

Behavioural interactions, kin and disease susceptibility in the bumblebee *Bombus terrestris*

EAMONN B. MALLON & PAUL SCHMID-HEMPEL

Ecology & Evolution, ETH Zürich, ETH-Zentrum NW, Zürich, Switzerland

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Abstract

Behavioural interactions are often analysed in terms of their costs and benefits to the actors [Hamilton, (1964) *J. Theor. Biol.* **7** 1–16; Gadagkar, (1993) *Trends Ecol. Evol.* **8** 232–234; Foster *et al.*, (2001) *Ann. Zool. Fenn.* **38** 229–238]. Using the bumblebee *Bombus terrestris*, we wish to distinguish between two possible determinants of interaction behaviour between conspecifics, namely kin-directed behaviour that reflects genetic distance between individuals, or, alternatively, interactions guided by a functional distance between individuals, specifically, with respect to disease susceptibility. We find no relationship between contact rate of individuals and the genetic distance of their respective colonies. Interestingly, we do find a significant negative correlation between contact rate and the distance between the two colonies in susceptibility to a spectrum of parasite strains. This cannot be explained by either of the *a priori* alternatives so we propose two further testable hypotheses to explain our results.

Introduction

Hamilton's theory of kin selection (Hamilton, 1964a,b) is an important and intensely researched area in evolutionary biology. It states that altruism can occur if the recipient of the help is related to the helper. The helper is then increasing his inclusive fitness. For kin-selected behaviour, the coefficient of relatedness (r) between two individuals is normally thought to be the relevant measure of the kin relationship. However, as Hamilton (Hamilton *et al.*, 1981) himself realized, if parasites drive the evolution of sexual reproduction and maintain genotypic diversity in their hosts, then the properties of the relatedness coefficient, which uses average genetic distance, fail to assess the special advantages or disadvantages represented by relatives. Whilst the coefficient of relatedness has been widely and successfully used, previous studies have, in fact, been unable to find a relationship between genetic distance and behaviour (Venkataraman & Gadagkar, 1992; Arathi *et al.*, 1997). We, therefore, considered Hamilton's objection to be a

serious possibility and asked whether behavioural interactions would reflect not the average genetic distance (as captured by r) but – as a working hypothesis – some other genetic relationship that more accurately reflects susceptibility to parasites. Such additional components of genetic relationship might be due to strong epistatic effects and the associated linkage disequilibria (Hamilton *et al.*, 1981). In this study, we measure the inter-individual contact rate as our token for behavioural interactions. This has been used previously as a measure for kin-directed behaviour (Mackenzie *et al.*, 1985; Carlin *et al.*, 1987; Fuller & Blaustein, 1990; Moritz *et al.*, 2000; Bull *et al.*, 2001).

Recent work on bumblebees has shown that there is indeed a high level of genetically determined specificity in the susceptibility to the trypanosomal gut parasite *Crithidia bombi* (Schmid-Hempel *et al.*, 1999; Brown *et al.*, 2000). Some colonies (representing different family lines with a different genetic background) are good at defending themselves against particular strains of *Crithidia* whereas other colonies do better against different strains. A parasite infecting one colony will pass easiest to another colony with a similar specificity profile as its host colony. It could, therefore, be imagined that workers from colonies with similar specificities should avoid each other as much as possible when foraging in order to prevent infection.

Correspondence: Paul Schmid-Hempel, Ecology & Evolution, ETH Zürich, ETH-Zentrum NW, CH-8092, Zürich, Switzerland.
Tel.: +41 1 633 6033; fax: +41 1 632 1271;
e-mail: psh@env.ethz.ch

This is not a trivial concern: *Crithidia* is a common parasite in the field (Imhoof & Schmid-Hempel, 1999) and has been shown in the laboratory to have a large mortality effect on noncompensating workers (Brown *et al.*, 2000) and exerts strong selection on the likelihood of colony-founding by infected queens (Brown *et al.*, 2003). In the wild, *C. bombi* is transmitted between colonies when a worker visits flowers that have previously been visited by infected workers (Durrer & Schmid-Hempel, 1994) and possibly by workers that erroneously enter foreign nests (Schmid-Hempel, 1998). Within colony, transmission occurs by contact with contaminated nest material or nectar. For successful infection, only a few cells of the parasite have to be imbibed (Schmid-Hempel & Schmid-Hempel, 1993).

We measured both average genetic and 'susceptibility' distance of the colonies. In line with normal procedures to estimate r , genetic distance is measured as Nei's genetic distance and is based on largely neutral microsatellite markers. Because the detailed genetic basis for resistance to parasites is currently unknown, susceptibility distance was measured by the colonies average response to a set of different parasite strains, i.e. how well the different parasite strains in the set were able to infect workers of a given colony, and how different the colonies were in this measure. Susceptibility distance thus reflects the similarity between two colonies with respect to their response towards the same parasites. We then compare these two measures to the individual workers contact rate, as observed with foreign workers in an experimental behavioural assay.

Methods

Mated queens were collected in the spring of 2002 in northwestern Switzerland and allowed to rear colonies in the laboratory with standard methods. All parasite strains used also originated from this location. No individual animals were reused for multiple assays. A total of 228 bees from eight colonies were used during the experiment.

Measurement of contact rate

Two bees each from two colonies were placed in a clean observation arena (115 mm × 90 mm). Bees from one of the two colonies were marked with Tippex® (randomly assigned). Interactions were filmed for 3 min on a digital video camera. For each bee it was noted how many times its antennae touched either its nest mate or one of the two foreign bees. To estimate the contact rate between workers of pairs of colonies, the number of foreign contacts was divided by the number of contacts with nest mates to control for background variation in general activity among colonies. These values were averaged over the four bees in a given arena to give a contact rate between individuals from this pair of colonies. The assay

was repeated for every colony pair combination. There were no obvious aggressive interactions between workers in any trial. We thus consider this contact rate as a rough measure for a propensity to congregate and interact with other individuals. Previous work had indeed found that, in a similar assay, workers on average had a higher contact rate with nest mates compared to non-nest mates (Shykoff, 1991).

Susceptibility distance

To prepare *C. bombi* strains for the experimental infections, faeces was collected from workers of naturally infected colonies, originating from the same locality as the test colonies (all test colonies were parasite naïve), and mixed with sugar water to create a standardized dose of 10 000 *Crithidia* cells per 20 µL of inoculum. Previous studies had shown that such inocula, prepared from different colonies, are genotypically different (P. Schmid-Hempel & C. Reber, unpublished results) and generate specific responses in novel hosts (Schmid-Hempel *et al.*, 1999). Each of the strains used here was similarly checked and differentiated from each other by microsatellite analysis (for methods see Schmid-Hempel *et al.*, 1999; data not shown) to verify that the used strains were indeed genotypically different from one another.

We then characterized, for each colony (total eight colonies), the susceptibility of its workers against the entire set of five *C. bombi* strains by separately measuring the infection intensities resulting from infections with each strain. Workers were 4 days old at the time of infection. Prior to infection, workers were starved for 2 h and then fed the strain-specific inoculum, i.e. were each infected by one strain only. The workers were then kept individually for a week before the intensity of the infection was assessed (Schmid-Hempel & Schmid-Hempel, 1993). Intensity was simply the number of *C. bombi* cells in the faeces (cells per 10 µL of faeces) of the infected workers. The response of a colony to a particular strain was calculated as the median infection intensity in the individual workers (on average 4.5 workers per colony/strain combination). The median infection intensities were used to calculate a distance in susceptibility between pairs of colonies. For this, we used the Euclidian distance between the two points characterizing a pair of colonies, i.e. the points given by the respective median infection intensities in the five-dimensional space defined by the five strains.

Genetic distance

Four workers from each of the eight colonies were tested against eight highly polymorphic microsatellite markers. The gel banding patterns found were analysed using the program GENALEX (Peakall & Smouse, 2001) to create for each colony pair a measure of genetic distance, specifically Nei's genetic distance value (Nei, 1972). Nei's

genetic distance is a measure of genetic distance between two samples as follows:

$$D_s = -\ln \left(\frac{\sum_1^n P[A(i)]P[B(i)]}{\sqrt{\sum_1^n (P[A(i)]^2 \times P[B(i)]^2)}} \right)$$

where $P[A(i)]$ and $P[B(i)]$, respectively, are the relative frequencies of allele i in populations A and B, and n is the total number of alleles. For the statistical analysis, Nei's distance was $\log(x + 1)$ -transformed to meet the normality assumptions of the coefficients in the partial Mantel's test (Thorpe, 1996).

Results

We confirmed the host line vs. parasite strain specificity found previously in the interaction between *C. bombi* strains and bumblebee colonies by the significant interaction term for infection intensity (two-way ANOVA: Interaction term $F[28, 140] = 1.815$, $P < 0.05$, Main colony effect $F[7, 28.86] = 2.921$, $P < 0.05$). We then tested the relationships between contact rate and susceptibility or genetic distance, respectively, using partial Mantel tests (Thorpe, 1996). This tested the relationship between two of the matrices, and keeping the third one constant, so as to remove any confounding effects.

There was no significant relationship between the two matrices describing genetic distances and contact rate (susceptibility distance is kept constant; $r = 0.089$, n.s.). Interestingly, we found a significant negative correlation between genetic distance and distance in susceptibility to *C. bombi* (contact rates is kept constant; $r = -0.354$, $P < 0.05$) (Fig. 1). When we compared susceptibility

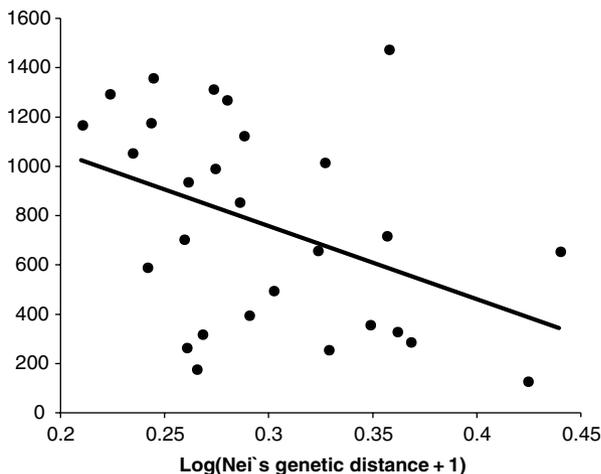


Fig. 1 Graph showing the negative correlation between distance in susceptibility between two colonies vs. the same two colonies' genetic distance measured by microsatellites. For measurement units, see *Methods*.

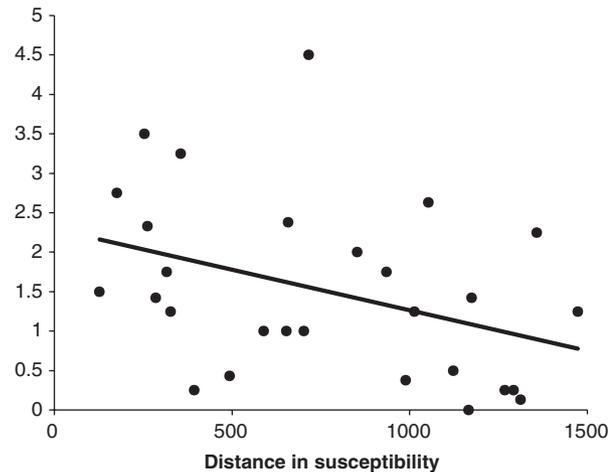


Fig. 2 Graph showing the negative relationship between the rate of contact between workers of two colonies in a behavioural assay vs. their respective distance in susceptibility to different strains of *Crithidia bombi*. For measurement units, see *Methods*.

distance to contact rate we found a significant negative correlation [genetic (Nei) distance is kept constant; $r = -0.393$, $P < 0.05$] (Fig. 2). In other words, contact behaviour was better explained by distance in susceptibility than by the average genetic distance.

Discussion

If closely related individuals preferred to spend their time together as expected from straightforward arguments of kin-selection theory, then we would have expected a negative relationship between contact rate and genetic distance. Yet, we found no significant relationship between contact rate and genetic distance. How about our susceptibility to disease hypothesis? For the hypothesis of avoidance of similarly susceptible individuals to be correct we would expect a positive correlation between susceptibility distance and contact rate. A bee should stay away from individuals who it is most likely to become infected by and interact mostly with those that do not represent a shared risk to contract the same parasite strains. But the actual correlation we observed was negative, indicating that the bees interacted most with workers from those colonies that would be infected by similar parasite strains (Fig. 2).

On the surface this seems very puzzling, why should bees preferentially interact with others who are susceptible to the same parasites as they are, that is bees that most easily pass a parasite on to them? It could be imagined that if the individuals were actually infected (the bees used in the contact rate assay were disease free) then we could see a reversal of the graph that would fit much better with our susceptibility hypothesis. However, this as yet untested possibility does not explain our

current result, since if it were the case we would have expected no relationship between susceptibility distance and contact rate for disease free bees, not the observed negative correlation.

Our two *a priori* hypotheses have been shown to be incompatible with our data. Here we present two further, albeit speculative, explanations, both of which would require more work to confirm their validity. The reciprocal of kin selection is Hamilton's spite. Here, an organism harms another without obtaining a direct benefit itself. It is important that the victim is negatively related (Grafen, 1985) to the attacker, that is, it is related less to the attacker than would be a randomly picked member of the population. It, therefore, relies on some kind of kin recognition system but with the reverse outcome. Very few examples have been found for this definition of spite (Foster *et al.*, 2001). A recent idea is that infected individuals could potentially infect and, therefore, seriously harm many nonkin at little direct fitness cost (Rozsa, 2000). Our results would agree with the idea that workers may try to pass disease on to their nonkin (Fig. 2).

Our second *post priori* hypothesis is if anything more speculative, but as with speculative ideas, very exciting. Kin recognition, as far as it is responsible for our observed contact behaviour, could be determined by genes that are linked to those affecting resistance against *C. bombi*. The analogy here is the major histocompatibility complex (MHC) in higher vertebrates. MHC genes on the one hand affect the interaction with parasites (Janeway *et al.*, 1997), and on the other appears to play an important role in 'kin recognition' mainly as a mechanism for outbreeding (McClintock *et al.*, 2002). The observed negative correlation between susceptibility distance and contact rate (Fig. 2) would then reflect the relevant 'kin' distance as determined by the linked susceptibility genes. This distance is not properly assessed by the microsatellite markers because of the nonadditive (epistatic) effects that may be important for susceptibility to disease. This would obviously take a lot more work to substantiate.

Our result that distance in susceptibility correlates inversely with genetic distance (Fig. 1) is counter-intuitive. We feel it can be explained as an artefact of the strong genotypic interactions between host and parasite in this system and corresponds to earlier, similar findings (see Fig. 3 of Schmid-Hempel & Schmid-Hempel, 1993). The effect results because extant lines of the host, by default, contain genetic variation that reflects their particular history of host-parasite interactions. Under strong host-parasite interactions (say, complete host-parasite 'matching' in the most extreme case) the exposure of genetically similar individuals to a random set of parasites (as done here) uncovers this variation and thus generates comparably large 'susceptibility' distances reflecting those interactions. By contrast, individuals that are genetically distant have very different histories and thus they each 'match' to a very different set of parasites. Hence, *on average*, they 'mismatch' to a similar degree

with the same set of parasites that thus generates an unexpected similarity in susceptibility.

In any event, our data show that contact behaviour and disease resistance are not independent and run counter to accepted, classical kin-directed behaviours. Clearly, more study is needed to disentangle the various elements of behavioural interactions.

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