PART I

Understanding within-host processes

CHAPTER ONE

Pollinator diseases: the *Bombus–Crithidia* system

PAUL SCHMID-HEMPEL, LENA WILFERT
AND REGULA SCHMID-HEMPEL

1.1 Introduction

Bee pollinators are critically important for ecosystem functioning and food security, and bumblebees are the most important large pollinators in temperate and alpine habitats, as well as for many crops (Goulson, 2003b; Velthuis & van Doorn, 2006). Today, some species are expanding their range (Inoue et al., 2010; Schmid-Hempel et al., 2013), but most are declining in range and abundance (Biesmeijer et al., 2006; Goulson et al., 2008; Cameron et al., 2011). Declines can be attributed to various reasons (Fitzpatrick et al., 2007), but parasites seem to play a role in many cases (Cameron et al., 2011). Hence, the study of host–parasite interactions in bumblebees is, on one hand, of importance for the monitoring and possible management of pollinators. On the other hand, the *Bombus–Crithidia* system (Figure 1.1) has proven an excellent test ground to scrutinise basic scientific questions surrounding host–parasite interactions.

Until the early 1980s, parasites had been the almost exclusive domain of traditional parasitologists. By then, behavioural ecologists started to realise that the selective pressures exerted by parasites affect a wide range of seemingly unconnected phenomena. Examples include sexual selection and the meaning of conspicuous male ornaments (Hamilton & Zuk, 1982), the maintenance of genotypic diversity in populations (Hamilton, 1980), or the manipulation of host behaviour to increase transmission success (Ewald, 1980; Moore, 1984). At the same time, students of evolutionary population biology started to wonder not only whether parasites could regulate their host populations (Anderson & May, 1978), but also why – beyond the obviously different mechanisms that are involved – some parasites have evolved to be very damaging to the host, whereas others only cause mild symptoms. A new approach was therefore taken, asking what might be the selective advantage for the parasite when harming its host (Anderson & May, 1982; Ewald, 1983). The question of what virulence towards its host a parasite should 'choose' to

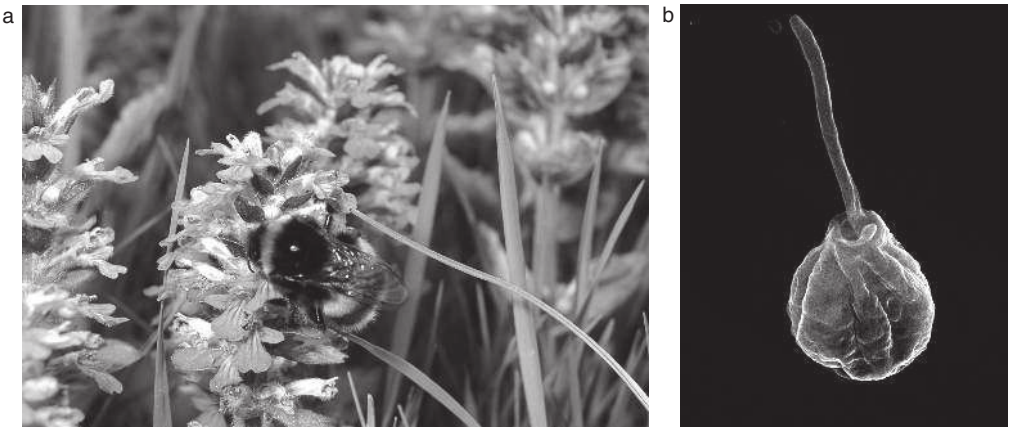

Figure 1.1 (a) Worker of *Bombus terrestris* visiting flowers of *Ajuga reptans* (photo: P. Schmid-Hempel). (b) SEM micrograph of *Crithidia bombi*. The length is around 10 μm (photo: Boris Baer, ETH Zürich). (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

maximise its fitness illustrates, in a nutshell, the concepts and ramifications of this approach, but also the difficulties and the kind of research needed to make progress. The *Bombus–Crithidia* system is a good example that shows what can and what cannot be asked and how natural host–parasite systems can be scrutinised to add to the theoretical concepts. Because the bumblebee hosts are social insects, a 'host' can either be the individual – where infection and immune defence unfold – or the colony as a whole, which is the tightly knit reproductive community. Colony members only gain fitness by raising close kin to become reproductives (daughter queens and sons, the drones) that go on to found the next generation. Colony success can also be understood as the founding female's (the queen's) success, which she achieves by building up a colony that ensures her reproductive success. Here, we generally focus on the individual host that is embedded in this background, but as far as the consequences of infection and defence go, these – evolutionarily speaking – accumulate at the colony level.

1.2 Natural history of the study system

Worldwide, some 250 species of bumblebees exist (genus *Bombus* Latreille 1802, Apidae). They inhabit temperate areas of the Holarctic, Neotropics, and South East Asia (Williams et al., 2008); four species were introduced to New Zealand and one to Tasmania. Bumblebees are social insects with an annual cycle. The queen on her own starts the colony at the beginning of the season (Figure 1.2). Once the first workers, the non-reproducing daughters, have hatched, the queen remains egg-laying in the nest as the colony grows in worker numbers.

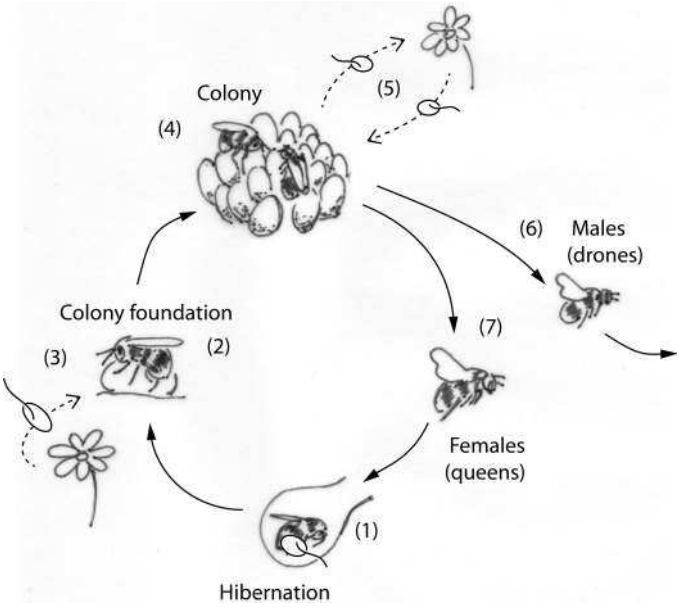

Figure 1.2 Sketch of host and parasite life cycle. The *Crithidia* parasites are directly transmitted, infect via ingestion of infective cells, and after passage through the bee, infective cells are shed with faeces. In bumblebees, (1) the queen hibernates alone and in soil. At this stage, some queens harbour an infection, which is the parasite's only way of passage through the hibernation period. (2) In spring, queens found their own colony. At this stage, they either have already carried an infection through hibernation, or may have acquired a new infection when foraging for food (3). When the colony has grown (4), the queen stays in the nest and an existing infection is passed on to other colony members inside the nest. As workers forage for food outside, they may carry a new infection back via contact on flowers (5). At the end of the colony cycle, sexual offspring are produced; both sexes can become infected inside the nest. (6) Males leave to mate and eventually perish, and do not pass on the infection. (7) Females (daughter queens) carry the infection into hibernation. They can also contract a new infection when foraging before going into hibernation.

Towards the end of the colony cycle, sexual offspring are produced – drones (males) and daughter queens (females, the reproductive daughters) which leave the colony and mate. Now, the colony's social fabric decays, no new worker brood is produced, and the colony's queen and her remaining workers perish. The males also die before the onset of winter, either having successfully mated or having unsuccessfully searched for females. Therefore, only the mated daughters enter hibernation (or other forms of a seasonal diapause), typically remaining buried in the soil. They emerge the next season as queens that attempt to found their own colonies (Goulson, 2003a).

Bumblebees have a number of parasites, such as viruses (McMahon et al., 2015; Manley et al., 2017), microsporidia (Li et al., 2012), neogregarines (Maharramov et al., 2013), and trypanosomes (Schmid-Hempel, 2001). Here, we illustrate the interaction between the host *Bombus terrestris* L. and its infectious gut parasite *Crithidia bombi* Lipa & Triggiani 1988 (Trypanosomatidae) (cf. Figure 1.1), which has been studied since 1985 and has effectively become a model system of host–parasite evolutionary ecology. The biology of two newly discovered species, *C. expoeki* (Schmid-Hempel & Tognazzo, 2010) and *C. mexicana* (Gallot-Lavallée et al., 2016), as well as other host *Bombus* species that are infected worldwide by these trypanosomes, are likely to be similar.

Taxonomically, *Crithidia* is a poorly defined paraphyletic genus. The taxa of *Crithidia* infecting bumblebees are closely related to *Leishmania* and *Leptomonas* (Schmid-Hempel & Tognazzo, 2010; Flegontov et al., 2015; Ravoet et al., 2015; Schwarz et al., 2015; Ishemgulova et al., 2017). The bumblebee-infecting *Crithidia* have no known vector or intermediate hosts; they are therefore classified as monoxenous (having only one host) (cf. Figure 1.2).

The life cycle of the parasite within the host starts with a primary infection of a host individual *per os* (i.e. the ingestion of infective cells via the mouth). The parasite cells pass through the digestive tract and eventually accumulate in the hind gut (the rectum). There, the cells attach to the gut wall, absorb amino acids from the gut lumen (Schaub, 1992), divide and eventually multiply to high numbers with infection intensities in the millions of cells. No evidence exists that *Crithidia* ever crosses the gut wall and spreads inside the host's body. As the parasite multiplies and the parasite population grows, infective cells are shed via the host's faeces as soon as 3–4 days post-infection. Peak intensity and peak shedding is typically reached around 10–12 days, where intensities often start to decline (Schmid-Hempel & Schmid-Hempel, 1993). Transmission from colony to colony is via flower visits (Durrer & Schmid-Hempel, 1994; Graystock et al., 2015) (Figure 1.2). Infected workers transfer the parasite's cells to either flower nectar or, more likely, the flower surface (Cisarovsky & Schmid-Hempel, 2014a), such that a bee visiting the same flower subsequently can become infected. The likelihood of transmission depends on the architecture of the inflorescence (complex ones lead to less transmission) and the bee species involved (Durrer & Schmid-Hempel, 1994; McArt et al., 2014). Within the nest, transmission occurs via contaminated surfaces or, for instance, via the nectar stores or infected larvae (Folly et al., 2017). In social insects with overlapping generations, such as bumblebees, vertical transmission between generations is equivalent to direct transmission within the nest (Imhoof & Schmid-Hempel, 1999), because bee-infecting *Crithidia* cannot be transmitted via eggs, in contrast to those in solitary insects (Dias et al., 2014). Bee-infecting *Crithidia* also have no durable stages that can persist outside their living host for the hibernation period

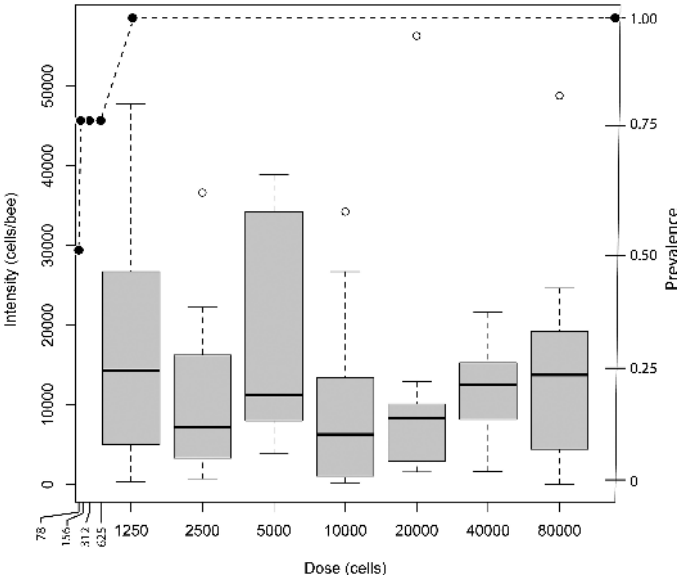

Figure 1.3 Dose–response curve. Box plots are shown of the resulting infection intensities (parasite cells per bee) when the inoculum (dose) varies from 78 to 80,000 cells (a logarithmic scale). The broken line is prevalence of infection (percentage infected individuals) as a function of dose. Each dose was applied to $n = 8$ bees. Data for doses below 1250 cells from Schmid-Hempel et al. (1999).

(Schmid-Hempel et al., 1999). Hence, during the winter months all infections are contained in the overwintering bumblebee queens.

From a conceptual stance, the infective dose, i.e. how many parasite cells are needed to start an infection, has recently been linked to the absence or presence of cooperation among infecting cells; without cooperation between the infecting cells, each cell can infect individually, with a low infectious dose being sufficient for infection, whereas cooperating cells necessitate a high infectious dose (Schmid-Hempel & Frank, 2007; Leggett et al., 2012). For cocktails of mixed *C. bombi* genotypes (which we also call 'strains'), experimental infections suggested that already with doses of around 1000 cells, infection is certain (i.e. prevalence – the fraction of hosts infected – among test bees is 100%) (Figure 1.3). However, doses as low as a few dozen cells seem sufficient to infect at least half of the exposed hosts (Schmid-Hempel et al., 1999); yet, regardless of dose, the resulting infection intensity (parasite cells/bee) also varies among colonies (Yourth, 2004). Unfortunately, little is known about quantitative differences in infective doses among different genotypes of the parasite, but it is clear that some strains do not infect well even at very high doses. Therefore, following the theoretical considerations outlined above, *C. bombi* does not seem to use cooperation tactics when infecting a host.

Likely, factors selecting against such cooperation are the suspected very low number of cells that can be transmitted via flowers (Durrer & Schmid-Hempel, 1994) and the small chance to encounter a suitable host genotype in the first place, as discussed below.

By definition, parasites gain fitness at the expense of their host's survival and reproductive success ('virulence' in the widest sense). Obviously, these effects vary with host condition, parasite type, and environmental conditions, among others. More generally, there are long-standing debates, for example, about how much virulence is necessary for host populations to evolve and maintain sex and recombination in defence (Hamilton et al., 1990). Against this background, *C. bombi* infections in workers of *B. terrestris* – disappointingly at first sight – cause only benign effects. Yet, effects appear when workers are stressed – for example, by food deprivation, where mortality increases 1.5-fold (Brown et al., 2000). Even more interestingly, infection reduces the ovary size of workers, likely by the sequestering of amino acids from the host's gut (Schaub, 1992). In addition, reproduction in the colony as a whole is delayed (Shykoff & Schmid-Hempel, 1991). Partial castration matters in particular when, instead of workers, spring queens (i.e. the emerging overwintered daughters that now start their own colony) become infected. Infected queens have poor chances to establish a successful colony, and lose roughly half of their average fitness (Brown et al., 2003b). This amounts to a considerable virulence effect, albeit only during a defined period of the host life cycle. However, contrary to the now 'classical theory' of virulence evolution (Frank & Schmid-Hempel, 2008), infection intensity, i.e. the number of potential propagules in the host, seems not to relate to survival (a virulence component) (Brown et al., 2000; Yourth, 2004). This raises the question whether bees have evolved the capacity to reduce the effects of infection – termed 'tolerance' (Råberg et al., 2007) – rather than preventing an infection in the first place ('resistance'). If anything, this would complement the underlying genotypic variation in 'resistance' as discussed below.

Beyond the immediately visible virulence effects, such as castration or increased mortality, *Crithidia* infection (as well as infestation by parasitic flies: Schmid-Hempel & Stauffer, 1998; Gillespie et al., 2015) also subtly changes the foraging behaviour of bumblebee workers. For example, steadiness – the tendency to visit the same species of flowering plant – decreases (Otterstatter et al., 2005; Gegear et al., 2006; Gillespie & Adler, 2013), with obvious consequences for pollination efficiency (Richardson et al., 2016) and pathogen dispersal in the environment. Infection does not seem to change behaviour within the nest though, nor do nest mates recognise infected workers (P. Schmid-Hempel, M. Brown & P. Korner, unpublished data), although the amount (but not the composition) of secretion from the Dufour's gland, which

is used in nest mate recognition, is significantly increased in experimentally infected workers (P. Schmid-Hempel, D. Morgan, & G. Jones, unpublished data).

1.3 Parasite prevalence in space and time

The patterns of how host–parasite interactions unfold in space and time shape the coevolution of, generally speaking, host defence and parasite virulence, as modelled for example in Gandon and Michalakis (2002) and Howard and Lively (2002) (see also Chapter 2). Hence, an important piece of natural history is to know how common parasites actually are in space and time. As far as *Crithidia* is concerned, infections are widespread in field populations but vary in prevalence (i.e. the fraction of infected bees) among localities, years, and host species. This situation is illustrated by a study in the Swiss Jura mountains (Durrer & Schmid-Hempel, 1995; Durrer, 1996). In this region, local communities of bumblebee species were surveyed in detail at 12 localities for two consecutive years. On average, 11.7% of all bees (n = 3481 bees checked) were infected with *Crithidia*, but prevalence varied on a rather small spatial scale and from one year to the next. Prevalence remained different among host species in both years, yet showed no consistent patterns (Figure 1.4). The *B. terrestris*-complex (*B. terrestris, B. lucorum*, and potentially two other cryptic species; Williams et al., 2012) may represent an exception, as these species show consistently elevated infection levels. In fact, *B. terrestris* is quite an opportunistic species, i.e. it feeds on a wide range of plant species and can be highly invasive (Schmid-Hempel et al., 2013). Host species might therefore simply accumulate infections depending on their ecology. In fact, the patterns of flower visitations that dominate a particular ecological community affects the genetic fabric of *Crithidia* genotypes present across the different host species (Salathé & Schmid-Hempel, 2011; Ruiz-Gonzalez et al., 2012). Together with the observation that infection is transmitted via flowers (Durrer & Schmid-Hempel, 1994; Graystock et al., 2015), niche overlap in flower usage among different host species seems to be a driver of the spread of *Crithidia* within bumblebee communities and ecological guilds – a pattern that may be of general relevance in pollinator populations (Fürst et al., 2014).

Similarly, taking spring queens of the most common host, *B. terrestris*, as an indicator shows that over a period of 15 years, infection prevalence is around 8–9%. The two sites differ somewhat and fluctuations between years occur (Figure 1.5). Moreover, prevalence is somewhat lower among spring queens than among the workers sampled in summer (cf. Figure 1.4). Indeed, also in experimental tests, queens are generally more refractory to becoming infected by *C. bombi* than either workers or males. As the season progresses, infections that were carried through the hibernation period by the spring queens rapidly spread in the local population (Imhoof & Schmid-Hempel, 1999). No infection

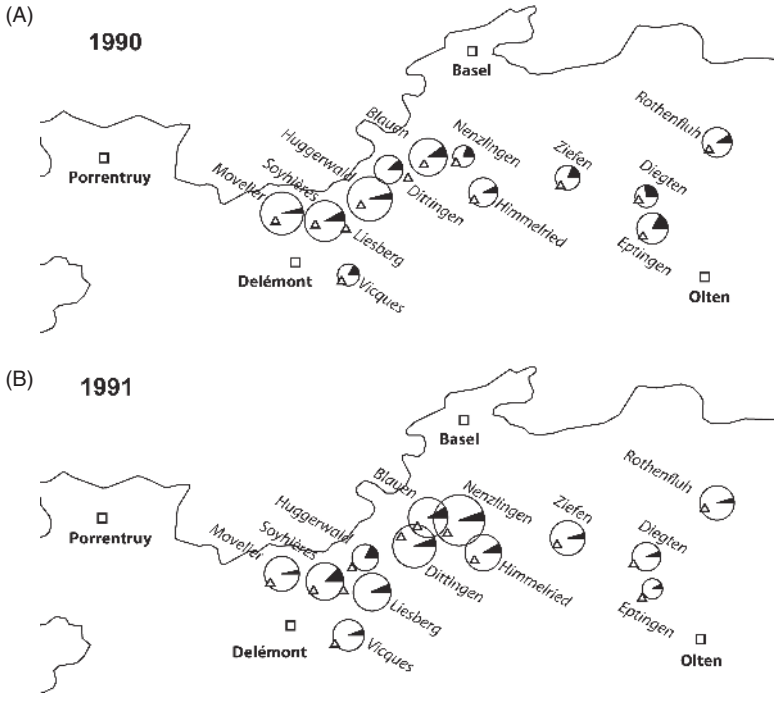

Figure 1.4 Prevalence of *C. bombi* infections in 12 study populations and in all host species. (A) Prevalence in 1990; $n = 2000$ bees checked (15 species). (B) Prevalence in 1991; $n = 1481$ bees checked (13 species). There is no correlation of prevalence in a site between the two years ($r = -0.11$, $p = 0.74$, $n = 12$ sites), but infection prevalence varies among host species in both years (for 1990: $^2 = 89.8$, $df = 14$, $p < 0.0001$; for 1991: $^2 = 230.8$ $df = 11$, $p < 0.0001$). Size of circles proportional to sample size; prevalence is black sector. Based on Durrer (1996).

was discovered in 2011 and 2013 at site Neunforn. Either the infection must have been at a very low level, and/or it was introduced by immigration of queens into this area somewhat later as the season progressed. The first possibility can be assessed by checking the likelihood that an infected queen was missed; in this case, with sample sizes around 150–200 bees, the population prevalence could be only a few percent with any confidence. On the other hand, the study of an ongoing invasion event, where *B. terrestris* was introduced in South America and keeps spreading by some 200 km per year, suggests that queens habitually disperse widely from their natal site every year (Schmid-Hempel et al., 2013). This is also suggested by occasional observations of long-distance migration of bumblebee queens in spring (Mikkola, 1984). Furthermore, the genetic structure of populations of *B. terrestris* in Central Europe suggests panmixis, with only some offshore islands being more isolated (Estoup et al., 1996; Widmer et al., 1998; Widmer & Schmid-Hempel, 1999).