Genetic investigation of species distribution within the *Bombus lucorum* complex across urban and rural habitats

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Lay person's summary

The humble bumble bee comes in the form of 21 different species in Ireland. Individual species prefer different habitats, flowers, altitudes and vary in their abundance. All bumble bees in Ireland, rare, threatened or common, are in decline. This worrying trend is a result of a combination of factors, but is mainly due to habitat loss, principally as a result of intensified farming practises, and also climate change. To further understand the abundance of some of the most common bumble bees in Ireland this study looked at the "*Bombus lucorum* cryptic complex" in urban sites in Dublin and rural ones in Fermanagh. The *Bombus lucorum* cryptic complex is a group of white tailed bumble bee species which cannot be distinguished from each other simply by visual inspection. The cryptic complex contains four species: *Bombus terrestris, Bombus lucorum, Bombus cryptarum* and *Bombus magnus*. Established techniques involving unique genetic markers were used to identify the species that were found at different sites. The results showed a marked difference in species composition in Dublin (urban) and Fermanagh (rural), a difference that would not have been possible to tell through visual identification. Dublin comprised *Bombus cryptarum* 4.2%, *Bombus lucorum* 8.3% and *Bombus terrestris* 87.5%; and Fermanagh comprised *Bombus cryptarum* 46%, *Bombus lucorum* 20% and *Bombus terrestris* 34%. This difference in species composition could indicate that these species are variable with regard to their preference for a rural or urban environment. *Bombus terrestris* appears most suited to an urban environment whereas *Bombus cryptarum* was most prevalent in rural areas. However, the study would need to be repeated at different times of the year, in different years and in a range of sites to confirm this finding. The driving factors causing differences in relative abundance are not clear, but this result suggests there is much to be revealed about these bumble bee species hidden in plain sight.

Abstract

Bumble bees are commercially and ecologically important pollinators. They are declining in Ireland due to climate change, land use change and habitat loss. To inform effective conservation it is vital that species distribution and abundance is known. Four of the most common bumble bees in Ireland, belonging to the *Bombus lucorum* cryptic complex, require molecular identification through PCR/RFLP digest to distinguish individual species, as morphological characteristics are shared in a cryptic manner. Our study used molecular techniques to identify bees from the urban and rural settings of Dublin city and County Fermanagh respectively. We were able to identify a total of 98 individuals, which revealed a unique species composition in each region. In Dublin, individuals were identified as *Bombus cryptarum* (4.2%), *Bombus lucorum* (8.3%) and *Bombus terrestris* (87.5%); and in Fermanagh,

Bombus cryptarum (46%), *Bombus lucorum* (20%) and *Bombus terrestris* (34%). No *Bombus magnus* individuals were found. This difference in species composition could indicate that these species are variable with regard to their preference for a rural or urban environment. More investigation is required to understand the ecology of each species uniquely and thus inform conservation strategies.

Introduction

Globally, the genus *Bombus* contains over 250 species of bumble bees. In Ireland, there are 21 bumble bee species, each with varying geographical ranges and conservation statuses. Bumble bees are of great ecological and economic importance as pollinators for natural and agricultural systems (Goulson 2003; Fitzpatrick et al. 2006). Pollination services, the majority of which is carried out by wild bees, are valued at €53 million for the Republic of Ireland (Bullock et al. 2008). Irish bees are in decline due to changing land use, habitat loss and harmful agricultural practises. This decline is a threat to the agricultural systems which rely on pollination, and to wild ecosystems. It is estimated that a third of bee species in Ireland are at risk of extinction (Fitzpatrick et al. 2006). Monitoring all of these species is crucial to informing conservation policy and trying to prevent further decline in the future.

Some of the most common bumble bees in Ireland and North West Europe are the species belonging to the subgenus *Bombus sensu stricto* (Fig. 1) (Stanley et al. 2013). In Ireland, this group comprises the species *Bombus terrestris, B. cryptarum, B. lucorum* and *B. magnus*. They share a highly similar banding pattern (Fig 2.) likely as a result of their monophyletic origins (Murray et al. 2008). Visual distinction of each species is near impossible because of the shared morphology, except for *B. terrestris* queens which display a unique buff tail. Banding pattern among workers (Fig. 2), males and queens of the species can vary slightly by colour and shape, but variation occurs within and between species meaning molecular analysis is the only reliable tool for species identification (Carolan et al. 2012).

Figure 2: A Bombus cryptarum worker caught at Killykeegan Nature Reserve in Co. Fermanagh. Its banding pattern of a yellow band on the thorax and abdomen and a white tail is shared by members of the *Bombus sensu stricto* subgenus.

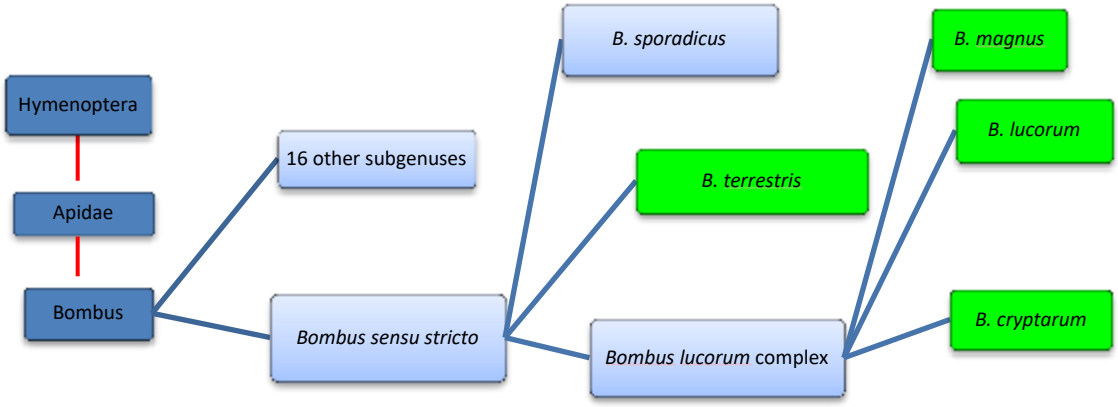

Figure 1: Taxonomy of the *Bombus sensu stricto* subgenus in Ireland. The species highlighted in green are species of bumble bee present in Ireland that are indistinguishable using normal morphological identification methods. *Bombus terrestris* queens are distinguishable by their buff tail, however workers usually have the colour banding pattern of the *lucorum* complex, and so are considered part of cryptic complex. Any ambiguous records for these 4 species are referred to *Bombus lucorum agg* (aggregate).

Materials and Methods

Sampling

The collection of individuals for this project was carried out between 5th June and 7th July 2018, during the period of peak bumble bee worker activity. Sites were visited in County Fermanagh, Northern Ireland, and Dublin city, Republic of Ireland. The Fermanagh sites included Monawilkin, Monastery Meadow, Castle Coole, Castle Archdale, Inis Davar Island, Devenish Island, Ferney Island and Killykeegan Nature Reserve. The Dublin sites included Glasnevin Botanical Gardens, Tolka Valley Park, Trinity Botanical Gardens, Collins Barracks, Broadstone Community Garden, Phoenix Park Walled Garden and Phoenix Park People's Garden. (For regional maps with species composition by site please refer to the Appendix and Figures 8 and 9)

Figure 3: Map of Ireland showing Dublin city and Fermanagh. Dublin city situated on the eastern seaboard of Ireland is shaded blue. County Fermanagh, part of Northern Ireland, in the north-west of Ireland is shaded green.

At each site, *Bombus lucorum* agg specimens were collected directly from flowers or by hand-netting, isolated in individual plastic vials and chilled until they could be safely handled. The terminal part of the tarsus from a mid-leg was removed from each bee and stored in a separate Eppendorf tube in 100% ethanol which was kept chilled until analysis. After tarsal removal the bee was released. Approximately every fifth whole bee was kept as a reference in case of DNA degradation in the tarsal samples. Most individual bees were also photographed and a note of the flower species they were foraging on at time of capture was made. GPS and estimated elevation using GPS was also recorded for where each bee was caught. This method of chilling and removal of a single tarsus has been shown to be non-lethal and appropriate for rare species (Holehouse et al. 2003). Precautions to avoid contamination of DNA between tarsal samples included cleaning forceps and scissors with ethanol in between individuals. In total 189 tarsi and 45 whole bees, visually identified as belonging to the *B. lucorum* agg cryptic complex, were collected across 7 Dublin sites and 8 Fermanagh sites. The number of samples collected at sites varied between 1 - 25 individuals and was dependent on the relative abundance of bees. Each site was visited only once and sampling took between 1-4 hours.

DNA extraction and RFLP genotyping

Two methods of DNA extraction were employed in this study. DNA was extracted from a full leg of a reference whole bee by the 10% Chelex method as per Murray et al. (2008). DNA was extracted from the tarsal samples using QIAGEN DNeasy tissue kit extraction. DNA extract from both methods was amplified via Tanaka PCR which amplified the region of the mitochondrial DNA (mtDNA) C01 barcode. PCR product was then digested using EcoN1 and Hinf1 enzymes to generate Restriction Fragment Length Polymorphisms (RFLP) within the mtDNA C01 barcode region. The RFLP digests were then loaded onto an agarose gel alongside previously identified reference samples for *B. lucorum, B. cryptarum, B. terrestris* and *B. magnus* thus allowing for identification of each individual. This follows protocol similar to that of Murray et al. 2008; and Stanley et al. 2013.

Results

Master Gels

Agarose gels (Figures 4 and 5) revealed the final product of DNA extraction, PCR and RFLP digest. Due to species differences in the base code of mitochondrial DNA in the region of the C01 barcode, it is possible to visually identify species from a gel. A species ID can be derived by a specific banding pattern caused by the separation of different lengths of DNA that is unique to each species. In Dublin, the majority of the banding patterns denote *B. terrestris* (annotated by a T, Figure 4). In Fermanagh, the double band of *B. cryptarum* is much more prevalent (annotated by a C) (Figure 5). *B. lucorum* (annotated L) also has a double band which sits higher in the gel than that of *B. cryptarum* (Figures 4 and 5).

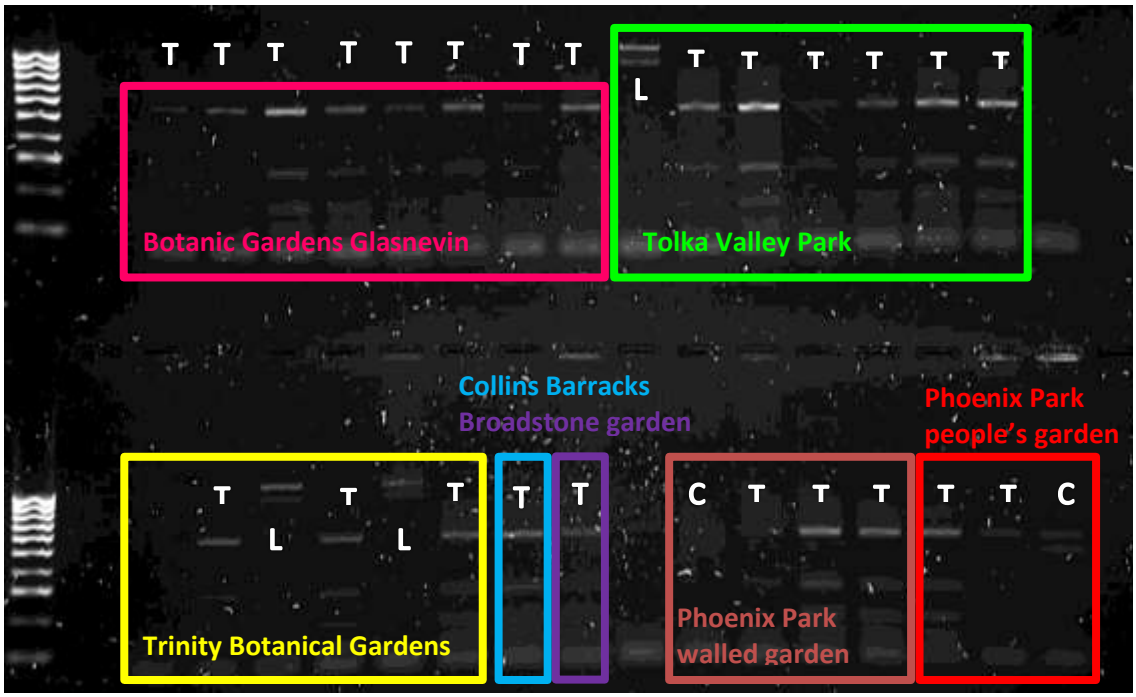

Figure 4: Agarose gel showing samples from Dublin city by site. This gel shows representative portions of species samples from each site. The relative position of each band in a lane indicates the species. Lanes marked with a T are *B. terrestris*, with an L are *B. lucorum* and C are *B. cryptarum*. As represented by this gel the majority of species identifications in Dublin were *B. terrestris*.

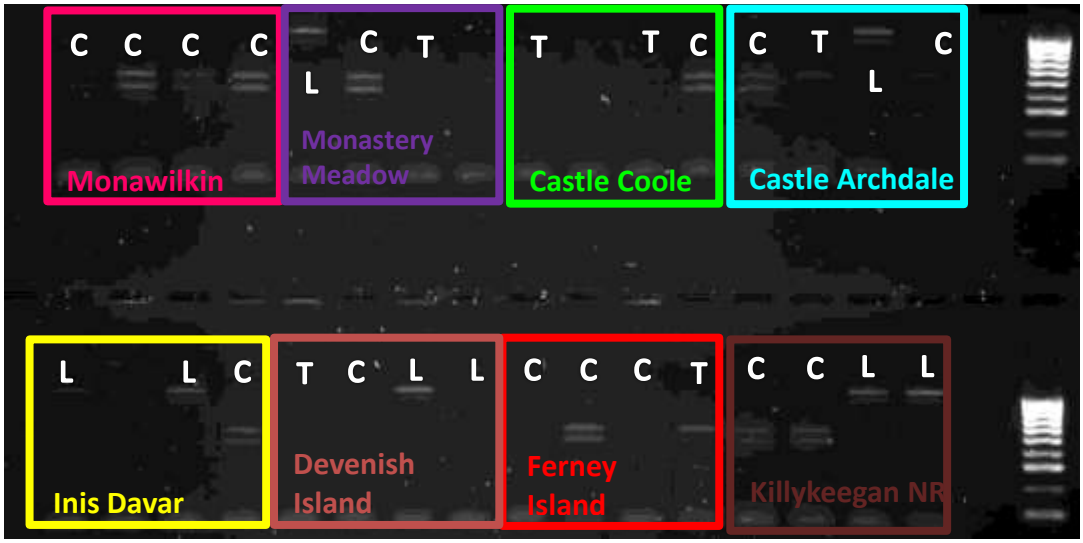

Figure 5: Agarose gel showing samples from County Fermanagh by site. This gel shows representative portions of species' samples from each site. The relative position of each band in a lane indicates the species. Lanes marked with a T are *Bombus terrestris*, with an L are *B. lucorum* and a C are *B. cryptarum*. Across the 8 sampling sites in Fermanagh, *B. cryptarum* was present at each. Overall there is greater variety per site than in Dublin.

All species identifications by site and region

A total of 48 positive species identifications were made from Dublin, and 50 from Fermanagh, totalling 98 identifications over both regions (Tables 1 and 2). *Bombus terrestris* made up 87.5% of samples from Dublin (Table 1, Figure 6), but only 34% of samples from Fermanagh (Table 2, Figure 6). Conversely, *B. cryptarum* was found in only 2 sites in Dublin (Table 1) representing 4.2% of all samples (Figure 6), but comprised 46% of individuals in Fermanagh (Figure 6) having been present at all sites sampled (Table 2). *B. lucorum* was least prevalent overall as it formed 8.3% of individuals in Dublin and 20% of individuals in Fermanagh (Figure 6).

Table 1: All species identifications for Dublin

Numbers of each species identified at each site via the PCR/RFLP identification method in Dublin. The majority of species identified are *Bombus terrestris* as 42 out of 48 of all identifications in Dublin belong to this species. *B. cryptarum* is overall the least prevalent in Dublin.

Dublin sites	*Bombus cryptarum*	*Bombus lucorum*	*Bombus terrestris*	Total
Broadstone community garden			1	1
Collins Barracks			1	1
Glasnevin Botanic gardens			16	16
Phoenix park people's garden	1		4	5
Phoenix park walled garden	1		10	11
Tolka Valley Park		1	6	7
Trinity Botanical Gardens		3	4	7
Dublin total	**2**	**4**	**42**	**48**

Table 2: All species identifications for Fermanagh

Numbers of each species identified at each site via the PCR/RFLP identification method in Fermanagh. All 3 species identified are present in more equal portions than in Dublin. *Bombus cryptarum* is the most prevalent of the 3 species in Fermanagh.

	Bombus cryptarum	*Bombus lucorum*	*Bombus terrestris*	Total
Castle Archdale	2	2	1	5
Castle Coole	1		3	4
Devenish Island	2	3	5	10
Ferney Island	6		1	7
Inis Davar	2	2		4
Killykeegan Nature Reserve	3	2	1	6
Monastery Meadow	1	1	6	8
Monawilkin	6			6
Fermanagh totals	**23**	**10**	**17**	50

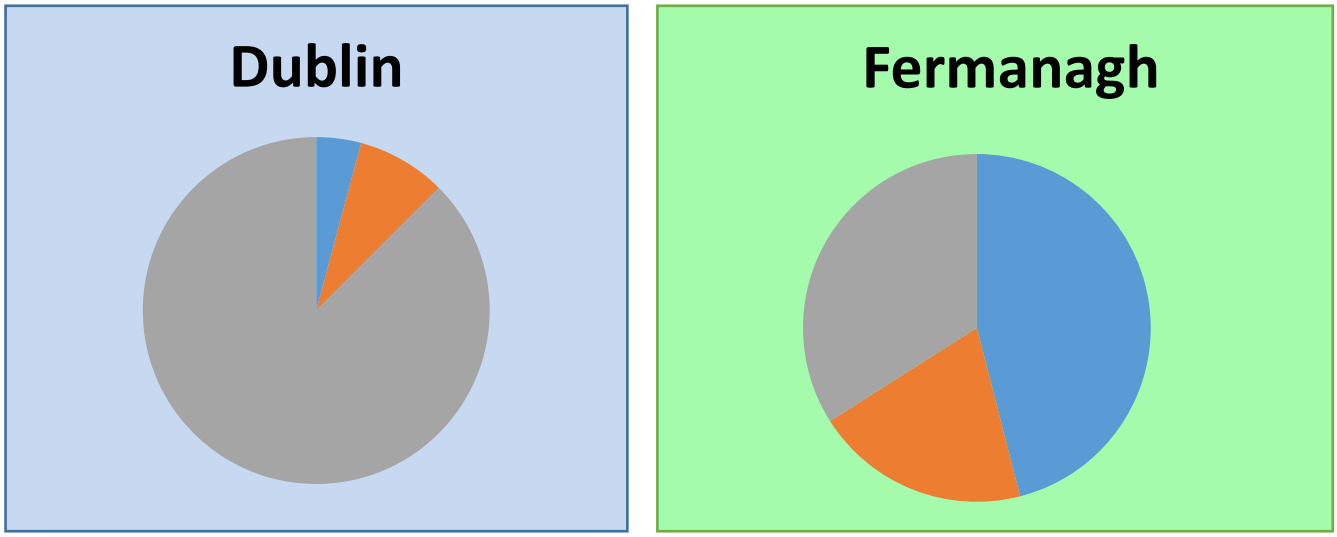

Figure 6: Species composition by region. The Dublin pie chart corresponds to the following percentages for each species: *B. cryptarum* (blue) = 4.2%, *B.lucorum* (orange)= 8.3% and *B. terrestris* (grey)= 87.5%. The Fermanagh pie chart shows species composition of: *B. cryptarum* (blue) = 46%, *B. lucorum* (orange) =20% and *B. terrestris* (grey) =34%.

Table 3: Plant species foraged on by members of B. lucorum cryptic complex. The 3 sections of the table show the common names that each individual bee species was found foraging on. The right hand column in each table section shows the number of each bee species found at each plant species. The ratio of bees sampled to flower species each foraged on was found to be similar (within 2.3-3.6) across all three species.

Bombus cryptarum	
Bramble	8
Meadow sweet	1
Pineapple broom	1
Snowberry	3
Thistle	1
White Clover	6
Yellow Hayrattle	1
Not foraging	4
Total = 7 species	25
Ratio of individuals : plant species 3.6 : 1	

Bombus lucorum	
Bramble	6
Orange Hawkweed	1
Pink shrub rose	1
Sea kale	1
White Clover	4
Yellow Hayrattle	1
Total =6 species	14
Ratio of individuals : plant species 2.3 : 1	

Bombus terrestris	
Beech	1
Birdsfoot Trefoil	4
Bramble	11
Catmint	1
Dahlia	1
Foxglove	1
Great Yellow Gentian	3
Lady Phacelia	5
Lamb's Ear	1
Lavender	1
Love In A Mist	6
Meadow Buttercup	2
Orange Hawkweed	1
Poppy	2
Raspberry	1
Sea Kale	2
Self-heal	2
Silky Phacelia	5
Thistle	1
Welted Thistle	1
White Clover	7
Total = 21 species	59
Ratio of individuals : plant species 2.8 : 1	

Aligning species with elevation above sea level

Dublin is situated on the east coast of Ireland and has noticeably lower elevation than Fermanagh (Figure 7). The majority of Dublin sites were at or below 37m elevation with the exception of one site which had an elevation of 50m. All Fermanagh sites had an elevation above 46m. Thus in this case species distribution by elevation is confounded with region and cannot be considered a separate variable which affects species proportion.

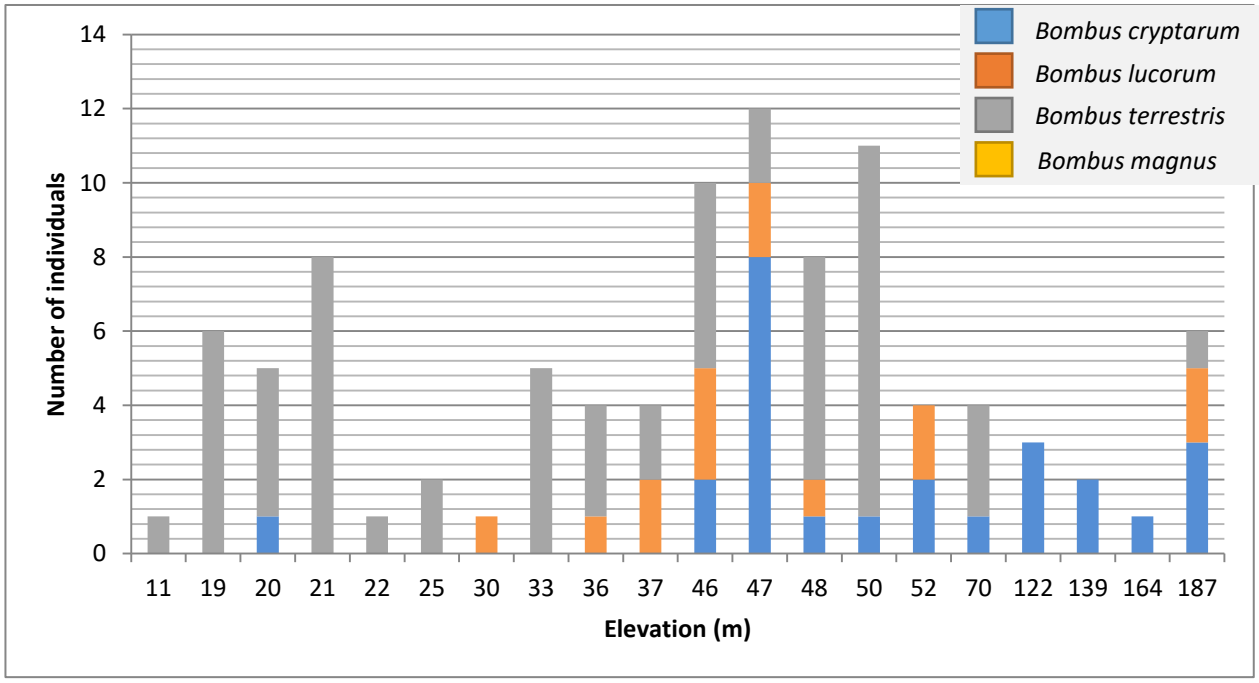

Figure 7: Species composition by elevation of sampling location. *B. terrestris* is most widespread but can be found mainly at 50m and below. *B. cryptarum* was primarily sampled between 46-187m, the upper half of elevation values. *B. lucorum* appears most commonly in the middle range of elevation values (30-52m). Elevation values from 11-37m are from sites in Dublin and 46-197m are from Fermanagh, except for 50m which is a Dublin site.

Discussion

We found a marked difference in species composition in the regions investigated. The majority (89%) of *B. lucorum agg.* bees in Dublin were *B. terrestris*. This could be a result of many different factors. It may be down to random chance; the timing of sampling; it may be that *Bombus terrestris* is more suited to an urban environment; or it is possible that species composition has been increased by the importation of commercial *B. terrestris* colonies for horticultural crop pollination into north county Dublin. From this study, we cannot ascertain which factors might be responsible. For the latter, the influence of commercial colonies could be assessed via haplotype comparison of *B. terrestris* individuals from the Dublin region. (Murray et al. 2008)

The samples take in County Fermanagh were also dominated by a single species, in this case, *B. cryptarum* (46%). Again, this may be due to many different factors, one of which is the different habitats available in the region. Fermanagh's landscape and habitats are mainly

considered rural and as a result may be more suited to this species of bee. The plants that bees were sampled from were mostly native species from mainly semi-natural habitats.

Differences in phenology of the different species may also explain our findings. We sampled during one month (5th June- 7th July), after an unusually cold spring which persisted through March into April in 2018 (Met Eireann 2018). *B. cryptarum* is noted to be an early spring species (Murray et al. 2008). It could be that *B. cryptarum* had reached peak colony numbers in Fermanagh during June, but *B. lucorum* was more prevalent at other times. *Bombus lucorum* is considered to be widespread and common even in urban areas (Murray et al. 2008), so timing may have played a role in the outcome of species abundance recorded in both Dublin and Fermanagh, where *B. lucorum* appeared to be neither common or widespread. If, however, *B. lucorum* is much less prevalent regardless of climatic changes, then perhaps its conservation status should be re-examined. Currently all visual identifications of bees with the cryptic complex banding pattern are recorded under *B. lucorum*, but as demonstrated by these findings only a fraction of those will be true *B. lucorum*. It is currently graded by the IUCN Red list as of Least Concern, but the records for *B. lucorum* could be significantly inflated by the inclusion of *B. cryptarum*, *B. magnus* and *B. terrestris* workers. Thus its conservation status may not be reliable, especially given that all bees in Ireland have been found to be in decline. However the conservation status of all *Bombus sensu stricto* species will only be apparent with further research into the cryptic complex in Ireland.

When the species identified are sorted by elevation (see Fig 7.) there is some separation in line with a previously proposed altitudinal gradient (Murray et al. 2008). The gradient of the whole complex would likely be more marked with the inclusion of *B. magnus* specimens, as this is considered a species of upland moor and heathland sites (Murray et al. 2008). However the proportion of each *B. lucorum* agg species is mainly affected by region and available habitats and is not a result of elevation alone. Rather in this case the urban environment of Dublin at sea level provides vastly different niches to those of rural County Fermanagh with its hilly profile.

Conclusion

This research project has shown differentiation in the species composition of *the B. lucorum* complex across a rural and urban setting. The species present varies between each region, but pooled regional data are mostly representative of each site within the region. While the findings show clear regional differences, the extent to which the *B. lucorum* complex reliably differentiates across all of Ireland can only be assessed by an all-Ireland study controlled for these rural and urban differences. As it stands, *B. cryptarum* and *B. magnus* are data deficient, however it could be noted that much of the data on *B. lucorum* is inconclusive as it too is also deficient on high quality (molecular) identifications.

Acknowledgements

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References

Bullock, C., Kretsch, C. & Candon, E., 2008. *The economic and social aspects of biodiversity : benefits and costs of biodiversity in Ireland*, Dublin : Stationery Office.

Fitzpatrick, Ú.; et al., 2006. *The state of Ireland's bees*, Available at: http://www.npws.ie/public [Accessed September 12, 2018].

Goulson, D., 2003. *Bumblebees: Their Behaviour and Ecology*, Oxford University Press.

Holehouse, K.A., Hammond, R.L. & Bourke, A.F.G., 2003. Non-lethal sampling of DNA from bumble bees for conservation genetics. *Insectes Sociaux*, 50(3), pp.277–285.

Met Eireann, 2018. *MARCH 2018*,

Murray, T.E. et al., 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. *Conservation Genetics*, 9(3), pp.653–666. Available at: http://link.springer.com/10.1007/s10592-007-9394-z.

Stanley, D.A., Knight, M.E. & Stout, J.C., 2013. Ecological Variation in Response to Mass-Flowering Oilseed Rape and Surrounding Landscape Composition by Members of a Cryptic Bumblebee Complex. *PLoS ONE*, 8(6).

Appendix

Supplementary maps with site diversity information

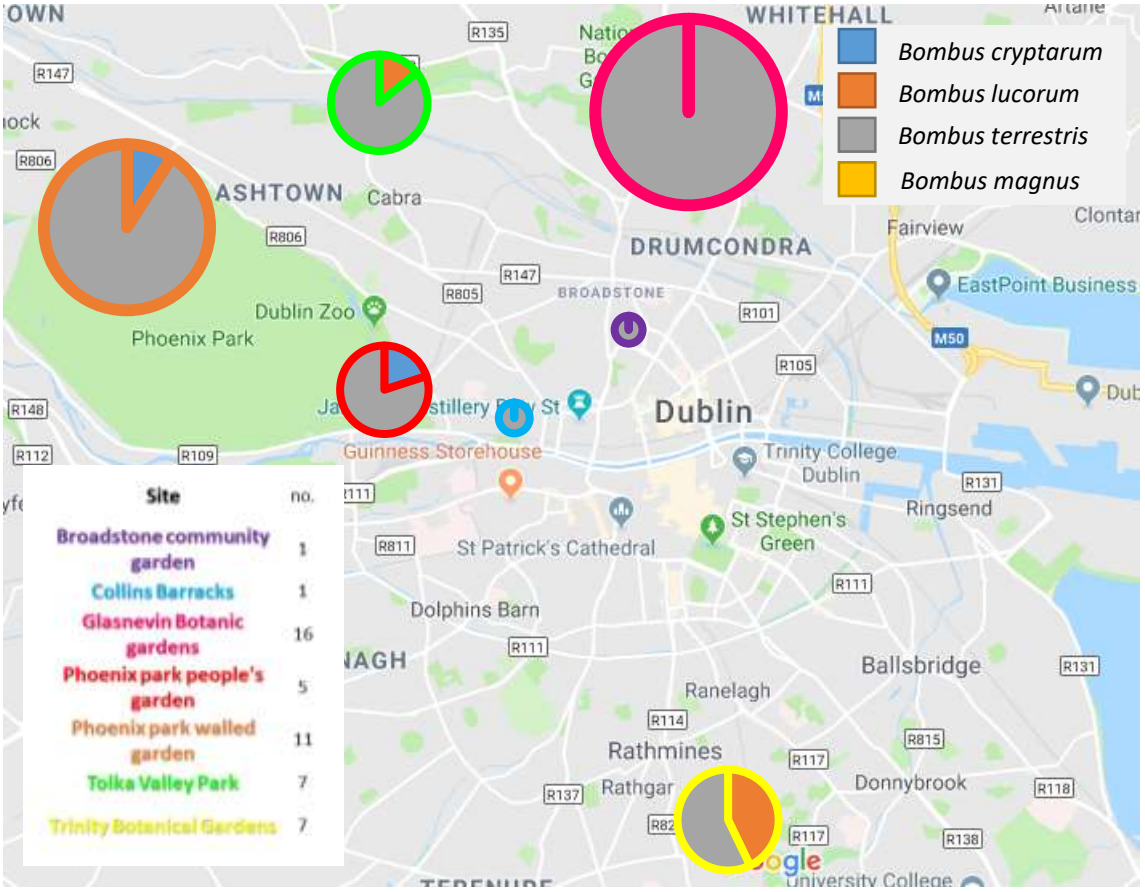

Figure 8: Species composition at sites in Dublin city. This map shows the species composition at each site, numbers sampled at each site and geographical position of each site within the region of Dublin city. The pie chart sizes are relative to the numbers sampled at the site. The border colour of the pie chart matches the colour of the site name in the key. Trinity Botanical Gardens (yellow) is comprised of *Bombus lucorum* and *Bombus terrestris*.

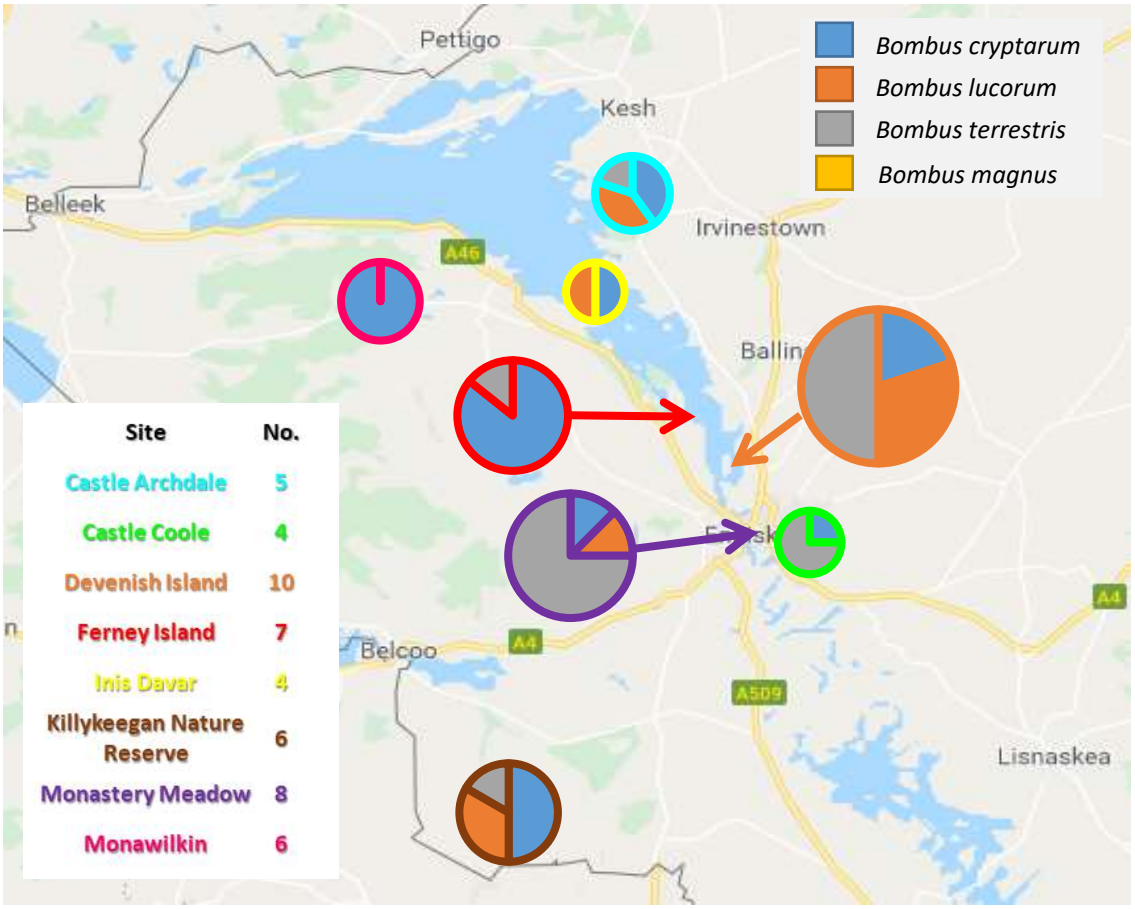

Figure 9: Species composition at sites in County Fermanagh. This map shows the species composition at each site, numbers sampled at each site and geographical position of each site within the region of County Fermanagh. The pie chart sizes are relative to the numbers sampled at the site. Inis Davar (yellow) was the smallest sample size and half of the species were *Bombus lucorum* and the other half were *Bombus cryptarum*. Devenish Island was the largest sample size and had *Bombus terrestris*, *Bombus lucorum* and *Bombus cryptarum*, with *Bombus terrestris* being the most prevalent.