al., 2008; Graystock, Blane, McFrederick, Goulson & Hughes, 2015; Graystock et al., 2013; Kraus et al., 2011).   In North America, an association has been drawn between commercial bumble bee operations and a fall in the relative abundance of four wild bumble bee species of up to 96% (Cameron et al., 2011). In turn, the industry has defended its position, and has criticized some of the scientific evidence as not representing the true picture (Biobest Group, 2013).

In this paper we review the scientific literature on the ecological risks from using managed, domesticated colonies of the Eurasian bumble bee *Bombus terrestris* L. (known commonly as the large earth bumble bee or the buff-tailed bumble bee) within its native range in Europe (see Supplemental Material S2 for a short description of the natural distribution and life cycle of *B. terrestris*). This was the first bumble bee to be mass-produced and it is also the most widely used. We explore the specific risks that have been put forward and we highlight the strengths, weaknesses and gaps in the current knowledge base. Evidence from studies conducted outside Europe and with other *Bombus* species is included where relevant. We are concerned primarily about countries within the European Economic Area although the review is relevant to all areas within the native range of *B. terrestris*. The review is intended to be accessible to a wide range of people including researchers, government representatives, horticultural growers and bee producers. We have included Supplemental Material which has contextual information on the biology of bumble bees, the ecological effects of non-native *Bombus* species outside Europe, and descriptions of the pathogens and parasites that can affect bumble bee health.

Use of domesticated bumble bees for crop pollination

The mass rearing of bumble bee colonies has only been possible in the last 30 years (Velthius & Van Doorn, 2006). The process is technically complex and a considerable number of challenges had to be overcome before it became economically viable, including storage of hibernating queens, initiation of new colonies, queen mating, the provision of good quality pollen as a food source, and prevention and management of disease (Ruijter, 1996; Velthius & Van Doorn, 2006). Greenhouse growers first used domesticated bumble bees for pollination on a commercial basis in Belgium in 1987, followed by growers in the Netherlands (1988), France and the UK (1989) (Velthius & Van Doorn, 2006). Commercially-reared bumble bees are now sold across Europe, in Asia and North America, predominantly for use on protected crops such as tomato, strawberry and raspberry (e.g. DEFRA, 2014a; Strange, 2015).

The stocking density of hives depends upon the type of crop and the time of year: for a tomato crop, for example, the published recommendation is for 5 – 7.5 colonies per ha (Peet & Welles, 2005). Five bumble bee species have been commercially developed for mass production (Velthius & Van Doorn, 2006): (i) within the subgenus *Bombus* (Williams et al., 2012a), *Bombus terrestris* (native to Eurasia, used commercially in Europe, N Africa, Asia and Australasia), *Bombus lucorum* (native to Eurasia, used in Asia), *Bombus ignitus* (used within its native range in east Asia), *Bombus occidentalis* (used in its native range in western North America); and (ii) within the subgenus *Pyrobombus*, *Bombus impatiens* (native to eastern North America but used across North America. *Bombus terrestris* is the most widely used species and is popular with growers because of the large boost provided to crop yield and quality (Lye, Jennings, Osborne & Goulson, 2011; Roldán Serrano & Guerra-Sanz, 2006). It is currently the only mass-produced bumble bee species deployed for crop pollination in Europe (Velthius & Van Doorn, 2006). Velthius and Van Doorn (2006) estimated that around one million *B. terrestris* colonies were sold for crop pollination in 2004 (Velthius & Van Doorn, 2006). In England, 21,799 hives were licensed for commercial pollination in 2013 (DEFRA, 2014a). It is reported that commercial colonies of *B. terrestris* have been used in at least 57 countries, of which 16 are outside of its native range including in East Asia, Mexico and Chile (Ings, 2007; T. C Ings, Ings, Chittka &, Rasmont, 2010; Velthius & Van Doorn, 2006). Imports of *B. terrestris* are now prohibited in the USA, Canada, Australia, China, South Africa, Argentina and Brazil because of evidence about its damaging effects as an invasive species (Velthius & Van Doorn, 2006; CABI. 2018) (see Supplemental Material S3).

Is commercial use of *B. terrestris* in Europe a risk to wild bumble bees?

Potential ecological risks to wild bee in Europe from managed B. terrestris

The main environmental concerns about commercial use of *B. terrestris* in Europe are to do with the effects of introducing large numbers of managed colonies into an area, with the managed bees subsequently interacting directly or indirectly with wild bees and causing them harm. The specific risks proposed include competitive displacement of wild bumble bees (Ings, Schikora & Chittka 2005; Ings, Ward & Chittka, 2006), introgression of genes from managed bumble bees to wild populations (Ings, Raine &

Chittka, 2005; Kraus et al., 2011) and the spread of infectious disease (Graystock, Goulson & Hughes, 2014; Graystock et al., 2013; Manley, Boots & Wilfert, 2015; Meeus, Brown, De Graaf & Smagghe, 2011; Murray, Coffey, Kehoe & Horgan; 2013; Otterstatter & Thomson, 2008; Whitehorn, Tinsley, Brown & Goulson, 2013). The size of the ecological risk is considered also to be affected by whether the managed *B. terrestris* subspecies is native or non-native within its area of use: the release of a non-native bumble bee subspecies has been described as presenting a greater risk of competitive exclusion, hybridization and gene introgression compared to releasing a native subspecies for example (Moritz, Härtel & Neumann, 2005; Schneider, DeGrandi-Hoffman & Smith, 2004; both cited in Ings et al., 2010). Some authors have proposed that these risks have already been realized. For example, Ings et al. (2006) concluded that establishment of feral colonies of non-native *B. terrestris* is very likely to have occurred in the UK, with a high risk of competitive displacement of native wild bumble bees. Meanwhile Kraus et al. (2011) reported that gene introgression from managed to wild bumble bees had occurred mainland Europe, while Murray et al. (2013) reported that pathogens had spilled over from managed *B. terrestris* to wild bumble bees in Ireland. These and other studies have been used to inform ecological risk assessments by government regulators. The main trend has been to prevent or severely curtail the use of non-native *B. terrestris* subspecies. The Sardinian endemic *B. t. sassaricus* was used for tomato crop pollination in southern mainland Europe between 1989 and 1996 but was subsequently discontinued by the industry in favor of *B. t. dalmatinus* (Velthius & Van Doorn, 2006; Ings et al., 2010). In the Canary Islands, the endemic *B. t. canariensis* is used for commercial pollination and the import of other subspecies is prohibited. In Great Britain, use of non-native *B. t. terrestris* and *B. t. dalmatinus* was permitted up until 2015, but the licensing regime was subsequently changed so that the indigenous subspecies *B. t. audax* became the default for commercial operations, while the use of *B. t. terrestris* / *dalmatinus* was restricted to circumstances in which crop pollination was threatened by lack of supply of *B. t. audax* hives (Natural England, 2013, 2014). Use *of B. t. terrestris* / *dalmatinus* hives is still permitted in the Republic of Ireland subject to safeguards (use of queen excluders and strict disposal protocols), however Bord Bia, the Irish state agency that promotes horticulture, does not favor the use of non-native bumble bees and hence bee suppliers have decided on a voluntary basis only to supply *B. t audax* hives (D. Foster, Koppert UK Ltd. personal communication, September 25, 2018). No restrictions have been placed yet in Europe on domesticated, native bumble bee subspecies. However, the situation is being monitored by government authorities in the light of concerns about disease risks (DEFRA, 2014b). In Norway, only locally-produced colonies of *B. t. terrestris* are permitted for commercial pollination (Velthius & Van Doorn, 2006). The trade in bumble bee colonies in the European Union and the European Economic Area is governed under animal health regulations (Council Directive 92/65/EEC) in which colonies sold between member countries must be accompanied by an approved health certificate and with a stipulation that they show no signs of disease (European Commission, 1992). The current regulations have been criticized as being inadequate, with recommendations to improve their effectiveness including the adoption of more stringent pathogen testing procedures (Graystock et al., 2015).

In the following sections, we examine the three main ecological risks from domesticated bumble bees that have been put forward, as they relate to use of *B. terrestris* within its native in Europe: (1) domesticated bumble bees outcompete wild bees for natural floral resources; (2) genes from domesticated bumble bees spread into wild bumble bee populations; (3) pests and pathogen levels are increased in wild bumble bee populations as a result of the presence of managed bumble bees.

Do domesticated bumble bees 'escape' from greenhouses into the wild?

Some managed bumble bees are used to pollinate crops grown in open field crops or in semi-open polytunnels, where they face no physical restrictions on them coming into contact with wild bees and, in these situations, they are known to forage outside of the target crop (Foulis & Gouslon, 2014; Trillo et al., 2019). A lot of managed bumble bees are used in enclosed greenhouses on protected edible crops such as tomato, but even here they may have access to the outside through unscreened vents and doors. In British Columbia, Canada, an analysis of the species identity of pollen collected from *B. impatiens* and *B. occidentalis* colonies housed within greenhouse tomato crops suggested that bees were foraging outside for a large amount of the time in late spring and summer (the proportion of non-tomato pollen collected on sampled foraging bees was as high as 73% in one case) although there were significant variations across time and at different greenhouse sites (Whittington, Winston, Tucker & Parachnowitsch, 2004). Similarly, in Ontario, Canada, workers of *B. impatiens* were observed leaving and entering greenhouses where managed colonies of this species were used in tomato and pepper production (Otterstatter & Thomson, 2008). The relative abundance of *B. impatiens* workers compared to other *Bombus* species observed on wildflowers declined with increasing distance from the study greenhouses, with > 90% of *B. impatiens* being collected within 200 m (Otterstatter & Thomson, 2008). The extent to which managed bumble bees leave European enclosed greenhouses has not been studied in detail, but if the examples from Canada are typical then outside foraging is likely to occur if preventative measures (screened vents and double doors) are not in place. In principle, movements of managed bees into the wider environment may be temporary (managed worker bees foraging outside and then returning to their home colony) or permanent, if managed gynes escape and set up new colonies outside. In Japan and South America, *B. terrestris* colonies imported from Europe are known to have escaped from greenhouses into the wild, established and spread (Matsumura, Yokoyama & Washitani, 2004; Morales et al., 2013). When use of non-native *B. terrestris* was still routinely permitted in the UK, growers were required to follow a set of practices to prevent gynes escaping, including use of queen locks in hives and destruction of colonies after use; however, an inspection of 20 growers by the UK government’s advisory body found that queen locks were being left open, and 60% of growers were not killing non-native colonies after use (Natural England, 2014). Both of the northern European subspecies *B. t. audax* and *B. t. terrestris* have lifecycles in which, at the end of season, colony workers and drones die and new, mated queens hibernate over the winter to emerge and form colonies the following spring (Woodard et al., 2015). In contrast, *B. t. dalmatinus* - which occurs in warmer, Mediterranean regions – forms colonies that are active throughout the winter in its native range. Following laboratory-based assessment of cold tolerance using standardized protocols, Owen, Bale and Hayward (2016) considered that *B. t. dalmatinus* would not be able to survive as active colonies under currently typical northern European winters, including the UK. The only large scale, long term monitoring study on establishment of managed non-native *B. terrestris* subspecies in Europe concerns the Sardinian endemic *B. t. sassaricus* which was used in southern mainland Europe for greenhouse pollination from 1989 – 1996 (Ings et al., 2010). *Bombus t. sassaricus* is morphologically distinct from mainland subspecies and can be readily identified in the field. Surveys carried out in southern France from 1988 – 2004 (i.e. before, during and after the period of commercial use) found *B. t. sassaricus* males and queens foraging outside of greenhouses (in 1991, 1993 and 1994), while several workers were observed foraging on native plants at a single location 30 km from the nearest commercial greenhouse in 1998, two years after import of *B. t. sassaricus* had stopped (Ings et al., 2010). However, no *B. t. sassaricus*, or hybrids with mainland subspecies, were observed in surveys done in 2004, suggesting that it had not been able to persist in detectable numbers, or had become indistinguishable from native subspecies following frequent hybridization (Ings et al., 2010).

Competition between domesticated bumble bees and wild bees

Managed *B. terrestris* bees that forage away from their target crop are likely to compete with wild bees for floral resources. A priori, the intensity and outcome of this competition will depend on a wide range of factors including whether the managed bumble bee is a native or non-native subspecies, the numbers of managed bumble bees entering the environment, whether or not they establish new colonies, the foraging-related attributes of the wild bee community in comparison to the managed bees, the availability and diversity of forage, the physical and ecological conditions of the landscape, and the action of natural enemies (Fontaine, Collin, & Dajoz, 2008; Herbertsson, Lindström, Rundlöf, Bommarco & Smith, 2016; Ranta & Lundberg, 1980; Stelzer, Raine, Schmitt & Chittka, 2010; Stout, Allen & Goulson, 1998). The area over which competition occurs will depend on the bees normal foraging range. The mean and maximum foraging distances for wild *B. terrestris* in forage-rich environments are reported to be c. 270 m and 600 – 800 m respectively (Darvill, Knight & Goulson, 2004; Osborne et al., 1999; Wolf & Moritz, 2008) with c. 40% of workers foraging within 100m of the colony (Wolf & Moritz, 2008), and it is reasonable to assume that managed bees will have a similar foraging range outside.

The different subspecies of *B. terrestris* show variation in traits which might influence competitiveness, including foraging behavior, learning, flower detection and color preference (Chittka, Ings & Raine, 2004; Ings et al., 2006; Skorupski, Döring & Chittka, 2007; Spaethe, Tautz & Chittka, 2001). In a reciprocal transplant experiment done in Sardinia and Germany, nectar foraging performance of three *B. terrestris* subspecies was found to differ consistently, with *B. t. canariensis* being superior to *B. t. sassaricus*, which in turn was superior to *B. t. terrestris*, with these differences being explained largely by the better foraging subspecies having a larger body mass (Ings et al., 2005). When body size was accounted for, native bumble bee colonies were inferior for nectar collection to at least one of the two non-native subspecies (Ings et al., 2005). A study of managed *B. terrestris* to aid pollination of raspberry crops grown in open ended polytunnels in Scotland found no evidence that the managed bees had negative effects on the species composition, abundance or diversity of other social bee species visiting raspberry flowers within the tunnels, although the authors recommended that more work was needed to satisfactorily rule out the possibility of competition between managed bumble bees and wild bee species (Lye et al., 2011). In a field experiment done in southern England, paired, matched colonies of *B. t audax* (reared from nest searching queens caught in the wild) and *B. t. dalmatinus* (obtained from a commercial supplier) were found to have different nectar foraging performances, with the commercial bees performing significantly better than native bees in four out of five study locations, attributed to the larger body mass of *B. t. dalmatinus* (Ings et al., 2006). The commercial colonies produced a mean of 24.7 gynes compared to 0.3 for native colonies (Ings et al., 2006). It is not known whether the superior foraging performance of *B. t. dalmatinus* would be maintained if individuals were able to establish their own colonies in the wild, nor whether this would result in competitive exclusion of native colonies. However, given the widespread use of managed colonies of *B. t. dalmatinus* in the UK at that time, Ings et al. (2006) concluded that it was highly likely that feral colonies had become established in the UK and presented a significant risk of competitive displacement of native *B. terrestris* and other bumble bees species (but see Owen et al., 2016, above). At this time, there has been no peer-review research published on whether non-native subspecies colonies of *B. terrestris* have established in the UK and hence this represents a gap in knowledge. It is technically difficult to investigate, given that wild nests are cryptic, but it should be possible to investigate using molecular ecology approaches (see Conclusions section later in this paper for recommendations).

Risks of hybridization and gene introgression

If non-native, managed bumble bees were to escape in the wild, then there could be genetic disturbance caused by mating with bees from wild populations. Hybridization between different subspecies that are normally geographically separate has potential to alter allele frequencies within and between populations and influence evolution (Balloux & Lugon-Moulin, 2002). Introgression of genes from managed bumble bees to wild populations could happen through three possible mechanism: (1) worker drift; (2) gynes and drones of managed bumble bees that leave greenhouses could mate with wild conspecifics; (3) greenhouse-mated queens could establish new colonies in the wild with subsequent mating of their offspring and wild bees. Goulson (2010) speculated that extensive introgression may have occurred already in the British Isles between managed *B. t. terrestris* / *dalmatinus* and *B. t. audax*, resulting in a single population. However, only a small number of studies have been done to date, and it is not yet possible to say definitively whether hybridization has occurred between managed and wild bumble bees.

Drifting is the process by which worker bees enter a new colony and are accepted by its occupants. It could contribute to gene introgression from managed bumble bees if workers enter wild nests and lay male eggs. Drifting occurs in a number of social bee species and has been explained in terms of errors in orientation and navigation (Free, 1958; Pfeiffer & Crailsheim, 1998). There is evidence that it is also associated with opportunistic worker reproduction and hence could be a mechanism for introducing genes from commercial bumble bees into wild populations. A study of drift behavior in colonies of *B. occidentalis* and *B. impatiens* used for greenhouse tomato pollination in Canada found that successfully drifting workers of *B. occidentalis* had more developed eggs than the resident bees in the host colony in the greenhouse (Birmingham, Hoover, Winston & Ydenberg 2004). The amount of drifting in greenhouses is affected by: (i) the size of the recipient colony, with workers drifting more to older colonies containing a larger number of resident workers (Birmingham et al., 2004); (ii) by the orientation and proximity of colonies. Stacked greenhouse bumble bee colonies have been reported to contain approximately 7 – 20% drifting workers (Birmingham & Winston, 2004; Lefebvre & Pierre, 2007), but the amount of drifting declines with increasing distance between colonies, and most greenhouse bees were found to drift no further than 30 m from their home colony (Birmingham & Winston, 2004). Worker drift appears to be less common between wild bumble bee colonies, presumably because they are naturally separated further apart than in greenhouses. An analysis of nearly 1500 individuals from 14 excavated wild *B. terrestris* colonies in Scotland, in which microsatellites were used to evaluate parentage of adults, eggs and brood, found six drifter workers and four drone offspring of drifters from a total of four colonies (O’Connor, Park & Goulson, 2013). It was concluded that drifting was not common in this case (O’Connor et al., 2013). In a field experiment in eastern England – done using marked workers in colonies founded by wild-caught queens and placed at set distances (2, 20 or 60 m) from each other - 2.7% of workers were accepted drifters, the majority of which (2.0%) came from nests sited 2 m apart (Zanette, Miller, Faria, Lopez-Vaamonde & Bourke, 2014). In a separate experiment in the same study, microsatellite genotyping of eight field-collected nests in southern England indicated that 3% of workers were not full sisters of their nest mates and were considered likely to be drifters (Zanette et al., 2014). To date, no studies have been published on investigations of drifting of managed bumble bees into wild nests and this represents a knowledge gap, therefore.

A small number of studies have been published on interbreeding between the different *B. terrestris* subspecies (Coppée, Terzo, Valterova & Rasmont, 2008; De Jonghe, 1986; Ings, Raine et al., 2005; Lecocq et al., 2013). Under laboratory conditions, members of the different subspecies of *B. terrestris* will interbreed but prefer to mate with their own subspecies (De Jonghe, 1986). Gynes of *B. t. dalmatinus* paired in the laboratory with males of their own subspecies or with *B. t. audax* showed preferential mating for their consubspecific in 71% of cases, which was considered insufficient to prevent the hybridization of escaped *B. t. dalmatinus* in Great Britain with endemic *B. t. audax* (Ings, Raine et al., 2005). However, the situation in the field might be more complex as mating behavior in bumble bees is strongly influenced by chemical cues from drones, which undertake territorial patrol flights to deposit scent marks (specifically, cephalic labial gland secretions, CLGS) that attract females. Laboratory experiments have shown that the drones of the different *B. terrestris* subspecies have specific CLGS profiles (Coppée et al., 2008; Lecocq et al., 2013). In laboratory bioassays, *B. t. dalmatinus* gynes were preferentially attracted to the CGLS from males of their own subspecies when given a choice between *B. t. dalmatinus* versus *B.t. canariensis* or *B. t. xanthopus*, but did not differentiate between *B. t. dalmatinus* and *B. t. sassaricus* (Lecocq et al., 2015). How these findings translate to field effects is not yet known.

Molecular ecology studies of *B. terrestris* have considerable potential to inform ecological risk assessments of managed bumble bees and should include baseline studies of the phylogeography of wild populations in Europe together with assessments of the impact of managed bees in regions where releases have occurred. Microsatellite loci analysis shows that the mainland European *B. terrestris* subspecies (*B. t. terrestris*, *B. t. dalmatinus*, *B. t. lusitanicus*) have a relatively uniform genetic structure characterized by natural gene flow between populations (Estoup, Solignac, Cornuet, Goudet & Scholl, 1996). In contrast, there are genetically distinct subspecies in Sardinia (*B. t. sassaricus*), Corsica (*B. t. xanthopus*) and the Canary Islands (*B. t. canariensis*) (Estoup et al., 1996), and it is reasonable to infer that these island endemics are at greater risk from the effects of introgression with commercial *B. t. terrestris* / *B. t. dalmatinus* compared to the mainland. The genetic structure of the British Isles subspecies *B. t. audax* is more complex. Mitochondrial COI sequence analysis from *B. terrestris* populations in Europe divided the species into two haplotypes, with the British and Irish populations forming a separate lineage from populations sampled from the contiguous mainland, with the latter also including commercially-reared *B. terrestris* from mainland Europe (Moreira, Horgan, Murray & Kakouli-Duarte, 2015). A microsatellite analysis done in the same study separated populations from both Ireland and the Isle of Man from those in Great Britain and mainland Europe, with populations from Great Britain showing significant levels of admixture with those in mainland

Europe (Moreira et al., 2015). It is not yet clear whether the use of managed *B. t. terrestris* / *B. t. dalmatinus* has contributed to this, although the evidence did not point to recent migration events (Moreira et al., 2015). Natural migration between wild *B. terrestris* populations in England and mainland Europe is possible, as bumble bees are capable of dispersing across the English Channel, evidenced by the natural colonization into southern England in 2001 by the tree bumble bee, *Bombus hypnorum*, from mainland Europe (Crowther, Hein & Bourke, 2014). Mitochondrial gene analysis identified *B. terrestris* individuals in the west of Ireland (where use of managed bumble bees is rare) with the COI haplotype normally associated with populations from the contiguous European mainland (Moreira et al., 2015). This could be evidence of a genetic disturbance associated with commercial use of continental *B. terrestris*, but further evidence is required to establish whether the COI haplotype from Britain and Ireland can also occur naturally in mainland European populations (Moreira et al., 2015).

Kraus et al. (2011) used microsatellite markers to look for evidence of gene flow between managed *B. terrestris* sampled from within three greenhouses in Poland and *B. terrestris* individuals sampled in the surrounding outside area, both within the immediate vicinity of the greenhouses and in separate areas at least 30 km away. Population genetic analysis was done to assign individual worker bees to putative mother colonies of both managed and wild bees. The amount of introgression was inferred by determining the number of individual workers sampled outside which could be assigned to managed bee populations. The authors concluded that they “found strong genetic introgression from the sampled greenhouse populations into the adjacent populations” (Kraus et al., 2011). A potential flaw in the approach is that individual bees caught outside and assigned to greenhouse colonies could have been escapes rather than the offspring of a mating between a commercial x wild bee, while the subspecies identities of the commercial and wild bee populations – which would have a strong influence on hybridization – were not determined. Only four microsatellite markers were available at the time, which failed to distinguish between some of the geographically distant (>30km) populations, suggesting some lack of power.

Managed stocks of bumble bees and increased disease threats to wild populations

Livestock can act as a source of infectious disease for wild, co-occurring animal populations, with endangered species reported to be at particular risk (Daszak, Cunningham & Hyatt, 2000; Tomley & Shirley, 2009). Bumble bees are susceptible to a range of macro- and microbial parasites that can be detrimental to both individual bees and their colonies (Allen, Seeman, Schmid-Hempel & Buttermore 2007; Brown, R. Schmid-Hempel & Schmid-Hempel, 2003; Lipa & Triggiani, 1988; Macfarlane, Lipa & Liu, 1995; Manley et al., 2015; Otti & Schmid-Hempel, 2007; Plischuk, Meeus, Smagghe & Lange, 2011; Poinar & Van der Laan, 1972; Rutrecht & Brown, 2008; Schmid-Hempel & Loosli, 1998) (see Supplemental Material S4 for details on the biology of the main pathogens of bumble bees). Concerns that stocks of managed bumble bees might pose a disease risk to wild populations date back to the 1990's, when colonies of *B. terrestris* and *Bombus ignitus* produced in Europe, and exported to Japan for use in commercial greenhouse crop production, were found to be infected with European strains of the tracheal mite *Locustacarus buchneri* (Goka et al., 2000; Goka, Okabe & Yoneda, 2006; Goka, Okabe, Yoneda & Niwa, 2001). An explicit association between managed bumble bees, infectious disease and population declines of wild bumble bees in North America was first raised by Thorp and Shepherd (2005) as part of a profile of the conservation status of North American *Bombus* species. Writing about rapid declines in *B. occidentalis, B. affinis* and *B. franklini*, they stated that "circumstantial evidence indicates that the principal cause for these population declines is the introduction of exotic disease organisms and pathogens via trafficking in commercial bumble bee queens and colonies for greenhouse pollination of tomatoes" (Thorp & Shepherd, 2005*)* although no data were presented in support. The topic was picked up by the US National Research Council in their report on the Status of Pollinators in North America (National Research Council, 2006), in which it was stated (again without hard evidence) that "A major cause of decline in native bumble bees appears to be recently introduced non-native protozoan parasites, including *Nosema bombi* and *Crithidia bombi*, probably from commercial bumble bees imported from Europe for greenhouse pollination. The bees frequently harbor pathogens and their escape from greenhouses can lead to infections in native species" (National Research Council, 2006). This view has been repeated elsewhere (Goulson, Nicholls, Botías & Rotheray, 2015). Following on from the statements from the USA, experimental studies were done in North America and Europe to investigate whether managed bumble bees act as reservoirs of infectious disease which then spread to wild populations. These studies are discussed below. While the focus of attention in the USA was initially about introductions of non-native pathogens from native managed bumble bees, in principal there could also be damaging effects from indigenous pathogen strains, and this has been the focus of studies in Europe (see below for details). Graystock, Blane, McFrederick, Goulson & Hughes (2015) proposed three mechanisms by which the presence of managed bumble bees could cause increased harm from infectious diseases in wild sympatric populations: (1) competition and other ecological stresses from managed bumble bees cause wild bumble bees to be more susceptible to infection; (2) stocks of managed bumble bees form a reservoir of pathogens, which then spill over into wild bee populations; (3) managed bumble bees acquire pathogens from wild bumble bees, with the pathogen population then multiplying within managed colonies and 'spilling back' to the wild in such a way that pathogen prevalence is increased in the wild population. The three commonest bumble bee pathogens - the trypanosome *Crithidia bombi*, the microsporidian *Nosema bombi* and the apicomplexan *Apicystis bombi* – affect all *Bombus* species and have a number of common features: (1) they are obligate parasites, and hence have evolved mechanisms for maintaining themselves within host populations; (2) they are transmitted fecal-orally between bees within the colony, and have potential to spread by direct contact (transmission between managed stocks and wild bees could occur through drifting, for example) or indirectly via flowers that have been fed upon previously by infected bees (see Supplemental Material S4 for details). The majority of studies on disease threats associated with managed bumble bees focus on these pathogens.

The premise underlying most of the published research is that pathogens associated with managed bees represent a significant threat to wild bumble bees (Cameron et al., 2016, 2011; Graystock et al., 2014, 2013; Meeus et al., 2011; Otterstatter & Thomson, 2008; Whitehorn et al., 2013). For example, Otterstatter & Thomson (2008), writing about their research on disease risks from managed bumble bees in North America, state that "it is probable that destructive pathogens have been spilling over into wild bee populations since the collapse of commercial *B. occidentalis* in the late 1990s, and this has contributed to the ongoing collapse of wild *Bombus sensu stricto*". Similarly, in the UK, Graystock et al. (2013) concluded that "commercially produced bumble bee colonies carry multiple, infectious parasites that pose a significant risk to other native and managed pollinators". However, when reviewing the available literature as a whole, a more complex picture is evident in which the effects of the commonest bumble bee pathogens is dependent upon sex, caste, environmental factors, and prevalence. Empirical studies on the effects of *C. bombi*, *N. bombi* and *A. bombi*, done under controlled laboratory conditions, show that: (1) *C. bombi* can damage individuals and colonies if they are subject to some other form of physiological or environmental stress, but otherwise it has low virulence; (2) *N. bombi* infection causes reduced survival of workers but this may not translate to reduced colony size, and while queen fecundity is not affected, the reproductive potential of sexual offspring (gynes and drones) is greatly reduced; (3) *A. bombi* has a lower natural prevalence than either *C. bombi* or *N. bombi*, but when present in queens it can cause a substantial reduction in life span (see Supplemental Material S4). Care needs to be taken when extrapolating the results of these laboratory studies to the field, where the impact of infections on individual bees and the colony are likely to be affected not only by the inherent virulence of the pathogen and the infection intensity, but also by a wide range of other factors including host genotype (Baer & Schmid-Hempel, 2003), genetic diversity within the colony (Baer & Schmid-Hempel, 1999), bee genotype x pathogen prevalence interactions (Manlik, R. Schmid-Hempel & Schmid-Hempel, 2017), host fitness components such as body size, age and foraging activity (Allen et al., 2007, and references therein), group size and structure (Schmid-Hempel, 2017), and gut-active, anti-parasitic effects of the bee microbiome (Mockler, Kwong, Moran & Koch, 2018), pollen (Locascio, Pasquale, Amponsah, Irwin & Adler, 2019) and phytochemicals acquired during foraging (Palmer-Young, Sadd, Stevenson, Irwin & Adler, 2016). For these reasons, the effects of infection on individual bees, colonies, populations and pollination service provision are likely to vary from situation to situation and are difficult to forecast using information from laboratory studies. An additional factor is a lack of systematic, baseline monitoring of natural pathogen prevalence over long time periods, across large geographical scales and in diverse landscapes, which makes it difficult to interpret individual studies of pathogen effects of managed bumble bees. The published studies on pathogen occurrence - which includes samples from wild bumble bees and experimental colonies - indicates that prevalence varies naturally in space and time for different pathogens: 11 – 80% for *C. bombi*; 1 – 71% for *N. bombi*; and 1 – 8% for *A. bombi* (summarized by Allen et al., 2007; see also Evison et al., 2012; Koch & Strange, 2012; Manlik et al., 2017; Vavilova et al., 2015).

Studying the pathogen risks from managed bumble bees is technically and logistically difficult, and so far, only a small number of studies have been conducted in Europe (they are discussed in detail below). Hence, for this review we have widened the evidence base to include studies on disease risks from managed, native bumble bees done in North America. This is a comparable situation to Europe (i.e. use of managed, native bumble bees). An ecologically different situation occurs where commercially produced, domesticated bumble bees are used outside of their native range. The use of bumble bees in this way has caused the introduction of non-native species or strains of parasites in Japan (*L. buchneri*) (Goka et al., 2000, 2006, 2001) and could have been responsible for pathogen introduction into South America (*A. bombi*) (Arbetman, Meeus, Morales, Aizen & Smagghe, 2013) and these are considered in Supplemental Material S3.

Disease threats from managed bumble bees: evidence from North America that is relevant to Europe

Commercial production of *B. occidentalis* and *B. impatiens* for pollination of greenhouse crops commenced in the USA and Canada in the early 1990s (Velthius & Van Doorn, 2006). This was done initially (from 1992 – 1994) using queens captured from the wild and exported to Europe for mass rearing of colonies; the colonies were then shipped to North America. This was followed a few years later by commercial colony rearing in facilities set up in eastern Canada and California (Velthius & Van Doorn, 2006; Cameron et al., 2016). Mass rearing of *B. occidentalis* was stopped in 1997. Velthius and Van Doorn (2006) reported that this was because of problems with infestations with *N. bombi* that first occurred in 1996, although no such problems occurred with *B. impatiens*.

The published evidence from North America on whether managed bumble bees are reservoirs of infectious disease is conflicting. The bumble bee producers maintain colonies at high densities within their rearing units, and hence any pathogen that establishes a foothold in the unit could spread rapidly with detrimental effects on production. Therefore, the companies have in place quality assurance (QA) systems intended to prevent pathogens from entering, being transmitted within their operations, and infected colonies being sold to customers. This involves evaluating colonies for the presence of parasites and disease. A detailed analysis has been published of the pathogen testing program at Koppert's *B. impatiens* production facility in the USA, co-authored by university scientists and Koppert staff (Huang, Skyrm, Ruiter & Solter, 2015). The QA system reported at the facility involves evaluating colonies at multiple stages of the production cycle. All colonies are assessed for development, overt disease and visible ectoparasites, and all colonies entering the queen production process are also screened for *N. bombi, C. bombi*, *A. bombi* and *L. buchneri*. This involves sampling a minimum of 10% of the workers per colony. Any colonies that test positive are destroyed, and if pathogens are detected, further tests are done on other colonies in the area. This assessment system was done originally using microscopic examination of gut contents for pathogens, but this was supplemented in 2013 by the introduction of a multiplex real-time PCR diagnostic of gut tissue (which included positive, negative and no-template controls) which was considered to have significant benefits in terms of efficiency and ease of use. Tests of the detection limits of the system were made, indicating that multiplex PCR was able to detect pathogens down to the limit of detection available with microscopic examination of gut contents. The QA system also involves an audit by a government inspector and an annual inspection by university scientists. Publication of the results of the pathogen screening program (queen rearing colonies) from 2008 – 2014 reported that 64 / 24,226 colonies tested positive for pathogens and parasites (colony prevalence = 0.26%, total number of bees tested = 127, 528) (Huang et al., 2015). However, these results are in contrast to a number of reports, dating from the early 2000's to 2015, that both *N. bombi* and *C. bombi* were present in managed bumble bee colonies of *B. occidentalis* and *B. impatiens* at high prevalence levels in North America. Whittington and Winston (2003) used microscopic examination of frass and abdomen contents to quantify *N. bombi* levels in *B. occidentalis* individuals from 49 newly-opened commercially reared colonies supplied to a commercial tomato greenhouse grower in British Columbia. Spores of *N. bombi* were recorded in bees from 51% of the colonies upon arrival, while after ten weeks all colonies contained infected bees. Infection intensity increased over this time to an average of 6×10^6 spores per bee, although these infections had no effect on adult population size, amount of brood or numbers of reproductives (Whittington & Winston, 2003). In Ontario, Gegear, Otterstatter and Thomson (2005) reported that approximately 30% of managed *B. impatiens* colonies obtained from a supply company contained individuals infected with *C. bombi*, while Otterstatter and Thomson (2007) identified infections of *C. bombi* in queens from newly opened managed colonies of *B. impatiens* (the proportion of colonies containing infected queens was not stated in the paper).

Otterstatter and Thomson (2008) evaluated the prevalence and intensity of pathogen infections in bumble bee species sampled at increasing densities from two commercial greenhouses in Ontario that deployed managed *B. impatiens* colonies for tomato and pepper crop pollination, and which was compared to a control site 50km from the nearest greenhouse. It was reported that both the prevalence and intensity of *C. bombi* infections among all sampled *Bombus* species decreased with increasing distance from the greenhouses, and this effect was still significant when *B. impatiens* was excluded from the analysis. From this it was concluded that the managed *B. impatiens* were acting as a focus for infections in wild bumble bees. Between 33 – 47% of sampled bees harbored *C. bombi* infections within 30 m of the greenhouses, while the zone of *C. bombi* infection extended for 2 – 6km from the greenhouses (Otterstatter & Thomson, 2008). Finally, in Mexico, a PCR screen of RNA collected from individual *B. impatiens* obtained from newly opened managed colonies from 120 different greenhouses found that 45% of locations tested positive for at least one pathogen, with *A. bombi* being the most common (32 /120 locations) (Sachman-Ruiz, Narváez-Padilla, & Reynaud, 2015).

Significant declines in range and relative abundance have occurred for four wild bumble bee species in North America: *B. occidentalis* (in the west) and *B. affinis, B. pensylvanicus* and *B. terricola* (in the east), with the fall in relative abundance occurring since the 1980s / 1990s and coinciding with the expansion of the managed bumble bee industry (Cameron et al., 2011, and summarized by Brown 2011). A fifth species, *B. franklini*, is potentially extinct (surveys in southern Oregon and northern California have failed to detect it since 2006) (Hatfield et al., 2015). It was hypothesized that the declines were caused by pathogen spillover from managed bees, specifically that a virulent strain of *N. bombi* transferred from *B. terrestris* to *B. impatiens* and *B. occidentalis* when these three species were being reared in the same production facilities in Europe, and then spilled over to the wild with bees exported to N. America and subsequently spread through wild bumble bee populations (Evans, Thorp, Jepsen & Black, 2008; Thorp & Shepherd, 2005; also see reviews by Goulson et al., 2008; Williams & Osborne, 2009). However, despite investigations (Cameron et al., 2011, 2016; Szabo, Colla, Wagner, Gall & Kerr, 2012), no causal evidence in support of the hypothesis has been found:

- Szabo et al.(2012) used logistic regression analysis of a large data set compiled for North American bumble bees, which compared occurrence records from 1980 – 1999 with those from 2000 – 2010 (i.e. before and after rapid declines in bumble bees were reported to have begun), and applied predictor variables for commercial greenhouse density (which was taken as a proxy for managed bumble bee use), pesticide use survey data, and change in human population density (as a proxy for habitat loss). Significant but weak relationships were observed between reductions in *B. terricola* and *B. pensylvanicus* and greenhouse density (P = 0.005, R^2 0.17; and P = 0.003, R^2 0.08) but not for *B. affinis*. This was interpreted as providing a direct link between pathogen spillover and bumble bee decline; however, we consider this a false conclusion, as the premise that greenhouse / hive density equates to levels of infectious disease in managed bees is not reliable. An alternative explanation proposed by the authors of effects of competition with escaped managed bees was considered to be less likely. No significant negative relationships were observed between bumble bee losses and pesticide use or human population density (Szabo et al. , 2012).
- An analysis of genetic variation of *N. bombi* from bumble bee specimens from the USA and Europe showed no support for the hypothesis that a non-native *N.*

bombi strain was introduced from commercial rearing operations in Europe (Cameron et al., 2016). Microscope analysis of bumble bees sampled from *B. occidentalis* and *B. pensylvanicus* showed a significantly higher prevalence of *N. bombi* (37% prevalence for *B. occidentalis*, and 15% prevalence for *B. pensylvanicus*) compared to species that were not in decline (Cameron et al., 2011). In addition, molecular analysis of museum specimens collected from 1979 to 2011 in a total of five declining North American bumble bee species (*B. affinis*, *B. franklini*, *B. occidentalis*, *B. terricola*, *B. pensylvanicus*) showed an increase in prevalence of *N. bombi* from the 1980s to after 1992 (prevalence of *N. bombi* in *B. occidentalis* increased from 5 to 14% in this period, for example) whereas control specimens of *B. terrestris* showed a constant prevalence over time (Cameron et al., 2016). It was concluded that the increased *N. bombi* prevalence in wild population, which coincided with a report of pathogen outbreaks in commercial stocks, was indicative of pathogen spillover from managed bees (Cameron et al., 2016, 2011). However, it is possible that these figures simply represent natural variation in the prevalence of *N. bombi* rather than an effect of managed bees. Populations of *B. occidentalis* sampled in Alaska, taken from collection sites geographically distant from agricultural areas using managed bumble bees, had an *N. bombi* prevalence of 45% (Koch & Strange, 2012), suggesting that populations can harbor naturally high levels of the pathogen.

Evidence on disease threats from managed bumble bees in Europe

To date, there is no causal evidence that measurable population declines of wild bumble bees have occurred in Europe as a result of disease outbreaks associated with managed bumble bees. However, researchers have reported that a high proportion of managed bumble bee colonies harbored infective microbial parasites (Graystock et al., 2013) and it has been argued that managed bumble bees are responsible for an increased prevalence of pathogens in local wild bumble bees (Murray et al., 2013; Graystock et al., 2014). Within the UK, these concerns are reflected in the policy action of the National Pollinator Strategy to monitor for spillover risks from managed *B. terrestris* and to act on evidence (DEFRA, 2014). The bumble bee producers have disputed the evidence on pathogen prevalence in commercially reared bees in Europe (BioBest Group, 2013). Because this evidence has proved controversial, it is worth reviewing in detail.

Graystock et al. (2013) conducted a laboratory study in the UK in which a PCR screen was used to determine presence / absence of bee pathogens in worker bees in *B. t. terrestris* / *dalmatinus* and *B. t. audax* colonies supplied by three commercial producers in mainland Europe. *Apicystis bombi*, *C. bombi* and *N. bombi* were detected in 35 / 48 colonies (73% colony prevalence), either alone or in combination. PCR analysis (which included appropriate controls) of the pollen supplied with the colonies detected the presence of at least one of these pathogens in 21 / 25 colonies (84% prevalence). Samples of pollen or bee frass from ‘pathogen positive’ colonies were then pooled to provide a mix of *N. bombi*, *N. ceranae, C. bombi*, *A. bombi* and deformed wing virus (frass) plus *Nosema apis* and the bee pathogenic fungus *Ascosphaera* sp. (pollen). These were fed within a sucrose solution to adult *B.t. audax* in a laboratory bioassay. Concentrations of *Nosema* and *Apicystis* spores were enumerated using hemocytometer counts. Survival of adult bumble bees was reduced for the frass (= 45% survival at 15 days) and pollen treatments (35% survival at 15 days) compared to the control (65% survival), while pathogens could also be detected in dead bees. The same laboratory bioassay was also used to establish infections in adult and larval honey bees (*Apis mellifera*) resulting in reduced survival. The authors concluded that managed bumble bees and their pollen feedstuff contained infectious pathogens. They described the implications of their findings for wild bee populations as "genuinely alarming" and made a series of policy recommendations including the adoption of methods to remove pathogens from pollen feedstuff and putting into place more effective screening procedures at the production site and at the point of delivery. However, the results of this study have been questioned by the commercial producers of bumble bees in Europe. In a written response to the paper, Biobest (Biobest Group, 2013) stated that the results contradicted independent tests for pathogen presence done on their bees over many years. They also stated that their production systems are designed to deliver disease-free bees to customers, including the use of sterilized pollen (which is therefore unlikely to be a source of pathogen contamination) and both in-house and independent pathogen screening, and have been visited by the UK government regulatory bodies (Biobest Group, 2013).

Pereira, Meeus & Smagghe (2019) performed a PCR-based pathogen screen on 17 samples of non-irradiated honey bee pollen obtained from companies in Romania and Spain who supply pollen to commercial bumble bee producers, with the intention of investigating whether this pollen represented an infection risk to bumble bee rearing. Sequences for eleven different insect pathogens were identified from pollen from both countries: the fungal pathogen *Ascosphaera apis* (the causative agent of chalk brood, prevalence on pollen samples = 47%); *A. bombi* (prevalence = 53%); *Microsporidium* sp. Oise (12%); *Crithidia* spp. (71%); *Nosema ceranae* (24%); *Nosema thomsoni* (18%); deformed wing virus (12%); Israeli acute paralysis virus (6%); chronic bee paralysis virus (6%); and sacbrood virus (59%). In addition, *Paenibacillus larvae* (the causative agent of American foul brood in honey bees) was found in a sample of pollen from Romania. Batches of pathogen-positive pollen (containing *A. apis, A. bombi*, *Crithidia* spp., *Microsporidium* sp. Oise, *N. ceranae, N. thomsoni*, and *P. larvae*) were then fed to *B. terrestris* micro colonies, and after 15 and 20 days, molecular diagnostics were done on gut and fat body tissue from adults and larvae. Of these, *A. apis* – infected larvae were observed in 3 / 4 treated colonies. No infections were observed in bees fed pollen treated with gamma radiation as a control. The authors concluded that unsterilized pollen represented an infection risk to bumble bee rearing facilities, and stated that effective diagnostic and control systems were needed by bumble bee producers as “risk of spillover to wild bee species is possible” (Pereira et al., 2019). However, if sterilization is used (see above) then infection is unlikely.

The small number of field experiments done so far on pathogen spillover in Europe show a complex picture. Whitehorn et al. (2013) evaluated the prevalence and abundance (= infection intensity) of *Crithidia* spp., *N. bombi* and *A. bombi* in fecal samples from bumble bees (*B. terrestris*, *B. pratorum*, *B. pascuorum*, *B. lapidarius*) sampled over four months from fruit farms where managed *B. terrestris* colonies were either present or absent. Both *A. bombi* and *N. bombi* occurred infrequently (overall prevalence of 0.7% and 2.0% respectively) while the overall prevalence of *Crithidia* spp. was 39%. *Crithidia* spp. exhibited a complex pattern of change that differed among bumble bee species. For *B. terrestris*, prevalence was lower overall on farms where managed bees were deployed, and while prevalence remained relatively level across the season in *B. terrestris* sampled on farms where managed bees were not used, there was an increase in prevalence at the end of the season on farms where managed bees were present. This was interpreted as possibly being a result of the reproduction and spread of the pathogen among managed hives associated with their high population density, however a similar pattern of increase in *Crithidia* spp. prevalence in the latter half of the season was observed in *B. pratorum* irrespective of whether farms used managed or not (Whitehorn et al., 2013). The authors concluded that there was no evidence from their study of spillover from managed bee colonies to other, wild bumble bee species, although Graystock et al. (2015) - in a review of disease threats from managed bees - reported that the *Crithidia* spp. results could suggest pathogen spillback.

Murray et al. (2013) investigated pathogen prevalence in *B. terrestris* at six sites in Ireland where managed bumble bees were used in strawberry crops grown in glasshouses, polytunnels and open fields for a minimum of ten years previously. Bumble bees were sampled at four distances from the center of each site (250m, 500m, 1km, 2km) and identified to species level using a PCR-RFLP diagnostic test. Individuals identified as *B. terrestris* were then examined by dissection for the presence of *A. bombi*, *Crithidia*, *N. bombi* and *L. buchneri*. Each site was paired with a control area 10km away that consisted of a comparable habitat but lacked a strawberry farm, and bees were collected within a 500 m radius of this control site. Samples were also taken of five worker bees from each of 68 managed colonies upon delivery at each farm. As part of the same study, pollen samples were taken from foraging workers returning to managed colonies, and the plant species identity of foraged plants was determined by microscopic analysis of individual pollen grains. The pollen analysis showed that managed bumble bees were foraging on plants other than strawberry crops, with the amount of foraging dependent upon the strawberry cropping system and the ease of access to other pollen sources. Meanwhile, examination for pathogens in newly opened managed bee colonies showed that *Crithidia* was present in 35% of colonies, *N. bombi* in 62%, and *A. bombi* in 1.5% of colonies. *Crithidia* and *N. bombi* occurred together in 25% of colonies, while no pathogens were observed in 26% of colonies. The prevalence of *Crithidia* in field caught *B. terrestris* workers declined significantly with increasing distance from greenhouses that contained managed colonies (*Crithidia* prevalence was approximately 15% at 250m and approximately 5% at 10km) but no pattern was observed for male *B. terrestris*. In contrast, *N. bombi* prevalence declined significantly in field caught *B. terrestris* males with increasing distance from the greenhouse (from approximately 35% at 250m to 10% at 10 km) but no significant pattern was observed for workers (where *N. bombi* prevalence remained at about 18% irrespective of distance from the greenhouse). The authors concluded that their results were indicative of the spread of infectious disease from managed *B. terrestris* to wild conspecifics up to a range of 10km from greenhouses. A legitimate criticism, raised by the authors themselves, is that the methods deployed were not able to distinguish between wild and managed *B. terrestris*, and hence did not provide definitive proof of pathogen spillover to the wild, but instead may have reflected sampling bias in favor of pathogen-infected managed bees that were more likely to be found near greenhouses than further away. To address this point, the authors stated that they did not detect higher abundance of *B. terrestris* closer to greenhouses (unpublished data in Murray et al., 2013). However, it is our view that without the ability to distinguish between wild and managed bees, the issue of potential sampling bias cannot be resolved in this case, while the different patterns observed for different pathogens makes it difficult to draw a generalized conclusion.

Graystock et al. (2014) published the results of a field survey on pathogen prevalence in field-caught bumble bees in relation to their proximity to managed bee colonies. Bumble bees were sampled at increasing distances (0.5, 3 and 5 km) from five commercial greenhouses in England, three of which used managed bumble bees (200 –

300 hives per site) and two where managed bumble bees were not used. Samples consisted of a range of *Bombus* species, the abundance and diversity of which varied between sites (*B. terrestris*, *B. hortorum*, *B. hypnorum*, *B. lapidarius*, *B. lucorum*, *B. pascuorum* and *B. pratorum* were all observed), with most samples consisting of *B. terrestris* (40%) or *B. lapidarius* (25%). PCR-based diagnostics were then used to screen for the presence / absence of a range of pathogens in a total of 764 individual bees, although there was no testing of bumble bees collected directly from managed colonies, while the methods used in the study were not able to distinguish between wild and managed *B. terrestris*, nor were pathogen prevalence patterns in relation to *Bombus* species investigated. Overall, bumble bees sampled from sites deploying managed hives had significantly more parasite species than those in which managed bees were absent. The prevalence of *A. bombi* declined with increasing distances from greenhouses in which managed bumble bees were deployed (from c. 48 % at 0.5km to c. 8% at 5 km), and no such pattern was found for greenhouses where managed bumble bees were absent. *Crithidia bombi* was more prevalent at sites containing managed bees compared to control sites, but there was no effect of proximity to the greenhouse. The authors urged that some caution in interpretation given that then study involved a small number of sites, but concluded that use of managed bumble bees appeared to increase pathogen prevalence in local bumble bees. As part of the same paper, an additional study was done in which pathogen prevalence was quantified in five commercial *B. t. audax* colonies positioned on the side of a field containing an apiary of 50 honey bee hives, and compared to five *B. t. audax* colonies sited 1 km away. In this case, average parasite richness was significantly higher in colonies close to the honey bee apiary, while the average prevalence of *C. bombi* was significantly higher in the bumble bee colonies close to the apiary (58% versus 30%) (Graystock et al., 2014).

Conclusions

The deployment of managed *B. terrestris* for crop pollination provides significant benefits for growers in terms of improved yield and quality of production, and it has become an integral part of the European horticultural industry. However, there are also plausible risks with potential for negative effects on wild bumble bees if appropriate safeguards are not in place. Having reviewed the scientific literature, we do not believe that there is sufficient evidence at present to support the statements by some authors that the current use of managed *B. terrestris* in Europe is harmful to wild bumble bees (Goulson et al., 2015; Graystock et al., 2015, 2014, 2013; Kraus et al., 2011; Murray et al., 2013). Given the uncertainty arising from what is a complex issue and a small evidence base, it is understandable that regulatory authorities are taking a precautionary approach to managed bumble bees. Effective regulation of managed *B. terrestris* requires reliable evidence on potential and actual risks, as well as good baseline information on bumble bee ecology, genetics, and diseases. We make the following recommendations to help fill the current knowledge and policy gaps:

(1) *Establishment of non-native B. terrestris subspecies and risks from gene introgression:* Gene introgression from non-native subspecies of *B. terrestris* is a particular concern because of the potential for long term or irreversible effects. Currently, there are government regulations that prevent the routine use of non-native, mainland European *B. t. terrestris* and *B. t. dalmatinus* in areas where island endemic subspecies reside (e.g. Canary Islands and GB), while the bee producers themselves have introduced a voluntary restriction on use of these non-native subspecies in the Republic of Ireland. This is a sensible precaution, given that cross breeding has been demonstrated under laboratory conditions, although there is no

strong evidence yet that hybrids of managed bumble bees x island endemics have established in the field. The regulatory position is complex for Great Britain, where *B. t. audax* is an endemic but where there is also evidence of natural gene flow with *B. terrestris* on the European mainland. In principle the situation is more straightforward in central mainland Europe, where the three recognized morphological subspecies (*B. t. terrestris*, *B. t. dalmatinus*, *B. t. lusitanicus*) show no significant differentiation in molecular markers and in all likelihood represent a monophyletic group. However, it would be worth conducting more baseline studies on the phylogenetic relationships of the *B. terrestris* subspecies to get a better understanding on patterns of evolution and geneflow to inform risk assessment for managed bumble bees. This could be used, for example, to inform the decision in Norway to use only locally produced colonies. Investigations are also warranted to better understand whether use of non-natives has impacted on local *B. terrestris* population structure. One suitable study area would be the Isle of Wight in southern England, which is geographically isolated and has a large glasshouse industry where *B. t. terrestris* / *dalmatinus* was used continuously for 30 years until 2015. Questions to be investigated would include whether or not colonies of non-native subspecies have become established (this would also provide useful policy information on risks of competitive exclusion of native bumble bees) and whether gene introgression into native populations has occurred.

(2) *Disease threats from managed bumble bees to wild populations:* There is a marked contrast between studies of pathogen prevalence levels in commercial bumble bee colonies conducted by university researchers in North America and Europe, which report colony prevalence levels in the range of 30 – 73%, compared to Koppert's independently audited QA data on their bumble bee production facility in North

America, which reported a colony prevalence level of 0.26%, with any pathogen-positive colonies being destroyed before dispatch (Huang et al., 2015). The commercial production of *B. t. audax* for sale to the UK market (which is done in facilities in mainland Europe) has been criticized by Graystock et al. (2015, 2013) for being essentially unregulated, with no legal requirement for pathogen screening, and for producing colonies with very high pathogen prevalence levels at the point of delivery, although this has been strongly refuted by the industry (Biobest Group, 2013). Data on pathogen screening has not been published by the industry for their production facilities in Europe, but if we assume that quality assurance protocols in the USA have been based on the systems that were already deployed by their parent company in Europe, then we would predict similar, low prevalence levels. The reasons for such a large disparity in these figures are not immediately apparent to us. This is clearly an important issue, because reports of high disease prevalence in managed bumble bees are quoted in government risk assessments (e.g. Natural England 2013, 2014). The case proposed by some researchers in the UK and Ireland for pathogen spillover from managed bumble bees rests largely on these colonies having a high pathogen prevalence (Graystock et al., 2014, 2013; Murray et al., 2013). This leads us to two recommendations: firstly, that a standardized method for quantifying pathogen prevalence, based on blind testing, is agreed upon by bee producers, independent researchers and government bodies, and secondly that QA data from bee producers in Europe is independently audited and published, as has been done in the USA (Huang et al., 2015). There is also a need for better baseline data on variation in natural pathogen prevalence in wild bumble bee populations, as well as research to determine whether pathogens are acquired by colonies from the field or glasshouse environment and increase to above-background levels during the season.

(3) *Best practice by end users:* At our current state of knowledge, the reduced risk scenario for managed *B. terrestris* is to use native bees from producers with trusted QA systems that prevent diseased colonies being dispatched to growers. Within this scenario, it would still be worth including management practices designed to minimize effects on wild bumble bees, such as ensuring that managed colonies are killed prior to disposal.

(4) *Dealing with uncertainty:* Evaluating the environmental risks from managed bumble bees is a difficult area. There are inherent challenges in extrapolating the findings of controlled laboratory experiments to the complex environment of the field, while field experiments themselves often have limited cause-and-effect explanatory power. Few of the papers published so far acknowledge these limitations fully, and we are concerned that some articles have made statements about managed bumble bees causing harm to wild bees that are not warranted on the basis of evidence, but which nevertheless have been reported widely in the general media. Moreover, it must be remembered that there are multiple interacting factors potentially associated with declines in bumble bee abundance, including habitat loss and degradation, pollution and climate change (Potts et al., 2010): disentangling these other drivers from the proposed effects of managed bumble bees on wild bumble bees at the landscape level is going to be extremely difficult. We currently have very limited knowledge on whether habitat degradation, for example, could affect the outcome of competition between managed and wild bumble bees or interact with pathogen infection. This all points to a need for more research to inform policy makers, but given the complexities and the scale of the challenges involved in conducting

individual experiments, there is also a need to draw on other, complementary methods such as use of structured expert panels (Barons et al., 2018). Government bodies at both national and European levels have a responsibility to deliver policies and regulations that allow managed bumble bees to be used in an environmentally sustainable way, but bumble bee producers, growers and environmental groups are also actively involved and should form part of a joint, consensus building program.

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