Infection, Genetics and Evolution 23 (2014) 129–137

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Rapid evolution of antimicrobial peptide genes in an insect host–social parasite system

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ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 8 January 2014
Accepted 7 February 2014
Available online 14 February 2014

Keywords:
Social insect
Co-evolution
Innate immunity
Bumblebee
Bombus
Host–parasite

ABSTRACT

Selection, as a major driver for evolution in host–parasite interactions, may act on two levels; the virulence of the pathogen, and the hosts' defence system. Effectors of the host defence system might evolve faster than other genes e.g. those involved in adaptation to changes in life history or environmental fluctuations. Host–parasite interactions at the level of hosts and their specific social parasites, present a special setting for evolutionarily driven selection, as both share the same environmental conditions and pathogen pressures.

Here, we study the evolution of antimicrobial peptide (AMP) genes, in six host bumblebee and their socially parasitic cuckoo bumblebee species. The selected AMP genes evolved much faster than non-immune genes, but only *defensin-1* showed significant differences between host and social parasite. Nucleotide diversity and codon-by-codon analyses confirmed that purifying selection is the main selective force acting on bumblebee defence genes.

1. Introduction

Host–parasite interactions are shaped by a wide range of biotic and abiotic factors. Under natural conditions, variations in parasite (pathogen) load and virulence, as well as variations in host susceptibility and immune responses, control host–parasite dynamics (Anderson and May, 1982; Gandon et al., 2001; Schmid-Hempel and Ebert, 2003). Co-evolutionary arms races are mostly characterized as reciprocal processes of adaptation and counter-adaptation between parasites and hosts (Dawkins and Krebs, 1979). Positive selection of the defence mechanisms clearly reflects the importance of pathogenic organisms in host evolution, because immunity related proteins are functionally important in the host immune system and play an important role in adapting to novel pathogens or pathogen genotypes.

Parallel evolution at the level of amino acid changes are characterized by parallel replacements between at least two related but distinct species possessing a common ancestor. Convergent evolution describes the same amino acid replacement with the same outcome, in two unrelated species without any common ancestor (Nei and Kumar, 2000). Both, parallel and convergent changes in amino acids are a sign for strong positive selection. However, under natural conditions parallel and convergent evolution have been very rarely observed (Doolittle, 1994).

Evolutionary forces acting on DNA can be characterized by measurable changes of synonymous (d_S) and non-synonymous (d_N) substitution rates in coding regions (Nielsen, 2005; Yang and Bielawski, 2000). The d_N/d_S ratio (ω) classifies possible occurring selection events in three different groups: $\omega > 1$, diversifying selection (positive selection); $\omega = 1$, no selection (neutral evolution) and $\omega < 1$, purifying selection (negative selection) (reviewed in Wagner, 2002).

Innate insect immune systems categorise the majority of parasites into four groups: viruses, gram-positive and gram-negative bacteria, and fungi or yeasts (Hultmark, 1993; Lemaitre and Hoffmann, 2007). Selection may take place separately at genes specific for each group, or globally against one of the groups. Social insects are model organisms for investigating adaptive evolution in the innate immune system, as group living increases their vulnerability to diseases, especially since the group is composed of closely related individuals (Schmid-Hempel, 1998).

Less attention has been paid to host–parasite systems where both host and parasite are closely related, sharing similar life his-

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http://dx.doi.org/10.1016/j.meegid.2014.02.002
1567-1348/

tory traits. This is known as social parasitism or brood parasitism and is found in both birds and social insects (e.g. ants and bumblebees, reviewed in Kilner and Langmore, 2011). Host bumblebees and their social parasites, cuckoo bumblebees (Fisher, 1988; van Honk et al., 1981) share the same, but timeshifted annual life cycle, and corresponding environmental conditions (Alford, 1975; Sladen, 1912). Host bumblebee colonies are comprised of drones (males), workers and a single bumblebee queen who initiates nest foundation in early spring. During colony development, workers are produced exclusively and only at the end of the season are new sexuals (drones and queens) produced for the forthcoming season (Alford, 1975; Sladen, 1912). Cuckoo bumblebee females invade host nests in spring, killing the host queen and leaving host workers to take care of the cuckoo female's brood. In contrast to the host, cuckoo females produce only male and queen offspring, lacking a worker caste (Alford, 1975; Sladen, 1912). This kind of parasitism is assigned to queen-intolerant inquilines (reviewed in Brandt et al., 2005). Within this asymmetric, inter-specific arms race (Dawkins and Krebs, 1979), cuckoo bumblebees may be specialist or generalist, being a mono- or multiple-host social parasite, depending on the host species range (Loken, 1984).

Multiple reports of plant and animal evolutionary adaptations suggest that the environment plays an important role in gene evolution and associated phenotypic shifts (reviewed in Levasseur et al., 2007; Salamin et al., 2010). Sharing the same environmental conditions (i.e. food source, homeostatic nest condition and symbionts, non-coevolving saprophytes, omnipresent microorganisms at nest and hibernation sites), including co-evolving parasites and pathogens, might force parallel evolution of parasite/pathogen defence mechanisms against common microbes and viruses, in bumblebee hosts and their cuckoo bee parasites. Environmental conditions (e.g., temperature, humidity and light) and shifts in the microhabitat or diets show a strong impact (37.5%; Fuller et al., 2011) on immunocompetence and pathogen susceptibility in social insects (Bulmer and Crozier, 2006; Fuller et al., 2011). Transitions to new habitats represent the exposure of the host to novel pathogens, which could direct rapid, adaptive changes in immune proteins (Bulmer and Crozier, 2006). As a sign of adaptive evolution, genes involved in the immune defences of various plants and animals, typically show a faster rate of nucleotide and amino acid substitutions (non-synonymous), than non-immunity related genes (McTaggart et al., 2012; Obbard et al., 2009; Sackton et al., 2007; Tiffin and Moeller, 2006; Trowsdale and Parham, 2004; reviewed in Bulmer, 2010).

Positive selection and rapid gene duplication as factors influencing evolution have been demonstrated in social insects for antimicrobial peptides (AMPs) (termicin – Bulmer and Crozier, 2004), gram-negative bacteria-binding proteins and relish in termites (Bulmer and Crozier, 2006) and several immune genes in ants (Viljakainen and Pamilo, 2008; Viljakainen et al., 2009). Positive selection was detected mostly in the mature region of the AMPs, whereas the signal and pro-regions seem to evolve neutrally (Lazzaro and Clark, 2003; Viljakainen and Pamilo, 2008). For termicin especially, the expressed mature peptide appears to have diverged more rapidly than the 3′UTR (Bulmer and Crozier, 2004). In addition, a population genetic analysis of nucleotide intra-specific polymorphism and inter-specific divergence indicated that a positive selection driven selective sweep reduced polymorphisms in the AMP termicin (Bulmer et al., 2010). Hence, if the immune system adapts to parasites/pathogens in similar ways in related species (i.e. host and cuckoo bumblebee species), we would expect to observe congeneric genes experiencing positive selection in different lineages of the same affiliation.

Social insects show a reduced number of immune genes relative to solitary species (Evans et al., 2006), and so may compensate for the reduction in immunity gene variance through group level 'social immunity' (Cremer et al., 2007; Richter et al., 2012; Traniello et al., 2002).

Social parasites and their hosts are frequently very close phylogenetic relatives that might influence the ease of evolutionary adaptations between host and social parasite on both sides (Davies et al., 1989). Here we tested whether parasite or pathogen driven evolutionary adaptations (parallel evolution of AMP genes) can be observed in closely related host-social parasite couples sharing the same environmental conditions i.e. parasite pressure (Erler et al., 2012). Six specialised host/social parasite bumblebee couples were used to determine the type and strength of selection on AMP genes, both within and between host and social parasite species.

2. Material and methods

2.1. Bumblebee samples

Bumblebee drones of six bumblebee hosts and their respective cuckoo bumblebee species were sampled in three locations across Europe (Table 1). Bumblebees have a haplo-diploid sex determination system; therefore the haploid drones provide a highly efficient model system for genetic studies as they present a single allele per locus. At each location, host and social parasite couples were caught during foraging flights and immediately stored in ethanol or at −80 °C until further processing. Bumblebee species were identified using the taxonomic key of Mauss (1994).

2.2. DNA isolation and target gene amplification

The thorax muscles of three individuals per species were used to isolate genomic DNA using the DNeasy Blood & Tissue Kit tissue protocol (Qiagen, Hilden, Germany). Tissue samples were homogenized, followed by proteinase K (600 mAU/mL) treatment for at least 2 h and final DNA elution was conducted twice in 50 μL AE Buffer. Quality and quantity of DNA was determined via NanoDrop ND-1000 (Peqlab, Erlangen, Germany).

AMP (*abaecin*, *defensin-1* and *hymenoptaecin*; all bumblebees) and non-immune reference gene – (*EF-1 alpha*, *arginine kinase*, *rhodopsin*, *PEPCK*; only for *B. perezi*) amplification was performed in a thermocycler, with denaturation at 95 °C for 4 min; 35 cycles at 95 °C for 40 s; 55 °C for 30 s, and 72 °C for 2 min 20 s, with final elongation at 72 °C for 10 min. Each reaction (10 μL) contained 2.0 mM dNTPs, 0.2 μM of each gene-specific forward and reverse primer (Table 2), 0.25 U of peqGOLD Taq-DNA-polymerase (Peqlab, Erlangen, Germany) and 1 μL of extracted genomic DNA.

PCR products were checked for correct amplicon size by automated multicapillary electrophoresis using the QIAxcel System with QIAxcel DNA High Resolution Kit (Qiagen, Hilden, Germany), purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) or SureClean (Bioline, Luckenwalde, Germany), before 96-well plate MTP sequencing by LGC Genomics (Berlin, Germany), based on traditional Sanger sequencing. All fragments were sequenced overlapping in both directions. *Abaecin*, *defensin-1* and *hymenoptaecin* were successfully amplified in all 12 host and social parasite bumblebee species listed in Table 1. The sequenced regions did not cover the entire coding region of the genes but lacked a few nucleotides of the coding regions in either the 3′ or 5′ end, or both.

A sequenced region of the 16S rRNA was used to confirm species identification of the cuckoo bumblebee species by comparison with reference sequences from GenBank (Cameron et al., 2007).

AMP gene sequences for all bumblebee species, non-immune and 16S rRNA gene sequences for *B. perezi* are available on GenBank, under the accession numbers: KC662127-38 (*abaecin*),