Received: 31 August 2022 | Accepted: 5 February 2023

DOI: 10.1111/1365-2664.14399

Journal of Applied Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Spillover of chalkbrood fungi to native solitary bee species from non-native congeners

Kathryn A. LeCroy[1,2] | **Erin Krichilsky**[2,3,4] | **Heather L. Grab**[2,5] | **T'ai H. Roulston**[1] | **Bryan N. Danforth**[2]

[1]Department of Environmental Sciences, University of Virginia, 400 Blandy Farm Lane, Boyce, Virginia 22620, USA

[2]Department of Entomology, Cornell University, Comstock Hall, Ithaca, New York 14850, USA

[3]Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York City, New York 10027, USA

[4]Division of Invertebrate Zoology, American Museum of Natural History, New York City, New York 10024, USA

[5]School of Integrative Plant Sciences, Plant Science Building, Cornell University, Ithaca, New York 14853, USA

Correspondence
Kathryn A. LeCroy
Email: kalecroy@gmail.com

Funding information
Garden Club of Virginia; Jefferson Scholars Foundation; National Science Foundation Graduate Research Fellowship Program, Grant/Award Number: 2013144751; UVA Blandy Experimental Farm; USDA NIFA AFRI Postdoctoral Fellowship, Grant/Award Number: 2021-08318

Handling Editor: Bret Elderd

Abstract

1. Introduced, managed bees such as mason bees (genus *Osmia*) can confer significant pollination benefits to agricultural systems, but a risk of introducing non-native species into new ecosystems is the co-introduction of pathogens along with them. Pathogen spillover to wild, native bees may then drive native bee species declines.
2. This study examined prevalence of the chalkbrood-causing fungal genus *Ascosphaera* in the nests of both non-native and native mason bee species. We conducted large-scale trap-nesting and pan-trapping efforts across the Mid-Atlantic United States with community scientists. Using molecular methods, nests were screened for all known *Ascosphaera* species in which genetic sequences have been published. After finding *Ascosphaera* species first described in Asia, we compared their local prevalence with the local abundance of mason bees from Asia. Lastly, we compared the prevalence of co-introduced *Ascosphaera* species across sites with a variety of landcover profiles.
3. Results indicate species originally described in Japan, *Ascosphaera naganensis* and *Ascosphaera fusiformis*, are now present in native Virginia mason bees, *Osmia lignaria* and *Osmia georgica*, with high prevalence of *A. naganensis* found in *O. georgica*.
4. We also found that the declining native mason bee *O. georgica* experienced higher prevalence of non-native *Ascosphaera* spp. at sites with larger numbers of non-native *O. cornifrons* and *O. taurus*, perhaps indicating greater likelihood of spillover of these *Ascosphaera* species with greater sources of transmission. Lastly, when the proportion of agricultural landcover surrounding bee nests was high, there was greater prevalence of non-native *Ascosphaera* in *O. georgica* compared to more natural landcover types.
5. *Synthesis and applications*. Through community science programming, we documented species of Japanese chalkbrood fungi inside native mason bee nests in North America. Native mason bees encounter non-native fungi more frequently

---

T'ai H. Roulston and Bryan N. Danforth joint senior authors.

with increasing abundance of non-native mason bees. Agricultural landscapes may exacerbate spillover of non-native fungi for native mason bees. Any use of non-native bee species in agriculture should involve monitoring native bees for pathogens in the surrounding area for detection of spillover and species declines.

KEYWORDS
agricultural pollinator, *Ascosphaera*, community science, disease spillover, introduced species, mason bee, Megachilidae, *Osmia*

1 | INTRODUCTION

The movement of bees is an integral part of commercial crop pollination services. Over 60% of commercially managed hives of the European honeybee *Apis mellifera* (Linnaeus, 1758) in the United States (US) are transported back and forth to the Central Valley of California to pollinate almonds each year (Glenny et al., 2017). The European alfalfa leafcutter bee, *Megachile rotundata* (Fabricius, 1787), is largely responsible for supporting alfalfa crop pollination in the US and Canada, and *M. rotundata* are shipped routinely from Canada to farmers all over the US (Pitts-Singer & James, 2008). The buff-tailed bumblebee, *Bombus terrestris* (Linnaeus, 1758), has been commercially imported by 57 countries for crop pollination, with 16 of those countries occurring outside of its native range (Murray et al., 2013). Imported bees do not travel alone: along with the transportation of bee species, without effective precautionary measures so too may their parasites, pathogens and commensal associates spread into new areas (Graystock et al., 2016; Schmid-Hempel et al., 2014). These associates may spill-over to native bee communities, which may not have any prior exposure nor immunity for a given parasite or pathogen (Arismendi et al., 2021; Kojima et al., 2011; Meeus et al., 2011).

Pathogens and natural enemies may spread from introduced bees through shared resources like flowers, providing evanescent routes of exposure (Keller et al., 2021; McArt et al., 2014; Vannette, 2020), and via nesting sites, which provide long-term routes of exposure (Park et al., 2009; Poinar & Van der Laan, 1972). In particular, many agriculturally important pollinators, including honey bees and commercially managed bumble bees (family: Apidae), leafcutter and mason bees (family: Megachilidae), typically nest in preexisting cavities, which may have been previously occupied by other cavity nesting insects, thus exposing the new occupants to parasites and pathogens of the previous occupants (Krombein, 1967).

Of the 80 known non-native bee species introductions worldwide, over 40% (33) are of cavity-nesting species in the family Megachilidae (Russo et al., 2021). In North America, this includes two mason bee species from Asia—*Osmia cornifrons* (Radoszkowski, 1887) and *Osmia taurus* Smith, 1873. *Osmia cornifrons* was intentionally introduced from Japan into Maryland, US for orchard pollination in 1977 (Batra, 1978). Following successful introduction, *O. cornifrons* has become broadly established in the Eastern United States (Hedtke et al., 2015). A second species from Asia, *O. taurus*, was first recorded in the US in 2002 in West Virginia and Maryland (Droege, 2020). Since then, it has spread quickly, becoming the most commonly collected mason bee in the Mid-Atlantic region of the US, with concurrent declines in six native congeners (LeCroy et al., 2020).

One major disease of cavity nesting bees is the specialist chalkbrood fungal genus *Ascosphaera* (L.S. Olive & Spiltoir, 1955) (Onygenales: Ascosphaeraceae; Wynns, 2012). *Ascosphaera* species are associated with a wide range of social and solitary bee species and attack both wild and managed bees, accounting for larval death rates up to 20% in present-day commercially managed *Megachile rotundata* (James & Pitts-Singer, 2013). Several *Ascosphaera* species are known to be associated with *Osmia* nests, including obligately pathogenic *Ascosphaera torchioi* (Youssef & McManus, 2001). When *O. cornifrons* was brought to the U.S. for release, S. Batra (Batra, 1978) noted the presence of dead bees exhibiting chalkbrood among the imported stock. Although the cadavers and contaminated nesting materials were destroyed when discovered (Batra, 1978), chalkbrood spores can travel through the air and on cocoons of nestmates that are otherwise asymptomatic (Stephen et al., 1981), thus providing the possibility that a novel disease organism was introduced to North America at the time of import.

In 2012, researchers (Hedtke et al., 2015) discovered *Ascosphaera naganensis* Skou, 1988, the holotype of which is from the same region of Japan from where *O. cornifrons* was introduced (Batra, 1978; Skou, 1988), in *O. cornifrons* nests in apple orchards in Ithaca, New York, US. Subsequent studies confirmed *A. naganensis* in *O. cornifrons* at several sites within the region and found that chalkbrood prevalence was highest at sites with high fungicide exposure and low natural habitat in the surrounding landscape (Krichilsky et al., 2021). Prior to our study, it was unknown if *A. naganensis* is also associated with the second species of introduced bee, *O. taurus*, or whether this or other non-native *Ascosphaera* species have spilled into native North American *Osmia* that are experiencing significant declines since the arrival of these non-native *Osmia* (LeCroy et al., 2020). Furthermore, much of what is known about this chalkbrood-causing genus is informed by studies within agricultural contexts, which may limit our inference for if and how disease spillover of *Ascosphaera* impacts wild bee communities beyond this habitat type (Anderson et al., 1998; Klinger et al., 2013; Krichilsky et al., 2021; Stephen et al., 1981), and these agricultural contexts are understood to be particularly stressful for bees (De Palma et al., 2015; Grab et al., 2019).

In this study we focus on the prevalence of non-native *Ascosphaera* among co-occurring *Osmia* species in North America to ask: (a) has non-native *Ascosphaera* invaded the nests of native *Osmia* species? If so, (b) is the likelihood of native *Osmia* being infected with non-native *Ascosphaera* related to the local abundance of non-native *Osmia*? (c) Are some species of native *Osmia* more likely to be associated with non-native *Ascosphaera*? Lastly, we asked: (d) does the prevalence of non-native *Ascosphaera* vary with landscape context?

2 | MATERIALS AND METHODS

2.1 | Bee collection from trap nests

Previously unused wooden trap nest blocks were deployed across 100 sites in Virginia, West Virginia, the District of Columbia, and Maryland with the help of community scientists (Figure 1). Written informed consent was granted by each community scientist that participated in this study through email correspondence. At each site, nine wooden nest blocks (three blocks drilled with 4-mm holes, three drilled with 6-mm holes and three drilled with 8-mm holes) were placed within a larger nesting frame, which was installed 1–2 m above the ground. These nest hole sizes have been previously used for studies of trap-nesting *Osmia* and cover the range of cavity diameters used by the various species of *Osmia* in this region (Krombein, 1967). All nests were installed by March 3, 2017 and retrieved between May 31–June 3, 2017 by Kathryn LeCroy and T'ai Roulston. Ethical approval for the use and collection of invertebrate specimens in this study was not required by researchers nor community scientists.

Following retrieval of nesting frames, each nest block containing bee or wasp nests was then placed in a storage shed with ambient temperature conditions for continued larval development and protection against adverse weather events until October 2017. We kept nests with evidence of wasp nesting because some wasp species have been found to usurp *Osmia* nests (Krombein, 1967). From October 2017, nests were maintained at 4°C in a refrigerator until nest contents were dissected and identified.

2.2 | Bee collection from pan traps

We evaluated abundance of non-native *Osmia* at each site using pan trapping at 43 of the 100 sites hosting nesting blocks during

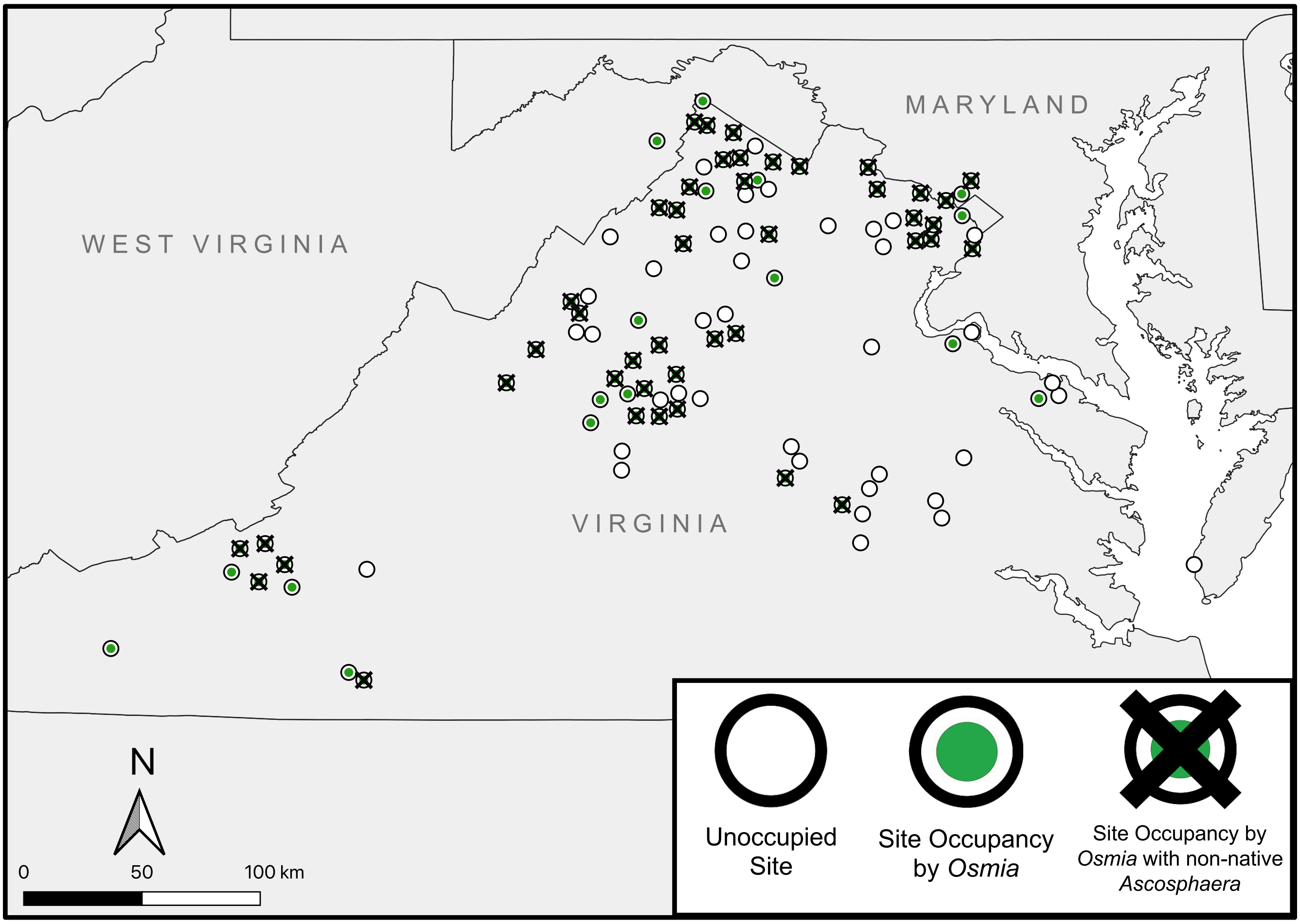

FIGURE 1 Map with all study sites. Sites marked with green indicate occupancy by *Osmia* spp. and underwent molecular screening for *Ascosphaera*; sites with additional "X" over green indicate *Osmia* occupancy with non-native *Ascosphaera*.

this study period (Figure S1). At each site, nine 354-mL (12-oz) plastic cups were set in 12.7-cm (5-inch) diameter PVC ring stands raised 1 m above the vegetation and spaced 5 m apart in a straight line. The nine stands held cups of three colours: fluorescent blue; fluorescent yellow; and white. These three colours have been found to collectively attract many bees, including *Osmia* (Hall, 2016; Kirk, 1984). Each cup was filled with 177.44 mL (6 oz) of a preservative solution (50:50 water: propylene glycol), and a drop of dish soap was added to break the surface tension for invertebrate capture. To reduce the chance of actively-nesting female *Osmia* bycatch, pan traps were placed as far as possible away from the corresponding trap nest, which ranged from 75 to 1135 m apart, with a median distance of 475 m apart. Community scientists were instructed on how to deploy pan traps and conduct weekly sampling with an introductory webinar, written handouts and in-person visits to every site by Kathryn LeCroy. At weekly intervals from March 1st 2017 to June 7th 2017, community scientists collected the trapped invertebrates weekly or biweekly from the cups, and Kathryn LeCroy retrieved these samples for further processing and identification.

2.3 | Dissection of nest contents

All springtime mason bee species in our region of study spin cocoons, morph into adults, and then overwinter as adults in their cocoons (Bosch et al., 2001). By October 2017, all live *Osmia* were adults inside cocoons, and processed nests were dissected to estimate *Ascosphaera* molecular prevalence among all life stages of *Osmia*, including larvae, pupae and adults. Using sterile techniques for extraction, brood cell contents were placed in 2-mL tubes and then stored at −80°C. Mortality attributed to chalkbrood was evaluated based on visual confirmation of diagnostic indicators of chalky cadavers with use of a dissection microscope. Nests drilled to 4-mm in diameter were unable to be dissected due to improper drilling, rendering nests inaccessible. All nest contents successfully collected were further subjected to molecular screening for *Ascosphaera* as described below.

2.4 | Bee identification and DNA extraction

The identifications of *Osmia* species from all trap nests and pan traps were made by Kathryn LeCroy and verified by Sam Droege (United States Geological Survey). Voucher specimens were accessioned at the field station insect collection of Blandy Experimental Farm at the University of Virginia (Boyce, VA USA). Following identification, DNA was extracted from the entire metasoma, interior and exterior, of all adult bees, wasps and half of the remains of non-adult nest occupants (and preserving other half for further study as needed). We utilized a phenol: chloroform extraction protocol (Danforth et al., 2004). Following extraction, PCR amplification was performed with the use of primers AscoAll-F and AscoAll-R, which cover the ITS regions 1 and 2 and a segment of the 5.8S ribosomal RNA gene (James & Skinner, 2005). All PCR-positive samples were then purified using ExoSAP (Exonuclease with Shrimp Alkaline Phosphatase, Affymetrix) and sequenced in at least one direction with an ABI 3740x1 capillary DNA Analyser with Big Dye Terminator chemistry. If sequence quality of a sample was poor (with overlapping reads) it was excluded from further analyses. High-quality sequence results were considered for inclusion in maximum-likelihood phylogenetic tree analysis (see methods in Appendix S1). For specimens with high-quality sequence results that otherwise failed to make the conservative alignment of the phylogenetic tree analysis, such sequences were determined from blastn searches in GenBank (NCBI) matching the GenBank reference species with at least 98% total coverage and at least a maximal percent identity of 98. Specimens that were assigned to a species within the genus *Ascosphaera* by either the phylogenetic tree construction or blastn search were then incorporated into further ecological analyses. Sequences used in subsequent analyses were accessioned in GenBank under accession numbers OQ581252–OQ581433.

2.5 | Site landcover analysis

To characterize the dominant land cover at each trap nest, the coordinates of each site were mapped onto the National Land Cover Database (Homer et al., 2015) using a 600-m radius for landcover analysis. We chose to use 600 m as the radius because this is the mean expected foraging range of *Osmia* species in this region (Greenleaf et al., 2007). We aggregated the land cover types of the NLCD into three categories for our analysis: “Natural”, which included NLCD classification types of Deciduous/Mixed/Evergreen Forest, Shrub/Scrub, Grassland/Herbaceous and Woody/Emergent Herbaceous Wetlands; “Agricultural”, with Pasture/Hay, Cultivated Crops; and “Developed”, with Open Space, Low Intensity Development, Medium Intensity Development, High Intensity Development and Barren Land. The dominant land cover type was determined as the category (Natural, Agricultural, or Developed) with the greatest number of pixels at each site.

2.6 | Statistical analyses

All statistical analyses were conducted in R version 4.0.4. The *Osmia* species included for all analyses were native *O. georgica* and *O. lignaria*, and non-native *O. cornifrons* and *O. taurus*. We excluded native *O. subfasciata* from all analyses due to its presence in only one nest at a single site. We first explored the pattern of association between *Ascosphaera* species and *Osmia* species by using a generalized linear mixed-effect model (GLMM) with binomial error distribution. The response variable was the number of nest cell occupants of a given *Osmia* species at a site with a positive screen for a given *Ascosphaera* species over the number of all nest cells screened at that site. For this analysis, we excluded *Ascosphaera* species for which there were five or fewer positive screens in the entire study (Table S1), and thus the *Ascosphaera* species included for this analysis were *A. naganensis*, *A. subglobosa* and *A. fusiformis*. The predictor variables were *Ascosphaera* species, *Osmia* species and their two-way interaction, with site location as a random effect. Significance of model terms was evaluated by comparison to a model excluding the interaction term via the 'drop1()' function, and pairwise differences were evaluated using post-hoc Tukey tests via the 'emmeans()' function.

Second, we utilized pan trap data and the corresponding trap nest data for evaluating whether abundance of non-native *Osmia* (as estimated from pan trap catch data) predicted non-native *Ascosphaera* prevalence in trap nests from those same sites using a GLMM. The response variable was the proportion of *Osmia* individuals with a positive screen (i.e. a positive PCR band and Sanger sequencing result) for the three non-native *Ascosphaera* species described originally in Japan (*A. naganensis*, *A. fusiformis* or *A. celerrima*) over the proportion of *Osmia* without this condition. The predictor variables were species identity of the *Osmia*, the number of *O. taurus* and *O. cornifrons* specimens captured in pan traps at a given site, and the interaction of these two variables, with site location as a random effect.

Lastly, we evaluated whether differences in dominant land cover type across sites were a significant predictor for prevalence of *Ascosphaera naganensis*, *A. fusiformis* and *A. celerrima* in *Osmia* species using a GLMM with binomial error distribution. The predictor variables were *Osmia* species, dominant landcover type and their interaction, with site location as a random effect. For all three aforementioned statistical models, the residuals of each model were inspected for any remaining spatial autocorrelation by constructing variograms, bubbleplots, correlograms and calculation of Moran's I (Zuur et al., 2009).

3 | RESULTS

3.1 | Trap nests

Of the 100 sites where trap nests were deployed, 61 sites were occupied by mason bees (Figure 1). Non-native *Osmia* (i.e. *O. cornifrons* or *O. taurus*) occupied nests at 50 of the 61 sites, while native *Osmia* utilized nests at only 19 of the 61 sites. Only 10 of the 61 occupied sites had native and non-native mason bee species co-occurring in nesting structures. From all sites, a total of 111 *Osmia* nests were found in nest blocks of 6-mm in diameter, and a total of 100 nests were recovered from 8-mm diameter nest blocks, for a total 211 nests (1198 cells, see Table S2). Adult ectoparasitoid wasps from the family Chrysididae were found in four nests of mason bees (two nests of *O. lignaria*, two nests of *O. cornifrons*; Table S1).

3.2 | Detection of *Ascosphaera* in *Osmia* nests

From nests across 61 occupied sites, 1198 nest cells were screened for *Ascosphaera* (Figure 1). Nine known and one unknown species of *Ascosphaera* were detected via molecular methods with phylogenetic tree construction and blastn searches (see Table S1; Figure S2). Of the 207 positive identifications of *Ascosphaera* species, 192 (93%) were identified to be one of the following: *A. naganensis* ($n=72$), *A. fusiformis* ($n=83$), or *A. subglobosa* ($n=35$). *Ascosphaera subglobosa* is a species first described in nests of non-native *Megachile rotundata* imported from Europe now used for alfalfa production in Western North America (Bissett, 1988; Wynns et al., 2012). *Ascosphaera fusiformis* was first described in the nests of *Osmia cornifrons* in Japan, and also *Ascosphaera celerrima* ($n=5$; Skou, 1988), which was additionally detected in this study (Table S1).

A total of five species of *Osmia* were identified from trap nests (see Table S2): non-native *O. taurus*, non-native *O. cornifrons*, native *O. lignaria*, native *O. georgica* and native *O. subfasciata*. Out of 61 sites

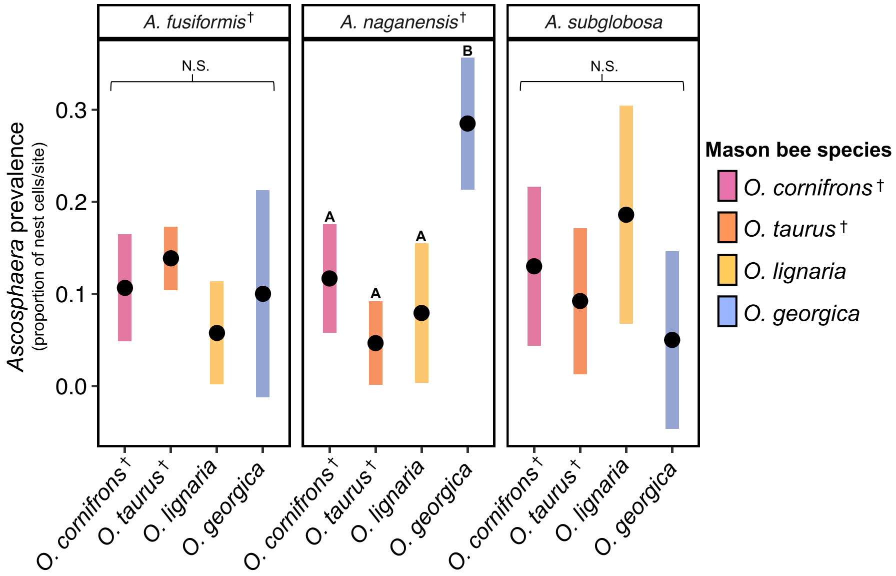

FIGURE 2 Estimated probabilities of *Ascosphaera* species associated with each *Osmia* species in trap nests. Shaded regions extending beyond each point estimate represent 95% confidence intervals. Fungi and mason bees with a dagger (†) denote species originally described from Asia.

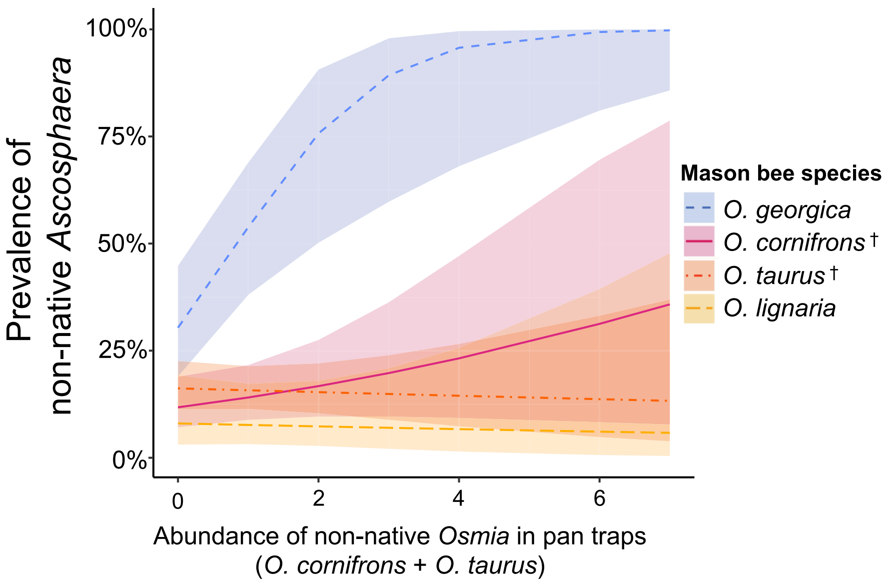

FIGURE 3 Estimated probabilities of non-native *Ascosphaera* species detection in relation to non-native *Osmia taurus* and *O. cornifrons* abundance in pan traps. Shaded regions extending beyond each line represent 95% confidence intervals. Daggers (†) denote mason bee species first described from Asia.

occupied by *Osmia*, 44 sites (72%) were found to have *Osmia* individuals in nests with *Ascosphaera* first described from Japan (*A. celerrima*, *A. fusiformis* and *A. naganensis*, Figure 1; Table S3). *Ascosphaera naganensis* was detected at 21 of the 44 sites; *A. fusiformis* was detected at 31 of the 44 sites. Of the 19 sites where native *Osmia* were found in trap nests, Japanese *Ascosphaera* species were detected at 16. Of the nine sites with exclusively nests of native *Osmia* species, *Ascosphaera naganensis* and/or *Ascosphaera fusiformis* were found in nest specimens of native *Osmia* at all but one site, even though we found no non-native *Osmia* species nesting inside those trap nests.

From analysing *Ascosphaera* prevalence in trap nests for each *Osmia* species, there was a significant difference in associations of *Ascosphaera* species with *Osmia* species ($\chi^2=18.745$, df$=6$, $p=0.004$). Specifically, native species *Osmia georgica* was more frequently associated with non-native *A. naganensis* than any other species of *Osmia* (Figure 2; Table S4). We found that the probability of non-native *Ascosphaera* species detection in native *O. georgica* significantly increased at sites with greater local abundance of non-native *Osmia* species, but this pattern was not found for other focal *Osmia* species (Figure 3; Table S5). In regard to landcover type, there was no significant variation in the prevalence of non-native *Ascosphaera* across the three dominant landcover categories ($\chi^2=10.564$, df$=6$, $p=0.1$), but there was greater non-native *Ascosphaera* prevalence in agriculturally dominant landscapes for native *O. georgica* compared to natural landcover types ($\chi^2=6.7751$, df$=2$, $p=0.034$, Figure 4). For all three models, no residual spatial autocorrelation was detected (Figure S3; Table S6).

4 | DISCUSSION

Our study documents a pattern of association consistent with the spillover of bee-specialist fungi first described in Asia into native mason bees in the region of study in North America. These non-native *Ascosphaera* species were frequently associated with native *Osmia* in trap nests, and we further documented that increasing abundance of Asian *Osmia cornifrons* and *Osmia taurus* at the site level was positively correlated with increased detection of Asian *Ascosphaera* in the native *Osmia georgica*. Lastly, this high prevalence occurs more frequently for *Osmia georgica* in agriculturally-dominated landscapes compared to more natural landscapes. These results generate hypotheses for biological mechanism(s) while simultaneously offering useful ecological information for agricultural and other commercial industries promoting the rearing and movement of mason bees.

As many as four species of *Ascosphaera* may have come into North America through intentional or inadvertent introductions of non-native bees. *Ascosphaera* species were likely co-introduced with *Osmia cornifrons* to North America in 1977 by way of importing diseased, spore-laden cadavers intermingled with cocoons containing healthy adults, despite best efforts to destroy the cadavers when discovered (Batra, 1978; Hedtke et al., 2015), with additional opportunities for co-introduction with *Osmia taurus*, which has not been proven to be same time or instance of introduction as *O. cornifrons*. The present study documents two more species originally described in Japan now present in North America, *Ascosphaera fusiformis* and *A. celerrima*, and we also report novel associations of non-native *A. naganensis* and *A. fusiformis* with native *Osmia lignaria* and *O. georgica* in North America.

In the present study, *A. naganensis* was found most frequently associated with native *Osmia georgica* (Figure 2), most of which were alive, otherwise asymptomatic adult individuals, despite a high proportion of host mortality for *A. naganensis* in *O. cornifrons* (Krichilsky et al., 2021). Determining negative effects of *Ascosphaera* from field data is challenging, as the relationships between the host insect, the host provision and the fungus can be complex. *Ascosphaera* is a genus with at least 28 species, and their effects on bees have been studied mainly through the lens of agricultural, commercial management of pollinators that experience chalkbrood from select *Ascosphaera* species (Evison & Jensen, 2018). Most species of

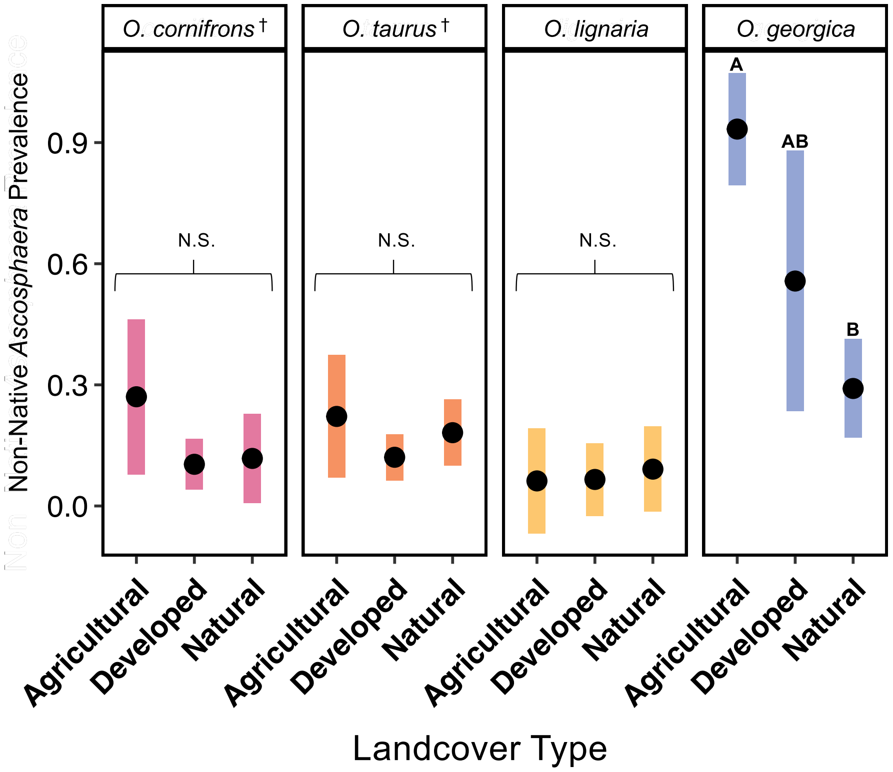

FIGURE 4 Probability of non-native *Ascosphaera* species prevalence for *Osmia* species across dominant landcover types. Error bars indicate 95% confidence intervals. Daggers (†) indicate *Osmia* species originally described from Asia.

this bee specialist fungal genus are poorly studied and their pathogenicities on bees in natural contexts virtually unknown (Klinger et al., 2013; Wynns, 2012).

Agriculturally-dominated landscapes yielded the greatest prevalence of *Ascosphaera* in the native mason bee *Osmia georgica* (Figure 4). This finding is in line with previous research of non-native *Osmia cornifrons* (Krichilsky et al., 2021). Floral food resources may be reduced in agriculturally-dominated areas because of significant phenological gaps in floral resources and/or reduced areas of available wildflowers in bloom (Di Pasquale et al., 2016). In these areas with flower dearth, there could be more concentrated areas of pathogen transmission on flowers that are available (McArt et al., 2014; McNeil et al., 2020; Otterstatter & Thomson, 2008). While a number of both native and non-native *Osmia* in this study are 'mesolectic' and forage on multiple plant families (sensu Cane & Sipes, 2006), *Osmia georgica* is understood to be a specialist pollinator of Asteraceae (Gibbs et al., 2017), and as such may be further restricted in these generalist floral resources available in these already low-resource areas.

4.1 | Future directions

Future experiments should evaluate *Ascosphaera* host range and specificity for a greater number of *Osmia* species, ideally involving native and introduced bee species. Specifically, larval bioassays provide a feasible way forward to test bee species' susceptibility to different *Ascosphaera* species in a controlled manner (Dharampal et al., 2018). To formally test for pathogenicity and sublethal impacts of *Ascosphaera* to *Osmia* in our study, we envision bioassays involving feeding spores from single-isolates *Ascosphaera* (e.g. *A. naganensis*) to native and non-native *Osmia* larvae, comparing their growth and development to nestmates that receive placebo doses, and further molecular and experimental validation that what actually killed the *Osmia* was indeed the *Ascosphaera* strain(s) in question (Jensen et al., 2013).

Furthermore, our methods could not detect co-incidence of multiple *Ascosphaera* species, but this may be a crucial aspect of evaluating their interactions with bees (McFrederick et al., 2014). Co-infection and co-occurrence of *Ascosphaera* species in solitary bee nests is thought to be common in nature but has never been formally evaluated in available peer-reviewed literature (Klinger et al., 2015). If interactions among *Ascosphaera* species are common, their interaction outcomes (e.g. competition, facilitation) should be incorporated into our understanding of host specificity and overall effective virulence of *Ascosphaera* infection (Klinger et al., 2015; Krichilsky et al., 2021; Vojvodic et al., 2012). There may be co-infection dynamics we know very little about that could improve best management practices in agricultural settings for managed bee species. Such improvements may have economic benefits, as agricultural pollination systems across the world rely on bees susceptible to *Ascosphaera* infestation (Glenny et al., 2017; Pitts-Singer & James, 2008).

4.2 | Implications

At present, there has been little monitoring of native megachilid communities to determine their population trajectories in the added presence of closely-related non-native competitors. Our results suggest the spillover of non-native fungi to multiple native mason bee species from co-introduced non-native mason bee species may offer insight regarding observed regional declines in six native *Osmia* species (Gutierrez et al., 2023; LeCroy et al., 2020). With increasing use of "bee hotels" by the public (Fortel et al., 2016; González-Zamora et al., 2021), without periodic cleaning, these nesting cavities could cause hotspots of *Ascosphaera* buildup and high rates of infection, serving as points of *Ascosphaera* spillover to native mason bees.

Additionally, companies in North America that sell mason bees to consumers should be better regulated and/or incentivized to check for unintentional spread of *Osmia taurus* and the spores of non-native species of *Ascosphaera* across North America. As of 2023, a number of these existing mason bee companies offer "buy-back" or "rental" programs, such that they receive occupied mason bee nests back from customers at the end of adult flight season (MacIvor et al., 2022). By doing this, companies are able to re-distribute these mason bee offspring across the continent, with little to no oversight. *Osmia taurus* and *O. cornifrons* are difficult to discern morphologically without a microscope, but there should be methods for spot-checking each nest received to monitor and prevent spread of the rapidly proliferating *Osmia taurus* (LeCroy et al., 2020). Regular molecular screening for various pathogens could be made possible by establishing relationships with academic or government agricultural research labs.

With increasing records of introduced and invasive species across the world, the co-introduction of diseases is still poorly documented but hypothesized to have enormous economic, public health and conservation implications (Aizen et al., 2019). It is more important than ever to elucidate mechanisms of disease-mediated invasions (Strauss et al., 2012), and we must understand these impacts and the intricacies of disease spillover to better manage risk and improve pollinator health.

AUTHOR CONTRIBUTIONS

Kathryn A. LeCroy and T'ai H. Roulston conceived the research ideas and designed bee collection methodologies. Erin Krichilsky, Heather L. Grab and Bryan N. Danforth designed molecular screening methodologies. Kathryn A. LeCroy and Erin Krichilsky collected the data. Erin Krichilsky led the phylogenetic tree construction and analyses. Heather L. Grab and Kathryn A. LeCroy conducted the statistical analyses. Kathryn A. LeCroy led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Michelle Prysby of the Virginia Master Naturalist Program for assistance in recruiting community scientists. We thank the following UVA undergraduate students involved in opening trap nests for data collection: Logan Ende, Sam Grimmelbein, Dat Ha, Shannon Hunter, Noah Jacobs, John Leahy, Nayoung Lee, James Lord, John McCormack, Swathi Mukkamala, Sara Richardson, Sebastien Simko, Hans Theiler and Jenny Wang. We thank Scott McArt, Tobias Mueller, Paige Muñiz, Benoît Geslin and anonymous reviewers for helpful comments on earlier versions of the manuscript. Consent has been given by all people featured in any photography. This work was made possible by funding from the National Science Foundation Graduate Research Fellowship (2013144751) to Kathryn LeCroy, a Garden Club of Virginia Fellowship to Kathryn LeCroy, a Blandy Experimental Farm Summer Graduate Fellowship to Kathryn LeCroy, a Jefferson Scholars Foundation Fellowship to Kathryn LeCroy, and a USDA NIFA AFRI Postdoctoral Fellowship (2021-08318) to Kathryn LeCroy. Our study involved 100 community scientists assisting with monitoring trap nests and collecting specimens from pan traps at study sites. Although they are not credited as authors on this publication, this work would not be possible without these community scientists volunteering their property, time and effort for data acquisition. All community scientists are each acknowledged by name here: Elisabeth Adams, Liz Alford, Chris Anderson, Stuart Ashton, Mindy Ashton, Katharina Bergdoll, Sarah Bingham, Chris Bowlen, Chip Brown, Bruce Bryant, Bobbie Burton, Jill Butler, Stephen Cassola, Stuart Clayman, Ellen Climo, Jane Colgan, Rachel Collins, David Cooper, Dera Cooper, Dana Crone, Kathryn Culbertson, Mary Loose DeViney, Kate Domanski, M'Shae Dunham, Ron Fandetti, Donna Fauber, Kathy Fell, Leslie Fellows, Char'Rese Finney, Stephanie Gardner, Diane Girgente, Barbara Glaser, Holly Glenn, Bland Goddin, Kristen Goff, Kate Guenther, John Hammer, Frances Han, Louis Harrell, Katie Hastings, Carol Heiser, Don Hess, Jennifer Hoover, Beverly Hovencamp, Jim Hurley, Atziri Ibanez, Roberta Jalbert, Erica Jeter, Nancy Joel, Bruce Jones, Dean Joyner, Marcia Kaetzel, Cindy Lambert, Thomas Letonja, John Lill, Charlotte Lorick, Mark Lovecky, Willow Lovecky, Marie Majarov, Lisa Matthews, Cathy Mayes, Bruce McClinton, Cindy McGregor, James Mello, Andy Miller, Margie Miller, Alex Newhart, Peggy Plass, Bill Plyler, Regina Prunty, Michelle Prysby, Ellen Reynolds, Laurel Riddle, Janet Rigoni, Dotty Rilee, Mary Rordam, Bob Ryan, Daniel Salatin, Emma Schneider, Lavinia "Vini" Schoene, Pamela Schoppee Bortz, Marilyn Schroeder, Beth Shatin, Elizabeth Sidamon-Eristoff, David Smith, Marilyn Smith, Elaine Smith, Alison Sowar, Riley Spahn, Dana Squire, Cynthia Stanley, Steve Twombly, Cyndi Walsh, Elizabeth Waters, Caroline Watts, Sandy Weber, Nikki White, Bradley Willet, Lynn Wilson, Jan Worthy, Kathleen Youngblood and Zita Zduoba.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dv41ns238 (LeCroy et al., 2023).

ORCID

Kathryn A. LeCroy https://orcid.org/0000-0001-8819-3350
Erin Krichilsky https://orcid.org/0000-0002-0739-5717
Heather L. Grab https://orcid.org/0000-0002-1073-8805
T'ai H. Roulston https://orcid.org/0000-0002-1220-1315
Bryan N. Danforth https://orcid.org/0000-0002-6495-428X

REFERENCES

Aizen, M. A., Smith-Ramírez, C., Morales, C. L., Vieli, L., Sáez, A., Barahona-Segovia, R. M., Arbetman, M. P., Montalva, J., Garibaldi, L. A., Inouye, D. W., & Harder, L. D. (2019). Coordinated species importation policies are needed to reduce serious invasions globally: The case of alien bumblebees in South America. *Journal of Applied Ecology*, *56*(1), 100–106.

Anderson, D., Gibbs, A., & Gibson, N. (1998). Identification and phylogeny of spore-cyst fungi (*Ascosphaera* spp.) using ribosomal DNA sequences. *Mycological Research*, *102*(5), 541–547.

Arismendi, N., Riveros, G., Zapata, N., Smagghe, G., González, C., & Vargas, M. (2021). Occurrence of bee viruses and pathogens associated with emerging infectious diseases in native and non-native bumble bees in southern Chile. *Biological Invasions*, *23*(4), 1175–1189. https://doi.org/10.1007/s10530-020-02428-w

Batra, S. (1978). *Osmia cornifrons* and *Pithitis smaragdula*, two Asian bees introduced into the United States for crop pollination. In Proceedings 4th international symposium on pollination. *Maryland Agricultural Experimental Station Special Publication*, *1*, 307–312.

Bissett, J. (1988). Contribution toward a monograph of the genus *Ascosphaera*. *Canadian Journal of Botany*, *66*(12), 2541–2560.

Bosch, J., Maeta, Y., & Rust, R. (2001). A phylogenetic analysis of nesting behavior in the genus *Osmia* (Hymenoptera: Megachilidae). *Annals of the Entomological Society of America*, *94*(4), 617–627.

Cane, J. H., & Sipes, S. (2006). Characterizing floral specialization by bees: Analytical methods and a revised lexicon for oligolecty. In N. Waser, & J. Ollerton (Eds.), *Plant-pollinator interactions: From specialization to generalization* (pp. 99–122). University of Chicago Press.

Danforth, B., Fang, J., Sipes, S., Brady, S., Almeida, E., Litman, J., & Cardinal, S. (2004). *Phylogeny and molecular systematics of bees (Hymenoptera: Apoidea)*. Cornell University.

De Palma, A., Kuhlmann, M., Roberts, S. P. M., Potts, S. G., Börger, L., Hudson, L. N., Lysenko, I., Newbold, T., & Purvis, A. (2015). Ecological traits affect the sensitivity of bees to land-use pressures in European agricultural landscapes. *Journal of Applied Ecology*, *52*(6), 1567–1577. https://doi.org/10.1111/1365-2664.12524

Dharampal, P. S., Carlson, C. M., Diaz-Garcia, L., & Steffan, S. A. (2018). In vitro rearing of solitary bees: A tool for assessing larval risk factors. *Journal of Visualized Experiments*, *137*, e57876.

Di Pasquale, G., Alaux, C., Le Conte, Y., Odoux, J.-F., Pioz, M., Vaissière, B. E., Belzunces, L. P., & Decourtye, A. (2016). Variations in the availability of pollen resources affect honey bee health. *PLoS One*, *11*(9), e0162818. https://doi.org/10.1371/journal.pone.0162818

Droege, S. (2020). *USGS PWRC—Native Bee Inventory and Monitoring Lab (BIML) [Data set]*. United States Geological Survey. https://doi.org/10.15468/6AUTVB

Evison, S. E., & Jensen, A. B. (2018). The biology and prevalence of fungal diseases in managed and wild bees. *Current Opinion in Insect Science*, *26*, 105–113. https://doi.org/10.1016/j.cois.2018.02.010

Fortel, L., Henry, M., Guilbaud, L., Mouret, H., & Vaissière, B. E. (2016). Use of human-made nesting structures by wild bees in an urban environment. *Journal of Insect Conservation*, *20*(2), 239–253. https://doi.org/10.1007/s10841-016-9857-y

Gibbs, J., Ascher, J. S., Rightmyer, M. G., & Isaacs, R. (2017). The bees of Michigan (Hymenoptera: Apoidea: Anthophila), with notes on distribution, taxonomy, pollination, and natural history. *Zootaxa*, *4352*(1), 1–160. https://doi.org/10.11646/zootaxa.4352.1.1

Glenny, W., Cavigli, I., Daughenbaugh, K. F., Radford, R., Kegley, S. E., & Flenniken, M. L. (2017). Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. *PLoS One*, *12*(8), e0182814. https://doi.org/10.1371/journal.pone.0182814

González-Zamora, J. E., Hidalgo-Matas, J. A., & Corell-González, M. (2021). Wild solitary bees and their use of bee hotels in Southwest Spain. *Journal of Apicultural Research*, *60*, 862–870. https://doi.org/10.1080/00218839.2021.1892416

Grab, H., Brokaw, J., Anderson, E., Gedlinske, L., Gibbs, J., Wilson, J., Loeb, G., Isaacs, R., & Poveda, K. (2019). Habitat enhancements rescue bee body size from the negative effects of landscape simplification. *Journal of Applied Ecology*, *56*(9), 2144–2154. https://doi.org/10.1111/1365-2664.13456

Graystock, P., Blane, E. J., McFrederick, Q. S., Goulson, D., & Hughes, W. O. (2016). Do managed bees drive parasite spread and emergence in wild bees? *International Journal for Parasitology: Parasites and Wildlife*, *5*(1), 64–75.

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, *153*(3), 589–596. https://doi.org/10.1007/s00442-007-0752-9

Gutierrez, G. M., LeCroy, K. A., Roulston, T. H., Biddinger, D. J., & López-Uribe, M. M. (2023). *Osmia taurus* (Hymenoptera: Megachilidae): A non-native bee species with invasiveness potential in North America. *Environmental Entomology*, nvad005. https://doi.org/10.1093/ee/nvad005

Hall, H. G. (2016). Color preferences of bees captured in pan traps. *Journal of the Kansas Entomological Society*, *89*(3), 273–276. https://doi.org/10.2317/JKESD1600022.1

Hedtke, S. M., Blitzer, E. J., Montgomery, G. A., & Danforth, B. N. (2015). Introduction of non-native pollinators can lead to trans-continental movement of bee-associated fungi. *PLoS One*, *10*(6), e0130560. https://doi.org/10.1371/journal.pone.0130560

Homer, C., Dewitz, J., Yang, L., Jin, S., Danielson, P., Xian, G., Coulston, J., Herold, N., Wickham, J., & Megown, K. (2015). Completion of the 2011 National Land Cover Database for the conterminous United States–representing a decade of land cover change information. *Photogrammetric Engineering & Remote Sensing*, *81*(5), 345–354.

James, R., & Skinner, J. (2005). PCR diagnostic methods for *Ascosphaera* infections in bees. *Journal of Invertebrate Pathology*, *90*(2), 98–103.

James, R. R., & Pitts-Singer, T. L. (2013). Health status of alfalfa Leafcutting bee larvae (Hymenoptera: Megachilidae) in United States alfalfa seed fields. *Environmental Entomology*, *42*(6), 1166–1173. https://doi.org/10.1603/EN13041

Jensen, A. B., Aronstein, K., Flores, J. M., Vojvodic, S., Palacio, M. A., & Spivak, M. (2013). Standard methods for fungal brood disease research. *Journal of Apicultural Research*, *52*(1), 1–20. https://doi.org/10.3896/IBRA.1.52.1.13

Keller, A., McFrederick, Q. S., Dharampal, P., Steffan, S., Danforth, B. N., & Leonhardt, S. D. (2021). (More than) Hitchhikers through the network: The shared microbiome of bees and flowers. *Current Opinion in Insect Science*, *44*, 8–15. https://doi.org/10.1016/j.cois.2020.09.007

Kirk, W. D. J. (1984). Ecologically selective coloured traps. *Ecological Entomology*, *9*(1), 35–41. https://doi.org/10.1111/j.1365-2311.1984.tb00696.x

Klinger, E. G., James, R. R., Youssef, N. N., & Welker, D. L. (2013). A multi-gene phylogeny provides additional insight into the relationships between several *Ascosphaera* species. *Journal of Invertebrate Pathology*, *112*(1), 41–48. https://doi.org/10.1016/j.jip.2012.10.011

Klinger, E. G., Vojvodic, S., DeGrandi-Hoffman, G., Welker, D. L., & James, R. R. (2015). Mixed infections reveal virulence differences between host-specific bee pathogens. *Journal of Invertebrate Pathology*, *129*, 28–35. https://doi.org/10.1016/j.jip.2015.05.003

Kojima, Y., Toki, T., Morimoto, T., Yoshiyama, M., Kimura, K., & Kadowaki, T. (2011). Infestation of Japanese native honey bees by tracheal mite and virus from non-native European honey bees in Japan. *Microbial Ecology*, *62*(4), 895–906. https://doi.org/10.1007/s00248-011-9947-z

Krichilsky, E., Centrella, M., Eitzer, B., Danforth, B., Poveda, K., & Grab, H. (2021). Landscape composition and fungicide exposure influence host-pathogen dynamics in a solitary bee. *Environmental Entomology*, *50*(1), 107–116. https://doi.org/10.1093/ee/nvaa138

Krombein, K. V. (1967). *Trap-nesting wasps and bees: Life histories, nests, and associates*. Smithsonian Press. http://archive.org/details/trapnestingwasps00krom

LeCroy, K., Krichilsky, E., Grab, H., Roulston, T., & Danforth, B. (2023). Spillover of chalkbrood fungi to native solitary bee species from non-native congeners. *Dryad, Dataset*. https://doi.org/10.5061/dryad.dv41ns238

LeCroy, K. A., Savoy-Burke, G., Carr, D. E., Delaney, D. A., & Roulston, T. H. (2020). Decline of six native mason bee species following the arrival of an exotic congener. *Scientific Reports*, *10*(1), 18745. https://doi.org/10.1038/s41598-020-75566-9

MacIvor, J. S., de Keyzer, C. W., Marshall, M. S., Thurston, G. S., & Onuferko, T. M. (2022). Establishment of the non-native horned-face bee *Osmia cornifrons* and the taurus mason bee *Osmia taurus* (Hymenoptera: Megachilidae) in Canada. *PeerJ*, *10*, e14216.

McArt, S. H., Koch, H., Irwin, R. E., & Adler, L. S. (2014). Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. *Ecology Letters*, *17*(5), 624–636. https://doi.org/10.1111/ele.12257

McFrederick, Q. S., Mueller, U. G., & James, R. R. (2014). Interactions between fungi and bacteria influence microbial community structure in the *Megachile rotundata* larval gut. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779), 20132653. https://doi.org/10.1098/rspb.2013.2653

McNeil, D. J., McCormick, E., Heimann, A. C., Kammerer, M., Douglas, M. R., Goslee, S. C., Grozinger, C. M., & Hines, H. M. (2020). Bumble bees in landscapes with abundant floral resources have lower pathogen loads. *Scientific Reports*, *10*(1), 22306. https://doi.org/10.1038/s41598-020-78119-2

Meeus, I., Brown, M. J. F., De Graaf, D. C., & Smagghe, G. (2011). Effects of invasive parasites on bumble bee declines: Parasites and bumble bee declines. *Conservation Biology*, *25*(4), 662–671. https://doi.org/10.1111/j.1523-1739.2011.01707.x

Murray, T. E., Coffey, M. F., Kehoe, E., & Horgan, F. G. (2013). Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations. *Biological Conservation*, *159*, 269–276.

Otterstatter, M. C., & Thomson, J. D. (2008). Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS One*, *3*(7), e2771. https://doi.org/10.1371/journal.pone.0002771

Park, Y.-L., Kondo, V., White, J., West, T., McConnell, B., & McCutcheon, T. (2009). Nest-to-nest dispersal of *Chaetodactylus krombeini* (Acari, Chaetodactylidae) associated with *Osmia cornifrons* (Hym., Megachilidae). *Journal of Applied Entomology*, *133*(3), 174–180. https://doi.org/10.1111/j.1439-0418.2008.01351.x

Pitts-Singer, T. L., & James, R. (2008). Past and present management of alfalfa bees. In R. James & T. Pitts-Singer (Eds.), *Bee Pollination in Agricultural Ecosystems* (pp. 105–123). Oxford University Press.

Poinar, G. O., & Van der Laan, P. (1972). Morphology and life history of *Sphaerularia bombi*. *Nematologica*, *18*(2), 239–252.

Russo, L., de Keyzer, C. W., Harmon-Threatt, A. N., LeCroy, K. A., & MacIvor, J. S. (2021). The managed-to-invasive species continuum in social and solitary bees and impacts on native bee conservation. *Current Opinion in Insect Science*, *46*, 43–49. https://doi.org/10.1016/j.cois.2021.01.001

Schmid-Hempel, R., Eckhardt, M., Goulson, D., Heinzmann, D., Lange, C., Plischuk, S., Escudero, L. R., Salathé, R., Scriven, J. J., & Schmid-Hempel, P. (2014). The invasion of southern South America by imported bumblebees and associated parasites. *Journal of Animal Ecology*, *83*(4), 823–837. https://doi.org/10.1111/1365-2656.12185

Skou, J. (1988). Japanese species of *Ascosphaera*. *Mycotaxon*, *31*(1), 173–190.

Stephen, W. P., Vandenberg, J. D., & Fichter, B. L. (1981). *Etiology and epizootiology of chalkbrood in the leafcutting bee*, Megachile rotundata (Fabricius), *with notes on* Ascosphaera *species*.

Strauss, A., White, A., & Boots, M. (2012). Invading with biological weapons: The importance of disease-mediated invasions. *Functional Ecology*, *26*(6), 1249–1261. https://doi.org/10.1111/1365-2435.12011

Vannette, R. L. (2020). The floral microbiome: Plant, pollinator, and microbial perspectives. *Annual Review of Ecology, Evolution, and Systematics*, *51*(1), 363–386. https://doi.org/10.1146/annurev-ecolsys-011720-013401

Vojvodic, S., Boomsma, J. J., Eilenberg, J., & Jensen, A. B. (2012). Virulence of mixed fungal infections in honey bee brood. *Frontiers in Zoology*, *9*(1), 5. https://doi.org/10.1186/1742-9994-9-5

Wynns, A. A. (2012). *The bee specialist fungus family Ascosphaeraceae and its allies: Systematics, ecology and co-evolution with solitary bees*. (PhD dissertation). University of Denmark.

Wynns, A. A., Jensen, A. B., Eilenberg, J., & James, R. (2012). *Ascosphaera subglobosa*, a new spore cyst fungus from North America associated with the solitary bee *Megachile rotundata*. *Mycologia*, *104*(1), 108–114. https://doi.org/10.3852/10-047

Youssef, N. N., & McManus, W. R. (2001). *Ascosphaera torchioi* sp. nov., a pathogen of *Osmia lignaria propinqua* (Hymenoptera). *Mycotaxon*, *77*, 7–14.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (Vol. *574*). Springer.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Map identifying pan-trapping sites.

Figure S2. Phylogenetic tree of *Ascosphaera* sequences.

Figure S3. Inspection of spatial structure in model residuals.

Table S1. Life stages of specimens screened for *Ascosphaera*.

Table S2. *Osmia* nest occupancy summary.

Table S3. Summary of *Ascosphaera* detection by *Osmia* species.

Table S4. Model contrasts for *Ascosphaera*-*Osmia* associations.

Table S5. Species-specific slope estimates for the probability of non-native Ascosphaera species.

Table S6. Moran's I estimates for all model residuals.

Appendix S1. Materials and methods for phylogenetic tree construction.

How to cite this article: LeCroy, K. A., Krichilsky, E., Grab, H. L., Roulston, T. H., & Danforth, B. N. (2023). Spillover of chalkbrood fungi to native solitary bee species from non-native congeners. *Journal of Applied Ecology*, *00*, 1–10. https://doi.org/10.1111/1365-2664.14399