Disentangling the heterogeneity of *Crithidia bombi* infections in bumblebee populations

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Chapter 1

General Introduction

Ecological Interactions

Interspecific interactions between individuals or populations can be either direct or indirect (at least a third species is involved; e.g. trophic cascades) and shape ecological communities. Interaction outcomes are classified by the net effect of the relationship on each partner (positive, neutral, or negative; Tab. 1.1) and are spatiotemporally dynamic owing to the variation of local biotic or abiotic conditions within the specific ecosystem (Schaefer 2003, Townsend et al. 2003, Holland & DeAngelis 2009, Moon et al. 2010). Continuous transitions and oscillations back and forth between different categories of species interactions are illustrated with the help of the interaction compass (reviewed in Holland & DeAngelis 2009; Fig. 1.1).

Table 1.1. Standard categories of direct effects denote the net effect of one species on the other species (Moon et al. 2010).

Interaction	Species A	Species B
Mutualism	+	+
Commensalism	+	0
Predation	+	-
Herbivory	+	-
Parasitism	+	-
Amensalism	0	-
Competition	-	-
Neutralism	0	0

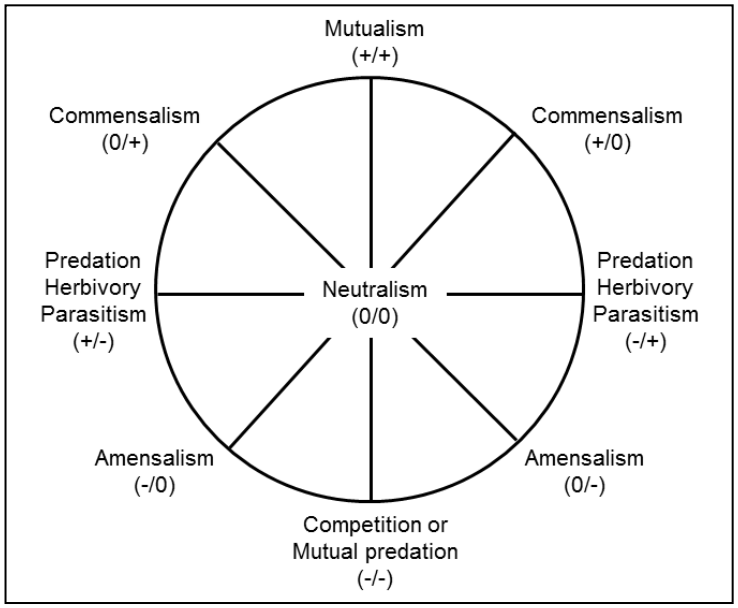

Figure 1.1. The interaction compass illustrates how changes in the sign of one or both interacting species reflect a continuum of transitions among the standard forms of interspecific interactions. The first sign represents the effect of species A on species B and the second sign vice versa (modified after Holland & DeAngelis 2009).

If both partners benefit from the relationship the interaction is termed mutualism. Plant-pollinator interactions are such a characteristic mutualism as for example bees receive nutrition (pollen and nectar) whereas the pollen is transferred from the anther to the stigma, thus facilitating pollination and reproduction of the flowering plants. In contrast, two organisms are

negatively affected if they compete for the same resources (e.g. food, space) (Schaefer 2003, Moon et al. 2010). Competition often determines the realized niche of a specific organism compared to its fundamental niche (i.e. an array of resources / habitats which could be used under ideal conditions) (Schaefer 2003, Townsend et al. 2003). Another example of antagonistic interactions is the relationship between hosts and parasites (cf. *Host-Parasite Interactions*) which represents the main subject of this thesis.

Ecological interactions induce the adaptation of life-history traits of the involved species, ideally enabling the coexistence of different species despite similar requirements (Schaefer 2003, Stuart et al. 2014). Hoehn et al. (2008) identified several functional guilds in a bee-pumpkin system as bees markedly differed regarding three flower visitation traits (preferred flower height, time of visitation, within-flower behaviour). The presence of competing species may also cause a shift of flower preferences (Fründ et al. 2013). In case of permanent reciprocal adaptation of two species or populations, coevolution occurs (Schaefer 2003, Zhang et al. 2012).

Host-Parasite Interactions

Parasites are thought to play a pivotal role in fostering biological diversity (Ebert & Hamilton 1996, Morgan et al. 2009). They are a driving force in the regulation of host population growth rate (Anderson & May 1978) and in structuring host communities as they influence the ecological competition between two host species (Schmid-Hempel 2001; e.g. parasite-mediated coexistence of susceptible and resistant species / genotypes, Morgan et al. 2009). In addition, parasites can be seen as a major trigger for the maintenance of sexual reproduction (Ladle 1992, Decaestecker et al. 2007) and potentially increase host genetic diversity in the long-term (Decaestecker et al. 2013), although theoretical evidence suggests various outcomes of coevolutionary dynamics (Woolhouse et al. 2002).

Parasites physiologically depend on another, larger organism (i.e. the host). Parasite species have a higher reproductive potential compared to their hosts and over the course of the infection their distribution within the host population tends to be overdispersed which causes a reduction of host fertility and / or lifespan. Consequently, heavily infected hosts are eventually killed by the parasite (Crofton 1971, Anderson & May 1978, Ebert & Hamilton 1996).

Virulence is a sophisticated trait (Ebert & Bull 2008) often regarded as an inevitable consequence of host exploitation (Gandon et al. 2001), and is broadly defined as parasite-mediated morbidity and mortality (Ebert & Bull 2008), ranging from avirulent (asymptomatic) to highly virulent (rapidly killing) levels (Myers & Rothman 1995, Ebert & Bull 2008). It may be related to either increased or decreased parasite fitness (Bull 1994, Nowak & May 1994,

Ebert 1994, Frank 1996), or might even be irrelevant to the pathogen at all (Bull 1994). Nonetheless, intermediate levels of virulence (i.e. the trade-off virulence model) may be preferred as they ensure the maximal lifetime transmission success of the parasite due to a balance of the parasite's transmission stage production and host exploitation (Frank 1996, Ebert & Bull 2008).

To complete their life cycle successfully, parasites need to be competent regarding host finding, infection (multiplication and reproduction; Wenk & Renz 2003) and finally transmission to the next host (Anderson & May 1979, Schmid-Hempel 1998). The latter is vital to the dynamics and evolution of host-disease relationships (Myers & Rothman 1995). Transmission occurs either directly (contact between hosts, inhalation of transmission stages, ingestion, skin penetration) or indirectly via one or several intermediate hosts (biting by vectors, e.g. flies, mosquitos, ticks; penetration by free-living transmission stages, e.g. produced by molluscs; parasite ingestion: predatory or scavenging primary host feeds on the intermediate host) (Anderson & May 1979). In contrast to the diverse procedures of horizontal transmission given above, disease can also be transferred from infected parents to their progeny (i.e. vertical transmission) (Anderson & May 1979, Myers & Rothman 1995). Whereas the former is thought to increase virulence, vertically transmitted diseases are expected to be less virulent to ensure host survival and reproduction (Lipsitch et al. 1996, Pagán et al. 2014), although there are exceptions (e.g. sublethal protozoan parasites and Cypoviruses in insects) (Myers & Rothman 1995).

Despite the great variety of parasitic forms living in or on the host (endo- and ectoparasites, respectively; Schaefer 2003), two classes are differentiated (Anderson & May 1979). Microparasites (viruses, bacteria and other prokaryotes, fungi, protozoa) have short generation times, tremendously high rates of reproduction within the host (to raise density and hence increase the likelihood of transmission), and tend to elicit immunity to reinfection in (vertebrate) hosts surviving the initial attack. Compared to the life expectancy of the host, microparasitic infections are of a transient nature (except e.g. the slow viruses) (Anderson & May 1979, Schmid-Hempel 1998). By contrast, macroparasites (nematodes, helminths, arthropods) lean towards considerably longer generation times than microparasites and rely on individual growth and viability within the host. Instead of direct multiplication within the host, a vast number of progeny is produced and released to infect new hosts. The induced immune responses usually depend on the number of parasites in a given host, and are likely to be impersistent. Consequently, infections by macroparasites are persistent, with hosts being repeatedly reinfected (Anderson & May 1979, Schmid-Hempel 1998).

Asexually reproducing individuals can spread their genes with half the costs compared to sexual conspecifics. However, host-parasite relationships are supposed to represent an adequate evolutionary power to compensate the inefficiency of sexual reproduction (Ladle 1992). The theory that sex is advantageous if rapidly evolving parasites are present (Jaenike 1978, Hamilton 1980) is known as the Red Queen hypothesis (RQH) (Van Valen 1973, Ebert & Hamilton 1996). Host-parasite interactions are characterized by antagonistic coevolutionary arms races with reciprocal selection (negative frequency-dependent selection) as the underlying mechanism (Ladle 1992). Strong selective pressure is mainly promoted by a parasite-induced decrease of host fitness (cf. virulence; Woolhouse et al. 2002). Parasites adapt to the most common host resistance genotypes to optimise host exploitation, whereas hosts continuously evolve to minimise fitness loss (Jaenike 1978, Hamilton 1980, Ladle 1992). Sexually produced host offspring usually possess new and rare genotypes, which is beneficial, as the resistance to coadapted parasites is enhanced (Ladle 1992, Ebert & Hamilton 1996). Increased numbers of different genotypes in a host population involve a smaller frequency of each and therefore a reduced probability that a parasite will encounter the identical genotype in consecutive hosts (Ebert & Hamilton 1996). Straight empirical evidence for Red Queen dynamics is hard to gain (Salathé et al. 2008) but was found for freshwater snails (Lively & Jokela 2002, Jokela et al. 2009), flour beetles (Fischer & Schmid-Hempel 2005), and water fleas (Decaestecker et al. 2007) and their respective coevolving parasites. Alternatively, Kidner & Moritz (2013) recently provided theoretical evidence that the RQH is not applicable to haplodiploid hosts (cf. *The specific system […]*), suggesting other explanations for the high recombination rates in Hymenoptera.

Under natural conditions host populations are faced with the omnipresence of various pathogens and parasites (Hart 2011) – either different species and / or different genotypes / strains of the same species (Schmid-Hempel 1998, Read & Taylor 2001, Woolhouse et al. 2002). Therefore hosts developed a great variety of additional defence strategies (Schmid-Hempel 1998, Wenk & Renz 2003, Hart 2011, Gray et al. 2012). Avoidance represents the most eminent and widespread behavioural disease control mechanism performed by animals. It involves, among others, grooming to eliminate ectoparasites or specific grazing strategies (avoidance of faeces) to reduce the exposure to infectious stages of intestinal parasites (Hart 2011). Herbal medicine may also be used – e.g. prophylactic self-medication in primates, Huffman 2011; increased resin foraging in honeybees after chalkbrood-challenge, Simone-Finstrom & Spivak 2012; altered food plant choice for oviposition in monarch butterflies, Lefèvre et al. 2012 – or controlled contact to pathogens to promote immunological competence (Konrad et al. 2012) (Hart 2011). Regarding social insects, collective immune defences (‘social immunity’) against parasites are derived from

collaboration of individual group members. These colony defence mechanisms operate both prophylacticly and activatedly when required and comprise behavioural, physiological and organisational modulation of the colony (Cremer et al. 2007).

Since most of the parasites are able to infect more than one host species (Cleaveland et al. 2001, Taylor et al. 2001) they appear to be rather generalists than specialists (Woolhouse et al. 2001, Rigaud et al. 2010). In addition to genotypic interactions between host and parasite (G_H x G_P), various abiotic and biotic components of the environment (E) potentially influence the expression of host and parasite traits (G_H x E / G_P x E / G_H x G_P x E interactions; Wolinska & King 2009). Rapid environmental changes (e.g. daily variation in temperature) may favour generalist strategies (phenotypic plasticity) over local adaptation (Via & Conner 1995, Vale et al. 2008a).

Diversity-Disease Relationship

In the face of a changeable environment, biodiversity is indispensable for the preservation of ecosystem functioning and ecosystem services (Naeem & Li 1997, Loreau et al. 2001). Biological diversity occurs at various organisational levels of biological systems from individuals (e.g. MHC loci in vertebrates, Wenk & Renz 2003) to populations to communities (Schaefer 2003). At the community level it buffers against the decline or extinction of single species (i.e. the insurance hypothesis, Yachi & Loreau 1999) as increased species richness per functional group (Naeem & Li 1997) safeguards the resilience of ecosystems, including the phenological synchrony of interacting species (Bartomeus et al. 2013). One of the underlying mechanisms is ‘response diversity’ (Elmqvist et al. 2003) of functionally redundant species.

Accordingly, global loss of biodiversity (Cardinale et al. 2012) and emerging infectious diseases (EIDs) (Jones et al. 2008) seriously threaten human and wildlife welfare (e.g. Binder et al. 1999, Siddle et al. 2007, Fürst et al. 2014). The diversity-disease relationship has recently gained increasing attention (Johnson & Thieltges 2010, Haas et al. 2011) as growing evidence infers close links between the two (Daszak et al. 2000, Johnson et al. 2008, Pongsiri et al. 2009, Keesing et al. 2010).

The net effect of increased biological diversity is thought to diminish specific disease risk in ecological communities, which is termed dilution effect hypothesis. Keesing et al. (2006) elucidate a simple susceptible-infected (SI) model containing five mechanisms (Fig. 1.2) through which changes in species richness potentially affect infection risk. Due to the challenge to unravel these mechanisms from field data alone, observed correlations between

species richness and disease risk may also be the result of several, jointly interacting proceedings (Johnson & Thieltges 2010).

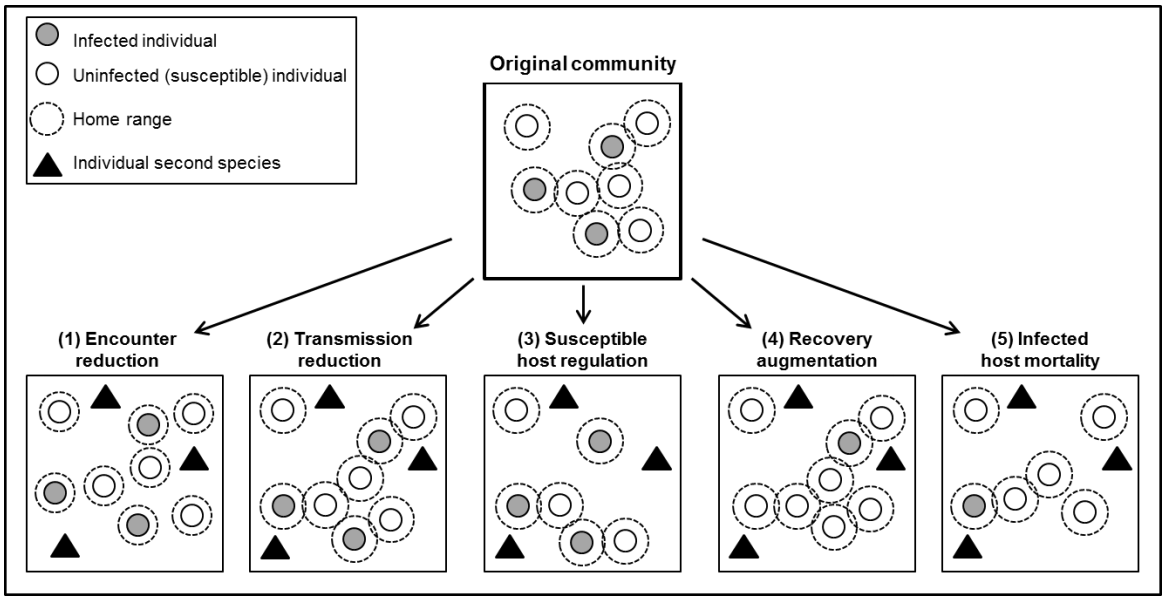

Figure 1.2. Conceptual model of the underlying mechanisms of the ***dilution effect*** in a specialist host-microparasite system (non-vector-borne transmission). The original community comprises a single species with infected and uninfected – and therefore susceptible – individuals, each with a specific home range. If a second species is added, five mechanisms are conceivable: **(1) Encounter reduction =** a reduction of space use by the host, hence reducing encounters between susceptible and infected individuals; **(2) Transmission reduction =** decreased probability of transmission given encounters, indicated here by no rise in the number of infected individuals despite contacts potentially leading to transmission; **(3) Susceptible host regulation** = decline in susceptible hosts; **(4) Recovery augmentation** = increased recovery rates of infected individuals, illustrated by some infected individuals becoming uninfected; or **(5) infected host mortality** = increased mortality rate of infected individuals is conceivable (modified after Keesing et al. 2006).

In contrast, increased diversity may also facilitate disease risk (i.e. the amplification effect). To reveal the conditions that either dilute or amplify the risk of infectious diseases in case of enhanced host species diversity, the dynamics of more complex systems (including vector-borne and multi-host single-pathogen systems) were analysed theoretically and empirically (Dobson 2004, Keesing et al. 2006). Finally, diversity-disease relationships proved to be multifaceted and scale dependent (Wood & Lafferty 2013).

Transmission is a complex feature of host physiology, immunity, behavior, and ecology and for multi-host parasites both within-species and between-species transmission have to be considered (Dobson 2004). Host species vary in quality, hence in their value to generalist parasites (Johnson et al. 2008, 2013b) and possess asymmetric inter- and intraspecific transmission potential (Ruiz-González et al. 2012). As a result, species identity and host community composition are key to conceive diversity-disease relations (LoGiudice et al. 2008,

Roche & Guégan 2011, Salkeld et al. 2013, Streicker et al. 2013) and potentially more important than biodiversity per se (Randolph & Dobson 2012).

Threatened Pollinators

Animal-mediated pollination is a key ecosystem service vital to human wellbeing (Klein et al. 2007), with insects playing a pivotal ecological and economical role in the effective pollination of wild plants and crops (Garibaldi et al. 2013). Nearly 90% of the global angiosperms rely on pollination by animals for seed set and sexual reproduction in the long-term (Ollerton et al. 2011). Therefore pollinators represent keystones for biodiversity, ecosystem functioning and human health (Kearns et al. 1998, Eilers et al. 2011, Ollerton et al. 2011, Fründ et al. 2013). Functionally diverse bee- and wild-insect assemblages proved to be particularly important for the maintenance of plant communities (Fründ et al. 2013) and high crop yields (Garibaldi et al. 2013).

Cultivated plants constitute <0.1% of all flowering plant species (Ollerton et al. 2011). Nonetheless, the estimated economic value of insect pollination amounted to €153 billion in 2005, corresponding to 9.5% of the value of global agricultural production used for human food (Gallai et al. 2009). In detail, pollinators enhance the fruit and / or seed set in 70% of the world's leading food crops (Klein et al. 2007), thereby providing most of the essential (micro-) nutrients (lipids, vitamin A, C and E, calcium, fluoride, folic acid, iron) compared to wind- or predominantly self-pollinated stable crops (reviewed in Eilers et al. 2011).

During the past decades evidence has accumulated suggesting a global decline of pollinators (Kearns et al. 1998), Potts et al. 2010), most notably in managed honeybees (vanEngelsdorp *et al.* 2010) and wild bees (Biesmeijer et al. 2006). In addition to habitat loss and fragmentation, which have been identified as key factors driving bee declines (Brown & Paxton 2009), decreased floral resources (Biesmeijer et al. 2006), pesticides (Whitehorn et al. 2012), climate change (Memmott *et al.* 2007), alien species (Thomson 2006), and emerging infectious diseases (EIDs) (Meeus et al. 2011, Fürst et al. 2014), are thought to threaten managed and wild bees. Recently, there is rising awareness concerning multiple interacting stressors and their potential negative impact on pollinator health, abundance and diversity (Vanbergen et al. 2013). On the other hand, first successes of conservation initiatives have become apparent at least in NW-Europe (Carvalheiro et al. 2013).

Due to enhanced dependency on pollination services in agriculture (Aizen et al. 2008) and increasing evidence of pathogen spillover from managed honey- and bumblebees to wild pollinators (Otterstatter & Thomson 2008, Murray et al. 2013, Fürst et al. 2014,

McMahon et al. 2015), the potential for disease-driven loss of key pollinators – like bumblebees (Goulson 2008a) – is one focus of current research.

The specific system: Bombus spp. and Crithidia bombi

Bumblebees (*Bombus* spp., Hymenoptera, Apidae) are primitively eusocial insects with an annual life cycle (cf. Fig. 1.5) headed by a single mated queen that founds a colony after hibernation in spring (Goulson 2010). As soon as the first batch of workers has hatched they take over foraging and participate in brood care, whereas the queen stays exclusively inside the nest and continues egg laying. Over the course of the season several generations of workers are raised until sexuals are produced (males and gynes – i.e. unmated queens), which will fly out and mate. After mating only the queens enter hibernation to start their own colonies in the following spring whereas the rest of the mother colony perishes (Sladen 1912, Goulson 2010).

Social Hymenoptera (ants, bees, wasps) usually exhibit parthenogenetic arrhenotoky (Crozier & Pamilo 1996), also known as haplodiploidy, with diploid females derived from fertilized and haploid males from unfertilized eggs (Lester & Selander 1979). More precisely, in many hymenopterans (including bumblebees) single locus complementary sex determination (CSD) is the underlying mechanism (Cook 1993), with heterozygous individuals becoming females whereas homozygous or hemizygous individuals develop into males (Paxton et al. 2000, Schmid-Hempel 2000). Consequently, haplodiploidy creates asymmetries in relatedness between colony members and increases relatedness between females under monogyny and monoandry ("hymenopteran full-sistership", Hamilton 1964; Schmid-Hempel 1998, 2000; Fig. 1.3). The high density of closely related commonly interacting individuals within a colony facilitates disease spread. Therefore social insects – like bumblebees – are particularly prone to a plethora of pathogens and parasites (Schmid-Hempel 1998, 2001).

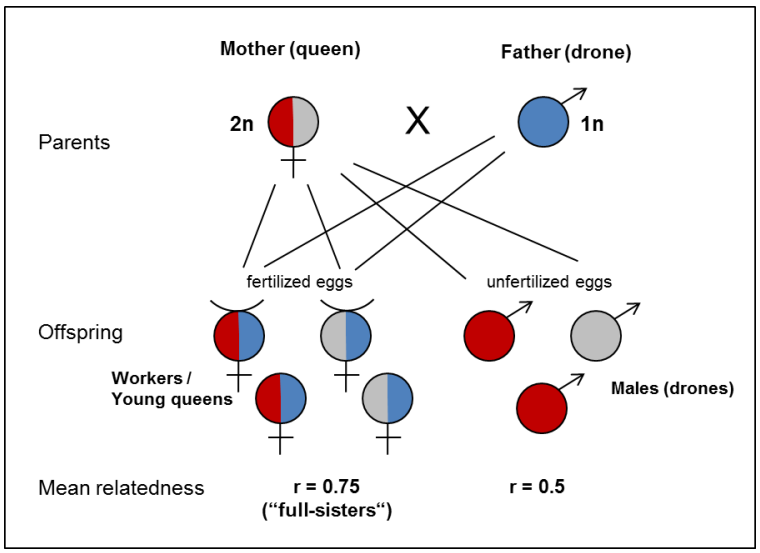

Figure 1.3. Mating system of a social hymenopteran colony with haplodiploid sex determination and one single mated queen (monogyny and monoandry). Diploid females and haploid males contribute asymmetrically to the next generation; different alleles are depicted with distinct colours. The female offspring (workers and young queens) share the same paternal allele whereby their relatedness is increased; r = coefficient of relatedness (modified after Schmid-Hempel 2000).

Endoparasitic trypanosomatids (Kinetoplastea) are uniflagellate protists predominantly found in the mid-gut and rectum of insects (reviewed in Maslov et al. 2013). Some genera are dixenous, which involves – in the case of Trypanosoma and Leishmania – the digestive tract of a bloodsucking insect vector and the blood and tissue of a vertebrate, the secondary host. Several representatives of dixenous parasites are of medical importance, as they cause Chagas disease (*Trypanosoma cruzi*), African trypanosomiasis (*T. brucei*) and leishmaniasis (*Leishmania* spp.) in humans, livestock and domestic animals (Wenk & Renz 2003, Maslov et al. 2013). In contrast, *Crithidia* spp. exclusively parasitizes invertebrates (mainly insects) and are usually regarded as harmless residents of the host's intestine (Schaub 1994, Maslov et al. 2013).

The flagellate parasite *Crithidia bombi* (Trypanosomatidae, Zoomastigophorea) (Gorbunov 1987, Lipa & Triggiani 1988) is widespread in natural bumblebee populations as it infects adults of all castes and sexes (Shykoff & Schmid-Hempel 1991a) of various bumblebee species (Shykoff & Schmid-Hempel 1991a, Kissinger et al. 2011, Cordes et al. 2012). Its cells attach to the wall of the mid- and hind-gut where they propagate and are shed with the host's faeces a few days post infection, ready to infect further individuals (Schmid-Hempel & Schmid-Hempel 1993, Imhoof & Schmid-Hempel 1999). *C. bombi* is a diploid organism (Schmid-Hempel & Reber Funk 2004) with the ability to reproduce clonally and, rarely, sexually (Schmid-Hempel et al. 2011). Successful parasite establishment rises with the dose of cells ingested by the host (Ruiz-González & Brown 2006). Microsatellite analyses revealed the coincident presence of

several *C. bombi* genotypes within populations, colonies and individuals (Schmid-Hempel & Reber Funk 2004, Erler et al. 2012, Popp et al. 2012).

Parasite transmission happens within colonies through contact with contaminated surfaces or infected nestmates (Schmid-Hempel & Schmid-Hempel 1993, Imhoof & Schmid-Hempel 1999, Otterstatter & Thomson 2007) and between colonies (intra- and interspecifically) via shared flowers during foraging (Durrer & Schmid-Hempel 1994; but see Fouks & Lattorff 2011; Fig. 1.4).

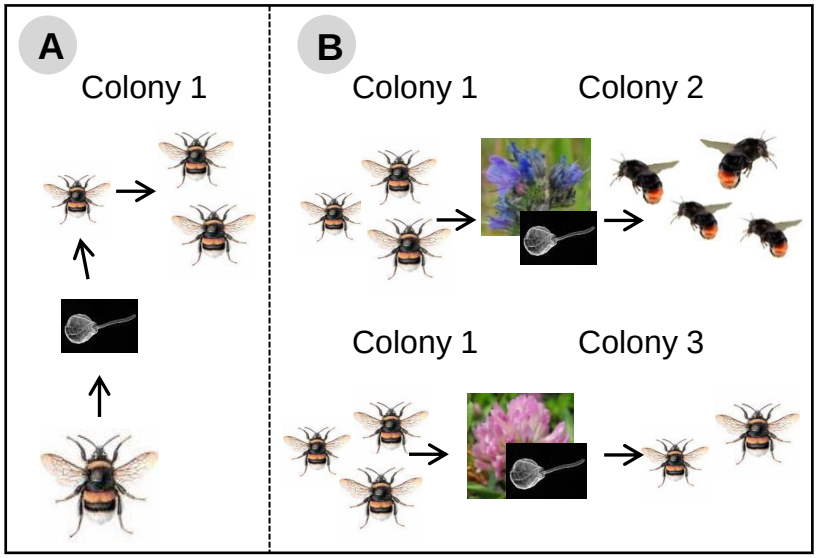

Figure 1.4. Transmission of *Crithidia bombi* (photo by E. Wehrli, EMEZ - Electron Microscopy ETH Zurich) via infectious faeces, **(A)** vertical and horizontal: within colonies **(B)** horizontal: between colonies (above: interspecifically, below: intraspecifically), photos by S. Parsche (*Echium vulgare* and *Trifolium pratense*). Corresponding references are given in the preceeding paragraph.

Under favourable conditions bumblebee colonies are able to cope with infections as *C. bombi* is usually a benign parasite (Brown et al. 2000). Nonetheless, the parasite affects its host as depicted in Fig. 1.5. Additionally, heavily infected individuals suffer from impaired learning and foraging competence (Otterstatter et al. 2005, Gegear et al. 2005, 2006).

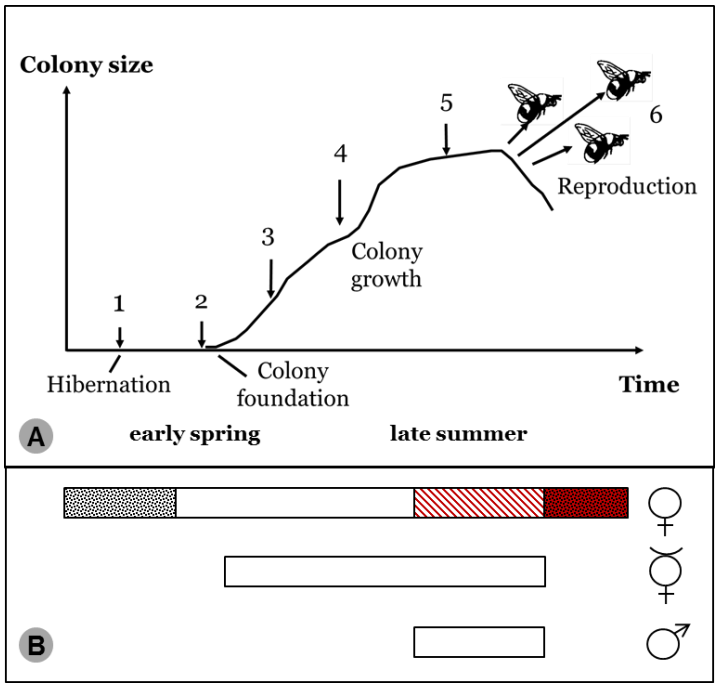

Figure 1.5. Life cycle of *Bombus* spp. and **(A)** impact of the intestinal parasite *Crithidia bombi* (Trypanosomatidae). **(1)** Infection with *C. bombi* considerably decreases the fitness of hibernating queens combined with **(2)** negative consequences for colony founding (Brown *et al.* 2003) and **(3)** colony size (Shykoff & Schmid-Hempel 1991b, Brown et al. 2003). **(4)** Worker mortality increased by 50% under food shortage (Brown et al. 2000), **(5)** the production of sexuals is delayed or reduced (Shykoff & Schmid-Hempel 1991b, Yourth et al. 2008) and **(6)** infections possibly affect the build-up of the queen's fat body for hibernation (Schmid-Hempel 2001) closing the circle to **(1)**. **(B)** Temporal occurrence of 'old' (white) and 'young' (red) queens, workers and males (top down). Dotted sections denote hibernation (modified after Schmid-Hempel 2001).

The *Bombus-Crithidia* system is a well-established, excellent model system which has proven to be equally convenient for both field and laboratory studies including experimental manipulation (Schmid-Hempel 2001). Compared with *Apis* spp. (Moritz et al. 1995), bumblebees are largely monoandrous (Schmid-Hempel & Schmid-Hempel 2000). As a consequence, kinship reconstruction via molecular markers is straightforward, thus enabling reliable information on host density and diversity as well as on the related infection status at the colony level in longstanding natural populations. This thesis mainly focuses on three abundant bumblebee species (*B. terrestris*, *B. lapidarius*, *B. pascuorum*; Fig. 1.6) because rare species often already suffer from fitness loss (inbreeding depression) and may be more susceptible to parasites (Whitehorn et al. 2011, 2014). Furthermore, obtained general patterns of transmission are possibly transferable to rare species but not vice versa. In case of *C. bombi*, more reliable prevalences can be gathered within a real world setting compared to *Nosema bombi* as the latter cause reduced activity in workers (reviewed in Shykoff & Schmid-Hempel 1991a) inducing a male-biased sample (Murray et al. 2013).

Figure 1.6. Three common bumblebee species (from left to right: *B. terrestris*, *B. lapidarius*, *B. pascuorum*; photos by S. Parsche) within the range of the multi-host parasite *C. bombi*.

Research objectives

This thesis deals with the manifold aspects of complex multi-host parasite interactions in order to unravel the individual contribution of various genetic, density-dependent and environmental effects. Therefore, replicated sampling of natural populations was conducted to answer the following principal questions of interest:

1) What are the interannual dynamics in the *Bombus*-*Crithidia bombi* system in terms of the predictability of disease outcome and the relationship between parasite diversity and prevalence? (Chapter 2)
2) How is the link between the spatial heterogeneity of floral resources and prevalence characterised? (Chapter 2)
3) Are there key factors (e.g. species identity) that contribute to *C. bombi* epidemiology? (Chapter 3)
4) Do host colony density and genetic diversity play a role in shaping the *C. bombi* infection landscape? (Chapter 3)
5) Do host species richness and diversity have a diluting or an amplifying effect with respect to *C. bombi* infections? (Chapter 4)
6) How important is the host community composition with respect to prevalences? (Chapter 4)

Chapter outline

Chapter 2 focuses on spatiotemporal disease dynamics. The genetic diversity of *C. bombi* served as proxy for the parasite's capability to infect different host species. Moreover, the horizontal transmission route via shared use of flowers was inspected to gain insights into the role of the environment.

Chapter 3 concentrates on the identification of potential key factors which have an impact on prevalence, type (single- vs. multiple-strain infection) and the intensity of infection. As a proxy for transmission potential, host colony density and genetic diversity were investigated. The latter also served as proxy for host species quality.

Whereas the preceding Chapter deals with the colony and population level, Chapter 4 focuses on the community level to examine hypotheses about local diversity-disease relationships.

Chapter 2

Environmental variability and its consequences for disease dynamics

Introduction

The setting of natural infection is highly volatile and disease epidemics come and go. Hosts and parasites interact in unpredictable environments, in which the effect of diseases frequently is context dependent (Lazzaro & Little 2008, Thieltges et al. 2008). The environment of the parasites is composed of various factors whereof the host's genotype (G_H) represents one such 'environment' (E) the parasite genotype (G_P) has to cope with (G_H x G_P interactions). Furthermore, manifold abiotic and biotic factors (e.g. temperature, precipitation and food, competitors, respectively; Schaefer 2003) potentially play a role in the expression of host and parasite traits (G_H x E / G_P x E or G_H x G_P x E interactions; Wolinska & King 2009), adding another degree of complexity (Vale et al. 2008a, Sadd 2011). This environmental dependence of the expression of quantitative traits has been accepted for a long time (Falconer 1952) and apparently, G x E interactions are common in natural systems (Lazzaro & Little 2008), modifying epidemiological dynamics across diverse host-parasite systems (Tseng 2006, Wolinska & King 2009).

Organisms are regularly faced with spatiotemporal variance of important environmental factors (Via & Conner 1995, Sadd 2011, Swei et al. 2011). Vale et al. (2011) provided experimental evidence that the severity of parasitism in the *Daphnia magna-Pasteuria ramosa* system is modified by both alternating food availability and temperature. Therefore, environmental variability is crucially important to current populations as it might entail the maintenance of polymorphism in natural populations (Lazzaro & Little 2008, Vale et al. 2008b), which is presumably essential in the evolution of resistance to infection (Lazzaro & Little 2008). In case of rapid environmental changes (e.g. daily variation in temperature), generalist strategies (phenotypic plasticity) may be favoured over local adaptation (Via & Conner 1995, Vale et al. 2008a). However, environmental fluctuations are often excluded as 'noise' from empirical studies and modelling (Altizer 2006, Lazzaro & Little 2008,

Wolinska & King 2009). Furthermore, evidence for environment-dependent interactions is mainly derived from laboratory host-parasite systems (Brown et al. 2000, Vale et al. 2008a, Sadd 2011, Vale et al. 2013) but the existence and role of the 'E' in G_H x G_P x E within natural settings remains less clear (Vale et al. 2008a, Sadd 2011).

One of the key factors in the life-cycle of parasites is the transmission to new hosts which is likely to be density-dependent in directly transmitted pathogens (McCallum et al. 2001). Amongst others, environmentally-mediated host density potentially affects the efficiency of transmission (reviewed in Wolinska & King 2009) typically underlying temporal fluctuations at different scales (daily, seasonal, interannual). Furthermore, the contact network of infected individuals is also closely linked to disease spread (Danon et al. 2011).

To increase the knowledge about environmental impact on host-parasite interactions we study spatiotemporal disease dynamics by sampling natural populations of bumblebees (*Bombus* spp.) and their intestinal parasite *Crithidia bombi* (Gorbunov 1987, Lipa & Triggiani 1988). The trypanosome is widespread in wild bumblebee populations and infects adults of all sexes and castes (Shykoff & Schmid-Hempel 1991a) of numerous species (Shykoff & Schmid-Hempel 1991a, Kissinger et al. 2011, Cordes et al. 2012). Its cells attach to the gut wall where they propagate and are shed with the host's faeces a few days post infection, ready to infect further individuals (Schmid-Hempel & Schmid-Hempel 1993, Imhoof & Schmid-Hempel 1999). *C. bombi* is directly transmitted within host colonies through contact with contaminated surfaces or infected nestmates (Schmid-Hempel & Schmid-Hempel 1993, Otterstatter & Thomson 2007). Owing to the annual life cycle of bumblebees (Sladen 1912, Goulson 2010) and the parasite's inability to survive outside the host for extended periods, parasite transmission between years only happens via hibernating queens (Schmid-Hempel et al. 1999, Ulrich et al. 2011). Transmission between colonies (intra- and interspecifically) takes place via shared flowers (Durrer & Schmid-Hempel 1994; but see Fouks & Lattorff 2011) and bumblebee species differ in terms of foraging preferences (Goulson & Darvill 2004, Goulson et al. 2008b). Thus, the resource overlap (i.e. niche overlap) – and therefore the probability of transmission – varies between host species.

With respect to spatial heterogeneity we focus on the host's food resources because of their potential role in the horizontal transmission of *C. bombi* (Durrer & Schmid-Hempel 1994). Specifically, (i) host species with the largest niche overlap are expected to suffer from higher infection risk, hence increased *C. bombi* prevalence. Furthermore, (ii) low diversity of flowering plants should increase the amount of shared resources which might result in enhanced transmission events and therefore higher prevalences compared to high plant diversity.

Temporal variations in parasite diversity (as proxy for the parasite's capability to infect various host species) were inspected and linked to infection outcomes. In detail, we hypothesised that (iii) low *C. bombi* diversity is associated with lower prevalence due to the reduced capability of the parasite to cope with different host species and to establish successfully in contrast to high parasite diversity.

Material and methods

Sampling

Workers (n = 1,761) and males (n = 401) of three bumblebee species (*Bombus terrestris, B. lapidarius, B. pascuorum*) were sampled during foraging in semi-natural and agricultural habitats, 2010 and 2011, in Germany (Tab. 2.1; cf. Tab. 3.1). Each of the eight locations was collected in a random order three times per year (June, July, August) during sunny weather. Time of day was also randomized to reduce biased data. Individuals were stored at -20°C prior to DNA extraction. After initial species identification in the field, individuals were double-checked for sex and species identity following the taxonomic key of (Mauss 1994). Details on species identification within the *B. terrestris* / *B. lucorum* complex are given in Appendix S3.1.

DNA analysis

CRITHIDIA BOMBI

Each bumblebee's gut was removed and the parasite's DNA was extracted following a modified Chelex protocol (Walsh et al. 1991, Erler & Lattorff 2010). Four polymorphic microsatellite loci were genotyped (Cri 4, Cri 1 B6, Cri 4.G9, and Cri 2.F10; Schmid-Hempel & Reber Funk 2004) using fluorescence labelled primers (Metabion International AG, Martinsried, Germany). All loci were amplified in one multiplex PCR following the protocol of Popp & Lattorff (2011). The final volume of 10 µl contained 1 µl template DNA, 5 µl PCR Master Mix (Promega Corporation, Madison/WI, USA), 0.3 µM (Cri 1 B6, Cri 4.G9), 0.6 µM (Cri 4, Cri 2.F10) per primer pair and 2.2 µl ddH_2O. PCR products were run with an automated DNA capillary sequencer (MegaBACE 1000, GE Healthcare, Munich, Germany) according to manufacturer's instructions and a standard protocol (Erler & Lattorff 2010). Allele sizes were scored using Fragment Profiler v1.2 after visual inspection of the processed raw data. *C. bombi* is a diploid organism (Schmid-Hempel & Reber Funk 2004). Therefore, more than two peaks per locus indicate an infection of the individual host with more than one strain (i.e. multiple infection).

Table 2.1. Sampling overview. Total number of bumblebees caught within three sampling periods of two consecutive years (2010: 15-25 June, 14-22 July, 10-24 August; 2011: 11-15 June, 11-22 July, 10-20 August); cf. Tab. 3.1 for location details. Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*. Individuals infected with *Crithidia bombi* are given in brackets (cf. Tab. S2.2 for prevalences including 95% Confidence Intervals).

Code Location	Longitude	Latitude	2010				2011			
			Te	La	Pas	∑	Te	La	Pas	∑
GW_KB	51°34'45.52"N	9°50'25.03"E	7 (-)	112 (8)	2 (-)	**121 (8)**	5 (1)	137 (25)	81 (18)	**223 (44)**
GE_BT	51°33'48.77"N	10° 0'40.08"E	4 (3)	95 (17)	6 (-)	**105 (20)**	4 (-)	85 (18)	14 (4)	**103 (22)**
Rö_SS	51°28'51.58"N	11°41'00.84"E	62 (20)	55 (26)	13 (-)	**130 (46)**	21 (-)	107 (11)	22 (13)	**150 (24)**
Rö_RÖ	51°27'47.21"N	11°41'53.57"E	87 (25)	45 (8)	1 (-)	**133 (33)**	18 (-)	65 (5)	9 (1)	**92 (6)**
Hal_HS	51°29'29.06"N	11°56'10.99"E	66 (26)	96 (26)	25 (-)	**187 (52)**	7 (1)	82 (2)	26 (0)	**115 (3)**
Hal_HE	51°27'36.70"N	12° 1'32.52"E	26 (13)	26 (5)	-	**52 (18)**	14 (-)	66 (1)	37 (3)	**117 (4)**
Fw_FF	52°21'38.81"N	14° 5'11.36"E	90 (2)	33 (2)	28 (-)	**151 (4)**	40 (2)	20 (3)	46 (1)	**106 (6)**
Fw_LW	52°19'54.98"N	14° 5'35.55"E	88 (12)	78 (4)	3 (-)	**169 (16)**	61 (2)	133 (23)	14 (4)	**208 (29)**
∑			**430 (101)**	**540 (96)**	**78 (-)**	**1,048 (197)**	**170 (6)**	**695 (88)**	**249 (44)**	**1,114 (138)**

Statistical analyses

All analyses – including the corresponding figures – were performed using R 2.15.3 (R Core Team 2013). Spearman's rank-order correlations were conducted with the package Hmisc (v3.13-0, Harrell et al. 2013).

TEMPORAL EFFECTS

Two-sample tests for equality of proportions were used to compare the mean *C. bombi* prevalence per location of two consecutive years with each other. Subsequently, the prevalences of the first year (2010) were divided into a 'low'- and a 'high'-prevalence group (>0.20 and ≤0.20; n = 4 each, respectively; cf. Tab. S2.8). The results of the former test (increase in / equality of / decrease in prevalence) were assigned to the respective group and a Fisher's Exact Test for r *x* c contingency tables, based on 100.000 replicates, was performed.

Population genetic parameters of the parasite – the number of alleles (A_N) and the observed heterozygosity (H_O) – were derived from a sub-sample of single-strain infections (2010: n = 128, 2011: n = 122; Tab. S2.2) using the Excel Microsatellite Toolkit (Park 2001). Local prevalences of this subsample are congruent with the overall sample (single- and multiple-strain infections; cf. Results: Temporal effects) and single infections alone are more conveniently to handle than additional multiple infections (Salathé & Schmid-Hempel 2011). Spearman's rank-order correlations were used to test for associations between each of the parameters and the mean prevalence per location separately for both years. A potential temporal change of A_N and H_O (mean over all four microsatellite loci and separately for the loci Cri 4.G9 and Cri 1.B6) was inspected via Wilcoxon matched pairs tests.

Furthermore, one-tailed Mann-Whitney U Tests of 'low'- vs. 'high'-diversity groups (cf. Tab. S2.2) were used to test if parasite diversity and prevalence are positively related to each other.

INTERACTION NETWORKS

The resource availability (i.e. the amount and species identity of flowering plants in bloom) and the actual resource usage (i.e. the amount and species identity of flowering plants visited per bumblebees species – pollen and nectar collection was not distinguished) were recorded three times per location in 2011. Both measurements were averaged across the season. Interaction networks were created with the R-package bipartite (v2.04, Dormann et al. 2008).

Four different one-way Analyses of Covariance (ANCOVAs; Field et al. 2012) were conducted. The explanatory variable of all ANCOVAs, plant family (the most abundant plant

families visited), comprised four levels: Asteraceae, Boraginaceae, Fabaceae, Lamiaceae. The response variable of the first ANCOVA was the mean prevalence and the covariate was the proportion per plant family. In the remaining ANCOVAs, the species-specific prevalence was the response variable and the corresponding species-specific visitation rate served as covariate.

The association between local plant diversity (resources available and used, both at family- and species level) and the mean prevalence was inspected using Spearman's rank-order correlations. Therefore, the Shannon diversity (hereafter 'obs D'), based on proportional abundance, was calculated per location as

$$\text{obs D} = \sum_{i=1}^{S} -(Pi * lnPi) \qquad (2.2)$$

where P_i is the fraction of the entire population made up of species *i* and S is the number of species encountered. The expected Shannon diversity (exp D) was obtained from a theoretical even distribution per plant family / species in order to determine the largest possible value as an orientation. Finally, a corrected value (corr D) served as proxy for local habitat quality and was calculated as

$$\text{corr D} = \frac{\text{obs D}}{\text{exp D}} \qquad (2.3)$$

and used for subsequent analyses (cf. Tab. S2.5).

At last, the niche overlap (i.e. similarity of resource usage per pair of bumblebee species) was calculated referring to Colwell & Futuyma (1971) and Goulson & Darvill 2004 as

$$1\text{-}0.5 * \textstyle\sum_A * (B_{1A}\text{-}B_{2A}) \qquad (2.4)$$

$$\text{where } B_{1A} = \frac{\text{n Bombus species 1 visiting flowering species A}}{\text{total n Bombus species 1}}$$

Niche overlap was tested vs. species-specific prevalences (Tab. S2.7). The latter were determined using the differences (absolute values) between particular species pairs. Additionally, potential associations between niche overlap and plant diversity were revealed (cf. Tab. S2.7).

Results

Temporal effects

High parasite prevalence in 2010 was linked to decreasing rates in 2011 (n = 4 locations) whereas principally no change between the years was found with respect to the 'low'-prevalence group (n = 3; Tab. S2.8). The observed change in prevalence differed significantly from the expected change (Fisher's Exact Test for r *x* c contingency tables: simulated P = 0.029; Tab. S2.9).

As local prevalences based on a sub-sample of single-strain infections are congruent with the overall sample (single- and multiple-strain infections) (Spearman correlation – 2010: S = 1.52, r_s = 0.98, P<0.0001; 2011: S = 1.02, r_s = 0.99, P<0.0001), population genetic parameters of *C. bombi* are derived from the former. The number of alleles (A_N) was positively related to prevalence (2010: S = 25.65, r_s = 0.69, P = 0.056; 2011: S = 8.55, r_s = 0.90, P = 0.002; Fig. 2.1) whereas no association between the observed heterozygosity (H_O) and the prevalence was found (2010: S = 50.80, r_s = 0.40, P = 0.333; 2011: S = 38.96, r_s = 0.54, P = 0.171).

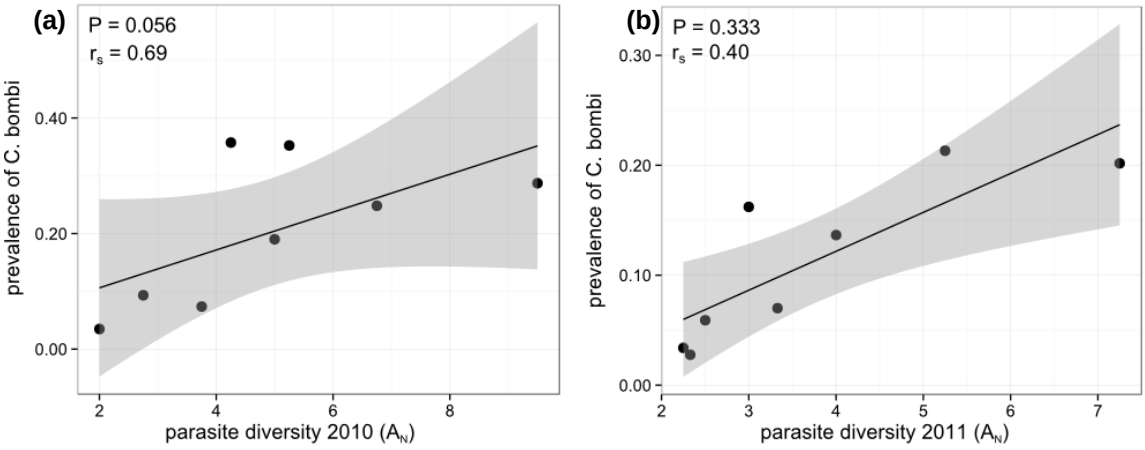

Figure 2.1. Parasite diversity (A_N) in relation to the mean prevalence per location (n = 8); **a)** 2010, **b)** 2011. Line of best fit with associated P-value and 95% CI (dark grey) are derived from Spearman's rank-order correlation. r_s = Spearman's correlation coefficient. Note different scales.

No temporal change of A_N was found (Wilcoxon test: V = 23, P = 0.547). H_O over all four loci was not different between both years (V = 26, P = 0.313). With respect to H_O of the two most informative loci, temporal differences were only marginally significant in the latter case (Cri4G9: V = 28, P = 0.195; Cri1B6: V = 20, P = 0.059).

A positive relationship between parasite diversity and prevalence was found (one-tailed Mann-Whitney U Tests, both years combined: A_N: W = 11, P = 0.015; H_O: W = 15.5, P = 0.046; Fig. 2.2).

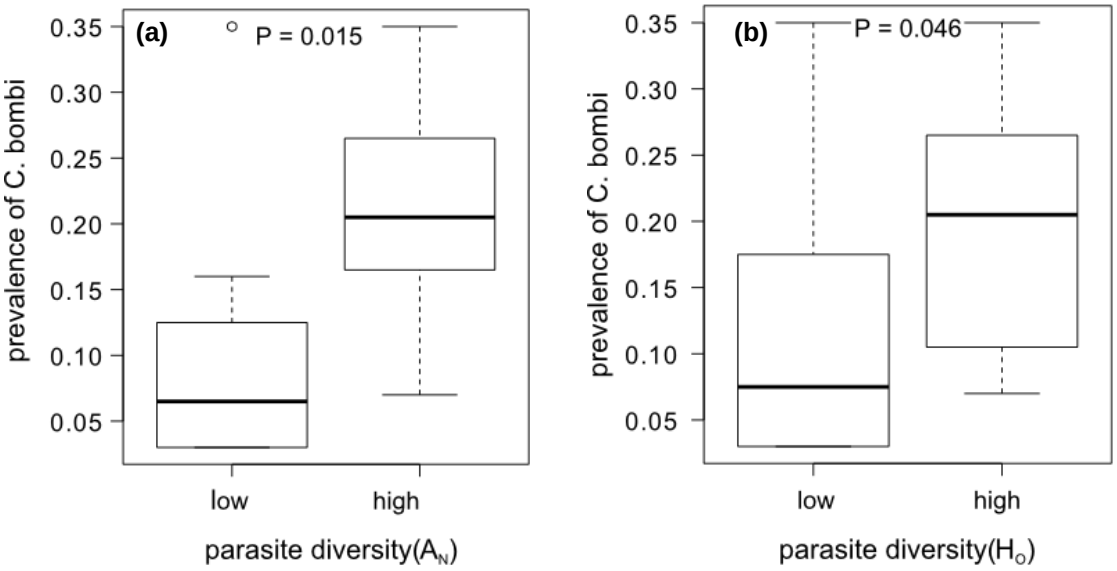

Figure 2.2. Prevalence of *C. bombi* in 'low'- vs. 'high'-diversity groups (n = 8 each); **a)** A_N and **b)** H_O. P-values are derived from one-tailed MWU Tests; data were pooled over two years. Boxplots: line = median, box = interquartile range, whiskers = data range.

Interaction networks

In total, 74 flowering species of 22 different plant families were recorded in 2011 (resource availability: Tab. S2.3). For 38 flowering species (14 families) flower visitation (n = 1,087) by at least one of the three bumblebee species was observed. Overall, *B. lapidarius* (n = 680) foraged at 30 different flowering species and *B. pascuorum* (n = 237) visited 19 species (nine families each). *B. terrestris* (n = 170) foraged at 22 different flowering species (ten families) (resource usage: Fig. 2.3, Fig. 2.4; Fig. S2.1, Tab. S2.4).

Strikingly, one of the flowering species, *Echium vulgare* (cf. Fig. 2.3: 'Ev'; Tab. S2.4) received 12.8% of all visits despite its minor proportion regarding the total amount of inflorescences available and potentially used (0.1%).

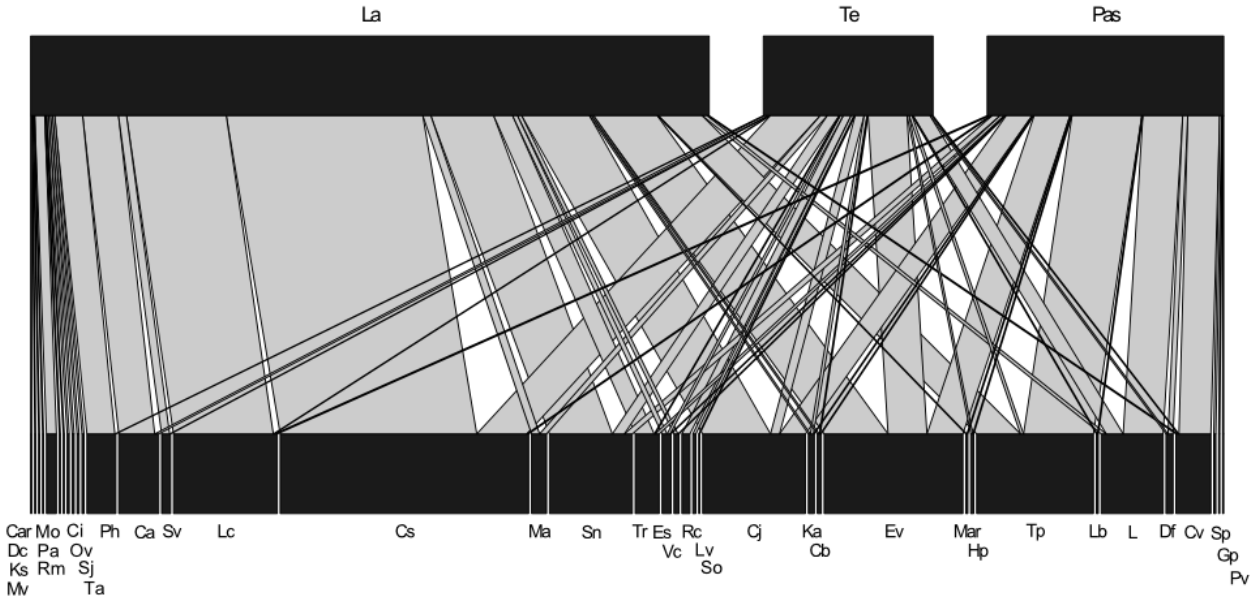

Figure 2.3. Pooled quantitative interaction network of three bumblebee species visiting 38 flowering plant species (letter code; see Tab. S2.4 for species names). La = *B. lapidarius*, Pas = *B. pascuorum*, Te = *B. terrestris*.

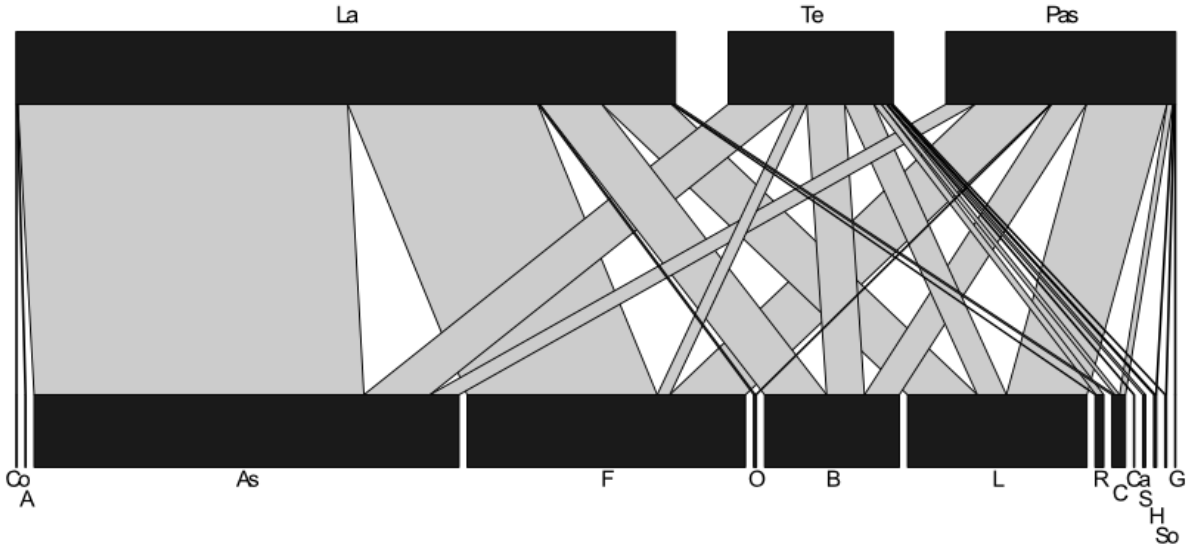

Figure 2.4. Pooled quantitative interaction network of three bumblebee species visiting 14 flowering plant families. A=Apiaceae, As=Asteraceae, B=Boraginaceae, C=Caprifoliaceae, Ca=Caryophyllaceae, Co=Convolvulaceae, F=Fabaceae, G=Geraniaceae, H=Hypericaceae, L=Lamiaceae, O=Orobanchaceae, R=Rosaceae, S=Scrophulariaceae, So=Solanaceae. La=*B. lapidarius*, Pas=*B. pascuorum*, Te=*B. terrestris*.

To reveal the relationship between the four most abundant plant families visited (Asteraceae, Boraginaceae, Fabaceae, Lamiaceae; n = 1,050 visits, i.e. 96.6%) and different levels of prevalence, several one-way ANCOVAs were conducted. Neither plant family nor the respective covariates (proportion per plant family and species-specific visitation rates) played a significant role in explaining local prevalences (Tab. S2.1).

Plant diversity (corr D) and mean prevalence were not associated with each other (Spearman correlation – plant families available: S = 53.82, r_s = 0.36, P = 0.382; plant species available: S = 68.91, r_s = 0.18, P = 0.670; plant families visited: S = 56.84, r_s = 0.32, P = 0.435; plant species visited: S = 46.78, r_s = 0.44, P = 0.272).

Niche overlap and species-specific prevalences were not correlated (Spearman – TeLa: S = 75.90, r_s = 0.096, P = 0.820; TePas: S = 120.00, r_s = -0.429, P = 0.299; LaPas: S = 78.44, r_s = 0.066, P = 0.876).

At first glance, no significant relationship between niche overlap and plant diversity (corr D of available floral resources) at all was found (Tab. S2.6). With respect to the niche overlap of *B. terrestris* and *B. lapidarius* (i.e. TeLa) vs. plant species diversity one location ('FF'; cf. Tab. S2.7) was identified as influential outlier (Bonferroni-P = 0.031). After removing the outlier, this association became significant (S = 8, r_s = 0.86, P = 0.024; Fig. 2.5).

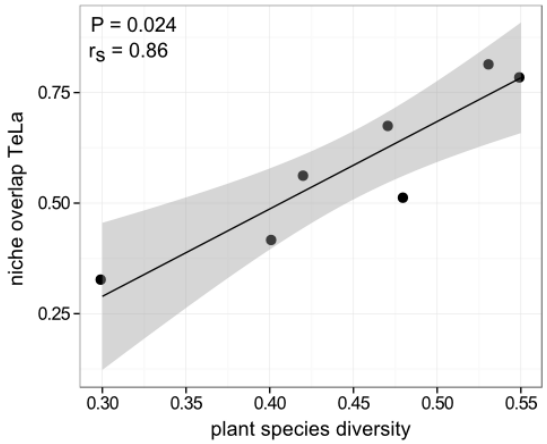

Figure 2.5 Niche overlap of *B. terrestris* and *B. lapidarius* (TeLa) in relation to plant species diversity (corr D of species available) per location (n = 7). Line of best fit with associated P-value and 95% CI (dark grey) are derived from Spearman's rank-order correlation. r_s = Spearman's correlation coefficient.

Discussion

With respect to temporal effects, we find that decreased prevalences in the second year were associated with high parasite prevalence in the first season, whereas usually no interannual change occurred in case of initially low prevalences. In total, the observed change in *C. bombi* prevalence markedly differed from the expected change. Intraspecific parasite diversity is positively related to prevalence.

B. terrestris and *B. lapidarius* exhibited the largest niche overlap (63%). However, no association between niche overlap (as proxy for transmission) and species-specific prevalence was detected. Likewise, no relationship was found between plant diversity and prevalence, no matter which mode (resources available or used – family- and species level) was inspected.

Temporal effects

The prediction of infection outcome from year to year is very challenging and becomes increasingly sophisticated with growing complexity of the host-parasite system, particularly within natural settings (Altizer et al. 2006, Knowles et al. 2014).

Transmission to the next host is one of the prerequisites of parasites to complete their life cycle (Schmid-Hempel 1998). Due to adverse conditions (UV radiation and desiccation), *C. bombi* is unable to survive outside its host for expanded periods of time (Schmid-Hempel et al. 1999). Combined with the annual life cycle of bumblebees (Sladen 1912, Goulson 2010), the parasite can only be successfully transmitted to the next season through overwintering queens (Schmid-Hempel et al. 1999). As a result, *C. bombi* populations face a bottleneck each autumn which becomes even more serious because only few bumblebee colonies contribute to the reproduction of young queens that additionally differ substantially in their probability to survive hibernation (Schmid-Hempel 2001). Furthermore, many colonies efficiently decrease the genetic diversity of the circulating infection by removing some parasite genotypes (i.e. "strain filtering", Ulrich et al. 2011) before the parasite is transmitted to young queens. Nonetheless, continuous screenings revealed that 5-10% of the spring queens are infected with one or multiple strains of *C. bombi* (reviewed in Ulrich et al. 2011). As *C. bombi* itself substantially reduces the fitness of hibernating queens and hamper colony founding (Brown et al. 2003, Yourth et al. 2008), parasite transmission and thus its survival might be at risk.

Consequently, initially high prevalences may decrease dramatically in the subsequent season which is in accordance with our findings and comparable to results reported by Salathé & Schmid-Hempel (2011). In the light of the above-mentioned interacting factors, the discrepancy we find between the observed and the expected change in *C. bombi* prevalence is not surprising. Moreover, empirical evidence of a highly dynamic parasite population structure across years, host species and sites (Salathé & Schmid-Hempel 2011, Erler et al.2012, Ruiz-González et al. 2012) is provided, which is likely attributable to environmentally-mediated interactions (G_H x G_P x E). In spite of G_H x G_P interactions (Schmid-Hempel 2001) and the annual life cycle of bumblebees (Sladen 1912, Goulson 2010), that jointly induce repeated bottlenecking of the *C. bombi* population ("filtering", Ulrich et al. 2011), the mixture of the parasite's clonal and sexual reproduction (Schmid-Hempel et al. 2011) counterbalances genetic impoverishment. Thus, the notably high diversity of different multi-locus genotypes is conceivable (Schmid-Hempel & Reber Funk 2004, Salathé & Schmid-Hempel 2011).

We also find that high intraspecific parasite diversity is related to higher prevalences. This is in accordance with our expectation and recent field observations (Salathé & Schmid-Hempel 2011) as well as with experimental evidence provided by Ganz & Ebert (2010) in the

Daphnia magna-Ordospora colligata system. Furthermore, increased *C. bombi* prevalence is linked to larger proportions of multiple-strain infections (cf. Chpt. 3). Hence, the association between a diverse parasite population and its increased performance regarding successful transmission between - and infection of different hosts (intra- and interspecifically) seems to be reasonable.

Interaction networks

Within natural settings, the transmission of *C. bombi* via shared floral resources is relevant (Durrer & Schmid-Hempel 1994) because natural bumblebee populations usually exhibit high *C. bombi* prevalences (Shykoff & Schmid-Hempel 1991a), although bumblebees are also able to avoid contaminated flowers (Fouks & Lattorff 2011). However, the specific mechanism behind this mode of horizontal transmission remains to be investigated (Cisarovsky & Schmid-Hempel 2014, McArt et al. 2014).

In contrast to our expectation, niche overlap and species-specific prevalences were not correlated. Moreover, even though *B. terrestris* and *B. lapidarius* shared 63% of their food resources which represents the largest niche overlap and is similar to values noticed by Goulson & Darvill (2004; ~70% niche overlap between *B. terrestris / lucorum* and *B. lapidarius*), this was not reflected by the species-specific prevalence observed. The absence of the expected relationship is most likely attributable to the small sample size of *B. terrestris* with only six individuals being infected in 2011. However, similarity regarding flower visitation already proved to be a good indicator of *C. bombi* genotype distribution between distinct host species (Salathé & Schmid-Hempel 2011, Ruiz-González et al. 2012).

The availability of food resources serves as a measure of habitat quality for bumblebees (Jha & Kremen 2013) but should also be related to the probability of picking up an infection. We hypothesized that low diversity of flowering plants increases the amount of shared resources, consequently augmenting transmission events, hence prevalence in contrast to high diversity of floral resources. Unlike our prediction, no relationship between plant diversity and prevalence was found. One possible explanation is the large diversity of flower architecture the bumblebees (as well as the parasites) are faced with. Distinct types of inflorescences (e.g. single / complex flowers) require different time effort while foraging, which should be linked to heterogeneous probability of both release and uptake of parasitic cells. However, in case of heavily infected individuals, parasite-mediated impairment of learning and foraging competence (e.g. increased time needed to handle a flower but also more grooming events on flowers, Otterstatter et al. 2005; Gegear et al. 2005, 2006) should also be considered. Additionally, a positive association between host health and parasite population growth

possibly serves as an alternative explanation because rich food resources may be equally beneficial to both host and parasite, thereby reducing the parasite-mediated harm (Brown et al. 2000, Vale et al. 2011, 2013).

Whereas we find no association between the four most preferred plant families (almost 97% of all visits) and prevalence, one flowering species appears to be remarkably attractive to bumblebees with potential consequences for disease outcome. Despite its small proportion (0.13% of all inflorescences potentially used), *Echium vulgare* receives almost 13% of the overall visits. Interestingly, its flower architecture and / or nectar seems to be advantageous for *C. bombi* in contrast to the flat and readily accessible flowers of *Rubus caesium* as the risk of infection was higher on *E. vulgare* (Durrer & Schmid-Hempel 1994). Therefore, the presumably crucial role of *E. vulgare* in parasite transmission as well as floral traits of different floral resources in general still call for further investigation (Cisarovsky & Schmid-Hempel 2014, McArt et al. 2014).

Conclusion

The present field study provides relevant insights into the relationship between a heterogeneous environment and spatiotemporal disease dynamics in the *Bombus-Crithidia* system. We show that intraspecific parasite diversity is positively associated with prevalence. Although we observed a decrease of initially high prevalences in the subsequent year, general predictions of interannual infection outcome remain sophisticated in complex host-parasite systems, especially within natural settings (Altizer et al. 2006, Knowles et al. 2014). Despite the large resource share between *B. terrestris* and *B. lapidarius*, no correlation between niche overlap and species-specific prevalence occurred, which is most likely due to the small number of infected *B. terrestris* in 2011. Even though we were unable to detect an association between plant diversity and prevalence, the underlying hypothesis appears conclusive. Therefore, a sharper focus is needed regarding the specific interplay of bumblebee foraging behaviour, various types of inflorescences and parasite transmission at flowers.

Manipulative field experiments represent the means of choice in order to control for a wealth of confounding factors and to directly ascertain the underlying mechanisms of transmission. Currently, little knowledge about the transmission of animal pathogens at flowers is available (but see Durrer & Schmid-Hempel 1994, Cisarovsky & Schmid-Hempel 2014). Hence, various floral traits along with host foraging behaviour (e.g. the time aspect) as well as parasite survival and transmission probability should be incorporated in future experimental studies to complement findings from observational surveys (McArt et al. 2014). Another future challenge is the development of forecast models also considering the role of environmental heterogeneity for the infection outome (e.g. Van der Werf et al. 2011).

Supporting Information for Chapter 2

Contents:

Table S2.1. Summary of the four different one-way ANCOVAs conducted; Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*; [1] response variable, [2] explanatory variable (four levels: Asteraceae, Boraginaceae, Fabaceae, Lamiaceae).

Variable	df	Sum of Squares	Mean Square	F	P
ANCOVA 1: mean prevalence[1]					
plant family (%)	1	0.00029	0.000288	0.041	0.842
plant family[2]	3	0.00195	0.000649	0.092	0.964
Residuals	20	0.14119	0.007059		
ANCOVA 2: *C. bombi* prevalence in Te[1]					
visitation Te (%)	1	0.00026	0.000257	0.041	0.841
plant family[2]	3	0.00146	0.000487	0.079	0.971
Residuals	20	0.12378	0.006189		
ANCOVA 3: *C. bombi* prevalence in La[1]					
visitation La (%)	1	0.00015	0.000150	0.030	0.864
plant family[2]	3	0.00302	0.001008	0.201	0.894
Residuals	20	0.10012	0.005006		
ANCOVA 4: *C. bombi* prevalence in Pas[1]					
visitation Pas (%)	1	0.0008	0.00075	0.019	0.891
plant family[2]	3	0.0103	0.00343	0.087	0.966
Residuals	20	0.7875	0.03938		

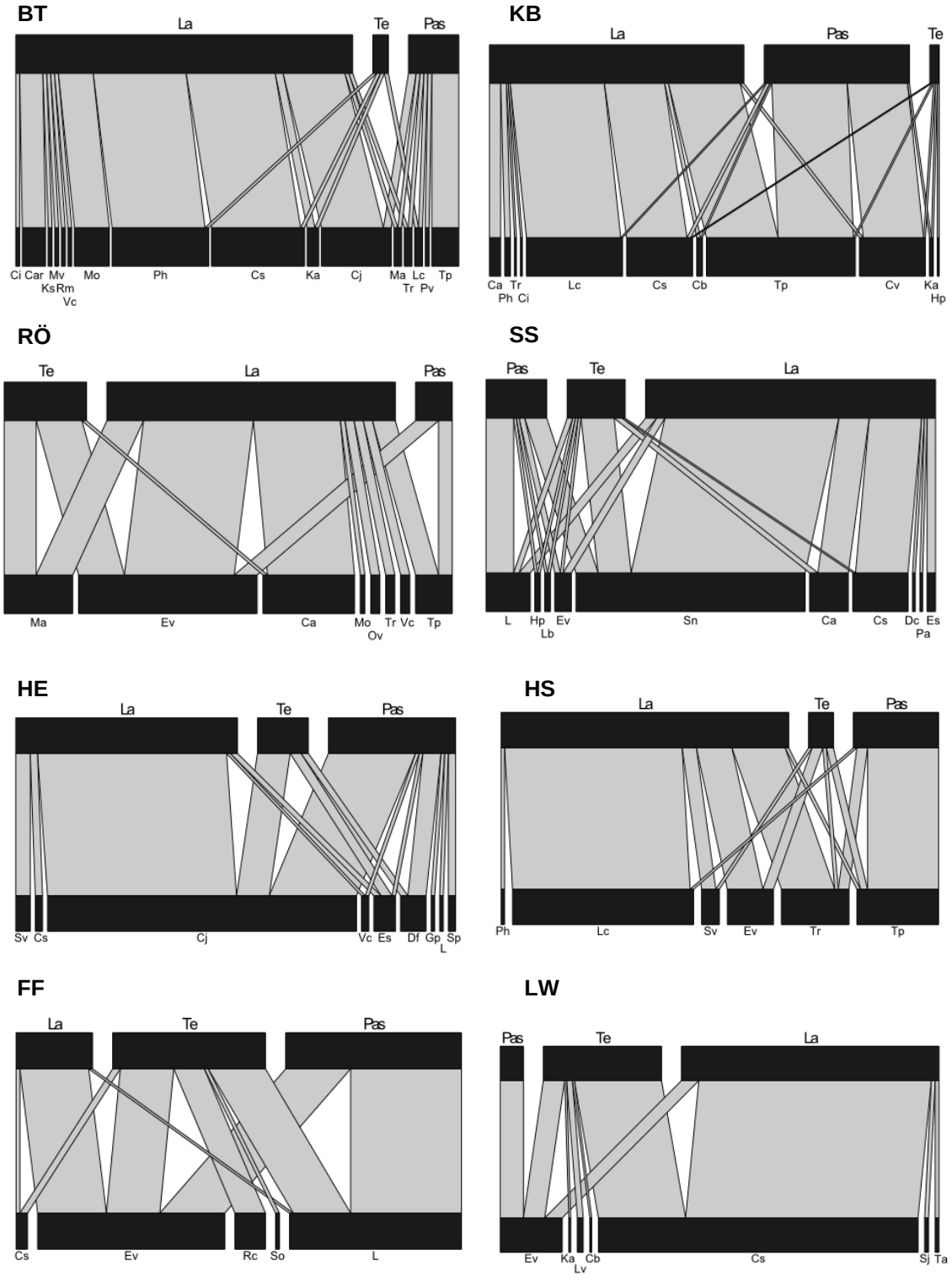

Figure S2.1. Quantitative interaction networks per location (cf. Tab. 3.1 for location details). Visitation of plant species by three bumblebee species (La = *B. lapidarius*, Pas = *B. pascuorum*, Te = *B. terrestris*) is pooled across three sampling events (June, July and August 2011). See Tab. S2.4 for species identity of the flowering plants (letter code).

Table S2.2. Population genetic parameters of *Crithidia bombi* (single-strain infections) of two consecutive years (cf. Tab. 3.1 for location details). N inf = bumblebee individuals infected with *C. bombi*, H_O = observed heterozygosity, A_N = number of alleles over all loci typed; non-bold / **bold** entries indicate assignment to the 'low'- / **'high'**-diversity group used for the one-tailed MWU tests. Means ± SD are shown.

Code Location	2010					2011				
	N inf	Loci typed	H_O ± SD	A_N ± SD	Prevalence [95% CI][1]	N inf	Loci typed	H_O ± SD	A_N ± SD	Prevalence [95% CI][1]
GW_KB	4	4	**0.81** ± 0.13	3.75 ± 2.36	0.07 [0.03, 0.13]	38	4	**0.55** ± 0.06	**7.25** ± 2.75	0.20 [0.15, 0.26]
GE_BT	12	3	0.42 ± 0.11	**5.00** ± 2.00	0.19 [0.12, 0.28]	19	4	**0.58** ± 0.08	**5.25** ± 2.22	0.21 [0.14, 0.31]
Rö_SS	34	4	**0.77** ± 0.05	**5.25** ± 2.63	0.35 [0.27, 0.44]	20	3	0.15 ± 0.07	3.00 ± 1.00	0.16 [0.11, 0.23]
Rö_RÖ	19	4	**0.68** ± 0.07	**6.75** ± 4.57	0.25 [0.18, 0.33]	5	3	**0.83** ± 0.14	**3.33** ± 1.53	0.07 [0.02, 0.14]
Hal_HS	36	4	**0.69** ± 0.05	**9.50** ± 1.73	0.28 [0.22, 0.35]	3	4	0.33 ± 0.15	2.25 ± 1.50	0.03 [0.01, 0.09]
Hal_HE	10	4	0.52 ± 0.09	4.25 ± 2.22	0.35 [0.22, 0.49]	4	3	0.00 ± 0.00	2.33 ± 1.15	0.03 [0.01, 0.07]
Fw_FF	3	3	0.28 ± 0.18	2.00 ± 1.00	0.03 [0.01, 0.07]	5	2	0.33 ± 0.21	2.50 ± 0.71	0.06 [0.02, 0.12]
Fw_LW	10	4	0.32 ± 0.11	2.75 ± 1.71	0.09 [0.06, 0.15]	28	4	**0.40** ± 0.08	**4.00** ± 2.00	0.14 [0.10, 0.19]

[1] mean prevalence comprising single- and multiple-strain infections; 95% Confidence Intervals [CI] for a binomial probability are calculated with Soper (2014)

Table S2.3. Resource availability 2011. Family=plant family: A=Apiaceae, As=Asteraceae, B=Boraginaceae, Br=Brassicaceae, Cp=Campanulaceae, C=Caprifoliaceae, Ca=Caryophyllaceae, Co=Convolvulaceae, F=Fabaceae, G=Geraniaceae, H=Hypericaceae, L=Lamiaceae, Li=Linaceae, On=Onagraceae, O=Orobanchaceae, P=Papavera-ceae, Pl=Plumbaginaceae, Pr=Primulaceae, Ra=Ranunculaceae, R=Rosaceae, S=Scrophulariaceae, So=Solanaceae. ∑ flowers=total amount of flowering plants available (based on three surveys per location; classification of abundance for calculations: <5; 5-10; 11-25; 26-55; 56-75; 76-100; >100; >1,000; 5,000; 10,000 inflorescences). Prop=proportion of all flowering species, N visits=number of visits per flowering species received by one of the three bumblebee species.

No	Scientific name	Family	∑ flowers	Prop [%]	N visits	(N) Locations
1	*Chaerophyllum* spec. L.	A	100	0.0	0	(1) BT
2	*Daucus carota subsp. carota* L.	A	221,241	16.9	1	(5) KB, BT, SS, RÖ, HE
3	*Arctium tomentosum* MILL.	As	208	0.0	0	(4) KB, BT, RÖ, HS
4	*Centaurea jacea* L.	As	20,100	1.5	103	(3) BT, HS, HE
5	*Centaurea scabiosa* L.	As	21,516	1.6	249	(6) KB, BT, SS, HE, FF, LW
6	*Cichorium intybus* L.	As	596	0.0	2	(6) KB, BT, SS, RÖ, HS, HE
7	*Cirsium arvense* (L.) SCOP.	As	20,425	1.6	40	(6) KB, BT, SS, RÖ, HS, HE
8	*Crepis biennis* L.	As	6,100	0.5	4	(2) KB, LW
9	*Echinops sphaerocephalus* L.	As	110	0.0	9	(3) SS, RÖ, HE
10	*Helianthus tuberosus* L.	As	41	0.0	0	(1) FF
11	*Helichrysum arenarium* (L.) MOENCH	As	288	0.0	0	(2) FF, LW
12	*Hieracium* spec. L.	As	106	0.0	0	(2) RÖ, HE
13	*Hypochaeris radicata* L.	As	100	0.0	0	(1) LW
14	*Leucanthemum* spec. MILL.	As	41	0.0	0	(1) BT
15	*Matricaria chamomilla* L.	As	473	0.0	0	(3) BT, SS, RÖ
16	*Picris hieracoides* L.	As	232,166	17.8	29	(7) KB, BT, SS, RÖ, HE, HS, FF
17	*Senecio jacobaea* L.	As	142	0.0	2	(2) RÖ, LW
18	*Tanacetum vulgare* L.	As	10,200	0.8	0	(2) RÖ, HE
19	*Echium vulgare* L.	B	1,272	0.1	139	(5) SS, RÖ, HS, FF, LW
20	*Berteroa incana* (L.) DC.	Br	15,000	1.1	0	(1) LW
21	*Brassica nigra* (L.) W.D.J. KOCH	Br	10,000	0.8	0	(1) SS
22	NA	Br	88	0.0	0	(1) RÖ
23	*Campanula patula* L.	Cp	18	0.0	0	(1) KB
24	*Campanula trachelium* L.	Cp	100	0.0	0	(1) KB
25	*Jasione montana* L.	Cp	618	0.0	0	(1) LW
26	*Dipsacus fullonum* L.	C	10	0.0	7	(2) KB, HE
27	*Knautia arvensis* (L.)	C	122	0.0	6	(3) KB, BT, LW
28	*Knautia sylvatica* KREUTZER	C	26	0.0	1	(2) KB, BT
29	*Scabiosa ochroleuca* L.	C	100	0.0	0	(1) LW
30	*Saponaria officinalis* L.	Ca	400	0.0	1	(2) FF, LW
31	*Silene dioica* (L.) CLAIRV.	Ca	1,136	0.1	0	(4) SS, HE, FF, LW
32	*Silene latifolia* MILL.	Ca	117	0.0	0	(4) KB, SS, RÖ, HE
33	*Convolvulus arvensis* L.	Co	119	0.0	1	(3) BT, FF, LW
34	*Convolvulus sepium* (L.) R. BR.	Co	106	0.0	0	(2) FF, LW
35	*Astragalus glycyphyllos* L.	F	100	0.0	0	(1) KB
36	*Lathyrus tuberosus* L.	F	99	0.0	0	(2) RÖ, HE
37	*Lotus corniculatus* L.	F	132,531	10.1	104	(5) KB, BT, HE, HS, FF
38	*Medicago lupulina* L.	F	12,672	1.0	0	(7) KB, BT, SS, RÖ, HE, HS, FF
39	*Medicago sativa subsp. varia* (MARTYN) ARCANG.	F	1,766	0.1	1	(4) BT, RÖ, HE, HS
40	*Melilotus albus* MEDIK.	F	159	0.0	15	(2) RÖ, FF
41	*Melilotus officinalis* (L.) PALL.	F	247	0.0	10	(2) BT, RÖ
42	*Onobrychis viciifolia SCOP.*	F	10,000	0.8	0	(1) HS

Table S2.3. Continued.

No	Scientific name	Family	∑ flowers	Prop [%]	N visits	(N) Locations
43	*Securigera varia* (L.) LASSEN	F	726	0.0	9	(2) HE, HS
44	*Trifolium arvense* L.	F	11,066	0.8	2	(2) BT, LW
45	*Trifolium pratense* L.	F	524,448	40.1	117	(6) KB, BT, RÖ, HE, HS, FF
46	*Trifolium repens* L.	F	8,456	0.6	24	(6) KB, BT, SS, RÖ, HE, HS
47	*Vicia cracca* L.	F	880	0.0	5	(7) KB, BT, RÖ, HE, HS, FF, LW
48	*Geranium pratense* L.	G	41	0.0	1	(1) HE
49	*Hypericum perforatum* L.	H	5,690	0.4	3	(6) KB, BT, SS, HE, HS, FF
50	*Clinopodium vulgare* L.	L	10,105	0.8	35	(2) KB, BT
51	*Lamium* spec. L.	L	1,448	0.1	62	(4) BT, SS, HE, FF
52	*Origanum vulgare* L.	L	59	0.0	2	(1) RÖ
53	*Prunella vulgaris* L.	L	136	0.0	1	(2) KB, BT
54	*Salvia nemorosa* L.	L	7,000	0.5	83	(1) SS
55	*Salvia pratensis* L.	L	66	0.0	2	(1) HE
56	*Stachys sylvatica* L.	L	41	0.0	0	(1) BT
57	*Thymus* spec. L.	L	100	0.0	0	(1) KB
58	*Linum usitatissimum* L.	Li	41	0.0	0	(1) HE
59	*Oenothera biennis* L.	On	81	0.0	0	(1) FF
60	*Melampyrum arvense* L.	O	300	0.0	2	(2) KB, BT
61	*Rhinanthus minor* L.	O	105	0.0	1	(1) BT
62	*Papaver rhoeas* L.	P	53	0.0	0	(2) BT, RÖ
63	*Armeria* spec. WILLD.	Pl	18	0.0	0	(1) FF
64	*Lysimachia punctata* L.	Pr	41	0.0	0	(1) HE
65	*Consolida regalis* GRAY	Ra	18	0.0	0	(1) RÖ
66	*Agrimonia eupatoria* L.	R	1,007	0.1	0	(5) KB, BT, SS, RÖ, HE
67	*Potentilla anserina* (L.) RYDB.	R	8	0.0	0	(1) BT
68	*Potentilla argentea* L.	R	12,166	0.9	1	(2) SS, LW
69	*Potentilla reptans* L.	R	1,261	0.1	0	(5) BT, SS, RÖ, HE, HS
70	*Rosa canina* L.	R	146	0.0	8	(2) BT, FF
71	*Rubus sectio rubus*	R	281	0.0	0	(3) BT, RÖ, HE
72	*Linaria vulgaris* MILL.	S	240	0.0	3	(4) RÖ, HS, FF, LW
73	*Verbascum* spec. L.	S	81	0.0	0	(3) RÖ, FF, LW
74	*Lycium barbarum* L.	So	88	0.0	2	(1) SS
∑	**74**	**22**	**1,306,956**	**100.0**	**1,087**	**8**

Table S2.4. Flowering plants visited by *B. terrestris*, *B. lapidarius* and *B. pascuorum* in 2011*.

No	Scientific name	ID	Englisch name	German name	Family (ID)
1	*Daucus carota* subsp. *carota* L.	Dc	Wild Carrot	Wilde Möhre	Apiaceae (A)
2	*Centaurea jacea* L.	Cj	Brown Knapweed	Wiesen-Flockenblume	Asteracea (As)
3	*Centaurea scabiosa* L.	Cs	*Greater Knapweed*	Skabiosen-Flockenblume	Asteracea (As)
4	*Cichorium intybus* L.	Ci	Common Chicory	Gemeine Wegwarte	Asteracea (As)
5	*Cirsium arvense* (L.) SCOP.	Ca	Creeping Thistle	Acker-Kratzdistel	Asteracea (As)
6	*Crepis biennis* L.	Cb	Rough Hawksbeard	Wiesen-Pippau	Asteracea (As)
7	*Echinops sphaerocephalus* L.	Es	Great Globe Thistle	Drüsenblättrige Kugeldistel	Asteracea (As)
8	*Picris hieracioides* L.	Ph	Hawkweed Oxtongue	Gewöhnliches Bitterkraut	Asteracea (As)
9	*Senecio jacobea* L.	Sj	*Tansy Ragwort*	Jakobs-Greiskraut	Asteracea (As)
10	Echium vulgare L.	Ev	Blueweed	Gemeiner Natternkopf	Boraginaceae (B)
11	*Dipsacus fullonum* L.	Df	Wild Teasel	Wilde Karde	*Caprifoliaceae (C)*
12	*Knautia arvensis* (L.) COULT.	Ka	Field Scabious	Wiesen-Witwenblume	*Caprifoliaceae (C)*
13	*Knautia dipsacifolia* KREUTZER	Kd	Wood Scabious	Wald-Witwenblume	*Caprifoliaceae (C)*
14	*Saponaria officinalis* L.	So	Common Soapwort	Echtes Seifenkraut	Caryophyllaceae
15	*Convolvulus arvensis* L.	Car	*Field Bindweed*	Ackerwinde	Convolvulaceae (Co)
16	*Lotus corniculatus* L.	Lc	Birdsfoot Trefoil	Gewöhnlicher Hornklee	Fabaceae (F)
17	*Medicago sativa subsp. varia (*MARTYN) ARCANG.	Mv	Sand Lucerne / Bastard Medic	Sand- / Bastard-Luzerne	Fabaceae (F)
18	*Melilotus albus* MEDIK.	Ma	White Sweet Clover	Weißer Steinklee	Fabaceae (F)
19	*Melilotus officinalis* (L.) PALL.	Mo	Yellow Sweet Clover	Echter/Gelber Steinklee	Fabaceae (F)
20	*Securigera varia* (L.) LASSEN	Sv	Purple Crown Vetch	Bunte Kronwicke	Fabaceae (F)
21	*Trifolium arvense* L.	Ta	Haresfoot Clover	Hasen-Klee	Fabaceae (F)
22	*Trifolium pratense* L.	Tp	Red Clover	Rot-Klee	Fabaceae (F)
23	*Trifolium repens* L.	Tr	White Clover	Weiß-Klee	Fabaceae (F)
24	*Vicia cracca* L.	Vc	Bird Vetch	Gemeine Vogel-Wicke	Fabaceae (F)
25	*Geranium pratense* L.	Gp	Meadow Cranesbill	Wiesen-Storchschnabel	Geraniaceae (G)
26	*Hypericum perforatum* L.	Hp	St John's Wort	Echtes Johanniskraut	Hypericaceae (H)
27	*Clinopodium vulgare* L.	Cv	Wild Basil	Gemeiner Wirbeldost	Lamiaceae (L)
28	*Lamium* spec. L.	Lsp	Deadnettle	Taubnessel	Lamiaceae (L)
29	*Origanum vulgare* L.	Ov	Oregano	Oregano / Echter Dost	Lamiaceae (L)
30	*Prunella vulgaris* L.	Pv	Common Self-Heal	Gewöhnliche Braunelle	Lamiaceae (L)
31	*Salvia nemorosa* L.	Sn	Woodland Sage	Steppen-Salbei	Lamiaceae (L)
32	*Salvia pratensis* L.	Sp	Meadow Sage	Wiesen-Salbei	Lamiaceae (L)
33	*Melampyrum arvense* L.	Mar	Field Cow-Wheat	Acker-Wachtelweizen	Orobanchaceae (O)
34	*Rhinanthus minor* L.	Rm	Yellow Rattle	Kleiner Klappertopf	Orobanchaceae (O)
35	*Potentilla argentea* L.	Pa	Hoary Cinquefoil	Silber-Fingerkraut	Rosaceae (R)
36	*Rosa canina* L.	Rc	Dog Rose	Hunds-Rose	Rosaceae (R)
37	*Linaria vulgaris* MILL.	Lv	Common Toadflax	Gewöhnliches Leinkraut	Scrophulariaceae (S)
38	*Lycium barbarum* L.	Lb	Matrimony Vine	Gewöhnlicher Bocksdorn	Solanaceae (So)

* visitation by at least one of the three bumblebee species

Table S2.5. Overview of plant diversity and prevalence per location 2011 (cf. Tab. 3.1 for location details); **a)** resource availability, **b)** resource usage. Prev = mean prevalence (cf. Tab. S2.2 for 95% CIs), ∑ flowers = amount of flowering plants available / visited, n = number of plant families / species, corr / obs / exp D = corrected / observed / expected Shannon diversity (corr D = $\frac{\text{obs D}}{\text{exp D}}$).

a) resource availability

Code Location	Prev	∑ flowers	Families available				Species available			
			n	corr D	obs D	exp D	n	corr D	obs D	exp D
GW_KB	0.20	971,314	10	0.46	1.06	2.30	25	0.40	1.27	3.22
GE_BT	0.21	42,668	10	0.43	0.98	2.30	36	0.48	1.72	3.58
Rö_SS	0.16	40,378	10	0.65	1.49	2.30	20	0.55	1.65	3.00
Rö_RÖ	0.07	23,674	11	0.21	0.50	2.40	29	0.42	1.40	3.37
Hal_HS	0.03	140,655	6	0.02	0.04	1.79	17	0.30	0.86	2.83
Hal_HE	0.03	39,462	11	0.38	0.91	2.40	29	0.47	1.57	3.37
Fw_FF	0.06	3,659	11	0.74	1.78	2.40	21	0.74	2.24	3.04
Fw_LW	0.14	46,135	10	0.61	1.39	2.30	19	0.53	1.56	2.94

b) resource usage

Code Location	Prev	∑ flowers	Families visited				Species visited			
			n	corr D	obs D	exp D	n	corr D	obs D	exp D
GW_KB	0.20	759,553	5	0.43	0.70	1.61	11	0.37	0.89	2.40
GE_BT	0.21	29,874	6	0.28	0.51	1.79	16	0.41	1.15	2.77
Rö_SS	0.16	29,452	7	0.61	1.18	1.95	10	0.58	1.27	2.20
Rö_RÖ	0.07	12,221	4	0.38	0.53	1.39	8	0.34	0.71	2.08
Hal_HS	0.03	127,844	3	0.01	0.01	1.10	7	0.27	0.52	1.95
Hal_HE	0.03	15,464	5	0.09	0.14	1.61	9	0.08	0.19	2.20
Fw_FF	0.06	1,905	8	0.65	1.36	2.08	5	0.85	1.36	1.61
Fw_LW	0.14	27,567	5	0.47	0.75	1.61	7	0.47	0.91	1.95

Table S2.6. Results of the Spearman's rank-order correlations of niche overlap versus plant diversity (corr D). Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*; also cf. Tab. S2.7.

Niche overlap	Plant diversity (corr D)[1]					
	Families available			Species available		
	S	P	r_s	S	P	r_s
TeLa (0.63)	56	0.428	0.33	38	0.171	0.55
TePas (0.43)	66	0.619	0.21	62	0.536	0.26
LaPas (0.38)	91.04	0.844	-0.08	79.98	0.910	0.05

[1] cf. Tab. S2.5 for formula; corr D = corrected Shannon diversity of families / species available

Table S2.7. Niche overlap, delta prevalence and plant diversity 2011. Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*.

Code Location	Niche overlap			Δ prevalence[1]			Plant diversity (corr D)[2]	
	TeLa	TePas	LaPas	TeLa	TePas	LaPas	families	species
GW_KB	0.41	0.44	0.35	0.02	0.02	0.04	0.46	0.40
GE_BT	0.52	0.08	0.18	0.21	0.29	0.07	0.43	0.48
Rö_SS	0.79	0.60	0.43	0.10	0.59	0.49	0.65	0.55
Rö_RÖ	0.56	0.56	0.46	0.08	0.11	0.03	0.21	0.42
Hal_HS	0.33	0.41	0.18	0.12	0.14	0.02	0.02	0.30
Hal_HE	0.68	0.81	0.73	0.02	0.08	0.07	0.38	0.47
Fw_FF	0.45	0.73	0.42	0.10	0.03	0.13	0.74	0.74
Fw_LW	0.81	0.18	0.07	0.14	0.25	0.11	0.61	0.53
overall	**0.63**	**0.43**	**0.38**	**0.04**	**0.13**	**0.18**		

[1] = difference (absolute values) between particular species pairs; [2] cf. Tab. S2.5 for formula; corr D = corrected Shannon diversity of families / species available

Table S2.8. Results of the two-sample tests. N = number of bumblebees caught, N inf = number of bumblebees infected with *C. bombi*, L / H = assignment to the 'low'- / 'high'-prevalence group.

Code Location	2010			2011			Two-sample test			Change[2]
	N	N inf	Prev[1]	N	N inf	Prev[1]	χ^2	df	P	
GW_KB	121	8	0.07 (L)	223	44	0.20	10.52	1	**0.0012**	↑
GE_BT	105	20	0.19 (L)	103	22	0.21	0.17	1	0.678	=
Rö_SS	130	46	0.35 (H)	150	24	0.16	13.96	1	**0.0002**	↓
Rö_RÖ	133	33	0.25 (H)	92	6	0.07	12.70	1	**0.0004**	↓
Hal_HS	187	52	0.28 (H)	115	3	0.03	30.36	1	**<0.0001**	↓
Hal_HE	52	18	0.35 (H)	117	4	0.03	30.94	1	**<0.0001**	↓
Fw_FF	151	4	0.03 (L)	106	6	0.06	1.51	1	0.219	=
Fw_LW	169	16	0.10 (L)	208	29	0.14	1.78	1	0.183	=

[1]mean prevalence, [2] change in prevalence from 2010 to 2011

Table S2.9. Results of the Fisher's Exact Test for r *x* c contingency tables, P = simulated P-value based on 100.000 replicates. L / H = 'low'- / 'high'-prevalence group.

observed change			expected change		
Prevalence	**L**	**H**	**Prevalence**	**L**	**H**
↑	1	0	↑	0.50	0.50
=	3	0	=	1.50	1.50
↓	0	4	↓	2.00	2.00
					P = 0.029

Chapter 3

Key factors of the infection outcome

Introduction

The majority of pathogens infects a range of host species (Cleaveland et al. 2001, Taylor et al. 2001) and appear to be rather generalists than specialists (Woolhouse et al. 2001, Rigaud et al. 2010). In recent years this issue has gained attention, as emerging infectious diseases (EIDs), threatening humans as well as wildlife populations (Binder et al. 1999, Siddle et al. 2007), often reside in reservoir host species (Woolhouse & Gowtage-Sequeria 2005). However, classical models of host-parasite interactions focus on single host species interacting with single parasite species. Multi-host parasite interactions have been studied less extensively, both theoretically and empirically (Rigaud et al. 2010). In the light of evolution of virulence and transmission of pathogens, studies of multi-host parasite systems seem to be crucial for the basic understanding of the spread and persistence of diseases in order to interfere in those systems to lessen disease risk (Roche & Guégan 2011).

Besides virulence, transmission to new hosts is one of the crucial factors in the life-cycle of pathogens (Schmid-Hempel 1998). It is assumed that transmission of directly transmitted pathogens is density-dependent (McCallum et al. 2001). Density-dependent transmission is a function of the probability of encounters of new susceptible hosts (Altizer et al. 2006). Additionally, the per-contact probability of transmission, but also the removal of pathogens due to recovery of infected hosts and the decay of infective particles within the environment will affect transmission and ultimately the prevalence of pathogens within host populations (Altizer et al. 2006). All those factors influencing transmission might vary due to seasonality of temperature, rainfall or bursts in birth rates of host species.

Likewise genetic factors of host species might influence transmission, but especially the establishment and reproductive rate of pathogens (King & Lively 2012). Evidence has accumulated that genetic diversity influences the spread of diseases at various levels of biological organisation, from individuals over populations up to communities (King & Lively 2012, Johnson et al. 2013b). Obviously, multi-host parasites are confronted with various levels of genetic factors as genetic differences occur between and within host species.

As pointed out by King & Lively (2012), the relative contributions of density-dependent and genetic effects are virtually unknown. In order to study those effects simultaneously, we use a well suited study system, the intestinal parasite *Crithidia bombi* infecting a wide range of bumblebee (*Bombus* spp.) host species (Shykoff & Schmid-Hempel 1991a, Sadd & Barribeau 2013).

Animal-mediated pollination is a key ecosystem service vital to human welfare (Klein et al. 2007) with wild insects, especially bumblebees (Goulson 2010), playing a pivotal ecological and economical role in the effective pollination of crops and wild plants (Garibaldi et al. 2013). Evidence has accumulated suggesting that parasites and notably EIDs (Meeus et al. 2011, Fürst et al. 2014) have contributed to a global decline of pollinators during the past decades (Biesmeijer et al. 2006). Social insects like bumblebees are threatened by disease spread due to the high intra-colonial density of closely related individuals frequently interacting with each other (Schmid-Hempel 1998)). Bumblebee colonies are headed by a single mated queen initially raising several generations of workers until sexuals (males and gynes (unmated queens)) are produced (Sladen 1912).

The trypanosome *Crithidia bombi* (Gorbunov 1987, Lipa & Triggiani 1988) infects numerous bumblebee species and appears to be widespread in natural populations infecting adults of all castes and sexes (Shykoff & Schmid-Hempel 1991a). The coincidence of a huge number of *C. bombi* genotypes within populations, colonies and individuals has been revealed by microsatellite analyses (Schmid-Hempel & Reber Funk 2004, Erler et al. 2012, Popp et al. 2012). The parasite is directly transmitted, within colonies through contact with contaminated surfaces or infected nestmates (Schmid-Hempel & Schmid-Hempel 1993, Otterstatter & Thomson 2007) and between colonies (intra- and interspecifically) via shared flowers during foraging (Durrer & Schmid-Hempel 1994; but see Fouks & Lattorff 2011). As transmission is dependent on the availability of new susceptible hosts and the probability for encounters, intra-colonial transmission might be a function of colony size, which clearly differs between species (Goulson 2010, Erler et al. 2012). However, species also differ with respect to the preference of floral resources (Goulson & Darvill 2004). Thus, the resource overlap – and consequently the probability of transmission – differs between host species. Furthermore, Ruiz-González et al. (2012) underpinned this providing evidence of inconsistent and asymmetric inter- and intraspecific transmission capability of different species, with higher prevalence in more common species (Gillespie 2010, Ruiz-González et al. 2012). Seasonal colony growth (Schmid-Hempel 1998) and high local colony densities should facilitate the transmission potential and therefore the risk of infection.

Genetic diversity affects disease spread in bumblebees at various levels of biological organization. Within colony genetic diversity is low and experimentally manipulated colonies with increased genetic diversity appear to be more resistant towards infections with *C. bombi* (Baer & Schmid-Hempel 1999). Similar effects have been demonstrated for population and species level (Cameron et al. 2011, Whitehorn et al. 2011, Jones & Brown 2014), potentially due to substantial amounts of standing genetic variation affecting *C. bombi* infections (Wilfert et al. 2007). The most obvious genetic difference is found between sexes due to the haplodiploid sex-determination system in Hymenoptera (Cook 1993). Haploid males might face a larger infection risk due to the lack of allelic diversity and the inability to compensate deleterious alleles, also known as the haploid-susceptibility hypothesis (O'Donnell & Beshers 2004).

In order to disentangle the relative contributions of various genetic, density-dependent and environmental effects, we conducted an extensive field study to (i) identify simultaneously potential factors contributing to *C. bombi* epidemiology. We have measured epidemiological characters as *prevalence* (proportion of infected bumblebees), *type* (single- vs. multiple-strain infection) and *intensity* (mean number of parasite cells per host) of infection and test the influence of genetic, density-dependent and seasonal factors.

We predict that (ii) higher genetic diversity reduces disease spread whereas (iii) density-dependent effects increase disease spread, both of them simultaneously measured at different levels of biological organisations.

Material and methods

Sampling

Workers (n = 1,847) and males (n = 422) of three bumblebee species (*Bombus terrestris, B. lapidarius, B. pascuorum*) were collected during foraging flights, in semi-natural and agricultural habitats (hedgerows, urban parks, grassland, field margins, fallow land), in Germany along a west-east transect with ten sites (max. distance: 311 km), each comprising three locations (Tab. 3.1). The mean distance between locations was 5.2 km ± 2.4 km (mean ± SD), exceeding the expected foraging ranges of different bumblebee species (Goulson 2010). Each location was sampled in a random order three times (June, July and August 2010) during sunny weather. Time of day was also randomized to reduce biased data. Individuals were stored at -20°C prior to DNA extraction. After initial species identification in the field, individuals were double-checked for sex and species identity following the taxonomic key of Mauss (1994).

Table 3.1. Sampling overview. Total number of bumblebees caught within three sampling periods (**1.** 13-26 June, **2.** 14-22 July, **3.** 19-25 August 2010). Individuals infected with *Crithidia bombi* are given in brackets. BB=Brandenburg, LS=Lower Saxony, SA=Saxony-Anhalt. Te=*B. terrestris*, La=*B. lapidarius*, Pas=*B. pascuorum*.

Code	Location	Longitude (N) / Latitude (E)	Te	La	Pas
GW_RB	Riesenberg, Güntersen, LS	51°32'44.65" /9°44'32.65"	24 (11)	49 (8)	24 (8)
GW_KB	Kuhberg, Emmenhausen, LS	51°34'45.52" / 9°50'25.03"	7 (-)	112 (8)	2 (-)
GW_AG	Am Graben, Lödingsen, LS	51°35'47.29" / 9°47'22.56"	2 (1)	69 (15)	38 (5)
GE_KK	Kleiner Knüll, Reinhausen, LS	51°28'33.67" / 9°59'55.49"	7 (3)	38 (15)	32 (8)
GE_BT	Bratental, Göttingen, LS	51°33'48.77" / 10° 0'40.08"	4 (3)	95 (17)	6 (-)
GE_LB	Lengder Burg, Groß Lengden, LS	51°30'27.44" / 10° 1'12.80"	21 (1)	47 (13)	11 (3)
Sgh_ML	Meuserlengefeld, SA	51°29'42.65" / 11°14'45.40"	5 (1)	27 (3)	1 (1)
Sgh_WR	Wettelrode, SA	51°30'37.86" / 11°17'18.20"	21 (2)	36 (6)	5 (1)
Sgh_OD	Obersdorf, SA	51°31'31.13" / 11°18'30.55"	15 (2)	36 (6)	11 (1)
Rö_SS	Salziger See, Seeburg, SA	51°28'51.58" / 11°41'00.84"	62 (20)	55 (26)	13 (-)
Rö_RÖ	Röblingen am See, SA	51°27'47.21" / 11°41'53.57"	87 (25)	45 (8)	1 (-)
Rö_WL	Wansleben, SA	51°27'51.46" / 11°45'32.14"	28 (8)	11 (2)	4 (-)
Bd_SM	Salzmünde, SA	51°31'23.70" / 11°49'15.62"	21 (1)	11 (2)	4 (-)
Bd_GB	Görbitz, SA	51°34'27.73" / 11°52'31.21"	40 (9)	25 (1)	4 (1)
Bd_BD	Beidersee, SA	51°33'58.03" / 11°53'51.88"	20 (5)	2 (-)	20 (3)
Hal_HS	Heide Süd, Halle, SA	51°29'29.06" / 11°56'10.99"	66 (26)	96 (26)	25 (-)
Hal_BG	Botanischer Garten, Halle, SA	51°29'21.36" / 11°57'37.73"	24 (3)	22 (1)	47 (8)
Hal_HE	Hufeisensee, Halle, SA	51°27'36.70" / 12° 1'32.52"	26 (13)	26 (5)	-
Ad_BÖ	Blösien, SA	51°19'33.93" / 11°54'16.62"	8 (-)	4 (-)	9 (-)
Ad_AD	Atzendorf (Merseburg), SA	51°20'17.29" / 11°57'59.46"	69 (26)	33 (2)	3 (-)
Ad_LÖ	Lössen (Schkopau), SA	51°22'21.05" / 12° 2'35.60"	21 (-)	44 (2)	2 (-)
Vr_WS	Waldersee (Dessau-Roßlau), SA	51°50'10.64" / 12°15'55.48"	5 (1)	8 (-)	12 (-)
Vr_VR	Vockerode, SA	51°51'6.43" / 12°20'22.96"	45 (11)	14 (1)	-
Vr_OB	Oranienbaum, SA	51°48'8.86" / 12°22'56.45"	18 (1)	3 (-)	4 (-)
Bs_RW	Reichenwalde, BB	52°16'4.94" / 14° 0'17.22"	4 (-)	1 (1)	1 (-)

Table 3.1. Continued.

Code	Location	Longitude (N) / Latitude (E)	Te	La	Pas
Bs_DB	Dachsberg, Bad Saarow BB	52°15'39.86" / 14° 1'56.84"	5 (-)	3 (-)	11 (-)
Bs_AH	Annenhof, Bad Saarow, BB	52°16'21.79" / 14° 5'34.20"	11 (2)	2 (-)	45 (1)
Fw_AP	Fürstenwalde/Spree Süc BB	52°20'7.68" / 14° 3'53.83"	17 (-)	5 (1)	12 (1)
Fw_FF	Fürstenwalde/Spree Ost BB	52°21'38.81" / 14° 5'11.36"	90 (2)	33 (2)	28 (-)
Fw_LW	Langewahl, BB	52°19'54.98" / 14° 5'35.55"	88 (12)	78 (4)	3 (-)
∑			**861 (189)**	**1,030 (175)**	**378 (41)**

DNA analysis

BUMBLEBEES

DNA was extracted from a single leg per individual following a modified Chelex protocol (Walsh et al. 1991; Erler & Lattorff 2010). Workers were genotyped at eight highly variable microsatellite loci (B11, B96, B124, B126, (Estoup et al. 1995, 1996); and BTMS0043, BTMS0045, BTMS0057, SSR0154_56i12, (Stolle et al. 2009, 2011). Several loci were amplified per multiplex PCR. Each reaction contained 1 µl template DNA, 5 µl PCR Master Mix (Promega Corporation, Madison/WI, USA), 0.4 – 0.75 µM per primer pair and made up to 10 µl with ddH_2O. The thermal profile of the PCR followed the protocol of (Erler & Lattorff 2010).

Additionally, novel primers for the unambiguous discrimination of the resembling species *B. terrestris* and *B. lucorum* (Sladen 1912) were designed (Appendix S3.1). Forward primers were labelled with different fluorescent dyes (Metabion International AG, Martinsried, Germany) and included in the multiplex PCR. The amplified fragments were visualized with an automated DNA capillary sequencer (MegaBACE 1000, GE Healthcare, Munich, Germany) according to manufacturer's instructions and a standard protocol (Erler & Lattorff 2010). Allele sizes were scored with the software MegaBACE Fragment Profiler v1.2 after visual inspection of processed raw data.

CRITHIDIA BOMBI

After the removal of each bumblebee's gut, DNA extraction was done according to the aforementioned Chelex protocol (Walsh et al. 1991; Erler & Lattorff 2010). Four polymorphic microsatellite loci were genotyped (Cri 4, Cri 1.B6, Cri 4.G9, and Cri 2.F10; (Schmid-Hempel & Reber Funk 2004) using fluorescence labelled primers (Metabion). All loci were amplified in one multiplex PCR following the protocol of (Popp & Lattorff 2011). The final volume of 10 µl contained 1 µl template DNA, 5 µl PCR Master Mix (Promega), 0.3 µM (Cri 1.B6, Cri 4.G9), 0.6 µM (Cri 4, Cri 2.F10) per primer pair and 2.2 µl ddH_2O. PCR products were run on a MegaBACE 1000 (GE Healthcare) and fragments were sized using Fragment Profiler v1.2. As *C. bombi* is a diploid organism ((Schmid-Hempel & Reber Funk 2004), more than two peaks per locus indicate an infection of the individual host with more than one strain (i.e. multiple infection). Due to the strong relationship between peak height and the mean number of *C. bombi* cells (log10-transformed) per host, we estimated intensity of infection on the basis of Cri 1.B6 and Cri 4.G9 peak height following the 'microsatellite method' of Fouks & Lattorff (2014). This method proved to be reliable as a positive correlation between increasing DNA amount and corresponding peak heights has been shown before (Moritz et al. 2003, Schulte et al. 2011).

Bumblebee kinship reconstruction and population genetics

As the colony represents the genetically relevant unit in social insects, it is crucial to identify the kinship relationships of the collected bumblebees, thus enabling estimation of both colony density and population genetic metrics. COLONY v. 2.0.5.0 (Wang 2004) was used to assign workers to matrilines according to their individual genotypes and the overall allele frequencies in the sample. Two replicate COLONY runs per location and species, each with a different random number seed, were conducted using the full-likelihood method. Locations with less than ten genotyped workers per species were excluded (included / excluded locations: *B. terrestris* – n = 17 / 13, *B. lapidarius* – n = 20 / 10, *B. pascuorum* – n = 14 / 16; cf. Tab. 3.3, Tab. S3.2). Error rates for allelic dropouts and other genotyping errors were set to 0.05 for all loci. The number of alleles (A_N) as well as the observed and expected heterozygosities (H_O, H_E) were obtained using the Excel Microsatellite Toolkit (Park 2001). COLONY is thought to be the most accurate software in assigning colonies, but only few multilocus queen genotypes were correctly reconstructed (Lepais et al. 2010). Hence, we calculated all population genetic parameters on the basis of real genotypes of the sampled workers using one randomly selected representative per reconstructed colony. In order to account for finite sample sizes, the non-sampling error (NSE) was calculated using the mark-recapture software Capwire

(Miller et al. 2005). Capwire allows for multiple sampling of an individual (or full-sib) and proved to be useful for estimating the number of colonies (e.g. Goulson et al. 2010). We ran the likelihood ratio test (LRT) to identify the best model (Tab. S3.2), either the Even Capture Model (ECM) or the Two Innate Rate Model (TIRM) per location and species (Miller et al. 2005). As with Stanley et al. (2013), the ECM was predominantly the better fit to our data (but cf. Goulson et al. 2010). Therefore, those estimates were used for further analyses. To ensure the comparability between studies we also provide the results of the TIRM method including the associated colony density estimates (Tab. S3.2). For locations with singletons only, no NSE and therefore no colony density estimation could be derived.

Statistical analyses of C. bombi infection

Generalized Linear Mixed Models (GLMMs) based on individual data were used to test the effects of the species identity, sex of bumblebees, the sampling period (SP) and their interactions (fixed effects / predictor variables) on the *prevalence* (presence-absence of infection) and the *type* of *C. bombi* infection (single- vs. multiple-strain infection). As both of the aforementioned response variables are binary, modelling was done with a binomial error distribution and the logit link function. For the main analyses (using workers only), colony was treated as a random effect and nested within location, which was in turn nested within site. When testing for potential sex-related differences, the first sampling period was excluded because male production started later. Sex was included in the model selection procedure together with species, sampling period and their interactions. Furthermore, colony was removed as a random effect since males were not assigned to colonies. The *dredge* function implemented in the R package MuMIn 1.9.5 (Bartoń 2013) was used to identify the best subset of fixed effects based on the full model. As a result the list of candidate models ranked by Aikake's Information Criterion (AIC) is provided (Tab. S3.1). The final model was compared to the null model (without fixed effects) using standard maximum likelihood (ML) for parameter estimation and subsequently fit with REML (restricted maximum likelihood) via Laplace approximation (Bolker et al. 2009). Goodness-of-fit (R^2) of the final model was calculated using *r.squaredGLMM* (MuMIn 1.9.5). Marginal ($R^2_{GLMM(m)}$: variance explained by fixed effects) as well as conditional R^2 ($R^2_{GLMM(c)}$: variance explained by both fixed and random effects) are provided (Nakagawa & Schielzeth 2013). In case of significant fixed effects, Tukey's HSD (Honest Significant Difference) post-hoc tests were used to test for significant differences between specific factors whilst simultaneously correcting for multiple comparisons.

For the analysis of *intensity* of the *C. bombi* infection, Linear Mixed Models (LMMs) were performed to account for the continuous data. The random effects structure and the model selection procedure remained the same, but R^2 was calculated using *r.squaredLR*. All analyses were performed using R 2.15.3 (R Core Team 2013) and the packages lme4 (v0.999999-2, function *lmer*; Bates et al. 2013), MuMIn 1.9.5 (Bartoń 2013) and multcomp (Hothorn et al. 2008).

Finally, multiple regression analyses were conducted (*B. terrestris* and *B. lapidarius;* the smallest dataset – *B. pascuorum* – was excluded to raise power and comparability) to understand the relationship between colony density and genetic diversity as predictors of the prevalence and infection intensity of *C. bombi*, respectively. The estimated colony density relies on the NSE derived from the ECM method in Capwire (Miller et al. 2005) and the species-specific flight ranges of workers reported in Knight et al. (2005). Expected heterozygosity (H_E) served as a measure of genetic diversity. Using linear models, F-tests were carried out (R packages MASS 7.3-23, Venables & Ripley 2002, and car 2.0-16, Fox & Weisberg 2011).

Results

Infection with Crithidia bombi

In total, 2,269 individuals of *B. terrestris* (n = 861), *B. lapidarius* (n = 1,030) and *B. pascuorum* (n = 378) were included in the analyses (Tab. 3.1). 405 bumblebees were infected (single / multiple infection: n = 266 / 139) on average with 32 *C. bombi* cells (median; 1^{st} – 3^{rd} quantile: 13 – 2,512 cells per host).

Here we present the results of the final models (i.e. the minimal adequate model) compared to the null models via likelihood ratio tests (LRT) and an overview of the contribution of each term (Tab. 3.2) – first for the main analyses with workers only and later for a subset of data which allows for a comparison of sexes. In case of significance, results of subsequent Tukey's HSD post-hoc tests are given (see Fig. 3.1 for P-values of significant interaction terms). A summary of the entire model selection statistics is provided in Tab. S3.1.

Species, sampling period and their interaction strongly influenced the *prevalence* of *C. bombi* (LRT: $\chi^2 = 105.25$, df = 12, $P < 0.0001$, $R^2_{GLMM(m / c)} = 0.16 / 0.34$; Fig. 3.1a, Tab. 3.2). All pairwise comparisons of species (including all sampling periods) revealed significant differences with *B. terrestris* showing the largest proportion of infected individuals (overall mean: 21.9%) compared to *B. lapidarius* (16.9%; Tukey's test: $z = 2.968$, $P = 0.008$; Fig. 3.1a) and *B. pascuorum* (10.8%; Tukey's test: $z = 5.100$, $P < 0.001$; Fig. 3.1a). *C. bombi* was also

significantly more prevalent in *B. lapidarius* than in *B. pascuorum* (Tukey's test: z = 3.375, P = 0.002; Fig. 3.1a). Concerning the sampling periods (including all species), markedly more individuals were infected in June (overall mean: 29.9%) than in July (15.4%; Tukey's test: z = 5.904, P < 0.001; Fig. 3.1a) and August (3.9%; Tukey's test: z = 3.045, P = 0.005; Fig. 3.1a). Furthermore, five out of 18 possible interaction terms were significant (Fig. 3.1a).

Species and sampling period, but particularly their interaction had a significant effect on the *type* of infection (LRT: $\chi^2 = 19.37$, df = 9, P = 0.002, $R^2_{GLMM(m/c)} = 0.09 / 0.26$; Tab. 3.2). The occurrence of multiple-strain infections was higher in *B. terrestris* than in *B. lapidarius* (Tukey's test: z = 2.698, P = 0.016) and marginally higher compared to *B. pascuorum* (z = 2.030, P = 0.093). Additionally, more multiple-strain infections were found in June than in July (z = 3.372, P = 0.0007). The *intensity* of infection differed between sampling periods (LRT: $\chi^2 = 6.28$, df = 7, P = 0.043, $R^2_{LR} = 0.08$) with fewer heavily infected individuals in July than in June (Tukey's test: z = -2.382, P = 0.040).

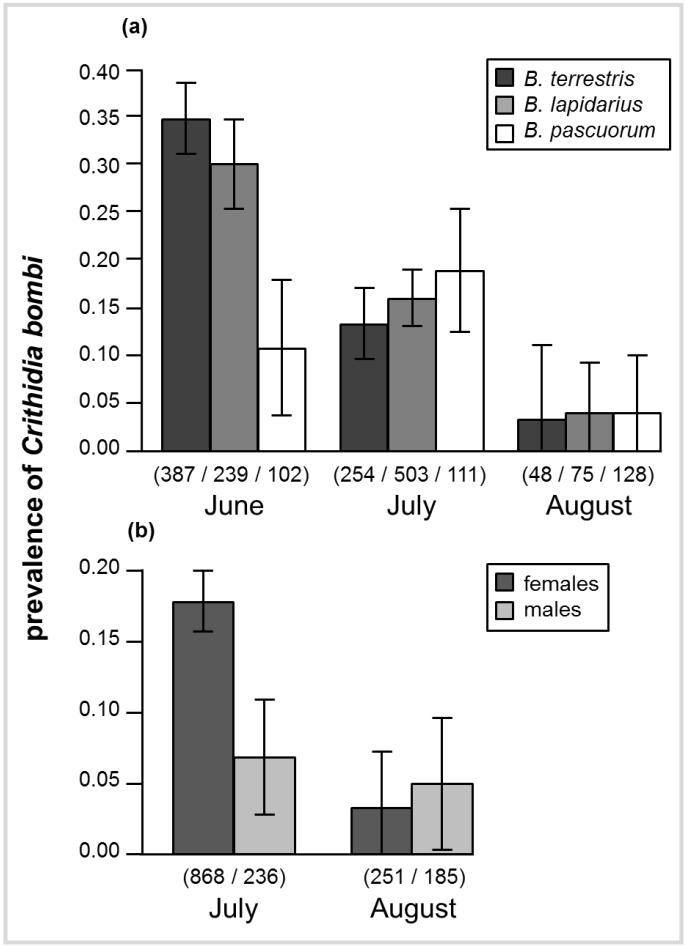

Figure 3.1. Results of Tukey's HSD post-hoc tests for the *prevalence* of *C. bombi*; **(a)** main analysis (females only), **(b)** comparison of sexes. Means ± SE are shown (n = 30 locations; individual sample sizes are given in brackets). Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*, f = females, m = males. P-values of interaction terms (significant values in bold) including direction of effect – **(a)**: TeJune > TeJuly **P < 0.01**, TeJune > TeAug **P = 0.044**, LaJune > LaJuly **P = 0.031**, TeJune > LaJune P = 0.057, TeJune > PasJune **P < 0.01**, LaJune > PasJune **P = 0.015**; **(b)**: fJuly > fAug **P < 0.001**, fJuly > mJuly P = 0.079.

Sampling period and sex markedly influenced *prevalence* and were found to interact (LRT: $\chi^2 = 43.39$, df = 6, $P < 0.0001$, $R^2_{GLMM(m)\,/\,(c)} = 0.11 / 0.27$; Tab. 3.2). More females (workers only) were infected compared to males (Tukey's test: $z = 2.359$, $P = 0.018$; means: 14.5% and 5.9%, respectively; Fig. 3.1b). Additionally, the prevalence of *C. bombi* was considerably higher in July than in August (Tukey's test: $z = 4.720$, $P < 0.0001$; Fig. 3.1b). One out of four possible interaction terms was significant (Fig. 3.1b).

With respect to the *type* of infection, no sex-specific differences in the distribution of single vs. multiple infections were detected. The final model including species was only marginally, but not significantly, better than the null model (LRT: $\chi^2 = 4.62$, df = 5, $P = 0.099$, $R^2_{GLMM(m)\,/\,(c)} = 0.04$). Species, sampling period and their interaction – rather than sex – significantly affected the *intensity* of infection (LRT: $\chi^2 = 14.88$, df = 9, $P = 0.011$, $R^2_{LR} = 0.09$; Tab. 3.2).

Table 3.2. Results of (Generalized) Linear Mixed Models of *C. bombi prevalence*, *type* (single vs. multiple strain(s)) and *intensity* of infection; **(a)** females only, **(b)** comparison of sexes. SP = sampling period; significant results are highlighted.

		***prevalence*[1]**			***type*[1]†**			***intensity*[2]**		
ANALYSIS	PREDICTOR VARIABLES*	**df**	**χ^2**	**P**	**df**	**χ^2**	**P**	**df**	**χ^2**	**P**
(a)	species : SP	8	19.58	**0.0006**	7	15.67	**0.0004**	-	-	-
	species	2	14.62	**0.0007**	2	1.46	0.483	-	-	-
	SP	2	55.63	**<0.0001**	1	1.62	0.203	-	-	-
(b)	sex : SP	5	5.03	**0.025**	-	-	-	-	-	-
	species : SP	-	-	-	-	-	-	7	5.31	0.070
	sex	1	2.63	0.105	-	-	-	-	-	-
	species	-	-	-	-	-	-	2	8.73	**0.013**
	SP	1	29.95	**<0.0001**	-	-	-	1	2.01	0.157

P-values were calculated from likelihood ratio tests following stepwise term removal from final models; * fixed effects, [1] GLMMs, [2] LMMs; † to ensure model convergence the third sampling period was excluded; dash = terms were not included in the final model / the final model did not contain an interaction term.

The effect of host colony density and genetic diversity on C. bombi infection

Only locations with at least ten genotyped workers were included in the population analyses. Based on 1,642 genotyped workers of *B. terrestris* (n = 605), *B. lapidarius* (n = 750) and *B. pascuorum* (n = 287), 396, 362 and 121 colonies could be reconstructed, respectively. All microsatellites were highly polymorphic in *B. terrestris* and *B. lapidarius* (except for BTMS0043 in *B. lapidarius* which was excluded) with an average of 9.44 ± 2.84 and 8.10 ± 2.92 alleles over all loci (means ± SD over all locations; Tab. 3.3), respectively. In *B. pascuorum*, an average of 4.01 ± 1.95 alleles was found (Tab. 3.3) which might be related to the small sample size. Observed and expected heterozygosities (H_O, H_E; overall means ± SD) are higher in *B. terrestris* and *B. lapidarius* (H_O : 0.75 ± 0.04 and 0.69 ± 0.05; H_E : 0.82 ± 0.04 and 0.79 ± 0.05, respectively; Tab. 3.3) compared to *B. pascuorum* (H_O : 0.57 ± 0.08; H_E : 0.62 ± 0.10; Tab. 3.3). The datasets for *B. terrestris* and *B. lapidarius* were similar in terms of the number of genotyped individuals, the overall distribution and presence of infected individuals per location (Tab. 3.1, Tab. 3.3), in contrast to *B. pascuorum* which was markedly smaller. To enhance power and comparability we excluded the smaller dataset, *B. pascuorum*, from multiple regression analyses investigating the impact of colony density and genetic diversity (H_E) on the prevalence and infection intensity of *C. bombi*.

Table 3.3. Summary of sampling data and derived genetic parameters per species, based on the female (i.e. worker) genotypes. Only locations with at least ten genotyped workers are included (cf. Tab S3.2). Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*, H_O / H_E = observed / expected heterozygosity, A_N = number of alleles over all loci. Means ± SD are shown.

Species (n locations)	**∑ Genotyped workers**	**Colonies observed (NSE*)**	**Colony density† (km²) ± SD**	**H_O ± SD**	**H_E ± SD**	**A_N ± SD**
Te (17)	605	396 (442)	27.38 ± 16.23	0.75 ± 0.04	0.82 ± 0.04	9.44 ± 2.84
La (20)	750	362 (125)	40.45 ± 13.84	0.69 ± 0.05	0.79 ± 0.05	8.10 ± 2.92
Pas (14)	287	121 (24)	16.77 ± 6.87	0.57 ± 0.08	0.62 ± 0.10	4.01 ± 1.95

*Non-sampling error = number of non-detected colonies (over all locations) based on the ECM method implemented in Capwire (Miller et al. 2005); †estimated colony density (km²) derived from the NSE and species-specific flight ranges of workers (Te: 758m, La: 450m, Pas: 449m; Knight et al. 2005).

Table 3.4. Results of multiple regressions on *prevalence* and *intensity* of *C. bombi* infection. Te = *B. terrestris* (n = 17 locations), La = *B. lapidarius* (n = 20 locations), **C** = Coefficient, cd = colony density (log10), H_E = expected heterozygosity, **R^2/ adj_R^2** = coefficient / adjusted coefficient of determination. Significant results are highlighted.

prevalence

Species	**C**	**Estimate ± SE**	**t-value**	**R^2**	**P**	**R^2**	**adj_R^2**	**F**	**df**	**P**
Te	cd	38.72 ± 10.07	3.843	0.22	**0.002**	0.63	0.58	11.93	2, 14	**0.0009**
	H_E	-342.93 ± 76.17	-4.502	0.41	**0.0005**					
La	cd	9.34 ± 17.88	0.522	0.05	0.608	0.15	0.05	1.49	2, 17	0.253
	H_E	43.96 ± 45.10	0.975	-0.12	0.343					

intensity

Species	**C**	**Estimate ± SE**	**t-value**	**R^2**	**P**	**R^2**	**adj_R^2**	**F**	**df**	**P**
Te	cd	2.65 ± 1.70	1.559	0.07	0.143	0.27	0.16	2.45	2, 13	0.125
	H_E	-23.21 ± 11.11	-2.089	0.20	0.057					
La	cd	1.50 ± 3.09	0.484	-0.01	0.634	0.06	-0.05	0.59	2, 17	0.566
	H_E	-8.30 ± 7.81	-1.063	0.07	0.303					

In *B. terrestris* (overall effects: $F_{2,14} = 11.93$, $P = 0.0009$, $R^2_{adjusted} = 0.58$; Tab. 3.4) high colony density was associated with high prevalence ($t = 3.843$, $P = 0.002$; Fig. 3.2a). Conversely, higher genetic diversity was related to lower prevalence ($t = -4.502$, $P = 0.0005$; Fig. 3.2c). In *B. lapidarius* (overall effects: $F_{2,17} = 1.49$, $P = 0.253$, $R^2_{adjusted} = 0.05$; Tab. 3.4), neither colony density ($t = 0.522$, $P = 0.608$; Fig. 3.2b) nor genetic diversity ($t = 0.975$, $P = 0.343$; Fig. 3.2d) was significantly associated with prevalence. Concerning the relationship of genetic diversity and prevalence, the correlation coefficients of *B. terrestris* and *B. lapidarius* (derived from the respective R^2-values in Tab. 3.4) were not different from each other (Fisher r-to-z transformation: $z = 1.09$, $P = 0.277$, two-tailed).

Regarding the intensity of infection no significant effects of either predictor could be shown (overall effects – *B. terrestris*: $F_{2,13} = 2.45$, $P = 0.125$, $R^2_{adjusted} = 0.16$; *B. lapidarius*: $F_{2,17} = 0.59$, $P = 0.566$, $R^2_{adjusted} = -0.05$; Tab. 3.4).

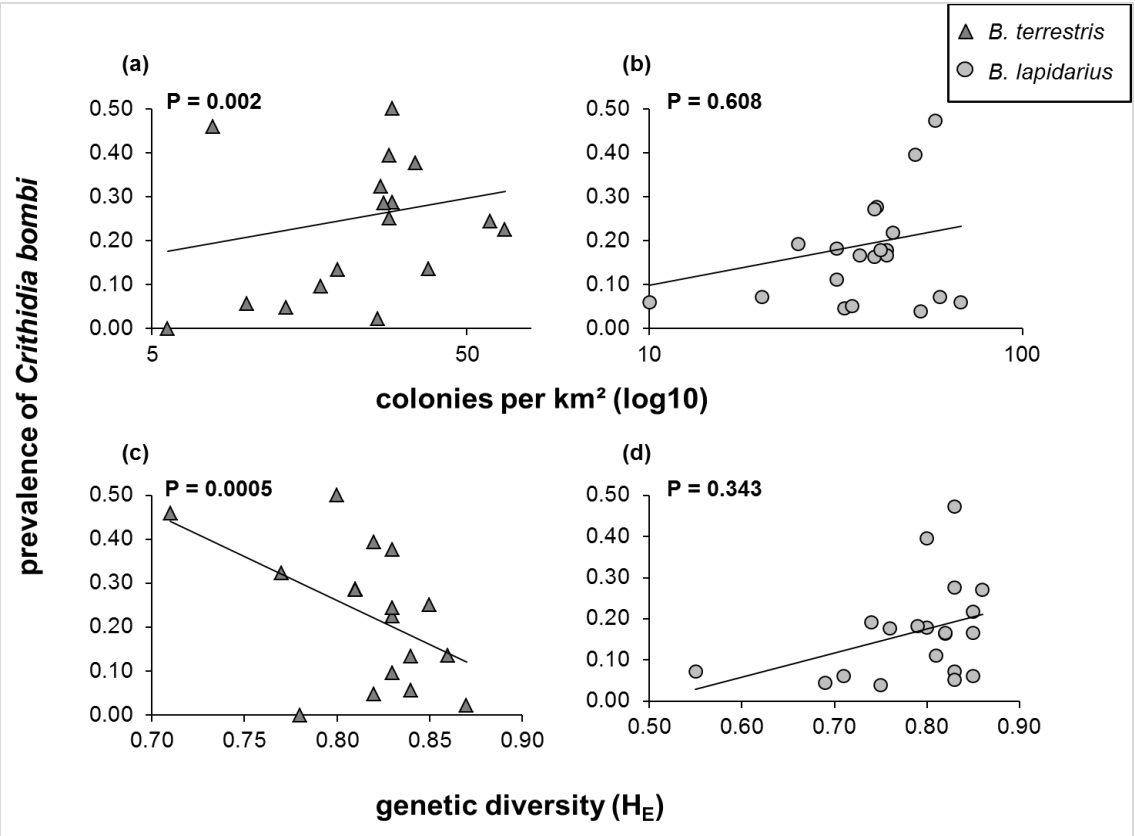

Figure 3.2. Prevalence of *C. bombi* in *B. terrestris* and *B. lapidarius* (n = 17 / 20 locations) in relation to **(a, b)** colony density (x-axes are log10-transformed) and **(c, d)** genetic diversity (H_E). Regression lines with associated P-values are derived from multiple regressions (Tab. 3.4).

Discussion

We find pronounced differences of *C. bombi* infections in natural bumblebee populations, with host species, sampling period and sex emerging as significant predictors of disease dynamics, particularly regarding *C. bombi prevalence*. With respect to *type* and *intensity*, no sex-specific differences could be detected, but we found the highest occurrence of multiple-strain infections in the early summer (June) and concentrated in *B. terrestris*.

Furthermore, for *B. terrestris* colony density was positively associated with prevalence whereas genetic diversity was negatively related to prevalence. Interestingly, these associations were not found for *B. lapidarius*. For both host species, neither colony density nor genetic diversity was linked to infection intensity. To our knowledge, this is the first study simultaneously showing an association of disease prevalence with colony density and genetic diversity, respectively.

Species, season and sex

Although *C. bombi* is a multi-host parasite of *Bombus* spp. (Shykoff & Schmid-Hempel 1991a, Schmid-Hempel & Tognazzo 2010, Erler et al. 2012, Ruiz-González et al. 2012) the parasite may encounter species-specific conditions that determine its growth rate and probability of transmission (Ruiz-González et al. 2012). In our study, *B. terrestris* and *B. lapidarius* were more abundant and showed higher prevalence rates (21.9% and 16.9%, respectively) compared to *B. pascuorum* (10.8%). Species-specific differences in *C. bombi* prevalence have been reported before (Shykoff & Schmid-Hempel 1991a) with higher prevalence in the more common species (Gillespie 2010, Ruiz-González et al. 2012), likely due to their higher probability to encounter parasites (Ebert 2008).

Additionally, parasite transmission via shared floral resources (Durrer & Schmid-Hempel 1994) could be accentuated in *B. terrestris* and *B. lapidarius* as they are short-tongued species and share many plant species for pollen and nectar provisioning, whereas *B. pascuorum* is equipped with a longer tongue and exhibits smaller resource overlap with the aforementioned species (Goulson & Darvill 2004). Recently, Salathé & Schmid-Hempel (2011) investigated whether the distribution of parasite genotypes is linked to ecological factors like resource overlap (i.e. the ecological hypothesis) or whether host species (i.e. the phylogenetic hypothesis) are a better predictor of host-parasite associations. In high-prevalence regions both factors equally contributed, but in low-prevalence regions shared floral resources were found to be more important. Ecological factors should therefore be considered in future studies examining the dynamics of host-parasite systems (Salathé & Schmid-Hempel 2011).

Furthermore, colony founding of *B. pascuorum* starts later in the season compared to *B. terrestris* and *B. lapidarius* (von Hagen & Aichhorn 2003) and the latter form larger colonies (Goulson 2010). As within-colony transmission increases with colony size (Schmid-Hempel 1998), this might also contribute to species-specific differences (Erler et al. 2012).

Alternatively, host-specific differences in disease prevalence may reflect differences in host susceptibility or resistance to *C. bombi* infection. Ruiz-González et al. (2012) demonstrated that almost half of the *B. terrestris* workers failed to establish an infection when inoculated with *B. pascuorum*-derived parasite cells, while strains gained from *B. terrestris* and *B. lucorum* successfully infected all *B. terrestris* workers. Under natural conditions the probability of self-infection was highest in *B. lapidarius*, lowest in the *B. terrestris* / *B. lucorum* complex, and intermediate in *B. pascuorum*. Thus, *B. lapidarius* may play a key role, since it served as infection source for the other host species (Ruiz-González et al. 2012). As *B. lapidarius* was abundant throughout the sampling locations exhibiting overall more homogenous prevalences, despite their larger ranges of colony densities and genetic diversity compared to *B. terrestris*, corroborates this species' role as disease reservoir.

Temporal effects appear to influence *C. bombi* prevalence, but the absence of general understanding concerning bumblebee colony development in natural populations (e.g. first occurrence of spring queens, foraging workers, males and gynes) or the impact of weather complicates meaningful comparisons between years and/or different studies. As a rule, infections build up over the course of a season in a density-dependent manner in accordance with the hosts' life cycle and colony performance, potentially showing a midsummer peak owing to a high proportion of colony mortality (Schmid-Hempel 1998). We found the highest proportion of infected bumblebees in June and the lowest in August. By contrast, Popp et al. (2012) detected the peak of infection in July, but comparisons between years are not straightforward. Furthermore, the population structure of *C. bombi* across years at a given location is highly dynamic (Salathé & Schmid-Hempel 2011, Ruiz-González et al. 2012, Erler et al. 2012). This might be due to the parasites' ability to reproduce clonally or sexually (Schmid-Hempel et al. 2011), and variability in transmission due to fluctuating floral resources and bumblebee communities (cf. Chpt. 2 / 4; Salathé & Schmid-Hempel 2011, Ruiz-González et al. 2012).

With respect to sex-related differences, we found 14.5% of the workers but only 5.9% of the males to be infected with *C. bombi*. These findings are in contrast to the haploid-susceptibility hypothesis that predicts a larger infection risk of males due to their lack of allelic variability at the individual level (O'Donnell & Beshers 2004). Though parasitism does not always differ between sexes (Ruiz-González & Brown 2006, Gillespie 2010), Murray et al. (2013) showed that males were more likely to harbour *Crithidia* infections. Nonetheless, our results are in accordance with those of Shykoff & Schmid-Hempel (1991a) which observed *C. bombi* prevalences of 39.6% and 26.3% in workers vs. males, respectively. Ruiz-González & Brown (2006) also found no empirical evidence to support the haploid-susceptibility hypothesis, in fact showing the opposite, that males were less susceptible and less likely to be infected. Interestingly, the reverse pattern was found for *Nosema bombi* – a harmful microsporidian parasite – in natural bumblebee populations (Shykoff & Schmid-Hempel 1991a, Gillespie 2010, Huth-Schwarz et al. 2012).

One explanation for those opposite sex-specific prevalences of the two parasites might be the reduced activity of workers infected with *N. bombi* (reviewed in Shykoff & Schmid-Hempel 1991a) which causes a male-biased sample (Murray et al. 2013), because males always leave the nest within a few days after eclosion (Sladen 1912, Goulson 2010). In contrast, workers infected with *C. bombi* continue foraging, but their flower visitation rate per minute declines with rising infection intensity due to increasing time needed to handle flowers (Otterstatter et al. 2005). Therefore, it may not be ploidy, but rather the sex-specific life-history differences of bumblebees (Shykoff & Schmid-Hempel 1991a) combined with the parasites' adaptation to the more frequent female hosts (Ruiz-González & Brown 2006) that may explain *C. bombi* prevalence. Longevity in workers and males is similar (Sladen 1912, Schmid-Hempel 1998) but males play a minor role in the colonies' everyday activities. Within-colony transmission of the parasite may therefore be attributable mostly to worker activity, as they have close contact with both infected nestmates and contaminated surfaces (Schmid-Hempel & Schmid-Hempel 1993, Otterstatter & Thomson 2007). Furthermore, worker infection risk is likely to increase due to the shared use of flowers during foraging (Durrer & Schmid-Hempel 1994), although foragers are also able to avoid flowers contaminated with cells of *C. bombi* (Fouks & Lattorff 2011).

Colony density and genetic diversity

We detected a positive association between colony density and *C. bombi* prevalence in *B. terrestris*, but the opposite pattern was recently shown for *B. terrestris* infected with *N. bombi* (Huth-Schwarz et al. 2012). We predict that high densities of suitable hosts at a given location may facilitate the transmission of *C. bombi*, perhaps due to enhanced contact at flowers during foraging (Durrer & Schmid-Hempel 1994).

Our study indicated that higher genetic diversity was associated with lower *C. bombi* prevalence in *B. terrestris* (but not in *B. lapidarius*). This is in agreement with Whitehorn et al. (2011), who found a similar pattern in *B. muscuorum*, and with several other studies showing that genetic diversity protects individuals, populations and communities from the spread of diseases (King & Lively 2012, Johnson et al. 2013b; i.e. the 'dilution effect' – Keesing et al. 2006, 2010; cf. Chpt. 4).

Conclusion

This study provides important insights into key factors – host diversity, host density and seasonal effects – and their relative contributions to disease prevalence of a widespread multi-host parasite in bumblebees. Here, we can show that genetic factors, like variation within and between species, differ resulting in decreased parasite prevalence when diversity is high. On the other hand, density effects promote parasite transmission, but may have a smaller effect than diversity. However, seasonality in prevalence might superimpose these effects, as high prevalence in *B. terrestris* and *B. lapidarius* is driven by high infection levels in early summer. Interestingly, for *B. lapidarius* neither associations for genetic diversity nor density effects were detectable, supporting its role as a species acting as a disease reservoir, maybe because it is rather disease tolerant.

Controlled experiments would help to disentangle the exact contribution of density- and diversity-mediated effects (Johnson et al. 2013b). Furthermore, profound knowledge about local species communities including the identification of key hosts dominating interspecific transmission would be highly beneficial for subsequent management of multi-host parasites (cf. Chpt. 4; Salkeld et al. 2013, Streicker et al. 2013).

Supporting Information for Chapter 3

Contents:

Appendix S3.1. Molecular discrimination of *B. terrestris* and *B. lucorum*.

Novel primers for the unambiguous discrimination of the resembling species *B. terrestris* and *B. lucorum* (Sladen 1912) were designed using the software Primer3 (Rozen & Skaletsky 2000) based on alignments produced by using Clustal X2 (Larkin et al. 2007) and utilizing all published sequences of cytochrome oxidase I (as of 21.09.2010). Species-specific primer pairs (BT: forward primer 5'-TTT ACC AGT ATT AGC CGG TG-3', reverse primer 5'-GCT GAT GTA AAA TAT GCT CGT-3' and BL: 5'-TTA ATT TTA TCT TTA CCA GTA TTA GCT G-3', 5'-TGG TGA GCT CAA ACA ATA AAT-3' forward and reverse primer, respectively) were selected based on the occurrence of SNPs in the 3'-end of the primer and on size differences of the final product (*B. terrestris* 290 bp; *B. lucorum* 330 bp).

Table S3.1. Summary of model selection statistics for *prevalence*, *type* (single vs. multiple strain(s)) and *intensity* of *Crithidia bombi* infection; **(a)** = females only, **(b)** = comparison of sexes. For each analysis a list of candidate models is given; SP = sampling period, ***** indicates main effects plus their interaction and **^2** means main effect plus all two-way interactions. **AIC** = Akaike's Information Criterion, **Delta** (ΔAIC) = difference in AIC value for model$_i$ compared to the top ranked model, **Weight** = Akaike weight of model$_i$, interpreted as the probability of model$_i$ being the best model (i.e. the minimal adequate model; lowest AIC) of the candidate models. Bold highlighted models are within 2 ΔAIC units of the best model and are considered to have substantial and equal model support ('top models').

(a) *prevalence*	**df**	**logLik**	**AIC**	**Delta**	**Weight**
SP * species	**12**	**-796.48**	**1616.97**	**0.00**	**1.00**
SP + species	8	-806.28	1628.55	11.58	0.00
SP	6	-813.58	1639.17	22.20	0.00
species	6	-834.09	1680.18	63.22	0.00
mnull	4	-849.11	1706.22	89.25	0.00
(b) *prevalence*					
sex * SP	**6**	**-506.87**	**1025.74**	**0.00**	**0.58**
sex + SP	5	-509.39	1028.77	3.03	0.13
sex * SP + species	8	-506.51	1029.02	3.28	0.11
SP	4	-510.70	1029.40	3.67	0.09
sex + SP + species	7	-509.07	1032.13	6.40	0.02
sex + SP + species + sex * SP + sex * species	10	-506.43	1032.85	7.12	0.02
sex + SP + species + sex * SP + SP * species	10	-506.44	1032.88	7.15	0.02
SP + species	6	-510.61	1033.21	7.48	0.01
sex + SP * species	9	-508.60	1035.21	9.47	0.01
sex * species + SP	9	-508.78	1035.57	9.83	0.00
SP * species	8	-510.28	1036.56	10.83	0.00
(sex + SP + species) ^2	12	-506.36	1036.72	10.98	0.00
sex + SP + species + sex * species + SP * species	11	-508.31	1038.63	12.89	0.00
sex * SP * species	14	-506.06	1040.11	14.38	0.00
sex + species	6	-521.08	1054.17	28.43	0.00
sex	4	-524.36	1056.72	30.99	0.00
sex * species	8	-520.50	1056.99	31.26	0.00
species	5	-526.14	1062.29	36.55	0.00
mnull	3	-528.56	1063.13	37.39	0.00
(a) *type*					
SP * species	**9**	**-219.13**	**456.25**	**0.00**	**0.97**
SP	5	-227.69	465.37	9.12	0.01
mnull	4	-228.81	465.62	9.37	0.01
species	6	-227.77	467.54	11.29	0.00
SP + species	7	-226.96	467.92	11.67	0.00
(b) *type*					
species	**5**	**-101.54**	**213.08**	**0.00**	**0.33**
mnull	**3**	**-103.85**	**213.69**	**0.62**	**0.24**
sex + species	**6**	**-101.18**	**214.36**	**1.28**	**0.18**
sex	**4**	**-103.27**	**214.54**	**1.46**	**0.16**
sex * species	8	-99.90	215.79	2.72	0.09

Table S3.1. Continued.

(a) *intensity*	**df**	**logLik**	**AIC**	**Delta**	**Weight**
SP	**7**	**-955.97**	**1925.94**	**0.00**	**0.45**
SP + species	**9**	**-954.54**	**1927.09**	**1.15**	**0.25**
species	7	-957.09	1928.18	2.24	0.15
mnull	5	-959.11	1928.22	2.28	0.14
SP * species	13	-954.07	1934.13	8.19	0.01
(b) *intensity*					
SP * species	**9**	**-462.25**	**942.49**	**0.00**	**0.24**
SP + species	**7**	**-464.90**	**943.81**	**1.31**	**0.12**
species	**6**	**-465.91**	**943.81**	**1.32**	**0.12**
sex + SP * species	**10**	**-462.10**	**944.20**	**1.71**	**0.10**
sex + SP + species + sex * SP + SP * species	11	-461.42	944.85	2.35	0.07
sex * species	9	-463.80	945.61	3.11	0.05
sex + species	7	-465.82	945.64	3.14	0.05
sex + SP + species	8	-464.89	945.78	3.29	0.05
sex * SP + species	9	-464.00	945.99	3.50	0.04
sex + SP + species + sex * SP + sex * species	11	-462.26	946.52	4.02	0.03
sex * species + SP	10	-463.30	946.60	4.10	0.03
sex + SP + species + sex * species + SP * species	12	-461.67	947.33	4.84	0.02
mnull	4	-469.69	947.38	4.88	0.02
sex * SP	7	-466.86	947.73	5.23	0.01
(sex + SP + species) ^2	13	-461.11	948.23	5.74	0.01
SP	5	-469.27	948.53	6.04	0.01
sex	5	-469.61	949.22	6.73	0.01
sex + SP	6	-469.27	950.53	8.04	0.00
sex * SP * species	15	-460.40	950.81	8.31	0.00

Table S3.2. Estimated colony density for **a)** *B. terrestris*, **b)** *B. lapidarius* and **c)** *B. pascuorum* per location. N_{ind} = number of genotyped individuals (workers); N_{obs} = number of colonies observed, derived from kinship reconstruction using Colony v. 2.0.5.0 (Wang 2004, Jones & Wang 2010). Model = best model (ECM or TIRM) identified by the likelihood ratio test (LRT) implemented in Capwire (Miller et al. 2005). N_{est} = number of estimated colonies, including the non-sampling error (NSE) from the TIRM / ECM method implemented in Capwire and 95% **C**onfidence **I**nterval; cd ECM = colony density per km², based on the NSE from the ECM method and species-specific flight ranges of workers (Knight et al. 2005). For locations with singletons only, no NSE and therefore no colony density estimation could be derived. NA = values without upper limit; locations with less than ten genotyped individuals per species were excluded from further analyses (non-bold entries).

a) *B. terrestris*

Location	N_{ind}	N_{obs}	Model	N_{est} TIRM [95% CI]	N_{est} ECM [95% CI]	cd ECM (km²)
GW_RB	**24**	**12**	**ECM**	**18 [12, 28]**	**14 [12, 19]**	**7.8**
GE_KK	6	4	ECM	6 [4, 15]	6 [4, 13]	3.3
GE_LB	9	7	ECM	NA	15 [7, 33]	8.3
Sgh_WR	**20**	**15**	**ECM**	**40 [19, 76]**	**31 [17, 88]**	**17.2**
Sgh_OD	**13**	**11**	**ECM**	**NA**	**35 [15, 74]**	**19.4**
Rö_SS	**61**	**35**	**ECM**	**59 [40, 78]**	**48 [36, 61]**	**26.7**
Rö_RÖ	**79**	**41**	**TIRM**	**70 [46, 88]**	**52 [42, 62]**	**38.9**
Rö_WL	**27**	**21**	**ECM**	**61 [31, 119]**	**49 [26, 108]**	**27.2**
Bd_SM	**18**	**13**	**TIRM**	**35 [14, 77]**	**24 [13, 71]**	**19.4**
Bd_GB	**23**	**21**	**ECM**	**NA**	**119 [43, 245]**	**66.1**
Bd_BD	**11**	**10**	**ECM**	**NA**	**51 [15, 51]**	**28.3**
Hal_HS	**61**	**36**	**ECM**	**61 [41, 84]**	**51 [38, 66]**	**28.3**
Hal_HE	**25**	**20**	**ECM**	**58 [28, 121]**	**52 [29, 142]**	**28.9**
Ad_AD	**53**	**36**	**ECM**	**72 [47, 101]**	**62 [44, 88]**	**28.9**
Vr_WS	4	3	ECM	NA	5 [3, 5]	2.8
Vr_VR	**35**	**30**	**ECM**	**119 [60, 302]**	**107 [54, 286]**	**59.4**
Vr_OB	**12**	**9**	**ECM**	**20 [9, 67]**	**18 [9, 62]**	**10.0**
Bs_AH	9	6	ECM	12 [6, 36]	9 [6, 33]	5.0
Fw_AP	**11**	**7**	**ECM**	**11 [7, 27]**	**10 [7, 24]**	**5.6**
Fw_FF	**53**	**32**	**ECM**	**63 [38, 84]**	**47 [36, 62]**	**26.1**
Fw_LW	**79**	**47**	**ECM**	**77 [54, 102]**	**68 [54, 86]**	**37.8**

Table S3.2. Continued.

b) *B. lapidarius*

Location	N_{ind}	N_{obs}	Model	N_{est} TIRM [95% CI]	N_{est} ECM [95% CI]	cd ECM (km²)
GW_RB	**46**	**21**	**ECM**	**28 [21, 43]**	**24 [21, 28]**	**40.0**
GW_KB	**77**	**32**	**ECM**	**40 [34, 54]**	**36 [32, 41]**	**60.0**
GW_AG	**65**	**25**	**ECM**	**30 [25, 39]**	**27 [25, 30]**	**45.0**
GE_KK	**34**	**21**	**ECM**	**39 [22, 59]**	**31 [23, 44]**	**51.7**
GE_BT	**61**	**24**	**ECM**	**28 [24, 38]**	**26 [24, 29]**	**43.3**
GE_LB	**41**	**21**	**ECM**	**31 [21, 44]**	**26 [21, 33]**	**40.6**
Sgh_ML	**21**	**13**	**TIRM**	**29 [13, 50]**	**19 [13, 35]**	**48.3**
Sgh_WR	**31**	**17**	**ECM**	**26 [17, 39]**	**22 [17, 31]**	**36.7**
Sgh_OD	**24**	**16**	**TIRM**	**36 [18, 68]**	**26 [16, 47]**	**60.0**
Rö_SS	**55**	**28**	**ECM**	**44 [29, 59]**	**35 [28, 45]**	**58.3**
Rö_RÖ	**44**	**21**	**ECM**	**29 [21, 45]**	**25 [21, 31]**	**41.7**
Rö_WL	9	8	ECM	NA	33 [9, 33]	55.0
Bd_SM	**10**	**8**	**ECM**	**NA**	**19 [8, 42]**	**31.7**
Bd_GB	**26**	**18**	**TIRM**	**44 [23, 71]**	**32 [20, 56]**	**73.3**
Hal_HS	**53**	**22**	**ECM**	**26 [22, 36]**	**24 [22, 28]**	**40.0**
Hal_HE	**22**	**12**	**ECM**	**18 [12, 29]**	**15 [12, 21]**	**25.0**
Ad_AD	**14**	**12**	**ECM**	**NA**	**41 [18, 86]**	**68.3**
Ad_LÖ	**43**	**18**	**TIRM**	**24 [18, 34]**	**20 [18, 23]**	**40.0**
Vr_VR	**10**	**7**	**ECM**	**13 [7, 46]**	**12 [7, 42]**	**20.0**
Bs_AP	4	3	ECM	NA	5 [3, 5]	8.3
Fw_FF	**18**	**6**	**TIRM**	**7 [6, 11]**	**6 [6, 6]**	**11.7**
Fw_LW	**55**	**20**	**ECM**	**24 [20, 32]**	**21 [20, 22]**	**35.0**

c) *B. pascuorum*

Location	N_{ind}	N_{obs}	Model	N_{est} TIRM [95% CI]	N_{est} ECM [95% CI]	cd ECM (km²)
GW_RB	**19**	**6**	ECM	**6 [6, 7]**	**6 [6, 6]**	**9.5**
GW_AG	**37**	**8**	TIRM	**8 [8, 8]**	**8 [8, 8]**	**12.7**
GE_KK	**29**	**14**	ECM	**18 [14, 30]**	**16 [14, 21]**	**25.4**
GE_LB	**10**	**4**	ECM	**4 [4, 6]**	**4 [4, 4]**	**6.3**
Sgh_OD	**11**	**6**	ECM	**10 [6, 20]**	**7 [6, 10]**	**11.1**
Rö_SS	**13**	**7**	TIRM	**12 [7, 23]**	**8 [7, 11]**	**19.0**
Rö_WL	4	3	ECM	NA	5 [3, 5]	7.9
Bd_BD	**12**	**8**	ECM	**16 [8, 33]**	**12 [8, 29]**	**19.0**
Hal_HS	**21**	**11**	ECM	**17 [11, 28]**	**13 [11, 16]**	**20.6**
Hal_BG	**41**	**18**	ECM	**23 [18, 34]**	**20 [18, 24]**	**31.7**
Ad_BÖ	9	6	TIRM	16 [6, 36]	9 [6, 33]	25.4
Vr_WS	**11**	**7**	ECM	**13 [7, 27]**	**10 [7, 24]**	**15.9**
Vr_OB	4	3	ECM	NA	5 [3, 5]	7.9
Bs_DB	**11**	**8**	ECM	**16 [8, 56]**	**15 [8, 51]**	**23.8**
Bs_AH	**30**	**10**	TIRM	**11 [10, 16]**	**10 [10, 10]**	**17.5**
Fw_AP	**10**	**6**	ECM	**10 [6, 22]**	**8 [6, 19]**	**12.7**
Fw_FF	**24**	**8**	TIRM	**11 [8, 17]**	**8 [8, 8]**	**17.5**

Chapter 4

The role of host species diversity and community composition for disease risk

Introduction

Biological diversity is vital to maintain ecosystem functioning and ecosystem services against the backdrop of a fluctuating environment (Naeem & Li 1997, Loreau et al. 2001). The insurance hypothesis (Yachi & Loreau 1999) states that biodiversity serves as a buffer in case of the decline or loss of single species. Enhanced species richness per functional group (Naeem & Li 1997) insures the resilience of ecosystems, including delicate species interactions (Bartomeus et al. 2013), through an increase of mean productivity and reduced variance of productivity over time (Yachi & Loreau 1999). The variability in response of functionally redundant species ('response diversity'; e.g. Elmqvist et al. 2003) towards different environmental changes is one of the underlying mechanisms.

Global loss of biodiversity (Cardinale et al. 2012) and emerging infectious diseases (EIDs) (Jones et al. 2008) are therefore key challenges of the 21st century, seriously jeopardizing human and wildlife wellbeing (Binder et al. 1999, Morens et al. 2004, Siddle et al. 2007, Fürst et al. 2014). Mounting evidence suggests tight links between the two issues (Johnson et al. 2008, Pongsiri et al. 2009, Keesing et al. 2010). Hence, the diversity-disease relationship has recently gained increasing attention (Johnson & Thieltges 2010, Haas et al. 2011).

The dilution effect hypothesis implies that the net effect of enhanced biological diversity (inclusive host and non-host species) decreases the risk of specific infectious diseases in ecological communities (Keesing et al. 2006). Likewise, the term 'monoculture effect', originating from agricultural research, describes the relationship between low diversity and high disease prevalence (Garrett & Mundt 1999; reviewed in King & Lively 2012). Crops are cultured to maximise yield, resulting in lack of genetic variability in contrast to their wild ancestors (Wolfe 2000, King & Lively 2012). Therefore, monocultures are particularly prone to epidemics with fatal consequences like the Irish potato famine in the 1840s (reviewed in

Yoshida et al. 2013) or rice blast (Zhu et al. 2000). Parallel evidence is gained from animal host-systems. In monoclonal *Daphnia* populations parasites spread faster (Altermatt & Ebert 2008) and prevalences were higher (Ganz & Ebert 2010) compared to host 'polycultures'. Moreover, monospecific communities of larval amphibians suffered from increased mortality, higher levels of limb malformation and a delay of metamorphosis when exposed to the virulent parasite *Ribeiroria ondatrae* (Johnson et al. 2008), whereas heterospecific communities strongly inhibited parasite transmission, thereby diminishing host pathology (Johnson et al. 2008, 2013b).

Conversely, increased diversity may enhance disease risk (i.e. the amplification effect), e.g. in cases with higher inter- than intraspecific transmission or when further species serve as an additional source of infection (reviewed in Keesing et al. 2006). Furthermore, positive correlations between host and parasite diversity were found (Hechinger & Lafferty 2005, Johnson & Thieltges 2010, Lafferty 2012), but one has to be cautious not to simply equate parasite diversity with disease risk (Johnson et al. 2013a). Although some studies ascertained a simple association between host diversity and infection (e.g. Altermatt & Ebert 2008, Ganz & Ebert 2010), the diversity-disease relationship is multifaceted and scale dependent (Wood & Lafferty 2013). The latter is specifically true for more complex host-parasite interactions (Roche & Guégan 2011).

Most parasites exploit a range of host species (Cleaveland et al. 2001, Taylor et al. 2001) and turned out not to be specialists but rather generalists (Woolhouse et al. 2001, Rigaud et al. 2010). Alternatively, host species differ in their worth to parasites as they vary in quality (i.e. competence: Johnson et al. 2008, 2013b; e.g. measured by parasite growth rate and the amount of transmission stages produced) and exhibit asymmetric inter- and intraspecific transmission potential (Ruiz-González et al. 2012). Therefore, species identity and host community composition are of crucial importance in order to understand diversity-disease relationships (LoGiudice et al. 2008, Roche & Guégan 2011, Salkeld et al. 2013, Streicker et al. 2013) and are thought to be even more appropriate than biodiversity per se (Randolph & Dobson 2012). As the relative abundance of the most competent host declines with rising host diversity, an increasing level of 'wasted' transmission takes place (Begon 2008, Johnson et al. 2008). Consequently, density- and diversity-mediated effects have to be disentangled carefully (Johnson et al. 2008) to ascertain the reason for reduced infection (Begon 2008).

Although theoretical framework (Dobson 2004, Begon 2008, Keesing et al. 2006) and experiments (e.g. Johnson et al. 2008; reviewed in Johnson & Thieltges 2010) recently shed light on the interrelationship of diversity and disease, insights into complex natural host-parasite systems are still rare (Rigaud et al. 2010; but see Ruiz-González et al. 2012, Johnson et al. 2013b). Empirical evidence suggests that dilution effects are common under

natural conditions, but more field studies are needed to assess the importance for other host-parasite systems (e.g. for non-vector-borne diseases; Johnson & Thieltges 2010). Due to the spatiotemporal heterogeneity in local species diversity, Roche & Guégan (2011) emphasize the need to incorporate many different communities at various periods of time to obtain considerable knowledge about the interaction between regional disease dynamics and host communities. Therefore, we performed an extensive field study using a well-established model system, the intestinal trypanosome *Crithidia bombi* (Gorbunov 1987, Lipa & Triggiani 1988) which infects multiple bumblebee species (*Bombus* ssp.; Shykoff & Schmid-Hempel 1991a, Sadd & Barribeau 2013).

Bumblebees are ecologically and economically relevant as they provide effective pollination of crops and wild plants (Kremen et al. 2007, Garibaldi et al. 2013) which is a key ecosystem service crucial to human wellbeing (Klein et al. 2007). Parasites and EIDs (Meeus et al. 2011, Fürst et al. 2014) have proved to contribute to the global decline of pollinators during the past decades (Biesmeijer et al. 2006, Potts at al. 2010, Cameron et al. 2011). Owing to the high density of closely related commonly interacting individuals within a colony, disease spreads quickly, posing a severe danger to social insects like bumblebees (Schmid-Hempel 1998, 2001).

C. bombi is widespread in natural bumblebee populations and infects adults of all castes and sexes (Shykoff & Schmid-Hempel 1991a). Successful parasite establishment rises with the dose of cells ingested by the host (Ruiz-Gonzaléz & Brown 2006), and microsatellite analyses revealed the coincidence of numerous *C. bombi* genotypes within populations, colonies and individuals (Schmid-Hempel & Reber Funk 2004, Erler et al. 2012, Popp et al. 2012). The parasite is directly transmitted within colonies through contact with infected nestmates or contaminated surfaces (Schmid-Hempel & Schmid-Hempel 1993, Otterstatter & Thomson 2007) as well as between colonies (intra- and interspecifically) via shared floral resources (Durrer & Schmid-Hempel 1994; but see Fouks & Lattorff 2011).

We aim at gaining further understanding about differences in disease risk along gradients of local species richness / diversity and species composition (Keesing et al. 2006, Roche & Guégan 2011). As postulated by Roche & Guégan (2011), we selected a generalist parasite and examined the diversity-disease relationship across various bumblebee species due to their potential as reservoir species.

We hypothesize that at the location level

i. Host species abundance, as a measure of density, is associated with increasing rates of *C. bombi* prevalence and a larger proportion of multiple-strain infections.

ii. Host species richness and diversity are negatively related to prevalence and the proportion of multiple-strain infections.

iii. Host community composition (including species identity) is related to disease outcome (i.e. prevalence).

Material and methods

Sampling

Workers (n = 1,953) and males (n = 461) of seven social bumblebee species (*Bombus lapidarius*, *B. terrestris*, *B. pascuorum*, *B. hortorum*, *B. lucorum*, *B. pratorum*, *B. ruderarius*) and males (n = 132) of two cuckoo bumblebee species (*Bombus* cf. *vestalis*, *B. rupestris*) were collected in semi-natural and agricultural habitats in Germany. A west-east transect was established with ten sites (max. distance: 311 km), each comprising three locations (Tab. S4.1; cf. Tab. 3.1). The distance between sampling locations is 5.2 km ± 2.4 km (mean ± SD), exceeding the expected foraging ranges of different bumblebee species (Goulson 2010). Each location was sampled during sunny weather in a random order three times (June, July, August 2010). Time of day was also randomized to reduce biased data. Individuals were stored at -20°C prior to DNA extraction. After initial species identification in the field, individuals were double-checked for sex and species identity following the taxonomic key of (Mauss 1994) and with the help of a sequenced subsample. Details on species identification within the *B. terrestris* / *B. lucorum* complex are given in Appendix S3.1.

DNA analysis

CRITHIDIA BOMBI

After the removal of each bumblebee's gut, DNA extraction was done following a modified Chelex protocol (Walsh et al. 1991, Erler & Lattorff 2010). Four polymorphic microsatellite loci were genotyped (Cri 4, Cri 1 B6, Cri 4.G9, and Cri 2.F10; Schmid-Hempel & Reber Funk 2004) using fluorescence labelled primers (Metabion International AG, Martinsried, Germany). All loci were amplified in one multiplex PCR following the protocol of Popp & Lattorff (2011). The final volume of 10 µl contained 1 µl template DNA, 5 µl PCR Master Mix

(Promega Corporation, Madison/WI, USA), 0.3 µM (Cri 1 B6, Cri 4.G9), 0.6 µM (Cri 4, Cri 2.F10) per primer pair and 2.2 µl ddH_2O. PCR products were visualized with an automated capillary sequencer (MegaBACE 1000, GE Healthcare, Munich, Germany) according to manufacturer's instruction and a standard protocol (Erler & Lattorff 2010). Allele sizes were scored using Fragment Profiler v1.2 after visual inspection of the processed raw data. As *C. bombi* is a diploid organism (Schmid-Hempel & Reber Funk 2004), more than two peaks per locus indicate an infection of the individual host with more than one strain (i.e. multiple infection).

Statistical analyses

C. BOMBI INFECTIONS, HOST SPECIES ABUNDANCE, RICHNESS AND DIVERSITY

Pearson's product-moment correlation and Spearman's rank-order correlation were applied to test for associations between host species abundance, richness and diversity (predictor variables) and the parasite prevalence as well as the proportion of multiple-strain infections (response variables). Beforehand, the data were inspected visually for normal distribution by means of histograms, boxplots, QQ-Plots and Shapiro-Wilk normality tests were performed. Moreover, Pearson correlations were run for the three most abundant species, *B. terrestris*, *B. lapidarius* and *B. pascuorum*, to inspect the relationship between the number of individuals caught (workers and males) and the number of colonies reconstructed (workers only; cf. Chpt. 3; Tab. S3.2) at the location level, thereby controlling for a potential sampling bias. In order to control for potential abundance-mediated effects on host species richness and / or diversity, Spearman and Pearson correlations were performed, respectively. All analyses were conducted using R 2.15.3 (R Core Team 2013) and the packages Hmisc (v3.13-0, Harrell et al. 2013) and vegan (v2.0-10, Oksanen et al. 2013).

The consideration of non-sampled species (hereafter 'unseen' species) due to finite sample sizes, also belonging to the local species pool, may be important to safeguard against spurious results. Therefore, the CHAO-method of the function *estimateR* (vegan, v2.0-10; Oksanen et al. 2013) was used for the calculation of the expected species richness (Tab. S4.2). The Shannon diversity index (hereafter 'D'), which simultaneously embraces richness and eveness (Poulin 2015), served as measure of species diversity and was calculated as

$$D = \sum_{i=1}^{S} -(Pi * lnPi) \quad (4.1)$$

where P_i is the fraction of the entire population made up of species *i* and S is the number of species encountered.

The association of local *C. bombi* prevalences with host species abundance, richness and diversity as well as the proportion of multiple infections (response variables) was inspected. Therefore, locations with 'low' versus 'high' prevalences (<0.10 and >0.25; n = 7 each, respectively) were grouped together (cf. Tab. S4.1). Beforehand, locations with less than 1% of individuals of the overall sample where excluded. Finally, each of the response variables of both groups ('low' vs. 'high' prevalence) was compared via one-tailed Mann-Whitney U Test.

C. BOMBI INFECTIONS, HOST COMMUNITY COMPOSITION AND HOST SPECIES IDENTITY

Prior to further tests, hierarchical clustering (function *hclust*, package fastcluster, v.1.1.13; Müllner 2013) was used to rule out geographical aggregation concerning the observed host community composition (the relative abundance per host species and location was used).

To investigate the relationship between local *C. bombi* prevalences and the respective host species composition, the same prerequisites as before – concerning 'low' vs. 'high' prevalences – were created (cf. Tab. S4.1). Afterwards however, the proportions of the three most common species (*B. terrestris*, *B. lapidarius*, *B. pascuorum*) and the pooled proportion of all less frequent bumblebee species (hereafter 'Other') (response variables) were explored using Principal Component Analysis (PCA, function *princomp*; R Core Team 2013) and subsequent Pearson correlations.

Results

A strong positive relationship between the number of individuals caught (workers and males) and the number of colonies reconstructed (workers only) per location was found for the three most abundant species (Pearson correlation: *B. terrestris* – $t = 16.88$, $df = 25$, $r = 0.96$, $P < 0.0001$; *B. lapidarius* – $t = 7.67$, $df = 22$, $r = 0.85$, $P < 0.0001$; *B. pascuorum* – $t = 7.29$, $df = 20$, $r = 0.85$, $P < 0.0001$). Therefore, the number of individuals sampled per location is supposed to be representative which was also transferred to the less abundant bumblebee species were no data on kinship relations is available.

Information on the number of unseen species (expected species richness) per location is provided in Tab. S4.2. As the observed and expected species richness are markedly related (Spearman correlation: $S = 116.51$, $r_s = 0.97$, $P < 0.0001$), observed species richness was used for subsequent analyses.

With respect to potential abundance-mediated effects, neither species richness (Spearman correlation: s = 3044.36, r_s = 0.32, P = 0.082) nor species diversity (Pearson correlation: t = 0.28, df = 28, r = 0.05, P = 0.78) increased with rising bumblebee abundance per location.

Infection with C. bombi

In total, 2,546 individuals of *B. lapidarius* (n = 1,030), *B. terrestris* (n = 861), *B. pascuorum* (n = 378), *B. hortorum* (n = 73), *B. lucorum* (n = 47), *B. pratorum* (n = 18), *B. ruderarius* (n = 7), *Bombus* cf. *vestalis* (n = 88) and *B. rupestris* (n = 44) were included in the analyses (Tab. S4.1, Fig. 4.1). 449 bumblebees were infected (single / multiple infection: n = 301 / 148).

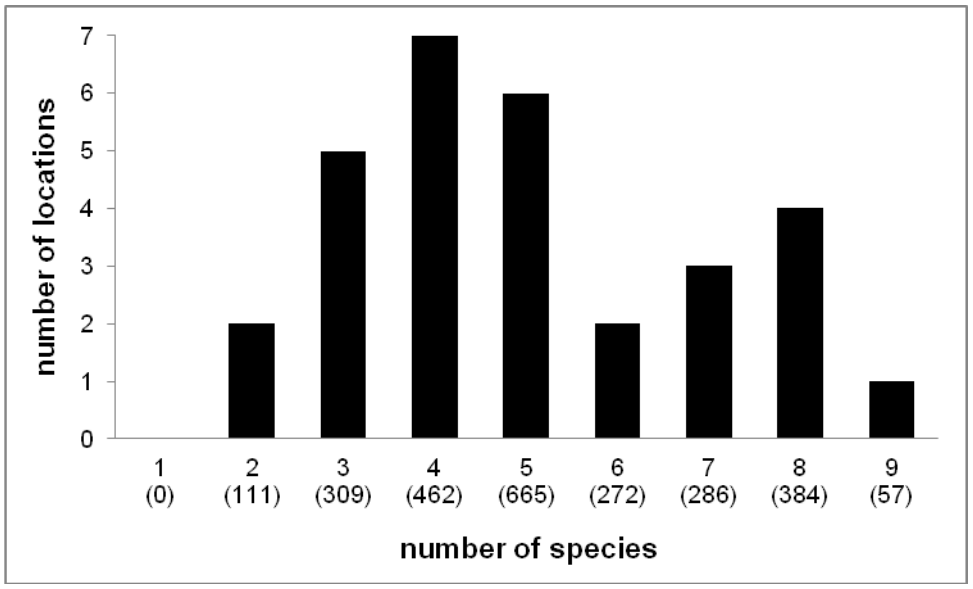

Figure 4.1. Number of sampled bumblebee species detailing the number of locations. N = 30 locations; individual sample sizes are given in brackets.

Host species abundance, richness and diversity

Except for a positive trend, bumblebee abundance was not associated with *C. bombi* prevalence (Pearson correlation: t = 1.26, df = 28, r = 0.23, P = 0.220). Likewise, no relationship was found between abundance and the proportion of multiple infections (t = 0.74, df = 28, r = 0.14, P = 0.465).

Regarding species richness, a negative relationship with prevalence occurred (Spearman correlation: S = 6082.77, r_s = -0.35, P = 0.056; Fig. 4.2a) whereas increasing species diversity was marginally – but not significantly – related to decreasing prevalence (Pearson correlation: t = -1.78, df = 28, r = -0.32, P = 0.086; Fig. 4.2b).

A weak negative association between species richness and the proportion of multiple infections (S = 5558.44, r_s = -0.24, P = 0.208; Fig. 4.2c) was found. Species diversity and the proportion of multi-strain infections were negatively correlated (t = -2.44, df = 28, r = -0.42, P = 0.021; Fig. 4.2d).

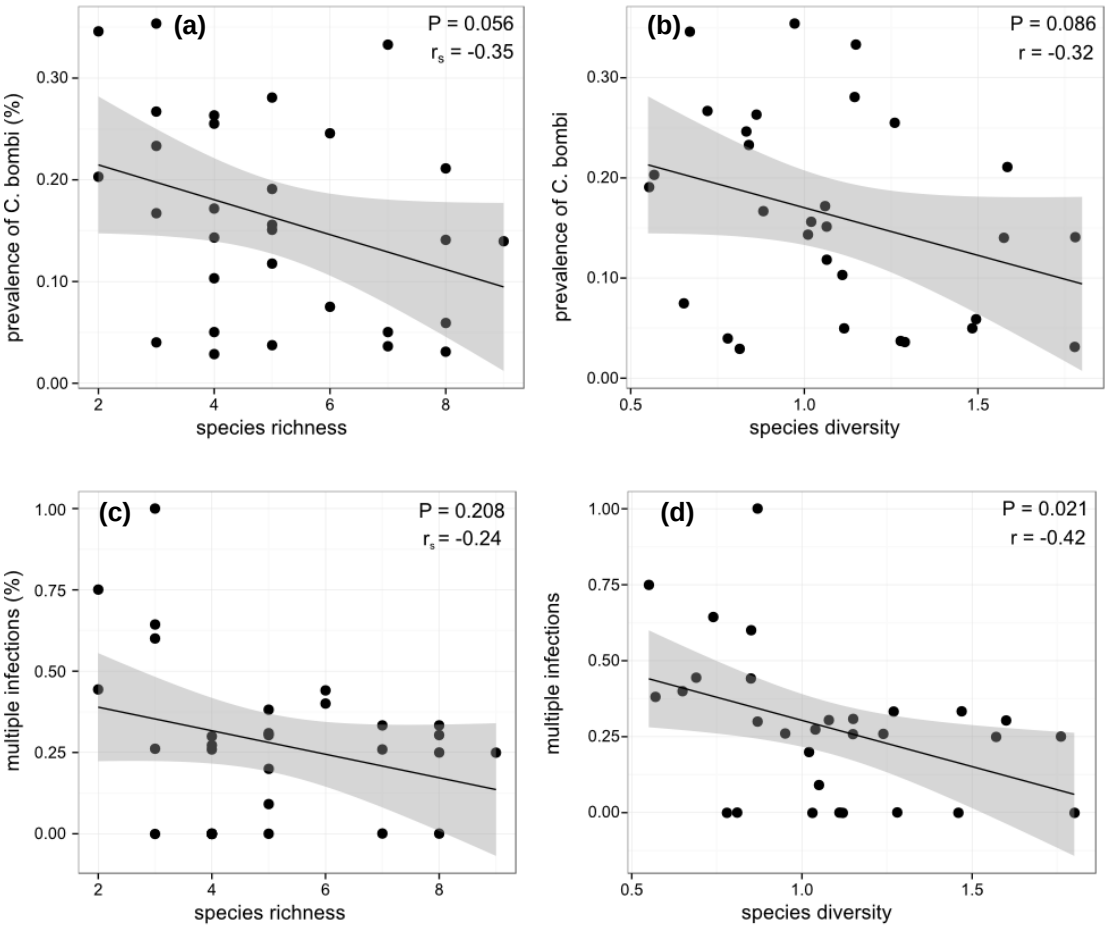

Figure 4.2. *C. bombi* prevalence **(a / b)** and proportion of multiple infections **(c / d)** in relation to species richness **(a / c)** and species diversity **(b / d)** per location (n = 30). Line of best fit with associated P-value and 95% CI (dark grey) are derived from Spearman's rank-order correlation / Pearson's product-moment correlation; r_s / r = Spearman's / Pearson's correlation coefficient.

Host community composition and host species identity

With respect to the observed host community composition of the 30 different locations, prior hierarchical clustering ruled out geographical aggregation. Inspecting the relationship between the proportion of the three most common bumblebee species and the respective *C. bombi* prevalence of the remaining species, no association was found for *B. terrestris* (Pearson correlation: t = -0.15, df = 28, r = -0.03, P = 0.879; Fig. 4.3a). In contrast, an increasing proportion of *B. lapidarius* was related to increased prevalences (t = 2.38, df = 28, r = 0.41, P = 0.025; Fig. 4.3b). With respect to *B. pascuorum*, a negative trend was observed (t = -1.40, df = 28, r = -0.26, P = 0.173; Fig. 4.3c). Additionally, again a negative trend occurred inspecting the proportion of the 'Other' bumblebee species and the prevalence of the three most common species (t = -1.85, df = 28, r = -0.33, P = 0.074; Fig. 4.3d).

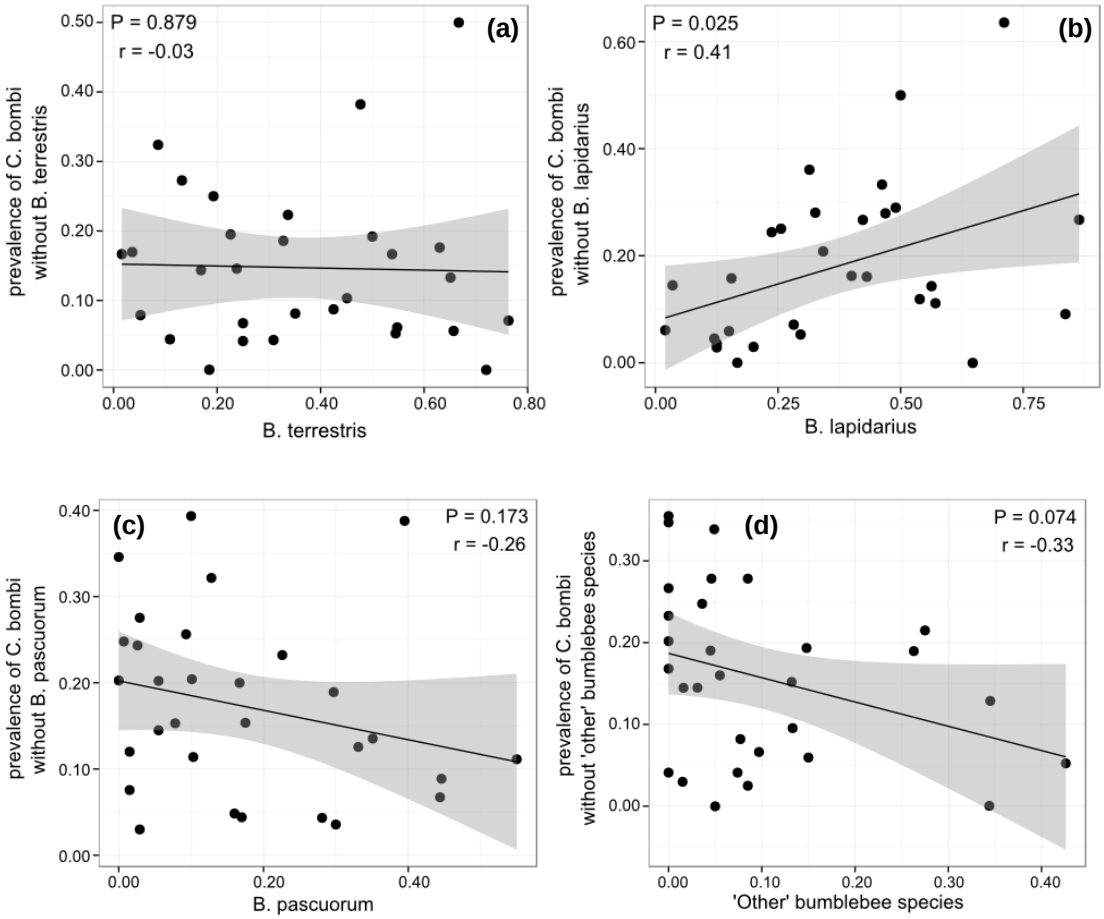

Figure 4.3. *C. bombi* prevalence in relation to the proportion of the three most common species, **(a-c)** *B. terrestris*, *B. pascuorum*, *B.* lapidarius and **(d)** the remaining bumblebees species per location (n = 30). Line of best fit with associated P-value and 95% CI (dark grey) are derived from Pearson's product-moment correlation; r = Pearson's correlation coefficient. Note different scales.

Low versus high prevalence

When comparing locations of 'low' versus 'high' prevalence (n = 7 each; Tab 4.1), the observed species richness was markedly smaller in the 'high'-prevalence group (Fig. 4.4a). This is also true when controlling for unseen species (expected species richness; Fig. 4.4b). Species diversity was marginally smaller in the 'high'-prevalence group (Fig. 4.4c).

The results for species abundance and the proportion of multiple infections were also in accordance with the expected direction but without showing significant differences between the 'low'- and 'high'-prevalence group (Tab. 4.1).

Table 4.1. Results of one-tailed Mann-Whitney U Tests comparing locations with 'low' (<0.10; n = 7) vs. 'high' (>0.25; n = 7) prevalences. Significant results are highlighted.

RESPONSE VARIABLES	Expected direction of effect ('low' vs. 'high')	**one-tailed MWU**	
		W	**P**
Species abundance	<	18	0.228
Observed species richness	>	42.5	**0.012**
Expected species richness	>	40	**0.027**
Species diversity	>	37	0.062
Multiple infections (%)	<	15	0.122

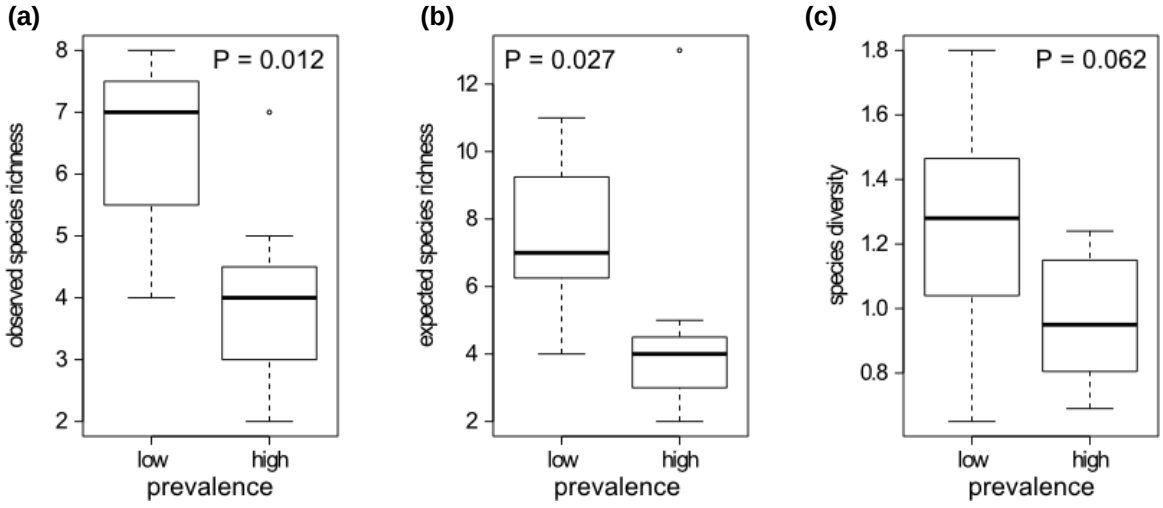

Figure 4.4. Comparison of locations with 'low' (<0.10; n = 7) vs. 'high' (>0.25; n = 7) prevalence rates, **(a / b)** observed / expected species richness and **(c)** species diversity. P-values are derived from one-tailed Mann-Whitney U Tests (cf. Tab. 4.1). Boxplots: line = median, box = interquartile range, whiskers = data range.

With respect to the host community composition, the principal component analysis (PCA) separated locations of 'low' vs. 'high' *C. bombi* prevalence according to their particular proportion of the three most common species (*B. terrestris*, *B. lapidarius*, *B. pascuorum*) and the pooled proportion of the 'Other' bumblebee species. The first and second components account for 57.1% and 31.8% of the variation in the host community composition, respectively (Fig. S4.1). Component one is negatively correlated with the proportion of *B. pascuorum* and the 'Other' bumblebee species (Pearson correlation: $t = -6.92$, $df = 12$, $r = -0.89$, $P < 0.0001$ and $t = -5.87$, $df = 12$, $r = -0.86$, $P < 0.0001$, respectively). In contrast, the proportion of *B. lapidarius* was positively correlated with the first component ($t = 4.03$, $df = 12$, $r = 0.76$, $P = 0.002$), whereas no association with *B. terrestris* was found ($t = 1.55$, $df = 12$, $r = 0.41$, $P = 0.147$). Component two is positively correlated with the proportion of *B. terrestris* but negatively associated with *B. lapidarius* ($t = 7.64$, $df = 12$, $r = 0.91$, $P < 0.0001$ and $t = -2.98$, $df = 12$, $r = -0.65$, $P = 0.01$, respectively). No correlation was found for both *B. pascuorum* and the 'Other' species and the second component ($t = -0.05$, $df = 12$, $r = -0.01$, $P = 0.964$ and $t = -0.45$, $df = 12$, $r = -0.13$, $P = 0.661$, respectively). Overall, locations of 'low' vs. 'high' prevalence are predominantly composed of distinct bumblebee communities. Consistent with prior analyses (cf. Fig. 4.3), high prevalences tend to be linked with high *B. lapidarius* abundance, whereas low prevalences tend to be linked with higher proportions of *B. pascuorum* and the 'Other' bumblebee species.

Discussion

In this study we tested the dilution effect hypothesis by investigating local diversity-disease relationships in natural bumblebee populations and their intestinal parasite *Crithidia bombi*. Furthermore, the role of host species abundance and community composition was inspected.

Bumblebee abundance was neither related to *C. bombi* prevalence nor associated with the proportion of multiple infections. Host species richness was negatively correlated with prevalence, whereas a weak negative relationship with the amount of multiple infections was found. While enhanced species diversity was marginally associated with parasite prevalence, a negative relationship with the proportion of multiple infections was detected. The additional comparison of 'low'- vs. 'high'-prevalence locations revealed pronounced differences with lower species richness in case of 'high' prevalences. In contrast, species diversity was only marginally smaller in the 'high'-prevalence group.

Concerning host community composition (including species identity), a high proportion of *B. lapidarius* was related to increased prevalences of the remaining host species whereas a

negative trend was found for *B. pascuorum*, and no association with *B. terrestris* occurred. Apart from a negative trend, an increasing proportion of 'Other' bumblebee species was not associated with *C. bombi* prevalence of the three most common host species. Furthermore, the comparison of 'low'- vs. 'high'-prevalence locations unfolds the dissimilarity of the respective bumblebee communities, further emphasising the aforementioned findings.

Host species abundance, richness and diversity

Due to the high density of closely related frequently interacting individuals within a colony, transmission is facilitated and disease spreads quickly, posing a severe danger to social insects like bumblebees (Schmid-Hempel 1998, 2001). This applies to *B. terrestris* at the colony / population level, but for a second species (*B. lapidarius*) only a positive trend occurred (cf. Chpt. 3). However, in contrast to our expectation, overall bumblebee abundance (i.e. the community level) was not related to prevalence. Likewise, no association with the proportion of multiple-strain infections was found. In addition to intra-specific transmission heterogeneity, multi-host parasites are often faced with host species that vary regarding susceptibility, contact rates and host competence causing interspecific transmission heterogeneity (Paull et al. 2012, Johnson et al. 2008, 2013b, Woolhouse et al. 1997). Recently, evidence of such asymmetric intra- and interspecific transmission potential was provided for the *Bombus-Crithidia* system (Ruiz-González et al. 2012) and might explain our findings.

Diversity-disease relationships turned out to be multifaceted and scale dependent (Wood & Lafferty 2013), particularly in more complex host-parasite systems (Roche & Guégan 2011). Therefore, increased biological diversity can either diminish or facilitate pathogen transmission, hence disease risk in ecological communities (dilution - vs. amplification effect; Keesing et al. 2006, 2010). Nevertheless, recently empirical evidence for increased transmission events and disease incidence, in case of biodiversity loss, accumulates across various host-parasite systems (Johnson & Thieltges 2010, Keesing et al. 2010, LoGiudice et al. 2003). For instance experimental evidence of increased disease spread and higher prevalences in monoclonal *Daphnia* populations as opposed to 'polycultures' was provided by Altermatt & Ebert (2008) and Ganz & Ebert (2010), respectively.

Our results are also largely in line with the dilution effect hypothesis rather than supporting the amplification effect, as a negative association between host species richness and prevalence of the multi-host parasite *C. bombi* as well as a marginal (but not significant) negative correlation between species diversity and prevalence occurred. The consideration of the number of unseen species locally (expected species richness) additionally underpins our results because overall the Chao method applied proved to be one of the least biased and most precise

measures, also with respect to rare species (reviewed in Poulin 2015). Concerning host species diversity, the Shannon diversity index was selected because it simultaneously embraces richness and evenness. Beside the Simpson's diversity, it belongs to the oldest and most widely used diversity indices and both are strongly correlated with each other when applied to the same species community (Poulin 2015), which is also true for our data (not shown).

Regarding the proportion of multiple-strain infections, a weak negative association with species richness was found, whereas increased species diversity was related to decreased amounts of multiple infections. Parasite transmission is concurrently influenced by host physiology, immunity, behaviour and ecology and in case of multi-host parasites, within-species as well as between-species transmission need to be incorporated (Dobson 2004). Additionally, genotype by genotype interactions of hosts and parasites (G_H x G_P) determine the success of parasite infection and transmission, (Mallon et al. 2003, Schmid-Hempel 2001) with several colonies of *B. terrestris* being resistant to the majority of *C. bombi* strains, while others are susceptible to virtually every strain (Schmid-Hempel & Schmid-Hempel 1993, Schmid-Hempel et al.1999, Mallon et al. 2003, Schmid-Hempel & Reber Funk 2004). Accordingly, heterogeneity in host susceptibility defines the subset of strains that will be transmitted to the next host (i.e. "strain filtering", Ulrich et al. 2011). If decreased parasite diversity is furthermore confronted with increased host diversity, the probability of a mismatch and therefore failure to establish in the new host rises.

Host community composition and host species identity

As already mentioned, G_H x G_P interactions are important in the *Bombus*-*Crithidia* system (Schmid-Hempel 2001), even in the presence of a single host species. However, within a natural setting, another level of complexity is added because different host species vary in quality, and thus in their value to generalist parasites (Johnson et al. 2008, 2013b) and may differ tremendously in their intra- and interspecific transmission potential (Ruiz-González et al. 2012). Consequently, species identity and the composition of the host community are vital to grasp a deeper understanding of diversity-disease relations (LoGiudice et al. 2008, Roche & Guégan 2011, Salkeld et al. 2013, Streicker et al. 2013) and may actually be more important than biological diversity itself (Randolph & Dobson 2012).

Our results support the importance of the specific composition of ecological communities – particularly the presence / absence of a certain 'key' host species (Johnson et al. 2008) – with respect to variable disease outcome. We found a positive relationship between the proportion of *B. lapidarius* and *C. bombi* prevalence in the remaining bumblebee species, emphasising its potential key role for transmission (cf. Chpt. 3), as Ruiz-González et al. (2012) already found

that both intra- and interspecific transmission potential is highest in *B. lapidarius*. In contrast, we found no such association with *B. terrestris* and a negative trend for the proportion of *B. pascuorum* and the prevalence of the remaining host species. Furthermore, a negative trend occurred when inspecting the relationship between increased proportions of 'Other' host species and the prevalence of the three most common bumblebee species. If we assume that *B. lapidarius* represents either, but not mutually exclusive, the most competent host, a reservoir species or an 'amplification host' (e.g. a 'superspreader', Paull et al. 2012), its relative abundance declines with higher host species diversity and causes an increase in 'wasted' transmission events (Begon 2008, Johnson et al. 2008). Therefore, density- and diversity-induced effects need to be unraveled thoroughly (Johnson et al. 2008) to identify the reason for reduced infection (Begon 2008).

Low versus high prevalence

The Results derived from the subsample of 'low' vs. 'high'-prevalence locations are in accordance with our overall findings already discussed above. Consequently, locations with high prevalence exhibited lower species richness / diversity and vice versa. This is also in line with findings derived from other host-parasite systems (*Daphnia*: Ganz & Ebert 2010; amphibians: Johnson et al. 2008, 2013b). We only found marked differences between 'low' and 'high'-prevalence locations in the case of richness, but indeed for both observed and expected species richness. Therefore, the general limitation of observational studies concerning sample size (number of locations) and sample breadth (the range of host richness observed) (Mihaljevic et al. 2014) most likely only applies to species diversity.

Locations of 'low' vs. 'high'-prevalence mainly comprised distinct bumblebee communities (cf. Johnson et al. 2008, 2013b). Consistent with prior analyses, high prevalences tend to be attributable to high proportions of *B. lapidarius*, whereas low prevalences may be linked to the higher abundance of *B. pascuorum* and 'Other' bumblebee species.

Conclusion

Our research adds to the body of evidence supporting the dilution effect hypothesis rather than the amplification effect at a local scale. When investigating natural bumblebee populations and their gut parasite *C. bombi*, we found that host species richness and diversity were negatively associated with parasite prevalence and the proportion of multi-strain infections. Furthermore, our results also highlight the importance of the specific composition of host communities – including species identity – for the increase in knowledge regarding diversity-disease relationships. In particular *B. lapidarius* is likely to play a key role in the maintenance and transmission of the multi-host parasite *C. bombi* (cf. Chpt 3; Ruiz-González et al. 2012).

Nonetheless, the specific underlying mechanisms of disease dynamics in species communities often remain obscure and may either occur due to variable host abundance (e.g. because of interspecific competition) or 'pure' diversity effects (reviewed in Johnson & Thieltges 2010). Therefore, controlled experiments precisely determining the contribution of density and diversity-mediated effects are highly recommended (Johnson et al. 2013b).

Supporting Information for Chapter 4

Contents:

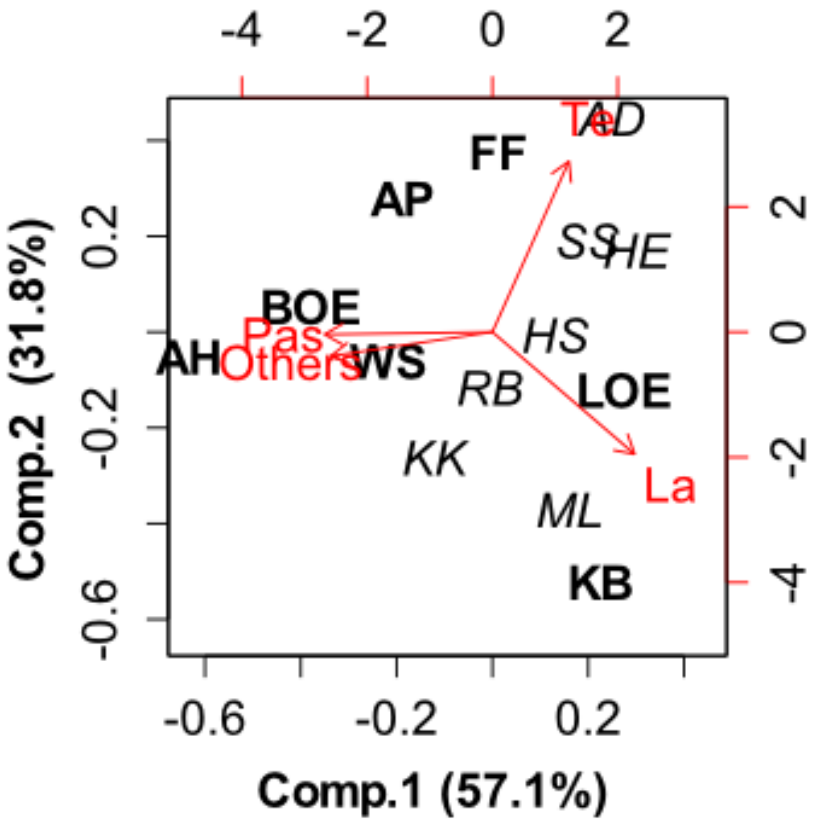

Figure S4.1. Biplot of the Principal Component Analysis (PCA) based on host species proportion data per location. **Bold** / oblique = locations with low / high prevalence of *C. bombi* (cf. Tab. S4.1 and Tab. 3.1 for location details), red = bumblebee species: Te = *B. terrestris*, La = *B. lapidarius*, Pas = B. pascuorum, Others = less frequent bumblebee species (pooled). The first and second component account for 57.1% and 31.8% of the variation in the host community composition, respectively.

Table S4.1. Sampling overview; cf. Tab. 3.1 for location details. Total number of bumblebees caught; individuals infected with *Crithidia bombi* are given in brackets; Te = *B. terrestris*, La = *B. lapidarius*, Pas = *B. pascuorum*, Ho = *B. hortorum*, Lu = *B. lucorum*, Pra = *B. pratorum*, Rud = *B. ruderarius*, cfVe = cf *B. vestalis*, Rup = *B. rupestris*; L / H = 'low'- / 'high'-prevalence group; [1] = observed species richness; [2] Shannon diversity index: $D = \sum_{i=1}^{5} -(Pi * lnPi)$.

Code	Te	La	Pas	Ho	Lu	Pra	Rud	cf. Ve	Rup	Mean Prevalence	∑ Species[1]	Shannon's diversity[2]
GW_RB	24 (11)	49 (8)	24 (8)	9 (-)	-	-	-	-	-	**0.26 (H)**	**4**	**1.24**
GW_KB	7 (-)	112 (8)	2 (-)	11 (2)	1 (-)	-	-	1 (-)	-	**0.08 (L)**	**6**	**0.65**
GW_AG	2 (1)	69 (15)	38 (5)	19 (1)	-	-	-	-	-	**0.17**	**4**	**1.04**
GE_KK	7 (3)	38 (15)	32 (8)	-	1 (-)	-	-	1 (-)	1 (1)	**0.33 (H)**	**7**	**1.15**
GE_BT	4 (3)	95 (17)	6 (-)	-	-	-	-	1 (-)	4 (1)	**0.19**	**5**	**0.57**
GE_LB	21 (1)	47 (13)	11 (3)	7 (1)	1 (-)	1 (-)	-	16 (5)	5 (-)	**0.21**	**8**	**1.60**
Sgh_ML	5 (1)	27 (3)	1 (1)	-	-	5 (5)	-	-	-	**0.26 (H)**	**4**	**0.87**
Sgh_WR	21 (2)	36 (6)	5 (1)	-	1 (-)	-	1 (1)	-	-	**0.16**	**5**	**1.02**
Sgh_OD	15 (2)	36 (6)	11 (1)	-	1 (-)	-	-	-	-	**0.14**	**4**	**1.03**
Rö_SS	62 (20)	55 (26)	13 (-)	-	-	-	-	-	-	**0.35 (H)**	**3**	**0.95**
Rö_RÖ	87 (25)	45 (8)	1 (-)	2 (1)	-	2 (-)	1 (-)	-	-	**0.25**	**6**	**0.85**
Rö_WL	28 (8)	11 (2)	4 (-)	-	-	-	-	-	-	**0.23**	**3**	**0.85**
Bd_SM	21 (1)	11 (2)	4 (-)	-	-	-	-	3 (1)		**0.10**	**4**	**1.12**
Bd_GB	40 (9)	25 (1)	4 (1)	-	-	-	3 (-)	-	1 (-)	**0.15**	**5**	**1.05**
Bd_BD	20 (5)	2 (-)	20 (3)	8 (0)	1 (-)	1 (-)	1 (-)	1 (-)	3 (-)	**0.14**	**9**	**1.57**

Table S4.1. Continued.

Code	Te	La	Pas	Ho	Lu	Pra	Rud	cf. Ve	Rup	Mean Prevalence	∑ Species[1]	Shannon's diversity[2]
Hal_HS	66 (26)	96 (26)	25 (-)	6 (2)	3 (1)	-	-	-	-	**0.28 (H)**	**5**	**1.15**
Hal_BG	24 (3)	22 (1)	47 (8)	7 (-)	1 (-)	6 (-)	-	25 (4)	10 (4)	**0.14**	**8**	**1.76**
Hal_HE	26 (13)	26 (5)	-	-	-	-	-	-	-	**0.35 (H)**	**2**	**0.69**
Ad_BÖ	8 (-)	4 (-)	9 (-)	1 (-)	1 (-)	1 (-)	-	5 (-)	3 (1)	**0.03 (L)**	**8**	**1.80**
Ad_AD	69 (26)	33 (2)	3 (-)	-	-	-	-	-	-	**0.27 (H)**	**3**	**0.74**
Ad_LÖ	21 (-)	44 (2)	2 (-)	-	-	-	1 (-)	-	-	**0.03 (L)**	**4**	**0.81**
Vr_WS	5 (1)	8 (-)	12 (-)	-	-	-	-	1 (-)	1 (-)	**0.04 (L)**	**5**	**1.28**
Vr_VR	45 (11)	14 (1)	-	-	-	-	-	-	-	**0.20**	**2**	**0.55**
Vr_OB	18 (1)	3 (-)	4 (-)	-	-	-	-	-	-	**0.04**	**3**	**0.78**
Bs_RW	4 (-)	1 (1)	1 (-)	-	-	-	-	-	-	**0.17**	**3**	**0.87**
Bs_DB	5 (-)	3 (-)	11 (-)	-	1 (1)	-	-	-	-	**0.05**	**4**	**1.11**
Bs_AH	11 (2)	2 (-)	45 (1)	1 (-)	3 (-)	1 (-)	-	27 (3)	11 (-)	**0.06 (L)**	**8**	**1.47**
Fw_AP	17 (-)	5 (1)	12 (1)	1 (-)	1 (-)	-	-	3 (-)	1 (-)	**0.05 (L)**	**7**	**1.46**
Fw_FF	90 (2)	33 (2)	28 (-)	1 (-)	6 (2)	-	-	4 (-)	3 (-)	**0.04 (L)**	**7**	**1.27**
Fw_LW	88 (12)	78 (4)	3 (-)	-	25 (7)	-	-	-	1 (-)	**0.12**	**5**	**1.08**
∑	**861 (189)**	**1,030 (175)**	**378 (41)**	**73 (7)**	**47 (11)**	**18 (5)**	**7 (1)**	**88 (13)**	**44 (7)**			

Table S4.2. Observed and expected host species richness[1] per location. The expected species richness and the number (N) of unseen species were estimated using the function *estimateR* implemented in the R package vegan (Oksanen et al. 2013). Results from the more conservative CHAO-method were used for the comparison with the observed species richness.

Code Location	Observed species richness	Expected species richness (CHAO)	N unseen species (CHAO)	Expected species richness (ACE)	N unseen species (ACE)
GW_RB	**4**	4	0	4	0
GW_KB	**6**	6.5	0.5	9.2	3.2
GW_AG	**4**	4	0	4	0
GE_KK	**7**	13	6	22.4	15.4
GE_BT	**5**	5	0	5.4	0.4
GE_LB	**8**	9	1	10	2
Sgh_ML	4	4	0	4.5	0.5
Sgh_WR	5	6	1	9	4
Sgh_OD	4	4	0	NA	NA
Rö_SS	3	3	0	NA	NA
Rö_RÖ	6	6.3	0.3	8	2
Rö_WL	3	3	0	3	0
Bd_SM	4	4	0	4	0
Bd_GB	5	5	0	5.5	0.5
Bd_BD	9	12	3	17.2	8.2
Hal_HS	5	5	0	5	0
Hal_BG	8	8	0	8.4	0
Hal_HE	2	2	0	NA	NA
Ad_BÖ	8	11	3	10.4	2.4
Ad_AD	3	3	0	3	0
Ad_LÖ	4	4	0	5	1
Vr_WS	5	6	1	7.2	2.2
Vr_VR	2	2	0	NA	NA
Vr_OB	3	3	0	3	0
Bs_RW	3	4	1	6.9	3.9
Bs_DB	4	4	0	4.6	0.6
Bs_AH	8	8.5	1	9.8	1.8
Fw_AP	7	10	3	11.5	4.5
Fw_FF	7	7	0	7.5	0.5
Fw_LW	5	5	0	6.1	1.1

[1] Central European bumblebee communities are composed of up to 16 species (Goulson 2010); cuckoo bumblebees depend on the occurrence of their hosts (*Bombus rupestris* = social parasite of *B. lapidarius, B. vestalis* = social parasite of *B. terrestris*; Sladen 1912, von Hagen & Aichhorn 2003).

Chapter 5

Synthesis

General Discussion

Bumblebees are pivotal pollinators providing effective pollination service (e.g. buzz pollination, Goulson 2010) to wild plants and crops (Goulson et al. 2008a, Kearns et al. 1998, Klein et al. 2007). Recently, Fründ et al. (2013) highlighted the functional complementarity among different bee species, further underpinning the ability of bumblebees to perform flower visitation under adverse weather conditions. In addition, also male individuals (drones) contribute considerably to pollination and show flower constancy comparable to those of the workers (Wolf & Moritz 2014). Functionally diverse bee- and wild insect assemblages are of particular importance for the maintenance of plant communities (Fründ et al. 2013) and high crop yields (Garibaldi et al. 2013). Therefore, global decline of pollinators during the past decades (Kearns et al. 1998, Potts et al. 2010) – mainly in honeybees (vanEngelsdorp et al. 2010) and wild bees (Biesmeijer et al. 2006, Goulson 2010, Cameron et al. 2011) – represent a serious threat for human and wildlife wellbeing. This also applies to the worldwide loss of biological diversity (Cardinale et al. 2012) and emerging infectious diseases (EIDs) (Binder et al. 1999, Siddle et al. 2007), whereas increasing evidence implies tight links between the two issues (Johnson et al. 2008, Pongsiri et al. 2009, Keesing et al. 2010). Furthermore, there is mounting evidence for the contribution of parasites and especially EIDs (Meeus et al. 2011, Fürst et al. 2014) to the pollinator decline (Cameron et al. 2011). Consequently, the potential disease-driven loss of key pollinators (like bumblebees, Goulson et al. 2008a) is one focus of recent research.

In this thesis, the manifold aspects of complex multi-host parasite interactions were addressed to disentangle the individual contribution of genetic, density-dependent and environmental effects. Hence, an extensive field study was conducted sampling natural bumblebee populations (*Bombus* spp., Hymenoptera, Apidae) and their gut parasite *Crithidia bombi* (Trypanosomatidae) (Gorbunov 1987, Lipa & Triggiani 1988). This specific system represents an excellent model system because it proved to be equally convenient for both field and manipulative (laboratory) studies (Schmid-Hempel 2001). In the recent past,

research concentrated almost exclusively on a single host species, *B. terrestris* (Ruiz-González et al. 2012). However, *C. bombi* infects numerous bumblebee species (Shykoff & Schmid-Hempel 1991a, Kissinger et al. 2011, Cordes et al. 2012). Therefore, the consideration of various host species better reflects natural conditions and is crucially important to gain basic understanding of the spread and perseverance of diseases (Rigaud et al. 2010, Roche & Guégan 2011), e.g. for subsequent effective conservation measures.

Transmission is a sophisticated feature of host physiology, immunity, behaviour and ecology. With regard to generalist parasites, both within-species and between-species transmission need to be incorporated (Dobson 2004). Host species vary in quality, hence in their value to multi-host parasites (Johnson et al. 2008, 2013b) and exhibit asymmetric intra- and interspecific transmission potential (Ruiz-González et al. 2012). Consequently, host species identity and local community composition are vital to conceive diversity-disease relationships (e.g. Salkeld et al. 2013, Streicker et al. 2013) and may be more important than biodiversity per se (Randolph & Dobson 2012, Wood & Lafferty 2013). Moreover, the identification of host species that excessively reduce or enhance pathogen transmission (‘dilution -’ vs. ‘amplification hosts’, Begon 2008) is highly beneficial for effective disease control (Johnson et al. 2008, Paull et al. 2012). Within the present thesis, we add to the body of work that finds a negative association between genetic diversity (at different levels) and parasite prevalence and the proportion of multiple infections, thereby supporting the dilution effect hypothesis. At the same time, we also provide evidence for the decisive role host species identity and local community composition play (cf. Chpt. 3 and 4). Particularly *B. lapidarius* appears to play a key role in the maintenance and transmission of *C. bombi* which is in line with recent findings of species-specific transmission heterogeneity (Ruiz-González et al. 2012). Communities of low diversity may be dominated by highly abundant, competent host species (Johnson et al. 2013b, Keesing et al. 2010) which tends to apply to *B. lapidarius* as it was abundant throughout the sampling locations and exhibited overall more homogeneous prevalences than *B. terrestris*, despite their larger ranges of colony densities and genetic diversity (cf. Chpt. 3). For that reason, controlled experiments would complement our observational data in order to determine the exact contribution of density- and diversity-mediated effects (Johnson et al. 2013b).

While other bumblebee species already represent a part of the environment, additional abiotic and biotic factors (e.g. temperature, food, competitors, predators; Schaefer 2003) potentially affect the expression of host and parasite traits (G_H x E / G_P x E / G_H x G_P x E interactions; Wolinska & King 2009), creating another degree of complexity (Vale et al. 2008a, Sadd 2011).

G x E interactions are common within a natural setting (Lazzaro & Little 2008), altering epidemiological dynamics across various host-parasite systems (Tseng 2006, Wolinska & King 2009). Complex environmentally-mediated interactions tend to be responsible for a highly dynamic *C. bombi* population structure across years, host species and sites (Salathé & Schmid-Hempel 2011, Erler et al. 2012, Ruiz-González et al. 2012). The mixture of the parasite's clonal and sexual reproduction (Schmid-Hempel et al. 2011) is likely to counterbalance genetic impoverishment jointly induced by the inability of *C. bombi* to survive outside its host for expanded periods of time (Schmid-Hempel et al. 1999), the annual life cycle of bumblebees (Sladen 1912, Goulson 2010), and "strain filtering" (Ulrich et al. 2011). Hence, the remarkably high diversity of different multi-locus genotypes is plausible (Schmid-Hempel & Reber Funk 2004, Salathé & Schmid-Hempel 2011). In line with recent evidence (Gant & Ebert 2010, Salathé & Schmid-Hempel 2011), we also find a positive association between intraspecific parasite diversity and prevalence, and increased *C. bombi* prevalence is linked to larger proportions of multiple infections. Accordingly, the relationship between a diverse parasite population and its enhanced performance (i.e. successful transmission and infection) in different host individuals and species is conceivable.

Shared floral resources are thought to be important for the horizontal transmission of pathogens in pollinators (e.g. *C. bombi*, Durrer & Schmid-Hempel 1994) but up to now little work inspecting the specific role of flowers and floral traits for pathogen transmission has been done (McArt et al. 2014; but see Cisarovsky & Schmid-Hempel 2014). Despite the large amount of jointly exploited resources we find for *B. terrestris* and *B. lapidarius*, no correlation of niche overlap and species-specific prevalence was found. Similarly, no association between flowering plant diversity and prevalence was detected. Both findings may be attributable to the limitation of observational field studies (Johnson & Thieltges 2010). Additional manipulative experiments are thus beneficial as they control for a wealth of confounding factors and allow to ascertain the underlying mechanisms of transmission.

General Conclusion and Future Directions

Taken together, general predictions of the interannual infection outcome remain challenging in complex host-parasite systems with respect to natural settings (Altizer et al. 2006, Knowles et al. 2014). In this connection, the development of more realistic forecast models incorporating ecological and epidemiological heterogeneity observed in generalist pathogen systems represents a promising approach (Van der Werf et al. 2011, Mihaljevic et al. 2014, Rudge et al. 2013), particularly in the light of ongoing global environmental change (Cardinale et al. 2012, Paull et al. 2012).

Intraspecific parasite diversity was positively correlated with *C. bombi* prevalence and increased prevalences were associated with larger proportions of multiple infections. So far, we neither found a relationship between niche overlap and species-specific prevalences nor between flowering plant diversity and prevalence. Host species identity, sex and season were identified as key factors contributing to *C. bombi* epidemiology, particularly regarding prevalence. Two common host species (*B. terrestris* and *B. lapidarius*) proved to be different concerning their respective association between colony density and prevalence and between genetic diversity and prevalence, indicating a key role of *B. lapidarius* which also applies to the community level. Overall, we provide further evidence of the dilution effect hypothesis simultaneously accentuating – in accordance with previous work – the importance of host species identity (cf. Chpt. 3 and 4) and the specific composition of communities (cf. Chpt. 4) regarding diversity-disease relationships (e.g. Johnson et al. 2008, 2013b, LoGiudice et al. 2008).

In addition to the precious insights derived from the extensive observational field study conducted, the establishment of a complex mesocosm experiment would be highly beneficial, allowing to clarify underlying transmission mechanisms under controlled, semi-natural conditions (Lively et al. 2014, McArt et al. 2014, Thieltges et al. 2008; cf. Nuismer & Gandon 2008). Specifically, data on host abundance and species composition from the field represent a valuable basis to ensure natural transmission dynamics in the experiment (Mihaljevic et al. 2014). Furthermore, information on the diversity of the three interacting partners (flowering plants, bumblebees and *C. bombi*) as well as flower availability and niche overlap should be incorporated. Therefore, large flight arenas should be provided with controlled resource availability (low- vs. high-diversity units) comprising a mixture of highly attractive (e.g. *Echium vulgare*) and less attractive flowering plants. Then a fixed number of host individuals (i.e. controlled host density) of several bumblebee species (different community compositions; i.e. controlled host diversity) are added. Finally, identical starting conditions concerning the multi-host parasite *C. bombi* (i.e. the amount of cells per inoculum and a specific number of different strains) should be created.

Summary

Biological diversity is indispensable for preserving ecosystem functioning and ecosystem services against the backdrop of a fluctuating environment. Bumblebees play a vital ecological and economical role in the effective pollination of wild and cultivated plants. Recently, awareness increases regarding multiple interacting stressors including the potential for disease-driven pollinator decline. Global loss of biodiversity and emerging infectious diseases (EIDs) seriously threaten human and wildlife welfare. As a result, they represent current research priorities.

In the present thesis, I address the multifarious aspects of complex multi-host parasite interactions in order to unravel the individual contribution of genetic, density-dependent and environmental impact. Therefore, I conducted an extensive field study sampling natural bumblebee populations and their intestinal parasite *Crithidia bombi*. *Chapter 2* and *3* focus on the three most common bumblebee species, *Bombus terrestris*, *B. lapidarius* and *B. pascuorum*, wheras *Chapter 4* also considers the less frequent species. By means of molecular tools, I mainly investigated the prevalence (the proportion of infected bumblebees) complemented by determining the type (single- vs. multiple-strain infection) and the intensity of infection (mean number of parasite cells per host). Furthermore, the biological diversity – predominantly of the hosts – was measured at different organisational levels. The corresponding scientific background is reviewed in *Chapter 1*.

The role of environmental heterogeneity, in space and time, and its ramifications for disease dynamics was examined in *Chapter 2*. Intraspecific parasite diversity (as a proxy for the parasite's capability to infect various host species) was positively associated with *C. bombi* prevalence. Moreover, increased prevalence was correlated with larger proportions of multiple-strain infections (cf. *Chapter 3*). Consequently, the association between a diverse parasite population and its enhanced performance, in terms of a successful intra- and interspecifical transmission and infection, seems to be conclusive. Except for a marked decline of initially high parasite prevalences in the subsequent year, general predictions of infection outcome from year to year remain challenging within natural settings. The latter is supported by the discrepancy found between the observed and the expected interannual change in *C. bombi* prevalence. Regarding spatial heterogeneity, the focus was on the host's food resources because of their potential role in the horizontal transmission of *C. bombi*. Therefore, I determined the availability, usage and diversity of floral resources and calculated the niche overlap (as a proxy for parasite transmission) per host species pair. Despite the large amount of shared resources between *B. terrestris* and *B. lapidarius*, no relationship of niche overlap and species-specific prevalence occurred, which is most likely due to the small number of infected *B. terrestris* in 2011. Likewise, no association was found between flowering plant diversity and prevalence. However, further (experimental) investigation is needed to obtain knowledge about the underlying mechanisms of transmission of the specific interplay of hosts, parasites and floral traits.

Chapter 3 focused on the identification of potential key factors that contribute to *C. bombi* epidemiology. Host species identity, sex and season emerged as important predictors of disease dynamics, especially concerning prevalence. No sex-specific differences occurred regarding type

and intensity of *C. bombi* infections, but the highest incidence of multiple infections was found in the early summer (June) and concentrated in *B. terrestris*. Furthermore, increased colony density facilitates parasite transmission, whereas genetic diversity was negatively related to prevalence in *B. terrestris*. Since these associations where not found for *B. lapidarius*, this species appears to play a particular role which confirms previous work that found host-specific variability in terms of host susceptibility or resistance and transmission potential (also cf. *Chapter 4*). To my knowledge, this is the first study concurrently demonstrating an association of disease prevalence with host colony density and genetic diversity, respectively.

Chapter 4 provides important insights into varying disease risks along gradients of local host species richness / diversity and community composition. This study presents additional evidence in support of the dilution effect hypothesis rather than the amplification effect as species richness and diversity were negatively related to parasite prevalence and the proportion of multiple infections. Moreover, the specific composition of host communities (including species identity) proved to be important with respect to prevalence. Notably *B. lapidarius* is likely to play a pivotal role (cf. *Chapter 3*) which should be investigated more intensively in controlled experiments disentangling the precise contribution of density- and diversity-mediated effects.

In conclusion, I provide precious empirical insights into the natural dynamics of the *Bombus-Crithidia bombi* system regarding environmental heterogeneity, by identifying key factors and by showing that diversity indeed matters for both hosts and parasites. Additionally, my results are in line with previous work, further underpinning the importance of host species identity and local community composition. Finally, considering the challenges of finding generalisable diversity-disease patterns in the field, I recommend the establishment of a mesocosm experiment to gain a deeper understanding in order to counteract pollinator and biodiversity loss, respectively as well as disease risk for human and wildlife health.

Zusammenfassung

Vor dem Hintergrund wechselnder Umweltbedingungen ist biologische Vielfalt unverzichtbar für die Aufrechterhaltung von Ökosystemfunktionen und Ökosystemdienstleistungen. Hummeln spielen eine entscheidende ökologische und ökonomische Rolle bei der wirksamen Bestäubung von Wild- und Kulturpflanzen. Seit kurzem steigt das Bewusstsein für Wechselwirkungen zwischen zahlreichen Stressoren einschließlich der Möglichkeit eines krankheitsbedingten Rückgangs von Bestäubern. Weltweiter Biodiversitätsverlust und (neue) Infektionskrankheiten stellen eine ernsthafte Gefahr für das Wohlbefinden von Mensch und Umwelt, einschließlich wildlebender Tiere und Pflanzen, dar und stehen daher im Fokus derzeitiger Forschungaktivitäten.

Die vorliegende Arbeit befasst sich mit den facettenreichen Aspekten komplexer Wirt-Parasit-Interaktionen mit dem Ziel, den individuellen Beitrag genetischer, dichteabhängiger und umweltbedingter Einflüsse kenntlich zu machen. Hierzu wurde eine umfangreiche Feldstudie zur Untersuchung natürlicher Hummelpopulationen und deren Darmparasiten *Crithidia bombi* durchgeführt. Während sich *Kapitel 2* und *3* auf die drei häufigsten Hummelarten (*Bombus terrestris*, *B. lapidarius* and *B. pascuorum*) konzentrieren, berücksichtigt *Kapitel 4* auch weniger häufige Arten. Mittels molekularbiologischer Methoden wurde hauptsächlich die Prävalenz (Anteil der infizierten Hummeln) untersucht. In Ergänzung wurden die Art (einfache vs. multiple Infektion) und Intensität der Infektion (durchschnittliche Anzahl von Parasitenzellen im Wirt) ermittelt. Des Weiteren wurde die biologische Diversität – vorwiegend der Wirte – auf unterschiedlichen organisatorischen Ebenen erfasst. Der entsprechende wissenschaftliche Hintergrund wurde in *Kapitel 1* zusammengefasst.

Mögliche Auswirkungen einer, räumlich und zeitlich, heterogenen Umwelt auf die Krankheitsdynamik wurden in *Kapitel 2* untersucht. Intraspezifische Parasitendiversität (als Indikator für die Fähigkeit der Parasiten, verschiedene Wirtsarten zu infizieren) war positiv mit *C. bombi*-Prävalenz assoziiert. Außerdem zeigte sich ein positiver Zusammenhang zwischen erhöhter Prävalenz und größeren Anteilen an Mehrfachinfektionen (vgl. *Kapitel 3*). Demzufolge erscheint der Zusammenhang zwischen diversen Parasitenpopulationen und deren verbesserter Leistungsfähigkeit – hinsichtlich einer erfolgreichen intra- und interspezifischen Übertragung und Infektion – schlüssig. Abgesehen von einem deutlichen Rückgang initial hoher Prävalenzen im darauffolgenden Jahr, bleiben generelle Vorhersagen über den Verlauf der Infektion von einem zum nächsten Jahr eine große Herausforderung unter natürlichen Bedingungen. Letzteres bestätigt sich durch die gefundene Diskrepanz zwischen der beobachteten und erwarteten zwischenjährlichen Veränderung der *C. bombi*-Prävalenz. Hinsichtlich der räumlichen Heterogenität lag der Fokus auf den Nahrungsresourcen der Wirte, da Blütenpflanzen eine potentielle Rolle bei der Übertragung von *C. bombi* spielen. Hierzu wurden Verfügbarkeit, Nutzung und Vielfalt der Blütenpflanzen ermittelt und der ’niche overlap’ (stellvertretend für eine potentielle Parasitenübertragung) je Wirtsartenpaar berechnet. Trotz des hohen Anteils gemeinschaftlich genutzter Resourcen bei *B. terrestris* and *B. lapidarius*, konnte kein Zusammenhang zwischen ’niche overlap’ und der artspezifischen Prävalenz hergestellt werden.

Eine mögliche Ursache ist die geringe Anzahl infizierter *B. terrestris* im Jahr 2011. Zwischen der Diversität der Blütenpflanzen und der Prävalenz konnte ebenfalls kein Zusammenhang ermittelt werden. Allerdings werden weitere (experimentelle) Untersuchungen benötigt, um Wissen über die zugrunde liegenden Übertragungsmechanismen des spezifischen Zusammenspiels von Wirten, Parasiten und Pflanzeneigenschaften zu erlangen.

Kapitel 3 widmet sich der Identifizierung potentieller Schlüsselfaktoren, welche die Epidemiologie von *C. bombi* beeinflussen. Artzugehörigkeit und Geschlecht des Wirts sowie Zeitpunkt der Probenahme traten als wichtige Einflussgrößen für die Krankheitsdynamik, insbesondere hinsichtlich der Prävalenz, hervor. In Bezug auf Art und Intensität der *C. bombi*-Infektionen zeigten sich keine geschlechtsspezifischen Unterschiede. Das höchste Vorkommen von Mehrfachinfektionen wurde im Frühsommer (Juni) in *B. terrestris* verzeichnet. Weiterhin begünstigt eine erhöhte Dichte von Hummelvölkern die Übertragung des Parasiten, wohingegen ein negativer Zusammenhang zwischen genetischer Vielfalt und *C. bombi*-Prävalenz bei *B. terrestris* bestand. *B. lapidarius* scheint eine besondere Rolle einzunehmen, da bei dieser Art keine signifikanten Zusammenhänge bezüglich Koloniedichte und genetischer Vielfalt gefunden wurden. Dieses Ergebnis bestätigt vorangegangene Arbeiten, welche wirtsspezifische Variabilität hinsichtlich der Anfälligkeit oder Resistenz und des Übertragungspotentials fanden (vgl. auch *Kapitel 4*). Dies ist die erste Studie, die gleichzeitig einen Zusammenhang zwischen Prävalenz und Koloniedichte des Wirts sowie Prävalenz und genetischer Vielfalt nachweist.

Kapitel 4 liefert wichtige Erkenntnisse über variierende Krankheitsrisiken entlang von Gradienten der Artenvielfalt und Artengemeinschaften. Diese Studie liefert zusätzliche Belege für die ‘dilution effect hypothesis’ und gegen den ‘amplification effect’, da hoher Artenreichtum und große Artenvielfalt mit niedriger Parasiten-Prävalenz und einem geringen Anteil von Mehrfachinfektionen einhergingen. Darüber hinaus bestätigte sich die Bedeutsamkeit der konkreten Zusammensetzung von Artengemeinschaften in Bezug auf die Prävalenz. Insbesondere *B. lapidarius* dürfte eine zentrale Rolle spielen (vgl. *Kapitel 3)*. Letzteres sollte im Rahmen kontrollierter Freilandversuche intensiver untersucht werden, um dichte- und diversitätsbezogene Effekten genau voneinander zu trennen.

Insgesamt werden wertvolle Einblicke in die natürliche Dynamik des *Bombus-Crithidia bombi* Systems – hinsichtlich umweltbedingter Heterogenität, durch die Identifizierung von Schlüsselfaktoren und durch den Nachweis, dass biologische Vielfalt tatsächlich (sowohl für Wirte als auch Parasiten) bedeutsam ist – gewährt. Darüberhinaus wird die Übereinstimmung mit früheren wissenschaftlichen Erkenntnissen deutlich, wobei die Bedeutung der Artzugehörigkeit der Wirte sowie die der konkreten Zusammensetzung von lokalen Artengemeinschaften zusätzlich untermauert wird. In Hinblick auf die bestehenden Herausforderungen beim Aufdecken generalisierbarer ’diversity-disease’-Muster im Freiland, stellen Mesokosmos-Experimente ein hervorragendes Instrument dar. Daraus gewonnene Erkenntnisse sollten zusätzliche Schutzmaßnahmen für Bestäuber – beziehungsweise für die biologische Vielfalt generell – initiieren, sowie der menschlichen Gesundheit dienen.

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Appendix

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht worden. Weiterhin erkläre ich, dass ich mich noch nicht zuvor um den Doktorgrad beworben habe und diese Arbeit weder der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Halle (Saale), den 30.03.2015

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