Immune stress and facultative sex in a parasitic nematode

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Abstract

It has been suggested that sexual reproduction in parasites may be advantageous because it helps evade genotype-specific host immune responses. Indirect support for this hypothesis has recently come from work on *Strongyloides ratti*, a parasitic nematode of rats that develops and reproduces sexually or asexually. In this species, host immune responses against *S. ratti* lead to a higher proportion of individuals reproducing sexually. However, an alternative explanation for these results is that sex is favoured by general environmental stress, including host responses against antigen sources other than *S. ratti*. Here we test this hypothesis, by determining how host immunity against two other parasitic nematode species (*Nippostrongylus brasiliensis* & *Strongyloides venezuelensis*) and commonly used mammalian antigens (sheep red blood cells) affects the likelihood of *S. ratti* larvae developing sexually. Our results show that increased levels of sex occur in response to immune responses generated against these other species, and not just host immunity elicited by *S. ratti*. This is consistent with the idea that sex is favoured under stressful conditions, and does not support the immune evasion hypothesis.

Introduction

One of the greatest challenges for evolutionary biology is explaining the widespread occurrence of sexual reproduction throughout the animal and plant kingdoms (Kondrashov, 1993; Hurst & Peck, 1996; West *et al*., 1999a). The currently most plausible explanations are that it either accelerates adaptation to a changing environment or allows deleterious mutations to be eliminated more efficiently. The most popular environmental hypothesis, the Red Queen, states that sex provides an advantage in biotic interactions (Bell, 1982; Bell & Maynard Smith, 1987). Red Queen models usually assume that selection by coevolving parasites against common host genotypes provides the antagonistic driving force that gives sex an advantage (Jaenike, 1978; Hamilton *et al*., 1990).

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An analogous version of the Red Queen hypothesis is that sexual reproduction in parasites may be advantageous because it helps evade host immune responses (Gemmill *et al*., 1997). The idea here is that hosts develop protective immunity specific to certain (common) parasite genotypes that they have encountered (Read & Viney, 1996), and that by having sex, the parasite is able to generate new genotypes which can evade this genotype-specific immunity. (It is well known that specific immune responses may be directed against a parasite species that has been previously encountered; here we are concerned with a different possibility, that a component of the immune response may be directed against specific parasite genotypes within a species; Read & Viney, 1996.) Indirect support for this hypothesis has recently come from work on *Strongyloides ratti*, a parasitic nematode of rats, larvae of which are able to develop and reproduce sexually or asexually. Gemmill *et al*. (1997) showed that larvae from hosts that have acquired immunity against *S. ratti* are more likely to develop into sexual adults.

However, there is an alternative explanation for these results. Stressful conditions frequently induce sexual

reproduction in a range of other species that only occasionally have sex, such as yeast, daphnids and algae (Bell, 1982; Dacks & Roger, 1999). Sex may be favoured under stressful, harsh or crowded conditions for a number of reasons, including: (a) it increases the likelihood of producing offspring with sufficiently few deleterious mutations to survive (Kondrashov, 1984); (b) it increases the likelihood of producing individuals with allele combinations that are adapted to the stressful environment; (c) the costs of sexual reproduction, such as the extra time taken, are reduced, and so if sex is favoured only occasionally, then this is the best time to do it (Burt, 2000); (d) sexual forms (e.g. zygotes) may be more resistant to stress (Vrijenhoek & Pfeiler, 1997; Rispe *et al*., 1998), and (e) stress may correlate with times when inbreeding is less likely. Several of these ideas could explain why anti-*S. ratti* immune responses favour sex in *S. ratti*. For example, immune stress will be greater when there are a larger number of parasites within the host, which may also be when the probability of inbreeding is reduced. Similarly, the fitness costs of a given number of deleterious mutations may be higher in immune hosts.

The Red Queen and Environmental Stress hypotheses make contrasting predictions about the type of immune responses which will influence the likelihood of *S. ratti* larvae developing sexually. Under a pure Red Queen view, sexuality should be triggered by immunity directed against particular *S. ratti* genotypes. In contrast, if sex is a response to general environmental stress, any protective host immunity, even that generated by different antigenic sources, should increase the likelihood of *S. ratti* larvae developing sexually. Here we test whether host immunity against two other parasitic nematode species (*Nippostrongylus brasiliensis* and *Strongyloides venezuelensis*) and a commonly used mammalian antigen (sheep red blood cells) affects the likelihood of *S. ratti* larvae developing sexually.

Methods

Study organisms and parasitology

S. ratti (Nematoda: Rhabditida) is a diploid parasite of rats. Parasitic adults inhabit the small intestine and are exclusively parthenogenetic females. These females mitotically produce eggs which are passed out in faeces (Viney, 1994). Development outside the host can be completed with or without sex. The eggs passed out develop into infective, skin-penetrating, third-stage larvae, either directly (asexual or homogonic development) or via free-living dioecious adults (sexual or heterogonic development). Free-living adults mate outside the host and reproduce by conventional meiosis and syngamy (Viney *et al*., 1993). All offspring from these matings develop into infective third-stage larvae. The proportion of larvae developing into sexual adults is under both environmental (Gemmill *et al*., 1997) and genetic (Viney, 1996) control. The host environment experienced by a parasitic female will predict the host environment that their offspring will encounter when: (a) territorial behaviour leads to the worm larvae infecting the same rat host, or (b) population structuring leads to the worm larvae infecting rats that have been infected previously with worms with similar genotypes.

The congeneric nematode species *S. venezuelensis* is also a natural parasite of rats, and has a life cycle similar to that of *S. ratti*. *N. brasiliensis* (Nematoda: Strongylida) is also a skin-penetrating gastrointestinal parasite of rats, but it has an obligate and conventional sexual reproduction inside the rat, and no replication outside the rat.

Hosts used were female 6-week-old Wistar rats (Banton and Kingman, UK). Food and water were provided *ad libitum* throughout the course of the experiment. The isofemale *S. ratti* line ED5 (Viney, 1996) was used throughout. These lines have been maintained for a large number of generations in Wistar rats. Infective larvae of *N. brasiliensis* were obtained from a laboratory line maintained at the University of Edinburgh, infective larvae of *S. venezuelensis* from Professor Haruhiko Maruyama (Nagoya City University Medical School, Japan) and sheep red blood cells were provided by the Scottish Antibody Production Unit (Carluke, UK). All innoculations were subcutaneous into the scruff of the neck and all worm immunizations involved the injection of live, untreated infectious larvae.

Lifetime fecundity (worm output) was estimated by collecting faeces from each rat overnight and dividing equally between three culture plates as described in Viney *et al*. (1992). These cultures were incubated for 2 days at 25 °C, after which mature worms were washed from culture plates, collected, counted and the relative proportion of sexual and asexual offspring determined as described by Gemmill *et al*. (1997).

Experiments

We conducted two experiments. The first involved immunization with *N. brasiliensis*, *S. ratti* and sheep red blood cells. In the second, we immunized with *N. brasiliensis*, *S. ratti* and *S. venezuelensis*. In both experiments, immunizing injections consisted of sheep red cells or live worms suspended in 0.4–0.5 mL of 0.8% w/v NaCl. Faecal egg counts by a Modified McMasters technique (Whitlock, 1948) were used to determine whether immunizing infections became patent. Immunizing infections were drug-cleared by dosing all rats (including uninfected rats) with 0.11 mL of 17.6% w/v thiabendazole suspension (Thibenzole, MSD AGVET, a broad-spectrum antihelminthic) by oral intubation on two consecutive days. All rats were subsequently monitored to confirm that the infections had been cleared, and subsequently challenged with 500 infective third-stage larvae *S. ratti* larvae (iL3s) suspended in physiological saline.

In the first experiment, five rats were inoculated with 10 *S. ratti* larvae (for how infection level varies with dose see Gemmill *et al*., 1997), five with 100 *N. brasiliensis* larvae, and five with 1000 *N. brasiliensis* larvae. Large numbers of *N. brasiliensis* were used in order to obtain a detectable increase in the immune response against subsequent infection by *S. ratti*. A further 15 rats were injected with saline. All infections became patent. Three weeks later, five of the 15 rats were inoculated with 10^6 red blood cells, and five with 10^7 red blood cells. At this time, all other animals in the experiment were injected with saline to control for any effects of injection. All animals were drug-treated 7 days later, and then challenged with *S. ratti* 2 weeks after that. Subsequent worm output was determined on days 6, 7, 8, 10, 11, 14, 18 and 21 post-challenge.

In the second experiment, 10 rats were infected with 10 *S. ratti* larvae, 10 with 2000 *N. brasiliensis* larvae, 10 with 1000 *S. venezuelensis* larvae, and 10 with physiological saline. Infections with *Strongyloides* spp. became patent, but the *N. brasiliensis* infections did not. Rats were drug treated 28 days post-infection and then challenged with *S. ratti* 2 weeks later. Subsequent worm output was determined on days 5, 6, 8, 9, 12, 13, 15, 16, 19 and 23 post-challenge.

Statistical analysis

The total number of offspring produced during the infections (total worm output) and the proportion of these that were sexual were estimated by numerical integration of the area under the nightly worm output by time curves (see Gemmill *et al*., 1997; Gemmill & West, 1998). Thus, each infection contributed a single degree of freedom to the analysis. The data for the two experiments were analysed separately, and also combined, whilst controlling for differences between the experiments; in all cases the same results were obtained. Data were analysed in the GLIM statistical package (Crawley, 1993). Proportion data frequently have non-normally distributed error variances. To deal with this problem, we first analysed our proportion data with a general linear model analysis of deviance, assuming binomial errors, using a logit link function available in GLIM. After fitting this model, the ratio of residual deviance to the residual degrees of freedom was >300, demonstrating considerable overdispersion and suggesting that the data did not fit the binomial error assumption (Crawley, 1993). All proportion data were therefore angular (arcsine square-root) transformed and analysed assuming a normal error structure. In all subsequent analyses, the residuals showed no obvious patterns, and plots of the ranked residuals against the standard normal deviates were straight lines, supporting the assumption of normal errors. All analyses were carried out with stepwise deletion from the maximal model (Crawley, 1993) and interaction terms are only given if significant.

Results

We first examined the consequences of immunization with *S. ratti*. Immunization with *S. ratti* led to a lower total worm output than the nonimmunized control rats ($F_{1,21} = 30.05$, $P < 0.01$) and a higher proportion of these worms were sexual ($F_{1,22} = 9.89$, $P < 0.01$). These results were also significant when analysing each of the two experiments separately, and confirm the phenomena reported by Gemmill *et al*. (1997). There were significant differences between the experiments in the total worm output ($F_{1,22} = 4.42$, $P < 0.05$), but not in the proportion developing sexually ($F_{1,22} = 0.84$, NS).

We then examined the consequences of immunization with *S. venezuelensis*, *N. brasiliensis* and sheep red blood cells. Overall, immunization with these led to a lower total worm output than nonimmunized control rats ($F_{1,52} = 17.14$, $P < 0.01$) and a higher proportion of these worms were sexual ($F_{1,52} = 4.06$, $P < 0.05$). The differences in total worm output and proportion sexual caused by these immunizations were smaller than those caused by immunization with *S. ratti* (Fig. 1), but show that increased sex will occur due to an immune response elicited by antigenic sources other than *S. ratti*. There results were obtained when controlling for significant differences between the experiments in the total worm output ($F_{1,52} = 8.93$, $P < 0.01$), and the proportion developing sexually ($F_{1,52} = 4.96$, $P < 0.05$).

The increased proportion developing sexually in the non-*S. ratti* treatments was due to the *S. venezuelensis* treatment in the second experiment. Considering only the rats immunized with *N. brasiliensis* and sheep red blood cells, the proportion of worms developing sexually did not differ significantly from the nonimmunized control rats ($F_{1,42} = 1.58$, NS). Analysing each of the treatments and experiments separately, only immunization with *S. venezuelensis* led to a significant increase in the proportion of worms developing sexually ($F_{1,18} = 4.49$, $P < 0.05$).

Interestingly, in both experiments, the greater the reduction in total worm output, the higher proportion of those worms that developed sexually (Experiment I: $F_{1,28} = 6.74$, $P < 0.05$; Experiment II: $F_{1,33} = 5.97$, $P < 0.05$; Fig. 1). This variation in the proportion of worms developing sexually is mainly due to between-treatment variation: when the significant variations between experiments ($F_{1,59} = 6.31$, $P < 0.05$) and treatments ($F_{4,59} = 4.54$, $P < 0.01$) are controlled for, there is no additional affect of total worm output ($F_{1,58} = 3.32$, NS). The negative correlation between proportion sexual and total worm output remains significant even when the data are analysed with each of the immunization treatments averaged to a single data point (Experiment I: $F_{1,4} = 33.34$, $P < 0.01$; Experiment II: $F_{1,2} = 19.27$, $P < 0.05$; Fig. 1), and the treatment with prior immunization by *S. ratti* larvae removed from the analysis (Experiment I: $F_{1,3} = 11.59$, $P < 0.05$).

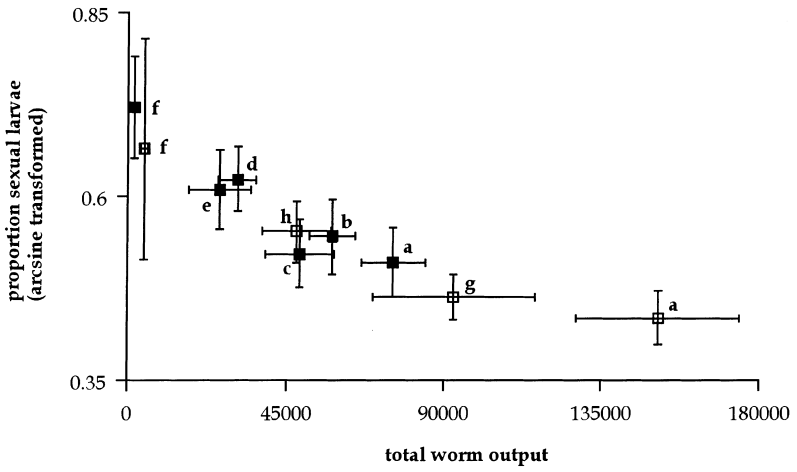

Fig. 1 The proportion of *S. ratti* larvae that develop sexually (arcsine transformed) plotted against the total number of worms emerging from the infection. Each data point represents the mean across rats given the immunization treatments: (a) control (saline); (b) 10^6 sheep red blood cells; (c) 10^7 sheep red blood cells; (d) 1000 *N. brasiliensis* larvae; (e) 100 *N. brasiliensis* larvae; (f) 10 *S. ratti* larvae; (g) 2000 *N. brasiliensis* larvae, and (h) 1000 *S. venezuelensis* larvae. Closed squares, expt 1; open squares expt 2. Error bars are ±1 SEM; some bars are smaller than the symbol.

Discussion

Our results show that, in *S. ratti*, the proportion of offspring that develop sexually is positively correlated with the strength of protective immunization (decrease in worm output) resulting from infections of *S. ratti*, other nematode species (*N. brasiliensis* & *S. venezuelensis*) and mammalian antigens (sheep red blood cells) (Fig. 1). This demonstrates that increased levels of sex occur due to immune responses elicited by different antigenic sources, and not just *S. ratti*. On the assumption that immunization with antigens other than *S. ratti* do not increase the ability of hosts to mount an immune response against specific *S. ratti* genotypes (although they can and do increase the ability to mount a response against *S. ratti* in general), these results: (a) are consistent with the idea that increased sex is favoured under stressful conditions, and (b) do not support the Red Queen hypothesis that sex allows offspring to avoid genotype-specific immune responses.

However, the Red Queen mechanism may still be important if: (1) worms do not have a perfect cue, and increase their amount of sex in response to any immune response that affects them because there is a reasonable likelihood that it will be in response to *S. ratti* infection, and/or (2) multiple mechanisms are at work (Howard & Lively, 1994; West *et al*., 1999a). In order to distinguish between these possibilities, and the variety of reasons why sex may be favoured under stressful conditions, future work must test the assumptions of several explanations, such as the relative importance of genotype-specific and nongenotype-specific immunity, and whether the cost of a high mutation load is greater under more stressful conditions.

Work on facultative variation in behaviour has provided some of the most conclusive evidence for adaptive explanations of life history evolution, such as sex allocation theory (Charnov, 1982; Godfray & Werren, 1996; West *et al*., 2000). Will facultatively sexual species, such as *S. ratti*, be similarly decisive regarding explanations of sex? Two lines of argument dampen our enthusiasm. First, as discussed earlier, the timing of sex may be determined by a number of factors and not necessarily to do with its function (see also Burt, 2000). This is particularly so in cases where only occasional sex has been argued to provide approximately the same benefits as obligate sex (Peck, 1994; Green & Noakes, 1995; Hurst & Peck, 1996; Otto & Barton, 1997) and so the timing of sex will not be important. A crucial point here is that mutational and some environmental models predict that the immediate result of sex is a decrease in fitness (Charlesworth & Barton, 1996; West *et al*., 1998), and that the advantages of sex take several generations to arise (Barton, 1995; but see Peters & Lively, 1999). Second, the strength of selection in favour of sex must be different in species which only occasionally undergo a sexual cycle (West *et al*., 1999a,b). For example, even if the Red Queen view of parasite sex is correct, parasites subject to relatively weak genotype-specific immunity may require only occasional sex, whereas obligatory sex might be favoured by strong genotype-specific immunity. All other parasitic nematodes of vertebrates are obligatory sexual (Poinar & Hansen, 1983). Thus, it may be that the strength of selection in favour of sex is greatest in obligatory sexual species, where it is hardest to measure the fitness consequences of not having sex.

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