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The biology and prevalence of fungal diseases in managed and wild bees

Sophie EF Evison[1] and Annette B Jensen[2]

Managed and wild bees, whether solitary or social have a plethora of microbial associations that vary in their influence on the health of the bees. In this review, we summarise our current knowledge of aspects of the biology and ecology of bee associated fungi. The biology of bees that fungi are associated with are described, and the likely influences on fungal transmission are discussed. There is a clear disparity in research on fungi associated with managed compared to wild bees, leaving gaps in our understanding of fungal pathogen epidemiology. Translocation of bees to meet global pollination needs will increase exposure of bees to exotic pathogens. Thus, filling these gaps is an important step towards mitigating the impact of fungal diseases in bees.

Addresses
[1] Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield S10 2TN, UK
[2] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C., Denmark

Corresponding author: Evison, Sophie EF (s.evison@sheffield.ac.uk)

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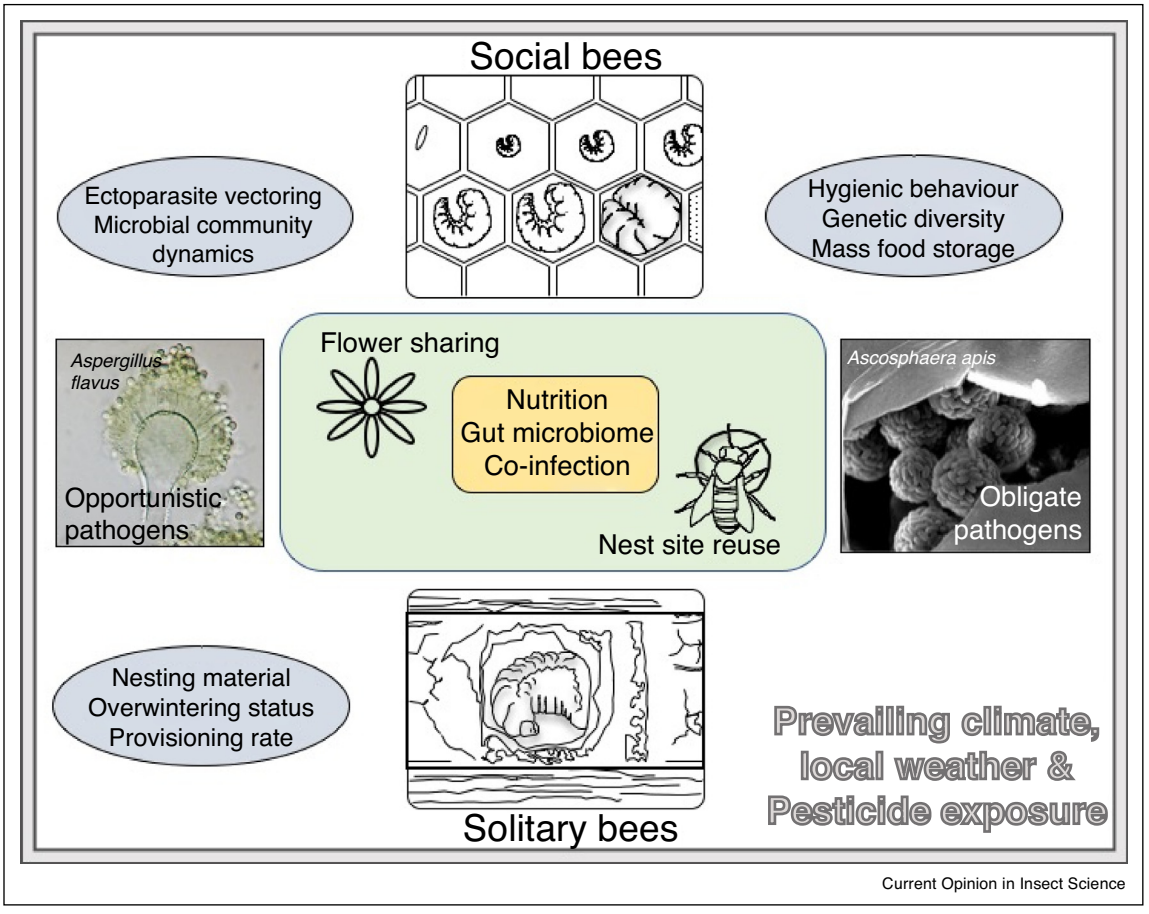
Introduction

Historically, fungi that are pathogens of or live in close association with bees have received very little attention, and generally are understudied [1••]. When bee associated fungi are pathogenic, their impact on the host, both ecologically and economically, varies considerably. Much of this variation depends on the underlying biological characteristics of both the host and the fungal pathogen, both of which influence the transmission potential of the disease. For example, whether the host bee has a solitary or social lifestyle, and whether the fungus is an obligate or opportunistic pathogen (summarised in Figure 1).

The genus *Ascosphaera* is by far the best studied fungal group. It consists of 28 species (Table 1), and all are specialists in the exploitation of bees or their nesting habitats, probably due to their osmophilic nature [1••]. Whilst many species in this genus are saprophytes of bee products (larval faeces, nesting materials, or pupal cocoons [2]), some species of *Ascosphaera* are obligate pathogens of the brood of both solitary and social bees, causing the fungal disease commonly referred to as 'chalkbrood' ([3•] and hereafter references therein; Table 1). In the honey bee *Apis mellifera*, outbreaks of chalkbrood caused by the obligate pathogen *A. apis* are rarely fatal for the colony; it is regarded as a common spring disease and most colonies can recover as they grow stronger over the summer. *Apis mellifera* colonies vary genetically in both individual-level and group-level susceptibility [3•], and a recommended management strategy is to requeen in order to replace the genetic stock of the colony [4]. Conversely, outbreaks of chalkbrood caused by *A. aggregata* in the alfalfa leafcutting bee, *Megachile rotundata* (also known as ragged brood disease), can cause huge economic losses when this species is raised commercially for alfalfa pollination [5]. As a solitary species, management is less straightforward than requeening, and outbreaks in field populations are common and persistent [6]. A fungal brood disease in *A. mellifera* symptomatically similar to chalkbrood is stonebrood, caused by facultative pathogens from the genus *Aspergillus* (Figure 1; Table 1). *Aspergillus* can infect and kill both larval and adult honey bees as well as many other organisms. It is a much rarer disease than chalkbrood, and considered of minor importance in apiculture [7]. However, *Aspergillus* is a zoonotic pathogen that can cause aspergillosis in humans, from mild types such as allergic reactions to true infections of the respiratory system, primarily in immune-compromised patients or those already suffering from other respiratory diseases [8].

In this review, we focus specifically on fungal brood pathogens in both social and solitary bee species. Our current understanding of the biology of bee associated fungi varies considerably (Table 1). The attention of the scientific community towards fungi reflects, to a certain extent, the level of economic losses to apiculture and agriculture as a result of pathogenesis. We include information on saprophytic fungi because there are several instances where species thought to be apathogenic in nature have been shown to be pathogenic when tested *in vitro* [9,10,11•]. We summarise our current knowledge of aspects of the biology of the fungi, the bee species they attack, and discuss likely influences on fungal transmission. We also highlight areas where improving our

Figure 1



Bees collect nutritious pollen and nectar and are themselves a nutritious source for fungal proliferation. Biological characteristics of both the host bee and the fungus will influence fungal pathogenesis and transmission. There are examples of specialised obligate pathogens like *Ascosphaera apis* and opportunistic pathogens like *Aspergillus flavus*. Adaptation to the bee habitat has evolved within the specialised fungi, for example, spore structures for optimal dispersal, whereas the opportunistic fungi rely on saprophytic proliferation on various substrates and their ubiquitous presence. The social bees have an additional line of group defense mechanisms (e.g. hygienic behaviour), however the social lifestyle introduces the risk of social transmission of fungal spores. Flowers, and nest site reuse are possible disease transmission routes for both social and solitary bees. Nutritional status, gut microbiota, co-infections as well as environmental and anthropogenic factors can influence the severity of the disease. The resilience to these factors across the spectrum of bees and fungal parasites is largely unknown and a greater understanding is of paramount importance for mitigating the effects of disease.

knowledge of fungal pathogen epidemiology could help to mitigate the effects of disease in bees.

Pathobiology and aetiology of fungal diseases

Ascosphaera spp. and *Chalkbrood disease*

Pathogenesis by species of *Ascosphaera* occurs only when spores are ingested by larvae, and infection proceeds across the gut lining [1••,3•]. This specific infection route of a live host is a feature of several species in this genus because the spores require high levels of CO_2 (that are found in the anaerobic environment of the gut) before they can germinate [12]. During pathogenesis, the host larva dies as a result of an invasive mycosis, and spores form external to (with the exception of *A. aggregata*, which form spores under) the cuticle [13]. Not all species are obligate pathogens; some grow as saprophytes within the host's nest sites, on faecal matter, pollen provisions or nesting material, but some of these have also been shown to be capable of infection *in vitro* (Table 1). *Ascosphaera* produces its transmission stage as spore ball structures within a unique double walled sporocyst (e.g. *A. apis;* Figure 1), which can persist in the environment for up to 15 years [3•]. There appears to be a close evolutionary relationship between pathogenic *Ascosphaera* and their primary hosts. Narrow host (species or genus) ranges, and no field records of social bee specialists infecting solitary bees or vice versa (Table 1), suggest adaptation by each pathogen to the specific environment of their host. Pathogenicity by *Ascosphaera* is always towards larvae; the adult bees act only as transmission vectors of the fungal

Table 1

Bee associated fungi from five different genera are listed, several are pathogens of bees while others live saprophytically in bee habitats. Their 'lifestyle' and their impact on the host, both ecologically and economically, varies considerably. The 'recorded host(s)' of bee associated fungi include both managed and wild bees, they vary in their 'reproductive structures', some have exclusive sexual reproduction while others have both sexual and asexual reproduction. Their 'Likely transmission routes' largely depend on the biology of the host, the fungal lifestyle and anthropogenic influence in managed systems. Many of the fungi's 'Recorded geographic distribution' is from a single record, thus they appear to have a narrow geographic distribution. Whether this reflects the frequency of studies conducted or is a true sign of host/habitat specialisation requires further study.

Species	Recorded hosts	Ref #	Reproductive structures	Lifestyle	Likely exposure/transmission routes	Recorded geographic distribution
Ascosphaera						
A. apis 'Chalkbrood'	*Apis mellifera* (primary host)	[3•]	Sporocysts	Obligate pathogen of social bees	Commercial beekeeping Flower sharing Honey robbing Social transmission	Worldwide
	Apis ceranae	[48]			Flower sharing Honey robbing Social transmission	
	Xylocopa californica	[49]			Flower sharing Nest site reuse	
	Bombus nevadensis	[14••]			Commercial rearing using contaminated honey bee pollen	
	Bombus vosnesenskii	[14••]				
	Bombus griseocollis	[14••]				
	Megachile rotundata	[11•]		Infrequently pathogenic *In vitro*	*In vitro* assessment	
A. acerosa	*Megachile rotundata*	[50]	Sporocysts	Saprophyte of bee cadavers	Flower sharing	Canada
	Megachile macularis	[51]			Nest site reuse	Australia
A. aggregata	*Megachile rotundata* (primary host)	[2,52]	Sporocysts	Obligate pathogen of solitary bees	Flower sharing Nest site reuse	Europe/N. America
	Megachile pugnata	[53]			Diseased nest mates during emergence	Canada
'Chalkbrood' or 'Ragged brood disease'	*Megachile relativa*	[54]				Canada
	Megachile pacifica	[52]			Sexual transmission via contaminated males	Denmark
	Megachile centuncularis	[52]				Denmark
	Osmia rufa	[52]				Denmark
	Osmia cornuta	[51]				Australia
	Osmia lignaria	[51]				Australia
	Coelioxys rufocaudata	[55]			Cleptoparasitism	Spain
	Apis mellifera	[11•]		Infrequently pathogenic *In vitro*	*In vitro* assessment	n/a
A. asterophora	*Megachile rotundata*	[56] [57] [33]	Sporocysts	Saprophyte	Flower sharing Pollen provisions Nest site reuse Diseased nest mates during emergence Sexual transmission via contaminated males	Denmark Australia USA
A. atra	*Megachile rotundata*	[58,59]	Sporocysts	Saprophyte of pollen provisions, pathogenic *In vitro* only	Flower sharing Nest site reuse	Widespread
	Apis mellifera	[10]			*In vitro* assessment	n/a

Table 1 (*Continued*)

Species	Recorded hosts	Ref #	Reproductive structures	Lifestyle	Likely exposure/transmission routes	Recorded geographic distribution
A. callicarpa	*Chelostoma florisomne*	[2]	Sporocysts	Saprophyte of nest reeds	Unknown	Denmark
A. cinnamomea	*Osmia cornifrons*	[60]	Sporocysts	Unknown	Unknown	Japan
A. celerrima	*Osmia cornifrons*	[60]	Sporocysts	Unknown	Unknown	Japan
A. duoformis	*Trigona carbonaria*	[51]	Sporocysts	Saprophyte of pollen provisions	Flower sharing Social transmission	Australia
	Apis mellifera	[51]		Detected in honey	Flower sharing	
A. fimicola	*Osmia rufa*	[2,52]	Sporocysts	Saprophyte of larval faecal pellets	Flower sharing Nest site reuse	Denmark
	Cacoxenus indagator	[2,52]			Cleptoparasitism	
A. flava	*Megachile* spp.	[51]	Sporocysts	Unknown	Unknown	Australia
A. fusiformis	*Osmia cornifrons*	[57,60]	Sporocysts	Unknown	Unknown	Japan
A. larvis	*Megachile rotundata*	[11•,13,61]	Sporocysts	Obligate pathogen of solitary bees	Flower sharing Nest site reuse Diseased nest mates during emergence Sexual transmission via contaminated males	Canada
	Apis mellifera	[11•]		Infrequently pathogenic *In vitro*	*In vitro* assessment	n/a
A. major	*Megachile centuncularis* *Apis mellifera*	[57,62]	Sporocysts	Saprophyte	Flower sharing Nest site reuse Flower sharing	Widespread
A. naganensis	*Osmia cornifrons*	[60]	Sporocysts	Unknown	Unknown	Japan
A. osmophila	*Megachile mystaceana*	[63]	Sporocysts	Obligate pathogen of solitary bees	Flower sharing Nest site reuse	Australia
A. parasitica	*Osmia cornifrons*	[60]	Sporocyts	Saprophyte	Unknown	Japan
A. pollenicola	*Megachile rotundata*	[13]	Sporocysts	Saprophyte	Flower sharing Nest site reuse	Canada
A. proliperda	*Megachile centuncularis* (primary host)	[57,62]	Sporocysts	Pathogen of solitary bees	Flower sharing Nest site reuse	Denmark
	Megachile rotundata	[9]		Pathogenic *In vitro*	*In vitro* assessment	n/a
	Apis mellifera	[10]		Infrequently pathogenic *In vitro*		
A. scaccaria	*Leioproctus* spp.	[64]	Sporocysts	Obligate pathogen of solitary bees	Flower sharing Nest site reuse	New Zealand
A. solina	Family Colletidae	[51]	Sporocysts	Unknown	Unknown	Australia
A. subcuticularis	*Megachile aethiops*	[51]	Sporocysts	Pathogen of solitary bees	Flower sharing Nest site reuse	Australia
A. subglobosa	*Megachile rotundata*	[33,65]	Sporocysts	Saprophyte	Pollen provisions Nest site reuse	USA

Table 1 (*Continued*)

Species	Recorded hosts	Ref #	Reproductive structures	Lifestyle	Likely exposure/transmission routes	Recorded geographic distribution
A. tenax	*Megachile willughbiella*	[66]	Sporocysts	Saprophyte	Unknown	Denmark
A. torchioi	*Osmia lignaria propinqua*	[67,68]	Sporocysts	Pathogen of solitary bees	Unknown	USA
A. variegata	*Megachile rotundata*	[13]	Sporocysts	Saprophyte	Unknown	Canada
A. verrucosa	*Osmia californica*	[60]	Sporocysts	Saprophyte	Unknown	Japan
A. xerophila	*Osmia cornifrons*	[60]	Sporocysts	Unknown	Unknown	Japan
Arrhenosphaera						
A. craneae	*Apis mellifera*	[16]	Sporocysts and asexual conidia	Obligate pathogen of social bees	Commercial beekeeping Flower sharing Honey robbing Social transmission	Venezuela
Bettsia						
B. alvei 'Pollen mold'	*Apis mellifera* *Melipona fasciata*	[52,52,69,70] [71]	Sporocysts and asexual conidia	Saprophyte of pollen provision and beebread	Commercial beekeeping Flower sharing Honey robbing Social transmission	Worldwide
	Osmia cornuta	[45]			Flower sharing	
Skoua						
S. fertilis	*Apis mellifera*	[1••]	Naked asci	Saprophyte	Commercial beekeeping Flower sharing Honey robbing Social transmission	Denmark
Aspergillus ('Stonebrood')						
A. flavus	*Apis mellifera* *Nomi melanderi*	[72] [73]	Cleistothecia and asexual conidia	Generalist Saprophyte and opportunistic pathogen of immunocompromised hosts	Ubiquitous presence	Worldwide USA
A. fumigatus	*Apis mellifera*	[7]				Worldwide
A. niger	*Apis mellifera*	[74,18••]	Asexual conidia Rarely sexual cleistothecia			
A. nomius	*Apis mellifera*	[18••]	Cleistothecia and asexual conidia			
A. oryzae	*Apis mellifera*	[74]				Egypt
A. phoenicis	*Apis mellifera*	[18••]				Worldwide
A. tamarii	*Nomia melanderi*	[73]				USA

spores [3•]. The only exception to this is the recent discovery of both vegetative and reproductive stages of *Ascosphaera* in adult queens of three bumblebee species ([14••]; Table 1). However, it is likely that this is similar to an *in vitro* exposure, that is, the result of commercial mass rearing protocols where spore contaminated pollen is fed to colonies [15], rather than an example of a true host-switch.

Arrhenosphaera, Bettsia, *and* Skoua

Arrhenosphaera, *Bettsia*, and *Skoua* are by far the least studied bee associated fungi. The genus *Arrhenosphaera* only includes one species, *A. cranae*, which has been reported only once (at the time of its description) as a problematic pathogen of *A. mellifera* ([16]; Table 1). It has similar aetiology to *A. apis* but a capacity to proliferate saprophytically on stored pollen. *Bettsia* also includes only one described species, *B. alvei* and it shares the same niche as several of the saprophytic *Ascosphaera* (Table 1). By contrast to *Ascosphaera*, both *Arrhenosphaera* and *Bettsia* produce asexual conidia as well as sporocysts [1••]. Recently, another pollen saprophyte was isolated from honey bee bread, *Eramascus fertilis*, but after taxonomic and phylogenetic analyses it was placed in its own genus and renamed *Skoua fertilis* [1••]. Even though *Bettsia* and *Skoua* are distantly related to *Ascosphaera*, they have both independently evolved unusual (naked) sporocysts, which suggests convergent evolution of spore dispersal mechanisms adapted for the bee habitat [1••].

Aspergillus spp. and Stonebrood disease

The primary infection route of *Aspergillus* is very similar to that of *Ascosphaera*, via ingestion of spores, which germinate in the gut leading to an invasive mycosis and host death. By contrast to *Ascosphaera*, both adults and larvae can be infected, and spores can also germinate on, and mycelia can penetrate, the external cuticle [7]. There are several species that can cause stonebrood disease ([7]; Table 1), but a key difference to *Ascosphaera* is that *Aspergillus* are primarily saprophytes, occurring almost ubiquitously in soils, and are only opportunistically pathogenic (Figure 1; Table 1). Prevalence of stonebrood disease is low as infection occurs only in colonies weakened by other factors, such as poor pollen diets [17]. *In vitro* assays on larvae show the virulence of *Aspergillus* is high, both with respect to speed of kill and sporulation when compared to *Ascosphaera apis* [18••], which suggests that the low disease prevalence in the field is due to other interacting factors. Vectoring by *Varroa* mites has been suggested as a potential transmission route (Figure 1), as *A. flavus* conidia appear to be carried readily on *Varroa* [19], but the effect *of Varroa* on stonebrood disease epidemiology remain to be tested.

Bee life history and fungal transmission

All the pathogenic fungi of bees described here have a semelparous life-history, producing transmission propagules only after the host has died and after the mycelia have penetrated the host cuticle from the inside. This has important consequences for disease transmission potential. One of the biggest factors influencing transmission is the life history of the bee, primarily because this determines what happens to the resulting sporulating larval cadaver. The diversity and abundance of fungi is greater in solitary bee nests than in eusocial bees ([1••]; Table 1). Social species such as *A. mellifera* can use group resistance mechanisms, such as hygienic behaviour, to remove the diseased brood ([20]; Figure 1), although this also has the potential to increase social transmission of spores [21]. However, use of the same nest site for several months or years can provide a more stable environment for fungal growth promoting disease persistence (Figure 1). Conversely, solitary bees are active for relatively shorter periods (as short as three weeks per year [22]), restricting the opportunity for fungal growth and transmission. This may be a reason that many of the solitary bee associated fungi are saprophytes (Table 1), because the nesting materials may often be more persistent than the bees themselves. The overwintering status of the bee species is also important. For example, alfalfa leafcutting bees nest in existing cavities in wood or hollow reeds and overwinter as larvae, providing an extended opportunity for within-host growth by fungi. In the early summer, emerging adults frequently must chew through dead, diseased siblings that block their exit, becoming contaminated with chalkbrood spores in the process and proliferating the disease [23]. A shared trait among bees is the use of a nest to rear offspring. Nest site reuse, either due to the overlapping generations of eusocial species, or natal nest preference in solitary species [24], is therefore a core transmission route for fungal spores (Figure 1; Table 1). Similarly, all bees collect pollen and nectar to rear their offspring, making flower sharing another core transmission route ([25,26]; Figure 1; Table 1).

Host and microbiome effects on disease prevalence

Apis mellifera shows significant genetic variation in resistance to chalkbrood at both the individual and colony level [4,27], but co-infection by multiple strains [28] or species [10,11•] of *Ascosphaera* can alter the outcomes of infection by augmenting virulence. There is evidence emerging that resistance to organisms whose key route of infection is via the gut can also be augmented by the resident gut microbiota ((e.g. [29]); Figure 1), and there is some evidence to suggest the honey bee gut microbiota may have antifungal activity [30]. In both honey bees and bumblebees, there appears to be a consistent gut microbiota consisting of nine different phylotypes [31]. However, there are also microbial symbionts in the hive of honey bees that appear important in the defence against fungal pathogens, particularly *Ascosphaera*. In honey bees, the primary mechanism of resistance to chalkbrood is hygienic behaviour (Figure 1), a secondary mechanism is

the addition during pollen collection and storage by bees of antagonistic moulds (mostly species belonging to the Mucorales and Aspergilli) and *Bacillus* spp. that inhibit the pathogen [32]. Very little is known about the gut microbiota of solitary species. However, although there is no evidence to suggest their gut microbiota are protective against fungal pathogens, it has been shown that the two can influence each other ([33]; Figure 1). To understand the epidemiology of fungal diseases it therefore appears increasingly pertinent to consider the entire community of microorganisms in which single pathogens operate, an approach that is also increasingly used in issues of human health and disease [10,34], as non-lethal synergists can have huge impacts on the evolution of the host–pathogen interactions [35]. Bees can also defend their provisions and larvae with a chemical arsenal. For example, colletid and halictid bees apply a dense lining to their nest using secretions from the Dufour's gland comprising a mix of terpenoid esters and macrocyclic lactones [36], which may inhibit microbial invasion [37,38]. Some bees use propolis to line their nest [34], which is known to have antimicrobial properties, against *Ascosphaera* at least [39]. The antimicrobial effectiveness of propolis varies by region suggesting that there are certain bee-preferred resinous plants, collected by bees to aid in defence against fungi [40••]. Host nutrition (Figure 1) is central to many of these processes, as nutritional limitation has a plethora of negative effects, including immunocompromising both larvae and adults [41], and increasing susceptibility to opportunistic pathogens such as *Aspergillus* [17]. Good host nutrition appears to require floral diversity, which extends to the microbial ecology of the entire colony in social species; polyfloral pollen mixtures increase resistance of honey bee larvae to *Aspergillus* [17], and honey bee colonies that have a higher concentration of symbiotic microbes in the pollen fed to their larvae are less susceptible to chalkbrood ([42]; Figure 1).

Conclusion

Our current understanding of the biology of bee associated fungi varies considerably (Table 1), but there appears to be bias in the attention of the scientific community towards fungi that are associated with economic losses to apiculture and agriculture. The historical research focus on honey bees should shift to include wild and solitary species, because they tend to be less resilient to environmental stressors (including pathogens; [43]) their importance as pollinators is increasingly evident [44], and they appear to act as a reservoir of honey bee disease [45]. As solitary bees are relied on more for commercial pollination [44], denser aggregations lead to higher prevalence of disease [46]. In addition, pollinators are regularly moved trans-continentally to meet global pollination needs, leading to transport of their associated fungal pathogens [47••]. As the potential for exposure to exotic pathogens increases, the potential for novel host-switching also increases. Therefore, a greater understanding of the biology and prevalence of fungal parasites across the spectrum of bees, social and solitary, native and exotic, is of paramount importance in mitigating disease.

Conflict of interest

No conflict of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Wynns AA: **Convergent evolution of highly reduced fruiting bodies in Pezizomycotina suggests key adaptations to the bee habitat**. *BMC Evol Biol* 2015, **15**:145.
Provides important phylogenetic analyses of rare fungal bee pathogens, and an interesting discussion of convergent evolution of an unusual spore structure likely adapted for dispersal by bees.

2. Wynns AA, Jensen AB, Eilenberg J: ***Ascosphaera callicarpa*, a new species of bee-loving fungus, with a key to the genus for Europe**. *PLoS ONE* 2013, **8**:e73419.

3. • Evison: **Chalkbrood: epidemiological perspectives from the host–parasite relationship**. *Curr Opin Insect Sci* 2015, **10**:65-70.
An overview of chalkbrood disease in honey bees, providing information on factors affecting disease establishment and spread.

4. Jensen AB, Pedersen BV, Eilenberg J: **Differential susceptibility across honey bee colonies in larval chalkbrood resistance**. *Apidologie* 2009, **40**:524-534.

5. Pitts-Singer TL, Cane JH: **The Alfalfa Leafcutting Bee, *Megachile rotundata*: the world's most intensively managed solitary bee**. *Annu Rev Entomol* 2011, **56**:221-237.

6. James RR: **Chalkbrood transmission in the alfalfa leafcutting bee: the impact of disinfecting bee cocoons in loose cell management systems**. *Environ Entomol* 2011, **40**:782-787.

7. Gilliam M, Vandenberg J: **Fungi**. In *Honey Bee Pests, Predators and Diseases,* edn 3rd. Edited by Morse RA, Flottum K.A I Root Co; 1997:81-110.

8. Seyedmousavi S, Guillot J, Arné P, de Hoog GS, Mouton JW, Melchers WJG, Verweij PE: ***Aspergillus* and aspergilloses in wild and domestic animals: a global health concern with parallels to human disease**. *Med Mycol* 2015, **53**:765-797.

9. Yousseff NN, Roush CF, McManus WR: ***In vivo* development and pathogenicity of *Ascosphaera proliperda* (Ascosphaeraceae) to the Alfalfa leafcutting bee, *Megachile rotundata***. *J Invert Pathol* 1984, **43**:11-20.

10. Vojvodic S, Boomsma JJ, Eilenberg J, Jensen AB: **Virulence of mixed fungal infections in honey bee brood**. *Front Zool* 2012, **9**:5.

11. • Klinger EG, Vojvodic S, DeGrandi-Hoffman G, Welker DL, James RR: **Mixed infections reveal virulence differences between host-specific bee pathogens**. *J Invert Pathol* 2015, **129**:28-35.
An experimental assessment of mixed infection by both pathogenic and saprophytic *Ascosphaera* spp. reveals host specificity as a key factor predicting competitive outcome of co-infections.

12. Bamford S, Heath LAF: **The effects of temperature and pH on the germination of spores of the chalkbrood fungus, *Ascosphaera apis***. *J Apicultur Res* 1989, **28**:36-40.

13. Bissett J: **Contribution toward a monograph of the genus Ascosphaera**. *Can J Bot* 1988, **66**:2541-2560.

14. •• Maxfield-Taylor SA, Mujic AB, Rao S: **First detection of the larval chalkbrood disease pathogen *Ascosphaera apis* (Ascomycota: Eurotiomycetes: Ascosphaerales) in adult bumble bees**. *PLoS ONE* 2015, **10**:e0124868.
Provides the first evidence of growth of *Ascosphaera apis* in an adult bee and a novel host, the bumblebee, and reinforces the issue of pathogen spread during commercial rearing protocols for pollinators.

15. Graystock P, Yates K, Evison SEF, Darvill B, Goulson D, Hughes WOH: **The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies**. *J Appl Ecol* 2013, **50**:1207-1215.

16. Stejskal M: ***Arrhenosphaera cranei*, Gen. Et Sp. Nov., a beehive fungus found in Venezuela**. *J Apicul Res* 1974, **13**:39-45.

17. Foley K, Fazio G, Jensen AB, Hughes WOH: **Nutritional limitation and resistance to opportunistic *Aspergillus* parasites in honey bee larvae**. *J Invert Pathol* 2012, **111**:68-73.

18. •• Foley K, Fazio G, Jensen AB, Hughes WOH: **The distribution of *Aspergillus* spp. opportunisitic parasites in hives and their pathogenicity to honey bees**. *Vet Microbiol* 2014, **169**:203-210.
Provides the first comprehensive apiary screening for agents of stone-brood disease, and *in vitro* virulence assays for multiple *Aspergillus* species.

19. Benoit JB, Yoder JA, Sammataro D, Zettler LW: **Mycoflora and fungal vector capacity of the parasitic mite *Varroa destructor* (Mesostigmata: Varroidae) in honey bee (Hymenoptera: Apidae) colonies**. *Int J Acar* 2004, **30**:103-106.

20. Wilson-Rich N, Spivak M, Fefferman NH, Starks PT: **Genetic, individual, and group facilitation of disease resistance in insect societies**. *Ann Rev Entomol* 2009, **54**:405-423.

21. Invernizzi C, Rivas F, Bettucci L: **Resistance to chalkbrood disease in *Apis mellifera* L. (Hymenoptera: Apidae) colonies with different hygienic behaviour**. *Neotrop Entomol* 2011, **40**:28-34.

22. Stephen WP, Bohart GE, Torchio PF: *The Biology and External Morphology of Bees*. Oregon Atate University; 1969.

23. James RR: **Chalkbrood transmission in the alfalfa leaf-cutting bee: the impact of disinfecting bee cocoons in loose cell management systems**. *Environ Entomol* 2011, **40**:782-787.

24. Pitts-Singer TL: **Olfactory response of Megachilid bees, *Osmia lignaria*, *Megachile rotunda*, and *M. pugnata*, to individual cues from old nest cavities**. *Environ Entomol* 2007, **36**:402-408.

25. Evison SEF, Roberts KE, Laurenson L, Pietravalle S, Hui J, Biesmeijer JC, Smith JE, Budge G, Hughes WOH: **Pervasiveness of parasites in pollinators**. *PLoS ONE* 2012, **7**:e30641.

26. Graystock P, Goulson D, Hughes WOH: **Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species**. *Proc R Soc Lond B* 2015, **282**:20151371.

27. Evison SEF, Fazio G, Chappell P, Foley K, Jensen AB, Hughes WOH: **Host–parasite genotypic interactions in the honey bee: the dynamics of diversity**. *Ecol Evol* 2013, **3**: 2214-2222.

28. Evison SEF, Foley K, Jensen AB, Hughes WOH: **Genetic diversity, virulence and fitness evolution in an obligate fungal parasite of bees**. *J Evol Biol* 2015, **28**:179-188.

29. Koch H, Schmid-Hempel P: **Socially transmitted gut microbiota protect bumble bees against an intestinal parasite**. *Proc Natl Acad Sci USA* 2011, **108**:19288-19292.

30. Omar MOM, Moustafa AM, Ansari MJ, Anwar, Fahmy BF, Al-Ghamdi A, Nuru A: **Antagonistic effect of gut bacteria in the hybrid carnolan honey bee *Apis mellifera carnica*, against *Ascosphaera apis*, the causal organism of chalkbrood disease**. *J Apic Sci* 2014, **58**:17-27.

31. Engel P, Martinson VG, Moran NA: **Functional diversity within the simple gut microbiota of the honey bee**. *Proc Natl Acad Sci USA* 2012, **109**:11002-11007.

32. Gilliam M: **Identification and roles of non-pathogenic microflora associated with honey bees**. *FEMS Microbiol Lett* 1997, **155**:1-10.

33. McFrederick QS, Mueller UG, James RR: **Interactions between fungi and bacteria influence microbial community structure in the Megachile rotundata larval gut**. *Proc R Soc B* 2014, **281**:20132653.

34. Anderson KE, Sheehan TH, Eckholm BJ, Mott BM, Degrandi-Hoffman G: **An emerging paradigm of colony health: microbial balance of the honeybee and hive (*Apis mellifera*)**. *Insect Soc* 2011, **58**:431-444.

35. Bonsall MB: **The impact of non-lethal synergists on the population and evolutionary dynamics of host–pathogen interactions**. *J Theor Biol* 2010, **262**:567.

36. Mitra A: **Function of the Dufour's gland in solitary and social Hymenoptera**. *J Hymenopt Res* 2013, **35**:33-58.

37. Batra SWT: **Behaviour of some social and solitary halictine bees within their nests: a comparative study (Hymenoptera: Halicitidae)**. *J Kansas Entomol Soc* 1968, **41**:120-133.

38. Batra SWT: **Ecology, behaviour, pheromones, parasites and management of the sympatric vernal bees *Colletes inaequalis, Colletes thoracicus* and *Colletes validus***. *J Kansas Entomol Soc* 1980, **53**:509-538.

39. Simone-Finstrom M, Spivak M: **Propolis and bee health: the natural history and significance of resin use by honey bees**. *Apidologie* 2010, **41**:295-311.

40. •• Wilson MB, Brinkman D, Spivak M, Gardner G, Cohen JD: **Regional variation in composition and antimicrobial activity of US propolis against *Paenibacillus larvae* and *Ascosphaera apis***. *J Invert Pathol* 2015, **124**:44-50.
Shows how variation in the botanical composition of propolis can influence its efficacy as defence against fungal pathogens, and highlights an underappreciated aspect of disease epidemiology.

41. Alaux C, Ducloz F, Crauser D, Le Conte Y: **Diet effects on honeybee immunocompetence**. *Biol Lett* 2010, **6**:562-565.

42. Gilliam M, Lorenz BJ, Richardson GV: **Digestive enzymes and microorganisms in honey bees, *Apis mellifera* — influence of streptomycin, age, season and pollen**. *Microbios* 1988, **55**:95-114.

43. Straub L, Williams GR, Pettis J, Fries I, Neumann P: **Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors**. *Curr Opin Insect Sci* 2015, **12**:109-112.

44. Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, Vanbergen AJ: **Safeguarding pollinators and their values to human well-being**. *Nature* 2016, **540**:220-229.

45. Ravoet J, De Smet L, Meeus I, Smagghe G, Wenseleers T, de Graaf DC: **Widespread occurrence of honey bee pathogens in solitary bees**. *J Invert Pathol* 2014, **122**:55-58.

46. Pitts-Singer TL, Bosch J: **Nest establishment, pollination efficiency, and reproductive success of *Megachile rotunda* (Hymenoptera: Megachilidae) in relation to resource availability in field enclosures**. *Environ Entomol* 2010, **39**: 149-158.

47. •• Hedtke SM, Blitzer EJ, Montgomery GA, Danforth BN: **Introduction of non-native pollinators can lead to trans-continental movement of bee associated fungi**. *PLoS ONE* 2015, **10**:e030560.
Reveals that several species of pathogenic fungi are moved trans-continentally from Japan to North America with their host, the commercially reared solitary bee *Osmia cornifrons*, highlighting another route of exposure of pollinators to exotic pathogens.

48. Gilliam M, Lorenz BJ, Prest DB, Camazine S: ***Ascosphaera apis* from *Apis cerana* from South Korea**. *J Invertebr Pathol* 1993, **61**:111-112.

49. Gilliam M, Lorenz BJ, Buchmann SL: ***Ascosphaera apis*, the chalkbrood pathogen of the honeybee, *Apis mellifera*, from larvae of a carpenter-bee, *Xylocopa californica arizonensis***. *J Invertebr Pathol* 1994, **63**:307-309.

50. Bissett J, Duke G, Goettel M: ***Ascosphaera acerosa* sp. nov. isolated from the alfalfa leafcutting bee, with a key to the species of *Ascosphaera***. *Mycologia* 1996, **88**:797-803.

51. Anderson DL, Gibson NL: **New species and isolates of spore-cyst fungi (Plectomycetes: Ascosphaerales) from Australia**. *Aust Syst Bot* 1998, **11**:53-722.

52. Skou JP: **Two new species of *Ascosphaera* and notes on the conidial state of *Bettsia alvei***. *Friesia* 1975, **11**:62-74.

53. Goerzen DW, Dumouchel L, Bissett J: **Occurrence of chalkbrood caused by *Ascosphaera aggregata* Skou in a native leafcutting bee, *Megachile pugnata* Say (Hymenoptera, Megachilidae), in Saskatchewan**. *Can Entomol* 1992, **124**: 557-558.

54. Goerzen DW, Erlandson MA, Bissett J: **Occurrence of chalkbrood caused by *Ascosphaera aggretata* Skou in a native leafcutting bee, *Megachile relativa* Cresson (Hymenoptera: Megachilidae) in Saskatchewan**. *Can Entomol* 1990, **122**: 1269-1270.

55. Hermoso de Mendoza M, Hermoso de Mendoza J, Puerta F, Asensio E, Bustos M, Padilla F, Pellin P: **Ascosphaeriosis of the parasitic bee, *Coelioxys rufocaudata*, by *Ascosphaera aggregata***. *J Apicul Res* 1989, **2**:61-65.

56. Skou JP: ***Ascosphaera asterophora* species nova**. *Mycotaxon* 1982, **14**:149-159.

57. Anderson DL, Gibbs AJ, Gibson NL: **Identification and phylogeny of spore-cyst fungi (*Ascosphaera* spp.) using ribosomal DNA sequences**. *Mycol Res* 1998, **5**:541-547.

58. Skou JP, Hackett K: **A new homothallic species of *Ascosphaera***. *Friesia* 1979, **11**:265-277.

59. Benny GL, Kimbrough JW: **A synopsis of the orders and families of Plectomycetes with keys to genera**. *Mycotaxon* 1980, **12**:1-91.

60. Skou JP: **Japanese species of *Ascosphaera***. *Mycotaxon* 1988, **31**:173-190.

61. Goettel MS, Duke GM, Goerzen DW: **Pathogenicity of *Ascosphaera larvis* to larvae of the alfalfa leafcutting bee, *Megachile rotundata***. *Can Entomol* 1997, **129**:1059-1065.

62. Skou JP: **Ascosphaerales**. *Friesia* 1972, **10**:1-24.

63. Skou JP, King J: ***Ascosphaera osmophila* sp. nov., an Australian spore cyst fungus**. *Aust J Bot* 1984, **32**:225-231.

64. Pinnock DE, Coles RB, Donovan B: **A new spore cyst fungus from New Zealand**. *Aust Syst Bot* 1988, **1**:387-389.

65. Wynns AA, Jensen AB, Eilenberg J, James R: ***Ascosphaera subglobosa*, a new spore cyst fungus from North America associated with the solitary bee *Megachile rotundata***. *Mycologia* 2012, **104**:108-114.

66. Skou JP, Holm SN: ***Ascosphaera tenax* species nova and a variant of *Ascosphaera aggregata***. *Mycotaxon* 1989, **35**:211-218.

67. Youssef NN, McManus WR: ***Ascosphaera torchioi* sp. nov., a pathogen of *Osmia lignaria propinqua* Cresson (Hymenoptera)**. *Mycotaxon* 2001, **127**:7-13.

68. Rust RW, Torchio PF: **Induction of *Ascosphaera* (ascomycetes, ascosphaerales) infections in field populations of *Osmia lignaria propinqua cresson* (hymenoptera, megachilidae)**. *Pan-Pacific Entomol* 1991, **67**:251-257.

69. Betts AD: **A beehive fungus, Pericystis alvei, gen. Et sp. Nov.**. *Ann Bot* 1912, **26**:795-799.

70. Gilliam M, Prest DB, Lorenz BJ: **Microbiology of pollen and bee bread: taxonomy and enzymology of molds**. *Apidologie* 1989, **20**:53-68.

71. Gilliam M, Roubik D, Lorenz BJ: **Microorganisms associated with pollen, honey, and brood provisions in the nest of a stingless bee, *Melipona fasciata***. *Apidologie* 1990, **21**:89-97.

72. Maassen A: **Die Aspergillusmykose der Bienen**. *Mitteilungen aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft* 1906, **2**:30-31.

73. Batra SWT, Bohart GE: **Alkali bees: response of adults to pathogenic fungi in brood cells**. *Science* 1969, **165**:607-608.

74. Shoreit MN, Bagy HM: **Mycoflora associated with stonebrood disease in honey bee colonies in Egypt**. *Microbiol Res* 1995, **150**:207-211.