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Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies

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Abstract In socially living animals, individuals interact through complex networks of contact that may influence the spread of disease. Whereas traditional epidemiological models typically assume no social structure, network theory suggests that an individual's location in the network determines its risk of infection. Empirical, especially experimental, studies of disease spread on networks are lacking, however, largely due to a shortage of amenable study systems. We used automated video-tracking to quantify networks of physical contact among individuals within colonies of the social bumble bee *Bombus impatiens*. We explored the effects of network structure on pathogen transmission in naturally and artificially infected hives. We show for the first time that contact network structure determines the spread of a contagious pathogen (*Crithidia bombi*) in social insect colonies. Differences in rates of infection among colonies resulted largely from differences in network density among hives. Within colonies, a bee's rate of contact with infected nestmates emerged as the only significant predictor of infection risk. The activity of bees, in terms of their movement rates and division of labour (e.g., brood care, nest care, foraging), did not influence risk of infection. Our results suggest that contact networks may have an important influence on the transmission of pathogens in social insects and, possibly, other social animals.

Keywords Epidemiology · Social insects · Disease ecology · Infection

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Introduction

Interaction networks are pervasive in biological systems. Food webs, neural nets, and metabolic pathways, in particular, have garnered much attention (May 2006; Montoya et al. 2006; Proulx et al. 2005); yet, the primary motivation for studying networks has often been to understand better the spread of disease (Keeling and Eames 2005; Newman 2003). Conventional epidemiological theory (Anderson and May 1991) assumes that hosts are randomly interacting entities, such that every susceptible individual is equally likely to encounter, and acquire infection from, an infected individual. Network theory (Newman et al. 2006), in contrast, assumes that contagious pathogens propagate via the highly structured interaction networks that exist among socially living organisms; thus, risk of infection varies among hosts according to their location in the social network. Although theoretical advances (Meyers 2007; Newman et al. 2006) have provided a firm basis for the study of contact network epidemiology, empirical, especially experimental, studies of disease spread on networks are lacking, largely due to a shortage of amenable study systems.

Although there is a clear connection between contact networks and the spread of sexually transmitted diseases in humans (Friedman et al. 1997; Klovdahl 1985; Liljeros et al. 2003; Neaigus et al. 2001), the relevance of network structure for the spread other diseases, particularly those of non-human animals, is uncertain. Recent work suggests that disease transmission might vary with social network structure in wildlife (brushtail possums, Corner et al. 2003; African buffalo, Cross et al. 2004). It is ironic, though, that almost no studies have investigated the contact network epidemiology of highly social species, such as the social insects. A notable exception is the study by Naug and Smith (2006), which showed that, in honey bees

(*Apis mellifera*), experimental manipulation of key epidemiological quantities (e.g., duration of infectiousness) leads to a significant change in the way pathogen-like tracers spread through a colony's social (trophallactic) network. Such studies demonstrate the value and amenability of social insects as an experimental model of disease dynamics.

In social insect colonies, individuals are thought to act on the basis of local information only, without a strict top-down chain of command (Wilson and Holldobler 1988). This mechanism of organization relies, to some degree, on information flow during encounters between individuals (Gordon et al. 1993; Nicolis et al. 2005; Pacala et al. 1996). Honeybee workers, for example, pick up pheromones on their bodies while attending their queen and then disperse these chemical signals throughout the colony via direct worker–worker contact (Naumann et al. 1991). In the context of infectious disease, pathogens might spread throughout a colony via these networks of physical contact (Schmid-Hempel 1998). Whereas such networks might separate nestmates socially, the natural division of labour (i.e., fidelity of individuals to specific tasks such as foraging or brood care) within social insect hives may also complicate transmission by separating individuals in space and time (Naug and Camazine 2002; Schmid-Hempel 1998). Thus, the dynamics of pathogen spread in social insects probably reflects a complex mix of behavioural, social, and spatial phenomena. Only direct, detailed observations of the behaviours and contacts of socially living individuals can fully clarify the role of contact networks in disease transmission. One promising approach is the use of automated video-tracking to monitor several social insects simultaneously inside a hive and extract their movement patterns and contact behaviour for social network analysis (Feldman and Balch 2004; Veeraraghavan and Chellappa 2005).

Bumble bees (*Bombus* spp.) are "primitively eusocial" insects (Wilson 1971) that live throughout temperate regions in relatively small annual colonies of 10–400 individuals (Alford 1975; Heinrich 1979). Bumble bee colonies exemplify many of the features of "simple" insect societies (Anderson and McShea 2001; Bourke 1999): queens and workers are morphologically and physiologically similar (although queens are larger), no physical castes exist within the worker force, and bees typically show no, or weak, age-related division of labour (Free 1955; O'Donnell et al. 2000). Although bumble bees are unusual among the eusocial insects in their absence of trophallaxis (direct food exchange between individuals), physical contacts are, nevertheless, common among nestmates during normal hive activities (e.g., Dornhaus and Chittka 2001; van Honk and Hogeweg 1981). For some pathogens of bumble bees, such as the gut protozoan *Crithidia bombi* Gorbunov (Trypanosomatidae: Kinetoplastida), direct physical interaction between infected and susceptible bees is sufficient for transmission (Otterstatter and Thomson, unpublished manuscript).

Infection of bumble bees by *C. bombi* has been well studied in Europe (Schmid-Hempel 2001); this pathogen can have serious effects on the fitness and survival of hosts (Brown et al. 2000, 2003). Although previous work has speculated on the role of contacts in spreading *C. bombi* (Mallon and Schmid-Hempel 2004; Shykoff and Schmid-Hempel 1991), no studies have investigated contact network epidemiology within hives. Here, we used automated video-tracking to determine if the within-colony spread of *C. bombi* occurs via networks of physical contacts among nestmates. Specifically, we asked if a bee's risk of infection varies with its rate of contact with infected nestmates in young *B. impatiens* hives that are naturally or artificially infected by *C. bombi*. We also investigated whether or not the time a bee spends engaged in various tasks (division of labour), or its general activity level, influences its risk of *C. bombi* infection.

Methods

Bumble bees and pathogens

For all experiments, we used *B. impatiens* colonies, each containing a queen and her first brood of larvae (prior to the emergence of any workers), provided by a commercial supplier in North America. Colonies were housed at ~23°C in clean plastic hives (29 cm length × 22 cm width × 13 cm height) fitted with transparent lids. Brood clumps were built on an elevated plastic "stage" (15 cm × 15 cm × 1.5 cm) surrounded by a mesh floor to prevent the build-up of faeces or debris. We initially supplemented hives with 30% sucrose solution and pollen every other day. After workers had emerged, each colony was connected to a separate screened cage (60 cm × 40 cm × 90 cm) containing a petri dish that supplied 30% sucrose solution via a cotton wick. We allowed workers to forage at will for sugar water, and we placed a small lump of pollen in each hive every other day. Each bee was tagged (workers, at emergence; queens, upon arrival from the supplier) on its thorax with a uniquely coloured paper disc (3 mm diameter), which allowed us to follow the activities of the colony (below) and know the age of workers.

We regularly screened the faeces of each bee in all experiments for *C. bombi* following previously established protocol (Otterstatter and Thomson 2006). Queen bees were screened upon arrival from the supplier and then approximately once a week, whereas workers were screened every 2–4 days. In each case, we temporarily removed a bee from its hive, placed it in a clean vial, and monitored it continuously until it defecated (usually within 10 min); we immediately returned the bee to its hive and collected the faeces

droplet with a graduated 5 µl microcapillary tube. The intensity of *C. bombi* (cells per microlitre) in a bee's faeces was determined with a Neubauer haemacytometer.

Within-colony transmission of *C. bombi*

To begin, we wished to determine how extensively pathogens spread in colonies founded by infected queens. We selected five colonies that contained an infected foundress (i.e., infection of the queen had occurred prior to the arrival of the hives from the supplier) and allowed *C. bombi* to spread naturally to first brood workers as they emerged. We monitored the transmission of infection by regularly screening the faeces of each worker. Given that few, if any, bumble bee workers survive beyond 6 weeks in the field (in temperate regions, reviewed by Rodd et al. 1980), we chose to terminate this experiment at 40 days (for each hive, starting from the emergence of the first worker), even though several first brood workers remained uninfected. At the end of the experiment, we starved workers for 3–4 h and weighed them to ±0.1 mg. In this initial study, we did not attempt to observe if social interactions were responsible for pathogen spread.

Next, we examined the spread of infection within seven new colonies in relation to social interactions and division of labour. Natural colonies may become diseased if they are founded by an infected queen or if workers bring in pathogens that they acquire during foraging (Durrer and Schmid-Hempel 1994; Imhoof and Schmid-Hempel 1999). We explored both routes of transmission. Infected queens founded five of these new colonies; as before, we monitored *C. bombi* spread from queens to first brood workers. In the remaining two colonies, both founded by uninfected queens, we artificially infected one forager per hive and monitored the spread of *C. bombi* to susceptible nestmates. We prepared two batches of inocula, one for each of the two colonies, using in each case the faeces of three bees from each of the five infected colonies from our preliminary study. We arbitrarily chose one forager from each of the two experimental hives, starved them for 2 h, and then fed them $\sim 1.6 \times 10^5$ *C. bombi* cells mixed in 10 µl of 30% sucrose solution. We held these bees in individual vials for 30 min post-inoculation, and then returned them to their hives. At the time of inoculation, the workers in these two hives were 12–14 days old. We ran this experiment for 40 days or until all first brood workers had become infected. As before, we weighed each bee at the conclusion of the experiment. For each of these seven hives, we monitored the social interactions among nestmates, using automated video-tracking.

Automated video-tracking of bumble bees

We tracked the within-hive movements of bees in our seven colonies using EthoVision Color-Pro software (version 3.1.16, Noldus Information Technology). The software is designed to recognize each individual according to its uniquely coloured tag and to record the *x*–*y* coordinates of all individuals simultaneously from a live video-feed (30 frames per second). From these data, the software can produce a variety of useful measurements of an individual's movements and social interactions. We ran the signal from our video-camera (Panasonic PV-GS180), centred approximately 50 cm above the hive, into a Pentium 4 PC fitted with a Picolo frame grabber. We used 24 h/day fluorescent lighting (5,500 K, Industrial True Lite), suspended 60 cm above the hive. For each colony, we tracked bees for approximately 12 h/day (half during the day, half during the night), starting on the day that first brood workers emerged.

The Ethovision software records a contact each time the coloured tags of two bees are closer than a user-specified threshold. In a preliminary study using tagged, freeze-killed bees, we had determined with calipers that a threshold value of 1.0 cm would ensure direct physical contact. The software's method of determining contacts does not account for variation in the size and shape of individuals; however, we found that varying the threshold value (0.60–1.50 cm) had little impact on network structure. In addition to contact frequency, the software also determines the duration of contact between pairs of bees and the distance that each bee moves inside the hive. One issue with automated tracking is that a bee's tag must be visible to the video-camera in order for the software to track its movements; if, for example, a bee crawls under the brood clump, or orientates its body sideways while incubating, the software temporarily assigns a "missing position" to that bee and no data are collected. Most missing position values arose while bees were in stationary incubating positions; thus, our data may underestimate the number of contacts that occurred during this time. However, during our daily observations of the colonies (described below), we noted that most contacts took place while bees moved about on the surfaces of the brood clump and hive, during which time the software was able to locate bees easily.

We also used automated video-tracking to determine the amount of time bees allocated to various tasks within the hive, i.e., division of labour. Regular observations (below) showed that bees engaged in three categories of labour: brood care, nest care, and foraging. Brood care was performed on the brood clump itself and primarily involved feeding and incubating larvae and constructing new wax cells. Nest care was performed away from the brood clump and involved guarding the nest (during which bees stood at the edge of the stage, facing outward, in a stereotypically "alert" posture that is easily recognizable) and carrying debris to the periphery of the hive. During foraging, bees made regular trips from the hive to the screened cage; they

imbibed sugar water from the feeder dishes and then regurgitated it into honeypots in the hive (wax containers built by bees for storing nectar within the nest). Although all bees had free access to the feeder dishes, only one or two workers per hive foraged. Based on these observations, we used the Ethovision software to delineate three zones in each hive: the brood clump, the "stage" on which the brood clump sat, and the entrance/exit from the hive to the foraging cage. The software recorded the amount of time a bee spent in each zone, which provided a crude estimate of how the bees allocated their time to different tasks. We set the software to treat the entrance/exit as a "hidden zone", i.e., from the time a bee entered the hidden zone (left the hive) until it re-emerged (returned from foraging), it was assumed to be out of the hive, and only the duration of time (not its position) was recorded.

Each day that we tracked a colony using Ethovision, we also observed the hive manually. During these periods, an observer sat next to the hive and successively followed each bee, recording its general location (e.g., brood clump, stage, outside of the hive) and behaviour for 10 min. By the end of the experiment, we had observed each bee for at least 1 h. We found that, on average, bees on the brood clump spent about 90% of their time incubating and feeding larvae, and bees on the "stage" spent about 80% of their time guarding or patrolling the hive and 10% cleaning the nest. Bees that were outside the hive spent all their time searching for, collecting, or returning with, nectar. Our manual observations also confirmed that bees made few contacts with one another during the times that they were hidden from the view of the video-camera.

Data analysis

Based on the faeces counts for each bee, we were able to estimate the rate at which *C. bombi* replicates in its host's intestine and then back-calculate the date of infection for each worker. At intervals, we counted the number of pathogen cells per microlitre of faeces for each of 16 infected, colony-dwelling bees originating from the five hives used in our preliminary study (Table 1). We regressed these counts [transformed as $\ln(x+1)$] against time (for each bee, the number of days since *C. bombi* had first been observed in its faeces) using a repeated-measures analysis, which accounts for co-variation among observations on the same individuals. For each bee, there was a marked deceleration in the growth rate of its faecal cell counts approximately 1 week after *C. bombi* first appeared in its faeces; thus, we restricted "time" in our analysis to a period of 7 days. We used the slope of this regression to determine the doubling time of *C. bombi* within bees [calculated as ln(2)/slope)]. We found that, following the first appearance of *C. bombi* in a bee's faeces, pathogen cell density doubled every 10.2 h (95% CI 9.0–11.7 h) under our laboratory conditions, which was similar to a previous estimate (~12 h, M. Ruiz-Gonzalez, pers. comm., calculated from the data of Wu 1994).

Table 1 Build-up of *C. bombi* infections within *B. impatiens* workers

Day	Average faecal counts[a]	Number	95% CI
1	3.798	2	1.858–5.739
2	4.938	7	3.133–6.743
3	6.931	6	4.8508–9.012
4	7.944	6	6.176–9.712
5	7.906	7	6.056–9.757
6	9.637	3	8.351–10.923
7	10.327	2	8.417–12.238

[a] Transformed as ln(cells/μl + 1)

In our seven experimental hives, we used the first observation (count) of *C. bombi* in a bee's faeces to back-calculate the density of pathogen cells expected in its faeces on each preceding day, assuming a constant doubling time of 10.2 h. Herein, we equate a bee's date of infection with the date when its estimated faeces count dropped to 1 cell per microlitre. Although we could not observe when *C. bombi* first began replicating in a bee's gut, previous work suggests that this occurs within 2 days prior to the first appearance of pathogen cells in the bee's faeces (Logan et al. 2005; Schmid-Hempel and Schmid-Hempel 1993). As a check on the accuracy of our method, we back-calculated the dates of infection for the two artificially infected workers used in this study; the estimated date was the same as the date of inoculation for the first bee, and 1 day after inoculation for the second bee.

We constructed a social network for each colony based on the overall pairwise contact rates that we attained with automated video-tracking. Automated video-tracking also allowed us to construct social networks based on pairwise durations of contact. In our networks, a node represents each bee, and edges (or "ties") directly connect pairs of nodes (bees) that had at least one contact. We used two measures of the "strength" of a tie: the observed rate (total number of contacts/total observation time) or duration (total time spent in contact/total observation time) of contact between the connected nodes (bees).

We used UCINET version 6.137 (Borgatti et al. 2002) to calculate two standard network statistics (Wasserman and Faust 1994) from our contact data: degree centrality and network density. The degree centrality of a node is the sum of the strength of all ties (representing either contact rate or duration) connected to that node. A bee's degree centrality indicates its potential for receiving or transmitting infection through the network; high centrality values signify highly social/active individuals who have many,

potentially transmissive, contacts with other bees. The network density is the sum of all tie strengths among nodes divided by the number of possible ties, which is equivalent to the average tie strength in our networks. Density reflects the amount of social activity among bees in a colony; a high density value indicates that nestmates made, on average, many contacts with one another. Although degree centrality and network density are non-independent (e.g., individuals with high centrality will tend to increase the density of their network), the latter is a group-level statistic whereas the former provides information about a single individual.

If bees within a hive encounter one another at random, and behave similarly with respect to other aspects of motion, such as velocity, nestmates should tend to have equal numbers (and rates) of contact. However, if numbers/rates of contact differ among nestmates, this would suggest that a colony's interaction network is non-random in some respect. Note that such non-randomness could arise from different patterns of movement and/or different rates of movement among nestmates. We used one-way chi-square tests (Proc FREQ, SAS Institute 2006) to explore whether or not certain pairs of nestmates interacted more or less frequently than expected (expected values assume that each pair of bees contributed an equal proportion to the total contacts observed in a hive). We excluded queens from this analysis, because our centrality statistics clearly showed that workers interacted more often with their queen than with other workers (i.e., we wished to know if contacts occurred randomly after excluding the effect of caste).

In our analyses of pathogen transmission, the main response variable was a bee's risk of infection, measured as the number of days that the bee remained free of *C. bombi*, starting from when it emerged as an adult (in colonies founded by infected queens) or starting from when we inoculated one of its nestmates (in colonies founded by uninfected queens). All bees were capable of becoming infected and, among nestmates, *C. bombi* infections increased at similar rates (as determined by regular faecal screening) and reached similar maximum intensities (see Fig. 1). In addition, our analyses also failed to find any effect of body size on infection risk (see Results). Thus, we assume that differences in risk of infection among nestmates reflect differences in exposure, rather than inherent differences in their ability to resist infection (e.g., immunocompetence). We point out, however, that nothing is known about the mechanisms of resistance in this host–pathogen system, and further experiments are needed to determine if individual immune function plays a role in patterns of *C. bombi* spread.

We used linear mixed models (Proc MIXED, SAS Institute 2006) to assess differences among hives in network characteristics and pathogen transmission. In all cases, we included "colony" as a random factor, to account for the non-independence of observations within hives. In addition, we pooled the data for each bee across all days of observation, because bees had similar daily rates of contact with their infected nestmate (non-significant effect of date, analysis of variance (ANOVA), $P > 0.14$ in all cases). We compared, among colonies, the degree centrality of queens versus workers, including colony and caste (queen or worker) as explanatory variables. In our analysis of the factors that influenced a bee's risk of infection, our dependent variable was the number of days a bee remained uninfected. Colony, bee mass, degree centrality, the distance per unit time that a bee moved inside the hive, the proportion of time a bee spent engaged in brood care, nest care, and foraging, and a bee's rate of contact with the colony's infected individual, were treated as explanatory variables. In all mixed model analyses, we sequentially removed non-significant factors from our model through backwards stepwise elimination. The assumptions of normality and equal variance were verified by analysis of residuals (Proc UNIVARIATE, SAS Institute 2006).

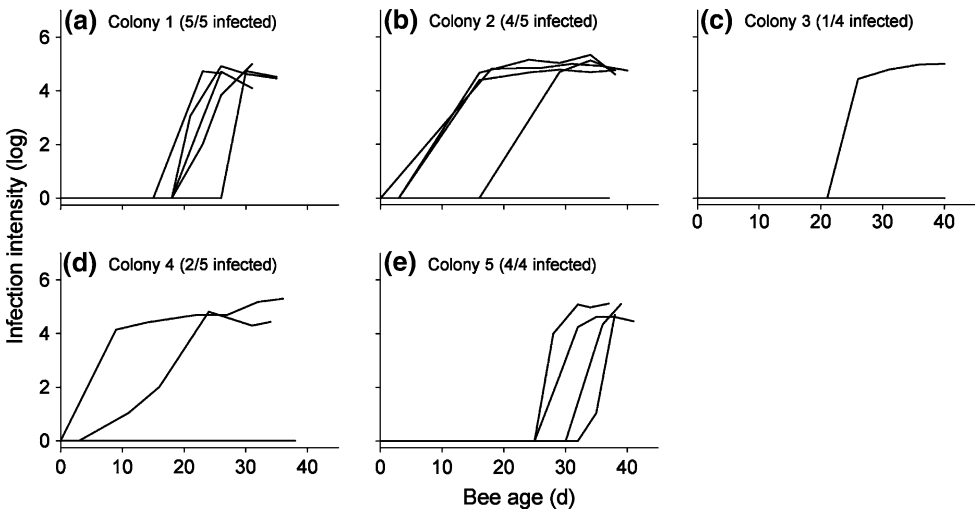

Fig. 1 Build-up of *C. bombi* infection among first-brood workers in five bumble bee (*B. impatiens*) colonies founded by infected queens. *Each panel* represents a single colony (with the number of infected and total workers indicated *in parentheses*) and *each trace* within a panel represents the intensity of infection (number of *C. bombi* cells per microlitre of faeces) of a single worker in relation to it age (days since emergence)

We similarly used mixed models to clarify whether a bee's rate of physical contact with an infected nestmate, or its spatial proximity to an infected nestmate, ultimately determined infection risk. As before, the dependent variable was the number of days a bee remained uninfected, and we included a bee's rate of contact with its colony's infected individual as an explanatory variable. However, in this analysis, we also included the time (as a proportion of the total observation time on an individual) that a bee spent within 3 cm, but no closer than 1 cm, of its colony's infected individual (i.e., in proximity to, but not physically touching, an infected individual). We confirmed that the variance inflation factor in this model (=1.35) was well below the acceptable limit of 10 (SAS Institute 2006); thus, our results were not biased by a correlation between the explanatory variables.

Results

Preliminary study

Our preliminary study of five colonies, each founded by an infected queen, showed that the spread of *C. bombi* differed greatly within and among hives (Fig. 1). Although some workers acquired infection from their queen almost immediately after emergence, others remained healthy for over a month (differences among colonies in age at first infection, $F_{4,14} = 7.9$, $P = 0.004$), and roughly one quarter (6/25) never became infected (differences among colonies in the proportion infected, $G = 10.9$, $P = 0.03$). The age at which workers acquired infection did not vary with body size ($F_{1,16} = 0.72$, $P = 0.41$), the average intensity of infection harboured by the colony's queen ($F_{1,16} = 0.73$, $P = 0.41$), or the number (=density) of bees in the hive ($F_{1,16} = 2.04$, $P = 0.17$); however, it should be noted that these explanatory factors varied little within or among colonies, and, hence, the power of these tests was very low (0.06, 0.06, 0.23, respectively). Following this preliminary study, we investigated whether or not a colony's social structure, in terms of social contacts and division of labour, could explain variation in the rate of pathogen spread.

Contact network structure within hives

Table 2 summarizes the contact network characteristics of our seven bumble bee colonies. Contact networks within hives (Fig. 2) were fully connected; every individual interacted with every other individual at least six times, but up to 187 times, per hour on average. The queen was the most "central" member of a colony's social network (highest degree centrality), regardless of whether or not we considered contact rate or duration (significant caste effect: rate, $F_{1,33} = 13.5$, $P < 0.001$; duration, $F_{1,33} = 12.5$, $P = 0.001$), whereas interactions among workers occurred infrequently by comparison. Certain workers in two colonies (QC10, QC12) had higher degree centralities than their queen, but not significantly so (non-significant caste × colony interaction: $F_{6,27} = 1.8$, $P = 0.13$). Chi-square tests (Fig. 2) clearly showed that, in all seven colonies, contacts did not occur

Table 2 Statistics describing the contact networks within seven *B. impatiens* colonies. For each statistic, except network density, we report the mean value for a colony's queen and the mean value for its workers; 95% CIs are shown in *parentheses*. For network density, we report the mean ± 1 SD of each colony

Colony	Caste	Number	Degree centrality	Contact rate[a]	Contact duration[b]	Network density
QC1	Queen	1	9.57	1.56 (0.23, 2.89)	0.40 (0.02, 0.78)	0.74 ± 0.83
	Workers	6	3.74 (1.55, 5.93)	0.61 (0.38, 0.84)	0.18 (0.11, 0.24)	
QC4	Queen	1	4.23	1.04 (0.47, 1.61)	0.34 (0.19, 0.50)	0.81 ± 0.29
	Workers	4	3.03 (2.09, 3.98)	0.75 (0.61, 0.89)	0.27 (0.23, 0.31)	
QC6	Queen	1	5.22	1.29 (0.51, 2.08)	0.58 (0.16, 1.00)	1.02 ± 0.39
	Workers	4	3.82 (2.39, 5.25)	0.95 (0.76, 1.14)	0.41 (0.30, 0.51)	
QC10	Queen	1	6.05	1.49 (−0.22, 3.20)	1.32 (0.28, 2.35)	1.31 ± 0.69
	Workers	4	5.14 (2.46, 7.82)	1.26 (0.93, 1.60)	1.29 (1.10, 1.49)	
QC12	Queen	1	1.78	0.36 (0.06, 0.65)	0.14 (0.04, 0.24)	0.38 ± 0.16
	Workers	5	1.96 (1.29, 2.62)	0.39 (0.33, 0.45)	0.15 (0.13, 0.16)	
UN1	Queen	1	7.95	1.56 (0.74, 2.38)	1.34 (0.63, 2.05)	1.10 ± 0.56
	Workers	5	5.07 (3.10, 7.04)	1.01 (0.80, 1.22)	1.06 (0.92, 1.21)	
UN2	Queen	1	8.01	1.31 (0.94, 1.68)	1.01, (0.73, 1.29)	0.88 ± 0.36
	Workers	6	4.90 (4.03, 5.76)	0.81 (0.70, 0.92)	0.50 (0.40, 0.59)	

[a] Rates shown as no. of contacts per minute

[b] Durations shown as percentages of total observation time

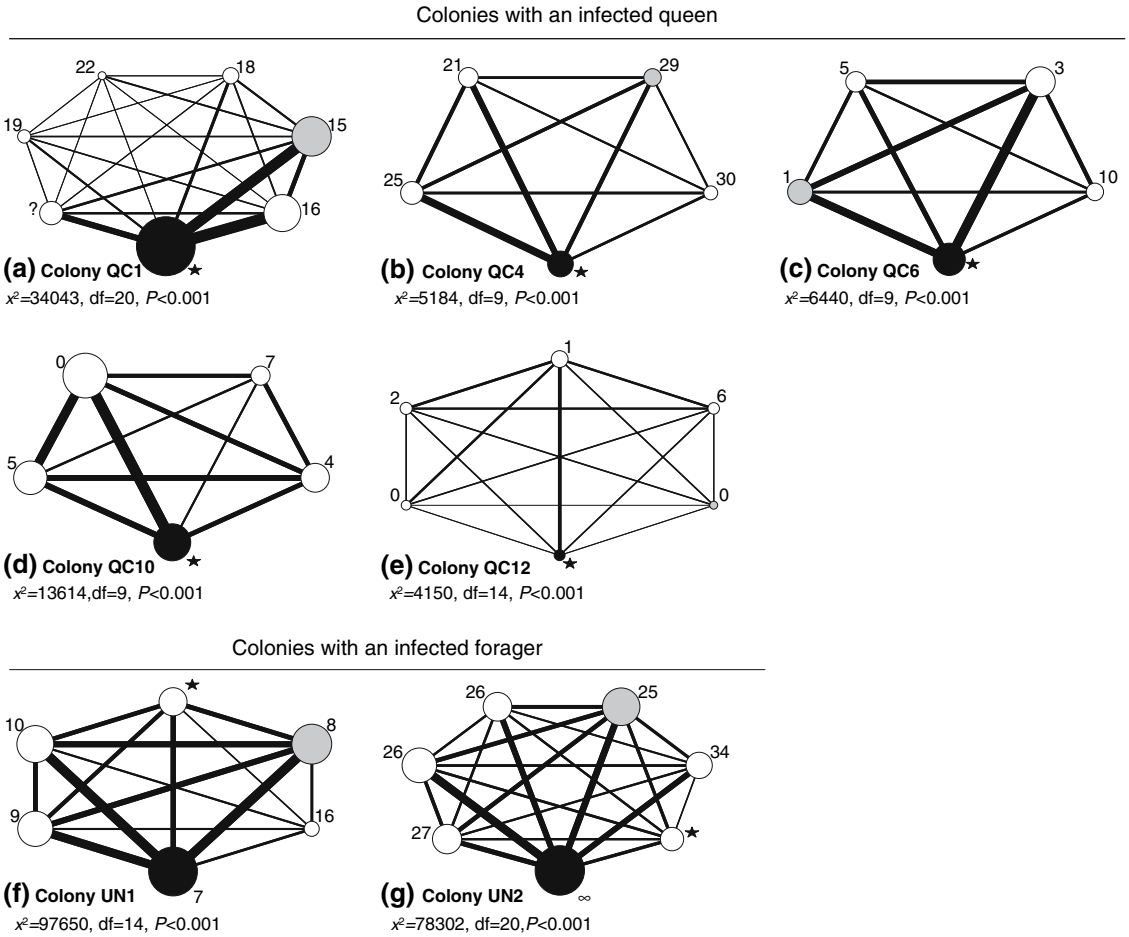

Fig. 2 Contact networks among nestmates in seven bumble bee colonies. Each *node* represents a bee (*black* queen; *white* nest worker; *grey* primary forager) and *lines* represent physical contacts between connected nodes. The size of a node is proportional to that bee's degree centrality (summed rate of contact with nestmates, see Methods), and the thickness of a line is proportional to the contact rate between connected nodes (networks based on contact duration revealed the same patterns). In each hive, the initially infected bee (i.e., the source of infection) is indicated with a *star*, either a naturally infected queen (panels **a**–**e**) or an artificially infected forager (panels **f**, **g**). Beneath each network are chi-square statistics that assess if frequencies of contact between pairwise combinations of nestmates occurred independently (workers only). The number of days (starting from the date of emergence for workers, and the date of inoculation for queens) that each bee remained uninfected is indicated beside the corresponding node; one bee (queen in colony UN2, indicated by the *infinity*) did not acquire infection, and another (worker in QC1, indicated by the *question mark*) was missed during regular screening

independently among workers, suggesting that contact networks within these hives were the result of non-random encounters.

We observed a similar division of labour in each of the seven colonies; data from a representative hive are shown in Fig. 3. Queens, plus one or two of the workers in each hive, spent virtually all (>90% on average) of their time tending broods (incubating, feeding, etc.). The remaining workers divided their time between brood care and either nest care (guarding, cleaning) or foraging. Colonies typically had a "primary" (46–64% of a colony's foraging time) and a "secondary" (14–38%) forager that, in combination, did ~80% of their hive's nectar collecting; because the colonies were young (first-brood only), two foragers supplied nectar using only ~10–20% of their total time budget. Colonies typically had at least one worker devoted to nest care; these bees spent, on average, ~15–25% of their time away from the brood clump engaged in guarding and cleaning the hive. Within hives, bees spent roughly the same proportion of their time each day engaged in brood care, nest care, and foraging (Fig. 3), i.e., we did not observe any obvious, age-related, changes in task allocation among the first brood.

Contact network structure and pathogen transmission

Infections spread more quickly in colonies that had dense social networks. Although the average duration that nestmates remained uninfected varied greatly among hives, most of this variation was explained by the colony's network density (average contacts per minute): bees became infected sooner in colonies that had frequent social contacts ($F_{1,5} = 8.8$, $P = 0.04$, $R^2 = 0.69$; Fig. 4). It should be noted

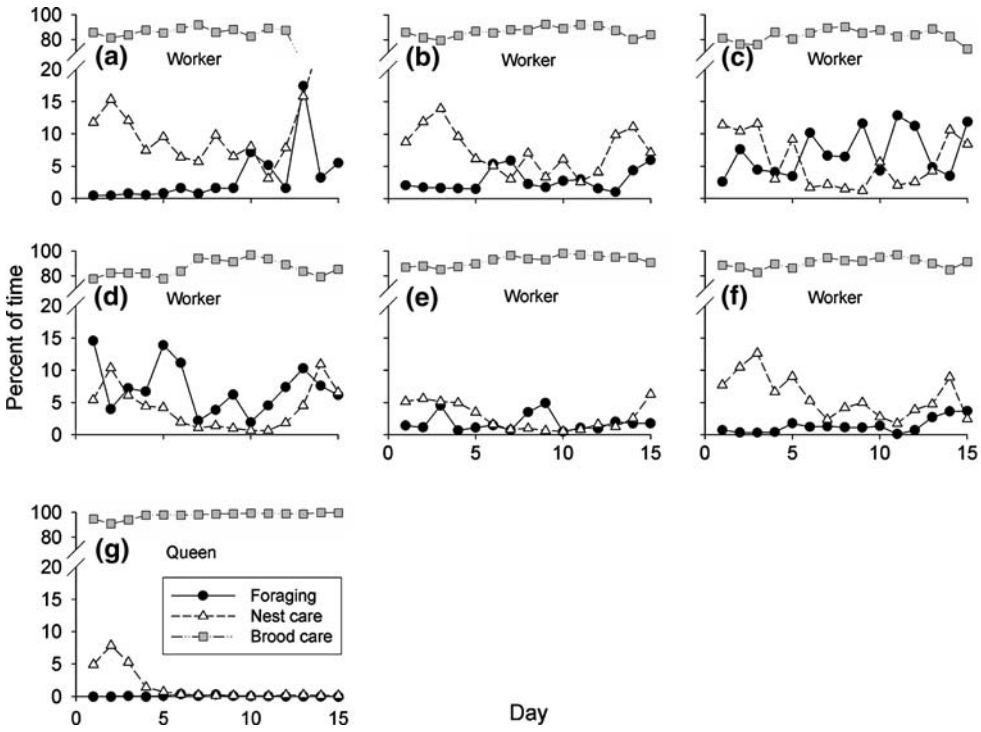

Fig. 3 Division of labour within a first-brood bumble bee colony. *Each panel* shows the amount of time each day, over the course of 15 days, that a single bee spent engaged in brood care (incubating and feeding larvae), nest care (cleaning and guarding the hive), and nectar foraging, as a percentage of total observation time per day (12 h). Only data from a single hive (UN2) with one queen and six workers are shown; all other hives showed similar divisions of labour (see Results)

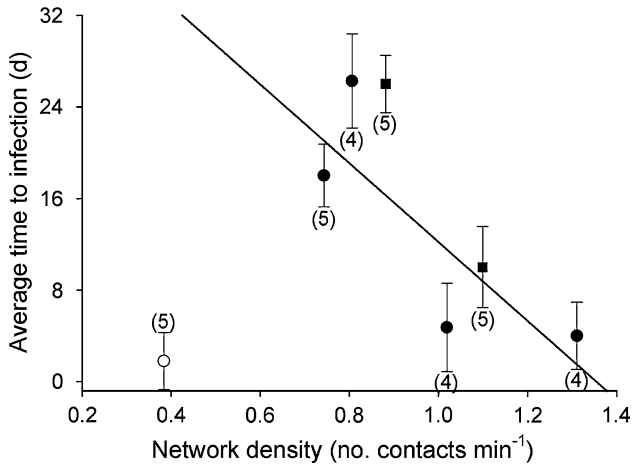

Fig. 4 Relation between a colony's network density (average number of contacts per minute among nestmates) and the rate of *C. bombi* infection (average ± SD time to infection across nestmates; the number of newly infected nestmates is shown *in parentheses*) in seven bumble bee hives. The *solid line* indicates the linear regression fit, $y = 51.86–37.91x$ (see Results for statistics). We artificially infected one worker in each of two colonies founded by uninfected queens (*squares*); infected queens founded the remaining five colonies (*circles*). One outlying colony (*open circle*) was excluded from the regression analysis because the queen probably contaminated the feeder while foraging (see Results)

that one of our colonies (QC12) did not conform to this pattern; in this hive, most workers acquired infection from the queen almost immediately after emergence, despite a low network density among nestmates. The queen in this hive was unusual in that she spent a large amount of time foraging for her colony (no other queens did this), and the pattern of infection suggests that she contaminated the hive's nectar feeder. For this reason, we excluded this colony from further analyses. In the remaining hives, the duration that nestmates remained uninfected did not vary with the number of bees in the hive, the infectiousness of the colony's infected individual, or if the infected individual was a queen or worker (ANOVAs, $P > 0.11$ in all cases). Thus, the amount of social contact between nestmates appears to be an important predictor of how quickly an infection builds up in a colony.

The only significant predictor of a bee's risk of infection was the amount of contact it had with the colony's infectious individual (Table 3). Figure 5 shows that bees became infected sooner (i.e., were at greater risk) when they made more contacts with their infected nestmate; this was true regardless of whether or not the infected individual was a queen or a worker (non-significant colony × contacts interaction, Table 3). In contrast, a bee's centrality in the network (summed contact rate with all nestmates) did not influence its risk of infection; thus, it was not simply the case that more "social" bees were at greater risk of infection. The distance that bees moved inside the nest, and the time they spent engaged in various tasks (brood care, nest care, foraging), had no influence on their risk of infection. There was insufficient time for secondary transmission to occur in most of our hives; nestmates typically became infected within 7 days of one another, which was equivalent to the latency period (duration between infection and infectiousness) of *C. bombi* under our laboratory conditions.

Table 3 Mixed model statistics assessing the influence of various factors on a bee's risk of infection by *C. bombi*. "Colony" was included as a random factor to account for the non-independence of observations with hives. The non-significant interaction term, "Colony × contacts with infected", is shown to illustrate that contacts had a similarly important influence on risk of infection across hives; all other interaction terms were non-significant and are not shown

Factor	*df*	*F*	*P*
Contacts with infected	1.21	17.91	0.001
Degree centrality	1.15	0.13	0.73
Bee mass	1.15	0.30	0.59
Bee activity (distance/time)	1.15	0.01	0.91
Percentage of time in brood care	1.15	0.47	0.50
Percentage of time in nest care	1.15	0.66	0.43
Percentage of time foraging	1.15	0.06	0.81
Colony × contacts with infected	5.15	0.49	0.78

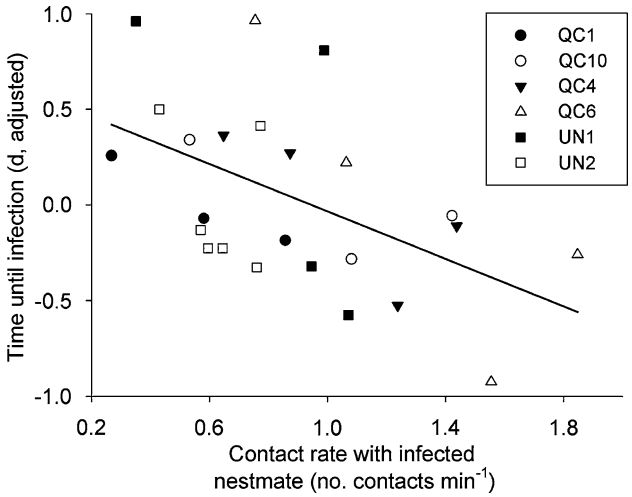

Fig. 5 The relation between an individual's risk of infection (time until infection was acquired) and its rate of contact with the infected nestmate for bees in six colonies (QC1, QC10, QC4, QC6, UN1, UN2). We present adjusted times to infection, i.e., the residuals of a one-way ANOVA with "colony" as an explanatory variable, to control for differences in the latency of infection among hives

It is possible that the observed correlation between contact rates and infection risk was the result of bees picking up pathogen cells from contaminated areas *near* infectious nestmates, and not from direct transmission per se; i.e., spatial proximity to an infectious individual might have been the true underlying risk factor for bees, and contact rates were simply acting as a proxy. We therefore examined the simultaneous contributions of direct contact and spatial proximity to risk of infection. Our statistical model revealed that a bee's risk of infection by *C. bombi* increased only with its rate of direct contact with an infected nestmate (contact rate, after controlling for duration in proximity, $F_{1,20} = 6.86$, $P = 0.017$), whereas duration spent in proximity to (within 3 cm, but not physically touching) an infected nestmate had no effect (duration, after controlling for contact rate, $F_{1,20} = 0.01$, $P = 0.92$).

Discussion

In socially living animals, individuals interact through complex networks of contact that may influence the spread of contagious disease. Here, we show for the first time that the spread of a contagious pathogen (*Crithidia bombi*) in colonies of a social insect (*Bombus impatiens*) is determined largely by the contact network characteristics of the host. Although colonies differed greatly in their rates of infection, most of this variation resulted from differences in network density among hives. Overall, a bee's rate of contact with an infected nestmate emerged as the only significant predictor of risk of infection within hives. The activity of a bee, in terms of its movement rate and the tasks it performed inside the hive (e.g., brood care, nest care, foraging), did not influence the risk of infection. Thus, the interaction networks within social insect colonies that are thought to function in information exchange (Pacala et al. 1996) and ergonomics (Gordon et al. 1993; Greene and Gordon 2003) might also serve to transmit contagious diseases (Schmid-Hempel 1998). Social insects and their pathogens are uniquely amenable to manipulative experiments and, thus, have the capacity to aid studies of infectious diseases when experimental approaches are otherwise impossible (Naug and Smith 2006).

The epidemiology of contact networks within bumble bee hives has wider implications for the study of pathogen spread in social groups. Whereas models of non-sexually transmitted infections often assume a fully mixed population of randomly interacting hosts (Anderson and May 1991), our data instead suggest that explicit knowledge of the contact network among hosts is needed to predict accurately how contagious diseases would spread. We also see that an individual's risk of infection cannot be deduced simply from its social behaviour, e.g., frequent social contact (normally equated with "high-risk" behaviour) does not necessarily increase the likelihood of infection. Rather, an individual's unique location in the social network determines its risk of infection. Studies of sexually transmitted infections of humans have reached similar conclusions through their focus on "who acquires infection from whom" (Anderson 1991; Friedman et al. 1997; Klovdahl 1985; Liljeros et al. 2003; Neaigus et al. 2001). Our results provide experimental evidence that contact network epidemiology is also relevant for non-human animals and non-sexually transmitted diseases.

Previous studies consider contact network epidemiology only when a disease is thought to spread through intimate

contact between hosts [e.g., networks of sexual contact and the spread of sexually transmitted diseases (STDs)]. However, gut pathogens also transmit readily through host-to-host contact; hence, their transmission might similarly reflect the contact structure of the host population. In humans, enteric infections by *Cryptosporidium parvum*, *Escherichia coli*, *Giardia lamblia*, and *Shigella dysenteriae*, for example, often spread directly from person to person (Birkhead and Vogt 1989; Eisenberg et al. 2005; El Bushra and Bin Saeed 1999; Keystone et al. 1978; Padhye and Doyle 1992; Ryan et al. 1997). Gut pathogens such as *C. bombi* probably spread when infective propagules cling to the body of an infected individual after it defecates. Autogrooming would distribute these cells over the host's body, and close physical contact between individuals would allow transmission to a new host. During feeding, a host might move infective cells to its mouth, allowing infection to occur per os. Our data support the notion that close physical contact between bees is an important mode of spread for an intestinal pathogen, and show that transmission reflects the network of contact among hosts.

We cannot exclude the possibility that, in our experiments, hosts also deposited and picked up infective cells from the surfaces of the brood clump and hive. Indeed, a few bees acquired infection sooner than would be expected on the basis of their contact rates. Based on our experimental design, these individuals may have ingested sugar water from a feeder contaminated by the faeces of an infected bee (foragers occasionally defecated on the petri dish feeders or crawled over the cotton wicks that supplied the nectar), or they may have spent time in contaminated areas of the nest. We are currently using pathogen-like tracers to determine how infectious cells would disperse inside a bumble bee hive, and whether multiple modes of transmission (e.g., direct and indirect) contribute to the spread of infection.

We examined, in our experiments, two routes by which a young bumble bee colony may acquire infection: via an infected foundress and via the import of pathogens by foraging workers. A benefit of this approach is that it mimics how bumble bee colonies naturally acquire disease; however, because we did not experimentally infect our founding queens (they were infected upon arrival from the commercial supplier), pathogen spread in these hives was not necessarily independent of queen/colony condition. Further, our study considered only young (first brood) bumble bee colonies with relatively simple social structures and divisions of labour. Thus, our data provide an indication of the primary spread of *C. bombi* during the early stages of colony growth but do not inform us about the dynamics of spread in large colonies, where secondary transmission may dominate. Nevertheless, the dynamics of infection during a colony's first brood stage might well determine the ultimate success of the hive. Young hives do not yet possess a strong worker force and, thus, rely on a small number of foragers for pollen and nectar. At this stage, the adverse effects of pathogens on worker foraging (Gegear et al. 2005, 2006; Otterstatter et al. 2005) may lead to food shortages within the hive and subsequent mortality among the infected bees (Brown et al. 2000). Unlike mature colonies, young hives do not easily absorb the loss of workers (Müller and Schmid-Hempel 1992). Hence, the cycle of reduced foraging, food shortage, and worker mortality may lead young colonies into a downward spiral from which they cannot recover.

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