Infection, Genetics and Evolution 56 (2017) 125–132

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

# Parasite infection of specific host genotypes relates to changes in prevalence in two natural populations of bumblebees

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ARTICLE INFO

*Keywords:*
Mitochondrial DNA
*Bombus terrestris*
Host-parasite interaction
Coevolution
Evolutionary genetics
Social insect

ABSTRACT

The antagonistic relationship between parasites and their hosts is strongly influenced by genotype-by-genotype interactions. Defense against parasitism is commonly studied in the context of immune system-based mechanisms and, thus, the focus in the search for candidate genes in host-parasite interactions is often on immune genes. In this study, we investigated the association between prevalence of parasite infection and host mitochondrial DNA (mtDNA) haplotypes in two natural populations of bumblebees (*Bombus terrestris*). The two most common haplotypes of the host populations, termed A and B, differ by a single nonsynonymous nucleotide substitution within the coding region of cytochrome oxidase I, an important player in metabolic pathways. We screened infection by *Nosema bombi*, a common endoparasite of bumblebees, and the corresponding host mtDNA-haplotype frequencies in over 1400 bumblebees between 2000 and 2010. The island population of Gotland showed lower mtDNA diversity compared to the mainland population in Switzerland. Over time, we observed large fluctuations in infection prevalence, as well as variation in host haplotype frequencies in both populations. Our long-term observation revealed that *N. bombi* infection of specific host genotypes is transient: We found that with increasing infection prevalence, proportionally more individuals with haplotype B, but fewer individuals with haplotype A were infected. This suggests that the presence of *N. bombi* in specific host genotypes relates to infection prevalence. This may be a result of parasite competition, or differential resilience of host types to ward off infections. The findings highlight the important role of host mtDNA haplotypes in the interaction with parasites.

## 1. Introduction

The genetic interaction between hosts and parasites is key to the dynamics of a host-parasite system and to understand how diseases spread. The relationship between susceptibility to parasite infection and genetic diversity was noticed by Elton (1958). Initially, this insight was based on the observation that crops grown in genetically homogeneous monocultures are typically more susceptible to the spread of diseases than genetically mixed cultures (e.g. Browning and Frey, 1969; Leonard, 1969; Wolfe, 1985; McDonald et al., 1988; Garrett and Mundt, 1999, Zhu et al., 2000, Mundt, 2002, Pilet et al., 2006). The relationship between genetic diversity of the host local population (i.e. the colony) and parasitic infection has also been shown in social insects (Hughes and Boomsma, 2004; Reber et al., 2008), including bumblebees (Liersch and Schmid-Hempel, 1998, Baer and Schmid-Hempel, 1999). Furthermore, colony fitness in bumblebees was found to be correlated with particular mtDNA haplotypes (Johnson et al., 2011).

In particular, Baer and Schmid-Hempel (1999) showed that infection load and prevalence of *Crithidia bombi* (Trypanosomatidae), a common parasite of bumblebees, is lower with higher genetic variation within colonies of its host, *Bombus terrestris*. In fact, genetically heterogeneous colonies of *B. terrestris* harbored generally lower pathogen loads, including lower loads of *Nosema bombi* (Microsporidia) (Liersch and Schmid-Hempel, 1998, Baer and Schmid-Hempel, 1999). Experimental infections of *Bombus* spp. with *N. bombi* have furthermore shown that the success of infecting different colonies depends on the source of the parasite's spores (Schmid-Hempel and Loosli, 1998). That study also showed that variation among conspecific colonies had a greater effect on susceptibility to *N. bombi* than variation across host species. Together, this suggests a genotype-genotype interaction between parasite strain (or genotype, respectively) and host colony (representing a host genetic background); this interaction affects the prevalence, infectivity and/or observed virulence effects of *N. bombi*. Further evidence has come from several additional studies (Schmid-Hempel and Schmid-Hempel, 1993; Schmid-Hempel et al., 1999; Imhoof and Schmid-Hempel, 1998; Schmid-Hempel, 2000). Yet, to date, variation in

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https://doi.org/10.1016/j.meegid.2017.11.019
Received 4 July 2017; Received in revised form 16 October 2017; Accepted 6 November 2017
Available online 16 November 2017
1567-1348/ 

parasite success could not be linked to specific host genotypes of *B. terrestris*. Here, we explore whether mitochondrial haplotypes, can be associated with the outcome of host-parasite interactions in a natural setting.

Variation in mitochondrial DNA (mtDNA) has been widely used in studies on population structure, evolution, and to reconstruct the phylogeny of various taxa (Avise, 1994). Yet, to date only few studies have linked mtDNA polymorphism to life-history traits, fitness or parasite defense. Considering that defense against parasites bears a considerable energy cost for the host (Moret and Schmid-Hempel, 2000), mitochondrial genes involved in energy metabolism, such as COx, could be a suitable target for further exploration. Genes on the mitochondrion are tightly linked (although some recombination occurs, see Stein and Sia, 2017), such that any marker could serve the purpose. Here, we focused on a 420-bp fragment mitochondrial DNA sequence, which includes the 5′-end of the coding region of cytochrome oxidase I (COI), as well as a non-coding intergenic sequence (IGS) between COI and COII (Crozier et al., 1989; Crozier and Crozier, 1993). This intergenic region is common in Hymenoptera (ants, bees and bumblebees) (Crozier et al., 1989; Crozier and Crozier, 1993; Kronauer et al., 2004) and varies considerably with respect to size and sequence across individuals (Cornuet and Garnery, 1991; Cornuet et al., 1991). Such variation defines different mtDNA haplotypes that can be used for population studies.

A few studies highlight the important roles of mtDNA haplotypes with respect to fitness and parasite defense. For example, Christie et al. (2004) observed differences in fitness traits among two common haplotypes of *Drosophila subobscura*. Under laboratory conditions one mtDNA haplotype outperformed the other by 10–20% with respect to longevity, egg-to-larva and larva-to-adult survival and resistance to desiccation. Furthermore, microsporidian infection prevalence was associated with specific haplotypes in the COI region of the amphipod host, *Gammarus duebeni* (Ironside et al., 2003). Two microsporidian parasites, *Microsporidium* sp. and *Nosema granulosis*, were shown to phenotypically feminize the male hosts (*Gammarus duebeni*). Ironside et al. detected five mtDNA haplotypes in a COI fragment in natural populations of *G. duebeni* sampled from three locations. Infection prevalence of both feminizing parasites was shown to differ significantly among the five haplotypes. For example, *N. granulosis* infection prevalence was significantly greater in one mitochondrial haplotype compared to all other haplotypes.

The fact that mtDNA is usually only transmitted maternally, is particularly relevant to the study of insects with the so-called complementary sex determination system. This is because female sexual offspring are not only more costly to produce due to generally larger body sizes, and are thus much less numerous than males, but also because they represent the diploid genetic state (whereas males in bees are haploid as a rule) (Johnson et al., 2011). Furthermore, in bumblebees, only few colonies are capable of producing daughter queens at all (Müller and Schmid-Hempel, 1992), which then leave, mate and overwinter to pass on their mtDNA haplotypes to their progeny, mostly the workers of their own colony and, eventually, the daughter queens and drones. Consequently, bumblebee populations should be dominated by only a few matrilines (Donovan and Wier, 1978; Müller and Schmid-Hempel, 1993; Imhoof and Schmid-Hempel, 1999), and the question arises whether variation in the frequency of haplotypes also relates to parasite pressure.

We here focus on *Nosema bombi* (Fantham and Porter, 1914), a microsporidian parasite that infects the gut of various bumblebee species and can cause a creeping disease (Schmid-Hempel, 1998; MacFarlane et al., 1995). *N. bombi* infection can be highly detrimental to the fitness of its host, *B. terrestris*. This has been shown in laboratory experiments, in which infected workers and males had significantly greater mortality than queens (Otti and Schmid-Hempel, 2007). Also, the size of colonies produced by infected queens was shown to be significantly smaller than those of uninfected ones in field experiments. The fitness of those infected colonies was effectively reduced to zero because none produced sexual progeny (Otti and Schmid-Hempel, 2008). Furthermore, Cameron et al. (2011) suggested that declining *Bombus* populations in North America have significantly greater infection levels of *N. bombi* than comparable stable populations. In our context, *Nosema* is of additional interest, as it lacks functional mitochondria, and is, thus, dependent on ATP production of its host (Weidner et al., 1999; Keeling and Fast, 2002; Burri et al., 2007). In fact, microsporidia have been observed to be surrounded by host mitochondria (Scanlon et al., 2004) and to disrupt the host mitochondrial network (Williams, 2009). Possible protein tethering between microsporidia and host mitochondria has been suggested (Sinai and Joiner, 2001; Scanlon et al., 2004; Williams, 2009). Considering that COx is part of the mitochondrial transmembrane complex, a possible interaction between infecting microsporidia and the COx proteins at the mitochondrial membrane of the host seems conceivable.

We here compared mtDNA sequence variation and haplotype frequencies of two natural *Bombus terrestris* populations with infection prevalence of the parasite *N. bombi*. This study expands on previous work by Johnson et al. (2011), who presented a mtDNA haplotype network for the Swiss population (2002) and linked extant haplotypes to fitness traits.

## 2. Materials and methods

### 2.1. Sampling and DNA extraction

Bumblebees (*B. terrestris*) were sampled at site ‘Neunforn’ (47.6° N 8.8° E), northeastern Switzerland, and on the island of Gotland, Sweden (57.5° N, 18.5° E) between 2000 and 2010 (supplementary Fig. S1). The collection site in northeastern Switzerland lies within the core range of *B. terrestris* in mainland Europe. Gotland, which is somewhat isolated from the mainland, and also represents the northern edge of the distribution of *B. terrestris* (Løken, 1973; Pekkarinen and Kaarnama, 1994). Spring queens were collected from the island of Gotland in 2001, 2002, 2003, 2004, 2005 and 2006. Workers were collected from the same site in the summers of 2002, 2003, 2006 and 2008. Queens in Neunforn were sampled during eleven consecutive spring seasons from 2000 to 2010. All bees were stored at − 80 °C and dissected prior to DNA extraction. DNA was extracted from guts and thoraces according to the *Qiaqen DNeasy™ 96* protocol (Qiaqen, July 2006) or alternatively, with the use of 10% Chelex (Walsh et al., 1991, Schmid-Hempel and Schmid-Hempel, 2000).

### 2.2. Haplotyping

Bumblebee mtDNA was sequenced to determine haplotypes for all available samples. A 500-bp mitochondrial fragment was amplified by polymerase chain reaction on a GeneAmp 9700 thermal cycler (Applied Biosciences). This sequence includes a coding region, the 5′-end of cytochrome oxidase I (COI) and a non-coding intergenic sequence between COI and cytochrome oxidase II (CO-II) genes (Crozier et al., 1989; Crozier and Crozier, 1993; Johnson et al., 2011).

The intergenic region varies in length and sequence in honeybees and bumblebees (Cornuet and Garnery, 1991; Cornuet et al., 1991; Johnson et al., 2011). The primer pair BB_IGSF1 (forward: 5′-GGA GCA ATA ATT TCA ATA AAT AG-3′) and BB_COIIB3 (reverse: 5′-TTA TGA AAT GAA ATT AAA TTA TCA G-3′), used for amplification, was also employed by Johnson et al. (2011). PCR reactions were performed using, 5 µl of dNTPs of 2 mM, 2 µl of each primer of 10 µM,2.5 U GoTaq DNA polymerase (Promega), 10 µl GoTaq reaction buffer (Promega) and water with an annealing temperature of 45 to 47 °C for 40 cycles. PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with GelRed™ (Biotium), or alternatively on PCR CheckIT gels (Elchrom Scientific).

The COx-IGS amplicons were sequenced using a 3730xl DNA