Selection for parasitoid resistance alters mating success in *Drosophila*

Jens Rolff^{1*} and Alex R. Kraaijeveld²

¹Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

²NERC Centre for Population Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK

* Author for correspondence (jor@sheffield.ac.uk).

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Parasite-mediated sexual selection is still a widely discussed hypothesis for the understanding of the evolution of secondary sex traits. Furthermore, it has sparked new fields such as ecological immunology. Despite this, most tests have been restricted to the manipulation of parasite loads. Here, we provide a new experimental approach, where resistance itself was manipulated. Parasitoid-resistant *Drosophila melanogaster* males achieved a higher mating success compared with non-resistant conspecifics. The underlying mechanism however remains elusive.

Keywords: parasite-mediated sexual selection; immunocompetence; correlated selection

1. INTRODUCTION

Parasite-mediated sexual selection is an important explanation for the evolution of sexual ornaments (Hamilton & Zuk 1982) and has been intensively studied for the last 20 years (Clayton & Moore 1997; Moore & Wilson 2002). Most studies have focused on the intraspecific prediction, namely that females mate with the most attractive males to obtain 'good genes' for parasite resistance and hence gain resistant offspring. The interspecific prediction envisages a positive correlation between male brightness and average parasite load, which could be explained by a genetic correlation between male attractiveness and resistance. As pointed out in a meta-analysis (Hamilton & Poulin 1997) the overall support for the mechanism put forward by Hamilton & Zuk (1982) is rather weak. However, most studies to date have used manipulations of parasite load to test the intraspecific prediction, and very few studies have attempted to manipulate resistance itself. The rationale underlying this study using selection lines is that selecting for increased resistance could result in a correlated response of attractiveness. This correlated response could be (i) negative, if a trade-off between resistance and attractiveness arises (*sensu* Verhulst *et al.* 1999); or (ii) positive, if parasite-mediated sexual selection shapes a positive genetic correlation between resistance and attractiveness. We tested this hypothesis using *Drosophila melanogaster* selected for resistance against a common parasitoid, a system where resistance is both heritable and costly (Kraaijeveld & Godfray 1997). This study revealed that resistant males have a higher mating success. However, the underlying mechanism remains elusive.

2. MATERIAL AND METHODS

The flies used in this study are derived from lines selected for resistance against *Asobara tabida* (four lines) and their corresponding control lines (four) at Imperial College London in 1996 (Kraaijeveld & Godfray 1997). They show a certain level of cross-resistance against another parasitoid species (Fellowes *et al.* 1999a). At least a substantial part of the underlying resistance mechanism is an increased haemocyte concentration in unparasitized larvae (Kraaijeveld *et al.* 2001). Haemocytes are involved in the encapsulation of the parasitoid egg (Carton & Nappi 2001) and form an important arm of immune defence in insects. Resistance has been shown to trade off against larval competitive ability (Kraaijeveld & Godfray 1997).

Mating success was assayed by confronting one resistant and one control male of controlled age (from a pair of lines) with a test stock female (Lyon). The mating arena was a plastic tube with a diameter of 15 mm and a length of 45 mm, closed with cotton wool. The triad was observed for 60 min and the identity of the successful male recorded. For each line 74–79 pairs of males were studied. To distinguish the males, they were cold anaesthetized and colour marked (24 h before the experiment). (Preliminary studies showed that colour marking had no effect on female choice. Additionally, we interchanged the colours between resistant and control males and again found no effect.)

Mating speed was recorded by placing one male and one female in a vial and observing them for 30 min (the sample size per replicate was 15–25). For males that mated within 30 min the time was recorded. Males that did not mate were censored in the analysis (see below).

Body size (thorax length) was measured under a dissecting microscope. All experiments were conducted at a constant temperature of 25 °C.

In studies using selection lines, the individual lines are the replicates. As we used four pairwise control and selected for resistance lines, we compared the data with a pairwise *t*-test (two-tailed). Mating speed was estimated using a survival analysis (Rybak *et al.* 2002), with censoring as implemented in S-PLUS. From this the mating speed in seconds is estimated for each replicate. The calculated mating speeds are compared with a pairwise *t*-test (two-tailed).

3. RESULTS AND DISCUSSION

Based on the hypothesis of parasite-mediated sexual selection we predicted that males resistant to *A. tabida* should have an altered mating success.

In pairwise comparisons resistant males are significantly more successful ($t = 4.17$, d.f. = 3, $p = 0.026$; figure 1). This is the first test, to our knowledge, using the new rationale outlined above, although Verhulst *et al.* (1999) already used selection lines to test the hypothesis of parasite-mediated sexual selection. They used poultry selected for an elevated response to sheep red blood cells and established a negative correlation between response ('immunocompetence') and the size of an important secondary sexual ornament, the comb. Our study differs in two crucial aspects. First, we studied lines that were resistant against a true parasite, where the mechanism of resistance is known (Kraaijeveld & Godfray 1997; Carton & Nappi 2001). Second, we studied mating success directly rather than a correlate.

However, so far we do not know the mechanism underlying the higher mating success of resistant males. We studied two other traits that are commonly affected in artificial selection studies: body size and mating speed. Body size did not differ between resistant and control lines ($t = -1.115$, d.f. = 3, $p = 0.346$). Mating speed, however differed significantly ($t = 3.384$, d.f. = 3, $p = 0.048$). Resistant lines mated significantly quicker than control lines. The design of the study, which is inherent to most selection line studies, does not allow us to estimate the amount of variance explained by mating speed. Whether mating speed alone is sufficient to explain the results remains to be unravelled by further mechanistic studies.

If mating speed is not sufficient to explain the success of resistant males, likely candidates are courtship song and

Figure 1. Mating success of resistant *Drosophila melanogaster* males (open bars) compared with the corresponding control males (filled bars). Resistant males are more successful.

cuticular hydrocarbons. Studies on the influence of these traits on mating success are numerous. However, few studies compare songs of individual males within a population. We are not aware of any study addressing the influence of cuticular hydrocarbons within a population. However, our results warrant further mechanistic analysis. Future experiments might also try to further disentangle female choice and male–male competition, which is not possible within our design. This is important, as the male–male competition is not part of the Hamilton and Zuk hypothesis.

If mating speed is sufficient to explain the reported patterns, the test of parasite-mediated sexual selection found no support for the hypothesis by Hamilton & Zuk (1982). Given that the generality of parasite-mediated sexual selection was cast in doubt by a recent meta-analysis (Hamilton & Poulin 1997), we suggest that the rationale proposed here is an advanced test.

There are at least three possible interpretations for the results of our study.

- (i) A genetic correlation between traits determining mating success and parasitoid resistance exists and was uncovered by the selection procedure. The reasons that these traits do not go to fixation in natural populations are twofold. First, resistance is costly in the study system (Kraaijeveld & Godfray 1997). Second, our experiment captures only an episode of the cycles of host resistance envisaged by Hamilton & Zuk (1982): when other parasites are becoming more important, the frequency of parasitoid resistant individuals reduces. A speculation here could be that different sexually selected traits are linked with different resistance mechanisms.
- (ii) In contrast to the study of Verhulst *et al.* (1999), our results are unlikely to be explained by invoking a resource-based trade-off. Differences in feeding rate underlie the trade-off between larval competitive ability and resistance in our study system (Fellowes *et al.* 1999b): resistant individuals feed slower. Jaw muscles and the haemopoietic organ, which pro-

duces haemocytes, are derived from the same embryonic structure (Tepass *et al.* 1994). Therefore it seems that resistance is already traded off.

- (iii) The increased mating success is just a by-product of the artificial selection.

At the current time we are not in a position to decide which explanation is correct, although we tend to the first explanation. If true, this could be intraspecific support for the interspecific hypothesis proposed by Hamilton & Zuk (1982). Parasite-mediated sexual selection is an important attempt to explain the existence of exaggerated secondary sexual traits. The study of such traits lies at the heart of evolutionary biology (Getty 2002). Parasite-mediated sexual selection received considerable interest but former attempts to test the predictions have not been very powerful by the very rationale they used. Tests using the manipulation of parasite load, as analysed in Hamilton & Poulin (1997), are not able to establish genetic correlations, whether caused by linkage disequilibrium or pleiotropy, between parasite resistance and ornament traits.

To conclude, we used a novel approach to test the hypothesis of parasite-mediated sexual selection put forward by Hamilton and Zuk in 1982. So far our data do not give unequivocal support for the idea that resistance and attractiveness are genetically correlated as envisaged by parasite-mediated sexual selection.

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