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Influence of urbanisation on the prevalence of protozoan parasites of bumblebees

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Abstract. 1. Increasing urbanisation is often cited as a cause of declining biodiversity, but for bumblebees there is evidence that urban populations of some species such as *Bombus terrestris* L. may be more dense than those found in agricultural landscapes, perhaps because gardens provide plentiful floral resources and nesting opportunities.

2. Here we examine the influence of urbanisation on the prevalence of the main protozoan parasites of bumblebees in west central Scotland. We would expect transmission rates and prevalence of parasites to be higher in high density host populations, all else being equal.

3. Workers of two bee species, *B. terrestris* and *B. pascuorum*, were sampled over a 45-day period in mid to late summer, and parasites were detected in faeces and via dissection. A comparison of the two methods suggests that faecal sampling is considerably less sensitive than dissection, failing to detect infection in 27.8%, 55.1%, and 80% of cases of infection with the parasites *Crithidia bombi*, *Nosema bombi*, and *Apicystis bombi*, respectively.

4. For all three parasites, broad patterns of prevalence were similar, with prevalence tending to increase with urbanisation in *B. terrestris* but not in *B. pascuorum.* The different patterns of seasonal prevalence in the two bee species suggest that intraspecific transmission is more important that interspecific transmission.

5. Our observation of greater parasite prevalence among *B. terrestris* in urban compared with rural areas suggests that urban habitats may present greater opportunities for parasite transmission. Greater bee densities in urban areas may be the driving factor; however, further study is still needed. For example, differences in disease prevalence between habitats could be driven by differences in the types and abundance of flowers that are available, or in exposure to environmental stressors.

Key words. *Apicystis bombi*, Apidae, *Bombus*, *Crithidia bombi*, Hymenoptera, *Nosema bombi*, population density, transmission.

Introduction

In addition to the important role that they play as pollinators, bumblebees and their nests support a diverse array of predators, parasites, and commensals (Schmid-Hempel, 1998). With widespread evidence for range contractions and local extinctions of various bumblebee species in Europe, North America, and Asia (Kosior *et al.*, 2007; Goulson *et al.*, 2008; Williams & Osborne, 2009), it seems likely that this could lead to declines in the species that depend upon bumblebees.

Several lines of evidence suggest that urban areas may support higher populations of bumblebees than farmland. Young nests of *Bombus terrestris* L. placed in suburban gardens have been found to grow more quickly when compared with nests placed in arable farmland, and analysis of the pollen loads of workers suggests that urban areas provide a greater diversity of pollen sources than farmland (Goulson *et al.*, 2002). McFrederick and LeBuhn (2006) found urban parks in San Francisco to have higher mean abundance of bumblebees compared with nearby ‘wilder’ areas. Osborne *et al.* (2008a) used a public survey to quantify bumblebee nest densities in

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both gardens and a range of rural habitats and found the highest nest densities in gardens. Goulson *et al*. (2010) found that the numbers of nests represented by foraging workers sampled in farmland was positively related to the area of gardens within 1 km of the sample site.

If bumblebee population density is higher in urban areas, we might expect associated organisms to also be more abundant. In particular, transmission rates of horizontally transmitted parasites are predicted to be linearly related to host density (e.g. Anderson & May, 1981; Goulson *et al*., 1995); if all else is equal this should lead to higher prevalence in high density host populations. Goulson *et al*. (2002) describe higher rates of infestation of experimental bumblebee nests with the bumblebee-specific wax moth, *Aphomia sociella* when placed in gardens compared with farmland, and they infer that this may be due to higher densities of bumblebee nests in gardens. Here, we test the prediction that the prevalence of protozoan parasites of bumblebees will be higher in more urbanised compared with more rural habitats, by sampling workers of *Bombus pascuorum* Scopoli and *B. terrestris* from sites in central Scotland. We also quantify the incidence of conopid fly larvae (parasitoids), since these too may be more abundant in areas with more hosts.

Materials and methods

Worker bumblebees were sampled between 24 July and 6 September 2010 from 10 sites in central Scotland (Fig. 1). Using data from OS MasterMap Topography Layer (EDINA Digimap) we calculated the percentage cover of urban areas (buildings, structures, and roads) within a 1-km radius of each sample site. This radius broadly corresponds to the maximum foraging range of worker bumblebees (Darvill *et al*., 2004; Knight *et al*., 2005, 2009; Osborne *et al*., 2008b). Sites were selected to span a broad range from 1.5 to 70.7% of urban area within a 1-km radius. At each site, bees were sampled from within an approximate 100 m radius of the given location. Sites were a minimum of 2 km apart. All workers of *Bombus terrestris* and *Bombus pascuorum* were caught until the sample size for both was approximately 20 (range 15–29 bees per species per site), with sites being visited repeatedly, as necessary, until sufficient bees were obtained. In total 201 *B. terrestris* and 203 *B. pascuorum* were sampled.

Once caught, bees were kept in ventilated plastic tubes for approximately 1 h. Many of the bees defecated during this time (224 out of 404). Faecal samples were collected using glass capillary tubes, stoppered with modelling clay, and brought back to the laboratory for microscopic analysis. The presence of *Crithidia bombi* Lipa & Triggiani, *Nosema bombi* Fantham & Porter, and *Apicystis bombi* Liu, MacFarlane & Pengelly were recorded. Once faecal samples had been obtained from as many bees as possible, all the bees were freeze killed and stored for dissection. Before dissection each bee was scored on a four-point scale of wing damage, a commonly used surrogate for age (e.g. Whitehorn *et al*., 2011a). During dissection, bees were examined for the presence of conopid fly larvae, and portions of the Malpighian tubules, fat body, and hind gut were removed and examined under a microscope for the three protozoan parasites. Bees were recorded as infected if a particular parasite was detected in either dissections or faecal samples, and as uninfected if the parasite was not detected by either technique.

Data were analysed separately for each parasite in PASW Statistic 18, using binary logistic regression with infection (presence/absence) as the dependent variable, species and age as explanatory factors, and percentage of urban habitat and date of capture as covariates. All two-way interactions were also included in initial models, and model simplification carried out by stepwise removal of explanatory factors that did not contribute significantly at $P < 0.05$.

Results

Of the 404 worker bees sampled, 167 (41.3%) were infected with *C. bombi*, 181 (44.8%) with *N. bombi*, and 50 (12.4%) with *A. bombi* (Table 1). There was no tendency for individuals infected with *C. bombi* to be more or less likely to be infected with *N. bombi* ($\chi^2_1 = 1.58$, $P > 0.05$), and similarly for *C. bombi* and *A. bombi* ($\chi^2_1 = 2.68$, $P > 0.05$) and *N. bombi* and *A. bombi* ($\chi^2_1 = 2.89$, $P > 0.05$), although it is perhaps worth noting that for all three comparisons the non-significant trend was towards a positive association between the incidence of parasites.

Predictably, dissection appears to be a more sensitive indicator of infection than examination of faecal samples, particularly for *N. bombi* and *A. bombi*. On the 224 bees for which both faecal and dissection data were obtained, faecal samples contained detectable levels of parasites in 91 of 126 infected bees (72.2%) for *C. bombi*, 48 of 107 (44.9%) for *N. bombi*, and 6 of 30 (20%) for *A. bombi*.

Average age did differ among sites (ANOVA, $F_{9,394} = 4.21$, $P < 0.001$), but there was no correlation between estimated age and the degree of urbanisation of the capture site (*Pearson product–moment correlation*, $r = -0.075$, d.f. $= 404$, $P = 0.130$).

Crithidia bombi

Prevalence increased through the season ($\chi^2_1 = 3.98$, $P = 0.046$). There was no significant main effect of species ($\chi^2_1 = 1.48$, $P = 0.224$) or percentage of urban area ($\chi^2_1 = 0.01$, $P = 0.939$), but there was a significant interaction between the two, with prevalence increasing slightly in more urban areas in *B. terrestris* but declining in *B. pascuorum* ($\chi^2_1 = 3.83$, $P = 0.049$, Fig. 2a).

Nosema bombi

Prevalence was significantly higher in *B. terrestris* (56.2%) than in *B. pascuorum* (33.5%, $\chi^2_1 = 11.7$, $P < 0.001$), and increased with bee age (48.5, 52.0, 61.3, and 69.8% for age categories 1–4, respectively, $\chi^2_3 = 8.83$, $P = 0.032$). There was a strong interaction between species and date, with prevalence increasing over time in *B. terrestris* but slightly

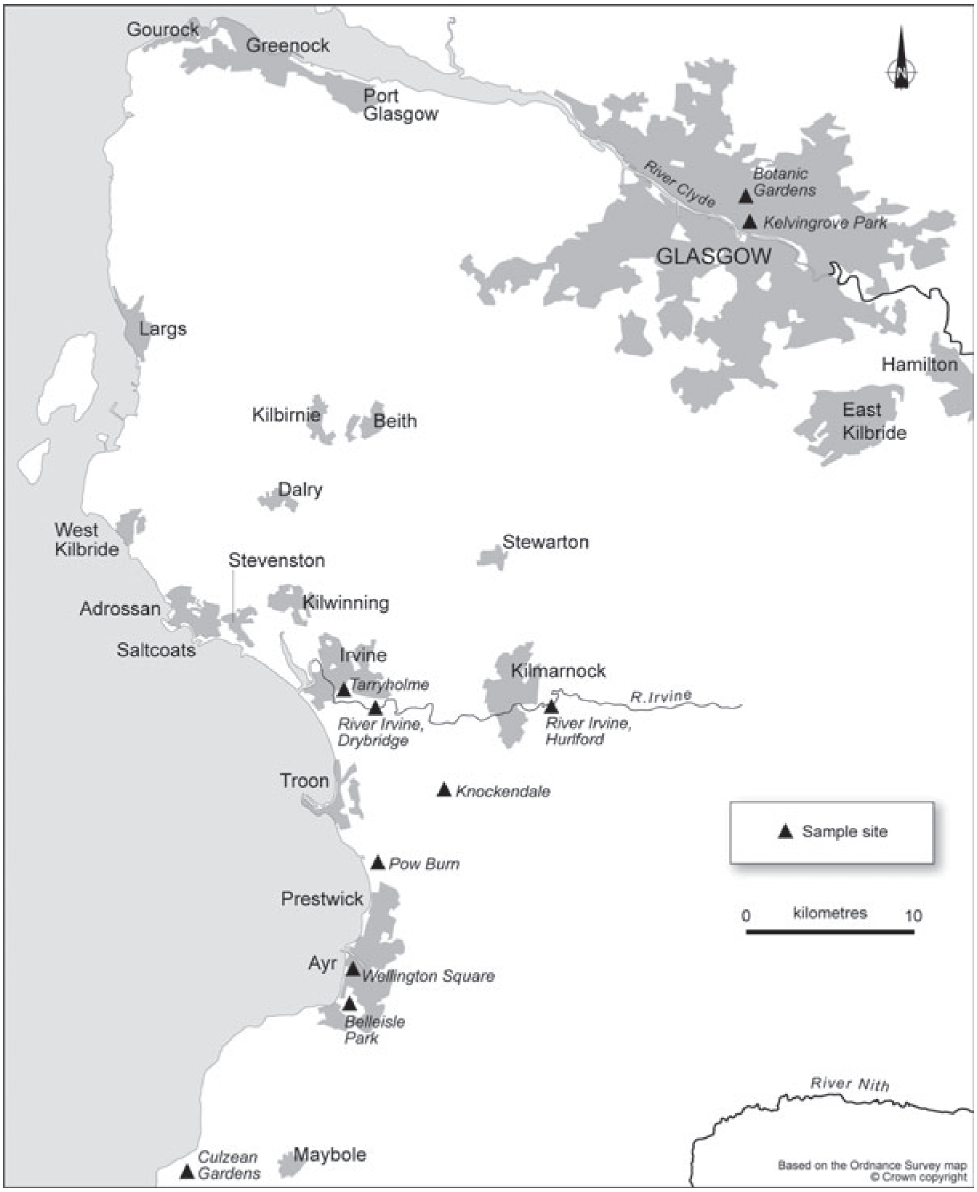

Fig. 1. Locations of sample sites in west Scotland, U.K.: Belleisle Park, Ayr (55°26.424′N, 4°38.488′W); Botanic Gardens, Glasgow (55°52.681′N, 4°38.488′W); Kelvingrove Park, Glasgow (55°52.052′N, 4°17.299′W); Tarryholme, Irvine (55°36.222′N, 4°39.230′W); Wellington Square, Ayr (55°27.679′N, 4°38.118′W); Culzean Gardens, Maybole (55°20.929′N, 4°46.555′W); Knockendale, Symington (55°33.498′N, 4°33.241′W); Pow Burn, Prestwick (55°30.914′N, 4°37.169′W); River Irvine, Drybridge (55°35.807′N, 4°37.609′W); and River Irvine, Hurlford (55°36.126′N, 4°27.268′W).

declining in *B. pascuorum* ($\chi_1^2 = 21.1$, $P < 0.001$, Fig. 3). There was no significant main effect of percentage of urban area ($\chi_1^2 = 1.85$, $P = 0.174$), but as in *C. bombi* there was an interaction between species and urban area, with prevalence being markedly higher in more urban areas in *B. terrestris* but showing no clear pattern in *B. pascuorum* ($\chi_1^2 = 5.61$, $P = 0.018$, Fig. 2b). There was also a significant interaction between date and percentage of urban area, such that the general but weak trends towards higher prevalence in more urban areas and at later dates resulted in a less than additive combined effect, i.e. prevalence is lower at late dates in more urbanised sites than would be predicted if these two covariates acted independently ($\chi_1^2 = 6.98$, $P = 0.008$).

Apicystis bombi

Overall prevalence of *A. bombi* was lower than for the other two parasite species. There was no significant main effect of species ($\chi_1^2 = 1.25$, $P = 0.263$), and prevalence did not change with degree of urbanisation ($\chi_1^2 = 0.106$, $P = 0.745$). As in both *C. bombi* and *N. bombi*, prevalence

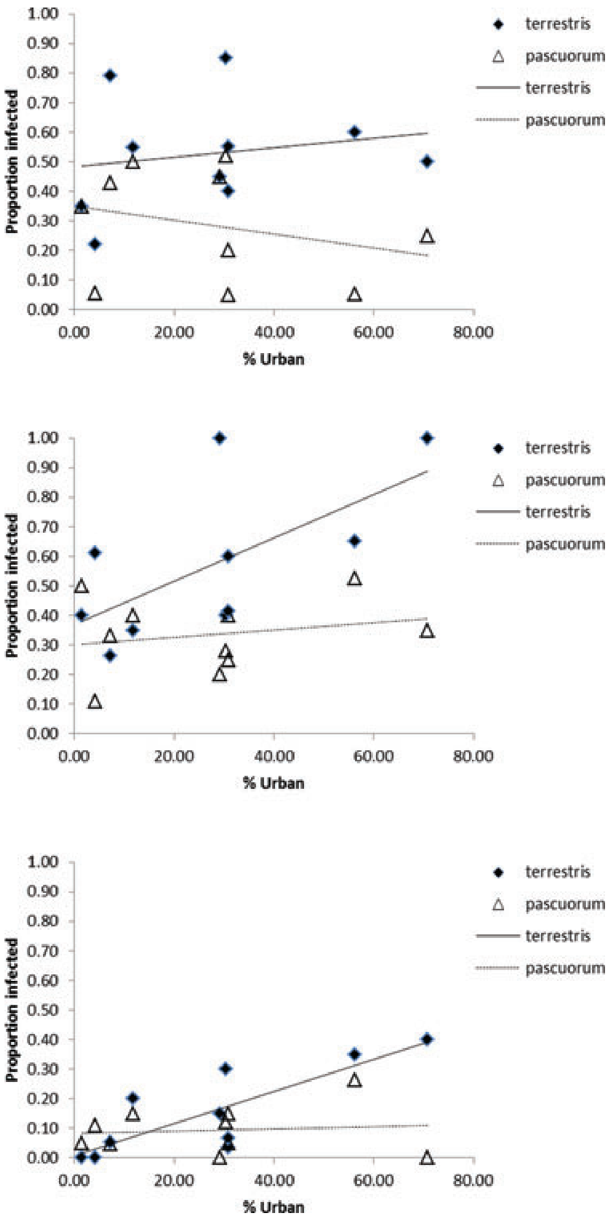

Fig. 2. Proportion of worker bees infected with parasites according to host species and the percentage of urban habitat within a 1-km radius. (a) *Crithidia bombi*, (b) *Nosema bombi*, (c) *Apicystis bombi.*

increased greatly in more urban areas in *B. terrestris*, but showed little clear pattern in *B. pascuorum*, resulting in an interaction between the two factors ($\chi^2_1 = 5.96$, $P = 0.015$, Fig. 2c).

Conopidae

Only 19 conopid fly larvae were recorded in total, providing too few for statistical analysis. However, they were more frequent in *B. terrestris* (13 larvae) than in *B. pascuorum* (six larvae).

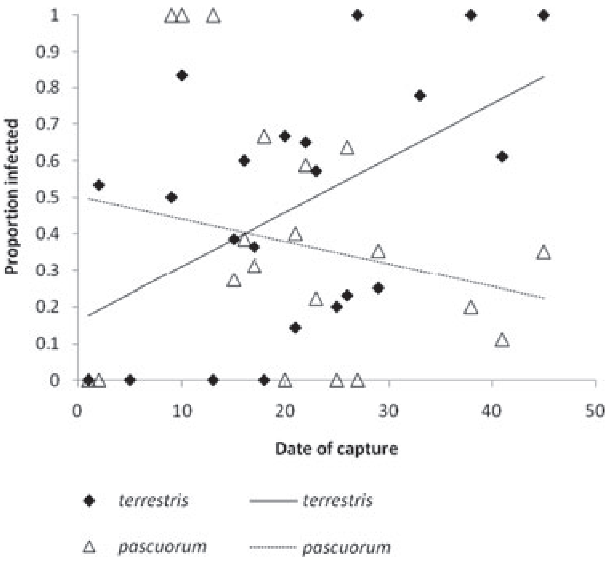

Fig. 3. Proportion of worker bees infected with *Nosema bombi* over time in *Bombus terrestris* versus *Bombus pascuorum*. Day 1 = 24 July 2010.

Discussion

Prevalence of the three protozoan parasites in our study (41.3% for *C. bombi*, 44.8% for *N. bombi*, and 12.4% for *A. bombi*) is broadly consistent with previous studies. Generally, *C. bombi* tends to be the most abundant parasite, particularly in late season workers where prevalence can approach 100%, while *N. bombi* is usually more scarce but prevalence varies widely (Goulson & Brown, 2010; Whitehorn *et al*., 2011a). As reported here, *A. bombi* tends to be the least common of the three (Rutrecht & Brown, 2008). Using faecal samples has the great advantage over dissection that it is non-destructive. However, our data suggests that it is an insensitive technique, particularly for *N. bombi* and *A. bombi*, leading to serious underestimation of prevalence. The low sensitivity for these latter two parasite species may reflect the fact that they are not confined to the gut of their host (Schmid-Hempel, 1998). Despite its low sensitivity, faecal sampling may still be valuable when working with rare species or when estimates of the number of infectious individuals are required (since pathogens in faeces are the primary source of new infections).

It is conceivable that our data are confounded by sampling of groups of sisters at single sites; infection rates of sisters would not be independent. However, genetic studies using these same bee species suggest that a sample of 20 bumblebees is unlikely to contain more than one or two sister pairs at most (Chapman *et al*., 2003; Darvill *et al*., 2004; Knight *et al*., 2005, 2009), so we suggest that this is unlikely to have led to substantial bias in the data.

Our results describe broadly consistent patterns in the prevalence of the three protozoan parasites. For all three parasites, prevalence was higher in *B. terrestris* than in *B. pascuorum*, although this difference was only significant

Table 1. Proportions of bees infected with different parasites at the 10 study sites.

		C. bombi		*N. bombi*		*A. bombi*		*n*	
	% urban	*Pasc*	*terr*	*pasc*	*terr*	*pasc*	*terr*	*pasc*	*terr*
Belleisle Park, Ayr	30.8	0.05	0.55	0.25	0.41	0.05	0.03	20	29
Botanic Gardens, Glasgow	29.2	0.45	0.45	0.20	1.00	0.00	0.15	20	20
Culzean Park, Ayr	4.2	0.06	0.22	0.11	0.61	0.11	0.00	18	18
Drybridge, River Irvine	11.9	0.50	0.55	0.40	0.35	0.15	0.20	20	20
Hurlford, River Irvine	30.9	0.20	0.40	0.40	0.60	0.15	0.07	20	15
Kelvingrove Park, Glasgow	70.7	0.25	0.50	0.35	1.00	0.00	0.40	20	20
Knockendale, Ayrshire	7.1	0.45	0.79	0.35	0.26	0.05	0.05	20	19
Pow Burn, Prestwick	1.52	0.35	0.35	0.50	0.40	0.05	0.00	20	20
Tarryholme, Irvine	30.4	0.52	0.85	0.28	0.40	0.12	0.30	25	20
Wellington Square, Ayr	56.3	0.05	0.60	0.50	0.65	0.25	0.35	20	20

pasc, *Bombus pascuorum*; terr, *Bombus terrestris.*

for *N. bombi*. Other studies have found marked differences between bumblebee species in the prevalence of *C. bombi* and *N. bombi* (reviewed in Schmid-Hempel, 1998; Gillespie, 2010; Cameron *et al*., 2011). In Sweden, *B. terrestris* had the highest prevalence of *N. bombi* in a comparison of eight bumblebee species that included *B. pascuorum* (Larsson, 2007), while Whitehorn (2011b) found higher prevalence of *C. bombi* in *B. terrestris* compared to *B. pascuorum* when sampling bumblebees on raspberry farms in Scotland, suggesting that this may be a general phenomenon.

For all three parasites, both host species and habitat had a significant interactive influence, with prevalence increasing in more urban areas in *B. terrestris*, but showing no clear response or a decline in *B. pascuorum*. Interpretation of our results would be greatly aided if we had measures of population density at our sample sites. However, there is no easy way to quantify density. Molecular markers can be used to identify sister groups and so count the number of nests that are represented (e.g. Knight *et al*., 2005), but this does not reveal how large the nests are or where they are. Bee counts on flower patches are largely determined by the size and quality of the patch, and perhaps by availability of alternative forage nearby, so it is unclear how they relate to population density. For parasites that are transmitted on flowers, the frequency with which different individual bees visit individual flowers (rather than the absolute density of bees) should determine transmission; this could be quantified across different habitats, but such data are currently lacking.

There is evidence to suggest that *B. terrestris* is relatively common, compared with *B. pascuorum*, in urban areas. *Bombus terrestris* is known to be very adaptable, readily incorporating a huge range of exotic flowers into its diet (reviewed in Goulson, 2003). Nests of 'two-banded white-tailed' bumblebees (a group usually dominated by *B. terrestris*) were by far the most common of those located by volunteers in gardens (Osborne *et al*., 2008a). In contrast, Darvill *et al*. (2004) estimated that the nest density of *B. pascuorum* in rural habitats in southern U.K. was approximately 15 times higher than that for *B. terrestris*. It may thus be that the difference in population density of *B. terrestris* between rural and urban areas is far greater than that found in *B. pascuorum*, which might explain the differential effect of habitat on parasite prevalence in the two species.

Alternatively, the differences in prevalence observed between urban and rural habitats in *B. terrestris* may be driven by factors other than host density. Urbanisation is accompanied by a multitude of environmental changes that could influence bumblebee health. For example, pollution might render bumblebees more susceptible to infection. Conversely, bumblebees in rural environments are more likely to come into contact with agrochemicals.

Conventional wisdom suggests that survival through the winter presents a challenge to these parasites as any negative influence on queen survival in hibernation and nest founding in the spring will greatly reduce prevalence (Schmid-Hempel, 1998). Queens infected with *A. bombi* rapidly die, leaving something of a mystery as to how the parasite survives to reappear each year in workers (Macfarlane *et al*., 1995; Rutrecht & Brown, 2008). During the spring and summer, all three parasites are probably transmitted horizontally by the oral–fecal route. *Crithidia bombi* is known to be transmitted between colonies via contamination of flowers (Durrer & Schmid-Hempel, 1994), and it seems likely that this also occurs in the other parasite species. Our finding that prevalence of *C. bombi* tends to increase over time is consistent with this hypothesis and with previous studies (e.g. Shykoff & Schmid-Hempel, 1991; Imhoof & Schmid-Hempel, 1999). *Nosema bombi* also tended to increase in prevalence over the season in *B. terrestris*, but interestingly its prevalence declined over time in *B. pascuorum*.

At present we can do little more than speculate as to the significance of high prevalence in certain species, habitats, and times of year. The generally high prevalence of parasites in *B. terrestris* may reflect low resistance to infection, or lower virulence of the parasite in *B. terrestris* compared with *B. pascuorum*, but it could also reflect high tolerance to infection; the same pattern would emerge if infection led to more rapid death in *B. pascuorum* than in *B. terrestris*. This could explain the declining incidence of *N. bombi* in *B. pascuorum* over the season, if infected nests of *B. pascuorum* tend to die out, while those of *B. terrestris* do not. These patterns may also reflect differences in ecology rather than resistance or tolerance: for example *B. terrestris* generally has larger nests than *B. pascuorum*, which might facilitate more rapid transmission.

At present we understand very little about the factors determining parasite prevalence in bumblebee communities. We do not know the relative importance of intra-colony transmission (presumed to be rapid), inter-colony transmission within species (on flowers and perhaps also due to drifting workers, Lopez-Vaamonde *et al*., 2004), and interspecific transmission (presumably mainly on flowers). The finding that these two bee species exhibit marked differences in the seasonal pattern of change in parasite prevalence, and in the response of prevalence to variation in habitat type, suggests that the majority of transmission is intraspecific, which we might expect since these two species have different tongue lengths and different (but overlapping) floral preferences (Goulson *et al*., 2005).

Should we be concerned at high levels of diseases in *B. terrestris* in urban areas? We know very little about the fitness implications for the hosts of infection with these parasites under field conditions, but certainly they can be fatal to both individual bees and to colonies (reviewed in Goulson & Brown, 2010). Recent studies in North America suggest that *N. bombi* is more prevalent in rare bumblebee species (Cameron *et al*., 2011), and it would be a concern if high prevalence of disease in urban areas were spilling over into populations of rare bees in nearby areas (for example, some of the few remaining U.K. populations of *B. sylvarum* are found on brownfield sites close to London). However, in the absence of evidence to the contrary we conclude that high prevalence of parasites in *B. terrestris* in urban areas simply reflects the presence of healthy, dense host populations, and should not be a cause for alarm.

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