Correlates of Rarity in Two British Bumblebee Species

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Introduction

Rare species often exist in recently fragmented, isolated populations[1]. It is expected that this isolation will cause a reduction in gene flow between populations, resulting in inbreeding and an increased risk of inbreeding depression. This may manifest itself as increased vulnerability to disease and parasitism.

Using one common (*Bombus pratorum*) and one declining species (*B. monticola*), this study investigated the relationship between rarity and fitness by measuring two components of the innate immune response (Fig. 1). Phenoloxidase (PO) plays a crucial role in melanisation, the process responsible for neutralising pathogens which penetrate the exoskeleton. Anti-microbial peptides circulate in the haemolymph and provide an antibacterial response.

Those data were then compared to the parasite load of each species, to determine the possible "real world" effects of a possible reduction in immunocompetence and the relationship of those variables to rarity.

Fig. 1a. *B. monticola*

Fig. 1b. *B. pratorum*

Methods

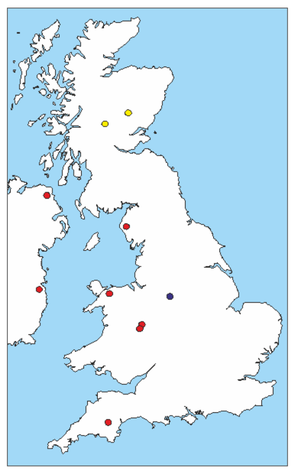

Fig 2. Map of sample sites. Red = both species collected. Blue = *B. pratorum* only; yellow = *B. monticola* only.

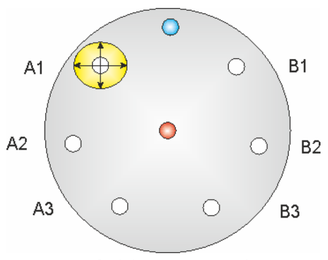

Fig 3. Zone of inhibition assay. Red = positive control; blue = negative control; yellow = measurements for zones of inhibition

SAMPLE COLLECTION

- Sampling sites were chosen based on records from the BWARS database[2]. Where possible 40 individuals of both *B. pratorum* and *B. monticola* were collected at each site (Fig. 2). The age of each individual was assessed using wing area adjusted for body size.

PO ACTIVITY

- PO activity was assessed by measuring the rate of conversion of L-DOPA to dopachrome[3].
- PO data was analysed using ANOVA.

ANTIMICROBIAL PEPTIDES

- Antimicrobial peptide activity was measured using standard zone-of-inhibition assays[4].
- Zones were measured at the maximum and minimum diameter and normalised to % of the positive control (Fig. 3)
- The occurrence of AMP activity was analysed using Chi-squared tests; zone diameter was analysed using ANOVA.

PARASITE LOAD

- Dissection and light microscopy were used for detection of *Apicystis bombi, Locustacarus buchneri,* and *Syntretus* larvae.
- The microparasites *Apicystis bombi*, *Crithidia bombi* and *Nosema bombi* were detected using species-specific PCR [5,6,7]
- All parasite data were analysed with Chi-squared or Fishers exact tests.

Results (1)

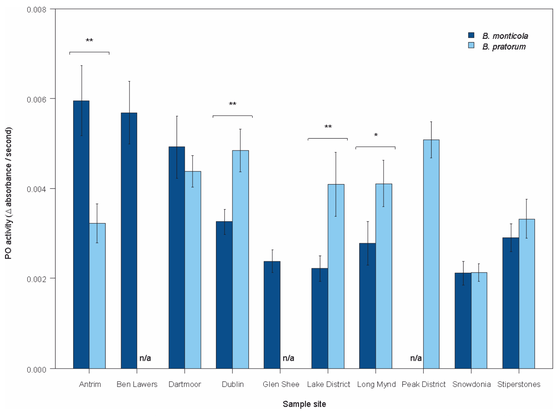

Fig. 4. Comparison of PO activity between study species. Significant differences are indicated with asterisks above the relevant bars (* = p<0.05; ** = p<0.01; *** = p<0.001).

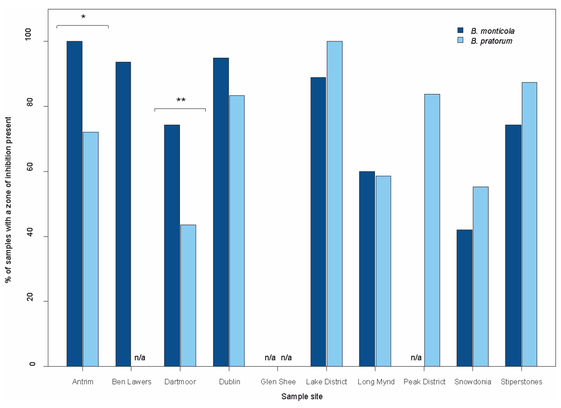

Fig. 5. Percentage of samples showing AMP activity. Significant differences between the study species are indicated with asterisks above the relevant bars (* = p<0.05; ** = p<0.01).

- PO activity was significantly negatively related with age in both *B. monticola* ($F_{1,274}$=15.61, p<0.001) and *B. pratorum* ($F_{1,237}$=4.47, p<0.05)
- PO activity was significantly different between species at the Antrim ($F_{1,41}$=13.09, p<0.001), Dublin ($F_{1,74}$=9.22, p<0.01), Lake District ($F_{1,51}$=9.10, p<0.01) and Long Mynd ($F_{1,39}$=4.24, p<0.05) sample sites (Fig. 4).
- The proportion of samples showing AMP activity was significantly different at the Antrim (Fisher's exact test, p<0.05) and Dartmoor ($X^2(1)$=7.03, p<0.01) sample sites (Fig. 5)
- There were no significant differences between study species in the size of zones of inhibition at any site.
- Zone of inhibition diameter was found to increase with age in both *B. monticola* ($F_{1,171}$=8.39, p<0.01) and *B. pratorum* ($F_{1,157}$=7.4-, p<0.01).
- This may be the result of a trade-off between the two mechanisms as individuals age, although further experimental work would be required to confirm this.

Results (2)

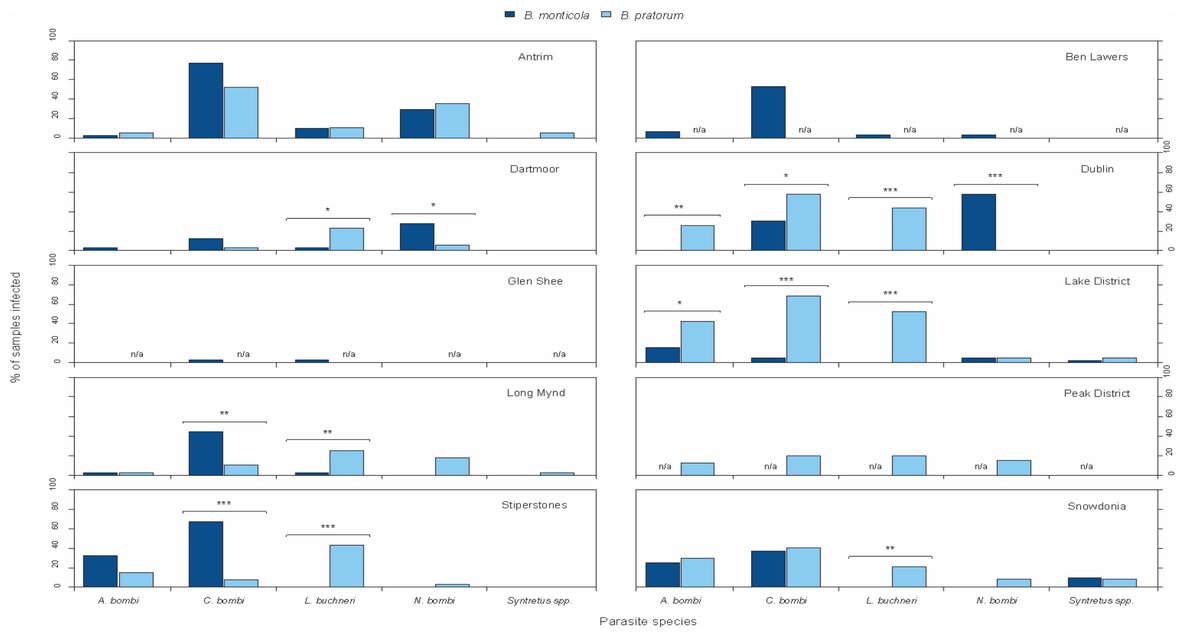

Fig. 6. Comparison of parasite prevalence between *B. monticola* and *B. pratorum* at all sample sites. Significant differences are indicated with asterisks above the relevant bars (* = p<0.05; ** = p<0.01; *** = p<0.001). All comparisons were performed using Chi-squared or Fisher's exact tests. For clarity, only significant results are reported.

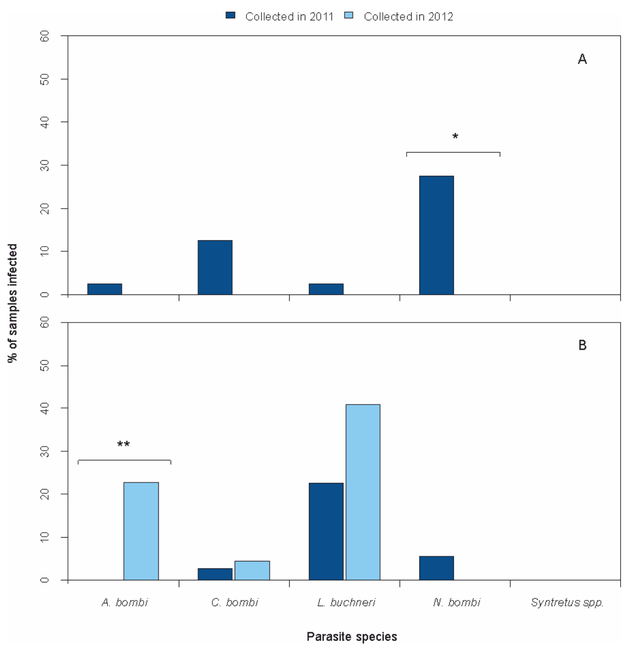

Fig. 7. Comparison of parasite prevalence between years in *B. monticola* (panel A) and *B. pratorum* (panel B) from the Dartmoor sample site. Significant differences are indicated with asterisks above the relevant bars (* = p<0.05; ** = p<0.01). All comparisons were made using Chi-squared or Fisher's exact tests.

- The presence of *L. buchneri* varied significantly between study species at all sites except Antrim, with prevalence always higher in *B. pratorum* (Fig. 6).
- There were significant differences in *C. bombi* prevalence at many sample sites, but no consistent pattern of one species being more readily infected (Fig. 6).
- *A. bombi* and *N. bombi* were found at low prevalence in both species at all sample sites. The most frequent infection in both species was by *C. bombi*.
- Prevalence of all parasite species showed significant variation depending on the year that samples were collected (Fig. 7).
- This calls into question the relevance of prevalence estimates based on a single sampling period, and highlights the need for long-term monitoring.
- There does not appear at this stage to be a correlation between PO or AMP activity and parasite prevalence in either of the study species.
- Variation in parasite prevalence may be due to genetic differences between species and populations.

Conclusions

- There is no consistent difference in PO activity or AMP activity between the two study species.
- There is no consistent difference in parasite prevalence between the two species, although *B. pratorum* seems to be preferentially chosen as a host by *L. buchneri.*
- Parasite prevalence is highly variable between sample sites and between years.
- The variability in parasite prevalence highlights the need for long-term monitoring – for meaningful estimates of the parasite prevalence and how it links to immune function, samples from multiple years are required.
- This study has so far found no evidence to support the theory that rarer species are likely to be less immunocompetent or suffer higher parasite loads than common species.
- Investigation of the genetic diversity of populations of both species would underpin these results, and give a greater insight into the possible link between rarity and fitness.

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