Infection, Genetics and Evolution 21 (2014) 192–197

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Few colonies of the host *Bombus terrestris* disproportionately affect the genetic diversity of its parasite, *Crithidia bombi*

Gabriel Cisarovsky *, Paul Schmid-Hempel

ETH Zürich, Institute of Integrative Biology (IBZ), Universitätstrasse 16, 8092 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 30 October 2013
Accepted 1 November 2013
Available online 18 November 2013

Keywords:
Crithidia bombi
Bumblebee
Trypanosomatid
Immune priming
Recombination in parasite
Parasite adaptation

ABSTRACT

Sex and recombination have long been considered as necessary means for hosts to keep up with and resist to their faster reproducing parasites. On the other hand, comparatively little attention has been paid to potential benefits of recombination for the parasites. Using as model organisms the bumblebee *Bombus terrestris* and its genetically highly variable trypanosomatid parasite *Crithidia bombi* we analysed the infection dynamics as well as the relative frequency of parasite recombinants over time, in colonies that were either immune-challenged with heat-killed bacteria or sham-inoculated. In addition, we used infective cells from a given colony to infect workers from other, untreated colonies, to investigate whether recombinant parasite strains may have a competitive advantage over the parental strains to infect the surrounding host population. We show that in our experimental setup the host immune status does not influence the proportion of recombinant parasite cells in the infection. Neither do recombinant parasite strains have an advantage over the parental ones at infecting workers unrelated to the host colony the infection originally came from. However, we found that the prevalence of recombinants was highly variable among colonies, with one particular colony producing significantly more recombinant strains than others. As the successful infection of daughter queens – the only individuals surviving the winter to the next year – is proportional to the number of circulating parasite strains in the colony, we suggest that such "super-producing" colonies may be responsible for most of the infections happening in the next year.

1. Introduction

The co-evolutionary race between host and parasite depends on the host preventing and fighting infection, and the parasite being able to infect, to resist the host's immune defence, and to become transmitted to the next host. Genetic diversity of host and parasite populations is an important element of this race. In particular, sex and recombination are considered to be beneficial, since these processes very efficiently increase genetic diversity of offspring (Hamilton, 1980; Hamilton et al., 1990; Wagner, 2011). Numerous mathematical models have been developed in order to define under which conditions sex and recombination could be selected under host-parasite co-evolution (Kerstes et al., 2012). Empirically, still very few experiment studies have been done so far, but several reported positive fitness effects of sexual reproduction and recombination for the hosts. For example, parasites were identified as important drivers of sexual reproduction in a mixed population of asexual and sexual *Potamopyrgus antipodarum* snails, particularly also as drivers of clonal diversity in the asexual snails (King et al., 2011). Similarly, the bacterial pathogen *Serratia marcescens* increases the outcrossing rate of its host, *Caenorhabditis elegans*, and its virulence decreases in outcrossed populations of the host (Morran et al., 2011). Also, coevolution between the red flour beetle *Tribolium castaneum*, and its microsporidian parasite *Nosema whitei* maintains genetic diversity in the host's population (Bérénos et al., 2011) and increases host recombination rate (Kerstes et al., 2012).

We here focus on the parasite side, which by implication should enjoy the same advantages of sex and recombination as the host. Comparatively little is known about genetic exchange in parasites (excluding bacteria and viruses), and fitness effects of sexual reproduction have so far only been analysed in the rat nematode parasite *Strongyloides ratti* (Gemmill et al., 1997; West et al., 2001). This is regrettable considering that co-infection by multiple strains of the parasite can potentially have important consequences on disease outcome (Read and Taylor, 2001). Moreover, research has mostly focused on pathogens causing human diseases (Heitman, 2006). For instance, a single cross is believed to have provided *Toxoplasma gondii* its high infectivity (toxoplasmosis; Boyle et al., 2006; Grigg et al., 2001; Su et al., 2003). Among the protozoans, and trypanosomatids in particular, exchange of genetic material has notably been demonstrated in *Trypanosoma brucei* (sleeping sickness; Gibson et al., 2006), *Leishmania major* (leishmaniasis;

* Corresponding author.
E-mail address: gabriel.cisarovsky@gmail.com (G. Cisarovsky).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.11.010

Akopyants et al., 2009), *T. cruzi* (Chagas disease; Gaunt et al., 2003), and *Plasmodium falciparum* (malaria; Mzilahowa et al., 2007).

In one of the rare studies in trypanosomatids non-pathogenic to humans, Votýpka et al. (2001) could not detect any signature of genetic exchange in the mosquito parasite *Crithidia fasciculata*. However, a recent study could demonstrate exchange of genetic material between co-infecting strains of the bumblebee parasite *Crithidia bombi*, thus generating recombinant strains (1% of all analysed parasite cells) as compared to the parental genotypes (Schmid-Hempel et al., 2011). *C. bombi* is an extracellular parasite residing in the hind gut of bumblebees of the genus *Bombus*, reducing by up to 40% its host's fitness (Brown et al., 2003). In the infected host, the parasite population grows in numbers and infectious cells are shed in the faeces starting a few days post-infection. These cells can quickly infect new workers inside a nest. *C. bombi* is highly prevalent in natural populations, with 10–70% of individuals infected (Cisarovsky et al., 2012; Gillespie, 2010; Shykoff and Schmid-Hempel, 1991; Tognazzo et al., 2012), up to 67% of them by more than one strain (Salathé and Schmid-Hempel, 2011; Tognazzo et al., 2012) as defined by multi-locus genotyping (Schmid-Hempel and Funk, 2004). Such multi-strain infections seem to be the rule more than the exception in nature (Balmer and Caccone, 2008; Keeney et al., 2007; Llewellyn et al., 2011; López-Villavicencio et al., 2007; Lord et al., 1999; Warren et al., 2004). In addition, Schmid-Hempel et al. (2011) estimated that approximately 11% of all infections generate a completely novel parasite type, thus raising the question of what fitness benefit(s) a parasite strain may get from exchanging genetic material with another, co-infecting strain, and what factors favour genetic exchange.

Here, we investigated the correlation between the frequency of recombinants in the infection and host immune status. Previous studies showed that priming with bacteria releases the pressure of the immune system on *C. bombi*. This may reduce the benefits of recombination and, therefore – if adaptive as a stress response, lower the frequency of recombinants observed in the infection (Sadd and Schmid-Hempel, 2009; Ulrich and Schmid-Hempel, 2012). Other factors included in the analyses were host colony identity (variation between host genetic backgrounds) and time since infection. As to the latter, we were also interested in the relative frequency of single recombinant clones, i.e., whether one or few clones would become dominant with time over all others, similarly to what is known, for example, for *Toxoplasma gondii* (Boyle et al., 2006). For this time line, we analysed the prevalence of recombinant strains of *C. bombi* during the life cycle of colonies of the bumblebee *B. terrestris*, in which workers were either artificially primed with heat-killed bacteria (and thus had increased immune activity), or in which workers had only received a sham treatment (injection of insect Ringer). In addition, we determined whether recombination provided the parasite with an advantage at infecting individual hosts from the general population that were never encountered before (i.e., whether the relative frequency of recombinants is higher in novel hosts). As we will show and contrary to expectations, irrespective of these factors, the main finding was that recombination only occurs in few colonies, and at different rates.

2. Material and methods

2.1. Bumblebee rearing and parasite culture

Bumblebee colonies used in this experiment were started in the laboratory by field-caught queens, collected during spring 2011 in Northwestern Switzerland (location Aesch, BL). Two sources for the parasite, *C. bombi*, were used (internal codes nos. 10.189 and 10.441, subsequently labelled A and B, respectively). These parasite isolates represented single-genotype clones extracted from infected queens collected in 2010 from the same field population and grown in culture medium following the method described in Salathé et al. (2012).

2.2. Within-colony dynamics

Seven days before the start of the experiment, six workers of each of 12 colonies were removed and infected with *C. bombi* by feeding them 10 μL 50% Apiinvert sugar water containing 5000 cells each of both parasite strains (10,000 cells in total). The workers were kept separately and after one week checked for infection by microscopy. Note that later molecular analysis (see Section 2.4 of Section 2) also confirmed that every colony had been successfully infected by both strains. The infected individuals (3–6 depending on the colony) were put back into their respective colony to serve as the source of the infection. Six colonies were then randomly assigned to the bacterial challenge group (colonies labelled with IDs B1–B6), and six to the control group (colonies labelled with IDs R1–R6). In the challenged group, a random 80% of the workers were challenged with 2 μL of a mixed culture of *Arthrobacter globiformis* and *Escherichia coli* in Insect Ringer. The protocol for cell culture followed Cisarovsky et al. (2012), with the exception of the final cell concentration, which was adjusted to 10^8 cells/mL per bacterium. The inoculum was injected into the abdomen between the second and third tergites with a fine glass micro-capillary. In the control group, workers only received a Ringer injection without parasite cells but in the same manner. These treatments were subsequently repeated every week throughout the whole experiment to maintain the priming effect. Additionally, and prior to the treatments, an experimental background mortality rate of 20% per week was applied to the workers by removing individuals at random.

Seven days *post* colony infection (DPI) and weekly until 35 DPI, faeces from all workers were collected, put separately in Eppendorfs filled with 20 μL *Crithidia* culture medium (Salathé et al., 2012), and checked for infection by light microscopy. To limit the workload, bees were put into the vials in pairs, and we assumed that both bees were infected if the faeces sample from the vial revealed the presence of parasite cells. All infected samples were then pooled and submitted to fluorescent activated single cell sorting (FACS) for cloning (Salathé et al., 2012) and later genotyping.

2.3. Between-colony dynamics: faeces transfers

We used the 12 colonies treated as described above as the "focal" colonies. In addition, one worker of each of 11 additional, untreated and unrelated "foreign" colonies was collected and all 11 individuals pooled into a set of workers representing the population sharing the same environment. Seven days *post* infection of a given focal colony, faeces of infected workers from the focal colonies were collected and used to infect two groups of workers (Fig. 1): (i) 12 workers of the same colony ("resident" workers) and (ii) 11 workers of the foreign colonies ("foreign" workers). These workers were all collected as callows (i.e., very shortly upon emergence from the pupa) and as such are known to be uninfected. Two weeks after infection, eight (infected) workers were randomly chosen from within each group, pooled (by group), and submitted to fluorescent activated single cell sorting (FACS; Salathé et al., 2012) for the analysis of clones contained in these test infections.

2.4. Molecular analysis

Single cells were sorted by FACS into 96-well plates and incubated for 10–14 days (Salathé et al., 2012). Three such plates were