Behav Ecol Sociobiol (2006) 60: 501–509
DOI 10.1007/s00265-006-0192-2

ORIGINAL ARTICLE

Mario X. Ruiz-González · Mark J. F. Brown

Males vs workers: testing the assumptions of the haploid susceptibility hypothesis in bumblebees

Received: 6 October 2005 / Revised: 20 February 2006 / Accepted: 1 March 2006 / Published online: 20 April 2006

Abstract The haploid state of males in eusocial Hymenoptera—the ants, bees, and wasps—has been proposed as a driving force in the evolution of social behavior under the assumption that haploidy results in higher susceptibility to pathogens. In this study, we present the first test of the assumptions of the "haploid male susceptibility hypothesis". We challenged males and workers of the bumblebee *Bombus terrestris* with its parasite *Crithidia bombi* but found no differences in either initial susceptibility or the intensity of infection between haploid males and diploid females. We reviewed observational studies on parasitism in haplodiploid insects and found that in 15 out of 26 cases, haploid males had lower parasite prevalence. However, the majority of available data related to nontransmissible parasites and thus any general statements about haploid susceptibility remain unclear. Using a simulation model, we studied how diverse genetic mechanisms could affect the values for resistance; results suggest that only a phenomenon that renders workers effectively haploid, e.g., imprinting, could explain our experimental results. A more likely explanation is that, in eusocial Hymenoptera with predominantly female populations, parasites may simply become more adapted to the more common female hosts and, thus, male haploid susceptibility may be hidden due to parasite adaptation. Our results do not support the idea that the haploid susceptibility hypothesis explains the origin or maintenance of social systems in the eusocial Hymenoptera.

Keywords *Bombus terrestris* · *Crithidia bombi* · Social Hymenoptera · Haploid susceptibility · Sex differences

Communicated by J. Traniello

M. X. Ruiz-González (✉) · M. J. F. Brown
Department of Zoology, School of Natural Sciences,
University of Dublin Trinity College,
Dublin 2, Ireland
e-mail: ruizgonm@tcd.ie

Introduction

Parasites are ubiquitous and exert strong selective pressures on their host populations. For example, parasite interactions may favor an increase in the level of host ploidy (Nuismer and Otto 2004), explain the evolution and maintenance of sexual reproduction (Hamilton et al. 1990), and select for recombination in their hosts (Otto and Michalakis 1998; Camacho et al. 2002; Fischer and Schmid-Hempel 2005). Furthermore, host genetic heterozygosity enhances resistance to pathogens (Penn et al. 2002), and high genetic variation in social groups reduces parasite load (Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999, 2001; Tarpy 2003). Thus, the host–parasite relationship implies a bidirectional selection pressure leading to coevolutionary Red Queen dynamics (Van Valen 1973; Lively et al. 1990; Camacho et al. 2002).

Sexual reproduction generates genetic variability, which is advantageous against parasites, but it has been pointed out that differences between sexes could lead to differences in resistance and susceptibility to parasites. In most vertebrate species, parasite infection rates, intensities of infection, or susceptibility to infection are higher in males than in females (Zuk and McKean 1996). Zuk and McKean (1996) suggested that this bias, or male disease susceptibility, could be due to sex-specific behavior or morphology, or to the relation between testosterone and the immune system. In contrast to vertebrates, arthropods show no such sex-biased pattern in susceptibility (Sheridan et al. 2000); perhaps because they lack testosterone and their immune system differs from the vertebrate one (Hoffmann et al. 1999). However, many arthropods are haplodiploid and it is possible that haploid males could be more susceptible than diploid females to diseases because we know that diploidy both masks deleterious mutations and improves the rate of adaptation to new environments (Mable and Otto 1998; Otto and Michalakis 1998).

O'Donnell and Beshers (2004) explored in a pioneering theoretical work how pathogen susceptibility could be linked to the evolution of sociality in haplodiploid insects. They proposed the haploid susceptibility hypothesis, which

stated that: (1) if haploid males are more susceptible to infections than their diploid worker sisters, then selection should have shaped the social behavior of these predominantly female societies in such a way to minimize interactions with males and to decrease the risk of colony infection, and (2) that male susceptibility is rooted in their haploid condition. Inasmuch as disease resistance is usually a multiple loci trait with epistasis being an important component of its expression (Kover and Caicedo 2001), and if resistance is controlled by many additive loci with many alleles, potential causes for haploid susceptibility include heterozygote and diploid females advantage and the buffering of recessive alleles that are covered up in heterozygosis.

Bumblebees and their trypanosome gut parasite *Crithidia bombi* (Gorbunov 1987) provide an excellent system to test the assumptions of the haploid susceptibility hypothesis. Bumblebees are haplodiploid species and have been widely used as model organisms to study the ecology and evolution of host–parasite interactions (reviewed by Schmid-Hempel 2001; Brown et al. 2003a; Schmid-Hempel and Reber Funk 2004) and the immune system of insects (Moret and Schmid-Hempel 2000, 2001; Doums et al. 2002; Brown et al. 2003b).

Most bumblebee species have an annual colony cycle. Queens emerge from hibernation, found a colony, and then produce one to several diploid worker broods before producing sexual (haploid males and diploid queens) adults. The sexual forms mate and the inseminated queens enter diapause before emerging the following spring to start the annual cycle again (Schmid-Hempel 2001).

C. bombi is a simple noninvasive flagellate trypanosome parasite that inhabits the gut of bumblebees (*Bombus* sp.), where it multiplies after attaching to the gut wall. After infection, the new cells can infect other hosts through the feces (Schmid-Hempel 2001) whether in the nest or through the shared use of flowers by foragers (Durrer and Schmid-Hempel 1994). At the individual level, *C. bombi* delays worker ovarian development and oviposition (Shykoff and Schmid-Hempel 1991c), allopatric infection causes worker mortality (Imhoof and Schmid-Hempel 1998a), sympatric infections increase by 50% the mortality rate of food-stressed workers (Brown et al. 2000), and the parasite inhibits early colony-founding by posthibernating queens (Brown et al. 2003a). Colonies produced by experimentally infected queens have fewer workers, and produce fewer males and gynes, with an overall fitness loss of 40% (Brown et al. 2003a).

There are strong genotype–genotype interactions between *C. bombi* and its *Bombus* hosts. Parasite transmission correlates with relatedness among hosts (Shykoff and Schmid-Hempel 1991b,d), genetically diverse colonies suffer lower prevalence and intensity of infections (Baer and Schmid-Hempel 1999, 2001), patrilines differ in their susceptibility to the parasite (Baer and Schmid-Hempel 2003) and colonies differ in the strains of the parasite by which they can become infected (Schmid-Hempel et al. 1999; Mallon and Schmid-Hempel 2004; Schmid-Hempel and Reber Funk 2004). Furthermore, recent work shows that the parasite elicits an immune response in its host (Brown et al. 2003b) and that resistance to *C. bombi* depends on at least several loci and shows epistatic interactions (Wilfert et al. 2004). These genotype–genotype interactions are presumably driven by the heavy fitness impact of the parasite on bumblebee colonies (see above).

In this study, we test the assumption of the haploid susceptibility hypothesis, that haploid males are more susceptible to infection than their diploid sisters, using males and workers from different colonies of the haplodiploid species *Bombus terrestris* L. and its gut trypanosomatid *C. bombi*.

Materials and methods

We used eight workers and eight males from each of four colonies of *B. terrestris* sourced from Koppert (Netherlands) and supplied by Hortico Industries (Ireland). To maintain productivity, Koppert periodically introduce new bumblebee queens to their rearing culture (A van Doorn, personal communication), so we have no reason to believe that the study colonies were inbred. Nevertheless, to check for heterozygosity, the animals used were genotyped using the B11 microsatellite primers and following the protocol of Brown et al. (2003c). To control for any effects of host age (Doums et al. 2002), animals were removed from the colonies on the day they eclosed and then transferred to plastic boxes (10.5×13×6 cm) with fresh pollen and sugar water (50% *v*/*v*) (Apiinvert) ad libitum. Bees were inoculated when they were 8-days old. Before the experiment, the feces of the bees were checked to confirm their parasite-free status.

To determine an appropriate experimental inoculation dose, we used previous studies (Brown et al. 2000, 2003b; Imhoof and Schmid-Hempel 1998a,b; Logan et al. 2005; Schmid-Hempel et al. 1999; Ruiz-González, unpublished data) to generate a curve of the relationship between dose and probability of infection (Fig. 1). These data were only available for workers. The dose chosen reflects a trade-off between methodological constraints (the number of individuals that could be handled per day) and the biology of infection. Too low a dose would require an unfeasibly large host sample size to generate sufficient data for analysis (see below) while too high a dose might swamp the effect of interest. Based on these criteria, we chose a dose of 10,000 cells per inoculum.

To prepare the inocula, feces were collected from *B. terrestris* queens and workers, naturally infected with *C. bombi*, caught in the spring of 2004 in Dublin, Ireland. The feces were mixed and the number of parasite cells was counted using an improved Neubauer slide. The feces were diluted with sugar water to produce an inocula concentration of 1,000 cells/μl. Before inoculation, the animals were starved for 3 h to facilitate ingestion. Each bee was then transferred to a vial where it was presented with a 10-μl drop of the inocula and observed until the drop was drunk. Thus, each animal ingested a total of 10,000 parasite cells.

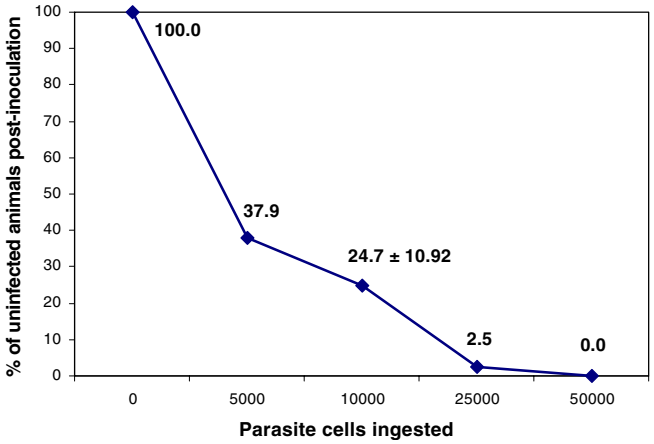

Fig. 1 Percentage of uninfected animals when challenged with increasing number of parasite cells. All data points were calculated from previous works (see text). The 10,000-parasite cell inoculum was used in four previous studies and we have given their mean±SD

Postinoculation, bees were individually transferred to sterilized plastic boxes with ad libitum pollen and sugar water. All the bees were kept in a dark room at 30°C and 59±6% relative humidity for the duration of the experiment.

For the next 15 days, feces were collected from each of the 64 bees. Fifteen days represents the majority of a worker's life span under natural conditions (Schmid-Hempel and Heeb 1991), male longevity under field conditions is unknown. Furthermore, *C. bombi* impacts bumblebee fitness from at least day 7 postinfection (Brown et al. 2000) and, thus, our experimental design encompasses the period when susceptibility would result in a fitness cost. The number of parasite cells per microliter was calculated using an improved Neubauer slide. This methodology—fecal counts rather than dissections—enabled us to track directly the development of the infection in individual bees.

To determine whether there is a relationship between the volume of feces defecated and the concentration of *Crithidia* cells, we used a separate sample of 56 infected *B. terrestris* workers. For each bee, we measured the volume of feces defecated during sampling on days 1–10 of the infection, and then conducted a Pearson correlation analysis between this and the concentration of parasite cells in the feces. We also examined whether there was any change across the duration of the infection in the amount of feces defecated at the sample times.

We used Fisher's exact test to check for differences in susceptibility to *C. bombi* between haploid males and diploid workers. We used repeated measures ANOVA to test for effects of caste, colony, day, and their interactions on levels of infection. Due to the death of some animals during the experiment, and to maximize the sample size (by including the data from the dead animals) of the infected animals per day, we analyzed only days 2 to 11 post inoculation (on day 1, no inoculated bee defecated parasite cells). Death of subject animals cannot be assigned to infection because there were no uninfected animals in the experimental design. Data were transformed using $\log(y+1)$. Because the assumption of homogeneity of variances was not met for all data days (only for days 6 to 11 was the assumption met), we also analyzed the data for each day using the nonparametric Mann–Whitney *U* test. *P*-values were corrected using the step-up sequential Bonferroni correction (Hochberg 1988). Finally, we used a two-way ANOVA with caste as a fixed factor and colony as a random factor to examine differences between haploid males and diploid females in their total amount of parasite cells produced across the whole experiment. Data were transformed using $y'=\log y$. Statistical analyses were done with SPSS 12.0.1 for Windows.

Literature review

We searched the literature (using ISI Web of Science and Schmid-Hempel 1998 as starting points) for studies which had examined parasite prevalence or intensity in haplodiploid insects for both males and females, to determine whether there was any general pattern of haploid susceptibility.

Simulation model

O'Donnell and Beshers (2004) describe the genetic mechanism behind haploid susceptibility as being linked to codominance, additive loci, and multiple alleles. To describe exactly the expectations for resistance of haploid and diploid individuals under a number of different assumptions, we constructed a multiloci simulation model for resistance. We wrote a program in C++ (Microsoft Visual C++ Standard Edition), which randomly generated three sets of 999 chromosomes with five loci each. The program randomly assigned to each locus one of 10 possible alleles with fixed values from 0.1 to 1.0 as potential values. Each of the 999 diploid animals were generated by combining two of the five loci chromosome sets, with the third set representing the 999 haploid males. In this study, we have defined resistance at the individual level as the mean of the sum of the combination of the alleles values present at the five loci, whether the animal is diploid or haploid. We calculated the mean resistance for diploid workers under three different assumptions: Hypothesis 1 assumed Mendelian expression, and a dominant/recessive allele structure, and so for each locus with two alleles only the allele with higher value was "activated" or chosen by the program; hypothesis 2 assumed random expression of alleles and for each locus with two alleles the program chose one randomly (this hypothesis acted as a null model control for H1 and H3); hypothesis 3 assumed imprinting (inactivation or silencing of one of the parental alleles, genes, or chromosomes) and the program calculated resistance based only on the first allele for each locus. We did not model either a mechanism assuming additive effects, e.g., the average resistance after summing the two paired values at each locus because its output will obviously be significantly bigger, or overdominance,

which would produce even higher mean resistance. We calculated the mean resistance for haploid males under three different assumptions: Males is the mean resistance for all the males; Males≥0.3 (soft purifying selection hypothesis) assumed that due to the male haploid condition, selective forces are acting on deleterious alleles, thus purifying those male genotypes with low fitness values—low fitness can happen when deleterious mutations in general are uncovered in haploids but also if resistance alleles are expressed as a general rather than specific response—resulting in haploid males with a mean resistance lower than 0.3 being unable to finish their development and, thus, removing them from the calculation of mean adult resistance (final N=973); in Males≥0.5 (strong purifying selection hypothesis) we removed those males with values lower than 0.5 (final N=662). We used the Kruskall–Wallis test to examine differences in simulated resistance among the different groups, and pairwise comparisons using the Mann–Witney U test corrected for multiple comparisons with the sequential Bonferroni procedure.

Results

Colony genetic variability

Microsatellite genotyping showed that all males were haploid. We found a total of six alleles at the B11 locus, indicating genetic variability across colonies. Heterozygous workers and males with different alleles were present in all colonies.

Susceptibility to *C. bombi* in haploid males and diploid workers

Bumblebee haploid males did not differ in their susceptibility to infection by *C. bombi* when compared with diploid workers (28 of 31 males and 31 of 32 workers became infected, Fisher's exact test, p=0.607). Parasite cells began to appear in the feces of some workers 1 day before (on day 2 postinoculation) the earliest infections in males and after day 8 postinoculation no further animals developed the infection.

Levels of infection in haploid males and diploid workers

Haploid males and diploid workers did not differ in their levels of infection (Fig. 2; $F_{1,47}$=3.45, p=0.07). There was a significant effect of day in the rmANOVA reflecting an increase across time in the average level of infection (Fig. 2; $F_{6,293}$=150.99, p<0.001) and in the interaction of day and colony ($F_{19,293}$=1.65, p=0.045) pointing out differences among the colonies in the levels of infection across time. In two out of four colonies, parasites appeared in day 3 rather than day 2 postinoculation and infection levels remained lower in these colonies for the first 7 days. There was no effect of colony or any other significant interaction effects on infection (Table 1).

The results of the nonparametric Mann–Witney U test showed a significantly lower level of infection in males for day 10 postinoculation (U=263, p=0.04; all other p>0.05), but after step-up sequential Bonferroni correction, there were no significant differences between haploid males and diploid workers in levels of infection.

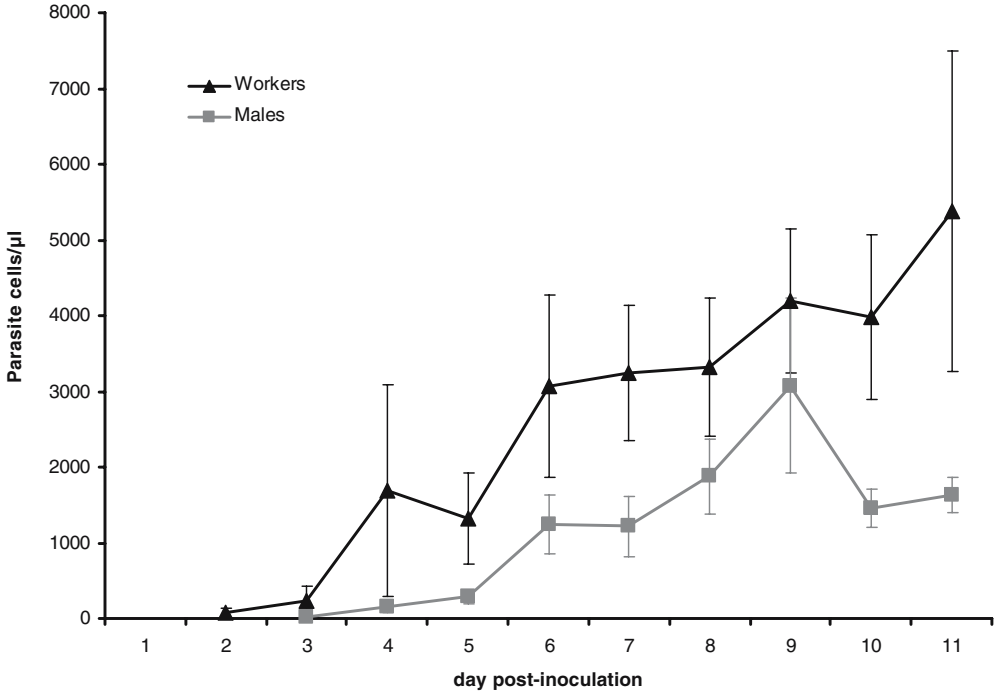

Fig. 2 Parasite intensities measured in haploid males and diploid workers across the 11 days of the experiment. Data are the mean±SE of parasite cells per microliter

Table 1 Results from the repeated-measures ANOVA of parasite infection intensities across the experiment

Factor	MS	*df*	*F*-value	*P*-value
Caste	13.47	1	3.45	0.070
Colony	6.33	3	1.62	0.197
Colony × caste	2.83	3	0.73	0.542
Error	3.90	47		
Day	135.31	6	150.99	<0.001
Day × caste	1.05	6	1.18	0.319
Day × colony	1.48	19	1.65	0.045
Day × colony × caste	1.36	19	1.52	0.080
Error	0.90	293		

Because the data did not meet the assumption of sphericity, statistics for the repeated measure (day) and its interactions are the Greenhouse–Geisser values (degrees of freedom have been rounded to the nearest whole number)

While haploid males shed fewer parasite cells across the infection than did diploid workers (males=10,764±1927.04, workers=26,516±7847.48), there were no significant effects of caste (MS=0.93, $F_{1,3.072}$=7.87, p=0.066), colony (MS=0.31, $F_{3,3}$=2.66, p=0.222), or their interaction (MS=0.12, $F_{1,48}$=0.52, p=0.668) on overall infection.

Results of these analyses were qualitatively the same if data for the whole 15 days were used.

There was no significant correlation between the day postinfection and the amount of feces produced at the daily sample (N=10, r=0.13, p=0.72). Similarly, in only one of the 10 days postinfection was there a significant correlation between feces volume and the concentration of parasite cells (day 6 postinfection, N=37, r=−0.53, p<0.01). Consequently, we believe that our sampling method produces a true reflection of the epidemiology of the parasite within its host.

Table 2 Prevalence of parasites in haplodiploid social hosts (honey bees, bumblebees, and Dolichoderine ants)

Haplodiploid host	Parasite	Prevalence (%)		Reference
		Males (*N*)	Workers (*N*)	
Apis mellifera	*Varroa destructor* (Acari)	90.07 (141)	49.12 (228)	4
Bombus bifarius, *B. californicus*, *B. flavifrons* and *B. occidentalis*	*Physocephala sp.* (Diptera: Conopidae)	**3.5** (431)	12.3 (1435)	6
B. terrestris/*B. lucorum*	(Diptera: Conopidae)	**16.7** (24)	22.9 (61)	2
	Crithidia bombi (Protozoa: Trypanosomatidae)	**54.2** (24)	80.3 (61)	2
	Nosema bombi (Microsporidia: Nosematidae)	50.0 (24)	14.8 (61)	2
B. pascuorum	(Diptera: Conopidae)	**8.6** (35)	30.3 (132)	2
	Crithidia bombi (Protozoa: Trypanosomatidae)	**0.0** (35)	8.3 (132)	2
	Nosema bombi (Microsporidia: Nosematidae)	8.6 (35)	2.3 (132)	2
	Locustacarus buchneri (Acarina: Podapollpodidae)	**5.7** (35)	6.1 (132)	2
B. lapidarius	(Diptera: Conopidae)	**7.7** (26)	27.2 (81)	2
	Crithidia bombi (Protozoa: Trypanosomatidae)	**15.4** (26)	53.1 (81)	2
	Nosema bombi (Microsporidia: Nosematidae)	15.4 (26)	0.0 (81)	2
B. pratorum	(Diptera: Conopidae)	**0.0** (6)	8.3 (12)	2
	Crithidia bombi (Protozoa: Trypanosomatidae)	**16.7** (6)	58.3 (12)	2
	Locustacarus buchneri (Acarina: Podapollpodidae)	**0.0** (6)	50.0 (12)	2
B. hortorum	(Diptera: Conopidae)	20.0 (10)	18.2 (11)	2
	Crithidia bombi (Protozoa: Trypanosomatidae)	50.0 (10)	36.4 (11)	2
	Nosema bombi (Microsporidia: Nosematidae)	**10.0** (10)	54.5 (11)	2
B. hypnorum	*Crithidia bombi* (Protozoa: Trypanosomatidae)	**50** (8)	100 (8)	2
	Nosema bombi (Protozoa: Microsporidia)	12.5 (8)	12.5 (8)	2
17 *Bombus* sp. from Canada	*Locustacarus buchneri* (Acarina: Podapollpodidae)	6.5 (598)	4.5 (3245)	7[a]
Bombus bifarius, *B. californicus*, *B. flavifrons* and *B. occidentalis*	*Apocephalus borealis* (Diptera: Phoridae)	~24 (144)	~13 (937)	3
	Physocephala texana (Diptera: Conopidae)	**1.8** (111)	~9.0 (896)	3
12 *Bombus* sp. and five *Psithyrus* sp.	*Sicus sp.* and *Physocephala* sp. (Diptera: Conopidae)	**7.1** (567)	13.2 (621)	1
	Syntretus sp. (Hymenoptera: Braconidae)	0.5 (567)	0 (621)	1
Dolichoderus bispinosus	(Strepsiptera: Myrmecolacidae)	17.5 (252)	8.99 (4,778)	5

We show results when data were available for both males and workers with at least five animals of each sex. Percentages in bold reflect lower prevalence in males than females. In 15 out of the 26 studies, the males showed lower prevalence

1 Schmid-Hempel et al. 1990, *2* Shykoff and Schmid-Hempel 1991a, *3* Otterstatter et al. 2002, *4* Santillán-Galicia et al. 2002, *5* Hughes et al. 2003, *6* Otterstatter 2004, and *7* Otterstatter and Whidden 2004

[a]The prevalence of the parasite was lower in males than in females [1.3% (N=310) males and 3.7% (N=2517) workers] in the best-sampled site (73.56% of all studied animals) out of the nine sites

Prevalence of parasites in haplodiploid social hosts for both sexes

We found data from more than 30 host species, all of them Hymenoptera, for around 10 parasites or parasitoids (Table 2). Most hosts were bumblebees, which have been the focus of most host–parasite studies in social Hymenoptera. However, only four of these parasites are transmitted within the nest (*Varroa destructor*, *C. bombi*, *Nosema bombi*, and *Locustacarus buchneri*) and, thus, are appropriate for assessing the importance of haploid susceptibility. Of these 15 independent data sets, males only had higher parasite prevalence in six cases (Table 2).

The impact of potential genetic mechanisms on resistance through *in silico* simulation

The results of the Kruskall–Wallis test to examine differences in simulated resistance among the different groups showed a significant overall effect $\left(\chi^2_5 = 1,179.03, p < 0.001\right)$, and pairwise comparisons using the Mann–Witney *U* test corrected for multiple comparisons with the sequential Bonferroni procedure showed no significant differences for the six pairwise comparisons among hypothesis 2, hypothesis 3, Males, and Males≥0.3, but detected differences ($p<0.001$) for all other pairwise comparisons. These results suggest a role for either random allelic expression or imprinting when haploid males and diploid workers show the same level of resistance or susceptibility to parasites.

Discussion

In the bumblebee *B. terrestris*, haploid males did not differ in susceptibility or levels of infection when compared to diploid workers. This shows that absolute levels of susceptibility and levels of infection are not rooted in the haploid condition and, thus, we find no evidence to support the assumption of the haploid susceptibility hypothesis. If anything, there was a trend for males to have lower susceptibility and lower intensities of infection than workers.

In this study, we have examined two measures of susceptibility—the initial susceptibility to infection and the within-host population growth of the parasite. Our inoculation dosage was chosen to maximize results for both of these measures. Initial susceptibility was high for both males and workers, and further work to investigate such susceptibility at lower inoculation doses is warranted. Population growth of the parasite as a measure of susceptibility depends upon the assumption that in resistant hosts, parasites will not be able to reproduce. As transmission of *C. bombi* is dependent upon the emission of parasite cells in the host feces, we believe that this is a good measure of susceptibility in this system. Previous work (Logan et al. 2005), combined with our data, suggest that the dynamics of successful infections are independent of initial dose. We did not measure host mortality due to parasitism, a further measure of susceptibility, which mainly occurs under starvation conditions in this system (Brown et al. 2000; but see Imhoof and Schmid-Hempel 1998a). Nevertheless, despite these caveats, we would reiterate the point made above that, if anything, haploid males appear to be less susceptible to *C. bombi* than diploid workers.

This is the first study to test the assumption lying behind the haploid susceptibility hypothesis (O'Donnell and Beshers 2004). In our literature review (Table 2), we found only correlational studies of parasite prevalence in male and female social Hymenoptera. These data suggested no general pattern of haploid susceptibility, but there is clearly an absence of studies outside the genus *Bombus*, so the generality of this observation is unclear. Moreover, we failed to find information from nonHymenopteran haplodiploid groups within the Thysanoptera, Coleoptera, Homoptera, Rotifera, and Acari. The dynamics of infection found for both males and females in our study match those from previous work in this host–parasite system (Schmid-Hempel and Schmid-Hempel 1993; Logan et al. 2005). Nevertheless, to some extent, our results are surprising, as female worker bumblebees have been demonstrated to have higher levels of constitutive immune defense than males (the prophenoloxidase system, Moret and Schmid-Hempel 2001; Gerloff et al. 2003). Previous work showed that *C. bombi* induces up-regulation of the prophenoloxidase system (Brown et al. 2003b), but our results suggest that differing initial levels of immune activity do not result in differences in susceptibility or parasite development within the host. In honey bees, males are more attractive to the *Varroa* mite than are workers, but this seems to be an adaptation to male developmental time rather than haploid susceptibility per se (Santillán-Galicia et al. 2002).

The concept of haploid susceptibility is rooted in the idea that disease resistance is based on genetic variation at the individual level, with codominant alleles at resistant loci leading to higher levels of resistance in diploid individuals (e.g., sickle cell anemia). The differences between haploid and diploid resistance should be greatest when many additive loci are involved and when each locus has multiple alleles (O'Donnell and Beshers 2004). Previous studies in the *Bombus–Crithidia* system have provided ample evidence for highly variable, genetically-based resistance (Shykoff and Schmid-Hempel 1991b,d; Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Schmid-Hempel et al. 1999; Baer and Schmid-Hempel 2001, 2003; Mallon and Schmid-Hempel 2004; Schmid-Hempel and Reber Funk 2004). So, why did we find no evidence for haploid susceptibility? We can think of at least three explanations.

First, what if resistance alleles are not codominant? In this case, a naïve expectation would be for adult haploid susceptibility to exist, e.g., haploid susceptibility could be found for polymorphic loci that harbor recessive alleles for susceptibility. However, if resistance loci are expressed as a general answer to parasitism, rather than being parasite

species-specific, males carrying recessive alleles may fail to reach adulthood by succumbing to diseases at the larval or pupal stage. As a consequence, adult males and workers might exhibit similar levels of resistance and the apparent absence of haploid susceptibility would be a result of looking at the wrong life-history stage of the host. While *C. bombi* cannot infect larvae, bumblebees host numerous larval diseases (Schmid-Hempel 1998). Schmid-Hempel and Loosli (1998) examined larval susceptibility to the microsporidian parasite *Nosema bombi* and found no evidence for differential infection in male or worker larvae. Furthermore, results from our simulation model suggest that even under high levels of larval failure (Males>0.5), adult male susceptibility should be greater than that of diploid workers (hypothesis 1 vs Males≥0.5; Fig. 3). Thus, we conclude that in the absence of other data, there is no evidence to support the idea that haploid susceptibility at the larval stage screens out differences among adult haploid males and diploid workers. However, even if such larval haploid susceptibility exists, this should not select for the specific social behavior suggested by O'Donnell and Beshers (2004).

A second explanation might be that only one allele at each locus is expressed, but due to a phenomenon like imprinting (Charlesworth 1996; Lloyd 2000; Bongiorni and Prantera 2003) rather than a dominant/recessive system. This is hypothesis 3 in our simulation and, apart from random expression of alleles, is the only outcome that predicts our experimental results. In this case, as shown by our simulation model, males and workers should, on average, have similar susceptibility to parasites, as their resistance alleles are both effectively haploid (Fig. 3). Although a genomic imprinting model, as proposed by Beukeboom (1995) to explain sex determination in Hymenoptera, appears to be quite unlikely (Kerr 1997), it has been shown for sex determination in *Nasonia vitripennis* (Dobson and Tanouye 1998) and there are strong theoretical expectations that it should occur in other haplodiploids (Queller 2003). While it should be noted that imprinting is only expected in conflict-related genes and imprinting of resistance loci might have substantial fitness costs, it remains the only likely purely genetic explanation for our results. We are currently working on determining whether imprinting is present in *B. terrestris*.

Our third explanation comes from the perspective of the parasite. In eusocial Hymenoptera, be they "primitive" like bumblebees, or "advanced" like honey bees, males are produced in relatively small numbers at a single point in time. In contrast, workers are produced in large numbers throughout the colony life cycle. Consequently, most parasites will utilize workers rather than males as their predominant host. Workers and males differ not only in their ploidy but also in their sex, and are consequently physiologically and morphologically different. As a result, parasites should be adapted to the female worker host, and should not necessarily be expected to perform well within the unfamiliar male host [a similar argument was developed independently by Schmid-Hempel (1998), p. 110]. This parasite adaptation would be enhanced by short life cycles relative to the host, and repeated passages through genetically related individuals, as we would expect for *C. bombi* in bumblebees. From this perspective, the trend from our results for *C. bombi* to do less well in males than in workers is not unexpected. While haploid susceptibility may still exist, and may perhaps explain the nonsignificant difference we found between males and workers in susceptibility (as if adaptation was the sole force, we would expect the parasite to have done significantly better in workers than in males), the adaptation of parasites to workers would make it unlikely for such susceptibility to play a driving role in the evolution of behavior in the colonies of social Hymenoptera.

There remains one way in which our results might be consistent with the haploid susceptibility hypothesis. Previous work (Rosengaus et al. 1998; Hughes et al. 2002; Pie et al. 2005) has shown that sociality may reduce susceptibility to parasitism. If workers, but not males, can

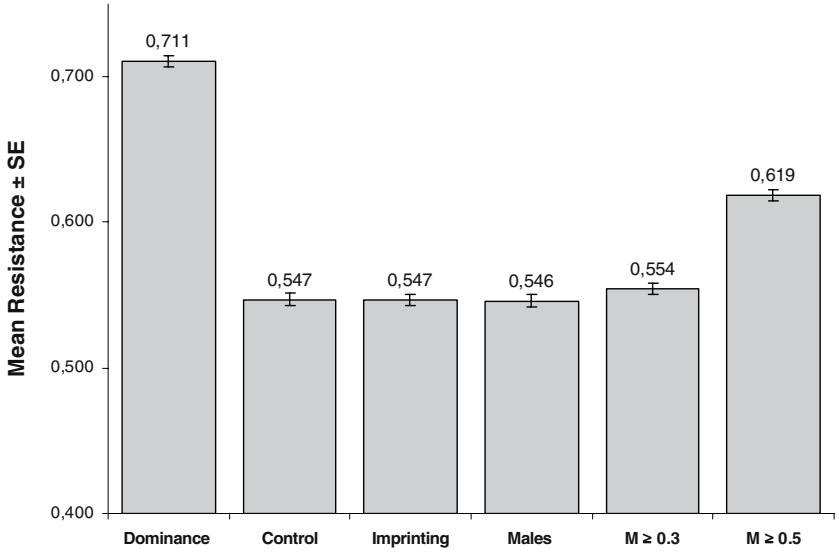

Fig. 3 Mean±SE resistance values obtained in a simulation of 999 haploid males and diploid workers. *Bars* represent the six tested hypothesis: dominance, random expression of alleles (null model control) and imprinting in diploid workers, and males, males under soft purifying selection (Males≥0.3), and males under strong purifying selection (Males≥0.5)

benefit from sociality in this way, the haploid susceptibility hypothesis would be supported. This remains to be tested.

O'Donnell and Beshers (2004) suggested that haploid susceptibility may have played a key role in the origin and maintenance of social behavior in the Hymenoptera. While we found no evidence for haploid susceptibility in an appropriate model system, further tests using other parasites and other hosts need to be done to confirm the generality of our results. In the absence of haploid susceptibility, the "design constraints" hypothesis (Baer 2003; O'Donnell and Beshers 2004) seems the most likely explanation for the evolution of predominantly female hymenopteran societies.

Acknowledgements We thank Rejane M. Falcao, Ailish M. Flanagan, and Peter Stafford for their help in the laboratory and technical support. This work was supported by a Basic Research Grant from Enterprise Ireland SC/2002/209 to MJFB. This work complied with the laws governing animal research in Ireland.

References

Baer B (2003) Bumblebees as model organisms to study male sexual selection in social insects. Behav Ecol Sociobiol 54:521–533. DOI 10.1007/s00265-003-0673-5

Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154

Baer B, Schmid-Hempel P (2001) Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. Evolution 55:139–1643

Baer B, Schmid-Hempel P (2003) Bumble bee workers from different sire groups vary in susceptibility to parasite infection. Ecol Lett 6:106–110

Beukeboom LW (1995) Sex determination in Hymenoptera: a need for genetic and molecular studies. Bioessays 17:813–816

Bongiorni S, Prantera G (2003) Imprinted facultative heterochromatization in mealybugs. Genetica 117:271–279

Brown MJF, Loosli R, Schmid-Hempel P (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91:421–427

Brown MJF, Schmid-Hempel R, Schmid-Hempel P (2003a) Strong context-dependent virulence in a host–parasite system: reconciling genetic evidence with theory. J Anim Ecol 72:102–994

Brown MJF, Moret Y, Schmid-Hempel P (2003b) Activation of host constitutive immune defence by an intestinal trypanosome parasite of bumble bees. Parasitology 126:253–260. DOI 10.1017/S0031182002002755

Brown MJF, Schmid-Hempel R, Schmid-Hempel P (2003c) Queen-controlled sex ratios and worker-reproduction in the bumble bee *Bombus hypnorum*, as revealed by microsatellites. Mol Ecol 12:1599–1605

Camacho JPM, Bakkali M, Corral JM, Cabrero J, López-León MD, Aranda I, Martín-Alganza A, Perfectti F (2002) Host recombination is dependent on the degree of parasitism. Proc R Soc Lond B 269:2173–2177. DOI 10.1098/rspb.2002.2135

Charlesworth B (1996) The evolution of chromosomal sex determination and dosage compensation. Curr Biol 6:149–162

Dobson SL, Tanouye MA (1998) Evidence for a Genomic Imprinting Sex determination Mechanism in *Nasonia vitripennis* (Hymenoptera; Chalcidoidea). Genetics 149:233–242

Doums C, Moret Y, Benelli E, Schmid-Hempel P (2002) Senescence of immune defence in *Bombus* workers. Ecol Entomol 27: 138–144

Durrer S, Schmid-Hempel P (1994) Shared use of flowers leads to horizontal pathogen transmission. Proc R Soc Lond B 258: 299–302

Fischer O, Schmid-Hempel P (2005) Selection by parasites may increase host recombination frequency. Biol Lett 1:193–195. DOI 10.1098/rsbl.2005.0296.

Gerloff CU, Ottmer BK, Schmid-Hempel P (2003) Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. Funct Ecol 17:582–589

Gorbunov PS (1987) Endoparasitic flagellates of the genus *Crithidia* (Trypanosomatidae, Zoomastigophorea) from alimentary canal of bumblebees. Zool Zhurnal 66:1775–1780

Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (a review). Proc Natl Acad Sci USA 87:3566–3573

Hochberg Y (1988) A sharper Bonferroni procedure for multiple test of significance. Biometrika 75:800–802

Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RAB (1999) Phylogenetic perspectives in innate immunity. Science 284:1313–1318

Hughes DP, Moya-Raygoza G, Kathirithamby J (2003) The first record among Dolichoderinae (Formicidae) of parasitism by Strepsiptera. Insectes Soc 50:148–150. DOI 10.1007/s00040-003-0637-9

Hughes WOH, Eilenberg J, Boomsma JJ (2002) Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. Proc R Soc Lond B 269:1811–1819

Imhoof B, Schmid-Hempel P (1998a) Patterns of adaptation of a protozoan parasite to its bumblebee host. Oikos 82:59–65

Imhoof B, Schmid-Hempel P (1998b) Single-clone and mixed-clone infections versus host environment in *Crithidia bombi* infecting bumblebees. Parasitology 117:331–336

Kerr WE (1997) Sex determination in honey bees (Apinae and Meliponinae) and its consequences. Braz J Genet 20. ISSN 0100-8455

Kover PX, Caicedo L (2001) The genetic architecture of disease resistance in plants and the maintenance of recombination by parasites. Mol Ecol 10:1–16

Liersch S, Schmid-Hempel P (1998) Genetic variation within social insect colonies reduces parasite load. Proc R Soc Lond B 265:221–225

Lively CM, Craddok C, Vrijenhoek RC (1990) Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature 344:864–866

Lloyd V (2000) Parental imprinting in *Drosophila*. Genetica 109:35–44

Logan A, Ruiz-González MX, Brown MJF (2005) The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. Parasitology 130:637–642. DOI 10.1017/S0031182005007304

Mable BK, Otto SP (1998) The evolution of life cycles with haploid and diploid phases. Bioessays 20:453–462

Mallon EB, Schmid-Hempel P (2004) Behavioural interactions, kin and disease susceptibility in the bumblebee *Bombus terrestris*. J Evol Biol. DOI 10.1111/j.1420–9101.2004.00717.x

Moret Y, Schmid-Hempel P (2000) Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1166–1168

Moret Y, Schmid-Hempel P (2001) Immune defence in bumble-bee offspring. Nature 414:506

Nuismer SL, Otto SP (2004) Host–parasite interactions and the evolution of ploidy. Proc Natl Acad Sci USA 101:11036–11039. DOI 10.1073/pnas.0403151101

O'Donnell S, Beshers SN (2004) The role of male disease susceptibility in the evolution of haplodiploid insect societies. Proc R Soc Lond B 271:979–983. DOI 10.1098/rspb.2004.2685

Otterstatter MC (2004) Patterns of parasitism among conopid flies parasitizing bumblebees. Entomol Exp Appl 111:133–139

Otterstatter MC, Whidden TL (2004) Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. Apidologie 35:351–357

Otterstatter MC, Whidden TL, Owen RE (2002) Contrasting frequencies of parasitism and host mortality among phorid and conopid parasitoids of bumble-bees. Ecol Entomol 27: 229–237

Otto SP, Michalakis Y (1998) The evolution of recombination in changing environments. Trends Ecol Evol 13:145–151

Penn DJ, Damjanovich K, Potts WK (2002) MHC heterozygosity confers a selective advantage against multiple-strain infections. Proc Natl Acad Sci USA 99:11260–11264. DOI 10.1073/pnas.162006499

Pie MR, Rosengaus RB, Calleri DV II, Traniello JFA (2005) Density and disease resistance in group-living insects: do eusocial species exhibit density-dependent prophylaxis? Ethol Ecol Evol 17:41–50

Queller DC (2003) Theory of genomic imprinting conflict in social insects. BMC Evol Biol 3:15

Rosengaus RB, Maxmen AB, Coates LE, Traniello JFA (1998) Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). Behav Ecol Sociobiol 44:125–134

Santillán-Galicia MT, Otero-Colina G, Romero-Vera C, Cibrián-Tovar J (2002) *Varroa destructor* (Acar: Varroidae) infestation in queen, worker, and drone brood of *Apis mellifera* (Hymenoptera: Apidae). Can Entomol 134:381–390

Schmid-Hempel P (1998) Parasites in social insects. Princeton University Press, Princeton, NJ

Schmid-Hempel P (2001) On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites. Naturwissenchaften 88:147–158

Schmid-Hempel P, Heeb D (1991) Worker mortality and colony development in bumblebees, *Bombus lucorum* (L.) (Hymenoptera, Apidae). Mitt Schweiz Entomol Ges 64:93–108

Schmid-Hempel P, Loosli R (1998) A contribution to the knowledge of *Nosema* infections in bumble bees, *Bombus sp*. Apidologie 29:525–535

Schmid-Hempel P, Müller C, Schmid-Hempel R, Shykoff JA (1990) Frequency and ecological correlates of parasitism by conopid flies (Conopiae. Diptera) in populations of bumblebees. Insectes Soc 37:14–30

Schmid-Hempel P, Puhr K, Kruger N, Reber C, Schmid-Hempel R (1999) Dynamic and genetic consequence of variation in horizontal transmission for a microparasitic infection. Evolution 53:426–434

Schmid-Hempel P, Reber Funk C (2004) The distribution of genotypes of the trypanosome parasite, *Crithidia bombi*, in populations of its host, *Bombus terrestris*. Parasitology 129:147–158. DOI 10.1017/S0031182004005542

Schmid-Hempel P, Schmid-Hempel R (1993) Transmission of a pathogen in *Bombus terrestris*, with a note on division of labour in social insects. Behav Ecol Sociobiol 33:319–327

Sheridan LAD, Poulin R, Ward DF, Zuk M (2000) Sex differences in parasitic infections among arthropod host: is there a male bias? Oikos 88:327–334

Shykoff JA, Schmid-Hempel P (1991a) Incidence and effect of four parasites in natural populations of bumble bees in Switzerland. Apidologie 22:117–125

Shykoff JA, Schmid-Hempel P (1991b) Genetic relatedness and eusociality: parasite-mediated selection on the genetic composition of groups. Behav Ecol Sociobiol 28:371–376

Shykoff JA, Schmid-Hempel P (1991c) Parasites delay worker reproduction in bumblebees: consequences for eusociality. Behav Ecol 2:242–248

Shykoff JA, Schmid-Hempel P (1991d) Parasites and the advantage of genetic variability within social insect colonies. Proc R Soc Lond B 243:55–58

Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc R Soc Lond B 270:99–103. DOI 10.1098/rspb/2002.2199

Van Valen L (1973) A new evolutionary law. Evol Theory 1:1–30

Wilfert L, Gadau J, Baer B, Schmid-Hempel P (2004) Population genomics and host–parasite interactions: investigating the evolution of resistance and host immunity in *Bombus terrestris*. In: Rigaud T, Ebert D (eds) Evolutionary ecology of host–parasite relationships. Conférence Jacques–Monod, p 59

Zuk M, McKean KA (1996) Sex differences in parasite infections: patterns and processes. Int J Parasitol 26:1009–1024