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# Hybrid vigour against parasites in interspecific crosses between two mice species

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The resistance and susceptibility to the intestinal pinworm *Aspiculuris tetraptera*, a natural parasite of the house mouse *Mus musculus*, is experimentally analysed using both the $F_1$ from wild-type mice of the two subspecies (*M. m. domesticus* and *M. m. musculus*) and the $F_1$ from different laboratory inbred mice. The results show that: (i) the $F_1$ from wild-type mice harbour a lower parasite load than the parental mice, suggesting a phenomenon of hybrid vigour; and (ii) the $F_1$ from inbred mice harbour parasite loads similar to the resistant parent, suggesting that resistance is inherited as a dominant feature in these laboratory mice. This analysis supports the hypothesis that recombinations occurring between the two mouse genomes (i.e. *M. m. domesticus* and *M. m. musculus*) are responsible for the hybrid dysgenesis observed in the natural hybrid zone between the two mice subspecies.

**Keywords:** hybrid vigour, interspecific crosses, mice, oxyuroids, parasitism.

## Introduction

Many authors have stressed the need for evolutionary biologists to study parasitism in host hybrid zones (Sage *et al.*, 1986a; Hewitt, 1988; Whitham, 1989; Coustau *et al.*, 1991; Moulia *et al.*, 1991, 1993; Le Brun *et al.*, 1992; Renaud *et al.*, 1992; Paige and Capman, 1993). Indeed, in one location and often the same environment, parasites are confronted on the one hand with the original parent genomes and on the other hand with a whole gradation of recombinant genomes. In this special situation, one can test hypotheses relating to the limits of parasite specificity, host susceptibility polymorphism, the determination (genetic vs. environmental) of parasite burden and the selective constraints exerted by parasite populations on the evolution of host populations.

The hybrid zone between the two mouse subspecies (*Mus musculus musculus* and *M. m. domesticus*), which crosses Europe from Denmark to Bulgaria (Boursot *et al.*, 1984), is characterized by a series of coincident clines about 40 km wide for autosomal enzyme markers and much narrower ones for sex chromosome markers (Hunt & Selander, 1973; Sage *et al.*, 1986b; Vanlerberghe *et al.*, 1986, 1988; Tucker *et al*, 1992; Dod *et al.*, 1993).

In addition to these genetic data, two investigations in the host hybrid zone have shown a high susceptibility to helminth parasites (cestodes, intestinal nematodes) of mice with recombinant genotypes when compared with parental mice (Sage *et al.*, 1986a; Moulia *et al.*, 1991). Furthermore, experimental infestations of parental and hybrid mice from the wild with the natural oxyuroid of the house mouse, *Aspiculuris tetraptera*, confirmed the genetic control of the resistance/susceptibility to those parasites, and thus reduced the importance of environmental parameters in the hybrid overinfection observed in nature (Moulia *et al.*, 1993).

These results support the 'evolutionary hypothesis' that during the adaptive radiation of the *Mus musculus* species, different coadapted gene systems have evolved between the two taxa, including those which control the resistance to parasites. As the genotypes of mice in the hybrid zone are 'mosaics' of recombined pieces of the *musculus* and *domesticus* genomes, and as $F_1$ hybrids no longer exist, we could not determine clearly when hybrid dysgenesis occurs. The current hypothesis is that genetic recombination may lead in some cases to the break-up of functional gene interactions.

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Consequently, in the present work, we studied the parasite susceptibility of $F_1$ mice, which contain both the parental haplotypes without any recombination between them. We thus performed experimental infestations of $F_1$ hybrids between wild-type *musculus* and *domesticus* mice for which resistance to *A. tetraptera* has been previously determined (Moulia *et al.*, 1993). Moreover, in order to obtain more information, we also repeated the same experimental study with five inbred strains and the $F_1$s from crosses of three of them. Individuals of the same strain are genetically identical, so even if their origins are uncertain (Bonhomme *et al.*, 1987), laboratory strains may allow us to understand the mechanisms occurring in natural populations.

## Materials and methods

### *Parasite*

The oxyuroid *Aspiculuris tetraptera* Schultz, 1924 (Nematoda, Oxyuridea) used in this study were taken from the wild and maintained through several successive cycles in 129/SV mice as described by Moulia *et al.* (1993).

### *Wild-type mice*

*Musculus* and *domesticus* mice were bred from pairs of wild-type mice of the two subspecies trapped outside the hybrid zone: MA, MRI and MRII are pairs of *musculus* mice, respectively, from Austria and Georgia; DFI and DFII are *domesticus* pairs of mice from France.

To obtain experimental $F_1$ hybrids, we produced crosses of young *domesticus* and *musculus* mice from the parental pairs above: DFI × MA, DFI × MRII, DFII × MRI and DFII × MRII.

### *Inbred mice*

We studied the parasite susceptibility of five strains of inbred mice (C3H/OU (C3), C57BL/6 (B6), DBA/2 (D2), BALB/C (C) (Iffa Credo, France), 129/SV (129) (Pasteur Institut of Paris)) and $F_1$ hybrids from crosses between three of them (129/SV× C3H/OU and 129/SV× C57BL/6).

### *Parasite phenotypes*

The phenotypes of resistance (or susceptibility) of these wild, inbred and $F_1$ mice to pinworms were determined according to the protocol described by Moulia *et al.* (1993). Briefly, the progeny of these parasite-free mice (treated with antihelminthic) were infested three times, using the same protocol, and their parasite burden was determined 24 days after the last infestation (in order to recover the adult parasites).

### *Data analysis*

Parasite loads of parental inbred or wild mice were compared with each other, and with those of their respective $F_1$s. Within each set of comparisons (i.e. parental mice and $F_1$), a parasite load threshold was determined in order to define categories analysed by the Fisher's exact test (Sokal & Rohlf, 1981), using the program GENEPOP (M. Raymond and F. Rousset, CNRS URA 327, Montpellier, France). The significance level for each test was adjusted to take into account the other tests using the sequential Bonferroni method as proposed by Holm (1979).

## Results

The parasite phenotypes of wild and inbred mice and their respective $F_1$s are presented in Table 1. From the data, it appears that: (i) the most resistant of the tested mice are found among inbred strains (i.e. C, C3 and B6); (ii) mice of strain 129 display the most susceptible phenotype of the five laboratory strains whereas B6 and C3 are the most resistant; and (iii) the $F_1$s from susceptible 129 and resistant C3 and B6 seem to show an intermediate phenotype between their two parents whereas wild $F_1$s between resistant mice appear more resistant than their *domesticus* and *musculus* parents.

Table 2 shows the results of Fisher's exact and sequential Bonferroni tests applied to wild parental mice and their $F_1$s. In the $F_1$s, the parasite distributions appear to be significantly different from the parental ones in seven of eight cases computed (Table 2). This confirms the higher resistance of these $F_1$ hybrids to parasites.

As far as inbred mice are concerned, we observed significant differences between the susceptible parent (i.e. 129) and the $F_1$s but not between the resistant parents (i.e. C3 or B6, respectively) and the $F_1$s (Table 3).

## Discussion

In previous work (Moulia *et al.*, 1993), we demonstrated the occurrence of a great variability in parasite loads of wild-type mice. Thus, the parasite loads observed in the studied inbred strains rank among the lowest, even for the strains which appear to be the most sensitive (i.e. 129 and D2, Table 1). To explain such a phenomenon, we can suggest that the 'resistant' allelic associations may have been selected in laboratory strains. Indeed, direct cycles of oxyuroids are favoured by contacts between animals. These contacts increase

**Table 1** Parasite phenotype of tested progeny of wild and inbred mice and their respective $F_1$ hybrids infested by *Aspiculuris tetraptera*

| | Pair of mice | Sample size | Mean parasite load | Standard error |
|---|---|---|---|---|
| *musculus* | MA | 13 | 60 | 9.0 |
| | MRI | 18 | 21 | 7.5 |
| | MRII | 8 | 31 | 8.3 |
| *domesticus* | DFI | 22 | 52 | 13.0 |
| | DFII | 22 | 72 | 20.8 |
| $F_1$ *domesticus* × *musculus* | DFI × MA | 7 | 12 | 6.2 |
| | DFI × MRII | 10 | 1 | 0.6 |
| | DFII × MRI | 20 | 8 | 5.5 |
| | DFII × MRII | 11 | 2 | 1.0 |
| Inbred strains | 129/SV (129) | 22 | 38 | 7.0 |
| | DBA/2 (D2) | 24 | 33 | 5.8 |
| | BALB/C (C) | 26 | 5 | 1.2 |
| | C3H (C3) | 11 | 2 | 1.6 |
| | C57BL/6 (B6) | 15 | 2 | 0.7 |
| $F_1$ of inbred mice | 129 × C3 | 31 | 11 | 2.3 |
| | 129 × B6 | 30 | 16 | 3.4 |

**Table 2** Statistical analyses of infestations of *Mus musculus musculus, M.m. domesticus* and their $F_1$s by *Aspiculuris tetraptera*

| | Threshold for Fisher's test (parasites per mouse) | Compared samples | Probability *P* of Fisher's exact text | Sequential Bonferroni test |
|---|---|---|---|---|
| DFI × MA | 50 | DFI/MA | 1(0) | NS |
| | | DFI/$F_1$ | 0.0174 (0.0007) | S |
| | | MA/$F_1$ | 0.0544 (0.0011) | NS |
| DFI × MRII | 10 | DFI/MRII | 0.1280 (0.0021) | NS |
| | | DFI/$F_1$ | 0.0004 (0.0001) | S |
| | | MR II/$F_1$ | 0.0119 (0.0005) | S |
| DFII × MRI | 20 | DFII/MRI | 0.7015 (0.0019) | NS |
| | | DFII/$F_1$ | < 0.0001 ( < 0.0001) | S |
| | | MRI/$F_1$ | 0.0030 (0.0003) | S |
| DFII × MRII | 20 | DFII/MFII | 0.1244 (0.0021) | NS |
| | | DFII/$F_1$ | 0.0008 (0.0002) | S |
| | | MRII/$F_1$ | 0.0182 (0.0007) | S |

Standard errors of *P* are given in parentheses; NS (nonsignificant), S (significant) results.

because of the promiscuity in captivity. Moreover, mice are maintained on litters soiled with faeces which contain parasite eggs: thus, exposure to parasites takes place daily. Because of the selective pressure of parasites in the laboratory, all the loci involved in resistance may actually act in inbred genomes as a group of loci inherited together as a single entity (i.e. a supergene).

The parasite loads of the $F_1$ hybrids from inbred strains contribute to our knowledge of the genetic determination of resistance to nematode parasites. They lead us to conclude that in an $F_1$, the resistance seems to act as a relatively dominant trait. These results are different from those of Wright *et al.* (1988) on resistance and susceptibility to *Schistosoma*

**Table 3** Statistical analyses of infestations of hybrid mice (129, B6 or C3, and their $F_1$s) by *Aspiculuris tetraptera*

| | Compared samples | Probability ($P$) of Fisher's exact test | Sequential Bonferroni test |
|---|---|---|---|
| 129 × B6 | 129/B6 | 0 (0) | S |
| | 129/$F_1$ | 0 (0) | S |
| | B6/$F_1$ | 0.0772 (0.0012) | NS |
| 129 × C3 | 129/C3 | 0.0002 (< 0.0001) | S |
| | 129/$F_1$ | 0 (0) | S |
| | C3/$F_1$ | 0.3021 (0.0017) | NS |

The threshold is 20 parasites per mouse for all the Fisher's exact tests. The abbreviated designations of inbred strains are given in the text. The standard errors of $P$ are shown in parentheses; NS (nonsignificant), S (significant) results.

*mansoni* and *S. japonicum*. Indeed, contrary to our results, the $F_1$ between resistant WEHI 129/J and susceptible BALB/C suggests that the inheritance of susceptibility is a dominant feature. However, these results on laboratory mice could not be found in wild animals. Roush & Croft (1986) showed that the determination of pesticide resistance in insects and mites was quite different in the field and in the laboratory.

This mouse hybrid zone is the only situation where a higher susceptibility to parasites of hybrids in comparison to the parental taxa has been described (Sage *et al.*, 1986a; Moulia *et al.*, 1991, 1993). Several studies in other systems have revealed that one of the two parental taxa is more sensitive to parasites than the other. Their natural hybrids display a gradual change in susceptibility correlated with their genome introgression. The *Mytilus edulis–M. galloprovincialis* and *Barbus barbus–B. meridionalis* hybridizing complexes are two good examples (Coustau *et al.*, 1991; Le Brun *et al.*, 1992). The parasite distributions clearly have a genetic basis (respectively, physiological and ethological).

According to the study of Whitham (1989) on a *Populus* hybrid zone based on the leaf morphology, hybrids seemed to be more susceptible to aphid parasites than parental trees. However, a more recent genetic study refutes Whitham's results and shows that, in this cottonwood hybrid zone, one parent is more susceptible than the other one and their hybrids (Paige & Capman, 1993).

Our present study clearly shows that not only are $F_1$s resistant to *A. tetraptera* but also that they are more resistant than their parents. We can deduce that there is hybrid vigour or heterosis. Behnke (1975) showed that immunity is involved in the control of the oxyuroid infrapopulations in mice. As many genes are known to act within the immune system, the control of this phenomenon is probably polygenic in wild mice. It is then possible that complementation between alleles at each locus belonging to those interactive gene systems would be involved in the origin of this hybrid vigour.

As far as we know, no example of heterosis in $F_1$ hybrids has been described in natural situations. Moreover, a parasitological study of natural $F_1$ hybrids of Cyprinidae fishes (Dupont & Crivelli, 1988) reveals, unlike our mouse model, a higher susceptibility to Monogenea of those $F_1$s when compared with their parents.

Our study clearly shows that when comparing parasitism in the hybrid zone between *musculus* and *domesticus* mice, hybrid dysgenesis does not appear in $F_1$s which are highly resistant to the parasite. If genetic complementation leads to this hybrid heterosis as far as resistance to parasites is concerned, does complementation also act in the expression of other phenotypic features of these peculiar hybrids? It is most probable that simple recombination between the two mouse genomes would explain the parasite susceptibility in $F_2$ hybrids. Thus, chromosomal rearrangements are not needed to be involved as was suggested for the $F_2$ breakdown of two taxa of the grasshopper *Caledia captiva* (the Moreton and Torresian subspecies) (Shaw *et al.*, 1985, 1993).

Thus, results of genetic and parasitological studies (Vanlerberghe *et al.*, 1986, 1988; Sage *et al.*, 1986a; Moulia *et al.*, 1991, 1993; Tucker *et al*, 1992; Dod *et al.*, 1993) lead us to propose a hypothesis that the recombinations between the two parental genomes might break down functional gene systems specific to each of them. The new allelic associations may then generate the reduced fitness of hybrids, which may be indicated by higher parasite loads.

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