THE ROYAL SOCIETY

The role of male disease susceptibility in the evolution of haplodiploid insect societies[†]

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Heterozygosity at loci affecting resistance against parasites can benefit host fitness. We predict that, in haplodiploid species, haploid males will suffer decreased parasite resistance relative to diploid females. We suggest that elevated susceptibility in haploid males has shaped the evolution of social behaviour in haplodiploid species. Male susceptibility will select for behavioural adaptations that limit males' exposure to pathogens and that limit male transmission of pathogens within and between colonies. The relatedness-asymmetry hypothesis that has been advanced to explain female-only workers does not make these predictions. We review the relevant evidence for genetic effects on parasite resistance in insects and summarize empirical evidence that relates to the haploid-susceptibility hypothesis.

Keywords: haplodiploidy; social evolution; parasite–pathogen resistance; Hymenoptera; male social behaviour

1. HAPLOID SUSCEPTIBILITY: A MODEL FOR THE EVOLUTION OF MALE BEHAVIOURAL ROLES IN HAPLODIPLOID SOCIETIES

A frequently cited component of heterozygote advantage (heterosis) is enhanced resistance to parasites and pathogens (Hamilton 1987; Ewald 1994; Brown 1997; Schmid-Hempel 1998; Baer & Schmid-Hempel 1999). Genetic variation at the individual level may confer fitness advantages, particularly when codominant alleles at resistance loci contribute to the defence against pathogens. In species with haplodiploid sex determination, such as Hymenoptera, males are haploid and therefore lack allelic variation at the individual level. We propose that males in haplodiploid species will suffer reduced disease resistance, or *haploid susceptibility*. Any resistance trait that is affected by heterozygosity, including behavioural and immunological responses, can contribute to haploid susceptibility. Average differences in resistance between haploid males and diploid females will be greatest when many additive loci are involved in resistance and when multiple alleles are present in a population at the resistance loci.

We further propose that haploid susceptibility has shaped the evolution of social behaviour in haplodiploid species. Although haploid males can expose recessive alleles to selection by pathogens, haploid susceptibility itself cannot be counteracted by evolution at resistance loci. Haploid susceptibility will instead favour the evolution of adaptations to diminish the risks imposed by susceptible males. The nature of the evolved responses to male susceptibility may vary among species or even among populations. Adaptive responses to haploid susceptibility will be influenced by the host species' unique set of parasites and by the nature of the alleles that affect resistance to those parasites. We will focus on eusocial Hymenoptera, because they are the most conspicuous and well-studied haplodiploid taxon. Our arguments apply equally well to other haplodiploid social insects (Jordal *et al.* 2000; Chapman *et al.* 2002).

2. THE IMPORTANCE OF TIMING IN HOST–PARASITE DYNAMICS

The dynamic course of pathogen–host interactions can be broken down into steps (figure 1). Genes for pathogen resistance could operate at any one or several of these steps. A given species' adaptive responses to haploid male susceptibility will depend, in part, on which steps are counteracted by codominant resistance loci.

(a) *Early steps: contact and infection*

Early steps involve initial contact between hosts and the infective stages of parasites, and successful colonization of the host's body (infection). Susceptible males may act as parasite vehicles, carrying infections into their social groups. We predict that male and female behaviours will evolve to reduce the rate of male contact with infective agents and/or to reduce the probability of infected males carrying parasites back to their social groups. The life stage at which males can be infected affects the adaptive behavioural responses to haploid susceptibility. If male larvae are more susceptible to early steps in infection, this could favour adult females' ability to discriminate the sex of a developing brood. Male larvae could be segregated within the nest, or at least removed from contact with foragers or other females that would be likely to carry the infective stages of the parasites.

We predict that it will be especially hazardous to allow males that have departed the natal nest to return. Many populations of eusocial Hymenoptera are outbred, which requires that males depart from their natal nests to seek mating opportunities (Page 1986). Males should remain in the natal nest until their mating flights. Once they have departed to mate, males should be excluded from their colonies. If parasite exposure or infection can be detected at the individual level, then males that have been exposed

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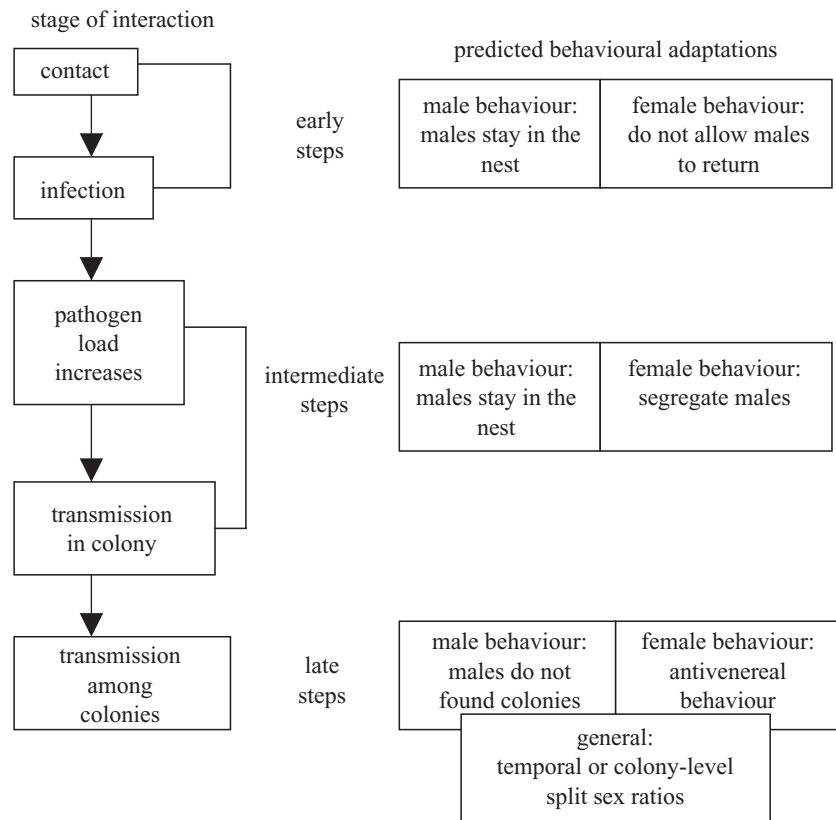DOI 10.1098/rspb.2004.2685

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Figure 1. Predicted behavioural and life-history adaptations of haplodiploid species in response to male haploid susceptibility. Adaptive responses are grouped according to the stage of the host–parasite interaction at which susceptibility loci are relevant.

to infective stages should be particularly excluded from their colonies.

Male genetic susceptibility during the early steps also has implications for the evolution of task performance and division of labour. Males should not contribute labour to their colonies if this exposes them to a significant risk of infection. Males are especially predicted to avoid tasks such as foraging that require them to go outside the nest, but they should also not perform in-nest tasks such as corpse removal and grooming of foragers that could expose them to pathogens. Haploid susceptibility does not preclude male performance of other tasks.

(b) *Intermediate steps: parasite multiplication and within-group transmission*

Intermediate steps in host–parasite interactions involve the multiplication of parasites within individual hosts' bodies, and transmission of parasites from infected to non-infected individuals within social groups (Rosengaus *et al.* 2000). Susceptible males may serve as pathogen incubators and distributors, increasing the parasite load in their colonies. Females should then behave in a manner that reduces their exposure to in-nest males. Adult males may be shunned or spatially segregated within the nest. Males that are exposed to pathogens should be ejected from the nest before an infection develops.

(c) *Late steps: transmission among colonies*

Intercolony transmission can be horizontal or vertical. Horizontal transmission is the infection of other mature colonies, which may or may not include genetically related individuals. In some species, mating occurs away from the

nest, limiting the risk of infection by males from other colonies. However, venereal transmission remains a risk to mating females and their natal or incipient colonies. Females of species that mate away from the nest are expected to exhibit mating behaviour that minimizes the risk of infection. This could include detection and avoidance of infected males, minimizing the time of contact with males and minimizing the number of males they contact. Most female eusocial Hymenoptera mate with only a single male (Strassmann 2001), which may reduce the risk of venereal infection. However, it has also been suggested that multiple mating could be favoured because it increases genetic diversity among the workers (Baer & Schmid-Hempel 1999). In other species of eusocial insect, females mate in or near their natal nests with males that have dispersed from foreign colonies (Hölldobler & Wilson 1990; van Veen & Sommeijer 2000). In these taxa, both venereal and direct infection of colonies by males is possible.

Vertical transmission involves the infection of offspring colonies, typically via infection of the founding adults. Contact between males and gynes in the same colony should be limited. Males should not participate in colony foundation (Kolmer & Heinze 2000; Smith *et al.* 2002). Workers that accompany reproductive swarms should avoid contact with males prior to departure. Split sex ratios may also have evolved as an adaptation to prevent vertical transmission of parasites. Colonies that specialize by producing only one sex of reproductives will be less likely to infect their own offspring colonies if transmission of infection occurs within the natal nest. Temporal splitting of sex production, such that only one reproductive

sex is present at a time in the nest, can also function to minimize contact between the sexes. Temporal separation of the sexes has been documented in some eusocial species (Bulmer 1983; Beekman & van Stratum 1998).

Selection should generally favour maximizing the number of diploids (females) present in the colony at any given time. Haploid males should be produced only when needed for mating, and males should be eliminated as soon as mating is accomplished. This could be achieved through protogynous reproduction late in the colony cycle, and through a short tenure of males in the nest.

3. EVIDENCE FOR PLOIDY EFFECTS ON PATHOGEN RESISTANCE

The haploid-susceptibility hypothesis rests on the assumption that heterozygosity at key genetic loci affects pathogen resistance. There are few empirical tests of the role of heterozygosity in disease resistance (Penn *et al.* 2002). Little is known about genetic variation of disease resistance in insects, or about the possible role of heterozygosity (Boucias & Pendland 1998). Genetic diversity contributes to parasite resistance in groups of bumble-bee workers (Baer & Schmid-Hempel 2003), with a lower transmission rate between unrelated individuals (Shykoff & Schmid-Hempel 1991). Genetic diversity has been shown to improve colony growth and resistance to infection in honeybees (Tarpy 2003).

4. DOES HYMENOPTERAN BEHAVIOUR MATCH THE PREDICTIONS OF HAPLOID SUSCEPTIBILITY?

Under the haploid-susceptibility hypothesis, male behaviour should evolve to balance the capacity for direct reproduction against the costs of transmitting parasites to kin. Males and females may be in conflict over male roles in their natal colonies.

(a) *Patterns of male behaviour in hymenopteran societies*

Some males remain in their natal nests for extended periods of several weeks or months, and the males are expected to contribute labour while present in the nest. Males should not forage, but the hypothesis does not preclude male in-nest work. To our knowledge, male foraging has not been documented in any eusocial Hymenoptera. However, males of some species contribute to in- or on-nest labour. Male bumble-bees and paper wasps perform thermoregulation and brood-care tasks (Cameron 1985; O'Donnell 1999). By contrast, male ants seem to provide labour very rarely (Hölldobler & Wilson 1990).

(b) *Treatment of males by females in hymenopteran societies*

A minimum requirement for adaptive female responses to males is that females can discriminate the sex of nest-mates. Males in many eusocial Hymenoptera are treated aggressively by their female nest-mates (Litte 1979, 1981; O'Donnell 2001). Aggression towards hymenopteran males is not universal, however. Males in some paper wasps are dominant over females (O'Donnell 1999). In

some bumble-bee species (*Bombus bifarius*, *B. huntii*) males are tolerated in their natal nests and are permitted to return after leaving on mating flights. By contrast, *B. californicus* and *B. appositus* males are ejected by workers soon after adult emergence. Males that attempt to re-enter their natal nests are often killed (R. Foster, personal communication). Males' roles and treatment by females also vary among *Mischocyttarus* paper wasps. *Mischocyttarus flavitarsis* males depart their nests to join mating aggregations and do not return (Litte 1979). By contrast, *M. mastigophorus* and *M. labiatus* males reside on their natal nests for periods of up to several weeks, and return daily from departures that include mating flights (Litte 1981; O'Donnell 1999). Diversity in female responses to males may result from the different parasite pressures that each species faces and depend on the genetic basis of disease resistance.

In swarm-founding *Polybia* wasps, males do not accompany their female nest-mates when the swarm moves to a new colony site (Bouwma *et al.* 2000). In *Apis mellifera*, drones can accompany swarms, but do so at a lower rate than expected from their numbers in colonies (Ratnieks & Miller 1993).

5. FURTHER TESTS OF THE HAPLOID-SUSCEPTIBILITY HYPOTHESIS

We predict several, as yet untested, patterns for rates of infection and genetic diversity in haplodiploid species. Perhaps most obviously, males should be more susceptible to pathogens than are females. Males of some eusocial hymenopterans are more susceptible to certain pathogens, for example *Varroa* mites in honeybees (Santillan *et al.* 2002), but whether this pattern is related to haploid susceptibility is not known. Field studies showing higher rates of infection or disease in males would provide a supporting but non-rigorous test. A stronger approach would be to challenge males and females with pathogens experimentally. These experiments could be used to test whether males are less immunocompetent than females (Adamo *et al.* 2001; Traniello *et al.* 2002). Some hymenopteran species produce diploid males (Page 1986), which could be included in immunocompetence experiments with the expectation that their resistance would match that of females.

Although the haploid-susceptibility model predicts general differences between males and females, not all species are expected to exhibit the same patterns of sex bias in infection by, or transmission of, pathogens. The correlational and experimental studies suggested in the previous paragraph should be repeated in diverse haplodiploid species. The resulting data could be analysed comparatively, with the prediction that male social roles in a given species (or population) will correspond to sex differences in infection or transmission rates.

The strongest tests of the hypothesis can be conducted in genetically well-characterized study organisms, when individual genetic loci with strong effects on immune function are known. It will then be possible to test directly for ploidy effects on resistance at a given locus, with the prediction that males and homozygous females will be equally susceptible, and both will be more susceptible than heterozygous females.

6. ALTERNATIVE EXPLANATIONS FOR MALE ROLES IN HYMENOPTERAN SOCIETIES

The haploid-susceptibility hypothesis appears to offer explanations for some widespread and conspicuous features of male behaviour in eusocial Hymenoptera. A strong application of the model to the evolution of eusociality would be to posit that male susceptibility has favoured the evolution of predominantly female societies, in other words female worker forces, in the eusocial Hymenoptera. A weaker application of the model would posit that male susceptibility has influenced the behavioural roles of males and the responses of females to males in predominantly female societies that evolved for other reasons.

We consider two alternative explanations that have been proposed to explain the evolution of predominantly female hymenopteran societies: the relatedness-asymmetry hypothesis and the design-constraints hypothesis. Relatedness asymmetry is rooted in kin-selection theory and predicts that males will behave less cooperatively than females in their natal colonies because they are less closely related to their sisters than they are to their daughters (Hamilton 1964). One prediction of relatedness asymmetry is that males should not function as workers in haplodiploid societies. Both the logic and the verity of the relatedness-asymmetry hypothesis have been criticized (reviewed in Bourke & Franks 1995), because, in abdicating work, males are potentially reducing their own fitness. Relatedness asymmetry makes no other specific predictions about the evolution of male behaviour.

The second alternative, the design-constraints hypothesis, is more likely to be correct, but is also difficult to test. This hypothesis states that hymenopteran males' behaviour and morphology are designed primarily by selection on mating ability (Wiernasz *et al.* 2001; Baer 2003). Sexually selected traits that favour male mating success may be incompatible with most forms of labour. Mating specialization would generally predict physical reduction of structures not directly associated with mating success, and enhancement or enlargement of mating structures. For example, large male body size may be required for producing large quantities of sperm, for strong flight, for storing enough nutrients to fuel flight and for scramble competition with other males to inseminate females (Hölldobler & Wilson 1990).

In basal groups of Hymenoptera the males are typically specialized for mating and are polygynous, and females provide substantial parental investment, provisioning and in some cases remaining with the larvae to feed and protect them. It may be that in the ancestors of the social Hymenoptera the characteristics that make social behaviour possible are present in females and not in males (Bourke & Franks 1995). This limits the importance of relatedness asymmetry, and should focus our attention on the design-constraints hypothesis and haploid susceptibility as mechanisms to explain the male–female division of labour within societies whose fundamental organization was already fixed along male–female lines.

7. CONCLUSIONS

Haploid susceptibility is a reasonable hypothesis that fits well with the current view that genetic diversity within insect colonies contributes to their ability to resist parasites and pathogens (Hamilton *et al.* 1990; Lewis 1998; Schmid-Hempel 1998). If experimental evidence supports the predictions that: (i) males are more susceptible to infections; and (ii) the susceptibility is rooted in their haploid condition, then we would conclude that haploid susceptibility may have played an important role in social evolution in the Hymenoptera. Since male haploidy is an ancient condition (Hunt 1999), haploid susceptibility would always have been a factor, even if haplodiploidy itself and design constraints resulting from sexual selection have also been factors, as seems likely. Some predictions of the haploid-susceptibility hypothesis are not made by the alternative explanations for male roles in haplodiploid societies. Haploid susceptibility may have an important and ongoing role in shaping the structure and behaviour of hymenopteran societies, even if the fundamental division along male–female lines has long been fixed.

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