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HOST IMMUNE STATUS DETERMINES SEXUALITY IN A PARASITIC NEMATODE

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Abstract.—We examine the hypothesis that sexual reproduction by parasites is an adaptation to counter the somatic evolution of vertebrate immune responses. This is analogous to the idea that antagonistic coevolution between hosts and their parasites maintains sexual reproduction in host populations. *Strongyloides ratti* is a parasitic nematode of rats. It can have a direct life cycle, with clonal larvae of the wholly parthenogenetic parasites becoming infective, or an indirect life cycle, with clonal larvae developing into free-living dioecious adults. These free-living adults produce infective larvae by conventional meiosis and syngamy. The occurrence of the sexual cycle is determined by both environmental and genetic factors. By experimentally manipulating host immune status using hypothyroid mutants, corticosteroids, whole-body γ -irradiation and previous exposure to *S. ratti*, we show that larvae from hosts that have acquired immune protection are more likely to develop into sexual adults. This effect is independent of the method of manipulation, larval density, and the number of days postinfection. This immune-determined sexuality is consistent with the idea that sexual reproduction by parasites is adaptive in the face of specific immunity, an idea which, if true, has clinical and epidemiological consequences.

Key Words.—Antagonistic coevolution, evolution of sex, facultative sexuality, immunology, Red Queen, *Strongyloides ratti*.

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Sex continues to puzzle evolutionary biologists. Despite the twofold reproductive advantage of parthenogenesis, sexual reproduction is common in nature. Numerous solutions of this paradox have been proposed (Kondrashov 1993). A popular hypothesis, at least in some circles, is that antagonistic coevolution between hosts and their parasites maintains sex in host populations (the Red Queen model; Jaenike 1978; Hamilton 1980; Bell and Maynard Smith 1987; Lively and Apanius 1995). The idea is that the short generation time of parasites relative to their hosts facilitates the selection of parasites closely adapted to common host genotypes. In these situations, clonal offspring are disproportionately vulnerable to parasitic attack, and the advantage of parthenogenesis is eroded. Sufficiently strong frequency-dependent selection of this type can maintain sex in hypothetical host populations (Hamilton et al. 1990; Howard and Lively 1994; Lively and Apanius 1995). Empirical evidence lends some support, but much of it is also broadly consistent with other hypotheses (Bell 1982; Kondrashov 1993; Hurst and Peck 1996).

The effects of host-parasite coevolution on sexuality have been studied almost exclusively in relation to reproduction by hosts. But why do so many parasites have sex? One possibility is that parasites are host to parasites of their own. Alternatively, sex might be an adaptation to combat facultative antiparasite defenses. Higher vertebrates have sophisticated systems of immune surveillance and protection. These can develop lasting resistance against an almost infinite succession of antigenic types within a single host generation. In many cases, rapid counteradaptation by parasites can occur because of their relatively short generation times and high population densities, and a variety of adaptations may assist this. In bacteria for example, various intragenomic mechanisms generate phenotypic variation relevant to immune evasion (Moxon et al. 1994). However, in other cases, the scope for sufficiently rapid counteradaptation may be limited. Many parasites, including most helminths, have relatively slow, or

even no, within-host replication. In these cases, a natural possibility arising from pathogen-driven models of host sex is that sexual reproduction in parasite populations is maintained as a diversity-generating mechanism to counter the rapidly changing selection imposed by acquired immunity (Bell 1982; Hamilton et al. 1990, footnote, p. 3567). A rare genotype is initially favored but selected against when it becomes sufficiently common to reinfect hosts previously exposed to it. Where large numbers of siblings infect the same host simultaneously, sexual reproduction may have an added advantage if clonal progeny are more likely to trigger density-dependent, genotype-specific immune responses (apparent competition between sibs). Suggestively, purely asexual life cycles are apparently rarer among eukaryotic parasites of higher vertebrates than among related taxa that are free-living or which parasitize plants or invertebrates (Bell 1982; Poinar and Hansen 1983).

The hypothesis that sexual reproduction in some parasitic species is an adaptation to counter the somatic evolution of vertebrate immune responses rests on two assumptions. First, that host immunity has severe effects on parasite fitness. This is well known. Second, that acquired immunity is most effective against the genotype that originally elicited it (genotype-specific immunity). This is known for microparasites such as those that cause malaria and influenza (Brown 1990; Webster et al. 1992). In helminth infections, genotype-specific immunity has been little studied, but there is arguably as much evidence of its existence as there is to the contrary. Isolate-specific responses have been reported for at least four helminth species (e.g., *Strongyloides ratti* [Carter 1986]); experiments which fail to find evidence of genotype-specific responses typically involve incomplete experimental designs and analyses of variation within single, highly inbred laboratory lines (Read and Viney 1996).

A number of testable predictions arise. Using the laboratory model described below, we are currently testing the cen-

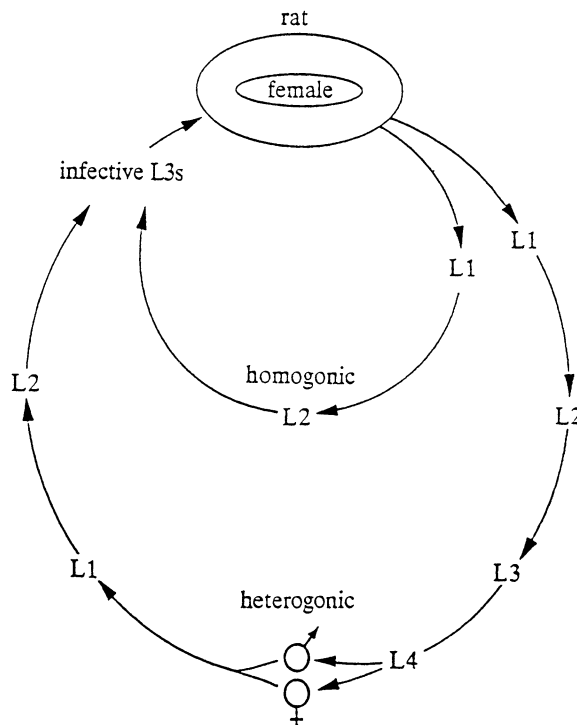


FIG. 1. Life cycle of *Strongyloides ratti*, showing the heterogonic (sexual) and homogonic (clonal) routes. Adult worms in the rat are always parthenogenetic females. Larval stages are denoted by L. Reproduced from Viney et al. (1993).

tral one: that sex and recombination enhance parasite fitness by allowing individuals to evade or survive immune responses acquired against parental genotypes. Here we report a test of another prediction. Where facultative sex is maintained as a strategy to counter genotype-specific immune responses, we expect greater investment in sexual reproduction when invading parasites are targeted by immune responses acquired against parental genotypes. Conversely, where no protective immune responses develop, sex should be less common.

Study System

The nematode *S. ratti* Sandground 1925 (Rhabditida: Strongyloididae) is a diploid parasite of rats (Fig. 1). Parasitic adults inhabit the small intestine and are exclusively parthenogenetic females. Mitotically produced eggs (Viney 1994) pass to the external environment in faeces and develop either as free-living, dioecious adults (heterogonic development) or as infective, skin-penetrating larvae (homogonic development). Free-living adults mate in the external environment and reproduce by conventional meiosis and syngamy (Viney et al. 1993). All offspring from these matings develop into infective third stage larvae (iL3s). Thus, in *S. ratti*, the life cycle can be completed with or without sex. The proportion of larvae developing into sexual adults is under both environmental (Schad 1989) and genetic control (Viney 1996). Some field isolates produce no sexual adults in the laboratory, whereas others are more likely to produce sexual morphs particularly towards the end of an infection (Viney et al.

1992). Artificial selection for heterogonic development is highly successful (Viney 1996) which, together with population differences in the occurrence of sexual morphs, provides good evidence of the "balance" argument: in natural populations, natural selection must be maintaining the sexual cycle (Williams 1975). Unlike some other facultatively sexual organisms (e.g., *Daphnia*; Bell 1982), sexual morphs of *S. ratti* are not specifically adapted for dispersal or for long-term survival. Free-living adults remain in close association with the faecal mass and are sensitive to environmental conditions. In the laboratory, they live for at best a week, typically less. In contrast, iL3s are nonfeeding (the mouth is completely sealed), migrate away from faeces, are environmentally resilient and can survive for months in this developmentally arrested state until contact with a suitable host. They (and the iL3s of other parasitic nematodes) are considered as analogous to the dauer stage of *Caenorhabditis elegans* (Hawdon and Schad 1991), which is highly resistant to desiccation, chemical attack, and all manner of environmental insult (Riddle 1988). Thus, all the evidence points to iL3s rather than free-living adults as being the dispersal stages.

In laboratory infections initiated with large numbers of iL3s, the number of worms emerging from the faeces of infected rats declines with time, and "self-cure" typically results in three to six weeks. Both iL3s and parasitic adults elicit immune responses that protect against subsequent infection, suppressing larval establishment as well as parasite survival and fecundity (reviewed by Dawkins 1989; Nawa et al. 1994). In the absence of reinfection, immunity gradually wanes (Bell et al. 1981).

Most work on environmental influences on development in *Strongyloides* spp. has involved manipulation of extra-host factors such as temperature, crowding, and food availability (reviewed by Schad 1989). This has led to the general conclusion that heterogonic development occurs when conditions outside the host are favorable and those inside are unfavorable (Moncol and Triantaphyllou 1978; Schad 1989). However, available evidence that host immunity influences developmental route is at best suggestive. Most comes from the observation that in infections of pigs with *Strongyloides*, sexual morphs become more frequent when hemagglutinating antibody levels rise and worm output declines. These correlations were confounded with the age of the infections and density of larvae in faeces as well as host morbidity and mortality from uncontrolled viral infections. Experimental manipulations have been performed (Varju 1966; Moncol and Triantaphyllou 1978) but involved unreplicated treatments. A comparison of *Strongyloides stercoralis* (a parasite of humans) in an immunosuppressed dog and an intact dog found no effect of host immune status on the frequency of sexual morphs (Shiwaku et al. 1988).

Immune Manipulations

To determine whether investment into sexual reproduction rises as host immunity develops, we measured the occurrence of free-living sexual morphs following experimental alteration of host immune status. Any particular method of immune manipulation may have additional physiological effects, not all of which are known or controllable. We therefore

used the following variety of techniques to minimize the chance of treatment effects being due to any factor other than alteration of immune status per se. (1) Corticosteroid treatment. This causes suppression of immune responses resulting in prolonged larval output, delayed expulsion of parasitic adults and reversal of immune-mediated damage to parasitic adults in *S. ratti* infections (Moqbel and Denham 1978; Olson and Schiller 1978). (2) Whole-body γ -irradiation. The procedure ablates lymphocytes and is routinely used by immunologists to immunosuppress rodents (Chan 1980). (3) Congenitally hypothyroid (nude) rats. Rats and mice homozygous for the hypothyroid condition have only a remnant thymic stump and are dysfunctional with respect to T-cell maturation. Heterozygotes do not express the hypothyroid condition and are otherwise normal. We are unaware of any studies of *S. ratti* in nude rats, but in nude mice, *S. ratti* infections are greatly prolonged, worm burdens are heavier, and the acquisition of protective immunity is diminished (Dawkins et al. 1982). (4) Previous exposure. Strong protective responses are elicited by infection with *S. ratti*. These reduce the size and duration of subsequent challenge infections, and the extent of this protection is dependent on the number of iL3s in immunizing inocula (e.g., Sheldon 1937; Uchikawa et al. 1989, 1991).

Differences in larval density unavoidably result from differences in host immune status. In *Strongyloides* spp., larval density can influence developmental route, possibly through its effects on per capita food availability (Hansen et al. 1969). In many nematodes, larval density influences sex ratio (Poinar and Hansen 1983). To determine whether developing larvae were responding to effects of immune status per se, we varied the number of iL3s in inocula to generate variation in larval density in faecal cultures while simultaneously varying host immune status.

MATERIALS AND METHODS

Parasitology

Unless otherwise stated, randomly bred, size-matched (100–150 g) female Wistar rats (Bantin and Kingman UK) were used in all experiments, with food and water provided ad libitum. Where hypothyroid or immunosuppressed rats were used, all animals (experimental and control) were given a wide-spectrum antibiotic (Baytril, Bayer) at a concentration of 0.01% w/v in drinking water. Worms were maintained by serial passage in female Wistar rats. The isofemale *S. ratti* line “Ed 5 Heterogonic” (Viney et al. 1992; Viney 1996) was used throughout. All individuals in an isofemale line are descended from a single parthenogenetic female. Infection of experimental animals was either via the percutaneous route, to mimic natural conditions (Tindall and Wilson 1988), or by subcutaneous injection. In percutaneous infections, rats were anaesthetized by intraperitoneal injection with 37 to 50 mg/kg body weight of pentobarbitone (Sagatal, Rhone Merieux), a patch of fur clipped from the flank and iL3s placed directly on dampened skin. For both infection routes, inocula of more than 100 iL3s were prepared by dilution. Smaller inocula were prepared by counting iL3s individually under a binocular microscope.

Faeces from experimental animals were sampled repeat-

edly at time points throughout the infections as follows. Faeces were collected overnight onto damp paper, weighed and a maximum of five 1.5 (\pm 0.1) g cultures made per animal and incubated for three days at 19°C (following Viney et al. 1992). After three days at 19°C, worms in a culture of infected rat faeces have matured into iL3s or free-living adults. At this point, offspring of free-living adults have not yet matured and the proportion of a parasite's offspring that have developed into sexual adults can be established. Worms were washed from each set of three-day cultures, and the number present estimated by calculation from a diluant of the collected worms. This number was adjusted for weight of faeces collected to give the number of worms produced per animal per night. Repeated samples of freshly agitated worm-containing suspension were examined until up to 250 worms from each set of cultures had been typed as either sexual adults or iL3s. Positions of collecting cages, the order in which cultures were made and subsequently processed, and their positions in incubators, were randomized throughout.

Experimental Manipulations

Corticosteroids.—Six rats were infected by subcutaneous injection of 500 iL3s and infections monitored from day 7 postinfection (PI) onwards. On day 18 PI, animals were arbitrarily assigned to control or treatment groups ($n = 3$ in each). Treated rats received 10 mg/kg mean body weight of betamethasone (Betsolan, Pitman Moore) by subcutaneous injection. Control animals received an equal volume of sterile saline. Treatment continued daily until day 27 PI (inclusive).

Whole-Body γ -Irradiation.—Three rats were exposed to 6.5 Grays from a $^{137}\text{Caesium}$ γ -emitting source, three days prior to percutaneous infection with 500 iL3s. This level of irradiation disrupts immune function until lymphocyte populations are replenished. Three control animals were treated comparably, but were not irradiated.

Congenitally Hypothyroid (Nude) Rats.—Infections in four homozygous (hypothyroid, HsdHan:NZNU-rnu^N) rats were compared with those in four heterozygous (thymic, HsdHan:NZNU-rnu/+) rats. Animals four to six weeks old (Harlan Olac, UK) were infected by subcutaneous injection with 500 iL3s.

Previous Exposure.—Groups of three rats were exposed to 0, 1, 10, or 50 iL3s by percutaneous infection. Infections were subsequently monitored to confirm patency. On days 27 and 28 PI, all rats were dosed with 0.11 ml of 17.6% w/v thiabendazole suspension (Thiabendazole, MSD AGVET) by oral intubation to clear any remaining parasites. Controlled experiments (Read, unpubl. data) demonstrated that this anthelmintic regime results in no subsequent worm output. One week later (35 d after initial infection) all rats were challenged by percutaneous infection with 250 iL3s.

Larval Inocula \times Corticosteroids.—Infections were initiated by subcutaneous injection of 100 or 500 iL3s and daily injections with betamethasone or saline administered on days 8 through 13 PI (inclusive) in a cross-factored experimental design with three animals per group.

Larval Inocula \times Whole-Body γ -Irradiation.—A similar cross-factored experiment was conducted, with irradiated or

nonirradiated rats infected percutaneously with 50 or 500 iL3s, again with three animals per group.

Statistical Analysis

We define "worm output" as the number of worms produced per animal per night. Total worm output, the total number of worms produced during an infection, was estimated by numerical integration of the area under the worm output by time curves for each infection. These total counts, and daily counts, were analyzed by conventional analysis of variance (ANOVA) following log transformation. Plotted values and standard errors of the proportion of sexual morphs are parameter estimates from logistic regression using Williams correction for overdispersion (Crawley 1993). Worm output towards the end of an infection drops dramatically. Where fewer than 50 worms from an infection could be found, estimates of the proportion of sexual morphs had uninformatively large standard errors, especially given the small number of infections per experimental group. Estimates based on fewer than 50 worms were therefore excluded from the analysis; in no case were the excluded estimates inconsistent with our conclusions. To ensure comparable standard errors, the mean daily proportion of sexual morphs for an experimental group was plotted only when worm counts from all rats in that group were ≥ 50 . Repeated measures of the proportion of sexual morphs from different time points in infections were used to calculate a mean, such that each infection contributed a single degree of freedom. These means were calculated as follows. Only sampling days on which all infections in an experiment produced sufficient worms to type at least 50 were included. This ensured that all treatment groups were represented by equal numbers of rats at all time points and eliminated noise due to low worm counts. For each infection, a mean logit score ($\log_e[S/(n - S)]$, where n = number worms counted, and S = number of those that were sexual morphs) across sampling days was estimated by logistic regression with Williams correction for overdispersion. This value is an average across sampling days allowing for nonconstant binomial variance and for differences in sample size (Crawley 1993). The mean logit score for each infection was used in conventional ANOVA. More than 95% of the proportions used in these analyses were based on counts of at least 200 worms and fell between 0.2 and 0.8, so the conclusions are unchanged if other methods of estimating average proportion of sexual morphs for an infection are used (e.g., unweighted arithmetic mean proportion).

RESULTS

Corticosteroids.—Prior to corticosteroid treatment, worm output from all rats fell steadily over time accompanied by the expected rise in the proportion of sexual morphs. After treatment began (day 18 PI), output from rats given corticosteroids increased, whereas output from rats given saline continued to fall (Fig. 2A). This resulted in greater total worm output from treated animals ($F_{1,4} = 16.23$, $P = 0.016$). Thus, treatment with corticosteroids was immunosuppressive. In line with our prediction, the rise in the frequency of sexual morphs was reversed in rats given corticosteroids (Fig. 2B).

Whole-Body γ -Irradiation.—Irradiation resulted in pro-

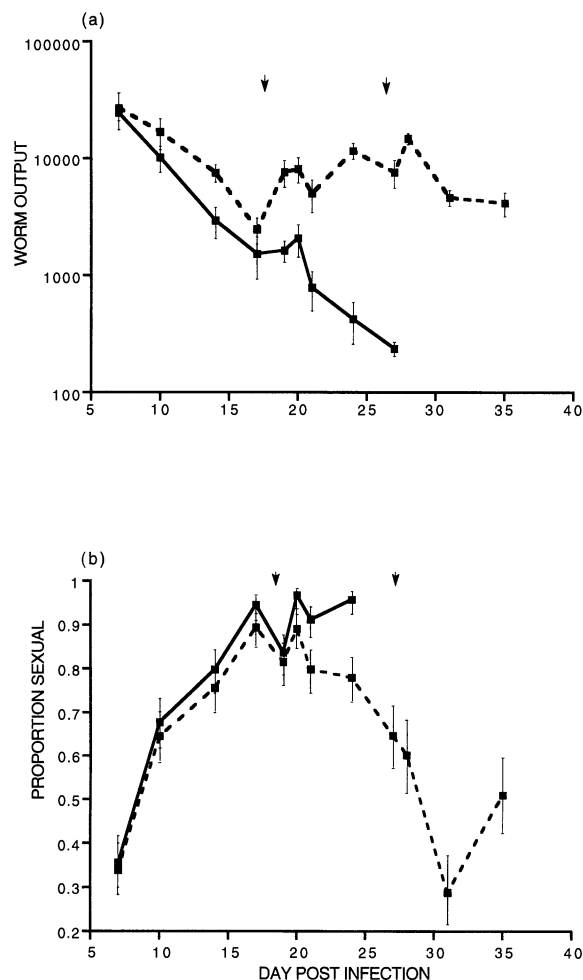


FIG. 2. The effect of corticosteroid treatment on (a) worm output (larvae per night), and (b) proportion of larvae developing into sexual morphs. Broken lines = rats treated with corticosteroid; solid lines = rats treated with saline. Arrows indicate first and last treatment days. Plotted values are means of three rats (± 1 SE).

longed and elevated worm output and consequently greater total worm output (Fig. 3A; $F_{1,4} = 17.46$, $P < 0.014$). In all infections, the proportion of sexual morphs rose through time, but it did so more gradually in irradiated rats (Fig. 3B). Thus, γ -irradiation was immunosuppressive and, like corticosteroid treatment, reduced the proportion of sexual morphs recovered on sampling days.

Congenitally Hypothyroid (Nude) Rats.—Worm output from heterozygous (thymic) rats fell rapidly and was barely detectable (fewer than 100 per day) four weeks after infection. In contrast, substantial numbers of worms continued to be recovered from the faeces of homozygous (hypothyroid) rats until day 214 PI and all hypothyroid rats harbored ongoing infections when the experiment was terminated on day 324 PI (Fig. 4A). Total worm output from thymic rats was about 7% of that from hypothyroid rats (geometric means: 0.24×10^6 and 3.21×10^6 worms, respectively; $F_{1,6} = 87.35$, $P < 0.0001$). Thus, any immune responses mounted by hypothyroid rats had a dramatically smaller impact on parasite fecundity than those mounted by thymic rats. The proportion

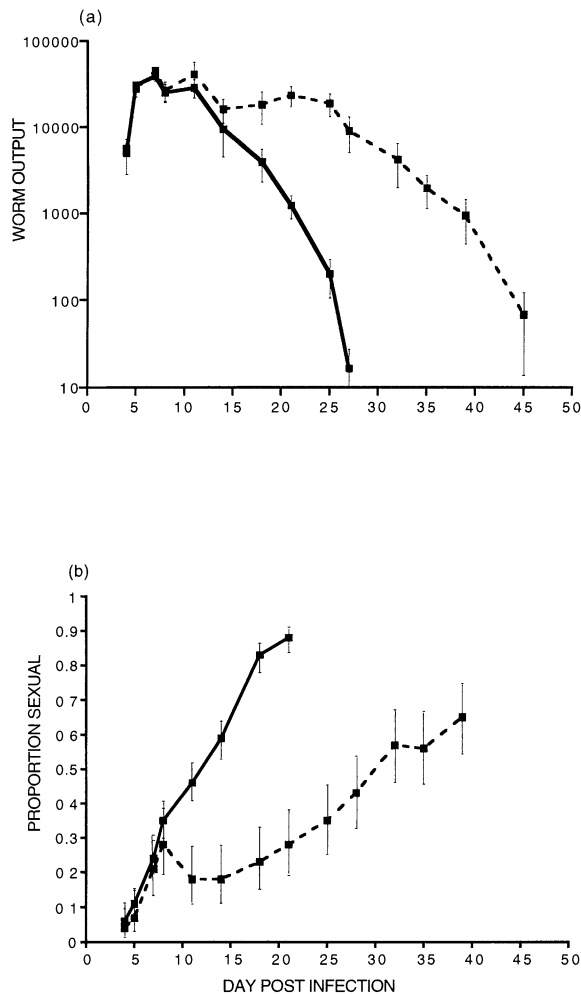


FIG. 3. The effect of whole-body γ -irradiation on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = irradiated rats; solid lines = nonirradiated rats. Plotted values are means of three rats (± 1 SE); in some cases, error bars are smaller than symbol.

of sexual morphs rose steadily during infections of thymic rats. In hypothymic animals this rise was considerably slower (Fig. 4B).

Previous Exposure.—One of the rats given a single iL3 in the primary inoculum did not develop a patent infection and was dropped from the experiment. Previous exposure to 0, 1, 10, or 50 iL3s generated dose-dependent reduction in total worm output from challenge infections with 250 iL3s (Fig. 5A). Total worm output from infections in previously exposed rats was inversely related to the level of previous exposure ($F_{3,7} = 22.54$, $P < 0.001$). In all groups, the proportion of sexual morphs produced rose during the infection (Fig. 5B). The mean proportion between days 5 and 11 postchallenge differed significantly between experimental groups, being lowest from rats not previously exposed and highest from rats previously exposed to 10 or 50 iL3s, with those exposed to a single iL3 producing intermediate values ($F_{3,7} = 3.25$, ordered heterogeneity test (Rice and Gaines 1994) $r_s P_c = 0.73$, $P < 0.05$). Much of this effect was due to previous exposure per se, rather than the level of that exposure: among

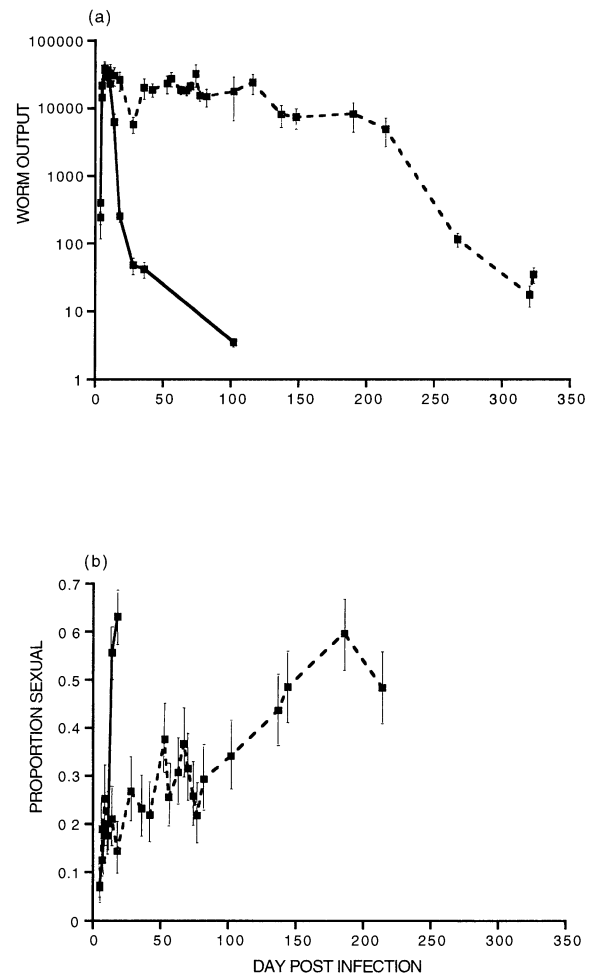


FIG. 4. The effect of congenital thymic deficiency on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = hypothymic (homozygous) rats; solid lines = thymic (heterozygous) rats. Plotted values are means of four rats (± 1 SE); in some cases, error bars are smaller than symbol.

previously exposed rats, there was no effect of primary inoculum size ($F_{2,5} = 0.82$, ordered heterogeneity test n.s.), whereas the mean proportion of sexual morphs differed between naive rats and those previously exposed ($F_{1,9} = 8.21$, $P = 0.02$).

Larval Inocula \times Corticosteroids.—Total worm output was greater from rats infected with more larvae and given corticosteroids ($r_s P_c = 0.0025$; Fig. 6A). The immunosuppressive effect of corticosteroids did not depend on whether infections were initiated with 100 or 500 iL3s (dose*treatment interaction: $F_{1,8} = 3.25$, $P > 0.1$). The fivefold difference in inoculum size produced a fivefold difference in initial worm output ($F_{1,10} = 21.44$, $P = 0.001$) and about a fourfold difference between the two treated groups in total worm output over the course of the infection (Fig. 6A; $F_{1,4} = 18.901$, $P = 0.015$; geometric means 1.27×10^5 and 5.72×10^5). The mean proportion of sexual morphs was affected by corticosteroid treatment ($F_{1,8} = 12.59$, $P < 0.01$; Fig. 6B), with a smaller proportion produced from treated rats. This effect was not altered by the size of the initial inoculum (dose*betamethasone effect, $F_{1,8} = 0.71$, $P > 0.2$, dose main

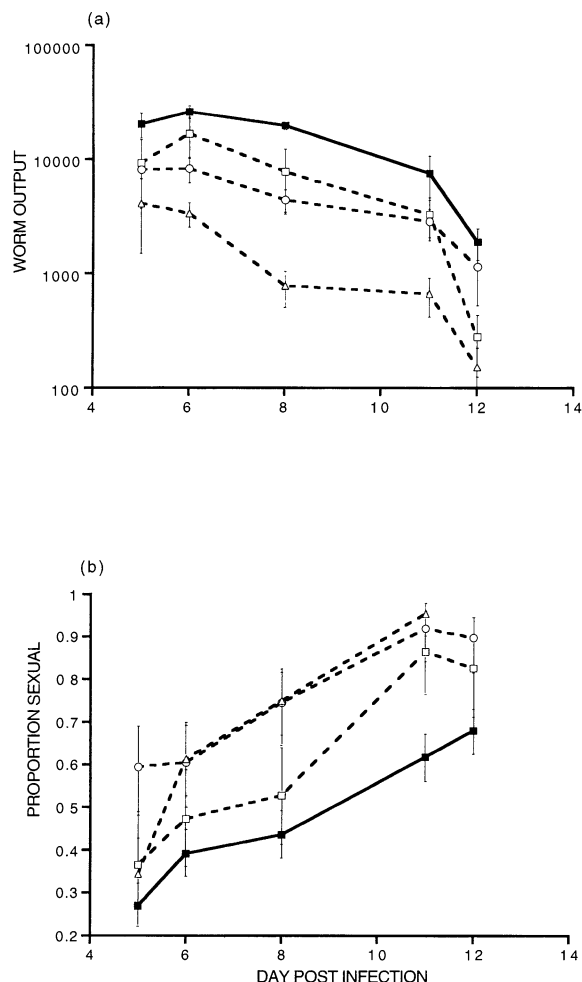


FIG. 5. The effects of varying levels of previous exposure to *Strongyloides ratti* on (a) worm output, and (b) proportion of larvae developing into sexual morphs. Broken lines = rats previously exposed to 1 (open squares), 10 (open circles), and 50 (open triangles) iL3s respectively; solid lines = previously unexposed rats. Plotted values are means of two or three (see text) rats (± 1 SE); in some cases, error bars are smaller than symbol.

effect: $F_{1,8} = 2.85$, $P > 0.1$). Thus, the proportion of sexual morphs emerging from rats treated with betamethasone was similar, despite fivefold differences in larval density (Fig. 6B).

Worm output from 500-dose infections in untreated rats converged with that of 100-dose infections in treated rats between 13 and 17 days PI (Fig. 6A). During this period, when worm output in the two groups was indistinguishable, relatively fewer sexual morphs were produced from treated rats (Fig. 6B).

Larval Inocula \times Whole-Body γ -Irradiation.—Two experimental groups failed to provide reliable estimates and are excluded from the analysis: the 50-dose nonirradiated group where worm output was too low to generate sufficiently large sample sizes and the 500-dose irradiated group where one rat died under anaesthesia and the other two (inexplicably!) lost their distinguishing marks. Despite this, the remaining experimental groups demonstrate that immune status per se rather than the resulting larval density is the key determinant

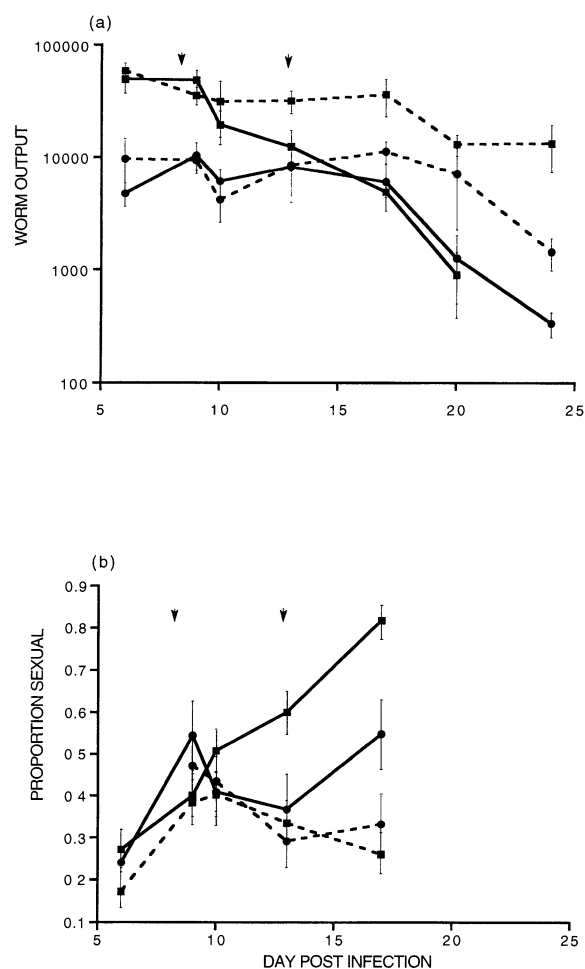


FIG. 6. The effects of corticosteroid treatment and number of iL3s in inocula on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = rats treated with corticosteroid; solid lines = rats treated with saline; circles = rats exposed to 100 iL3s; squares = rats exposed to 500 iL3s. Arrows indicate first and last treatment days. Plotted values are means of three rats (± 1 SE); in some cases, error bars are smaller than symbol.

of the proportion of sexual morphs produced. During the period 18 to 20 days PI, worm output from the 500-dose nonirradiated group and 50-dose irradiated group converged ($P > 0.1$ on both days; Fig. 7A). Despite this convergence in density, worms from irradiated rats were less likely to be sexual morphs than those from nonirradiated rats (Fig. 7B; $F_{1,4} = 12.26$, $P < 0.05$). Thus, at the same stage of infection and at the same larval density, fewer sexual morphs were produced from infections in irradiated animals.

DISCUSSION

The production of sexual morphs in *S. ratti* is strongly influenced by environmental conditions. If sex benefits parasites in the face of genotype-specific immunity, larvae should be more likely to develop into sexual adults when hosts develop immunity against parasitic females. Consistent with this prediction, our results show that larvae from an immune or intact host are more likely to develop into sexual

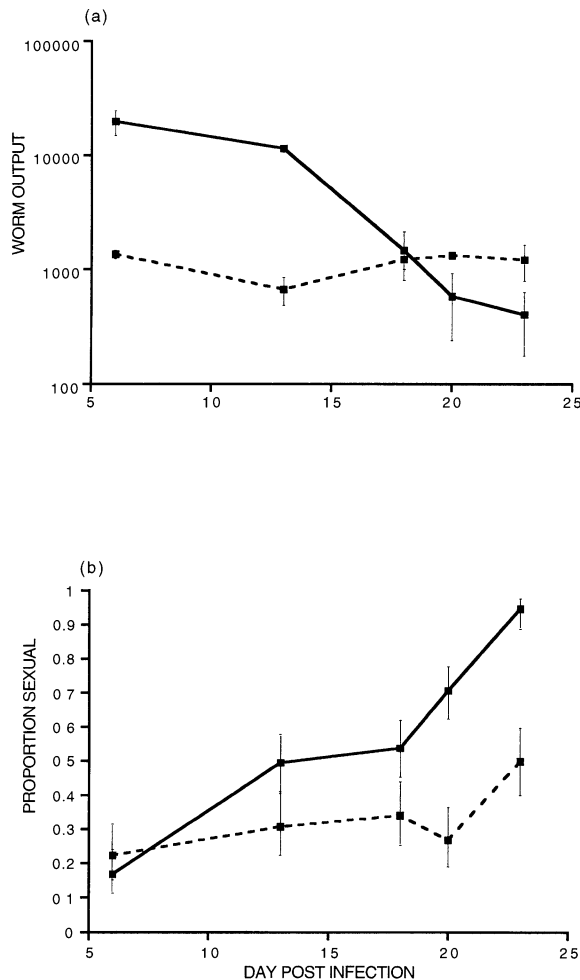


FIG. 7. The effects of whole-body γ -irradiation and number of iL3s in inocula on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = irradiated rats given 50 iL3s; solid lines = nonirradiated rats given 500 iL3s. Plotted values are means of three rats (± 1 SE); in some cases, error bars are smaller than symbol.

morphs (heterogonically) than larvae from a naive or immunosuppressed host. This effect is independent of the method of immune manipulation used.

Our experiments were not designed to reveal the mechanism controlling facultative developmental switching, but they demonstrate that some proximate cue(s) associated with host immune status other than larval density and age of infection must be involved. Several immune effectors or physiological alterations could predict host immune status, either in the gastrointestinal tract prior to and just after hatching, or in voided faeces. Immune responses have profound effects on the size and structural integrity of adult worms (Moqbel and McLaren 1980), and parasitic females might alter the developmental routes of their offspring in response to such damage. There is good evidence that larvae alter their own developmental route in response to extra-host environmental conditions. Like all nematodes, *Strongyloides* larvae possess sensory structures called amphids. In *C. elegans*, these play a central role in integrating environmental signals when larvae switch facultatively between developmental routes (Rid-

dle 1988; Ashton and Schad 1996). Temperature sensitivity of development begins two to four hours after *S. ratti* larvae exit the host, with commitment complete after 24 hours (Viney 1996). It should be possible to determine experimentally whether larvae respond to factors correlated with host immunity during this same period.

Wherever the mechanism acts, current immune status of a host should reliably predict the level of immune challenge larvae will encounter if they reinfect the same host and, depending on the epidemiological situation, perhaps any host in the same population. Given current levels of ignorance about the nature of host immunity to *S. ratti*, it is possible that antagonistic coevolution maintains the sexual life cycle in wild populations. Density-dependent acquired immune responses are elicited by both invading iL3s and established parasitic adults (e.g., Fig. 5). A large number of effector mechanisms, some of which are antibody mediated, are involved in these responses and generate strong protection against incoming worms (reviewed by Dawkins 1989). Reciprocal cross-challenge experiments show that these responses are more effective against challenge with the inbred line that elicited them than against challenge with other conspecific lines. This specific protection can be transferred passively with immune mesenteric lymph node cells (Carter 1986). Whether acquired specific protection is targeted against antigens encoded by genes at unlinked loci, and whether protection against antigens encoded by genes at single loci is less effective are, to our knowledge, open questions for any sexually reproducing parasite.

The natural history of *S. ratti* almost certainly gives the worms the potential to outcross. Mixed-genotype infections are common in wild caught rats (Fisher and Viney in prep.). Faecal marking of abutting territorial boundaries and use of shared latrines could provide opportunities for crossing between worms from different rats. These same features will also promote reinfection of the same and related hosts. Transmammary transmission also occurs such that early exposure to particular genotypes is highly likely (Zamirdin and Wilson 1974).

Immune-dependent parasite sexuality is consistent with Red Queen models of sex. Failure to find it despite strong evidence of conditional sex in *S. ratti* would certainly have challenged the idea. However, as with much data on the occurrence of sexuality (Bell 1982; Kondrashov 1993, Hurst and Peck 1996), the phenomenon can be interpreted in the context of other hypotheses for the evolution of sex. Deterioration of intrahost conditions may be a reliable cue that a host no longer offers suitable habitat and that genetically diverse offspring are required to maximize the chance of establishment in hosts whose genotype or previous worm exposure is unpredictable (lottery models). Sexual reproduction in facultatively sexual organisms is frequently associated with a response to stress (Bell 1982). Sex may also be more beneficial if its mutation-purging consequences have sufficiently large fitness benefits only when the worms are under immune-imposed stress (Howard and Lively 1994).

Alternatively, heterogonic development of *S. ratti* larvae may be adaptive not because it involves sexual reproduction but rather because free-living adults generate extra fecundity. However, this extra fecundity is not without cost. Heterogonic

development delays the potential for reinfection by about a week at 13°C. In our laboratory, daily fecundity early in an infection is substantially higher for a parasitic female (> 50 offspring, coefficient of relatedness = 1.0) than for a free-living female (< 20 offspring, coefficient of relatedness = 0.5–1.0). Thus, early in an infection, selection may favor homogenic development and rapid reinfection to maximize reproductive output. As immunity develops and reinfection becomes increasingly difficult, free-living reproduction may be a strategy for further increasing the number of infective larvae in the environment. There is a snag with this scenario. It does not explain why free-living females reproduce sexually. Free-living reproduction in *S. ratti* is exclusively sexual yet this species is clearly capable of parthenogenesis. The cost of sex is substantially reduced if mating with nonsibs is avoided, but we have not found the consistently female-biased sex ratios expected if sib mating is the rule (Hamilton 1967).

Discriminating these alternatives from the idea that sex is an adaptation to counter host immunity is possible experimentally and may have implications outside of evolutionary biology. Even if the fitness benefits in terms of immune evasion are insufficient to explain the evolutionary maintenance of sex in *S. ratti*, there are clinical and epidemiological consequences if these benefits are not zero. For example, more parasites will be found in populations in which outcrossing is more frequent. That would raise the prospect of (mis-)managing disease by altering parasite mating patterns. Intervention strategies that reduce the possibility of outcrossing, such as the selective treatment of hosts harboring the majority of worms in a population, could have a disproportionate effect on parasitic disease, even if parasites themselves are not wholly eliminated.

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