



Research article

Individual variation in immune function in the ant *Formica exsecta*; effects of the nest, body size and sex

LIISA VAINIO¹, HARRI HAKKARAINEN², MARKUS J. RANTALA^{3,*}
and JOUNI SORVARI²

¹Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FIN-40014 Jyväskylä, Finland; ²Department of Biology, Section of Ecology, University of Turku, FIN-20014 Turku, Finland; ³Department of Biology, University of California, Riverside, CA 92521, USA (*author for correspondence, e-mail: marrant@dodomail.jyu.fi)

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Abstract. It has been generally thought that sex differences in the immune system are the result of the differing life history strategies of the sexes, although the available data are not entirely consistent with the hypothesis. In this study, we studied the variation in the immune function in the mound-building wood ant *Formica exsecta*. *F. exsecta* has two forms of males, distinguished by size: the small males (micraners) and the large males (macraners), which die after the mating period, whereas females live tens of years laying their eggs. We found that in general males have a lower encapsulation response against nylon monofilament (i.e. lower immune function) than queens. Among males, the micraners had a lower encapsulation rate than the macraners. However, in queens, there was no correlation between size and encapsulation rate. The origin nest had an effect on the encapsulation rate of males: males from the large nests had a stronger encapsulation rate than males from small nests. However, in queens, nest size did not have any effect on encapsulation response. The observed variation between sexes and individuals in the encapsulation rate is discussed in the context of reproductive strategies and parasite-mediated sexual selection.

Key words: encapsulation rate, immune function, immunocompetence handicap hypothesis, male dimorphism, sex differences

Introduction

In many vertebrate species, males tend to suffer more from parasitic infections and to have a reduced immune response when compared with females (Poulin, 1996; Zuk and McKean, 1996; Møller *et al.*, 1998, 1999). Reduced immunocompetence in male vertebrates has been discussed in the context of the evolution of life histories, especially with reference to selection on life span and on sexual selection (e.g. Folstad and Karter, 1992; Wederkind and Folstad, 1994; Sheldon and Verhulst, 1996; Møller *et al.*, 1999). There is some empirical evidence that female arthropods have a more effective immune function than males (Rheins and Karp, 1985; Nigam *et al.*, 1997; Gray, 1998; Radhika *et al.*, 1998;

Kurtz *et al.*, 2000; Adamo *et al.*, 2001; Kurtz and Sauer, 2001; Rolff, 2001; Siva-Jothy *et al.*, 2001). However, this result is confounded by other studies where no such relationship was found in many other studies (Gillespie and Khachatourians, 1992; da Silva *et al.*, 2000; Yourth *et al.*, 2001) and by others where the opposite has been observed (Siva-Jothy and Thompson, 2002). Thus, the available data are not entirely consistent with the hypothesis that males are less immune competent than females. If the observed sex differences in immune function are the result of different life history strategies of different sexes, ants would be an ideal model system to test sex differences in the immune system, due to the remarkable life history differences in males and queens.

Formica exsecta Nylander 1846, is a mound-building ant species, living in open woodlands, rough pastures, moor and heathlands. *F. exsecta* is characterized by a highly male biased sex ratio and colony specialization on the production of male or female reproduction (Pamilo and Rosengren, 1983; Brown and Keller, 2000). As in most ants, *F. exsecta* queens mate only during a mating flight at the beginning of their adult lives. Queens store a lifetime supply of sperm. After the mating flight, the queen tries to establish her colony and may live up to 20 years laying eggs (Pamilo, 1991). In contrast to the queens, all males die after the mating flight (Hölldobler and Wilson, 1990). Thus, females may gain fitness through increased longevity, whilst males gain fitness by increasing mating rate (see Trivers, 1972). These differences in the life history strategies between sexes may lead to sex differences in immune function (see e.g. Rolff, 2001).

F. exsecta males have two forms, distinguished by size (Fortelius *et al.*, 1987). The small males (micraners) appear to mature later, display a sharper circadian activity peak, and disperse farther than the larger males (macraners). Micraners are more prevalent in crowded, multiqueen colonies characterized by strongly biased sex ratios (Fortelius *et al.*, 1987). It has been suggested that macraners are more common during the early stages of growth of local populations, when there is an advantage to the reduction of dispersal to enhance the build-up of the local population (Fortelius *et al.*, 1987). Later, the shift to micraners favor dispersal, which is the better strategy when local resources have become strained by a larger colony size (Fortelius *et al.*, 1987).

This study addresses multiple questions: (1) whether there are gender differences in immune function in *F. exsecta*; (2) whether micraners have a lower immune function than macraners; (3) whether the origin nest has an effect on the immune function.

Materials and methods

The queens and the males in this study were collected from nests located in the vicinity of Jyväskylä (62°16'N, 25°30'E) in central Finland on July 10, 2002.

From each nest the basal diameter (mean of min. and max. diameter) of nest mound and the height of the trees within a radius of 10 m from nest mounds (as an estimate of colony size and shadiness of habitat, respectively) were measured. We collected pupae and workers with some nest material into plastic bags and transported them to the laboratory in a cool thermobox. In the laboratory, pupae, workers and nest material from each nest were placed separately into a glass aquarium (30 cm × 40 cm × 45 cm in size). Workers were allowed to take care of their offspring at a constant room temperature (28 ± 1 °C). We waited until queens and males hatched and started swarming at the aquarium. In total, we had 261 individuals (164 males and 97 queens) from 17 different nests.

In insects, one of the most common and informative ways to assay the immune function is to measure the magnitude of the cellular encapsulation response to a novel and standardized antigen, such as a nylon monofilament, that mimics a novel parasitoid (e.g. König and Schmid-Hempel, 1995; Rantala *et al.*, 2000, 2002, 2003; Ryder and Siva-Jothy, 2000; Siva-Jothy, 2000; Rantala and Kortet, 2003, in press; Koskimäki *et al.*, in press). Encapsulation is a cellular response through which insects defend themselves against multicellular pathogens, for example, nematodes, mite parasitoids and fungi (e.g. Gillespie *et al.*, 1997), but it also plays a role in defence against viruses (Washburn *et al.*, 1996). In cellular response, circulating cells in the haemocoel recognize an object as foreign and form a capsule surrounding it that melanises and hardens. This results in the death of the intruder by asphyxiation (Fisher, 1963) or through the production of necrotizing compounds (Nappi *et al.*, 1995). The rationale for measuring the encapsulation rate as a general measure of immune function is that it is complex and requires the co-ordination of many cellular and humoral immune effector systems (Ratcliffe, 1993) into a single, easily measured response: failure to encapsulate is the equivalent of failure of the immune system (Webb and Luckhart, 1996).

Encapsulation rate assay

To measure encapsulation rate, ants were individually CO₂-anaesthetized and put upside down onto separate object glasses firmly taped. We inserted a 1 mm long piece of nylon monofilament (0.1 mm diameter) through a puncture in the pleural membrane between the second and third sternites (see Rantala *et al.*, 2002). The ants' immune system was allowed to react to this object for 3 h, while they were kept individually in small plastic jars (diameter: 3 cm, height: 5 cm) at a constant room temperature (28 ± 1 °C). The implant was then removed and dried. The encapsulation rate was measured using the method of Rantala *et al.* (2002). The removed monofilament was examined under a light microscope and photographed from three different angles using a digital

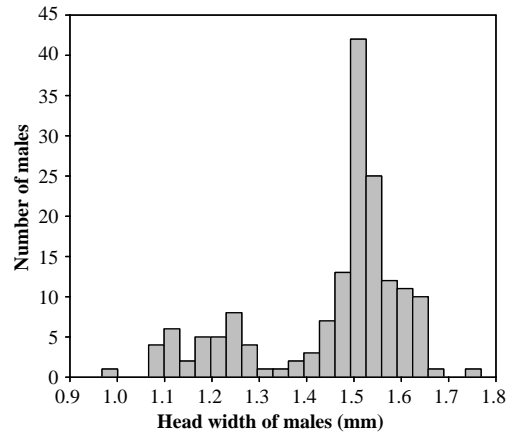


Figure 1. Head width (mm) distribution among males.

camera. The pictures were then analyzed using an image analysis program (Image Pro). The degree of encapsulation was analyzed as the grey value of reflected light from the implants. As a measure of encapsulation rate, we used the average grey values of the three pictures. The scale was calibrated to indicate that the darkest grey received the highest encapsulation rate (total black). Previously, it has been shown that the repeatability for this measurement is very high ($R = 0.997$, Rantala *et al.*, 2002). After the encapsulation rate assay, the head width was measured as an estimate of body size by using a dissection microscope with ocular micrometer units. We divided males into two groups on the basis of head width (macranes > 1.3 mm; Fig. 1).

Statistical methods

We used ANOVA and ANCOVA models in tests. A nested ANOVA with nest as a random factor (nested under sex) and head width as a covariate was used in the test for gender differences in encapsulation. The head width information of one queen was missing. Its encapsulation rate, however, was measured, and the information was included into analysis of interaction between gender and the basal diameter of nest mound. The encapsulation variable was normalized natural logarithm (ln) transformation so that parametric tests could be used. All analyses were performed with SPSS 11.0 statistical software (SPSS inc.).

Results

Queens had a higher encapsulation rate than males, even when the size (head width in mm) was used as a covariate (Table 1; Fig. 2). The head width and the

Table 1. Effect of gender on encapsulation rate

	df	<i>F</i>	<i>p</i>
Gender	1, 46.1	4.73	0.03
Nest	18, 239	5.18	< 0.001
HW	1, 239	0.12	0.76

Nested ANOVA with nest as a random factor (nested under sex) and head width (HW) as a covariate.

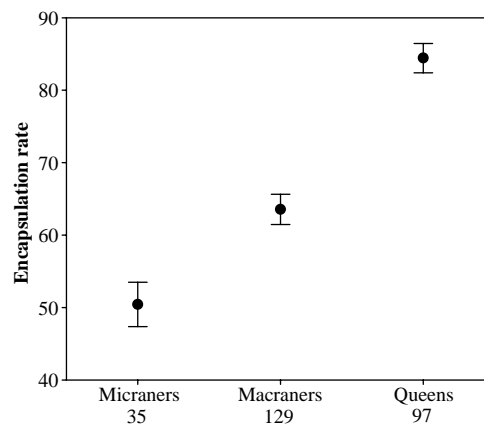


Figure 2. Mean encapsulation rate (artificial units) (\pm SE) of micraner and macraner males and queens.

basal diameter of the nest mound positively affected encapsulation in males but not in queens (Table 2). Among males, micraners had a lower encapsulation rate than macraners (male size groups: $F_{1,164} = 8.11$, $p = 0.005$; basal diameter of nest mound as a covariate: $F_{1,164} = 4.79$, $p = 0.03$) (Fig. 2). The probability of a colony to produce sexual offspring was significantly affected by the basal diameter of the nest mound and marginally by the height of the trees around

Table 2. The effect of head width (HW) and the basal diameter of nest mound (diameter) on encapsulation in both sexes

	df	<i>F</i>	<i>p</i>
<i>Queens</i>			
Diameter	1, 96	0.92	0.34
HW	1, 96	0.31	0.58
<i>Males</i>			
Diameter	1, 164	5.20	0.02
HW	1, 164	3.84	0.05

Table 3. Results of the logistic regression analysis of the probability (producing/non-producing) to produce sexual offspring (fit of the model 91.4, Nagelkerke $r^2 = 0.41$, $G = 9.9$, $df = 2$, $p < 0.007$)

	β	SE	Wald χ^2	p
Mean diameter	0.135	0.06	5.20	0.023
Tree height	-0.212	0.127	2.78	0.095

the nest mounds (Table 3). An increase in the basal diameter of the nest mound increased the probability of colonies to produce sexual offspring, whereas an increase in the tree height decreased it (Table 3).

Discussion

Gender differences in immune function have been thought to arise from the differing life history strategies between sexes (e.g. Rolff, 2002). Male fitness is generally limited by the number of mates fertilized, whereas female fitness is limited by the number of offspring produced and reared (Trivers, 1972). Males are thus expected to have a ‘live hard, die young strategy’, at least where polygyny is common and where males that take large risks may also accrue large gains (Zuk and Stoehr, 2002). Thus, selection on investment in reproduction at the expense of parasite resistance may cause differences in immune function between sexes (e.g. Rolff, 2002). In this study, we found that queen *F. exsecta* ants have a higher encapsulation rate than males. This is consistent with many previous studies with insects which have found gender differences in immune function. For example, in studies in *Gryllus texensis* (Adamo *et al.*, 2001) and *Acheta domesticus* (Gray, 1998), it has been found that male crickets are more susceptible than females to infection with the bacteria *Serratia Liquefaciens*. Similarly, scorpion fly females *Panorpa vulgaris* were better able to phagocytose injected particles and had higher levels of antibacterial compounds and a higher phenoloxidase activity (PO) than males (Kurtz *et al.*, 2000; Kurtz and Sauer, 2001). Consistent with those studies, Rolff (2001) found that mature female damselflies had a higher phenoloxidase activity than mature males. A few other studies on gender differences in invertebrate immunity also showed lower male immunocompetence (Rheins and Karp, 1985; Nigam *et al.*, 1997; Radhika *et al.*, 1998; Siva-Jothy *et al.*, 2001). However, in the grasshopper *Melanoplus sanguinipes*, there were no sex differences in resistance to the fungus *Beauveria bassiana* (Gillespie and Khachatourians, 1992) and in PO activity in *Acheta domestica*, although females had significantly higher haemocyte counts than males had (da Silva *et al.*, 2000). Likewise, there were no sex differences in encapsulation of mite feeding tubes in four species of dam-

selfies (Yourth *et al.*, 2001). Surprisingly, in the mealworm beetle, *Tenebrio molitor*, males had an even higher PO activity than females (Siva-Jothy and Thompson, 2002). Furthermore, Sheridan *et al.* (2000) did not find any differences in parasite infections among arthropod hosts. Thus, the available data are not entirely consistent with the hypothesis that males are less immune competent than females. Clearly, more studies are needed to reveal whether the gender differences in immune function exist also in insects.

Micraners were found to have a lower encapsulation rate than macraners. This is consistent with many studies in insects which have found that larger individuals have a stronger immune function (e.g. Suwanchaichinda and Paskewitz, 1998; Rantala *et al.*, 2002; but see Rantala and Kortet, in press). Micraners may occur more prevalently in crowded, polydomous colonies characterized by strongly biased sex ratios and a high nest density (Fortelius *et al.*, 1987). The high nest density may decrease the amount of food available in the colonies. It has been found that expression of immune function is condition dependent (e.g. Feder *et al.*, 1997; Suwanchaichinda and Paskewitz, 1998, Siva-Jothy and Thompson, 2002; Rantala *et al.*, 2003). Thus, the micraners may be poor quality males produced under resource limitation. It is possible that colonies producing micraners use 'make the best of a bad situation' strategy rather than using different morphs for dispersal (see Fortelius *et al.*, 1987). We found that larger colonies produced males with a better encapsulation rate than smaller colonies. It is possible that larger colonies can produce males with a greater body condition due to a good resource availability. In our data set, however, there was no specialization in micraners in small colonies and macraners in large colonies.

On the other hand, Brown and Keller (2000) found that macraners were produced in queen producing colonies, whereas micraners were produced in colonies with no queen production. In our study, micraners were produced in all-male producing colonies often with a low proportion of macraners, whereas only macraner males were found in colonies that produced queens. This may be related to the queen replenishment theory (Brown and Keller, 2000). If a good encapsulation ability is a heritable trait, males of a high quality (macraners) may have been produced as mates for new queen generation to ensure good immunocompetence of the colony. This intranidal mating, however, may lead to inbreeding if queens in the queen pool of colonies are related. This inbreeding may lead to a lower immunocompetence of colonies (see e.g. Acevedo-Whitehouse *et al.*, 2003).

In queens, no correlation was found between size and encapsulation rate. It is possible that the different life history strategies of sexes affected the correlation between size and encapsulation rate. In addition, no correlation was found between colony size and encapsulation in queens. This correlation was found only within size dimorphic males.

In conclusion, in the mound-building ant, *F. exsecta* encapsulation rate of queens was higher than that of males, suggesting that queens have a higher immunocompetence than males. Since insects lack male-specific hormones, such as testosterone (Nijhout, 1994), observed sex differences in the immune system are probably the result of differences in life history traits (see Zuk and Stoehr, 2002). To our knowledge, this is the first study on the immune ecology of ants.

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