Genetic diversity and parasite prevalence in two species of bumblebee

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Abstract

Many bumblebee species have been suffering from significant declines across their ranges in the Northern Hemisphere over the last few decades. The remaining populations of the rare species are now often isolated due to habitat fragmentation and have reduced levels of genetic diversity. The persistence of these populations may be threatened by inbreeding depression, which may result in a higher susceptibility to parasites. Here we investigate the relationship between genetic diversity and prevalence of the parasitic mite *Locustacarus buchneri* in bumblebees, using the previously-studied system of *Bombus muscorum* and *Bombus jonellus* in the Western Isles of Scotland. We recorded *L. buchneri* prevalence in 17 populations of *B. muscorum* and 13 populations of *B. jonellus* and related the results to levels of heterozygosity. For *B. muscorum*, we found that prevalence of the mite was higher in populations with lower genetic diversity but there

was no such relationship in the more genetically diverse *B. jonellus*. In contrast to population-level measures of genetic diversity, the heterozygosity of individual bees was not correlated with infection status. We suggest population-level genetic homogeneity may facilitate parasite transmission and elevate prevalence, with potential consequences for population persistence.

Introduction

The role bumblebees have as pollinators makes them a vital component of ecosystems and also gives them great economic value. Over recent decades many bumblebee species have been declining across their range in the Northern hemisphere, predominantly due to the intensification of agriculture and the resultant loss of habitats (Goulson *et al.*, 2008; Williams & Osborne, 2009). These declines have been particularly severe in the UK where 3 of the 27 native species have become extinct and 10 species have undergone severe range contractions (Goulson, 2010). The remaining populations of the rarer species have become isolated in habitat patches where suitable forage and sites for nesting still exist. There are instances of these populations going extinct, despite the continuing presence of good habitat. For example, Wicken Fen in central England supported 14 species of *Bombus* in the 1920s but by 1978 only six remained (Williams, 1986).

In order to implement the appropriate conservation strategies for bumblebees it is important to understand what is driving these remaining populations to extinction. Research has suggested that genetic factors might have a role; rare species with fragmented populations, such as *B. sylvarum* and *B. muscorum* in the UK and *B. occidentalis* and *B. pensylvanicus* in North American have much lower genetic diversity than common, widespread species such as *B. terrestris* and *B. pascuorum* in the UK and *B. bifarius* and *B. impatiens* in North America (Ellis *et al.*, 2006; Darvill *et al.*, 2006; Cameron *et al.*, 2011; Lozier *et al.*, 2011). Detailed study of the genetic diversity and population structure of *B. muscorum* has provided further information. *B. muscorum* has become rare across its range in the UK and is now predominantly found in the Western Isles of Scotland. Darvill *et al.* (2006) found that the more isolated island populations of *B. muscorum* were genetically differentiated from those closer to the mainland and had substantially reduced genetic diversity. These studies suggest that habitat fragmentation and population isolation have led to a loss of genetic diversity in rare species of bumblebees. If the populations with reduced levels of genetic diversity also have lower fitness, inbreeding depression may be occurring. This might be the mechanism driving these populations towards extinction, as has been demonstrated in other invertebrate species (Saccheri *et al.*, 1998; Reed *et al.*, 2007).

One form of inbreeding depression, which may lead to an increased extinction risk, is higher susceptibility to parasitism (de Castro & Bolker, 2005). Increased homozygosity can increase both the prevalence of parasites at the population level and susceptibility to parasites at the individual level (Frankham *et al.*, 2010). At the population level, the more

genetic diversity present, the more likely it is that some individuals can resist a pathogen. If this genetic diversity is lost due to inbreeding, pathogen epidemics may spread more efficiently in the genetically homogenous population. Previous work by Whitehorn *et al.* (2011) has supported this theory, revealing a negative correlation between genetic diversity of island populations of *B. muscorum*, and prevalence of the intestinal microparasite *Crithidia bombi*. Further support comes from North America, where declining bumblebee populations have lower levels of genetic diversity and a significantly higher prevalence of the pathogen *N. bombi* compared to stable bumblebee populations (Cameron *et al.*, 2011). Similar relationships have been found in other invertebrates (e.g. Ebert *et al.* 2007), but not universally (Trouve *et al*. 2003; Field *et al.* 2007).

Experimental work with Drosophila in the laboratory suggests that inbreeding can decrease the immunity of invertebrates at the individual level through the loss of specific resistance alleles (Spielman et al. 2004) or reduced defensive behaviour (Luong et al. 2007). Knowledge of the individual inbreeding co-efficient (*f*) is informative when establishing whether such relationships exist between inbreeding and parasite susceptibility at the level of the individual. This is calculated using detailed pedigree information, but this is rarely available for wild populations (Marshall *et al.*, 2002). As an alternative, microsatellites have been increasingly used to provide a measure of multi-locus heterozygosity (MLH), which is then used to infer relative levels of inbreeding among individuals. Correlations between MLH and fitness related traits are known as heterozygosity-fitness correlations (HFCs). HFCs based on a small number of

microsatellite loci have been found in many different species for a range of fitness traits, including parasite susceptibility (Coltman *et al.*, 1999) and lifetime reproductive success (Slate *et al.*, 2000). Support for the existence of HFCs in invertebrates comes from research on the damselfly *Calopteryx splendens*, where a negative correlation between an individual's inbreeding coefficient (estimated from AFLP markers) and its parasite burden has been found (Kaunisto *et al.* 2013).

This study aims to further investigate the relationship between genetic diversity and parasitism in bumblebees. We consider two bumblebee species, *B. muscorum* and *B. jonellus*, that live sympatrically in the Western Isles of Scotland but have different levels of genetic diversity. Investigating their levels of parasitism allows us to compare the impacts that inbreeding and population differentiation have on parasites. *Bombus muscorum* belongs to the subgenus *Thoracobombus* and is considered threatened. It has been placed on the UK Biodiversity Action Plan (UKBAP) along with three other species belonging to its subgenus. *Bombus jonellus* is a member of the subgenus *Pyrobombus* and has a widespread but local distribution and is not thought to be threatened (Benton, 2006). Darvill *et al.* (2010) found that the two species differed significantly in overall heterozygosity with *B. muscorum* exhibiting much lower genetic diversity. *B. muscorum* also shows markedly higher population structuring and isolation by distance than *B. jonellus* ($\theta = 0.13$ compared to $\theta = 0.034$). *B. jonellus* has evidently retained genetic cohesion over greater distances and it was estimated that they are able to disperse >50km relatively frequently. In contrast, *B. muscorum* were estimated to disperse >8km only infrequently and the species also showed an increased frequency of population

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bottlenecks (Darvill *et al*. 2010). These differences in dispersal abilities suggest that *B. muscorum* is more susceptible to population isolation due to habitat fragmentation.

This study tests how genetic diversity differences between host species, populations and individuals impact on parasite prevalence. To do this we quantify the prevalence of the tracheal mite, *Locustacarus buchneri*, in the *B. muscorum* and *B. jonellus* individuals collected by Darvill *et al.* (2010).

Methods

During the summers of 2003 to 2005, individuals of *B. muscorum* and *B. jonellus* were collected from islands in the Inner and Outer Hebrides and stored in 100% ethanol. In a previously published study (Darvill *et al.*, 2010), *B. muscorum* were genotyped at 8 microsatellite loci (B10, B11, B96, B118, B124, B126, B131, B132) and *B. jonellus* were also genotyped at 8 microsatellite loci (B10, B11, B96, B100, B121, B124, B126, B132). This gave each bee a measure of individual heterozygosity (the number of heterozygous loci divided by the number of genotyped loci). Tests for genotypic linkage disequilibrium and departure from Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP version 3.4 (Raymond and Rousset, 1995). Darvill et al. (2010) excluded loci with null alleles from their analysis. The presence of sisters within each population was checked using KINSHIP v 1.3.1 (Goodnight & Queller, 1999), which assigned workers to colonies, allowing all but one representative from each nest to be removed. A measure of average (unbiased) heterozygosity (H_E) for each population was also calculated using

FSTAT and these figures are published in Darvill *et al.* (2010) (table 1). For the present study, the width of the thorax of each bee was measured using electronic digital callipers and the bee's age was estimated by assessing the extent of wing wear, using a four point scale (modified from Mueller & Wolfmueller, 1993). Each abdomen was then dissected in order to quantify the number of adult *L. buchneri* present.

Statistical analyses

All analyses were performed in R, version 2.15.3 (R Core Team 2013). Binomial generalised linear mixed effect models were used to investigate whether *L. buchneri* prevalence was influenced by the level of genetic diversity at the population level. *Bombus muscorum* and *B. jonellus* were analysed separately as island heterozygosity measures are different for the two species. Population-level heterozygosity, bee age (entered as a covariate with a four point scale), bee size (thorax width), sampling date (entered as a covariate, numbered continuously from June 1st through to September) and finally island area (as a proxy for bumblebee population size) were entered as fixed effects. Island and sampling year were entered as random factors and individual bee was the unit of replication. Binomial generalised linear mixed effect models were also used to analyse determinants of *L. buchneri* infection on an individual level. Fixed effects included: bumblebee species, individual heterozygosity, bee age, bee size and sampling date. Island and sampling year were entered as random factors. Models were fit with lmer in the lme4 package (ver. 1.0-4; Bates *et al.*, 2013). *Locustacarus buchneri* infection intensity was also analysed, but no variables were found to significantly influence the

number of adult mites infecting the bees and so only the presence/absence results are presented here. All statistical tests were two-tailed and models were selected and simplified according to Akaike's Information Criterion (AIC). All two-way interactions were investigated, but as none of these were significant they are not reported here.

Results

A total of 506 *B. muscorum* and 360 *B. jonellus* workers were dissected. The *B. muscorum* samples came from 17 island populations with a mean sample size of 29.8 (range: 20 to 41) from each island. The *B. jonellus* samples came from 13 island populations with a mean sample size of 27.7 (range: 18 to 30) from each island. The tracheal mite *L. buchneri* was present in 15 out of the 17 populations of *B. muscorum* and had an overall prevalence of 28% in this species. The parasite was present in all populations of *B. jonellus* and had an overall prevalence of 39% (table 1). The mean number of mites per infected bee was 6.45 (range: 1 to 68).

	Longitude	Latitude	Island size km^2	*Bombus muscorum* H_E	*Locustacarus buchneri* prevalence n	*Locustacarus buchneri* prevalence Mean	*Bombus jonellus* H_E	*Locustacarus buchneri* prevalence n	*Locustacarus buchneri* prevalence Mean
Barra	7° 28′ 0″ W	56° 59′ 0″ N	67	0.393 (0.113)	30	0.50 (0.32-0.68)	0.766 (0.048)	30	0.67 (0.47-0.82)
Canna	6° 32′ 44.3″ W	57° 3′ 28.4″ N	14	0.433 (0.086)	30	0.23 (0.11-0.43)	0.758 (0.070)	30	0.23 (0.11-0.43)
Coll	6° 33′ 26″ W	56° 38′ 0″ N	73	0.499 (0.091)	29	0.34 (0.19-0.54)	0.738 (0.073)	30	0.30 (0.15-0.50)
Colonsay	6° 13′ 0″ W	56° 4′ 0″ N	44	0.416 (0.086)	20	0.15 (0.04-0.39)	-	-	-
Eigg	6° 10′ 0″ W	56° 54′ 0″ N	30	0.533 (0.094)	30	0.00 (0.00-0.14)	0.757 (0.066)	30	0.37 (0.21-0.56)
Lunga	6° 25′ 18″ W	56° 29′ 27″ N	1	0.507 (0.108)	30	0.00 (0.00-0.14)	0.742 (0.076)	30	0.33 (0.18-0.53)
Mingulay	7° 38′ 15″ W	56° 48′ 41.4″ N	6	0.374 (0.115)	30	0.33 (0.18-0.53)	0.696 (0.048)	18	0.22 (0.07-0.48)
Monachs	7° 40′ 0″ W	57° 31′ 0″ N	4	0.305 (0.092)	30	0.13 (0.04-0.32)	-	-	-
Muck	6° 14′ 56″ W	56° 50′ 3″ N	6	0.425 (0.088)	30	0.20 (0.08-0.39)	0.751 (0.056)	30	0.20 (0.08-0.39)
Muldoanich	7° 26′ 35″ W	56° 55′ 9″ N	1	0.421 (0.103)	26	0.62 (0.41-0.79)	-	-	-
N. Uist	7° 20′ 0″ W	57° 36′ 0″ N	308	0.404 (0.113)	20	0.25 (0.10-0.49)	-	-	-
Pabbay	7° 34′ 21.4″ W	56° 51′ 31.7″ N	3	0.399 (0.118)	30	0.53 (0.35-0.71)	0.729 (0.046)	22	0.82 (0.59-0.94)
Rum	6° 21′ 0″ W	57° 0′ 0″ N	109	0.451 (0.077)	29	0.21 (0.09-0.40)	0.749 (0.079)	28	0.32 (0.17-0.52)
S. Uist	7° 19′ 0″ W	57° 16′ 0″ N	309	0.404 (0.113)	25	0.40 (0.22-0.61)	0.755 (0.054)	22	0.32 (0.15-0.55)
Sandray	7° 31′ 0″ W	56° 53′ 36″ N	4	0.367 (0.111)	30	0.57 (0.38-0.74)	0.763 (0.054)	30	0.47 (0.29-0.65)
Staffa	6° 20′ 25″ W	56° 26′ 10″ N	0.5	0.484 (0.091)	46	0.09 (0.03-0.22)	0.697 (0.082)	30	0.53 (0.35-0.71)
Tiree	6° 49′ 0″ W	56° 31′ 0″ N	75.25	0.499 (0.086)	41	0.27 (0.15-0.43)	0.715 (0.076)	30	0.27 (0.13-0.46)
Overall				**0.437 (0.015)**	**506**	**0.28 (0.24-0.32)**	**0.743 (0.005)**	**360**	**0.39 (0.33-0.43)**

Table 1: **Population means for host genetic diversity and parasite prevalence.** The figures in parentheses are the standard errors for genetic diversity and the 95% C.I. for parasite prevalence. Measures for heterozygosity (H_E) are taken from Darvill et al. (2010).

Population level results

There was a significant negative correlation between the prevalence of *L. buchneri* and *B. muscorum* population heterozygosity (Z = -2.78, P = 0.005, figure 1). There was also a significant positive correlation between island size and *L. buchneri* prevalence (Z = 3.15, P = 0.002). There was no correlation between these two explanatory variables (r = -0.052, P = 0.843). Sampling date significantly influenced *L. buchneri* infection; bees sampled later in the year were more likely to be infected (Z = 2.33, P = 0.020). Neither bee age nor size significantly affected *L. buchneri* infection in *B. muscorum* (table 2). No variable significantly influenced the overall prevalence of *L. buchneri* in *B. jonellus* populations (table 2).
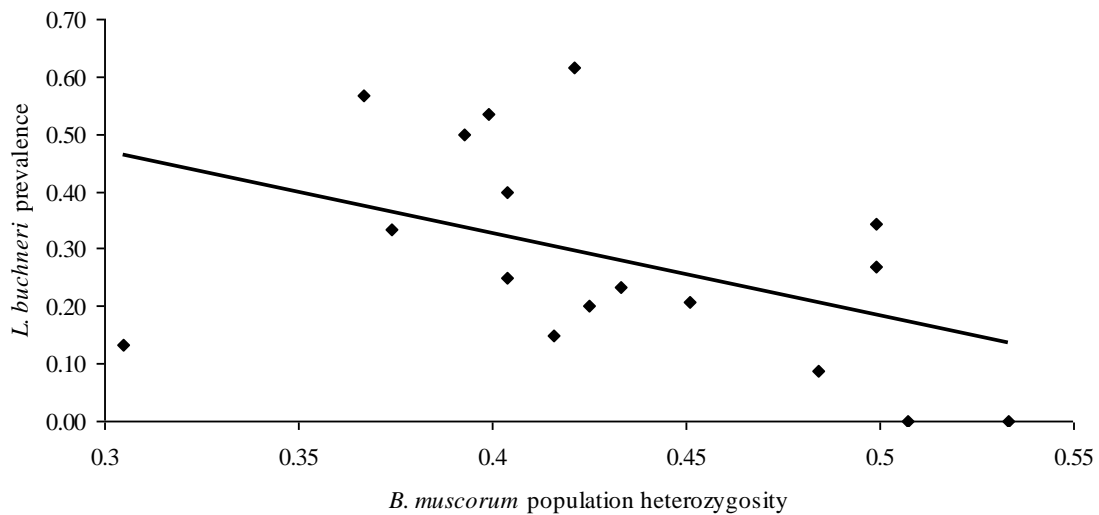

Figure 1: Relationship between *L. buchneri* prevalence and heterozygosity of the host population.
Each point represents an island population. Islands with higher heterozygosity had significantly lower prevalence of *L. buchneri* (P = 0.005, table 2).

	Bombus muscorum				*Bombus jonellus*			
	Co-efficient esimate	SE	Z	P	Co-efficient esimate	SE	Z	P
Heterozygosity of population	-8.760	3.149	-2.78 (1)	**0.005**	1.223	10.050	0.12 (1)	0.903
Date	0.026	0.011	2.33 (1)	**0.020**	0.008	0.014	0.61 (1)	0.543
Age	0.104	0.112	0.93 (1)	0.350	0.157	0.127	1.24 (1)	0.217
Bee size	-0.022	0.261	-0.09 (1)	0.932	0.097	0.349	0.28 (1)	0.782
Island Area	0.006	0.002	3.15 (1)	**0.002**	-0.001	0.003	-0.31 (1)	0.755

Table 2: Output of binomial generalised linear mixed effect models for the prevalence of L. buchneri in B. muscorum and B. jonellus populations. Degrees of freedom are given in parentheses and significant results are highlighted in bold.

Individual level results

Bombus jonellus were more frequently infected with *L. buchneri* than *B. muscorum* (χ^2 = 11.85, df = 1, p < 0.001). Bees sampled later in the season were more likely to be infected (χ^2 = 5.51, df = 1, p = 0.019). Individual heterozygosity, bee age and size did not significantly predict whether bees were infected with *L. buchneri* (table 3).

	Co-efficient esimate	SE	χ^2	P
B. muscorum compared to *B. jonellus*	-0.558	0.162	11.85 (1)	**<0.001**
Individual heterozygosity	-0.017	0.540	0.001 (1)	0.975
Age	0.135	0.082	2.665 (1)	0.103
Bee Size	0.112	0.202	0.305 (1)	0.581
Sampling date	0.025	0.009	5.507 (1)	**0.019**

Table 3: Output of binomial generalised linear mixed effect models for the presence/absence of L. buchneri. Degrees of freedom are given in parentheses. Likelihood ratio tests provide χ2 and p values for each term. Significant results are highlighted in bold.

Discussion

This study demonstrates that *B. muscorum* populations with lower levels of heterozygosity have higher prevalence of the tracheal mite *L. buchneri*. This builds on previous work by Whitehorn *et al.* (2011), who also studied Hebridean island populations of *B. muscorum* and found a significant negative relationship between parasite prevalence and host population genetic diversity in the gut trypanosome parasite *Crithidia bombi*. While this earlier study also examined *L. buchneri*, sample sizes were too low to detect meaningful biological relationships. Together with the recently discovered higher

prevalence of *N. bombi* in US bumblebee populations that had reduced genetic diversity (Cameron *et al.*, 2011), our results suggest a general relationship between parasite prevalence and genetic diversity in bumblebee populations. These findings support previous experimental work that found genetic heterogeneity within colonies to be negatively correlated with parasitic infections in social insects (Baer & Schmid-Hempel, 2001; Hughes & Boomsma, 2004; Seeley & Tarpy, 2007).

Although there was a population-level relationship between genetic diversity in *B. muscorum* and prevalence of *L. buchneri*, there was no such relationship between individual heterozygosity and infection. This could be because heterozygosity is not affecting susceptibility to parasites at an individual level, which is supported by Whitehorn *et al.* (2011) who found that individual immune measures were unaffected by genetic diversity. We hypothesise that the population-level effect that we observed results because particular parasite genotypes can spread to high prevalence in populations that lack genetic diversity at relevant pathogen susceptibility loci. Another explanation for the absence of a heterozygosity-infection association in individuals is that heterozygosity at the neutral markers genotyped may not be a good indicator of underlying inbreeding at the individual level. This is possibly due to the relatively small number of loci genotyped: a study by Slate & Pemberton (2002) concluded that, in order to reliably detect Heterozygosity Fitness Correlations (HFCs), a panel of ten or more microsatellite markers were needed. Other studies have also found that multi-locus heterozygosity is an unreliable predictor of individual genetic diversity at loci influencing fitness (for example, Hedrick, 2001; Pemberton, 2004; Slate *et al.*, 2004).

Relatively little research has been conducted on *L. buchneri* but limited data suggest that heavy infections might be associated with lethargy and reduced foraging (Husband & Sinha, 1970). In contrast, *Acarapis woodi*, the tracheal mite of honey bees *Apis mellifera*, has been studied in more detail. For example, experimental work has found that infection with *A. woodi* causes a reduction in the metabolic rate of individual bees and this may constrain activity, particularly in cool weather (Harrison *et al.*, 2001). Additionally, a recent review (McMullan & Brown, 2009) concluded that honey bee colonies infected with tracheal mites exhibit increased temperature dependent mortality. It is certainly possible that *L. buchneri* inflicts similar costs on bumblebees. Parasitic infection may also have indirect effects on fitness simply by stimulating the immune system (Brown *et al.*, 2003; Bashir-Tanoli & Tinsley, 2014) and *L. buchneri* infection does indeed trigger a melanisation response in the host's trachea (pers. obs.). Bumblebee colonies whose workers are immune challenged may have lower reproductive output, an effect that is exacerbated by harsh environmental conditions (Moret & Schmid-Hempel, 2001; Moret & Schmid-Hempel, 2004). Therefore, parasitism is likely to exert fitness costs on the hosts and as prevalence is higher in less genetically diverse populations, it may increase their risk of extinction, as suggested by de Castro & Bolker (2005).

In contrast to the observations in *B. muscorum*, there was no relationship between the prevalence of *L. buchneri* and the genetic diversity of *B. jonellus* populations. This may be a result of the appreciably lower range in the measures of population heterozygosity (a range of only 0.019 compared to a range of 0.228 for *B. muscorum*), which may limit our

ability to detect any influence that genetic diversity has on parasite prevalence. Interestingly, *B. jonellus* had consistently higher infection rates compared to *B. muscorum*, something its greater heterzyogosity would not lead us to expect. This could reflect the inability of the less genetically diverse *B. muscorum* to survive high levels of infection, meaning that high parasite prevalence was not observed. Alternatively, this observation may be due to an inter-specific difference in the parasitism rates of these two species, as such differences are commonly found in bumblebees (for example, Shykoff & Schmid-Hempel, 1991; Korner & Schmid-Hempel, 2005). The reasons behind these differences remain unknown but are likely to relate to inter-specific variation in transmission opportunities, host genetics and parasite defence, environmental factors or parasite virulence.

In conclusion, this study has demonstrated that low genetic diversity in *B. muscorum* populations is associated with a higher prevalence of the tracheal mite *L. buchneri*. This supports theories that suggest parasite species can spread to higher prevalence in populations that are more genetically homogeneous. Therefore, the persistence of small, isolated populations of bumblebees may be threatened due to inbreeding and the associated effects on levels of parasitic infection.

Acknowledgements

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