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REVIEW AND SYNTHESIS

Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens

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Abstract

Several floral microbes are known to be pathogenic to plants or floral visitors such as pollinators. Despite the ecological and economic importance of pathogens deposited in flowers, we often lack a basic understanding of how floral traits influence disease transmission. Here, we provide the first systematic review regarding how floral traits attract vectors (for plant pathogens) or hosts (for animal pathogens), mediate disease establishment and evolve under complex interactions with plant mutualists that can be vectors for microbial antagonists. Attraction of floral visitors is influenced by numerous phenological, morphological and chemical traits, and several plant pathogens manipulate floral traits to attract vectors. There is rapidly growing interest in how floral secondary compounds and antimicrobial enzymes influence disease establishment in plant hosts. Similarly, new research suggests that consumption of floral secondary compounds can reduce pathogen loads in animal pollinators. Given recent concerns about pollinator declines caused in part by pathogens, the role of floral traits in mediating pathogen transmission is a key area for further research. We conclude by discussing important implications of floral transmission of pathogens for agriculture, conservation and human health, suggesting promising avenues for future research in both basic and applied biology.

Keywords

Bacteria, fungus, host, plant–animal interactions, pollinator decline, protozoan, systematic review, vector, virus.

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INTRODUCTION

Flowers are beacons of colour and scent that attract a wide range of floral visitors. Besides diverse pollinator communities, flowers may attract herbivores that feed on floral tissue (McCall & Irwin 2006), predators in search of prey (Louda 1982) and act as aggregation sites for mate finding (Pellmyr & Thien 1986). Indeed, the density of invertebrates on flowers can be ten thousand times higher than on surrounding foliage (Wardhaugh *et al*. 2012). This density of animals is noteworthy, as important pathogens of both plants and animals are transmitted in the warm and sugar-rich environment of flowers. In plants, pathogens vectored by floral visitors can infect floral tissues locally or become systemic infections; such pathogens are common in the wild (e.g. Thrall *et al*. 1993; Roy *et al*. 1998) and can be economically devastating in agricultural systems (Batra 1983; Farkas *et al*. 2011). For pathogens of pollinators and other floral visitors, transmission at flowers may occur via faecal contamination (Durrer & Schmid-Hempel 1994), shared use of pathogen-inoculated resources, such as pollen (Singh *et al*. 2010), or other possible mechanisms.

Traditionally, floral evolution has been attributed largely to selection by pollinators, although the role of a range of plant antagonists has also been recognised (e.g. Strauss & Whittall 2006). Pollinators that also transmit pathogens may reduce the benefits of pollinator attraction, depending on the plant fitness benefits of pollination and the costs of pathogens. Flower-transmitted plant pathogens have fitness impacts ranging from mild (e.g. Lara & Ornelas 2003) to complete sterilisation or death (Sasu *et al*. 2010a; Schafer *et al*. 2010). What floral traits determine the likelihood that pathogens are transmitted to plants and animals? Is infection by pathogens an inevitable consequence of pollinator visitation? Plant pathologists have made great strides in identifying floral traits that mediate host plant resistance to floral pathogens in individual systems; synthesising this literature can provide generality in identifying traits that mediate plant–pathogen dynamics. From the pollinator's perspective, there has been surprisingly little work elucidating the role of flowers and floral traits for pathogen transmission. Given recent concerns about pollinator declines caused in part by pathogens (e.g. Cameron *et al*. 2011), understanding the role of floral traits in disease transmission is a key missing element.

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The purpose of this review is to synthesise the disparate literatures on flower-transmitted pathogens of plants and animals and the traits that mediate transmission, introduce areas where an understanding of flower-associated microbes can be applied to address current issues and suggest areas for future research. We confine our review to pathogens that are transmitted by floral visitors (rather than abiotic vectors such as wind) to highlight systems where traits that attract pollinators may also play a central role in pathogen transmission (e.g. Stephenson 2012). We define pathogens broadly, including examples of microbes that may be beneficial rather than costly under some ecological conditions, but we exclude external parasites (such as mites that are transmitted between bird pollinators at flowers) and parasitoids that attack insect hosts at flowers. While external parasites and parasitoids could transmit microbes at flowers, the literature addressing this topic is limited to our knowledge. Our intent is to stimulate new interest in elucidating the numerous roles that flowers and floral traits play in mediating multispecies interactions, with consequences for ecological and evolutionary dynamics of both plants and animals.

SYSTEMATIC REVIEW METHODS

To find literature pertaining to plant pathogens deposited in flowers and floral traits important for transmission, we searched via the Thomson Reuters Web of Knowledge. We employed the following search string: Topic = (flower OR floral) AND (pathogen OR microb* OR disease* OR fung* OR bacteria* OR vir*) AND (pollinat* OR transmi* OR vector* OR host). This search returned 2324 results (all records until August 26, 2013). We then used the title and abstract to assess whether each study was relevant according to the following criteria: (1) floral visitors must be implicated in transmission, and (2) transmission must occur at flowers or pathogen-induced pseudoflowers. When this information was not clear from the title or abstract, we evaluated the full text of the article. In each relevant article, we searched the literature cited for additional references that may have been missed. In total, we found 187 studies published between 1947 and 2013 that fit our criteria. Across all studies, we identified 26 plant pathogens known to rely on animal vectors for transmission between flowers or pathogen-induced pseudoflowers (Table S1), including 18 fungi, two bacteria and six viruses.

To search the literature for animal pathogens, we used the following search string: Topic = (flower* OR floral OR nectar OR pollen) AND (pathogen* OR parasit* OR disease* OR microb* OR microorganism* OR bacter* OR vir* OR fung* OR microsporid* OR protist OR protozoa* OR trypanosom*) AND (animal* OR pollinator* OR bee OR *fly OR hummingbird* OR beetle* OR bat OR lepidopt* OR hymenopter* OR insect*) AND (transmi* OR infect*), returning 618 results (all records until September 6, 2013). We examined this literature using the same criteria as for the plant pathogen literature. In addition, the references cited by and citing each relevant paper were examined for additional publications missed by our strategy. In total, we found eight major groups of animal pathogens that are potentially transmitted at flowers, including a trypanosomatid, fungi, bacteria and RNA viruses (Table 2).

PLANT PATHOGENS

Fungal plant pathogens

The life histories of fungal pathogens that exploit flowers are remarkably diverse, and these pathogens can be transmitted by invertebrates (Jennersten 1988; Roy 1993) as well as vertebrates (Lara & Ornelas 2003). The most well-studied floral fungal pathogen is *Microbotryum violaceum*, which infects plants in the family Caryophyllaceae. Spores are vectored by insect visitors from diseased to healthy flowers (Jennersten 1988; Shykoff & Bucheli 1995), where, following germination and meiosis, the fungal cells conjugate and directly penetrate the plant epidermis (Schafer *et al.* 2010). Mature flowers on which spores are deposited do not typically become diseased; rather, the fungus grows into the plant meristem and destroys developing pollen mother cells, replacing anther sacs with fungal spores (Schafer *et al.* 2010). A more manipulative tactic used by fungal pathogens involves the induction of pseudoflowers to attract floral visitors. For example, primary infection by mummyberry disease (*Monilinia vaccinii-corymbosi*) causes infected shoots of blueberry (*Vaccinium spp.*) to exude a sugar-rich solution, reflect UV light (Batra & Batra 1985) and produce floral odour compounds (McArt *et al.* unpublished data). Insects visit the pathogen-induced pseudoflowers, acquire asexual conidia and vector this infectious stage to the stigmas of blueberry flowers. Conidia morphologically and chemically mimic pollen grains, and hyphae ingress down the stylar canal in a manner similar to pollen tube growth (Ngugi & Scherm 2004), culminating in fruit infection.

Bacterial plant pathogens

Only two bacterial pathogens of plants are known to rely on pollinators as vectors, but both cause extensive agricultural losses. *Erwinia amylovora* (fire blight) infects plants in the family Rosaceae, including fruit crops such as apple and pear (Farkas *et al.* 2011). The most common site of *E. amylovora* infection is the hypanthium, where nectar is secreted. The pathogen then gains entry to inner floral tissues via the nectar-secreting stomata (Farkas *et al.* 2011). Bees are common vectors of *E. amylovora*, moving the pathogen from diseased to healthy flowers (Alexandrova *et al.* 2002). *Erwinia tracheiphila*, the causative agent of bacterial wilt disease in cucurbits, is transmitted via the frass of cucumber beetles that have fed on infected vegetative tissues. While infection via beetle-damaged leaves is well-studied, pollen-feeding beetles can also infect plants when frass falls onto the nectary and bacteria pass into the xylem (Sasu *et al.* 2010a).

Viral plant pathogens

All viral plant pathogens known to be vectored by floral visitors are transmitted in pollen (Card *et al.* 2007). These viruses are located in or on pollen grains, occasionally cause the pollen to become inviable and typically lead to systemic plant infections. *Prunus* necrotic ringspot virus, prune dwarf virus, tobacco streak virus and sowbane mosaic virus are all pollen vectored by thrips (Card *et al.* 2007 and references therein). In each case, infected pollen attaches mechanically to the

insect exoskeleton during foraging in flowers. The disease is vectored to additional plants when the pollen-associated virus detaches and enters feeding wounds caused by thrips in various plant tissues. Several additional pollen viruses are vectored by larger floral visitors, such as bees. For example, blueberry shock ilarvirus is transmitted by honey bees during foraging for pollen and nectar (Bristow & Martin 1999).

Nectar yeast and bacteria

Nectar itself is prone to microbial colonisation by yeast and bacteria that can tolerate high sugar concentrations, and several studies suggest that pollinators vector these nectar microbes (e.g. Herrera *et al*. 2009; Schaeffer & Irwin in press). Nectar-inhabiting microorganisms can negatively affect plants through both indirect and direct pathways. For example, nectar microbes can alter nectar pH, H_2O_2 concentration and sugar concentration and composition, thus altering floral attractiveness and pollination (Vannette *et al*. 2013). Alternatively, nectar microbes can directly reduce seed production by drawing carbohydrate resources away from developing ovaries (Golonka 2002) or inhibiting pollen germination and pollen tube formation (Eisikowitch *et al*. 1990). It is important to note, however, that nectar microbes do not always harm plants. In some systems nectar microbes increase pollinator visitation to flowers (e.g. Herrera *et al*. 2013; Schaeffer & Irwin in press). This increase in pollinator visitation increases pollen donation, a component of male plant reproduction, in *Delphinium nuttallianum* (Schaeffer & Irwin in press). A major challenge for future research is to understand how ecological factors shape conditionally mutualistic or antagonistic interactions between nectar microbes and plants.

Plant pathogens remaining to be studied

Understanding interactions between floral microbes and vectors has important implications for both natural and managed systems. In managed systems, six additional *Monilinia* species can be problems in blueberry and cranberry orchards, and each is likely to be vectored by insects (Batra 1983). However, no studies have investigated the effect of pollinator abundance on infection. This lack of information is surprising considering that orchards are often managed with variable densities of bees, and bee density can strongly influence transmission dynamics of other pollinator-vectored diseases, including *M. vaccinii-corymbosi* (Dedej *et al*. 2004). Similarly, of the 39 plant viruses known to infect pollen, 17 can be horizontally transferred and each of these viruses infect agriculturally important crops (Card *et al*. 2007). However, to date, studies have only identified how six pollen viruses are vectored by floral visitors (Table S1), and we are aware of no studies that have addressed the role of pollinator abundance, identity or diversity on transmission.

In natural systems, an understanding of pathogen–vector interactions is pertinent to topics such as pathogen host shifts, hybridisation and speciation. For example, the extent of hybridisation and emergence of host-specific cryptic species of *Microbotryum violaceum* across the Caryophyllaceae (Le Gac *et al*. 2007) and *Puccinia monoica* across the Brassicaceae (Roy *et al*. 1998) may largely depend on host visitation patterns of vectors. Indeed, vector-specific interactions appear to reduce gene flow of *M. violaceum* between *Silene dioica* and *S. latifolia* (van Putten *et al*. 2007), while vector overlap may be facilitating a host shift of *M. violaceum* from *S. latifolia* onto *S. vulgaris* (Antonovics *et al*. 2002).

FLORAL TRAITS INFLUENCING TRANSMISSION OF PLANT PATHOGENS

There are at least four major avenues by which floral traits could influence plant pathogen transmission: (1) traits that influence floral attractiveness of healthy plants, which affect the frequency of vector/pathogen arrival at flowers, (2) traits that affect pathogen establishment in flowers, (3) traits that influence the floral attractiveness of diseased plants, which affect the frequency of vector visitation, and (4) traits that affect pathogen acquisition by vectors upon visiting diseased flowers. A full list of traits that have been evaluated in each category is presented in Table 1. Meta-analytic comparisons among traits were not advisable due to low sample sizes in each trait category.

(1) Attraction of vectors to healthy plants

Floral traits that influence the attraction of vectors to healthy plants include floral longevity and phenology, floral morphology, nectar rewards and floral volatiles. A wealth of evidence shows that delayed flowering phenology is associated with reduced vector visitation, spore deposition and infection of *Microbotryum violaceum* across four plant species (Jennersten 1988; Alexander 1989; Jennersten & Kwak 1991; Thrall & Jarosz 1994; Biere & Antonovics 1996; Biere & Honders 1996b). Reduced floral longevity is associated with decreased infection by *M. violaceum* in *Silene latifolia* and *S. dioica* (Shykoff *et al*. 1996), although increases in spore deposition and no differences in infection have also been observed in *S. latifolia* (Thrall & Jarosz 1994). While reduced floral longevity decreases a flower's potential exposure to disease vectors, the mechanisms driving increased infection of early flowering plants are less clear. Two dominant hypotheses have emerged. First, there is typically a higher density and frequency of spore-producing flowers in populations early in the season (Alexander 1990). Second, experienced bees prefer uninfected flowers compared with infected flowers later in the season, whereas naïve bees do not discriminate early in the season when diseased plants begin to appear (Jennersten 1988). Thus, potential vectors are more likely to encounter the pathogen as well as interact with diseased plants earlier vs. later in the season, both of which could increase transmission to healthy plants. Since *M. violaceum* sterilises the plant, there is presumably strong selection on floral traits that prevent the attraction of vectors. Accordingly, natural selection by the vector/pathogen has been shown to drive the evolution of delayed flowering in *S. latifolia* (Biere & Antonovics 1996).

Variation in floral morphological traits also affects visitation by pathogen vectors. For example, plants with larger flowers and longer stigmas, styles and ovaries received greater *M. violaceum* spore deposition, resulting in increased infection

Table 1 Floral traits influencing the transmission of vectored plant pathogens

Trait category	Floral trait	Pathogen–plant interaction	References
Attraction of vectors to healthy plants			
Longevity	Flower duration	Greater, less and no difference in attraction of *Microbotryum violaceum* vectors to longer flowering *Silene latifolia* and *S. dioica*	Thrall & Jarosz (1994), Shykoff *et al.* (1996)
Phenology	Flower timing	Greater and no difference in attraction of *M. violaceum* vectors to earlier flowering *S. latifolia*, *S. dioica*, *Melampyrum pratense* and *Visceria vulgaris*	Jennersten 1988, Alexander (1989), Jennersten & Kwak (1991), Thrall & Jarosz (1994), Alexander & Antonovics (1995), Biere & Antonovics (1996), Biere & Honders (1996b)
Morphology	Corolla width/petal length	Greater attraction of *M. violaceum* vectors to larger flowered *S. dioica*, *S. latifolia* and *Dianthus sylvester*	Elmqvist *et al.* (1993), Shykoff *et al.* (1997), Biere & Honders (2006)
	Stigma/style/ovary length	Greater and no difference in attraction of *M. violaceum* vectors to *S. dioica*, *S. latifolia* and *D. sylvester* with longer stigmas/styles/ovaries	Elmqvist *et al.* (1993), Shykoff *et al.* (1997), Biere & Honders (2006)
	Number of flowers per inflorescence	Greater and no difference in attraction of *M. violaceum* vectors to *S. latifolia* and *V. vulgaris* with more flowers per inflorescence	Alexander & Antonovics 1988, Jennersten (1988), Alexander (1989), Thrall & Jarosz (1994), Shykoff & Bucheli (1995), Biere & Antonovics (1996), Biere & Honders (2006)
Reward	Nectar volume	No difference in attraction of *M. violaceum* vectors to *D. sylvester* with greater nectar volume	Shykoff *et al.* (1997)
	Nectar sugar concentration	Greater attraction of *M. violaceum* vectors to *S. latifolia* with greater nectar sugar concentration	Shykoff & Bucheli (1995)
Chemistry	VOCs	Greater attraction of *M. violaceum* vectors to *S. latifolia* with greater amounts of VOCs; no difference in attraction to qualitative differences in VOC composition	Dotterl *et al.* (2009)
Pathogen establishment in flowers			
Longevity	Flower duration	Greater infection of *M. violaceum* in *S. latifolia* with later-abscising flowers	Kaltz & Shykoff (2001)
Phenology	Flower age	Greater infection of *Erwinia amylovora*, *Monilinia vaccinii-corymbosi* and *Botrytis cinerea* in younger flowers of *Malus pumila*, *Vaccinium corymbosum* and *Vitis vinifera* respectively	McClellan & Hewitt (1973), Ngugi *et al.* (2002), Thomson & Gouk (2003), Pusey & Smith (2007)
Morphology	Style length	Greater infection of *M. vaccinii-corymbosi* in *V. corymbosum* with longer styles	Lehman *et al.* (2007)
Reward	Nectar sugar concentration	Greater growth of *E. amylovora* in nectar with less concentrated nectar	Pusey (1999)
	Nectar sugar composition	Greater growth of *E. amylovora* in nectar with higher proportion of disaccharides: monosaccharides	Pusey (1999)
Chemistry	Antimicrobial nectar	Greater inhibition of *Erwinia tracheiphila* by *Cucurbita pepo* nectar vs. controls	Sasu *et al.* (2010b)
	Nectar H_2O_2 via nectarins	Greater and no difference in inhibition of *B. cinerea* and *E. amylovora* growth, respectively, by *Nicotiana* nectar vs. controls	Thornburg *et al.* (2003), Carter *et al.* (2007)
Attraction of vectors to diseased plants			
Longevity	Flower duration	Greater and less attraction of *Fusarium verticillioides* and *M. violaceum* vectors to longer flowering infected *Moussonia deppeana* and *V. vulgaris* respectively	Jennersten (1988), Jennersten & Kwak (1991), Lara & Ornelas (2003)
Phenology	Flower timing	Reduced attraction of *M. violaceum* vectors to earlier flowering infected *V. vulgaris*	Jennersten & Kwak (1991)
Morphology	Ovary length/width	Reduced attraction of *M. violaceum* vectors to infected *S. latifolia* with shorter and thinner ovaries	Biere & Honders (2006)
	Number of flowers per inflorescence	Greater attraction of *M. violaceum* and *F. verticillioides* vectors to greater numbers of flowers on infected *S. dioica*, *S. latifolia* and *V. vulgaris,* and *M. deppeana* respectively	Jennersten (1988), Jennersten & Kwak (1991), Shykoff & Bucheli (1995), Lara & Ornelas (2003), Biere & Honders (2006)

(continued)

Table 1. (continued)

Trait category	Floral trait	Pathogen–plant interaction	References
Reward	Nectar volume	Reduced attraction of *M. violaceum* vectors to lower reward infected *V. vulgaris*; greater attraction of *F. verticillioides* vectors to higher reward infected *M. deppeana*	Jennersten (1988), Jennersten & Kwak (1991), Lara & Ornelas (2003)
	Nectar sugar concentration	Reduced attraction of *M. violaceum* and *Uromyces pisi* vectors to lower reward infected *V. vulgaris* and *S. latifolia,* and *Euphorbia cyparissias**	Jennersten & Kwak (1991), Shykoff & Bucheli (1995), Pfunder & Roy (2000)
Chemistry	VOCs	Greater and no difference in attraction of *Puccinia monoica* and *P. arrhenatheri* vectors to infected *Arabis drummondii** and *Berberis vulgaris** VOCs respectively	Roy & Raguso (1997), Naef *et al.* (2002)
Visual	Colour	Greater attraction of *P. monoica* vectors to yellow vs. white *A. drummondii** artificial pseudoflowers	Roy & Raguso (1997)

*denotes fungal-induced pseudoflowers

(Elmqvist *et al.* 1993; Shykoff *et al.* 1997; Biere & Honders 2006). In a naturally infected population of *D. sylvester*, vector preference for larger flowers drove natural selection for decreased flower size (Shykoff *et al.* 1997). Similarly, Elmqvist *et al.* (1993) found a strong signature of local adaptation for flower size and style length, where *S. dioica* plants from islands without *M. violaceum* had larger flowers and longer styles than plants from islands where the disease was present. Importantly, in a common garden, the larger flowering plants from unexposed islands received nine times as many spores and were more likely to become infected compared with plants from islands exposed to *M. violaceum*. This within-species pattern of greater infection in larger flowered plants also occurs across plant species, suggesting that there may be broad consistency in the floral traits important for vector visitation and/or infection. Across 160 species in the genus *Silene*, there was a trend for species with larger flowers to be infected by *M. violaceum* (Thrall *et al.* 1993). More generally, this relationship between flower size and infection is consistent with a broad pattern of balancing selection on flower size by invertebrate floral mutualists and antagonists (Strauss & Whittall 2006).

Finally, floral volatiles and nectar rewards can also affect vector attraction to healthy plants. Vectors were more attracted to flowers that emitted greater amounts of scent compounds, whereas qualitative differences in scent composition had little effect on visitation (Dotterl *et al.* 2009). In addition, increased nectar sugar concentration of male vs. female *S. latifolia* plants was associated with increased vector visitation to males (Shykoff & Bucheli 1995). However, the one study that investigated natural selection by the vector/pathogen on nectar traits found that morphological traits were under much stronger selection than nectar rewards in *D. sylvester* (Shykoff *et al.* 1997).

(2) Pathogen establishment in flowers

Flower duration, age and morphology can affect pathogen establishment in multiple hosts. Male *Silene latifolia* plants that dropped flowers more quickly following inoculation with *M. violaceum* were less likely to become infected (Kaltz & Shykoff 2001). In addition, infection was more likely in young vs. old flowers of *Vitis vinifera* by *Botrytis cinerea* (McClellan & Hewitt 1973), *Malus pumila* by *Erwinia amylovora* (Thomson & Gouk 2003; Pusey & Smith 2007) and *Vaccinium corymbosum* by *Monilinia vaccinii-corymbosi* (Ngugi *et al.* 2002). Interestingly, style length was associated with susceptibility to *M. vaccinii-corymbosi* across several *V. corymbosum* cultivars (Lehman *et al.* 2007). Hyphal growth rate within styles tended to be higher in the longer styles of more susceptible cultivars, suggesting a portion of resistance is expressed during fungal growth in the gynoecial pathway.

Floral nectar and the nectary are important for resistance to pathogens in several ways. In artificial nectar experiments, population growth of *E. amylovora* generally decreased with increasing sugar concentration and the ratio of disaccharides (sucrose) to monosaccharides (fructose and glucose) (Pusey 1999). This suggests that the concentration and composition of primary metabolites in nectar can affect pathogen establishment. Several studies have speculated on the importance of secondary metabolites in nectar and other floral tissues for pathogen establishment (e.g. Biere & Antonovics 1996; Ngugi & Scherm 2004; Farkas *et al.* 2011). One study has shown that nectar from *Cucurbita sp.* inhibited *Erwinia tracheiphila* growth *in vitro*, although the specific mechanism was unknown (Sasu *et al.* 2010b). Finally, volatile secondary metabolites may affect pathogen establishment in flowers. Emission of the sesquiterpene (E)-β-caryophyllene from *Arabidopsis thaliana* stigmas increased resistance to floral infection by *Pseudomonas syringae* (Huang *et al.* 2012). As (E)-β-caryophyllene is one of the most common volatile compounds in floral scents across angiosperms (Knudsen *et al.* 2006), similar inhibition may occur for vectored floral pathogens.

Nectar proteins, or nectarins (reviewed in Heil 2011), were discovered more than 80 years ago and may also play a pivotal defensive role in protecting against microbes. For example, in ornamental tobacco, 21% of nectary-based cDNAs were related to defence and many defence genes were more strongly expressed in nectary than foliage tissue (Thornburg *et al.* 2003). Nectarins can create high levels of hydrogen peroxide via the nectar redox cycle, with the potential to protect against nectar-inhabiting microbes (e.g. yeasts) as well as pathogens that gain access to plants through nectaries. Necta-

rins have demonstrated antimicrobial function in a few systems; for example, nectarins in *Nicotiana spp*. nectar can inhibit *Botrytis cinerea* (Thornburg *et al*. 2003), *Escherichia coli, Pseudomonas fluorescens, Pseudomonas syringae* and *Salmonella typhimurium* (Carter *et al*. 2007). Conversely, nectarin activity had no effect on *Erwinia amylovora* population growth, suggesting potential adaptations of this plant pathogen to tolerate nectar redox conditions (Carter *et al*. 2007). Characterising genes underlying nectarin production provides an exciting avenue to understand the evolution of traits that play a major role in floral pathogen defence, with potential to engineer resistance traits into agricultural plants.

(3) Attraction of vectors to diseased plants

Several flower-exploiting pathogens manipulate plant traits to attract vectors, although positive effects on transmission are not universal. For example, infection by *M. violaceum* caused *Visceria vulgaris* to bloom earlier and have flowers that remained open longer than healthy plants (Jennersten 1988; Jennersten & Kwak 1991). The earlier blooming of infected plants caused naïve pollinators to visit infected plants and become vectors, even though they preferred healthy plants later in the season (Jennersten 1988). *Moussonia deppeana* flowers infected with *Fusarium verticillioides* were also retained on average 2 days longer than healthy flowers, and this increased floral longevity was associated with increased visitation by hummingbird vectors (Lara & Ornelas 2003). Altered phenology has also been observed in pseudoflower-producing pathogens. For example, instead of 'flowering' at the same time as healthy plants, *Arabis drummondii* infected with *Puccinia monoica* produce pseudoflowers at the same time as *Ranunculus inamoenus,* which they resemble visually (Roy 1994). Greater insect visitation to pseudoflowers occurs when they are next to *R. inamoemas*, suggesting the pathogen may exploit the phenology of co-occurring plants to increase attraction of insect vectors.

Floral morphological traits are also affected by plant pathogens, with various consequences for vector visitation. *Silene latifolia* and *S. dioica* infected with *M. violaceum* produce smaller and more irregularly shaped flowers (Alexander & Maltby 1990; Biere & Honders 1996a; Shykoff & Kaltz 1998), which is associated with reduced vector visitation to *S. latifolia* (Biere & Honders 2006). While infection generally increases the number of flowers produced by plants (Lee 1981; Jennersten 1988; Shykoff & Bucheli 1995), potential vectors still discriminated against infected inflorescences (Jennersten 1988; Jennersten & Kwak 1991; Shykoff & Bucheli 1995; Biere & Honders 2006). The opposite pattern was found for *F. verticillioides* infection of *M. deppeana* (Lara & Ornelas 2003). Here, diseased plants produced more flowers than healthy plants, which was associated with more than twice as many hummingbird visits to flowers on diseased vs. healthy plants.

Altered nectar rewards of infected plants can also affect vector visitation. The volume, concentration and energy content of nectar are typically reduced on *Visceria vulgaris, S. latifolia, S. latifolia* and *S. dioica* plants infected by *M. violaceum* (Jennersten & Kwak 1991; Shykoff & Bucheli 1995; Biere & Honders 1996a; Shykoff & Kaltz 1998). This reduction in floral rewards caused pollinators to discriminate against flowers from diseased plants (Jennersten 1988; Jennersten & Kwak 1991; Shykoff & Bucheli 1995). By contrast, for *F. verticillioides* infection of *M. deppeana* (Lara & Ornelas 2003), infected plants produced more nectar over a longer period than healthy plants, which likely contributed to the increased visitation of hummingbirds. Vectors are also highly attracted to nectar rewards produced by pseudoflowers. Sugar-rich exudates that closely resemble the composition of nectar are produced in the pseudoflowers of *Arabis spp*. infected by *Puccinia monoica* (Roy 1993) and *Vaccinium spp*. infected by *Monilinia vaccinii-corymbosi* (Batra & Batra 1985), as well as several other systems (e.g. Patt 1992; Pfunder & Roy 2000; Naef *et al*. 2002).

Finally, infected plants can attract vectors via olfactory and visual cues. In a series of elegant experiments, Roy & Raguso (1997) showed that both olfactory and visual cues were important for vector attraction to pseudoflowers induced by *Puccinia monoica* on *Arabis spp*. Interestingly, the relative importance of olfactory vs. visual cues depended on the vector; olfactory cues were more important for flies, whereas visual cues tended to be more important for bees (Roy & Raguso 1997). Subsequent work with *P. monoica* and *P. arrhenatheri* found that pseudoflower compounds mimic both host plant floral fragrance and insect pheromones (Raguso & Roy 1998; Naef *et al*. 2002), suggesting a highly efficient mechanism of vector attraction. Olfactory and visual cues are utilised by several additional pseudoflower-inducing fungi, suggesting their importance (Batra & Batra 1985; Patt 1992; Pfunder & Roy 2000).

(4) Pathogen acquisition by vectors on diseased plants

While floral traits that affect vector attraction and pathogen establishment have been described in several systems, we are aware of no studies addressing how floral traits affect pathogen acquisition by vectors upon visiting diseased plants. One study has speculated on such a trait. Roy (1993) noted that visits by pollinators to pseudoflowers were much longer than to co-occurring flowers, and speculated this was due to the diffuse presentation of nectar across the large pseudoflower surface (as opposed to concentration in a small organ such as a nectary). As insects were detained for longer periods of time, the likelihood of pathogen acquisition by vectors potentially increased. Pathogen acquisition is a major component of disease transmission, and further studies are clearly warranted to fill this gap in our knowledge.

ANIMAL PATHOGENS

Compared with plant pathogens, relatively few animal pathogen studies have investigated transmission at flowers (Table 2). This is an important gap in our knowledge, as horizontally spread pathogens have been implicated in recent declines in several pollinator species, including bumble bees in North America (Cameron *et al*. 2011). Only one study, to our knowledge, has experimentally shown the transmission of a naturally occurring animal pathogen at flowers. The bumble bee pathogen *Crithidia bombi* (Trypanosomatidae) is transmitted between hosts through the shared use of flowers (Durrer

& Schmid-Hempel 1994). *Crithidia bombi* cells are shed in the liquid faeces of infected bumble bees, which may contaminate floral surfaces and nectar and lead to infection of novel hosts through inadvertent consumption of the pathogen (Durrer & Schmid-Hempel 1994). As a consequence, *C. bombi* is frequently transmitted within and across different bumble bee species, and similarity in flower visitation patterns is a good predictor for the distribution of pathogen genotypes among hosts of different species (Salathe & Schmid-Hempel 2011).

For other microorganisms, floral transmission between hosts may be inferred on the basis of more indirect evidence. Indistinguishable strains of *Spiroplasma* bacteria, for example, have been found on the surface of flowers and in the haemolymph of honey bees, suggesting horizontal transmission at flowers (Raju *et al*. 1981). Molecular genetic surveys of pathogens provide a powerful tool for assessing disease prevalence within networks of pollinators (Singh *et al*. 2010; Evison *et al*. 2012; Li *et al*. 2012). For example, using genetic techniques, Evison *et al*. (2012) found that deformed wing virus (DWV), *Ascosphaera* fungi, and microsporidia including *Nosema* exhibited broad overlap among 17 field-collected pollinator species that utilise similar floral resources. Similarly, based on pathogen sequence data from pollinators and the pollen loads they were carrying, Singh *et al*. (2010) found evidence for pollen-mediated exchange of a range of picorna-like viruses between 12 hymenopteran pollinators. Using phylogenetic techniques, the authors showed that there was little evidence for clustering of viruses among specific pollinator species; rather, viruses were shared, presumably via shared use of pollen resources. Singh *et al*. (2010) further demonstrated transmission of Israeli acute paralysis virus (IAPV) between honey bees and bumble bees in a greenhouse environment, likely through shared flower use. Such surveys provide a glimpse of the largely unstudied diversity and transmissibility of animal pathogens deposited at flowers. Further manipulative experiments offer the opportunity to more directly reveal mechanisms of transmission.

Similar to the effects of nectar-inhabiting microorganisms on plants, it is important to note that microbes acquired by animals at flowers can vary from pathogenic to beneficial (Table 2). For example, pollinators may acquire 'probiotics' such as lactic acid bacteria at flowers (McFrederick *et al*. 2012; Vasquez *et al*. 2012). Lactic acid bacteria found in honey bee

Table 2 Animal pathogens transmitted at flowers

Microbe	Hosts	Effect on host	Plant tissue implicated	References
Floral transmission shown experimentally				
Crithidia bombi (Excavata, Trypanosomatidae)	Bumble bees (*Bombus* spp.)	Pathogenic, reduced colony founding success, increased mortality under stressed conditions	General floral surface, nectar	Durrer & Schmid-Hempel (1994), Salathe & Schmid-Hempel (2011)
Indirect evidence of floral transmission				
Ascosphaera spp. (Fungi, Ascomycota)	Honey bees (*Apis* spp.), alfalfa leafcutting bee (*Megachile rotundata)* and other bee species	Pathogenic to bee brood (chalkbrood disease)	Horizontal transmission suggested via spores on floral surfaces	Batra *et al*. (1973), Stephen *et al*. (1981), Evison *et al*. (2012)
Aspergillus flavus and other *Aspergillus* spp. (Fungi, Ascomycota)	Honey bees, diverse solitary bees	Pathogenic moulds of bee brood (stone brood disease)	Present in nectar, but spores may also originate from other environmental sources (e.g. soil)	Batra *et al*. (1973)
Diverse ascomycetous yeast species (Fungi, Ascomycota)	Honey bees, bumble bees, stingless bees (Meliponini), diverse solitary bees	Commensals, but have been implicated both in the preservation and spoilage of bee brood provisions	Nectar	Batra *et al*. (1973), Ganter (2006)
Nosema apis, *N. ceranae*, *N. bombi* (Fungi, Microsporidia)	Honey bees, bumble bees	Pathogenic to adult bees	Horizontal transmission suggested via spores on floral surfaces	Imhoof & Schmid-Hempel (1999), Colla *et al*. (2006), Li *et al*. (2012)
Spiroplasma apis, S. melliferum (Bacteria, Mollicutes)	Honey bees, bumble bees	Pathogenic, increased mortality in honey bees	Floral surfaces	Raju *et al*. (1981), Mouches *et al*. (1984), Meeus *et al*. (2012)
Lactic acid and acetic acid bacteria (Lactobacillales & Acetobacteraceae)	Honey bees, bumble bees, stingless bees, halictid bees (Halictidae)	Commensals? May either preserve or spoil bee brood provisions; protection against larval pathogens in honey bees	Pollen, nectar	Batra *et al*. (1973), McFrederick *et al*. (2012), Vasquez *et al*. (2012)
Hymenopteran picorna-like viruses: Deformed wing virus (DWV), Black queen cell virus (BQCV), Sacbrood virus (SBV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV)	Honey bees, bumble bees, diverse other bee species (e.g. *Xylocopa virginica Andrena* sp., *Ceratina dupla*, *Augochlora pura*), wasps (e.g. *Bembix* sp., *Vespula vulgaris*, *Polistes spp*.)	Pathogenic to honey bees, effect on other Hymenoptera unclear	Pollen	Bailey (1975), Genersch & Aubert (2010), Singh *et al*. (2010), Evison *et al*. (2012), Li *et al*. (2012)

crops and floral nectar were shown experimentally to reduce mortality from infections with European foulbrood (*Melissococcus plutonius*) in honey bee larvae (Vasquez *et al*. 2012). On the other hand, lactic acid bacteria appeared to spoil pollen provisions of ground-nesting solitary bees, suggesting detrimental effects to other hosts (Batra *et al*. 1973). Thus, the type of interaction between flower-associated microbes and animal hosts can depend on host species and ecological context.

FLORAL TRAITS INFLUENCING TRANSMISSION OF ANIMAL PATHOGENS

In contrast to the relative wealth of evidence for the role of floral traits in plant pathogen transmission (Table 1), there is an almost complete lack of knowledge on how floral traits mediate the transmission of microorganisms in animals. Based on a study of *Crithidia bombi* and bumble bees, the morphological complexity of inflorescences may influence floral transmission (Durrer & Schmid-Hempel 1994). Bees were more likely to become infected at simple, linear inflorescences than at complex, spiral ones of the same plant species. The same study also showed that infection probability differed between two plant species, *Rubus caesius* and *Echium vulgare*. *Crithidia bombi* was less likely to be transmitted on the randomly distributed, flat and readily accessible flowers of *Rubus caesium*, than on the spirally arranged flowers with long corolla tubes of *Echium vulgare*. However, the specific underlying mechanisms of these transmission differences between plant species were not investigated.

In addition to morphological traits, plant chemical traits may be important for animal pathogens transmitted at flowers. One study has examined the role of nectar secondary chemistry on pathogen establishment. Bumble bees that were fed *C. bombi* inoculum in artificial nectar with or without gelsemine, the primary alkaloid from *Gelsemium sempervirens* nectar, did not differ in subsequent pathogen load, suggesting that this secondary compound does not initially influence pathogen establishment in hosts. However, nectar gelsemine consumed post-infection did reduce pathogen loads, indicating anti-pathogen activity when consumed during infection (Manson *et al*. 2010). The importance of self-medication across animal taxa is beginning to be recognised (de Roode *et al*. 2013), and this study suggests pollinators may benefit from ingesting plant secondary compounds when challenged with disease. Thus, floral chemistry may play a significant but largely unrecognised role in pollinator–pathogen infection dynamics.

Although few studies have addressed how floral traits mediate pathogen transmission to animals, we hypothesise that the same four mechanisms by which floral traits affect plant pathogen transmission also influence animal pathogen transmission. All these hypothesised mechanisms could be tested in future research.

(1) Floral attractiveness of uninoculated plants

For plant pathogens, traits such as floral longevity and phenology, floral morphology and nectar rewards are important for vector attraction. These same traits and others, such as UV reflectance and floral volatiles, are likely to affect the attraction of hosts depositing animal pathogens, thus increasing the probability of inoculating flowers.

(2) Pathogen acquisition and viability in flowers

The probability that infectious material is deposited on a flower during visitation by an infected animal may be influenced by several floral traits. For example, complex flowers or nectar rewards necessitating long handling times by pollinators may increase the likelihood of inoculation. Conversely, faecally transmitted pathogens may rarely be deposited on flowers that, due to their morphology, are only contacted by the anterior part of a pollinator. Such a mechanism may be important for *C. bombi* transmission among bumble bees (Durrer & Schmid-Hempel 1994).

Following deposition on flowers, animal pathogens must remain viable until they are acquired by a new host. For microorganisms adapted to living in association with animals, flowers represent an alien and potentially hostile environment. In contrast to plant pathogens, however, active growth within the flower would not be necessary, and animal pathogens may simply be found as spores or resting stages within flowers. Still, survival time on flowers is a crucial variable for successful transmission to novel hosts, and a range of floral traits could affect microbial survival, such as antimicrobial volatiles, primary and secondary compounds in pollen and nectar or the exposure of floral surfaces to UV radiation and desiccation.

(3) Floral attractiveness of inoculated plants

Similar to plant pathogens, animal pathogens may alter floral traits and thereby alter the attractiveness of flowers. For example, bumble bees were shown to avoid flowers experimentally inoculated with *C. bombi* (Fouks & Lattorff 2011). Bees frequently extended their proboscis and tasted floral nectar before landing; thus, they may be able to detect chemical changes in nectar or headspace volatiles due to microbial presence. Pathogen-mediated changes in nectar chemistry and floral volatiles are known to impact the attraction of plant pathogen vectors, and these mechanisms could also apply to the attraction of hosts for animal pathogens. While it would benefit animal pathogens to attract as many hosts as possible, the ability to discriminate between inoculated vs. uninoculated flowers would clearly benefit pollinators. Further work will likely reveal how floral traits mediate this intriguing yet almost completely unexplored aspect of animal pathogen transmission.

(4) Pathogen acquisition and establishment in hosts upon visiting inoculated flowers

Just as floral rewards and morphology may alter pollinator behaviour in ways that increase the likelihood of depositing inoculum, these traits could influence the probability that visitors acquire inoculum from infected flowers. For example, traits that affect animal contact with infected floral surfaces and the time spent to access floral rewards would likely influence the probability of acquisition. Furthermore, once animals acquire inoculum from flowers, floral traits may still influence the successful establishment of infection. For example, antimi-

crobial substances ingested with nectar or pollen can reduce infection loads in pollinators (Manson *et al*. 2010). In addition, components of nectar or pollen may modulate the animal host's immune system, as has been shown for *p*-Coumaric acid (a component of pollen grains), which activated immune and detoxification pathways in honey bees (Mao *et al*. 2013).

APPLICATIONS

The study of how floral traits affect disease transmission in plants and animals provides several opportunities to improve pathogen control in agricultural systems as well as address topics pertinent to human health. We summarise three examples below.

Pollinator vectoring of biocontrol agents

Because honey bees and bumble bees are often managed in agricultural settings, there is growing interest in using these pollinators to vector microbial biocontrol agents to flowers. Biocontrol agents are typically non-pathogenic species or genotypes of bacteria or fungi that suppress target pathogens in flowers. Hive-mounted dispensers that permit honey bees and bumble bees to acquire biocontrol agents have been used successfully to limit *Monilinia vaccinii-corymbosi* in blueberry (Dedej *et al*. 2004) and *Erwinia amylovora* in apple and pear (reviewed in Farkas *et al*. 2011), as well as other systems. However, the success of using vectored biocontrol agents to suppress target diseases can vary widely depending on environmental conditions, timing of implementation and the cultivars present (Farkas *et al*. 2011). We suggest that an understanding of how floral traits affect vector attraction to flowers and microbial interactions within flowers will likely facilitate increased effectiveness of pollinator-vectored biocontrol. For example, some bacterial strains that combat *E. amylovora* are more susceptible to high nectar sugar concentration than *E. amylovora* (Pusey 1999). Fluctuations in nectar sugar concentration in apple and pear are highly dependent on precipitation patterns, humidity and host plant genotype (reviewed in Farkas *et al*. 2011). Thus, considering the influence of cultivar and environmental context may help predict when this vectored biocontrol agent is likely to be effective. Similarly, flower age can affect the relative population growth rates of *E. amylovora* vs. biocontrols (Thomson & Gouk 2003). Thus, using vectored biocontrol agents at the most appropriate phenological stage of flowering is also likely to influence effectiveness. Improving basic knowledge of floral traits may therefore have important applications for improving management decisions.

Pesticides and pathogens of pollinators

Insecticides such as neonicotinoids can be found in nectar and pollen of flowering plants (Blacquiere *et al*. 2012). Analogous to plant secondary compounds in nectar or pollen, the presence of pesticides in flowers may have a considerable effect on the infection dynamics of flower-visiting animals. For example, at otherwise non-lethal concentrations, neonicotinoids have been shown to weaken the immune system of honey bees, increase infection loads with parasites or viral pathogens and lead to increased mortality in the face of pathogen infections (Alaux *et al*. 2010; Di Prisco *et al*. 2013). Neonicotinoids can also impair cognitive functions in honey bees, including olfactory learning (Palmer *et al*. 2013). As olfactory cues may aid in bee avoidance of pathogen-inoculated flowers (Fouks & Lattorff 2011), cognitive impairment by pesticides could potentially increase the likelihood of bees becoming infected in the field. Soil application of various neonicotinoids are a popular method to introduce these systemic pesticides into crops, and plant species and cultivars within species vary in their ability to take up and concentrate neonicotinoids in pollen and nectar (Stoner & Eitzer 2012 and references therein). A greater understanding of the plant traits responsible for this variation in neonicotinoid concentration in pollen and nectar could have important management and conservation implications. For example, if neonicotinoids will be applied via soil, utilising species or cultivars that accumulate little pesticide in pollen and nectar could minimise exposure to pollinators.

Mosquito nectar feeding and human diseases

Although no human diseases to our knowledge are acquired by insects at flowers, floral traits have the potential to affect the capacity of mosquitoes to vector human diseases. For example, sugar feeding is essential for adult mosquitoes (Foster 1995). Preferences for nectar from certain plant species correlate both with sugar availability and mosquito survival and egg laying, suggesting a role for floral traits mediating population dynamics that affect vectoring capacity (Manda *et al*. 2007). Access to sugar-rich plant sources in the field can increase mosquito survival and longevity, which could increase vector capacity (e.g. Stone *et al*. 2012), although reduced biting rates in sugar-rich environments may counteract these effects. One avenue to combat mosquito-transmitted pathogens is the use of genetically modified gut bacteria to deliver a pathogen-inhibiting effector molecule; nectar feeding may be an effective way to introduce these bacteria into wild mosquito populations (Lindh *et al*. 2006). Alternatively, secondary compounds in floral nectar have the potential to affect feeding rates and pathogen loads in mosquitoes. For example, one study has shown that nectar secondary compounds can reduce pathogen loads in bumble bees (Manson *et al*. 2010); we are aware of no work exploring this phenomenon in mosquitoes. A greater understanding of how floral traits mediate pathogen transmission dynamics could lead to effective, low-cost methods to reduce mosquito-borne diseases.

FUTURE DIRECTIONS

Numerous exciting topics are emerging in the study of how floral traits impact plant and animal pathogens. We highlight three areas that we believe are particularly promising avenues for future research.

Linking floral traits to pathogen transmission in animals

Pathogens are one of the contributing factors implicated in recent declines in several pollinator species, including bumble

bees (e.g. Cameron *et al*. 2011). Dynamics of within-colony pathogen transmission are being examined for social bees, but for social as well as solitary bees we know surprisingly little about where pathogens are first acquired or the factors that play a role in transmission. *Crithidia bombi* infects healthy *Bombus* foraging at flowers where infected bees have defecated (Durrer & Schmid-Hempel 1994). *Nosema* also infects honey and bumble bees that feed on spores deposited in the faeces of infected nestmates. It has been speculated that transmission on flowers is a likely mode of horizontal transmission for *Nosema* (Imhoof & Schmid-Hempel 1999), but this has not been demonstrated. Aside from a pioneering study conducted nearly two decades ago (Durrer & Schmid-Hempel 1994) and one more recent examination of nectar chemical traits (Manson *et al*. 2010), we know little about plant traits that facilitate or impede animal infection. Are bees more likely to become infected at certain plant species, and if so, why? Do plant species, or individuals within species, differ in the amount of pathogen inoculum they harbour? Is the likelihood of infection strictly a function of visitation rate (which would presumably affect both the deposition and acquisition of pathogen cells), or do floral morphological, chemical or other traits affect the likelihood of infection after controlling for visitation rate? Understanding the distribution of pathogens across and within plant species and the role of floral traits mediating transmission to new hosts are critical first steps for predicting where pathogen impacts are likely to be greatest and how they could be mitigated, in both ecological and agricultural settings.

Chemical ecology of plant–pathogen–vector interactions

To our knowledge, no studies to date have directly linked floral secondary compounds to the establishment of a vectored floral pathogen. One recent study has found that a major floral volatile, (E)-β-caryophyllene, acts as an antimicrobial defence against a bacterial plant pathogen, *Pseudomonas syringae* (Huang *et al*. 2012). The ubiquity of (E)-β-caryophyllene in angiosperm floral scents (Knudsen *et al*. 2006) raises several intriguing questions. Do additional antimicrobial floral volatiles exist? Do different volatiles function in pollinator attraction vs. disease resistance? Floral volatiles are also important for the attraction of disease vectors (Raguso & Roy 1998; Naef *et al*. 2002; Dotterl *et al*. 2009); can plants balance volatile production to maximise antimicrobial defence and pollination while minimising visitation by pathogen vectors? In addition to volatile secondary compounds, little work has addressed the importance of non-volatile floral defences. The importance of antimicrobial enzymes in nectar is beginning to be recognised (Heil 2011), yet the expansion of this research beyond the laboratory or to vectored floral pathogens has been minimal (Thornburg *et al*. 2003; Carter *et al*. 2007). As two of the most economically devastating floral pathogens infect plants through the nectary (Sasu *et al*. 2010a; Farkas *et al*. 2011), a greater understanding of how nectar defences function will be beneficial to both basic and applied biologists. Overall, further work on the chemical ecology of plant–pathogen–vector interactions has the potential to greatly increase our knowledge regarding mechanisms of disease transmission.

Selection on floral traits by floral visitors and microbes

Natural selection on floral traits has typically been studied via pairwise interactions between plants and animals (e.g. McCall & Irwin 2006; Strauss & Whittall 2006). However, the selective pressures of animals become more complicated when they vector microbes. To our knowledge, three studies have considered the selective pressures of animals that vector plant pathogens to flowers, and each has found that floral traits evolve rapidly to reduce attractiveness to pollinators (Elmqvist *et al*. 1993; Biere & Antonovics 1996; Shykoff *et al*. 1997). We note that each of these studies considers *Microbotryum violaceum*, which sterilises the plant and therefore exerts a strong antagonistic effect on plant fitness. Several vectored floral pathogens have fitness impacts that are comparatively mild (e.g. Batra 1983; Lara & Ornelas 2003). How do floral traits evolve in systems where the fitness impacts of the plant pathogen are less severe? Conversely, plants vary markedly in their reliance on pollinators for reproduction, and this reliance on pollinators can be linked to the evolution of floral traits such as defensive chemistry (Adler *et al*. 2012). How does reliance on pollinators affect the evolution of attractive/defensive floral traits in systems where plant mutualists vector microbial antagonists?

Further complicating matters, not all microbes transmitted by pollinators are plant pathogens. For example, the presence of some nectar yeasts can increase pollinator visitation (Herrera *et al*. 2013; Schaeffer & Irwin in press), and pollen donation (Schaeffer & Irwin in press), a component of plant fitness. In addition, animal pathogens that are transmitted at flowers can also affect pollinator visitation, which may affect plant fitness. For example, bumble bees avoid flowers containing *C. bombi* (Fouks & Lattorff 2011). Can floral traits evolve in response to pollinator-transmitted microbes that are not plant pathogens? Finally, we know that some floral traits can affect both pollinators and microbes. For example, the nectar alkaloid gelsemine reduces both pollinator preference for *Gelsemium sempervirens* (Adler & Irwin 2005) and the establishment of *C. bombi* in *Bombus* (Manson *et al*. 2010). Is such trait multi-functionality common? Does multi-functionality constrain or accelerate trait evolution? The recognition that floral microbes are common in nature and can be important agents of selection on floral traits through both direct and indirect pathways presents numerous exciting opportunities for evolutionary biologists.

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AUTHORSHIP

All authors researched and contributed to writing the manuscript.

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