An interaction between host and microbe genotypes determines colonization success of a key bumble bee gut microbiota member

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There has been a proliferation of studies demonstrating an organism's health is influenced by its microbiota. However, factors influencing beneficial microbe colonization and the evolution of these relationships remain understudied relative to host–pathogen interactions. Vertically transmitted beneficial microbes are predicted to show high levels of specificity in colonization, including genotype matching, which may transpire through coevolution. We investigate how host and bacterial genotypes influence colonization of a core coevolved microbiota member in bumble bees. The hindgut colonizing *Snodgrassella alvi* confers direct benefits, but, as an early colonizer, also facilitates the further development of a healthy microbiota. Due to predominantly vertical transmission promoting tight evolution between colonization factors of bacteria and host lineages, we predict that genotype-by-genotype interactions will determine successful colonization. Germ-free adult bees from seven bumble bee colonies (host genotypic units) were inoculated with one of six genetically distinct strains of *S. alvi*. Subsequent colonization within host and microbe genotypes combinations ranged from 0 to 100%, and an interaction between host and microbe genotypes determined colonization success. This novel finding of a genotype-by-genotype interaction determining colonization in an animal host-beneficial microbe system has implications for the ecological and evolutionary dynamics of host and microbe, including associated host-fitness benefits.

KEY WORDS: Bumble bee, genotype-by-genotype interactions, host colonization, microbiota, *Snodgrassella alvi*.

Recent advances have shed light on the important contributions of microbial communities for the health and evolutionary fitness of their hosts. These microbiota have implications for host defense (Dong et al. 2009; Oliver et al. 2012; Parker et al. 2017), development (Visick et al. 2000; Palm et al. 2015), and physiology (Kikuchi et al. 2012; Brune 2014; Kešnerová et al. 2017). The role of an organism's microbiota in determining fitness-relevant host traits necessitates an understanding of ecological and evolutionary determinants of the composition of these microbial communities, including a focus on factors that drive the colonization and establishment of particular microbes.

Colonization represents an essential component in host-beneficial microbe systems (Visick et al. 2000; Vivas et al. 2003; Lee et al. 2013), with the beneficial effects dependent upon successful microbial colonization. Many factors, such as environmental influences (Yellowlees et al. 2008; Bourne et al. 2009; Kikuchi and Yumoto 2013), host-species specificity

(Kwong and Moran 2015; La Rivière et al. 2015), and the host or symbiont genotypes (Chong and Moran 2016; Goodrich et al. 2016; Parker et al. 2017; Wang et al. 2017), may determine colonization success. The influence of these factors on the success of colonization also depends upon the route of microbe transmission between hosts (Bright and Bulgheresi 2010). Where symbionts are environmentally acquired, abiotic factors can serve as the primary driving force behind microbial colonization (Thursz et al. 1999; Sison-Mangus et al. 2018). Such symbionts are facultative and experience a free-living stage between hosts, making survival in both their host and external environment paramount. The environmental influences on these systems are twofold, as the environment serves as the source of these microbial organisms, but also abiotic factors influence survival and colonization success. Abiotic factors, such as temperature and salinity, can influence microbial growth within hosts and when free-living (Soto et al. 2009). Although environmental influences

are involved, systems where microbes are acquired through vertical transmission are predicted to show a greater degree of coevolution and host–microbe specificity (Poisot et al. 2011).

The vertical transmission of beneficial microbial symbionts from mother to offspring facilitates interactions that can shape host specificity (Moran et al. 1995; Schardl et al. 1997; Poisot et al. 2011; Powell et al. 2014; Moeller et al. 2018). Relationships between hosts and microbes may become highly specialized, with genotype matching of hosts and vertically transmitted microbes (Poisot et al. 2011). Such specificity may have consequences for subsequent ecological and evolutionary interactions related to host and symbiont fitness (Gundel et al. 2012). In animals, studies of the effects of host genotype on colonization include examples in multiple systems, such as aphids (Chong & Moran 2016), mice (Moeller et al. 2018), stink bugs (Hosokawa et al. 2016), and humans (Blekham et al. 2015). Moreover, reciprocal genotype-level specificity (Poisot et al. 2011) is predicted to lead to host genotype by microbe genotype interactions, but it has not been investigated if host and bacterial genotypes interact to determine colonization success in animal host and beneficial microbe systems.

Despite a lack of research investigating the role of host and beneficial microbe genotypes in determining colonization, there are studies examining genotype-dependent functional outcomes. Functional outcomes, including pathogen defense, digestion, and host development, may depend upon host genotype, beneficial microbe genotype, or interactions between the two. Host genotype affects the formation of microbe-induced nitrogen fixing nodules in plants (Smith and Goodman 1999) and how host immune function determines mammalian gut microbiota composition (Spor et al. 2011). Additionally, beneficial microbial genotypes may differentially determine host defense against pathogens (Ford et al. 2017), ecological invasion success (Gueguen et al. 2010; Rudgers et al. 2010), and resistance to pesticides (Kikuchi et al. 2012; Hosokawa et al. 2016). Furthermore, interactions between host and beneficial microbial genotypes in aphids can mediate pathogen susceptibility (Parker et al. 2017) and the fitness costs of possessing defensive symbionts (Vorburger and Gouskov 2011). Although the interactions between genotypes have been studied in regard to such functional outcomes, the ability for these interactions to determine the successful colonization of beneficial symbionts remains uninvestigated outside of plant systems (Wang et al. 2017).

The contribution of host and symbiont genotypes to colonization have not been well studied in host-beneficial microbe systems, but work in host–parasite systems provides potential insights into the factors involved, and the implications of genotype-level specificity. Within host–parasite systems, host genotypes are implicated in differential immune responses (Lazzaro et al. 2006) and susceptibility to infection (Samuel 2002). However, the influences of genotypes in colonization is not constrained to hosts,

as parasite genotypes differ in their abilities to infect (de Roode et al. 2008). Furthermore, the differential immune expression of host individuals in response to different parasite genotypes is evidence of the importance of parasite genotype in these systems (Barribeau and Schmid-Hempel 2013). In systems with diverse host and parasite genotypes, infection outcomes may be determined by the combination of host and parasite genotypes, and there are numerous examples of such genotype-by-genotype interactions (Carius et al. 2001; Lambrechts et al. 2005; Sadd and Schmid-Hempel 2009; Barribeau et al. 2014). In these host–parasite systems, genotype-level specificity is a prerequisite for the existence of such genotype-by-genotype interactions. It is thought that these interactions are the consequence of coevolution, but they will also feedback on coevolutionary dynamics (Sadd and Schmid-Hempel 2009).

Social bees are a useful model system for understanding host–microbe evolution and its outcomes, and corbiculate bees possess a relatively simple gut bacterial community with 11–15 core species (Martinson et al. 2011). These core species have been implicated in host defense (Koch and Schmid-Hempel 2011; Schwarz et al. 2016), digestion (Kešnerová et al. 2017; Zheng et al. 2017), and development (Näpflin and Schmid-Hempel 2016; Kwong et al. 2017a). Further underlining the vital function of these microbes are the negative consequences following their perturbation by pathogens (Schwarz et al. 2016), pesticides (Kakumanu et al. 2016), and antibiotics (Raymann et al. 2017). Vertical transmission of many of these microbes creates a situation where microbial fitness is intrinsically linked with host health and sets the stage for potential coevolution between host lineages and their microbes (Powell et al. 2016). This is expected to be greatest where genetic variability within host lineages is low, such as in many bumble bee species with high relatedness of worker bees from a single colony (Hines 2008), and sociality may amplify vertical transmission and the potential for coevolution (Koch et al. 2013). Subsequently, specificity and genetic differences within hosts and microbes influencing colonization success could result.

Snodgrassella alvi is a key gut colonizer within social bees, including bumble bees. This microbe is highly prevalent in the hindguts of adult bees and is the first microbial species to begin settling (Powell et al. 2014; Li et al. 2015; Lim et al. 2015). Because of this, direct interactions between the host and *S. alvi* are highly relevant to understand. The biofilm that *S. alvi* forms, directly on the host epithelium (Katsnelson 2015), mediates many environmental hindgut conditions (Zheng et al. 2017). These modifications may be critical in allowing other gut symbionts to colonize the nutrient-poor hindgut.

The importance of *S. alvi* for the health of their pollinator hosts make the benefits of studying *S. alvi* colonization twofold. First, studies in this system will provide novel insights into

the principal factors of beneficial symbiont colonization more generally and highlight potential implications for host fitness and subsequent evolutionary dynamics. Additionally, understanding the patterns and factors influencing successful colonization can shed light upon potential implications for pollinator health.

Previous research has demonstrated that *S. alvi* can display host genus level specificity, where *S. alvi* isolated from specific host genera, bumble bee (*Bombus*) and honey bee *(Apis)*, do not colonize hosts of the foreign genus as well as their native hosts (Kwong et al. 2014). The limited number of bumble bee strains used in the study were from a different species than the inoculated host and phylogenetic analyses suggest some sharing of strains across species, but many appear more narrowly specialized (Powell et al. 2016). Transmission of *S. alvi* in social bumble bees may facilitate evolution with host lineages and potentially genotype-level matching for certain strains (Poisot et al. 2011), which would result in colonization being restricted to related host types with the matching factors relevant for colonization. However, the extent of specificity in this system has not been experimentally examined on the level of host and bacterial genotypes.

We ask if the colonization success of the beneficial gut symbiont *S. alvi* into its bumble bee host *Bombus impatiens* is influenced by the bacterial strain genotype and the genotype of the host, delimited by the host genotypic units of different colonies of origin. We hypothesize that some *S. alvi* strains exhibit narrow specificity to host genotypes, which will stem from interactions between host genotypes and their vertically transmitted bacteria. Thus, we predict that genotype-by-genotype interactions will determine successful colonization.

Materials and Methods
OVERALL EXPERIMENTAL DESIGN
Three *B. impatiens* colonies (A–C) were raised from wild collected queens from the Mackinaw River Study Area (Lexington, IL). Queens were collected with the permission of the ParkLands Foundation (http://www.parklandsfoundation.org/) in April 2018. Three additional colonies (D–F) were raised from lab-reared and mated queens originally derived from distinct parental commercial colonies from Koppert Biological Systems. One colony (G) was obtained directly from Koppert Biological Systems. Colonies were held under red-light at $26 \pm 1.5°C$ and provided with sugar water (1 g cane sugar, 1 mL boiled tap water, and 0.1% cream of tartar [potassium bitartate] to partially invert the sugars) and pollen (Brushy Mountain Bee Farms, Moravian Falls, NC). Founding queens and a subset of workers were checked by fecal screening and confirmed free of detectable infections of known gut pathogens. Six strains of *S. alvi* (three from colonies of commercial origin [C7, C11, C14] and three from colonies of wild origin [I1, I2, I49]) were used to create inoculums. Gut-microbe-free

adult worker bees from each colony were assigned to one of seven treatments. All combinations of host genotypic background and *S. alvi* strain were created, with five replicates per genotype treatment combination for a total sample size of 210. An additional 35 bees across all colonies were used to check the gut microbe-free status of uninoculated bees. All strains used were foreign to the host colonies. Native strains could not be used due to restrictions of the colony life-cycle and the time intensive nature of isolating and confirming *S. alvi* identity. However, *S. alvi* strains were isolated from three of the colonies before worker production ceased and a limited number of inoculations showed that colonization in native hosts was achievable, as would be predicted.

SNODGRASSELLA ALVI STRAIN ORIGIN, CULTURING, AND IDENTIFICATION
Original hosts (*B. impatiens*) of wild origin strains were sourced from Central Illinois as described above. Original hosts of strains designated commercial were from colonies obtained from Koppert Biological Systems. Bees were isolated for 24 hours after collection and fed gamma-irradiated pollen and sterile sugar water ad libitum. This isolation period ensures any environmentally acquired microbes just passing though the bees' guts are cleared, preventing environmental contaminates from overgrowing *S. alvi* on agar plates and hindering *S. alvi* isolation. After the 24-hour isolation period, bees were chilled on ice, their guts removed aseptically, and hindguts homogenized for 30 seconds in 200 µL of ringer saline with 2.4 mm metal beads in a Bead Ruptor (Omni International) on high. Serial dilutions of homogenized samples were spread plated on brain heart infusion agar (Sigma-Aldrich [53286]) supplemented with food coloring for visualization. Plates were incubated at 37°C and 10% CO_2 for 48 hours (Kwong and Moran 2013). Isolated colonies from plates were inoculated into brain heart infusion broth and grown at 37°C and 10% CO_2 for 48 hours. Then that 400 µL of each culture was mixed with 400 µL of sterile 50% glycerol solution in a 1.5-mL screw top tube, and these stock cultures were slow frozen (1°C per minute) and stored at −80°C.

16S rRNA gene sequences were used to confirm isolates as *S. alvi*. Briefly, cells were cultured as above, pelleted (8000 rpm, 10 minutes, Micro 200R microcentrifuge, Hettich Zentrifugen), and suspended in lysis buffer (20 mM Tris-Cl, 2 mM EDTA, 1% Triton X-100, 20 mg lysozyme/mL) for 30 minutes at room temperature. Following lysis, DNA was extracted using an IBI Bacterial DNA Extraction Kit following the manufacturer's protocol. 16S rRNA gene sequences were amplified with the primers 27F and 1492R (Kwong and Moran 2013), amplicon purity checked using gel electrophoresis, and sample concentration and quality quantified using a µDrop plate in a Thermo Scientific MultiSkan GO. Sanger sequencing was performed at the University of Illinois Roy J Carter Biotechnology Center,

Urbana-Champaign, Illinois. Sequences were manually curated using Sequencher software (Gene Codes Corp, Ann Arbor, MI), and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to confirm isolates as *S. alvi*.

Partial single copy genes *minD* (Powell et al. 2016) and *phage repressor protein C* (525 bp, locus SALWKB2_RS03510 of GenBank accession CP007446) were used to assess *S. alvi* strain relatedness. Primers RND1_MinDF1 and RND1_MinDR2wbl2, minus Illumina platform primers, were used for *minD* (product 514 bp, Powell et al. 2016), with initial denaturing of 5 minutes at 95°C, 35 cycles of 95°C for 45 seconds, 54°C for 30 seconds, and 72°C for 60 seconds, followed by a final 5-minute extension at 72°C. The reported parameters vary from the previous study (Powell et al. 2016) because of the exclusion of the deep-sequencing portion of amplification. *Phage repressor protein C* (product 593 bp) was amplified with novel primers PRPFor (ATGACTATGGCCGGTTTCAG) and PRPRev (CG-GCCAAACCACAATACTTT), with PCR conditions the same as for *minD* except annealing at 52°C. Sequencing was as above, with alignments of trimmed sequences (492 bp for *minD* and 526 bp for *phage repressor protein C*) carried out using MUSCLE (Edgar 2004) through the Sequencher version 5.4 DNA-Seq tools plugin. Sequences have been deposited in the GenBank under the accession numbers MN385152 to MN385157 for *minD* and MN385146 to MN385151 *phage repressor protein C*.

PREPARATION OF SNODGRASSELLA STRAINS FOR INOCULATION

Inoculation cultures were initiated from stocks using agar and broth culture parameters as above. After 48 hours in liquid culture, tubes were shaken vigorously to resuspend cells, and optical density (OD) at 600 nm measured in four technical replicates to estimate cell number based on predetermined OD-cell number relationships. Dilutions were performed to create stock inoculums of 300 μL containing an estimated 10^6 cells of *S. alvi* per 10 μL. As cell to OD measurements can be variable (Francois et al. 2005), a 1:10,000 dilution of 10 μL of each stock solution was plated to verify realized stock cell concentrations through counting the colony forming units (CFUs) and multiplying by the dilution factor. The mean realized inoculation size was 1.26×10^6 cells per 10 μL (standard deviation = 0.46×10^6). We used the realized inoculum size as a covariate in analyses to control for any variation caused by varying inoculum sizes.

Stocks were held at 4°C to limit cell division and reductions in the inoculum viability over time. However, as low temperatures can change bacterial gene expression, stress responses, and metabolism (Liu et al. 2002), fresh stocks were recreated every two to three days to reduce any influence of longer refrigeration on *S. alvi* colonization. However, ages of the stock solutions were tracked and also included in analyses.

REARING AND INOCULATION OF GERM-FREE BEES

Cohorts of developing bumble bee larvae will usually be spatially grouped within the colony, with clumps of well-defined individual pupae. Clumps of late stage pupae were identified, removed, and housed in sterilized individual containers until a single worker emerged. Containers had been soaked in 10% bleach for 30 minutes, rinsed with ultrapure water, and dried at 60°C. The emergence of a single worker was used to age the isolated clump, as once a single worker has emerged the remaining individuals should be late-stage pupae or pharate adults and individuals in these stages are better able to survive the germ-free treatment. Aged clumps were soaked in a 3% bleach solution for 90 seconds (Näpflin and Schmid-Hempel 2016), sterilizing the outer pupal casing. After soaking, pupal clumps were dried with kimwipes, placed into new germ-free containers, and checked daily for emergence. During the pupal stage, bees shed their gut lining, which, combined with casing sterilization, creates adult bees lacking gut microbiota. This has been confirmed in other studies (Näpflin and Schmid-Hempel 2016) and replicated in our laboratory.

Emerged adult workers were isolated in individual vials processed using the same procedures as the sterile housing. After 1 hour, bees were presented with 10 μL of sugar water with the estimated 10^6 *S. alvi* cells. This same technique is used for parasite exposures in this system (Sadd 2011). Inoculums were created by pelleting stock inoculums (2500 rpm for 5 minutes), removing the supernatant, and resuspending cells in sugar water. Workers were monitored to ensure inoculum consumption and isolated in sterile housing with autoclaved sugar water and gamma-irradiated honey bee pollen provided ad libitum.

After four days, bees were chilled on ice, their guts removed aseptically, and hindguts homogenized in 200 μL of ringer saline. Following homogenization, guts were serially diluted to 1:100, 1:10,000, and 1:1,000,000, plated onto brain heart infusion agar, and grown for four days as above. The four-day cut-off allowed sufficient time for visible colonies to form, even from slower growing strains. Plates were imaged (Canon EOS 7D and Canon Remote Capture software) and CFUs counted as a proxy for viable cell number on the dilution plate that allowed individual CFUs to be discerned from one another (i.e., were nonoverlapping). This dilution was then included in the conversion of counted CFUs on that plate to the viable number of cells per bee hindgut. Forewings were also removed from each bee and the radial cell measured as a surrogate for body size to use as a covariate in analyses (Palmer-Young et al. 2018).

STATISTICAL ANALYSIS

Analyses were carried out in R 3.4.2. (R Core Team 2017). Initially, estimated viable cells per hindgut were used, but these count data contained a large proportion of zeros and models designed to address such distributions, zero-inflated and hurdle,

Table 1. Hypothesis testing model selection.

Model	Residual d.f.	Residual deviance	AICc	*P*-value
Full	158	162.06	278.43	
Body size removed	159	162.06	275.13	0.9793
Inoculum age removed	159	164.96	278.03	0.0885
Inoculum size removed	159	168.89	281.96	**0.0089**
Final model	160	164.98	274.78	0.0875

Table showing the degrees of freedom, residual variance, and *P*-value for tested models during the model selection process. Models were tested for the null hypothesis of no significant difference until the final model was obtained. Significant value (*P* < 0.05) is in bold.

did not converge. Therefore, to further analyze the data, cell estimates were converted to prevalence data, with 0 being individuals with no apparent bacterial colonization and 1 being individuals with *S. alvi* colonization. Absence represents samples that were actually 0 or below the detection threshold of 100 cells. A generalized linear model with a binomial distribution was used to analyze presence/absence data. Wing size, inoculum size, and stock age were included in the model as covariates. The initial model included microbe strain and host colony as fixed effects, with their interaction. Terms were sequentially removed from the model and hypothesis testing, with likelihood ratios, used to detect significant differences between models due to removed terms. Additionally, Akaike Information Criterion (AICc) values were calculated for each of the models. The final model was determined using the combination of this hypothesis testing and information criteria, so that the final model did not differ significantly from the full model, while also having the lowest associated AICc (Table 1). The final binomial model was considered appropriate as no overdispersion (dispersion value = 0.96) was indicated by the R package *msme* (Hilbe and Robinson 2013).

Results

Uninoculated control bees across all colonies exhibited no evidence of *S. alvi* colonization, indicating the effectiveness of the germ-free treatment (Fig. 1A).

Based on pairwise distances (Fig. 1B) between partial sequences of the *minD* and *phage repressor protein C* gene, the six *S. alvi* isolates represent six distinct *S. alvi* strains. The colonization levels of *S. alvi* strains at four days post-inoculation ranged from 0 (below the detection limit of 100 cells) to 252,000,000 viable cells in a single bee's hindgut (Fig. 1). Due to 66% of samples having zero cell counts, colonization was analyzed as presence or absence for each sample. Coded in this way, colonization ranged from 0 to 100% across replicates of host colony and bacterial strain combinations (Fig. 2). The best fitting model for the binomial colonization data included strain, colony, realized inoculum size, and the strain by colony interaction (Table 1). Realized

inoculum size had an unsurprising positive effect on colonization (Table 2). Indicative of a genotype-by-genotype interaction, the colonization of *S. alvi* was also determined by a significant interaction between strain and host genotypic unit (Table 2).

Discussion

Although predominant vertical transmission of mutualistic symbionts is predicted to result in high levels of specificity and genotype matching (Poisot et al. 2011), the interaction between host and microbial genotypes in determining colonization success within animal-host and beneficial-microbe systems has remained largely unexplored. The few studies investigating potential interactions in these systems do not explicitly test the influence of genotypic interactions in microbial colonization in an experimental manner (Gomez et al. 2016; Moeller et al. 2016). Our results provide evidence for a genotype-by-genotype interaction determining the colonization success of the beneficial bumblebee symbiont *S. alvi* in its *B. impatiens* host. This shows that specificity can be at a much finer scale than the previously reported genus level specificity (Kwong et al. 2014). The demonstration of a genotype-by-genotype interaction in colonization is also intriguing as previously the effects of host and microbial genotype have mostly been studied within the context of host–parasite systems (Carius et al. 2001; Barribeau et al. 2014). Such genotype-by-genotype specificity will have important implications for the ecological and evolutionary outcomes of interactions in host and beneficial microbe systems.

The vertical transmission of microbes across generations is widespread (Funkhouser and Bordenstein 2013), making understanding the evolutionary processes and patterns in these systems of critical importance. Social bees and their gut microbiota offer an amenable system in which to do this (Kwong and Moran 2016), including studies of specificity of interactions and fitness-relevant outcomes of differential colonization. It is hypothesized that vertical transmission and associated coevolution of hosts and microbes will lead to narrow specificity in some strains, with sociality potentially amplifying the potential for coevolution (Koch
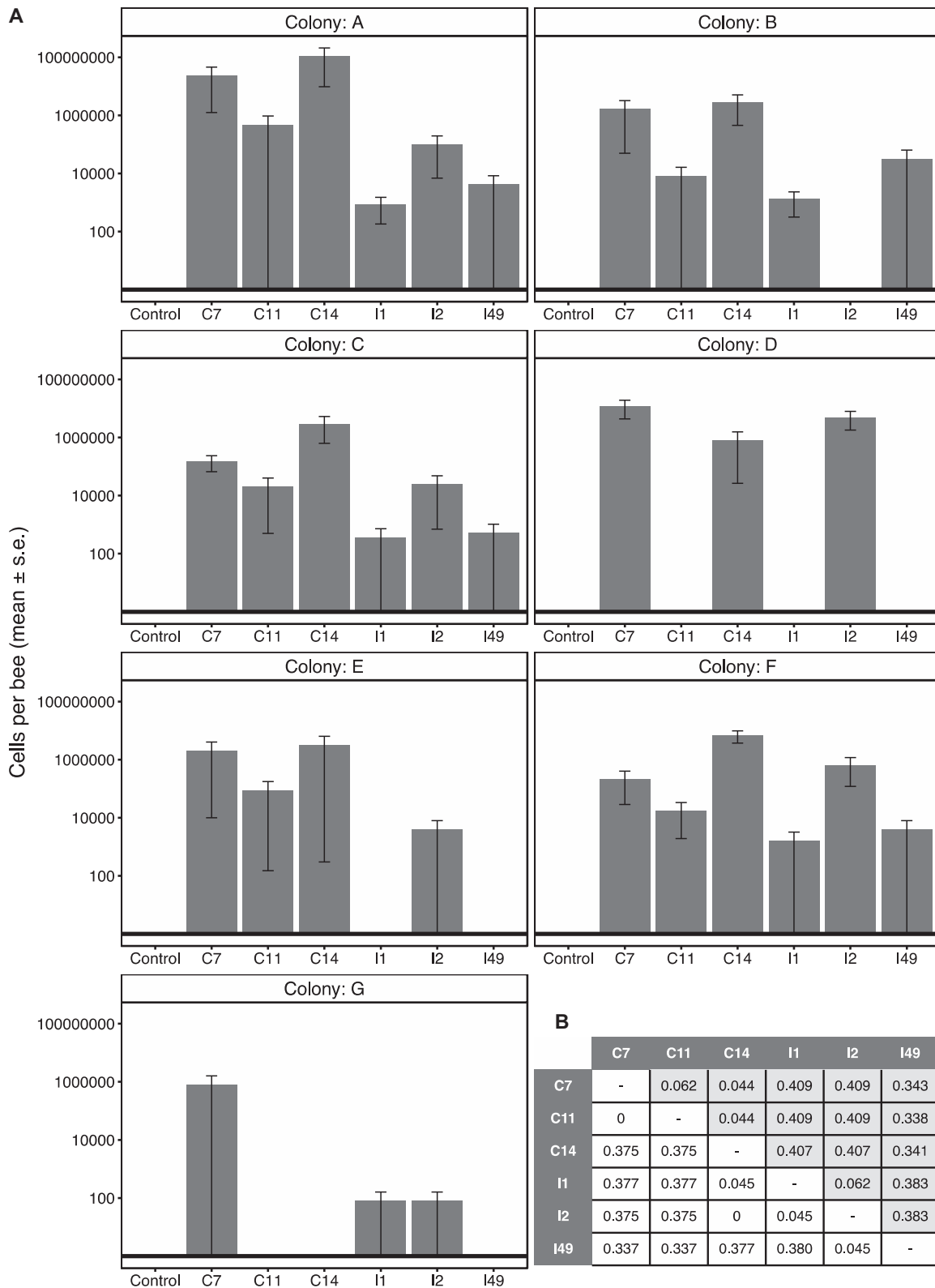
Figure 1. **(A)** Viable cells of *S. alvi* colonizing bee guts 4 days following inoculation of different *S. alvi* genotypes (*x*-axes) across multiple host colonies (genotypic units, panels A–G). Different *S. alvi* strains used to inoculate germ-free bees of these host genotypes are on the *x*-axis. Control individuals (first column in each panel) received the germ-free bee protocol, but were not given *S. alvi*. **(B)** Pairwise between strain distance matrix for *minD* (below diagonal, unshaded) and *phage repressor protein C* (above diagonal, shaded). Dissimilarity was calculated with *seqinr* package in R and represents the square root of the pairwise proportional distance.
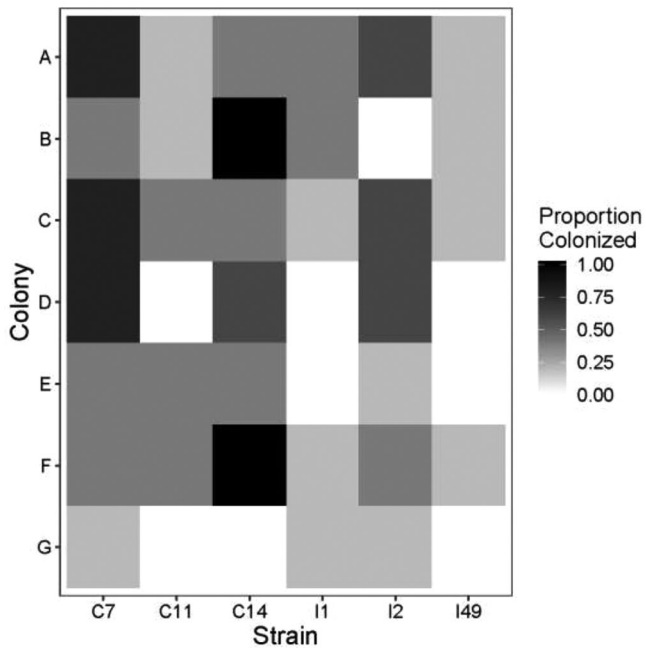
Figure 2. Proportion of inoculated bees colonized by *S. alvi* based on the host genotypic unit (colony) and *S. alvi* strain genotype. There were five samples per treatment type combination.

Table 2. Factors in the final binomial generalized linear model of *S. alvi* colonization.

Effect	d.f.	LR χ^2	*P*-value
Strain	5	12.50	**0.0285**
Colony	6	9.58	0.1435
Inoculum size	1	6.93	**0.0084**
Strain × colony	30	44.51	**0.0428**

Significant values (*P* < 0.05) are in bold.

et al. 2013). Although this study does not directly test coevolution between host and microbe, the finding of a genotype-by-genotype interaction may be indicative of its action (Poisot et al. 2011). However, it is important to acknowledge that other evolutionary processes can determine symbiont genome evolution, including genetic drift (McCutcheon and Moran 2012), and, much like in host–parasite systems (Antonovics et al. 2013), specificity may not only be the result of coevolution. A determinant of colonization that is not mutually exclusive of the explanations above and could contribute to colonization patterns observed is the presence of generalist and specialist strains of *S. alvi*. Previous work highlights the presence of some *S. alvi* strains able to colonize across host species (Kwong et al. 2014, 2017b) and clusters of both narrow species specialists and more generalists based on phylogenetic data (Powell et al. 2016). Our results, however, indicate that even if some strains are generalists across host species, a level of within-species specificity could be common in bumble bee *S. alvi* interactions. In fact, only one strain (C7) successfully colonized in at least one replicate bee across all host backgrounds.

Ideally, native strains to the bee colonies would have been used as one of the inoculation treatments, but this was not possible due to logistical constraints. Therefore, only a small number of native inoculations took place to confirm they do indeed colonize. Under the scenario of coevolution between host and microbe, in comparison to the foreign strains used, native strains would be predicted to be the best colonizers in general. However, even without these native combinations, the described genotype-by-genotype

interaction shows specificity, with colonization determined by an apparent matching of factors between host and microbe. These same factors could be those associated with the factors determining colonization success of native strains.

In addition to the mechanisms behind these interactions, there are implications for the presence of genotype-by-genotype interactions that extend beyond beneficial symbiont colonization phenotypes into the functional and evolutionary outcomes. Different patterns of *S. alvi* diversity have been found between honey bee and bumble bee individuals and colonies (Powell et al. 2016). The different ecologies of these bees, along with the genotype-by-genotype interactions demonstrated here, could contribute to these patterns. Bumble bee species typically possess a single strain of *S. alvi*, whereas honeybees more often possess multiple strains (Powell et al. 2016). This observation was previously attributed to colony founding events. Bumble bees have an annual social life cycle and colonies are founded by a single queen after hibernation (Goulson et al. 2008), whereas honey bees found colonies through swarming of a queen and many workers (Villa 2004). Although these founding events may contribute to the maintenance of strain diversity within a colony, genotype-by-genotype interactions may also be involved. Genetic variation within honey and bumble bee colonies will typically differ as a result of different levels of polyandry. Although it can vary by species, bumble bee queens are predominantly singly mated (Estoup et al. 1995), whereas honeybee queens can mate upwards of 10 times (Tarpy and Nielsen 2002; Mattila and Seeley 2007). Genotype matching between *S. alvi* strains and genotypes of their bee hosts could subsequently drive the observed strain variation within colonies. Polyandry of honeybees increases the genetic variation between workers in a colony (Mattila and Seeley 2007) and multiple strains may be maintained due to differential colonization across the multiple host genotypes present. In bumble bees, the low number of starting strains due to colony foundation by a single queen (Powell et al. 2016) and high level of relatedness between bumble bee workers within a colony (Hines 2008) would restrict *S. alvi* strain diversity.

In aphids, symbiont titers determined by host genotype have been shown to be correlated with host fitness (Chong & Moran

2016). For the bumble bee and *S. alvi* system, further studies are require to elucidate fitness consequences of differential colonization. If an association with host fitness exists, as the benefits of colonization with *S. alvi* elude to (Kwong and Moran 2016), a potential implication of the genotype-by-genotype interaction relates to compatibility mismatches, and hence negative consequences for host fitness (Gundel et al. 2012). As *S. alvi* is acquired through vertical transmission (Martinson et al. 2011; Koch et al. 2013), with the mother queen passing the bacteria to her daughter offspring, matings creating incompatible offspring genotypes could reduce *S. alvi* colonization success in the subsequent colony, depriving offspring of the bacteria's benefits. These offspring, with low or absent levels of *S. alvi* colonization, would experience negative functional outcomes (Raymann et al. 2017), such as increased pathogen susceptibility or reduced levels of nutrition. These effects could result directly from *S. alvi* abundance, but also indirectly due to overall microbiota dysbiosis because of the foundational role of *S. alvi* in the adult bee gut (Zheng et al. 2017). The influence of host outcrossing creating incompatible host genotypes has been demonstrated in plant-symbiont systems (Gundel et al. 2012; Sneck et al. 2019) and has been suggested as a mechanism underlying imperfect vertical transmission resulting in the loss of symbionts in certain host lineages (Sneck et al. 2019). There could also be consequences for beneficial microbe colonization and bumble bees due to the introgression of genes from commercial bumble bee colonies into wild populations (Kraus et al. 2011). The importance of genotype-by-genotype interactions and their implications in determining colonization patterns in social bees demonstrates the need for future studies aimed at disentangling underlying mechanisms.

Genotype-by-genotype interactions within a host-beneficial microbe colonizer system have potential consequences for host and microbe evolution. These interactions can determine the beneficial outcomes a host receives from vertically transmitted microbial species, and hence evolutionary fitness, as discussed above. Additionally, as outlined for host–parasite interactions, genotype-by-genotype interactions can provide a basis for local adaptation (Lively and Dybdahl 2000; Zhan et al. 2002). They have also been shown to not only be a consequence of coevolution, but also to feedback into evolutionary dynamics (Thompson and Burdon 1992; Lambrechts et al. 2006). Furthermore, these genotype-by-genotype interactions can result in differing host biotypes (Chu et al. 2011), with the potential to lead to future speciation (Brucker and Bordenstein 2012). These elements require further thought concerning the implications of genotype-by-genotype interactions for host-beneficial microbe evolution.

By demonstrating the importance of host and beneficial symbiont genotypes in determining colonization, the results presented here advance our understanding of host-beneficial microbial colonization and support predictions relating to genotype matching

in such systems (Poisot et al. 2011). The further implications of such interactions for ecological and evolutionary processes, and the crucial roles of beneficial microbes in host health, highlight the need for subsequent studies to understand the underlying factors determining such patterns.

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