Experimental and comparative analyses of the evolutionary ecology of parasitic nematodes

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DECLARATION

I declare that this thesis has been composed by myself and is entirely my own work, except where otherwise stated.

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Gemmill, A. W., Viney, M. E. and Read, A. F. (1997). Host immune status determines sexuality in a parasitic nematode. *Evolution* **51**(2), 393-401.

Gemmill, A. W. and Read, A. F. (1998). Counting the cost of disease resistance. *Trends in Ecology & Evolution* **13**(1), 8-9.

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Elvis on Worm Size *This essay was one of ten runners-up in the 1998 Wellcome-New Scientist Millennial Science Essay Competition and recently appeared in the magazine* BBSRC Business.

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ABSTRACT

Causes and consequences of plasticity in parasite life histories were investigated using a gastrointestinal nematode species, *Strongyloides ratti*, a natural parasite of rats. Empirical work focused on three putative instances of adaptive and non-adaptive plasticity in the life history of *S. ratti*: host-specificity, facultative sexuality, and immune-dependent maturation time. Host-specificity (the differential success of a parasite on alternative host types) represents non-adaptive plasticity in fitness and is commonly viewed as an unavoidable outcome of parasite specialisation - an intuitive conclusion that has rarely been questioned. While the lifetime reproductive success of two *S. ratti* lines was unaffected by host (rat) genotype, the frequency and timing of sampling was crucial in quantifying host-specificity accurately. Proximate processes generating the differential performance of *S. ratti* in rats and mice were then characterised and quantified. Reduced parasite fitness in mice resulted from lower parasite establishment, more rapid expulsion and suppressed fecundity. Differences in the efficacy of thymus-dependent (T-dependent) immunity between host species were insufficient to explain this variation in parasite fitness. Experimental natural selection and reciprocal fitness assays were used to discriminate between alternative models of host-specific specialisation. Selection failed to modify host-specificity suggesting either a lack of genetic variation among parasites or the action of unidentified factors underlying the different performance of *S. ratti* in rats and mice.

The *S. ratti* life-cycle incorporates a facultative sexual phase and the frequency of sex depends on the strength of host acquired immunity. Immunisation of rats with infective larvae of other nematode species or with mammalian antigens reduced the reproductive success of parasites but only immunity acquired against *S. ratti* induced a facultative increase in the frequency of sexual reproduction indicating antigen-specificity of this plasticity.

The time between infection and the onset of reproduction (maturation time) is a key fitness component in parasitic nematodes. An optimality model for maturation time was developed centred on pre-maturational growth, the duration of which has opposing consequences for fecundity and for survival to reproductive age. The maturation time favoured by natural selection was found to be inversely proportional to pre-maturational mortality rate. The product of maturation time and mortality rate was predicted to be an invariant number equal to the allometric slope linking daily fecundity to maturation time. Predictions were tested using comparative data on mammalian gastrointestinal nematode taxa, and half the cross-species variation in observed maturation time was accounted for by this adaptive trade-off hypothesis. For individual parasites, the pre-maturational mortality rate and hence the time available for

growth depends on the strength of host immune responses. In theory therefore, optimal maturation time varies with immune-imposed mortality. The hypothesis that maturation time is facultatively adjusted by parasitic nematodes in response to variation in host immunity was examined experimentally using immunisation and immune-suppression. Initial reproductive activity was inversely related to the strength of host immunity. Tissue migration times were significantly longer in the absence of T-dependent immunity and the onset of parasite egg production (maturity) was delayed. However, the prediction of the "adaptive delay" hypothesis concerning body size at maturity was not supported by the data. An extensive literature survey confirmed that slowed development is a common feature of immune-suppression studies with parasitic nematodes and is not explained easily in terms of non-adaptive plasticity.

Results are discussed in relation to nematode biology; possible evolutionary outcomes of medical intervention and control programmes; models of phenotypic plasticity and future prospects for the study of variation in endoparasite life histories at the level of individual organisms.

Chapter 1

General Introduction

"**plastic** (*pläs'tik*). having power to give form: ...mouldable: modifiable:...capable of permanent deformation without giving way."

Chambers 20th Century Dictionary
New Edition

1.1 Phenotypic Plasticity

(i) Adaptive and non-adaptive plasticity

Adaptation takes time. At the very least, a single generation is required for natural selection to alter the genetic composition of a population. For a neonate, endowed only with the genetic assets bequeathed by its parent (s), adaptation is therefore not an option in the biological armoury. Each individual is born, lives and dies with only a single genotype at its immediate disposal. It is the central insight of Darwinism that adaptations exist because large numbers of individuals have died, or have failed to reproduce sufficiently, due to fatal inadequacies of their genetic legacy. But this is not the whole story of life.

Natural selection bears down directly on phenotypes, not genotypes. Only as an indirect consequence of differential survival values attached to individual phenotypes do genes themselves become exposed to differential rates of extinction and replication. The potential contribution of each individual's genotype to its phenotype may be fixed at conception. Phenotypes however are far from immutable.

In fact, an individual's phenotype can be highly sensitive to all manner of environmental factors throughout its entire ontogenetic history. This is why gardeners can spend time and effort meddling with soil nutrient levels and photoperiod cycles in their greenhouses all the time confident that such exercises will prove ultimately

profitable. The environmental sensitivity of a trait is also known as its 'plasticity' or 'reaction norm' (Woltereck 1909; Bradshaw 1965; Falconer and McKay 1996). The simplest reaction norm is a straight line linking the phenotypic values expressed by a single genotype in two environments. Even the simplest reaction norm has both a height (intercept) and a shape (slope). Somewhat ironically, the reaction norm was first conceived by Woltereck (1909) as a workable measure for distinguishing between genotypes (Woltereck worked with clonal isolates of *Daphnia* spp.). Advances in genetical research have made the recognition of genotypes less problematic since Woltereck's time, but the example remains instructive - genetic variation can underlie variation in the plasticity of phenotypes.

Heritable variation in plasticity does indeed exist (Scheiner and Lyman 1989) and artificial selection on plasticity generally produces a measurable response (e.g. Scheiner and Lyman 1991; further examples in Scheiner 1993). Simultaneous, opposing selection on the phenotypic value of a trait in separate environments can lead to changed plasticity of that trait - an altered reaction norm (e.g. Thompson and Rook 1988). The direction of this change is predictable from quantitative genetic theory, these predictions having been borne out by the results of many artificial selection experiments (the 'Jinks-Connolly rule': Falconer 1990).

Discussion of plasticity is hindered by inexact, overlapping or competing nomenclatures. Gotthard and Nylin (1995) developed the following scheme for navigating the associated terminological quagmire. In the broadest sense, the plasticity of a trait encompasses all environment-dependent phenotypic variation. This variation can be labelled either adaptive or non-adaptive as assessed by its effect on fitness. However, because plasticity is adaptive this need not imply that the plasticity is an adaptation (i.e. produced and maintained by natural selection for a purpose). Conversely, plasticity which has been evolutionarily derived and maintained might yet

yield a non-adaptive outcome depending on the prevailing environment. As an illustration of these distinctions consider the following, hypothetical example.

In a (purely imaginary) population of arctic foxes, most individuals grow a thicker pelt with the approach of winter. This seasonal plasticity in pelt thickness is an adaptation (derived and maintained by selection) and is also adaptive, providing increased body insulation in winter while avoiding the energetic costs of maintaining an unnecessarily thick pelt all year round. One fox in this population (a mutant) exhibits a different plasticity and actually shortens her pelt very slightly as winter nears. The plasticity displayed by this individual is neither an adaptation nor is it adaptive since it results in a heightened risk of death during winter. Now, imagine that the foxes' territory is overrun one winter by a band of fur trappers who place great value on thick fox pelts. If the trappers' hunting is intensive enough, thick winter coats become a selective liability to their possessors - an adaptation is rendered non-adaptive by a change in the environment in which it is expressed. The mutant fox on the other hand is at a greatly lowered risk of being hunted for her pelt. If this lowered risk outweighs the disadvantages of a thin winter coat, the mutant fox is at a selective advantage (at least until the trappers are gone). Thus, a plasticity which is not an adaptation can nevertheless be adaptive in the current environment. These distinctions are really no different from those which can apply to any trait, plastic or not (Williams 1966) but greatly simplify discussion and are used in this thesis.

In the real world, plasticity in major life history traits is extremely common and much of this plasticity appears to be adaptive. For example, in the coho salmon *Oncorhynchus kisutch* mature males exhibit two alternative morphologies - so-called "jacks" which leave the ocean aged two years and the much larger "hooknoses" which spend three years growing at sea (Gross 1985). At breeding, individuals of the hooknose morph secure matings by a policy of physical aggression. The smaller jacks avoid fighting and instead use a "sneaking" strategy to access females at the moment of

egg laying. Jacks have an average lifetime reproductive success less than half of that of hooknoses but have about double the chance of reaching reproductive age (Gross 1985). The decision to adopt either life history strategy appears to be dependent on the quality of juvenile growing conditions (Bilton 1980). Young males that achieve a better than average body size after only two years at sea do better by leaving the ocean and breeding as jacks.

Among insects, the Lepidoptera show a variety of apparently adaptive plasticities. For example, butterflies in the genus *Lasiommata* adjust larval developmental rate in response to seasonal cues, notably photoperiod (Nylin *et al.* 1996). North-European species diapause as larvae during winter and resume growth in spring. In autumn, before diapause, daylength is decreasing with the opposite being true in spring. Accordingly, larval adjustment of growth rate responds to changes in photoperiod quite differently before and after diapause. Before diapause, shorter photoperiods induce a quickening of growth. Once diapause is broken however, decreasing photoperiod produces modulation of growth rate in the opposite direction. Developmental rate therefore shows different reaction norms before and after diapause and this has been shown to be consistent with theoretical expectations from optimality models of growth and reproduction in a seasonal environment (Nylin *et al.* 1996; Gotthard 1998)

Perhaps some of the best understood examples of adaptive phenotypic plasticity surround local adjustments of sex ratio through environmental sex determination (Charnov 1982). In the Mermithidae, nematode parasites of insect larvae, both host body size and the level of infection affect the sex allocation decisions made by individual parasites. In the majority of these cases, poor growing conditions (small hosts and/or high infection levels resulting in crowding) constrain size at maturity and favour development to the sex (usually male) whose fitness is least affected by small body size. In field studies of the North-American species

Romanomermis nielseni, a parasite of mosquitoes, local sex ratio variation has been shown to depend on host size and in-host crowding in precisely the manner predicted from sex ratio theory (Blackmore and Charnov 1989).

(ii) Theoretical models of plasticity

From the foregoing examples it should be clear that there is no shortage of examples of adaptive plasticity in nature and there can be little doubt that plasticity as an adaptation exists. However, considerable questions remain as to when such plasticity is governed by loci other than those contributing to the trait itself - so-called 'plasticity genes' (Schlichting and Pigliucci 1993; Via 1993). Plasticity genes are envisaged as being environmentally sensitive loci with regulatory functions in structural gene expression (Scheiner 1993). For example, De Jong (1990) presented population genetic models of plasticity in which discrete loci control the slopes of reaction norms. Others (beginning with Via and Lande 1985) have taken a quantitative genetic approach by considering a single trait in multiple environments as multiple, genetically correlated characters. Studies using both approaches, as well as models that consider the maintenance of alternative morphologies as an optimality problem (e.g. Moran 1992), show that plasticity can evolve readily and be maintained in response to natural selection (plasticity as an adaptation).

1.2 Plasticity in the genus *Strongyloides*

Several taxa in the phylum Nematoda exhibit uniparental (parthenogenetic or self-fertilising hermaphroditic) reproduction. In some species parasitising vertebrate hosts, uniparental reproduction alternates with free-living, dioceous generations (e.g. *Rhabdias* spp: see Poinar and Hansen 1983 for a review of reproductive modes in nematodes).

Nematodes in the genus *Strongyloides* (Rhabditida:Strongyloididae) parasitise land-dwelling hosts from every major tetrapod group (Anderson 1992). Morphologically, these species are extremely conservative and are distinguished taxonomically only by minor structural differences, differences in physical proportions, or by reference to their common host species (Speare 1989). Within species however, life history variation following environmental change appears to be common (Schad 1989).

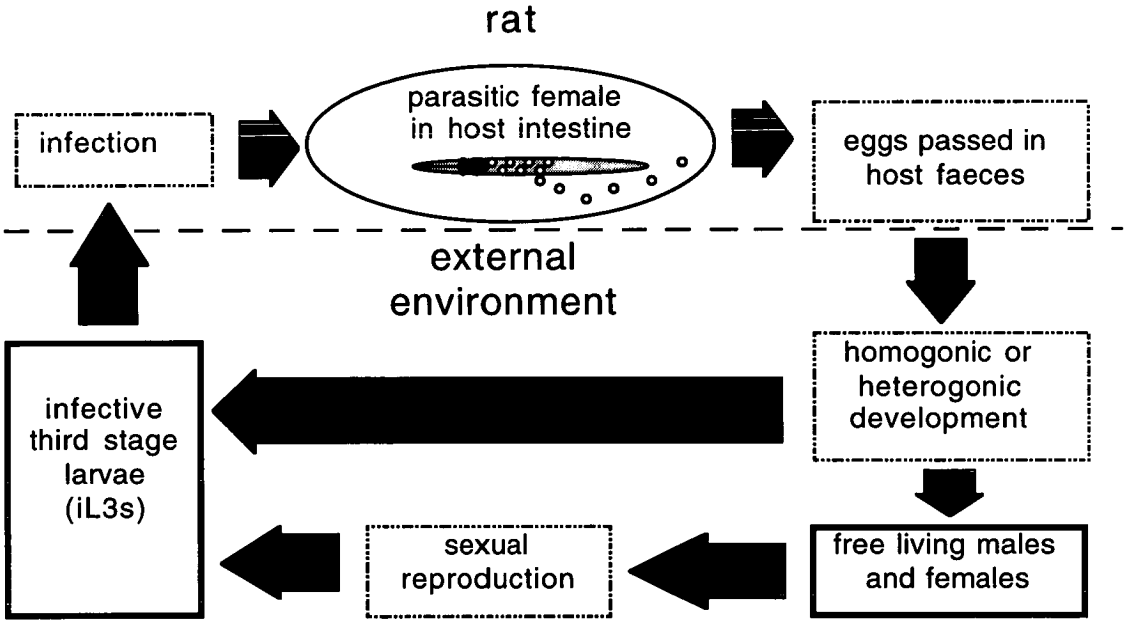
Strongyloides spp. exhibit a rare form of conditional sex in the simultaneous production of sexual and asexual offspring by the same parthenogenetic mother. The ratio of these alternative morphs varies under the influence of various environmental factors. In the case of extra-host factors, temperature; partial pressures of O_2 and CO_2; concentrations of fatty acids; pH and larval density in host faeces have all been reported as affecting the ratio in which sexual and asexual morphs occur in various *Strongyloides* spp. (e.g. Graham 1939; Moncol and Triantaphyllou 1978; Minematsu *et al.* 1989; Taylor and Weinsein 1990). The production of sexual morphs in *Strongyloides* spp. is therefore a plastic trait in the broadest sense (Schad 1989), the phenotypic expression of which is sensitive to the environment in which larvae undergo development.

1.3 Plasticity in the life history of *Strongyloides ratti*

(i) Life cycle

Enough is known of the sequence of major developmental events in the life cycle of *Strongyloides ratti* to enable a general, phenomenological life cycle to be drawn (Figure 1). Adult parasites are exclusively female, inhabit the mucosa of the small intestine of rats and reproduce by functionally mitotic parthenogenesis (apomixis; Viney 1994). However, the *S. ratti* life cycle also incorporates a free-living, sexual phase as follows. Parasitic females shed their eggs into the host intestine from where

Figure 1. Life cycle of *Strongyloides ratti*. Parasitic females reproduce by functionally mitotic parthenogenesis (Viney 1994) and eggs pass to the external environment in host faeces. After hatching, larvae pass through either two moults to become infective third stage larvae (iL3s) or four moults in the case of free living adults. Free living adults mate and all offspring from these mating develop as iL3s. The iL3s infect rats by skin penetration or transmammary transmission and moult twice inside the host to become adult parasitic females.



they pass with faeces to the external environment. After egg hatching, larvae develop via one of two ontogenetic routes, termed homogonic and heterogonic development (Schad 1989).In homogonic development larvae moult twice to become infective third stage larvae (iL3s) which infect hosts and continue the parasitic life cycle. In heterogonic development, larvae pass through four moults and mature as dioecious, rhabditiform, free-living adults. Free-living adults mate outside the host and produce eggs by conventional sexual reproduction (amphimixis) which develop into iL3s, and only iL3s (Viney *et al.* 1993). Hence, parasites are invariably parthenogenetic and free-living adults are invariably sexual.

This inflexible coupling of habitat with reproductive mode therefore has an interesting consequence. The parent of a free-living adult is always an apomictic

parasite whereas parasites have either apomictic (parasitic) or amphimictic (free-living) parentage. Thus, the *S. ratti* genome encodes a range of adult morphologies, adaptations for survival in two distinct niches, amphimictic and apomictic reproductive modes, and both male and female sexual function.

The iL3s produced by either developmental route (heterogonic or homogonic) must infect a rat in order to continue development towards reproductive maturity. How host contact is made in the wild situation is unclear. Host-location mechanisms are a fascinating feature of parasitic nematode life histories but have been little studied in the case of *S. ratti*. However, in *S. robustus* (a parasite of North American flying squirrels), iL3s actively migrate to host-body temperature on an experimental thermal gradient (Wietzel and Wiegl 1994). In some skin-penetrating and entomopathogenic nematodes, a behaviour called nictation may play an important role in maximising the chances of physical contact with mobile hosts (Campbell and Gaugler 1993). In nictation, infective larvae raise all but the posterior tip of the body from the substratum, assume a vertical posture and move the head in wide, three-dimensional, elliptical motions (Croll and Matthews 1977). This is not an energetically cost-free behaviour since in standing upright larvae must first overcome considerable surface tension forces. These forces have been estimated as being 10^4 to 10^5 stronger than gravity (Crofton 1954). Nictation behaviour is a common sight in laboratory cultures of *S. ratti* (personal observation) but its potential role as a host-location mechanism is, to my knowledge, entirely uninvestigated in this species.

Once iL3s contact a rat, they actively burrow through host skin, probably facilitated by the secretion of powerful proteases (McKerrow *et al.* 1990; Brindley *et al.* 1995), enter subcutaneous vasculature and begin migrating towards their eventual breeding site in the intestine. Whether there exists a well defined migration route via which larvae actively navigate the parenteric host tissues is still an open question in this and other species of tissue-migratory nematodes (Tindall and Wilson 1990; Wilson

1994). Little is known regarding the cues (if any) that migrating larvae might use to find their way to the gut. For many years it was believed that skin-penetrating rhabditid and strongylid nematodes reached the gut via a 'blood-lung' route. In this model of tissue migration, larvae penetrate the skin, enter the venous blood supply and are swept to the heart. From here they are supposedly transported via the pulmonary artery to the lungs. Here they penetrate alveoli, migrate through bronchioles and bronchi to the trachea from where they acccess the host alimentary tract by being swallowed. However, this traditional model has come in for severe criticism, not least because of the highly equivocal nature of most of the supporting data, based on numbers of parasites present in various host tissues at autopsy (Wilson 1994). Nonetheless, in *S. ratti* large numbers of migrating iL3s can be recovered from various tissues of infected rats notably the lungs and the cranio-frontal region of the head and there is now detailed evidence that this last region is part of a route through which most establishing parasites pass in the case of *S. ratti* (Tindall and Wilson 1990; Wilson 1994). In infections of lactating female rats, some iL3s reach the mammary glands and are passed to nursing pups with milk (Wilson *et al.* 1978). The adaptive value of this transmammary transmission route has not yet been quantified empirically.

Larvae arrive in the intestine beginning roughly 36 hours after skin penetration (Tindall and Wilson 1990). Here larvae pass through a further two moults before commencing reproduction as adult female parasites. Eggs are first detectable in host faeces four to five days postinfection.

(ii) Conditional sexual reproduction

Identifying the presumed selective advantage of sexual reproduction is a longstanding and famous puzzle in evolutionary biology (Kondrashov 1993; Hurst and Peck 1996). A parthenogenetic female enjoys a twofold reproductive advantage over a sexually reproducing rival (Bell 1982). What then is the advantage of sexual

reproduction? Currently, two resolutions of this apparent paradox are fashionable (although more than twenty separate theories have been advanced to date: Kondrashov 1993). In an asexual lineage deleterious mutations accrue relentlessly at each generation; mutation accumulation avoidance is thus one possible benefit of amphimixis (Kondrashov 1988). Alternatively, under frequency-dependent selection imposed for example by parasites (Hamilton 1980), genetic recombination may give fitness benefits sufficient to outweigh the cost of sexual reproduction (Howard & Lively 1994).

These possibilities are not mutually exclusive; indeed pluralist explanations have been advanced which combine them and stress that there may be many reasons for the maintenance of sexual reproduction. These reasons may be specific to particular species and/or ecologies (West *et al.* in press).

In *S. ratti*, the frequency with which free-living adults occur varies between field isolates (Viney *et al.* 1992) and also within isolates following variation in the environment. Notably, both host immune status and the temperature at which larvae develop outside the host can affect the production of sexual morphs dramatically (Viney 1996; Gemmill *et al.* 1997). Apparently therefore, the differential production of sexual and asexual morphs is influenced by both genetic and environmental factors.

A situation where discrete adult morphologies are expressed in genetically identical individuals is referred to as a polyphenism. In taxa such as the Homoptera and Lepidoptera, seasonal polyphenism associated with a switch between asexual and sexual reproduction is a common form of phenotypic plasticity (Shapiro 1976; Moran 1992). In *S. ratti* the adaptive value of this polyphenism is not known. However, one possibility is that sexual reproduction by this species is a strategy to evade acquired specific immune responses mounted by the host against particular parasite genotypes (Gemmill *et al.* 1997).

(iii) Host-specificity

All else being equal, the host-specificity of parasites constitutes a case of non-adaptive plasticity in the following sense. Most macroparasites are restricted to one or a few host species in which they can complete their normal life cycle. Variation in parasite reproductive success in different host species and even in different genotypes of the same host species, can be very substantial (Wakelin & Blackwell 1988). In this case the trait whose value varies in an environment-dependent manner is fitness itself. Since any plasticity in fitness implies that an organism is doing badly in at least some environments, any plasticity in fitness can be labelled non-adaptive. Identification of the underlying causes of host-specificity is a key issue in parasitology and parasite ecology (Rohde 1994). In *S. ratti*, little is known about the factors determining the range of hosts infected in nature but recorded host species include several species of rat and, less certainly, mice (Speare 1989; Dawkins 1989).

(iv) Maturation time

Most animals begin life with a period of somatic growth. In many if not most species, fecundity is a function of mature female body size (Peters 1983). Determining the optimum moment at which to commence reproduction is therefore one of the most important decisions facing any juvenile (Cole 1954; Gadgil and Bossert 1970). If, for example, rapid development bestows a competitive advantage on juveniles but shows a negative correlation with adult egg output, natural selection should favour the most profitable compromise of rates of growth and offspring production - the gene combinations that maximise the combined contribution of both traits to individual fitness.

In *S. ratti* and other parasitic helminths the possibility of adaptive plasticity in in-host maturation time has never, to my knowledge, been addressed. However, some interesting and somewhat puzzling phenotypic variation in maturation time has been

reported in *S. ratti*. Sheldon (1937) studied differences in infection kinetics between rats infected by subcutaneous injection and those infected by application of iL3s directly to host skin (percutaneous infection). Sheldon measured the time between infection and the shedding of eggs in host faeces (prepatent period). What he found was that subcutaneous infection resulted in higher parasite establishment, which he suggested was due to subcutaneous infection directly bypassing the "skin barrier" and so reducing larval mortality (Sheldon 1937). The effect of infection route on prepatent period was quite the opposite however, eggs first being shed more than a day later from animals infected subcutaneously. This is precisely the opposite of what one might expect if bypassing the skin barrier speeds parasite development.

1.4 Aims

This thesis focuses on three putative instances of adaptive and non-adaptive plasticity in the life history of *S. ratti*:- host-specificity, conditional induction of sexual morphs, and in-host maturation time. The results chapters have been written as free-standing papers and each introduces the necessary biological and theoretical background information.

Chapters 2 and 3 concentrate on host-specificity, in particular the problems inherent in measuring this trait (Chapter 2) and in using it as an indicator of ecological specialisation (Chapter 3). Chapter 4 investigates the role of acquired immunity in the induction of alternative phenotypes (sexual and asexual morphs). Chapters 5 and 6 deal with optimal rates of in-host maturation time, motivated by results arising from the work on thymus-dependent immunity described in Chapter 3. Chapter 7 provides a general summary of the main findings and discusses their evolutionary aspects as well as implications for parasite control measures.

CHAPTER 2

Influence of rat strain on larval production by the parasitic nematode *Strongyloides ratti*

This chapter is a re-formatted version of Gemmill and West (1998). See Appendix.

2.1 **Summary:** The course of infection with *Strongyloides ratti* in a range of rat strains was assessed by monitoring the production of larvae. To our knowledge, this is the first such study of *S. ratti* using its natural host *Rattus norvegicus*. Host strain influenced the pattern of larval production. The results were qualitatively the same for two *S. ratti* lines of North American and Japanese origin.

2.2 Introduction

Nematodes in the genus *Strongyloides* are important gastrointestinal parasites of humans and domestic livestock (Dawkins 1989). *Strongyloides ratti* is a parasite of rats and is used extensively as a convenient laboratory model of strongyloidiasis. The influence of host genetics on the course and kinetics of infection has received little attention in *S. ratti*. This is despite its possible significance in various aspects of parasitic disease in general (Grenfell and Dobson 1995). With *S. ratti*, the majority of work in this area has been conducted using mice, (*Mus musculus*), in which a number of inbred and mutant strains have been shown to differ in susceptibility to infection with this nematode (e.g., Dawkins *et al.* 1980; Nawa *et al.* 1985; Nawa *et al.* 1988). Studies with the natural host (*Rattus norvegicus*) have been confined to examination of the effects of gender (e.g. Katz 1967) and a single immunologic mutation (Gemmill *et al.* 1997). Our aim here was to quantify the pattern of larval production by *S. ratti* in

various inbred and random bred strains of the natural host *R. norvegicus*. The rat strains used were chosen on the basis of differing profiles of antibody production in response to another gastrointestinal nematode, *Nippostrongylus brasiliensis* (Kennedy *et al.* 1990), and differ in haplotype at an MHC (RT1) locus.

We carried out 2 experiments. In the first we compared larval production by a single *S. ratti* line (ED5 Heterogonic; Viney 1996; referred to here as ED5) in a random bred rat strain (Wistar) and 4 inbred strains. The second was designed to provide greater detail on patterns of larval production in 2 rat strains that differed consistently in the first experiment. Specifically, we sampled infections more frequently, and used larger numbers of rats. In addition, in this second experiment, we examined whether the same patterns were observed for a second *S. ratti* line (ED279) of different geographic origin. ED5 and ED279 are isofemale lines descended from single parasites from North American and Japanese isolates respectively (Viney 1996: ED279 derives from 'isofemale line 132').

2.3 Methods

Methodology was the same in both experiments. Six weeks old male rats (Harlan UK) were used, with food and water provided *ad libitum*. Parasite lines were maintained by serial passage in laboratory rats. Experimental animals were infected by subcutaneous injection with 500 infective third stage larvae (iL3) and the numbers of parasite offspring emerging from faeces (nightly worm output) were monitored throughout infection. Faeces were collected onto damp paper overnight, cultured in glass Petri dishes and incubated for 2 days at 25 °C or 3 days at 19 °C. Cultures were then washed extensively in distilled water to collect worms into a known volume and the numbers present determined by counting worms in repeated samples under a binocular microscope. The positions of animals in collecting apparatus, the order in which cultures were made and subsequently processed as well as the positions of

cultures in incubators, were randomised anew at each sampling point. The total number of parasite offspring produced over the course of infection (total worm output) was estimated by numerical integration under the worm output by time curves. Data were analysed by conventional Analysis-of-Variance (ANOVA).

2.4 Results and Discussion

Nightly worm outputs from the first experiment are shown in Figure 1. Total worm output was highest in Wistar rats and lowest in F344 rats. One-way ANOVA using Bonferroni/Dunn multiple means comparison revealed that total worm output was higher in Wistar rats than in PVG and F344 rats (Wistar*PVG, $p = 0.049$; Wistar*F344, $p = 0.03$). The same comparison yielded p-values of 0.07 and 0.22 for the LOU and LEW strains, respectively.

In our second experiment we examined patterns of larval production in Wistar and PVG rats with 2 lines of *S. ratti*. Nightly worm outputs from the 4 groups are shown in Figure 2. Two-way ANOVA on total worm output revealed the following. Total worm output was not significantly greater in Wistar rats than in PVG rats ($F_{1,18} = 3.1$, NS). Infection with ED279 led to lower total worm output regardless of rat strain ($F_{1,18} = 42.2$, $p < 0.0001$; worm line X rat strain interaction $F_{1,18} = 0.047$, NS).

At first sight, the results of the 2 experiments appear contradictory. In the first experiment, there was a significant difference in total worm output between PVG and Wistar rats infected with ED5. In the second, there was not. By analysing the early and late parts of our second experiment separately, the reason for this inconsistency becomes clearer. Nightly worm output from rats infected with ED5 was highest in PVG rats prior to day 11 PI and highest in Wistar rats thereafter. Total worm output until and including day 10 PI did not differ significantly between rat strains ($F_{1,9} = 0.3$, NS) but total worm output from day 11 PI onwards did ($F_{1,9} = 12.6$, $p < 0.01$).

Figure 1. Nightly worm output from five strains of rat infected with 500 iL3 of the *Strongyloides ratti* line ED5. For each rat strain, haplotype at the MHC RT1 locus is given in parentheses following the sample size. Open triangles = Wistar (n = 2, variable); open circles = PVG (n = 4, $RT1^c$); open squares = LOU (n = 4, $RT1^u$); closed circles = F344 (n = 4, $RT1^{lv1}$); closed squares = LEW (n = 4, $RT1^l$). Errors are ± 1 S.E.M. In some cases, error bars are smaller than symbol.

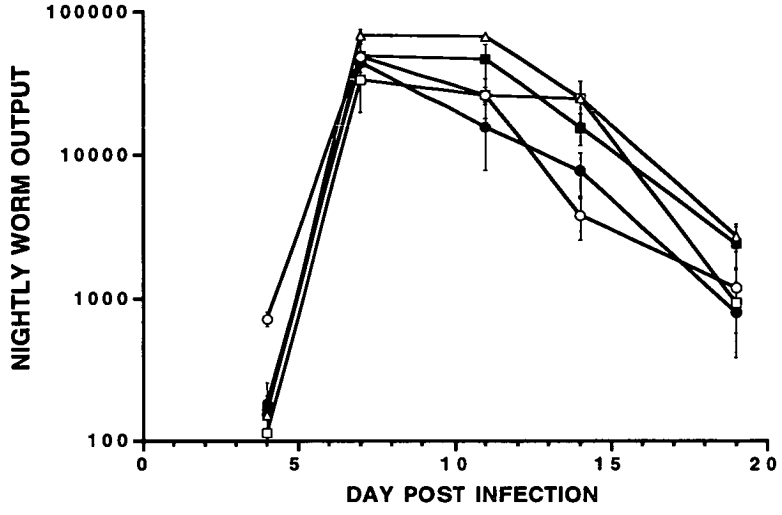
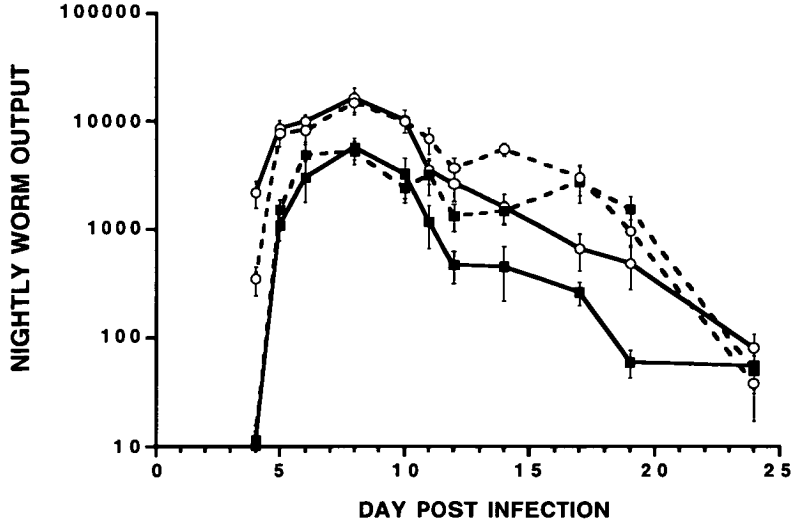


Figure 2. Nightly worm output from PVG and Wistar rats infected with 500 iL3 of the *Strongyloides ratti* lines ED5 or ED279. Open circles, broken line = Wistars infected with ED5 (n = 5); closed squares, broken line = Wistars infected with ED279 (n = 5); open circles, solid line = PVGs infected with ED5 (n = 6); closed squares, solid line = PVGs infected with ED279 (n = 6). Errors are ± 1 S.E.M. In some cases, error bars are smaller than symbol.



This difference is given undue weight in the first experiment. In the first experiment only 2 time points were sampled prior to day 11 PI, the period when nightly worm output was at its highest. The difference in the timing of sampling leads to an underestimate of total worm output prior to day 11 PI and to the (false) conclusion that total worm output differs significantly between the PVG and Wistar strains. Furthermore, if only those sampling points common to both experiments are used to calculate total worm output in the second experiment, the effect of rat strain becomes significant for infections with both ED5 and ED279 ($F_{1,18} = 12.17, p < 0.01$; worm line X rat strain interaction $F_{1,18} = 0.51$, NS).

In the second experiment, there is also a difference in the pattern of worm output in the latter period. This is apparent as an interaction of rat strain X day PI in a repeated measures ANOVA ($F_{5,45} = 2.55, p = 0.041$). Thus, from day 11 PI on, ED5 behaved differently in PVG and Wistar rats without significantly affecting total worm output across the experiment as a whole.

Our results illustrate 2 points. First, rat strain had no overall effect on total worm output. Second, rat strain did have subtle effects on the pattern of larval production. The observed magnitude of these effects depends crucially on when and how often infections are sampled. This observation is not surprising but is nevertheless important in quantitative studies of host-parasite interactions. Much work in this area relies on measurements taken at a fraction of possible sampling points, (e.g. examine relevant figures in Wakelin and Blackwell 1988, Chapters 4 and 5).

The fact that patterns of larval production by *S. ratti* differ between rat strains is a novel finding in this host-parasite system. These differences may stem from divergent aspects of immunology and parasite reproductive strategies in different host strains. The overall difference in total worm output between ED5 and ED279 could be the result of environmental variance or it may have a genetic cause.

In summary, we report that genetic variation in the host (*R. norvegicus)* leads to subtle alteration in patterns of larval production by *S. ratti.* In addition, our results caution that experiments employing incomplete sampling regimes can fail to determine accurately the outcome of host-parasite interactions. Certainly, much remains to be clarified concerning the host-parasite relationship in the system studied here.

CHAPTER 3

The host-specificity of *Strongyloides ratti*

> "...we assume that the existence of a narrow and restricted range of hosts ... represents some sort of evolutionarily optimal condition. We presume that natural enemies, chemical defences or some other force would reduce the fitness of any parasites that extended the range. These are rash assumptions that beg to be tested."
>
> *Begon, Harper and Townsend*
> *Ecology, Third Edition (1996)*

3.1 **Summary:** Factors determining the host-specificity of the parasitic nematode *Strongyloides ratti* were investigated. Differences in the efficacy of thymus-dependent immunity between host species (rats and mice) were insufficient to explain the bulk of observed variation in parasite establishment and reproductive success. The role of natural selection in producing host-specific adaptation was addressed using experimental selection followed by reciprocal fitness assays in both host species. Experimental selection failed to modify the host-specificity of *S. ratti* to any measurable degree, suggesting either a lack of genetic variation among parasites or the involvement of as yet unidentified factors underlying the different performance of *S. ratti* in rats and mice respectively. Results are discussed in relation to existing models of ecological specialisation, host immunology and previous attempts to experimentally alter the host-specificity of parasitic nematodes.

3.2 Introduction

An enduring idea in ecology is that an organism which is a "jack-of-all-trades" is, as a necessary consequence, a "master-of-none" (Van Tierden 1991). That adaptation to one ecological niche might limit an organism's performance in others is an intuitively appealing notion. Identifying the circumstances in which natural

selection favours the evolution of ecological generalists over specialists is a central problem in ecology and evolutionary biology.

Parasites are sometimes seen as the ultimate ecological specialists (Thompson 1994). Their differential fitness on potential host species ("host-specificity") and their success in exploiting the resources of other organisms' bodies, strongly suggest the existence of host-specific adaptations. The presence of such adaptations is, however, more often inferred than demonstrated directly (Secord and Kareiva 1996; Tompkins and Clayton in press). Understanding the basis of host-specificity is therefore a key issue in parasitology (Rohde 1994).

Phylogenetic study of host-parasite co-speciation has been the main focus of research into parasite specialisation (Thompson 1994; Hoberg *et al.* 1997). The main assumption underpinning this approach is that parasites tend to speciate when their hosts speciate - a vicariance event in a host population might result not only in newly isolated host populations, but also in newly isolated parasite populations. Where hosts go, parasites follow. This is the underlying logic of 'Farenholz Rule' (see Klassen 1992). Near-perfect congruences of host-parasite phylogenies continue to be uncovered (e.g. Hafner and Nadler 1988; see Klassen 1992 for a history of this macroevolutionary tradition). Nonetheless, a salient feature of these studies is the ubiquity of phylogenetic non-congruences, often interpreted as reflecting "host-switching" events (Brooks 1988). Thus, while host-specificity evolution is viewed profitably at a macroevolutionary level, host-switching requires explanation in terms of microevolutionary processes.

In evolutionary ecology, the arguments most frequently advanced to account for ecological specialisation are based on the concept of trade-offs (Templeton and Rothman 1974; Via and Lande 1985; Futuyma and Moreno 1988). A trade-off exists when genetic coupling (linkage disequilibrium or pleiotropy) produces a negative correlation in the phenotypic values of two or more traits, thus constraining the

possible range of their simultaneous responses to natural selection (Roff 1992). This simple idea is applicable to many biological scenarios and has been successfully elaborated in numerous theoretical studies (see Stearns 1992 for discussion).

Meanwhile, a large body of empirical data on specialisation has accumulated, mainly concerning phytophagous arthropods (Jaenike 1990; Via 1990). Reciprocal cross transfer of organisms between habitats, as well as artificial selection and other controlled breeding designs are the standard experimental tools in this field (e.g. Fry 1990; Rausher 1984; Mackenzie 1996; Scheck and Gould 1996). Trade-offs require that negative genetic correlations exist between habitat-specific fitness components. In this sense, existing data are disappointing since negative genetic correlations are almost never found (Rausher 1988; Via 1990; Fry 1996; Whitlock 1996, but see McKenzie 1996).

Accordingly, recent models of the evolution of specialisation abandon a role for trade-offs and consider instead the effects of population sub-division among habitats on the strength of selection in each sub-habitat (Kawecki 1996, 1997, 1998; Whitlock 1996). The major findings common to these theoretical studies are as follows. First, adaptation to a specific habitat (fixation of beneficial alleles with habitat-specific effects) is both more probable and more rapid in populations confined to that habitat than in populations spread across several environments. This is because a higher proportion of loci with habitat-specific effects are exposed to natural selection at any one time in habitat-restricted populations so that the strength of selection is greater. Second, populations which are sub-divided over several habitats suffer higher habitat-specific mutation and drift loads (the accumulation of deleterious alleles with habitat-specific effects) resulting in lowered average fitness (Whitlock 1996; Kawecki 1997).

In support of habitat-specific selection models the results of at least two published selection experiments can be adduced, one employing artificial and the other natural, selection. First, in an experiment combining backcrossing with artificial

selection, Scheck and Gould (1996) showed that in the hybridising, phytophagous moth species *Heliothis viriscens* and *Heliothis subflexa*, loci affecting host-plant utilisation success were genetically independent. Scheck and Gould moved loci controlling utilisation of soybean from the polyphagous *H. viriscens* onto the genetic background of the monophagous *H. subflexa*. The ability to utilise soybean in these selected lines did not confer success on other plants in the host repertoire of *H. viriscens* suggesting multiple, independent, habitat-specific loci as the genetic basis of polyphagy. Second, Bergeijk *et al.* (1989) reared three hundred generations of a parasitoid wasp, *Trichogramma maidis*, on larvae of an unnatural host (the moth *Ephestia kuehniella*). Selected lines of *T. maidis* showed no increase in ability to exploit larvae of *E. kuehniella* but did show a substantial (almost total) loss of capacity to exploit their natural host, *Ostrinia nubilalis*. This result is consistent with mutation and drift at *Ostrinia*-specific loci, if such loci exist in the *T. maidis* genome.

Here I investigate factors constraining host-specific adaptation using a gastrointesinal parasitic nematode, *Strongyloides ratti*. *S. ratti* is a natural parasite of rats (*Rattus norvegicus*) but can also complete its life cycle (with reduced success) in mice (*Mus musculus*: Dawkins *et al.* 1980; Ovington *et al.* 1998).

Proximate causes of host-specificity were first quantified using normal and immune-deficient rats and mice. Experimental selection and reciprocal cross infection were then used to assess how quickly and in what direction natural selection might act to change host-specificity. If selection leading to increased fitness on one host involves a contemporaneous decrease in fitness on another, a negative genetic correlation (trade-off) is implicated in maintaining host-specificity. In contrast, if restriction to one host is accompanied by a negligible or delayed decrease in fitness on others, this suggests mutation and drift due to relaxed selection on loci with host-specific effects, as possible causes.

3.3 Methods

(i) Parasites and parasitology

Adult females of *S. ratti* are parthenogenetic and inhabit the host small intestine (Viney 1994). Eggs are shed into the intestine and pass out with host faeces. Parasite offspring then develop in the external environment. The isofemale *S. ratti* line ED 5 (Viney 1996) was used in all experiments and was maintained by serial passage in female Wistar rats (Banton and Kingman, U.K.). Food and water were provided to animals *ad libitum*. Unless otherwise stated, experimental infections were initiated by subcutaneous injection of inocula containing 500 infective third stage larvae (iL3s) suspended in physiological saline (0.8% w/v NaCl solution). Inocula were prepared by serial dilution from a worm-containing suspension of known volume. For assessment of parasite reproductive output (worm output), faeces were collected overnight and divided equally between two culture plates (mice) or three culture plates (rats). Cultures were prepared as described in Viney *et al.* (1992) and incubated for two days at 25 °C. Mature worms were then washed from culture plates, collected and counted under a binocular microscope as described elsewhere (Gemmill *et al.* 1997).

Intestinal parasite numbers (gut burdens) were determined as follows. Animals were sacrificed, the small intestine between stomach and caecum excised, opened longitudinally and rinsed briefly in tap water to remove excess digesta. These initial rinses were subsequently checked for the presence of parasites with none being found on any occasion. Each small intestine was then divided into three roughly equal parts and each part incubated separately in gradated 50ml conical tubes (Corning, U.S.A.) containing approximately 25ml of physiological saline. After two hours incubation at 37 °C, each portion of small intestine was vigorously rinsed and backwashed with fresh physiological saline in order to detach any remaining parasites. Parasites from

each individual animal were then concentrated into a single suspension and counted under a binocular microscope.

Throughout the study, the order in which experimental animals were infected, the position of collecting cages, the order in which intestinal contents and cultures were processed and counted, as well as the positions of cultures in incubators, were randomised anew at each sampling point using random numbers generated by the Excel software package (Excel 5.0).

(ii) Establishment and expulsion

To determine the kinetics of parasite arrival in and expulsion from, the small intestine, the following experiment was conducted. Experimental animals were six week old male Wistar rats and C57/BL/6J mice (Banton and Kingman, U.K.). Gut burdens were determined in groups of five rats and five mice on days 4, 5, 7, 9, 11 and 14 postinfection (PI). Faeces from the animals sacrificed on day 14 PI, were collected overnight prior to each day of sacrifice, cultured and worm outputs determined as detailed above. This design also allowed an estimate of parasite fecundities (offspring per parasite per night), calculated by dividing nightly worm outputs by gut burden values determined on the morning following faecal collection. To the extent that gut burdens are dynamic, and can change during a period of faecal collection, these fecundity estimates can only be taken as rough approximations.

(iii) Thymus dependent immunity

Thymus-dependent (T-dependent) lymphocyte activation and proliferation play a major role in the control of parasitic nematode infections (Maizels *et al.* 1993; Maizels and Holland 1998). To determine whether T-dependent immunity is involved in the different performance of *S. ratti* in rats and mice, an experiment was conducted using congenitally thymic and athymic host animals. In both *R. norvegicus* and *M.*

musculus, single recessive mutations exist which result in complete non-development of a functional thymus in homozygous individuals. Homozygous ("nude") individuals are thus incapable of mounting effective T-dependent immune responses, typically resulting in exacerbation and/or prolongation of experimental infections (e.g. Gemmill *et al.* 1997). In both host species, individuals heterozygous for the nude condition display a normal phenotype.

Six nude mice (BALB/cOlaHsd-*nu/nu*) and six heterozygous mice (BALB/cOlaHsd-*nu*/+), along with six nude rats (Hsd:RH-*rnu/rnu*) and six heterozygous rats (Hsd:RH-*rnu*/+) were infected with ED5. All animals were 6 week old males (Harlan U.K.). To prevent bacterial infections, all 24 animals (nude and heterozygous rats and mice) received a wide-spectrum antibiotic (Baytril, Bayer) at a concentration of 0.01% w/v in drinking water. Worm output was monitored from day 4 PI until day 33 PI.

(iv) Selection

Some strains of *M. musculus* are almost entirely refractory to infection with *S. ratti* (Dawkins *et al.* 1980). To ensure the continuance of viable population sizes in parasite lines selected in mice, four commercially available mouse strains - MF-1; C57/BL/6J; TO and ICR (Banton and Kingman, U.K.) - were first assessed for resistance to *S. ratti* (data not shown). Only in the C57/BL/6J strain was worm output sufficient to support continuing population sizes of around 500 individuals.

At each generation host animals were age-matched male Wistar rats (Banton and Kingman, U.K) and C57/BL/6J mice (from a colony maintained at the University of Edinburgh or from Harlan, U.K.) six to seven weeks old. Five "rat" lines were established and maintained independently in a single rat at each generation. A further five "mouse" lines were maintained separately in pairs of mice. Mice were infected in pairs to ensure the collection of enough parasite offspring to parent subsequent

generations. Faeces collected from both mice within each line were pooled. These initial infections were designated as generation zero (G0). On each infection day, each animal in the experiment (rat or mouse) was infected with 500 iL3s collected from the previous generation of the relevant line. If, at any generation, a selected line failed to produce sufficient viable iL3s, inocula sizes in all selected lines were reduced to the number available in the line with the fewest viable worms. On day 5 PI, faeces were collected overnight from pairs of mice or from single rats, cultured and incubated for two days. Mature worms were then washed from culture plates and the number present in each set of plates determined. It was an arbitrary sample of these worms, collected on day 5 PI, that were chosen as parents of the next generation. In this experimental design parents are not selected on the basis of their individual phenotypic trait values:- all iL3s collected from culture plates on the day of infection have a roughly equal probability of being chosen. Thus, no artificial selection is operating (Falconer and McKay 1996). Any response to selection must result from the action of natural selection alone.

(v) Estimation of variance components

To quantify differences in day 5 worm output due to variation between host species and among selected lines, as well as sources of statistical "noise", the following experiment was carried out. At G10 duplicated infections were initiated for all ten selected lines. Then, worm numbers in each individual culture plate (rather than the number from each set of pooled plates) were counted separately. Finally, for three rat lines a further set of duplicated infections were initiated using the same stock of iL3s, but with inoculations being carried out on a different day. These data were used in several "nested" or "hierarchical" analyses-of-variance (nested ANOVAs; Sokhal and Rolf 1995). These give the variances within and among:- host species; selected

lines; days of inoculation; single rats and mouse pairs as well as the variance among culture plates.

(vi) Reciprocal infection

To assess the effects of selection in one host on the fitness of selected parasites in that or the other host species, a reciprocal cross infection design was employed. For practical reasons, not all selected lines could be assayed in both host species simultaneously. At G14, two "rat" lines and two "mouse" lines were chosen randomly. For each line thus chosen, infections were initiated and worm output assayed in both rats and mice (n = 3 animals per host species per selected line) on days 5, 8 and 11 PI.

(vii) Statistics

The total number of parasite offspring produced across infection (total worm output) was estimated by numerical integration of the area under the nightly worm output by time curves. Where necessary, data were $\log_{10}$ transformed ($\log_{10}$ [value+1]) prior to analysis. One- and two-factor ANOVAs were carried out in the Statview program (Statview release 4.5). Analyses involving nested ANOVA were conducted by fitting generalised linear models in the GLIM statistical package (Crawley 1993). Where faeces were collected from pairs of mice (the selection experiment), numerical values of nightly worm outputs were halved for the purposes of graphical presentation.

3.4 Results

(i) Relative success of S. ratti *in rats and mice*

Nightly worm outputs from animals sacrificed for gut burden counts on day 14 are plotted in Figure 1a. Parasite offspring were first detected in the faeces of all animals on day 4 PI. Mean worm output from rats on day 5 PI was about three times larger than that from mice (Fig. 1a: $F_{1,8} = 22, p < 0.01$). A repeated-measures ANOVA across the experiment revealed significant effects of host species and day postinfection, and a significant interaction of host species with day postinfection (main effects: host species, $F_{1,40} = 17.16, p < 0.01$; day postinfection, $F_{5,40} = 2.9, p < 0.05$; interaction term, $F_{5,40} = 3.17, p < 0.05$). Output in rats remained high throughout sampling but was barely detectable in mice by day 13 PI. One-way ANOVA on total worm output revealed a sixteen-fold difference in total worm output between rats and mice (Figure 1b: $F_{1,8} = 72, p < 0.0001$). Thus, *S. ratti* established and reproduced in mice, but reproductive success was substantially less than that achieved in rats.

(ii) Establishment, expulsion and fecundity

Gut burden kinetics in rats and mice paralleled the nightly worm output curves. Gut burdens were significantly higher in rats compared to mice at every point (Figure 2a). There was a five-fold difference in peak gut burden between rats (peak at day 11 PI) and mice (peak at day 5 PI) ($F_{1,8} = 36, p < 0.001$).

While all parasites were expelled from the intestines of mice by day 14 PI, gut burdens in rats remained high (despite an unexplained dip at day 9 PI, probably due to experimental error). Parasite fecundity estimates (excluding estimates based on day 9 gut burdens) are plotted in Figure 2b and were significantly different in rats and mice, being four to five times greater in rats.

Figure 1. Reproductive success of *S. ratti* in rats (closed squares; $n = 5$) and mice (open circles; $n = 5$). In (a), plotted values are nightly worm outputs per host animal. In (b), plotted values are total worm outputs per host animal. Error bars are ± 1 S.E.M. In some cases, error bars are smaller than symbol.

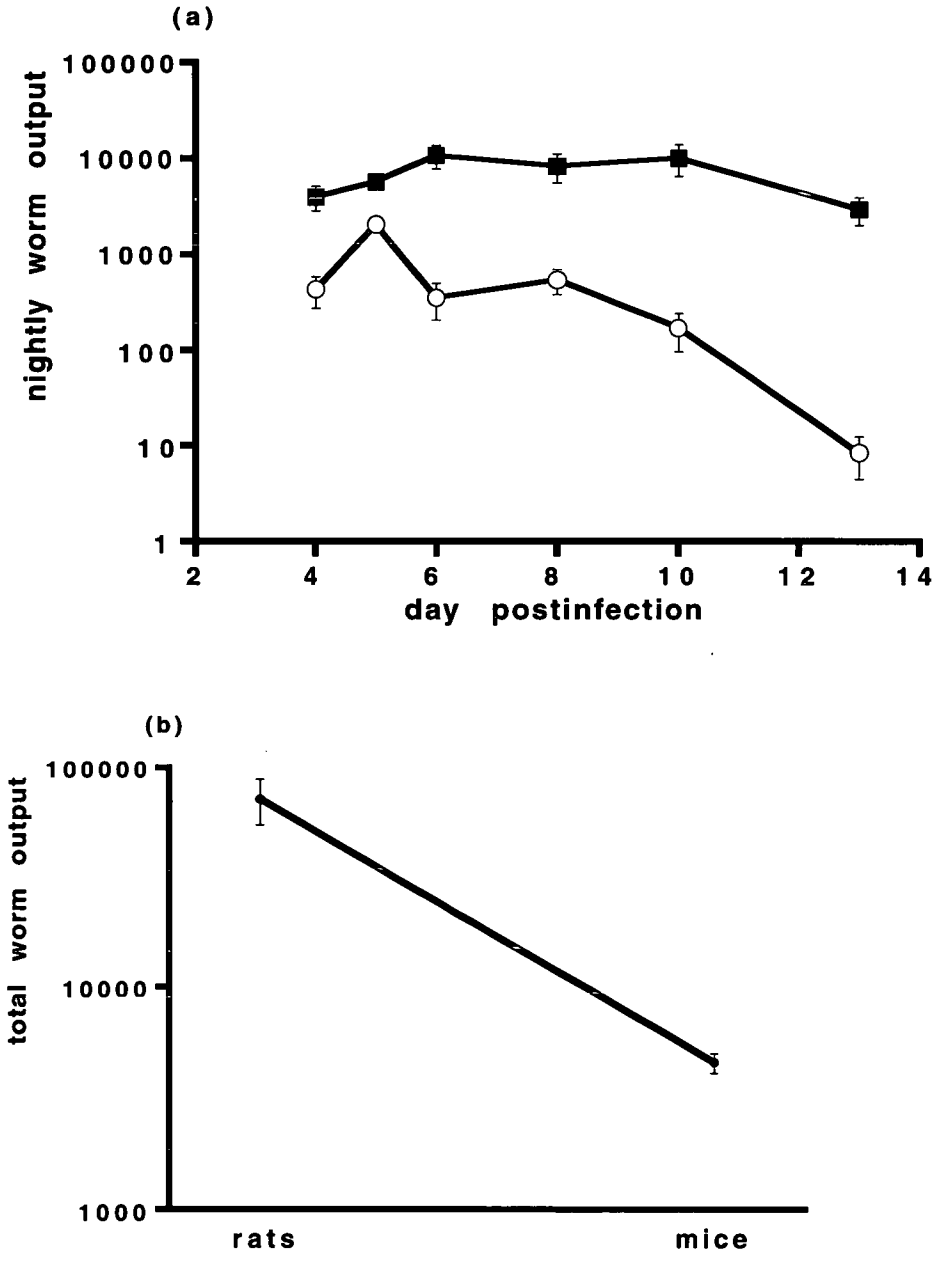
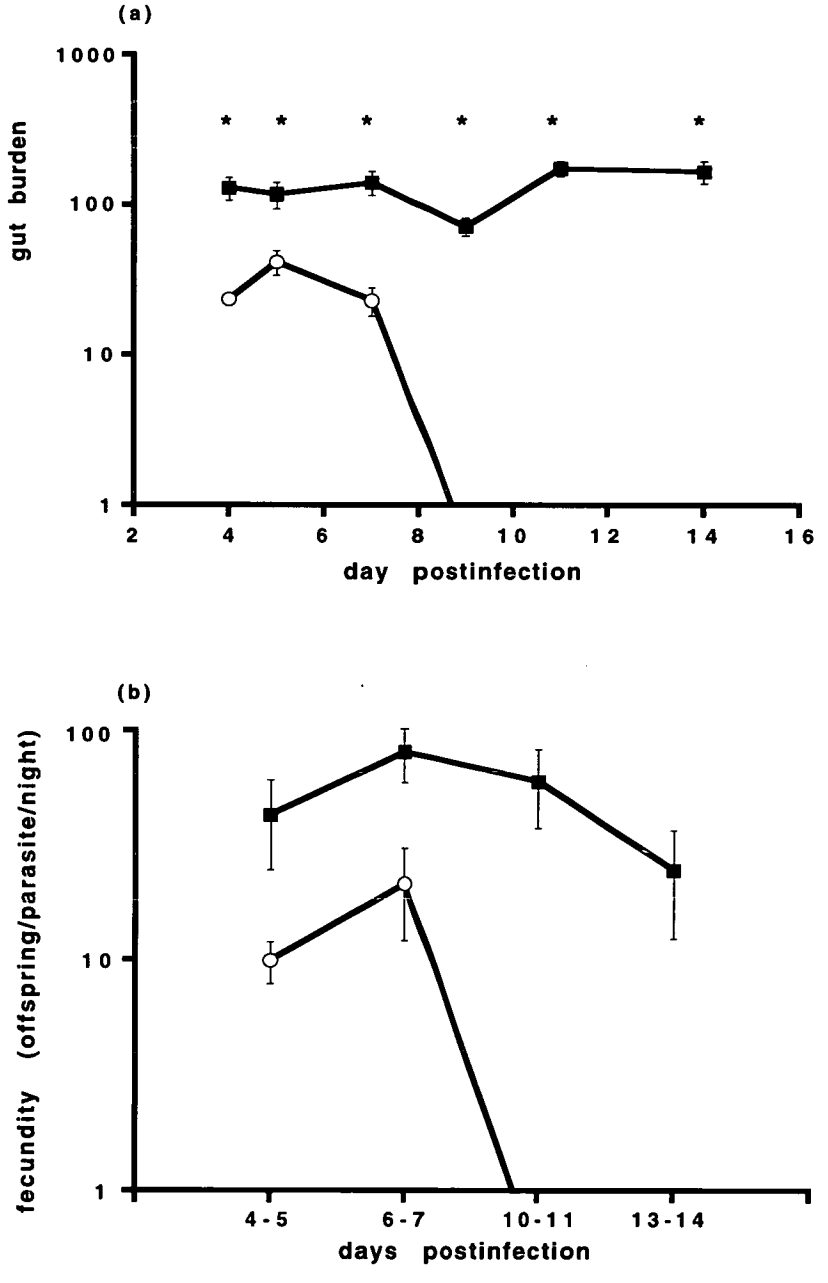


Figure 2. Kinetics of gut burdens and parasite fecundity in rats (closed squares; n = 5 at each data point) and mice (open circles; n = 5 at each data point). In (a), plotted values are gut burdens. In (b), plotted values are estimated parasite fecundities. Asterisks denote significant differences ($p < 0.01$) between rats and mice as assessed by ANOVA on $\log_{10}$ transformed values. Error bars are ± 1 S.E.M. In some cases, error bars are smaller than symbol.



(iii) The role of thymus-dependent immunity

Nightly worm outputs from nude and normal rats and mice are shown in Figure 3a. Worms were found in cultures from all nude rats as well as all normal rats and mice, on day 4 PI. Only one nude mouse had a patent infection at this time, the remaining five nude mice producing no worms until day 5 PI. Worm outputs from nude animals of both species remained elevated for a prolonged period compared to that from normal, conspecific animals. Although this led to a more than six-fold difference in total worm output between nude and normal rats (Figure 3b: $F_{1,10} = 48$, $p < 0.0001$), nude mice did not produce significantly more worms than normal mice ($F_{1,10} = 2.1$, N.S.). Thus, there was a significant interaction of thymic status with host species (main treatment effects: thymic status, $F_{1,20} = 24$, $p < 0.0001$; host species, $F_{1,20} = 200$, $p < 0.0001$; interaction term: $F_{1,20} = 6.1$, $p < 0.05$). However, while total worm output from mice was not affected significantly by host thymic status, there was some evidence that once larval production reached its peak it declined thereafter less rapidly in nude mice. Using log-log regressions of nightly worm output on day postinfection to calculate a rate of decline (slope) for each individual mouse allowed these values to be used as independent data points in a one-factor ANOVA. This revealed that larval output declined about half as quickly in nude compared to normal mice ($F_{1,10} = 9.85$, $p < 0.05$).

(iv) Outcome of selection and sources of variance

The trait under selection was worm output on day 5 PI. Selection was continued for eighteen generations. The mean day 5 worm outputs of "rat" and "mouse" lines between G0 and G17 are shown in Figure 4a. At some generations (G3, G11, G15 and G16), day 5 output was not measured for logistical reasons. Tables 1 (a), (b), and (c) show the results of nested ANOVAs used to estimate

variance components (at G10) in the selection experiment. Not surprisingly, host species was the largest single source of variance in day 5 worm output. There was no significant variation among selected lines, indicating that by G10 no appreciable genetic divergence had occurred. Variation between culture plates, infection days and duplicate infections all accounted for substantial portions of the total variance in day 5 worm output.

Reciprocal cross infection at G14 showed no effect of selection on day 5 worm output (Figure 4b). There was no significant interaction between the host species in which lines had been maintained and the host species in which they were measured (main treatment effects: selection regime, $F_{1,20} = 0.03$, N.S.; host species, $F_{1,20} = 31$, $p < 0.0001$; interaction term, $F_{1,20} = 0.39$, N.S.). Thus, worm performance in mice and rats was unaffected by the host species which had harboured the previous 14 generations (Fig. 4b). Virtually identical results were obtained on days 8 and 11 PI (data not shown). Thus, there had been no measurable response by G14.

Figure 3. Effect of host thymic status on reproductive success of *S. ratti* in rats (closed squares: $n = 6$ nude; $n = 6$ normal) and mice (open circles: $n = 6$ nude; $n = 6$ normal). In (a), plotted values are nightly worm outputs and solid lines = normal animals; broken lines = nude animals. In (b), plotted values are total worm outputs. In both (a) and (b), error bars are ± 1 S.E.M. In some cases error bars are smaller than symbol.

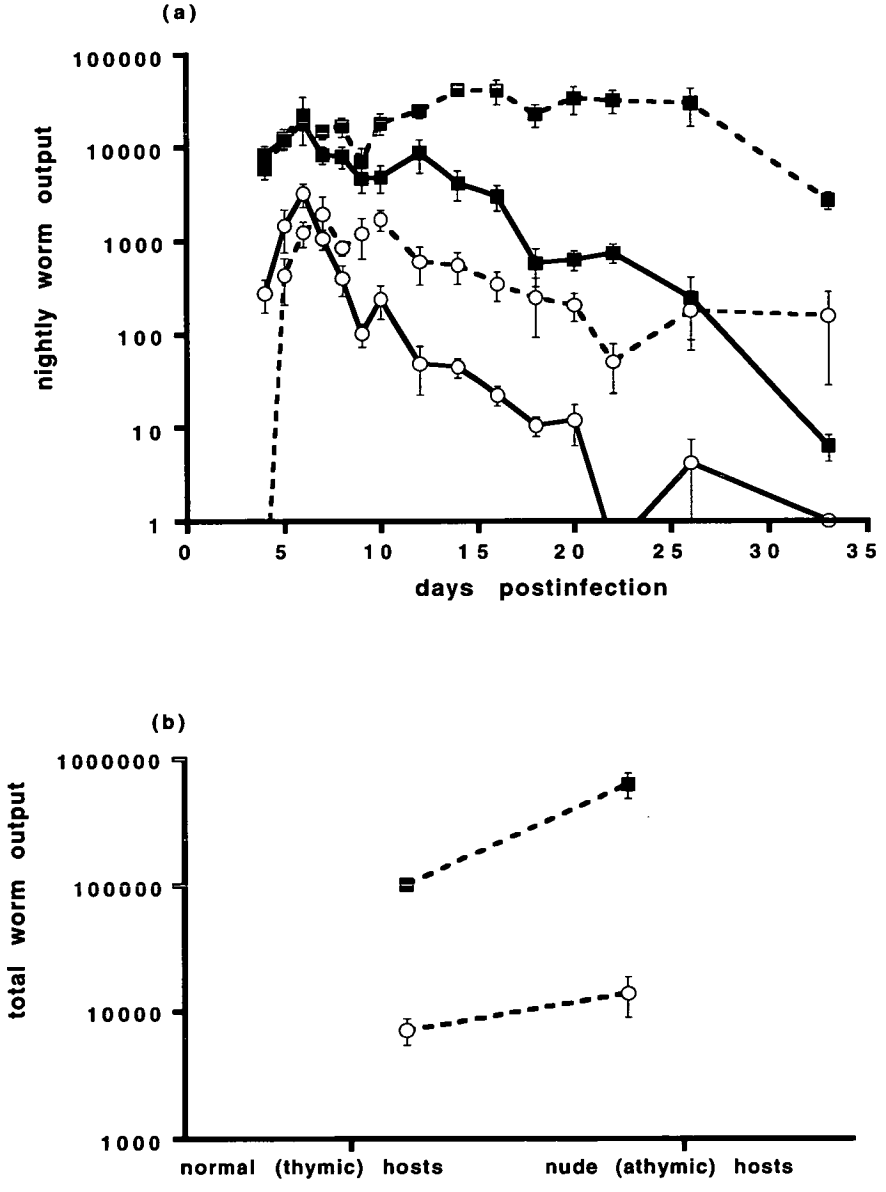


Table 1. Nested ANOVAs showing sources of variance at generation 10 of the selection experiment. All data were $\log_{10}$ transformed. S.S. = sums of squares, d.f. = degrees of freedom and M.S. = mean square. In (a) and (b) respectively, each duplicate infection or each culture plate was nested within selected line, nested within host species . In (c), each duplicate infection in "rat" lines was nested within each day of infection, nested within selected line. In each table, percentages of the total variance accounted for by each source are shown.

(a)

source of variance	S.S.	d.f.	M.S.	*F*	% of total	*p*-value
host species	26.81	1	26.81	134	79.9	< 0.05
selected line	1.59	8	0.2	0.4	4.7	> 0.05
duplicate	5.16	10	0.52	-	15.4	-
total	33.55	19	-	-	-	-

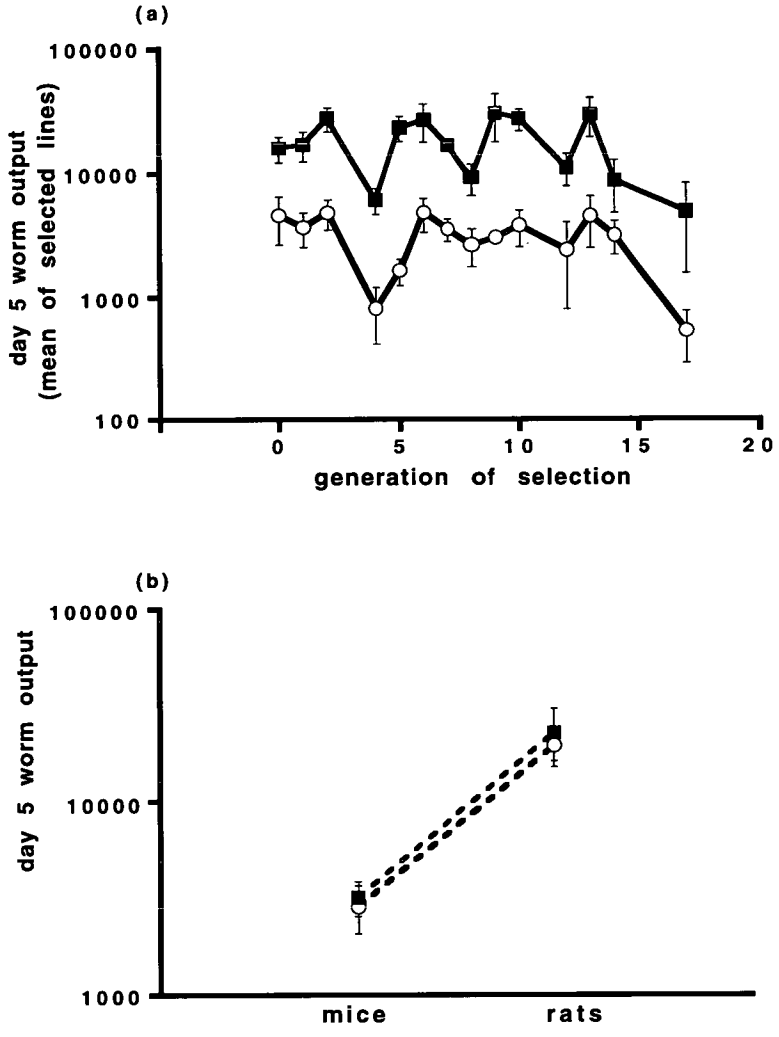
(b)

source of variance	S.S.	d.f.	M.S.	*F*	% of total	*p*-value
host species	12.5	1	12.5	39.06	58	< 0.05
selected line	0.64	2	0.32	0.31	3	> 0.05
culture plate	8.33	8	1.04	-	39	-
total	21.47	19	-	-	-	-

(c)

source of variance	S.S.	d.f.	M.S.	*F*	% of total	*p*-value
selected line	0.74	2	0.37	1.1	44	> 0.05
day infected	0.67	3	0.34	7.17	40	< 0.05
duplicate	0.28	6	0.05	-	16	-
total	1.69	11	-	-	-	-

Figure 4. Outcome of selection in rats and mice on day 5 worm output in *S. ratti*. In (a), plotted values are mean day 5 worm outputs of "rat" lines (closed squares: $n = 5$) and "mouse" lines (open circles; $n = 5$) respectively. In (b), plotted values are mean day 5 worm outputs of 2 "rat" lines and 2 "mouse" lines each assayed in rats ($n = 3$ per line) and mice ($n = 3$ per line). Error bars are ± 1 S.E.M. In some cases error bars are smaller than symbol.



3.5 Discussion

Even where the ecology of an organism is very habitat-restricted, this need not imply a proportionate degree of specialisation. The problem is partly terminological (Futuyma and Moreno 1988; Berenbaum 1996). To some, the word "specialisation" implies the possession of habitat-specific adaptations. Undoubtedly, such adaptations are maintained in many parasitic taxa, but their existence is usually inferred indirectly (Secord and Kareiva 1996; Tompkins and Clayton in press). In the study of phytophagous arthropods there are parallel problems concerning the identification of phylogenetically derived, habitat-specific adaptations ("key innovations") which have now begun to be redressed (Berenbaum *et al* 1996).

The experiments reported here give an empirical account of some proximate factors shaping host-specificity in *S. ratti.* To my knowledge this is the first study to compare directly the performance of *S. ratti* in rats and mice. On average, unselected worms had substantially higher reproductive success in rats compared to mice. This resulted from lower establishment rates, earlier expulsion of established parasites and reduced per parasite fecundity in mice. The majority of these differences can not be ascribed to the mouse T-dependent immune system, since worm output was significantly lower in nude (thymus deficient) mice than in normal rats. Further, total worm output did not differ significantly between nude and normal mice. These findings confirm that T-dependent immunity is not the major cause of reduced parasite fitness in mice. Particular compartments of the mouse T-dependent immune system may nevertheless be more or less important than others in this regard. For example, a recent study on the cytokine IL5 reported a significant difference in worm output between congenitally IL5 deficient and IL5 sufficient mice (Ovington *et al.* 1998). This cytokine forms part of the "'type 2" T-dependent immune pathway and is involved in the recruitment of eosinophils, immune effector cells that target and destroy tissue-phase larvae of some parasitic nematodes (Maizels and Holland 1998). The

relative importance of IL5 (or any cytokine) in mediating the control of *S. ratti* infections in rats and mice has not yet been investigated directly, however.

Conceivably, the small size of mice compared to rats may physically limit space and/or other resources available to parasites, with observed host-specificity arising as an effect of scale. However, previous studies have shown that density dependence in the reproductive output of *S. ratti* in C57/BL/6 mice is absent, even when inocula sizes are increased up to three thousand worms or more (Dawkins *et al.* 1980). Resource limitation is therefore unlikely with inocula sizes of five hundred parasites. Clearly, one or more factors must be responsible for generating the difference in fitness of *S. ratti* in these two host species. Perhaps the most likely candidate mechanisms are other components of the rat and mouse immune systems (e.g. T-independent antibody production and non-specific inflammatory responses), or non-immunological factors stemming from the different physiologies of rats and mice.

In the present study, eighteen generations of experimental selection failed to modify the fitness of *S. ratti* in mice. Reciprocal fitness assays of selected lines in both host species confirmed that the original pattern of host-specificity displayed by this parasite remained intact. It is feasible that continued selection would result in a response. Artificial selection experiments sometimes fail to produce a response in the first few generations, the usual problem being small population size leading to genetic drift which retards a response (Falconer and McKay 1996). However, experience from the bulk of published artificial selection experiments is that if a response occurs at all it does so within eighteen generations (Falconer 1989, 1992). Artificially imposed selection differentials may of course be larger than those imposed by natural selection and it may be the case that given enough time, *S. ratti* could be adapted to a new host species. Alternatively, the use of an established laboratory line derived from a single female may have limited genetic diversity in the base population. Again, this explanation does not seem entirely satisfactory. The same line has responded rapidly

to selection in the laboratory for other, apparently complex, life history traits (conditional sexuality; Viney 1996).

S. ratti infections are common in wild rats (Wertheim and Lengy 1964; Fisher and Viney 1998). However, since the late nineteenth century, there have been at least two reports of *Strongyloides* spp. occurring in wild-caught mice (Grassi and Serge 1887; Prokopic and Del Valle 1966). In one of these cases, recovered worms have been classified taxonomically as *S. ratti* (Prokopic and Del Valle 1966). The absolute non-dispersal of *S. ratti* infective larvae to wild mice is therefore open to doubt. If such occurrences are at all frequent, what prevents this nematode from expanding its host range?

Currently, two broad classes of explanation exist for the evolution of ecological specialisation - models based on trade-offs and models of habitat-specific selection (Kawecki 1998). As mentioned in the introduction to this chapter, trade-off based hypotheses are hampered by a lack of data showing the necessary negative genetic correlations. In the case of parasitic nematodes three published studies using experimental natural selection followed by reciprocal fitness assays have been conducted, all with *Nippostrongylus brasiliensis* (Haley 1966a; Solomon and Haley 1966; Wescott and Todd 1966). *N. brasiliensis* is a cosmopolitan parasite of rodents (Anderson 1992). Haley (1966a) selected *N. brasiliensis* (using a base population isolated from rats) for forty generations in hamsters (*Mesocricetus auratus*). By the eighth generation, "hamster" lines showed a significant increase in establishment in hamsters with an apparent selection limit being reached between the sixteenth and twenty fourth generations. After thirty nine generations these lines were no less successful in rats than rat-selected control lines. Solomon and Haley (1966) selected lines of *N. brasiliensis* (again, isolated from rats) for success in mice. The response to selection was substantial and reached its limit after only seven generations. A further forty seven generations of selection failed to reduce the fitness of these lines in rats

compared to control lines. In contrast, Westcott and Todd (1966) conducted a very similar experiment. They too reported a rapid increase in the performance of "mouse" lines. However, this response was accompanied by a decrease in the fitness of "mouse" lines in rats. Thus, what evidence there is regarding genetic correlations between fitness components on alternative hosts in *N. brasiliensis* is mixed. Other studies have attempted to adapt parasitic nematodes to new host species, but few involve adequate controls or reciprocal fitness assays (e.g. Bracket and Bliznick 1949; Lindquist 1950; Thatcher and Scott 1962; Haley 1966b; Lichtenfels 1971; Forrester 1971; Vincent *et al.* 1982; Lyons *et al.* 1987).

An understanding of the evolutionary routes by which pathogens alter their host repertoire may prove valuable in designing successful control measures. Concern over newly emerging diseases, many of which are zoonotic in origin, underlines the need for continued research in this field (Murphy 1998). Controlled breeding experiments are not easy with parasites. Assigning phenotypic values and measuring genetic correlations (especially in endoparasitic species) are awkward propositions, but can be accomplished (Mackinnon and Read in press). This approach offers a powerful tool for studying the microevolutionary processes surrounding host-switching events in nature and so, by direct implication, the factors maintaining and constraining the host-specificity of parasites.

Chapter 4

Immunity acquired against another nematode does not induce sexuality in *Strongyloides ratti*

4.1 **Summary:** The specific nature of host immune responses required to induce conditional sex in the parasitic nematode *Strongyloides ratti* were investigated. The frequency of sexual morphs was monitored in infections of rats previously immunised with either *S. ratti*, a second nematode species (*Nippostrongylus brasiliensis*) or a mammalian antigen (sheep red blood cells). Immunisation with both *S. ratti* and *N. brasiliensis* conferred substantial resistance to reinfection. However, the proportion of parasitic offspring that developed into sexual morphs was significantly affected only by existing host immunity acquired against *S. ratti*. Results are discussed in relation to alternative explanations for the maintenance of conditional sexual reproduction.

4.2 Introduction

All else being equal, a parthenogenetic female replicates her genes at twice the rate of a sexually reproducing rival (Bell 1982). Thus, the selective value of sexual reproduction remains a central and unresolved question in evolutionary biology (see West *et al.* in press for a recent discussion). Currently, two general types of theory dominate investigations of this important issue. First, "Red Queen" models stress the potential of frequency-dependent selection to favour rare genotypes, the production of which is one consequence of sex and recombination (e.g. Jaenike 1978; Bell and Maynard Smith 1987). Second, "Mutational" models concentrate on the power of sexual reproduction to facilitate the eradication of deleterious mutations from a lineage

by exposing different gene combinations to the action of natural selection (Kondrashov 1982, 1984). These two general classes of theory are nevertheless united by the fact that in both cases sexual reproduction is believed to be favoured through the generation of genetically variable offspring.

In a previous study we suggested that immune-dependent conditional (facultative) sex in the parasitic nematode *Strongyloides ratti*, a natural parasite of rats, represents an immune-evasion strategy aimed at countering frequency-dependent selection generated by genotype-specific host immune responses (Gemmill *et al.* 1997, see Appendix). If host immune responses are targeted against particular parasite genotypes, rare genotypes will be favoured initially but selected against when they become relatively common. Sex and recombination produce new, rare genotypes. This is a Red Queen class of explanation.

Here we attempt to discriminate between this and an alternative hypothesis, namely that conditional sex is favoured as a response to increased selection against deleterious mutations under increased environmental stress. This alternative explanation is based on two assumptions: - that there exists a "critical number" of mutations above which no individual reproduces (truncation selection) and that this critical number itself depends upon environmental conditions. If the critical number drops as environmental stress increases, a smaller proportion of the population (the proportion comprised of those individuals carrying the smallest numbers of mutations) survives to reproduce. In this situation, sexual reproduction may be favoured if it results in a larger distribution of mutation numbers per offspring. In theory, this applies because a larger proportion of sexually produced offspring carry mutation numbers below the new critical value (Kondrashov 1984). This was first suggested as an explanation for seasonally occurring, cyclical parthenogenesis as occurs in some aphids, *Daphnia* spp. etc. (Bell 1982), but can just as readily be invoked to explain conditional sexuality (Kondrashov 1984).

In the case of the conditional sexuality displayed by *S. ratti*, a rise in the frequency of sexual offspring coincides with an increase in a major environmental stress (the host immune response). If this mutational "stress" model explains conditional sex in *S. ratti*, then host immunity acquired against a variety of antigens should be equally effective in inducing the production of sexual morphs. If this explanation is wrong, only host immunity acquired against *S. ratti* can be expected to act as a cue for conditional sexual reproduction by this parasite. In fact, the previously advanced Red Queen explanation for conditional sex in this nematode predicts unequivocally that only immunity acquired against *S. ratti* should elicit an increase in sexuality.

Here we use previous exposure to *S. ratti*, to another nematode (*Nippostrongylus brasiliensis*) and to a commonly utilised mammalian antigen (sheep red blood cells, here abbreviated to RBCs) to conduct an empirical test of the mutational hypothesis. The aim of immunisation with RBCs was to stimulate innate immunity (perhaps by elevating levels of non-specific effector cells; neutrophils; complement etc.) not acquired specifically against parasite antigens. Dosages of antigens were varied in order to maximise the range of resulting acquired immune responses.

4.3 Methods

(i) Parasitology

The isofemale *S. ratti* line ED5 (Viney 1996) was used. Infective larvae of *N. brasiliensis* were obtained from a laboratory line maintained at the University of Edinburgh. Experimental animals were female Wistar rats six weeks old (Banton and Kingman, U.K.). Food and water were provided to animals *ad libitum*. Experimental infections were initiated by subcutaneous injection of inocula containing 500 infective third stage larvae (iL3s) suspended in physiological saline (0.8% w/v NaCl solution).

Inocula were prepared by serial dilution from a worm-containing suspension of known volume. For assessment of parasite reproductive output (worm output), faeces were collected overnight and divided equally between three culture plates. Cultures were prepared as described in Viney *et al.* (1992) and incubated for two days at 25 °C. Mature worms were then washed from culture plates, collected, counted and the relative proportion of sexual and asexual offspring determined as described elsewhere (Gemmill *et al.* 1997).

(ii) Immunisation

Five weeks prior to infection with 500 iL3s of ED5, fifteen rats were exposed to immunising nematode infections and fifteen rats sham-inoculated with sterile physiological saline. Five rats were immunised by subcutaneous injection of 10 iL3s of ED5 suspended in sterile physiological saline. A second group of five rats was infected with 100 larvae and a third group with 1000 larvae of *N. brasiliensis*, also administered by subcutaneous injection. These immunising infections were subsequently monitored to confirm that patent infections had established, this being achieved via faecal egg counts carried out by a Modified McMasters technique (Whitlock 1948).

Fourteen days prior to infection, five sham-inoculated rats were immunised by subcutaneous injection with 10^6 RBCs (Scottish Antibody Production Unit, Carluke, U.K.) and five with 10^7 RBCs suspended in physiological saline. This left a group of five non-immunised rats to serve as controls. At this time, all other animals in the experiment were injected with a similar volume of physiological saline to control for possible effects of injection *per se*. All fifteen rats in the nematode-immunised treatment groups were dosed with 0.11ml of 17.6% w/v thiabendazole suspension (Thibenzole, MSD AGVET, a broad-spectrum antihelminthic) by oral intubation to remove any remaining parasites on both the twenty eighth and twenty ninth days

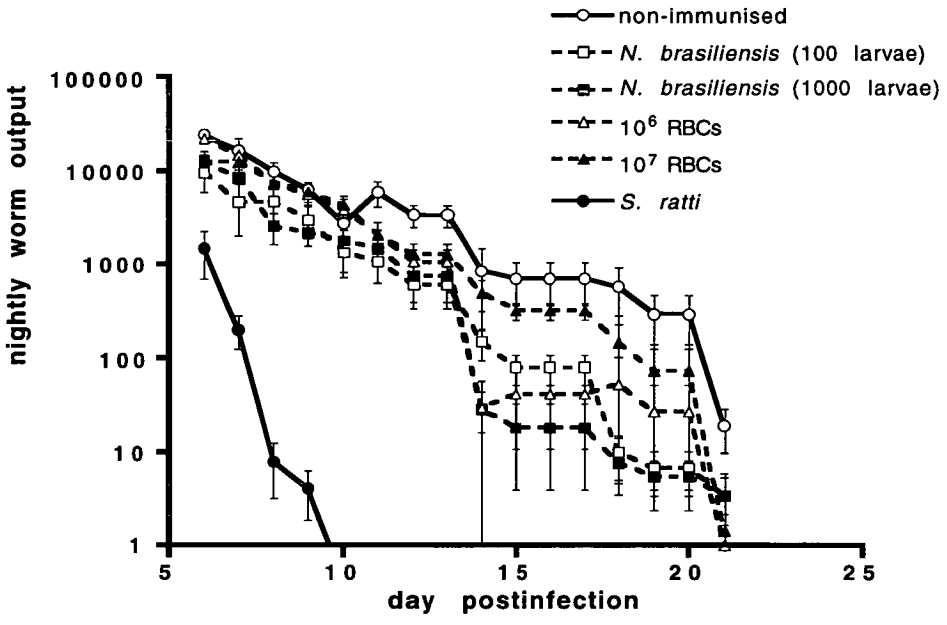
following immunising infection. The remaining fifteen animals (control and RBC-treated groups) were identically treated to control for possible effects of thiabendazole treatment.

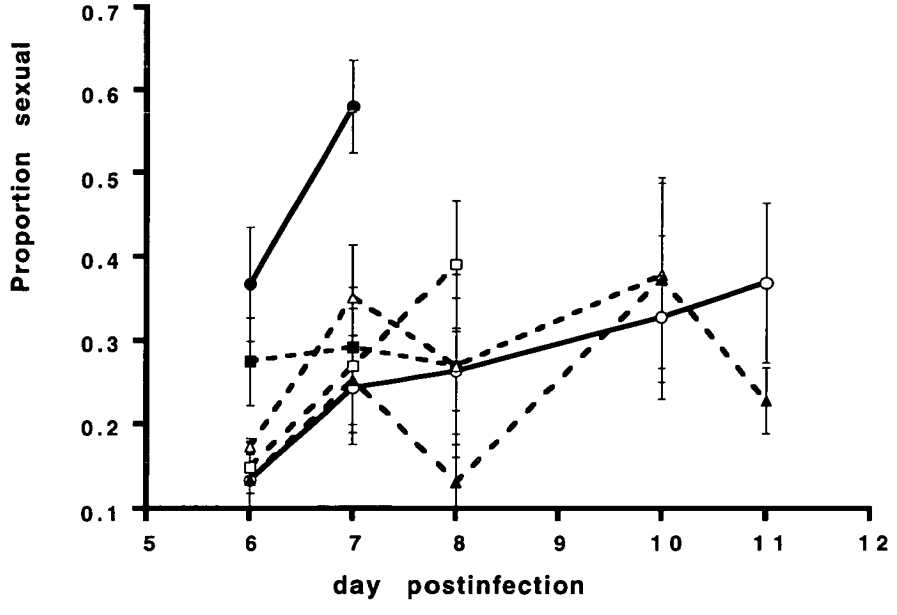
(iii) Statistical analysis

The total number of parasite offspring produced across infection (total worm output) was estimated by numerical integration of the area under the nightly worm output by time curves. Similarly, the total numbers of both sexual and asexual offspring were determined by numerical integration. These latter estimates were then used to calculate the overall proportion of sexual morphs produced from each rat, such that each infection contributed a single degree of freedom to the analysis. Data were analysed in the GLIM statistical package (Crawley 1993). First, full models were fitted including all six experimental groups. Terms were then grouped by a step-wise process to test for significant differences between treatments or groups of treatments (Crawley 1993). Whether the grouping of particular treatments caused a significant increase in deviance (thereby indicating a significant difference between those treatments) was assessed with *F*-tests.

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 395, demonstrating considerable overdispersion and suggesting that the data did not fit the binomial error assumption (Crawley 1993). All proportion data were therefore angular (arcsin [square-root]) transformed and analysed assuming a normal error structure. Only sampling days on which all infections produced positive worm counts were included. This ensured all treatment groups were equally represented at all time points and eliminated noise due to proportions based on.

Figure 1. The effect of immunisation regime on (a) nightly worm output and (b) the proportion of larvae developing into sexual morphs. In both (a) and (b), plotted values are the means of five rats per group. Solid line, open circles = non-immunised controls; solid line, closed circles = rats immunised with 10 iL3s of ED5; broken line, open squares = immunisation with 100 larvae of *N. brasiliensis*; broken line, closed squares = immunisation with 1000 larvae of *N. brasiliensis*; broken line, open triangles = immunisation with 10^6 RBCs; broken line, closed triangles = immunisation with 10^7 RBCs. Errors are ± 1 S.E.M.





low worm counts (Gemmill *et al.* 1997). For graphical purposes, the mean daily proportion of sexual morphs was plotted only where ≥ 50 worms could be typed from each rat in an experimental group.

4.4 Results

(i) Immunisation and worm output

Nightly worm outputs are shown in Figure 1a. Over the course of the experiment as whole, immunisation led to significant differences in total worm output between treatment groups ($F_{5,24} = 9.71, p < 0.01$).

Outputs from rats given 10^6 and 10^7 RBCs were not significantly different from each other or from non-immunised controls ($F_{1,26} = 2.22$, NS) indicating that immunisation with RBCs had failed to evoke any immune responses that were effective in controlling infection with *S. ratti*. Similarly, worm outputs in the groups immunised with 100 and 1000 larvae of *N. brasiliensis* differed neither from each other ($F_{1,24} = 0.36$, NS) nor from rats immunised with *S. ratti* ($F_{1,27} = 1.61$, NS). However, output from rats immunised with *S. ratti* and *N. brasiliensis* was significantly lower than that from control rats and RBC-treated rats combined ($F_{1,28} = 26.17, p < 0.01$).

(ii) Immunisation and conditional sex

The frequency of sexual morphs differed significantly between treatment groups (Figure 1b; $F_{5,24} = 19.65, p < 0.01$). However, this effect was entirely due to the group of rats immunised with *S. ratti* ($F_{1,28} = 19.73, p < 0.01$). Thus, while previous exposure to *N. brasiliensis* conferred measurable resistance to reinfection as assessed by its effect on parasite reproductive success, it failed to raise the frequency of sexual offspring above that observed in controls.

4.5 Discussion

The occurrence of sexually reproducing morphs in *S. ratti* is powerfully influenced by the strength of host immunity (Gemmill *et al.* 1997). The results of the experiment described here confirm this finding. Further, these results indicate that immunity acquired through previous exposure to *S. ratti* antigens is singularly effective in promoting conditional sexuality. While previous infection with *N. brasiliensis* conferred protective immunity to subsequent infection with *S. ratti*, it failed to invoke any measurable change in the frequency of *S. ratti* sexual morphs.

This result is not expected if conditional sex in this parasite is maintained as a mechanism for reducing mutation load in harsh environments. If this were the case, any acquired host immune response that affected parasite fitness should induce an opposite plastic response in the frequency of sex. Thus, a critical assumption of the Red Queen explanation for conditional sex in *S. ratti* is strongly supported by these results. However, this conclusion is not without its own caveat. Specifically, it is possible that the spectrum of acquired immunity generated here may be insufficient to reveal the potential of each antigen to affect immune-dependent parasite sexuality. For example, it could be the case that immunisation with *N. brasiliensis* produces insufficient stress to induce increased production of sexual morphs.

It has recently been argued with some force that more than one mechanism may be at work maintaining sex in nature (West *et al.* in press). Red Queen and mutation accumulation avoidance processes, far from being mutually exclusive, may in fact interact synergistically in counteracting the two-fold cost of sexual reproduction in at least some species (Howard and Lively 1994; West *et al.* in press). A resolution of these questions is the aim of current empirical studies with *S. ratti.* by A. F. Read and S. A. West.

Chapter 5

Optimal timing of first reproduction in parasitic nematodes

A slightly re-formatted version of this chapter has been accepted for publication in The Journal of Evolutionary Biology.

5.1 **Summary**: The time between infection and the onset of reproduction (maturation time) is a key determinant of body size, fecundity and generation time in parasitic nematodes. An optimality model for maturation time is developed centred on pre-maturational growth, the duration of which has opposing consequences for fecundity and for survival to reproductive age. The maturation time favoured by natural selection is found to be inversely proportional to pre-maturational mortality rate. The product of maturation time and mortality rate is predicted to be an invariant number equal to the allometric slope linking daily fecundity to maturation time. Predictions are tested using comparative data on mammalian gastrointestinal nematode taxa. Half the cross-species variation in prepatent period (the time from infection until propagules are shed from the host) is accounted for by this adaptive trade-off hypothesis, even though many biological details are not explicitly modelled. These results are discussed in the light of previous studies and in the context of general models of life history evolution.

5.2 Introduction

Maturation time in the final host is a major determinant of generation time, body size and reproductive output in parasitic nematodes (Skorping *et al.* 1991; Read

and Skorping 1995; Morand and Sorci 1998). These parameters not only represent some key components of parasitic nematode fitness, they also affect levels of infection and pathology experienced by hosts (Read and Skorping 1995). An improved understanding of how selection acts on maturation time is of applied as well as theoretical interest since medical and veterinary intervention programs are expected to alter selection on parasite life history schedules (Medley 1994; Read and Skorping 1995; Buckling *et al.* 1997, Poulin 1998, Skorping and Read 1998).

As in many organisms (Peters 1983), adult female body size is closely linked to reproductive success in parasitic nematodes (Skorping *et al.* 1991). Across species there is a positive correlation between fecundity and prepatent period (the time from infection until parasite propagules are shed from the host; Skorping *et al.* 1991; Morand 1996). This almost certainly arises because bigger worms take longer to grow and are more fecund than smaller worms. Where somatic growth either ceases or slows at maturity, as seems to be the case in nematodes (Malakhov 1994), age and size at maturity must, in general, be positively correlated (Stearns 1992). Thus, *Trichinella spiralis* is a few millimetres long and commences reproduction less than a week after infection, while *Ascaris lumbricoides* is about 30 cm in length and can spend several months growing before beginning to produce eggs.

On a cross-taxa level, the relationship between daily fecundity and prepatent period in parasitic nematodes is well described by an allometric relationship (Skorping *et al.* 1991) $Y = cX^{\beta}$, where X is prepatent period, Y is daily fecundity, and the exponent β is the allometric slope (the slope of the regression of log Y on log X). The allometric slope of daily fecundity with prepatent period is greater than +1 across gastrointestinal nematode taxa that parasitise mammals; Skorping *et al.* (1991) reported a slope of 2.66 across 19 genera. This has the immediate implication that daily fecundity increases disproportionately (i.e. more than linearly) for a given delay in the onset of reproduction. Since a female's future reproductive output can be substantially

enhanced by postponing maturation, it is natural to ask what constrains the evolution of ever longer maturation times.

Here we develop an optimality model incorporating a trade-off between the size-mediated fecundity advantage of delaying reproduction and the potential disadvantage of doing so: longer growth phases entail increased exposure to the risk of pre-maturational mortality. Natural selection should act to optimise the fitness consequences (costs and benefits) of this trade-off, an intuitive expectation that has been supported in several theoretical studies (e.g. Bell 1980; Kozlowski and Wiegert 1987; see Stearns 1992 for discussion). We ask how well the model explains quantitative variation in prepatent period across gastrointestinal nematode taxa and conclude by discussing results in relation to more general life history frameworks.

5.3 Model

Maturation time (α) is defined as the time (postinfection) at which a female first reproduces. We wish to know how the optimal maturation time of a female parasitic nematode, α^*, varies as a function of mortality rate and potential fecundity.

We make the following assumptions (see Table 1 for notation), and the validity of each is dealt with in the discussion.

(i) Lifetime reproductive output (total number of offspring produced by a parasitic female in her lifetime) is an appropriate measure of fitness (ω). This assumption holds if R_0, the average number of parasitic females produced by a parasitic mother which then survive to reproduce is, on average, 1. When $R_0 = 1$, the population is neither increasing nor decreasing. This is believed to be approximately true of helminth populations in general (Anderson and May 1985).

(ii) Average per unit time fecundity (m) depends on maturation time (α) according to the relationship $m = c\alpha^{\beta}$, where c is a constant and the exponent β is the allometric slope (the slope of the regression of log m on log α).

(iii) Upon entering a host, parasites are first subject to a constant pre-maturational mortality rate, M_i, until maturation (at time α) followed by a constant post-maturational mortality rate, M_a.

(iv) Per unit time fecundity, m, is determined by body size at maturation (time α) and is independent of the time since maturation and of any post-maturational growth.

Under assumptions *(i)* to *(iv)*, we can write

$$\omega = c\alpha^{\beta} e^{-M_i\alpha} \frac{1}{M_a}, \qquad (1)$$

since $e^{-M_i\alpha}$ is the proportion of worms surviving at maturity (time α), daily fecundity is $c\alpha^{\beta}$, and life expectancy after time α equals $\frac{1}{M_a}$. This function has a single maximum at α^*, the maturation time that maximises fitness (ω). Figure 1 shows fitness (ω) plotted against maturation time (α) for a range of values of pre-maturational mortality rate (M_i). As shown, α^* becomes progressively smaller as pre-maturational mortality rate increases.

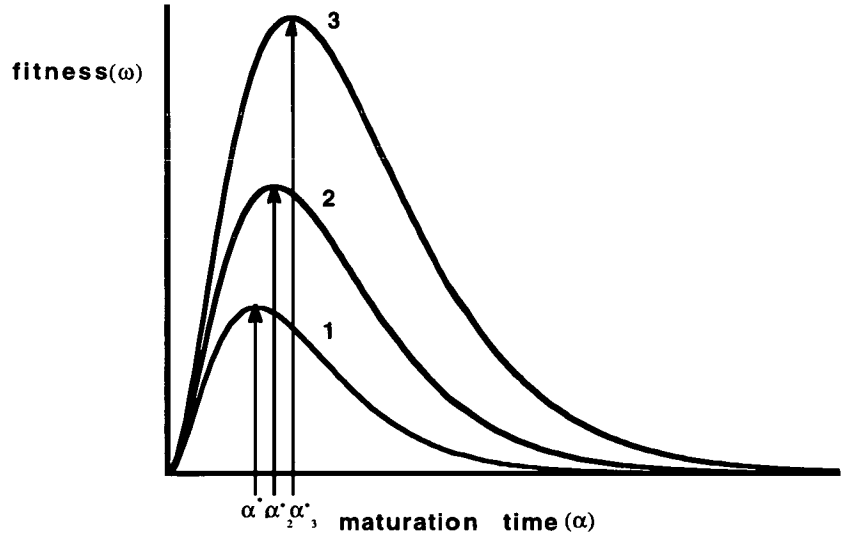
Differentiating equation (1) with respect to α gives

$$\frac{d\omega}{d\alpha} = \beta \frac{1}{M_a} c\alpha^{(\beta-1)} e^{-M_i\alpha} - M_i \frac{1}{M_a} c\alpha^{\beta} e^{-M_i\alpha}.$$

Table 1. List of symbols used in the text and their definitions.

α	maturation time, time from infection until production of propagules (eggs or larvae) by parasites
α^*	optimal maturation time, the α that maximises fitness (ω)
b	adult female body size
β	allometric slope, slope of log-log regression of m on α
m	average per unit time fecundity
g	factor by which L_{max} overestimates L. When $M_i = M_a$, $g = \ln N$ (see text)
L	life expectancy of parasites (average age at death measured from time of infection)
L_{max}	maximum lifespan, age at death (measured from infection) of longest lived individual in a cohort, here equal to $P + \alpha$
L_{adj}	estimated L, calculated as $\frac{L_{max}}{g - \beta}$ (see text)
M_i	pre-maturational mortality rate
M_a	post-maturational mortality rate
N	starting number of individuals in a cohort
P	patent period, duration of (parasite) egg or larval production from infected hosts, here equal to L_{max} - α
R_o	net reproductive rate
ω	fitness, taken to be lifetime reproductive output under the condition $R_0 = 1$

Figure 1. Fitness (ω = lifetime reproductive output) as a function of maturation time (α) under three different pre-maturational mortality rates (M_i) The curve labelled 1 was generated with the highest value of M_i and the curve labelled 3 with the lowest. The peak of each fitness curve corresponds to the optimal maturation time (α^*) as indicated by arrows. Scale and units arbitrary.



To find α^* we set $\frac{d\omega}{d\alpha} = 0$.

Rearrangement gives

$$\frac{\alpha^{\beta}}{\alpha^{(\beta-1)}} = \frac{\beta}{M_i},$$

which reduces to

$$\alpha^* = \frac{\beta}{M_i} \qquad (2).$$

Hence the model predicts optimal maturation time (α^*) to be proportional to the inverse of pre-maturational mortality rate (M_i). The constant of proportionality is β,

Figure 2. Phylogenetic relationships of species used in the analysis of prepatent period and daily fecundity data. Depicted branch lengths are arbitrary.

the allometric slope of per unit time fecundity with maturation time. Note that the fitness costs of longer maturation time arise solely because delaying reproduction results in fewer worms surviving to reproductive age. Post-maturational mortality rate (M_a) which is not a function of α in the model, affects the height of the fitness function but not the position of the optimal maturation time (α^*).

Under the assumptions listed above, equation (2) is also given by expansion of either $\sum_{x=0}^{\infty} l(x)m(x)$, or $\int_{x=0}^{\infty} l(x)m(x)$ (where $l(x)$ is the probability of survival to time x and $m(x)$ is fecundity if alive at time x) using geometric series (see Roff 1992, Bulmer 1994).

5.4 Testing the model

We now test how well the model explains observed maturation time in a range of mammalian gastrointestinal nematodes. To do this, we use life history data compiled from the literature for 66 species (Skorping *et al.* 1991). First we estimate β, the allometric slope linking per unit time fecundity to maturation time. As a measure of maturation time we use data on prepatent period, the time from infection until parasites' propagules are shed from the host. Per unit time fecundity is represented by data on daily fecundity (offspring per female parasite per 24 hours). The resulting estimates of β are then used to generate predicted values. Next, mortality rates are estimated from data on patent period to allow a test of the model's predictions. Observed and predicted values are compared by regression.

(i) Estimating β

maturation time and fecundity Determining the relationship between fecundity and maturation time among individual parasitic nematodes is, for obvious practical reasons, not easy and we know of no relevant estimates based on intraspecific data. We therefore used cross-taxa data to estimate an average value of β. Arguably, the relationship between maturation time and fecundity measured at a cross-taxa level more closely reflects the relationship that natural selection acts on within those taxa, spanning as it does, greater variance in both variables and being less influenced by environmental variation faced by individual worms.

phylogeny Statistical non-independence of species values due to shared ancestry needs to be controlled for when estimating slopes of allometric relations from

comparative data (Harvey and Pagel 1991). Several methods (and associated computer programs) are available which use phylogenetic information to calculate standardised differences (independent contrasts, here abbreviated to ICs) between pairs of sister taxa as first advocated by Felsenstein (1985). While the values of sister taxa themselves are not independent, ICs are and can therefore be used to test for relationships between variables by standard statistical methods.

Daily fecundity and prepatent period data were first $\log_{10}$ transformed. Sets of ICs were generated with the CAIC computer package (Purvis and Rambaut 1995) using a phylogeny based on that given in Read and Skorping (1995). The phylogeny (Figure 2) is based largely on morphological systematics (see Read and Skorping 1995 for source taxonomic references). Additional resolution was provided by molecular data in Blaxter *et al.* (1998) and Newton *et al.* (1998) at the nodes marked 1 and 2 respectively.

Data on the majority of branch lengths in this phylogeny (Fig. 2) are unavailable and in this situation several approaches are possible. The assumption that branch lengths are equal (punctuated evolution model) has proved the most robust option in simulation studies (Purvis *et al.* 1994) and was used here. All regressions involving ICs were forced through the origin (Garland *et al.* 1992).

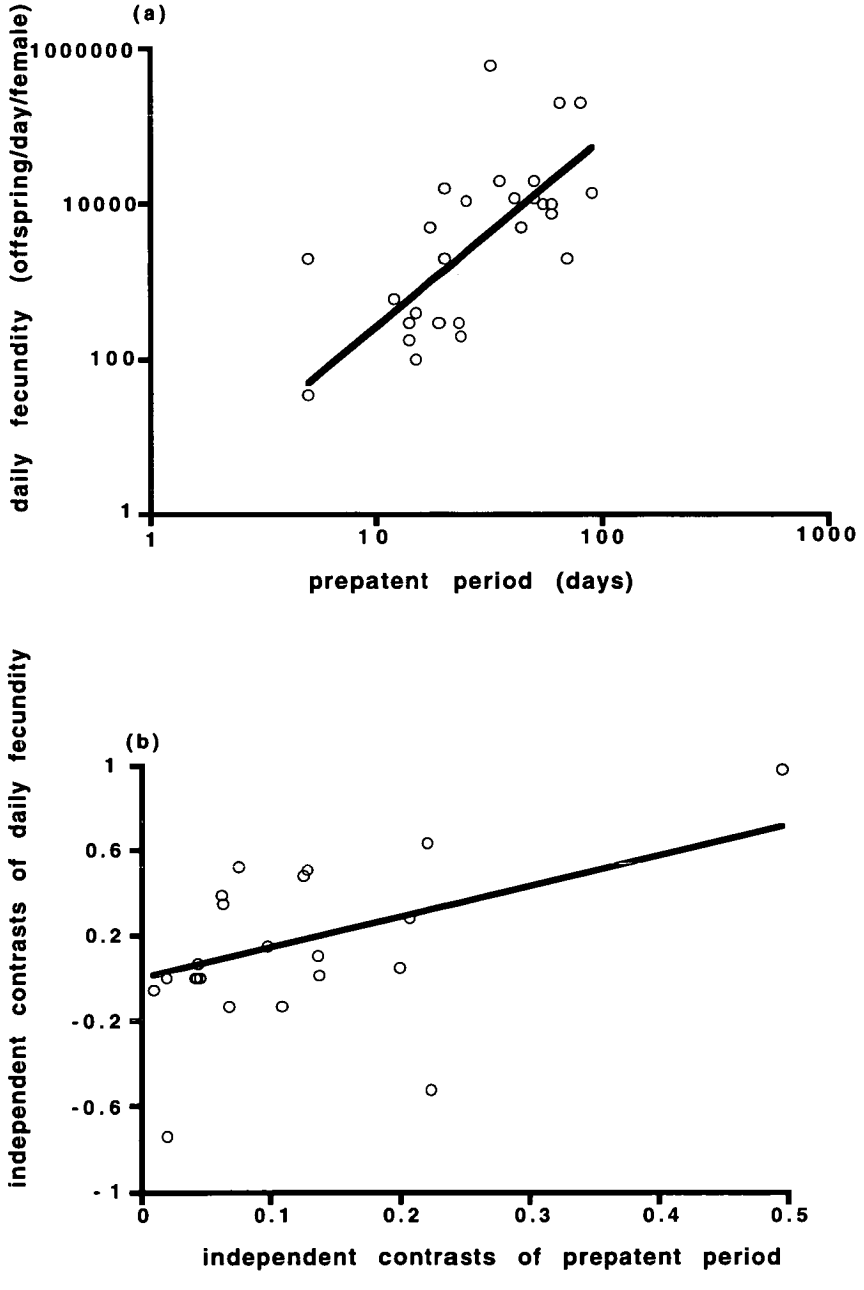
regression To estimate the value of a cross-taxa regression coefficient, a choice of regression model has to be made. Ideally, where the error rates in both variables (or their ratio, $\frac{Y\sigma_e^2}{X\sigma_e^2}$) are known, the structural relation (SR) should be used (Rayner 1985; Harvey and Pagel 1991; Sokal and Rohlf 1995). So-called Model I (ordinary least squares or OLS) and Model II (major axis and reduced major axis) regression are particular cases of the SR (Rayner 1985). The OLS slope estimate converges on the SR as $\frac{Y\sigma_e^2}{X\sigma_e^2}$ becomes very large (Kuhry and Marcus 1977). In Model II methods,

major axis (MA) assumes equal X and Y error variances ($\frac{Y\sigma_e^2}{X\sigma_e^2} = 1$) and reduced major axis (RMA) assumes the ratio of error variances to be equalled by the ratio of the variances ($\frac{Y\sigma_e^2}{X\sigma_e^2} = \frac{Y\sigma^2}{X\sigma^2}$). These different regression models will produce different estimates of the slope. Therefore, the extent to which the assumptions of each model are violated is the primary consideration in choosing between them.

Since the error rates in estimates of our Y-variable, daily fecundity (offspring per female parasite per day), are almost certainly far more substantial than the error rate in estimates of prepatent period, the equal error variance assumption of MA seems least appropriate. OLS may be an adequate model if $\frac{Y\sigma_e^2}{X\sigma_e^2}$ is sufficiently large (Rayner 1985). Since we have no reason to expect OLS or RMA to be more or less successful estimators of the functional relationship, results are reported for analyses employing both regression models.

Daily fecundity and prepatent period data are available for 28 species from six nematode orders. The OLS and RMA slopes of the cross-species regression of fecundity on prepatent period (±95% c.i.) are 2.42 (±0.21) and 2.48 (±0.22) respectively (Figure 3a). The CAIC analysis yields 22 pairs of ICs. Five of the Y values are negative and therefore some care is required before proceeding to a slope estimate. In the present case, negative values indicate that an increase in X (prepatent period) is associated with a decrease in Y (daily fecundity). If negative values of Y are as likely as positive values, there is no evidence of an association of X with Y and little point in fitting a regression line. Consequently, a binomial (or similar) test should first be applied to determine whether the frequencies of positive and negative Y values are significantly different from the 50:50 ratio expected under the null hypothesis of no association of X with Y (Harvey and Pagel 1991). This is indeed the case (one-tailed binomial probability <0.01) indicating that a positive relationship of daily fecundity

Figure 3. Regressions through the origin of $\log_{10}$ daily fecundity on $\log_{10}$ prepatent period across gastrointestinal nematodes. In both cases plotted line is the OLS regression. In (a), data are average species values. In (b), data are independent contrasts controlling for phylogeny (see text). In (a), OLS slope = 2.42 (95% c.i. ±0.21), $r = 0.97$, $p < 0.0001$; RMA slope = 2.48 (95% c.i. ±0.22). In (b), OLS slope = 1.45 (95% c.i. ±0.95), $r = 0.58$, $p < 0.005$; RMA slope = 2.5 (95% c.i. ±1.6).



with prepatent period exists across gastrointestinal nematode taxa once phylogenetic effects are accounted for. OLS and RMA regressions through the origin give slopes (±95% c.i.) of 1.45 (±0.95) and 2.5 (±1.6) respectively (Figure 3b). As expected, the OLS slope is lower than the RMA, but neither estimate is significantly different from the other or from the cross-species slope estimates of 2.42 and 2.48.

(ii) Estimating mortality rates

Direct estimates of mortality rates for parasitic nematodes are extremely rare. However, if we make the additional assumption (assumption *v*) that mortality rates do not change significantly after maturity (i.e. $M_i \approx M_a$) an estimate can be made using data on patent period (P), the duration of egg or larval shedding from an infected host. The mortality schedule inferred from assumption *v* is illustrated in Figure 4. We can write $M_i = M_a = M = \frac{1}{L}$, where M is the average mortality rate and its reciprocal, L, is average life expectancy. Substituting $\frac{1}{L}$ for M_i in equation (2), the model's prediction can then be written as

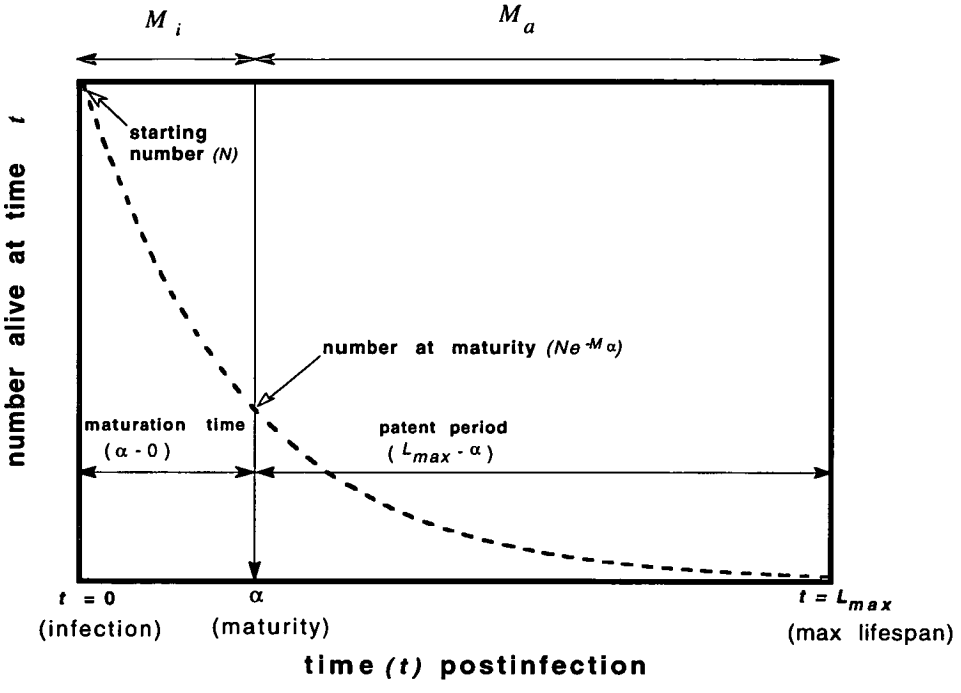
$$\alpha^* = \beta L \qquad (3a)$$

or

$$\alpha^* M = \beta \qquad (3b).$$

Our life history data ultimately derive from parasitological studies in which large numbers of parasites are inoculated into hosts. Thus, maximum lifespan (prepatent period + patent period, here called L_{max}), records the age of the longest lived parasite in a cohort. As discussed by Beverton and others (Beverton 1992 and

Figure 4. Mortality schedule implied by the assumption of equal pre-maturational (M_i) and post-maturational (M_a) mortality rates (assumption *v*, see text). The proportion of the starting number (N) alive at time (t) postinfection is plotted (broken line). Under the constant mortality assumption, $M_i = M_a$, so that the number of parasites reaching maturity is simply the product of the starting number, N, and the proportion alive at time = α (which we can write as $e^{-M_i\alpha}$). Since maximum lifespan is the sum of maturation time (α) and maximum reproductive lifespan (patent period), patent period is itself equal to $L_{max} - \alpha$ under assumption *v*.



references therein) L_{max} overestimates L (the average life expectancy) by a factor g such that $L_{max} = gL$ (and hence $g = ML_{max}$).

In studies involving other taxa, indirect estimates of g have been obtained and applied with some success (e.g. Hoenig 1983; Beverton 1992; Charnov 1993). These methods rely on obtaining independent estimates of L_{max} and M in other closely related species or populations and, consequently, are not of use here. However, given assumption *v*, we can estimate g from the starting number in a cohort (N) as follows.

Writing the standard equation for a survival curve under constant M we have

$$S(t) = e^{-Mt},$$

where $S(t)$ = proportion surviving at time t. In the case of $t = L_{max}$, a single individual achieves age L_{max}, so that

$$S(t) = \frac{1}{N},$$

and we can write

$$\frac{1}{N} = e^{-ML_{max}},$$

and so

$$\ln\left(\frac{1}{N}\right) = -ML_{max}.$$

Beverton's relation states that $g = ML_{max}$ and so

$$g = -\ln\left(\frac{1}{N}\right) = \ln N \qquad (4).$$

Thus for parasitic nematodes in experimental infections, g approximates to ln N. Patent period (P) can be written as $L_{max} - \alpha$ (Fig. 4). Under assumption v, just as $\frac{L_{max}}{L} = ML_{max}$, so also $\frac{L_{max} - \alpha}{L} = M(L_{max} - \alpha)$. The factor by which patent period (P) overestimates L is therefore ML_{max} - αM which [from equations (3b) and (4)] we can write as ln$N$ - β. We will refer to $\frac{P}{\ln N - \beta}$ as L_{adj} - the adjusted estimate of life expectancy. Data on the dose of parasites administered are not available for every species in the dataset. Most of the life history data are from monographs which cite numerous original studies when discussing general life history attributes of particular species. To estimate the dose in a typical infection, as many original references as could be matched with a specific estimate of patent period were compiled (Table 2). The average value of g in this compilation is 10 (95 % c.i.± 0.75). Accordingly, estimated life expectancy (L_{adj}) for a given species was calculated as $\frac{P}{10 - \beta}$.

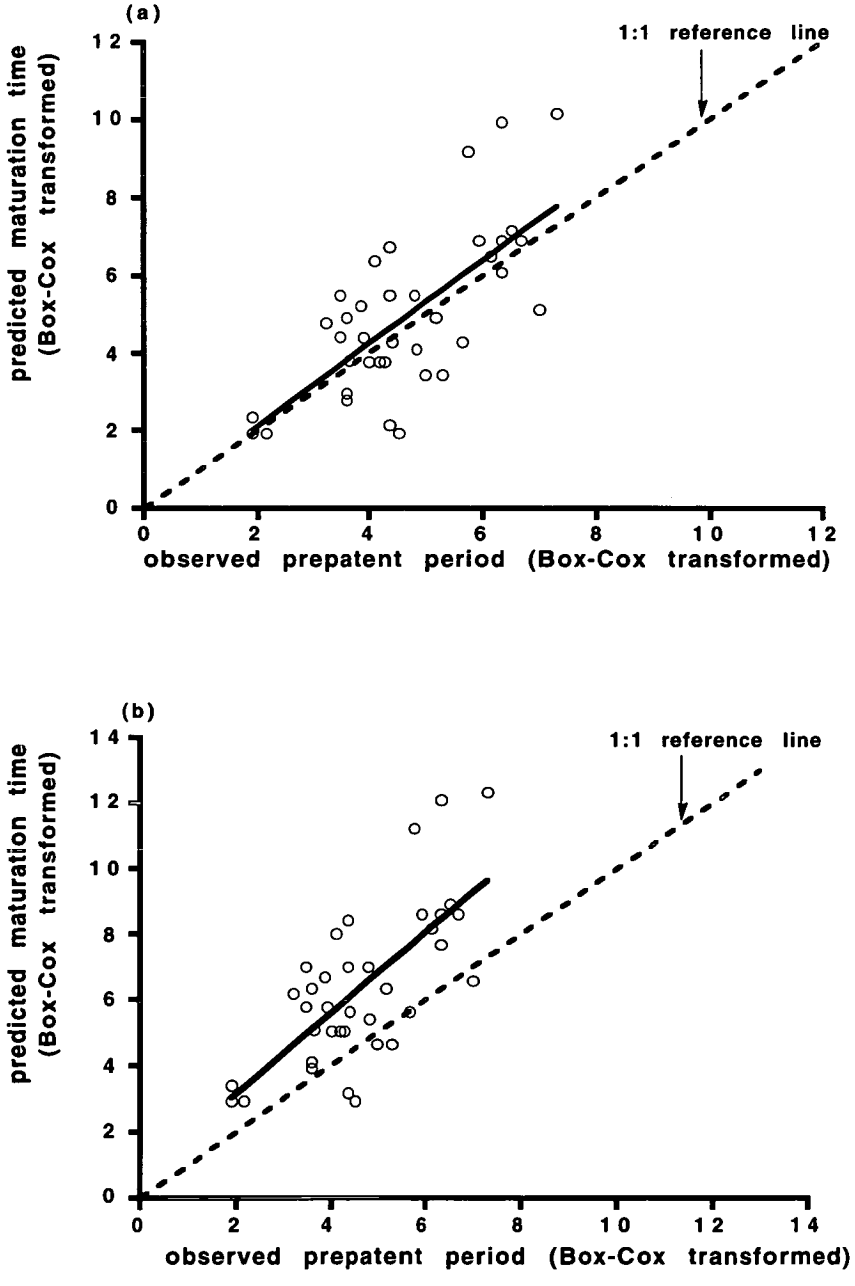
Table 2. Doses of parasite infective stages and source references.

reference	doses administered (x10^3)
Herlich 1954	0.37; 0.62; 16; 28; 100; 140; 1000
Mayhew 1962	6.16; 9.18; 9.88; 13.02
Bizzel and Ciordia 1965	20; 22; 25; 25; 25; 35; 35; 38; 38; 40; 62

5.5 Observation and prediction

Data on patent period and prepatent period in natural hosts are available for 37 species. Applying equation (3a), with $\beta = 1.45$ or $\beta = 2.5$ (the phylogenetically controlled allometric slope estimates of daily fecundity with prepatent period), the optimal maturation times predicted by our model are calculated as βL_{adj}. An obvious test of the predicted values' fit with observation is by regression. This will show a significant 1:1 relationship if observation and prediction agree. The distributions of observed and predicted values are right-skewed (data not shown) so that prior to testing their fit an appropriate transformation is required for both variables (Roff 1992). Box-Cox transformation (Sokal and Rohlf 1995) with $\lambda = 0.2$ results in maximal normality of both variables and Figures 5a and 5b show the regression plots of the transformed data for $\beta = 1.45$ and $\beta = 2.5$ respectively. As shown in Fig. 5, the slopes and intercepts of these plots are not different from +1 and zero respectively. A value of $\beta = 1.45$ produces a tighter visual fit than $\beta = 2.5$. Nevertheless, for either value of β, prediction and observation are highly correlated ($r = 0.71$, $p < 0.0001$ in both cases). In fact, so long as β lies between 0.5 and 3.1 the data are a reasonable fit to either set of predicted values (slopes and intercepts not significantly different from 1:1 expectations).

Figure 5. Plots of predicted versus observed values of maturation time (n = 37 species). In both plots, broken line is the 1:1 reference line. All data are Box-Cox transformed with $\lambda = 0.2$ (see text). In (a), $\beta = 1.45$; slope between observed and predicted values = 1.07 (95% c.i. ±0.37); intercept = -0.02 (95% c.i. ±1.79). In (b), $\beta = 2.5$; slope between observed and predicted values = 1.23 (95% c.i. ±0.42) , intercept = 0.70 (95% c.i. ±1.65). In both regressions (OLS), $r = 0.71$, $p < 0.0001$.



There is another way to test the model's capacity for describing the data. Since $\alpha * M = \beta$ [equation (3b)], the model yields a dimensionless number, αM, and predicts that independent of separate values of α and M, their product must always equal β. The number αM (or aM) is a known life history invariant in a wide range of animal taxa (Charnov 1993). Where αM is truly invariant (proportionality), the slope of a log-log regression of L on α is equal to unity (Charnov 1993). Our model predicts this relationship. Taking logs of both sides of equation (3b)

$$\log L = \log \alpha * - \log \beta \qquad (5a)$$

or

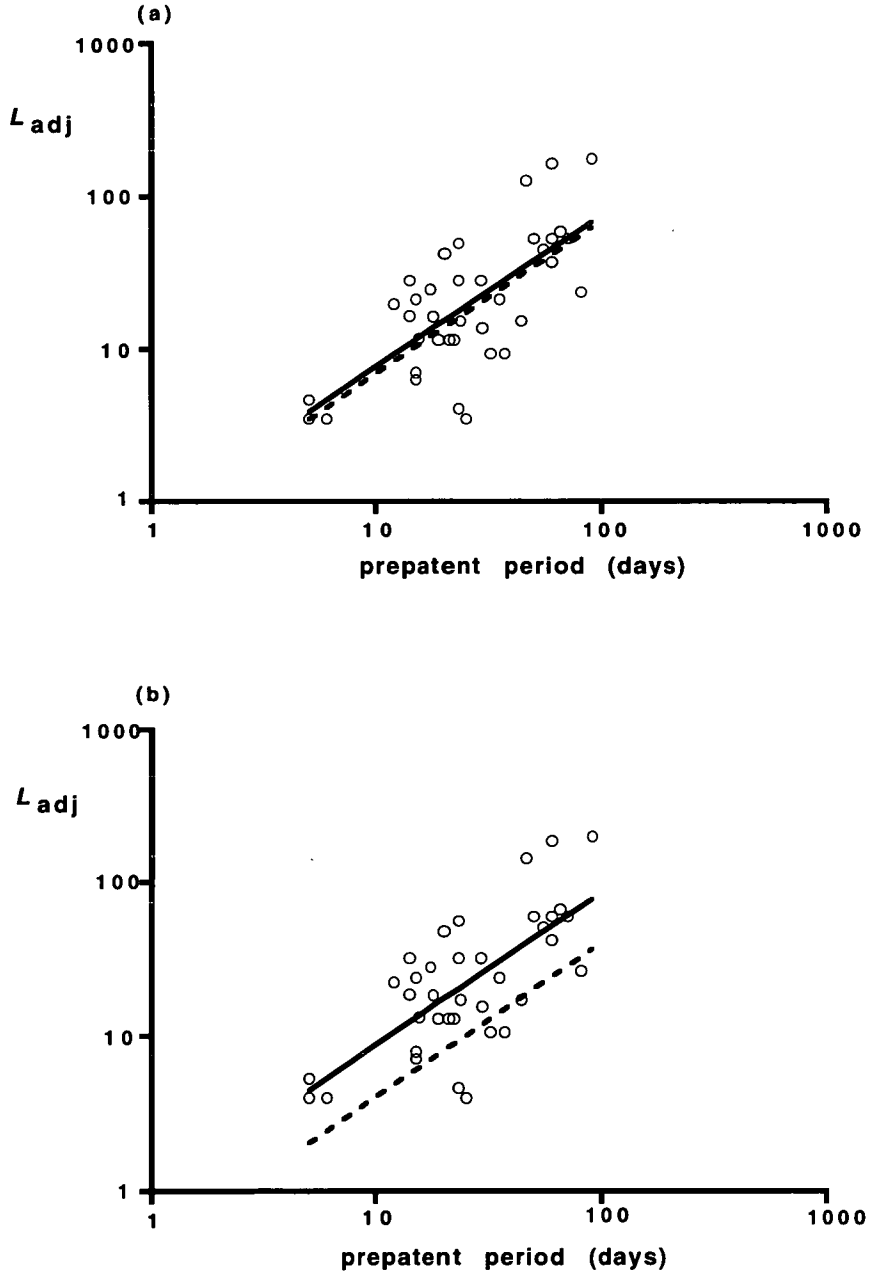
$$\log \alpha^* = \log L + \log \beta \qquad (5b).$$

Equations (5a) and (5b) define straight lines with slopes of +1 (proportionality) and intercepts at $\log \beta$ and $- \log \beta$ respectively. We can ask whether parasitic nematode life histories conform to these lines by testing their goodness of fit with the observed log-log regressions. As with the data on fecundity and prepatent period, OLS and RMA regression can be used to estimate the functional relationship between prepatent period and L_{adj}. Since L_{adj} is likely measured with far greater error than prepatent period, when using OLS the regression of life expectancy on prepatent period, equation (5a), is the most appropriate (regression of prepatent period on L_{adj} involves a major violation of the error variance assumption of OLS and may severely underestimate the slope). If equation (5a) successfully describes parasitic nematode

life histories, the regression will have an intercept at - log β and a slope of +1.

Figures 6a and 6b, show the OLS regressions of log L_{adj} on log prepatent period for β = 1.45 and β = 2.5 respectively. The slopes are not different from +1 (invariance). The regression lines fall close to the predicted lines and the intercepts are not statistically different from either predicted value (-0.16 and -0.39). RMA regressions give slopes of 1.41 (±0.48), again not different from the predicted value of +1. As in the comparison of observed and predicted values (Fig. 5), the regression lines account for around 50 percent of the variance (r^2 = 0.497).

Figure 6. Log_{10}-$\log_{10}$ plots of life expectancy (L_{adj}) and observed prepatent period with g = 10 (n = 37 species). The OLS regressions for β = 1.45 and β = 2.5 (solid lines) are plotted alongside the lines predicted by the model (broken lines). In (a), β = 1.45, OLS slope = 0.99 (95% c.i. ±0.34), intercept = -0.10 (95% c.i. ±0.49). In (b), β = 2.5, OLS slope = 0.99 (95% c.i. ±0.34), intercept = -0.05 (95% c.i. ±0.49). For both values of β, r = 0.71, $p < 0.0001$; RMA slopes = 1.41 (±0.48).



5.6 Discussion

This study attempts to explain variation in a central life history trait in parasitic nematodes, in-host maturation time, in terms of a trade-off mediated by the opposing effects of development time on fecundity and mortality. Worms that grow for longer periods before reproducing are less likely to survive until reproductive age but have potentially higher per day fecundity. The optimality model is simple and makes clear predictions about relationships between life history variables, explaining around 50 percent of the variation in prepatent period (Figs 5 and 6).

None of our model's assumptions apply exclusively to interspecific variation. If individual parasitic nematodes were able to assess the prevailing in-host parasite mortality rate – and it may be a relatively straightforward matter to assess rate of immune attack for instance – natural selection should favour those individuals that modify their maturation time accordingly. It would be extremely interesting to know to what extent, if any, facultative modulation contributes to the observed variation in prepatent period within species. In any case, the framework used here should prove useful in investigating the potential effects of medical and veterinary interventions on the evolution of size, fecundity and other life history traits in these parasites. Many of these latter traits are highly correlated with prepatent period and with each other across taxa (Skorping *et al.* 1991; Morand and Sorci 1998).

The explanatory power of the model is perhaps surprising given the somewhat crude methods used to estimate parameters. For example, in testing our predictions we assumed that all gastrointestinal nematode species share approximately the same growth curve and estimated the allometric slope β from data showing considerable scatter (Figs 3a and 3b). Indeed, some taxa in our sample (the Ancylostomatidae, Bunostominae and Toxocarinae) show patterns of life history traits which contradict the assumption that development time is positively associated with fecundity (i.e. the negative values in Fig 3b). Similarly, the assumption (assumption *v*; Fig. 4) of an

unchanging mortality rate throughout infection is probably an oversimplification for at least some of the species in the analysis.

Further, as a measure of maturation time in gastrointestinal nematodes, prepatent period is not perfect: the first eggs or larvae to appear will be those of the earliest maturing individuals, not those of the average individual whose behaviour the model predicts. The time lag between the production of eggs by parasites and their eventual exit from the host is also ignored. Other than making some modest across-the-board adjustment to the observed values of prepatent period, there is currently little we can do to rectify these problems. It would of course be of interest to know how the model performs when more direct estimates of average maturation time become available.

Several of the modelling assumptions are likely to be violated in nature. For example, we assume that once reproduction begins, per unit time fecundity remains constant until death (assumption *iv*) which is valid if growth ceases at time α and there is no senescence. But growth continues after maturity in many nematodes (Anderson 1992), and a fall in reproductive output with time is common among gastrointestinal species (Wakelin 1996). It may be that any gains in fecundity accruing through post-maturational growth are approximately balanced by a declining fecundity in later life.

Despite the above limitations, the model makes quantitatively successful predictions. Such predictions have been noticeably absent from the literature on parasite life histories (Skorping *et al.* 1991; Read and Skorping 1995; Morand and Sorci 1998; Poulin 1995). Roff (1984) used a similar modelling approach to that employed here to predict age at maturity in teleost fishes. Despite containing some equally simplified assumptions (such as determinate growth), his model successfully described the pattern of maturation events across a large number of teleost species. Like nematodes, most teleosts are indeterminate growers whose fecundity is closely

linked to their body size. It may be that a model similar to Roff's (it incorporates a Von Bertalanffy growth curve and assumes fecundity is proportional to length^3) would also be successful in describing nematode life histories. However, we are unaware of any of the parameter estimates needed to incorporate such growth curves in the model. It would be of interest to know how well our model could describe teleost life histories if relevant data on per unit time fecundity were applied.

In the present study, the product of mortality rate (M) and maturation time (α) is predicted to be invariant and equal to β, the allometric slope of per unit time fecundity with α. Morand (1996) first estimated αM (his aM) in nematode parasites of vertebrates as 0.23. This implies that a parasitic nematode maturing at one month postinfection has, on average, about four months left to live. We find a very different value of αM (1.45 - 2.5) suggesting that worms devote a substantially larger portion of total lifespan to maturation than previously suspected. However, Morand's estimate is based on a comparison of prepatent period with patent period (the maximum duration of egg or larval production). As recognised elsewhere, the use of maximum reproductive lifespan as a measure of life expectancy in these organisms tends to overestimation (Anderson and May 1985). The use here of the correction factor g may be an improvement in this regard.

Our estimate of αM in nematodes is similar to that of other poikilothermic indeterminate growers, such as fish and shrimp ($\alpha M \approx 2$ in both cases) at the other end of a life history spectrum from birds and mammals ($\alpha M \approx 0.4$ and 0.71 respectively; Charnov 1993 his Figure 1.9). Charnov (1993) developed ESS models for the αM number in determinate growers, the most general formulation of which led to the prediction $\alpha M = 3(1-\delta^{0.25})$, where δ is relative size at independence (offspring

size/maternal size). When we apply this equation, calculating δ as egg volume/female volume (n = 38 species), we find that for mammalian gastrointestinal nematodes, $3(1-\delta^{0.25}) = 2.52$ (95% c.i. ± 0.11). This is not statistically different from the cross-species estimates of β or from the phylogenetically controlled estimate from RMA regression (Fig 3). It may be that Charnov's equation is so fundamental that the complications of indeterminate growth prove to be of relatively minor importance. In fact, there is recognition that the distinction between determinate and indeterminate growth is somewhat artificial, a more useful distinction being the one between organisms which approach their asymptotic size slowly and those which approach it abruptly (Beverton 1992).

Charnov (1993) postulated that $V(\alpha) \propto \alpha^d$, where $V(\alpha)$ is the reproductive value of a female who attains maturity (at age α), and assuming that mortality is unchanged after maturity derived the result $\alpha M = d$. Thus αM was found to be equal to the exponent linking fitness to age at maturity, similar to our own finding for nematodes that $\alpha M = \beta$ [equation (3b)]. That so general a relation should so closely predict the life histories of parasitic nematodes may point to some relatively simple facts governing nematode reproductive biology and to some very broad generalities underpinning the evolution of major life history traits.

Chapter 6

Host immune status determines maturation time in a parasitic nematode

6.1 **Summary:** The maturation time of parasitic nematodes determines adult body size which in turn determines fecundity. The mortality rate of parasites and hence the time available for growth, depends on the strength of host immune responses. In theory, optimal maturation time varies with immune-imposed mortality. The hypothesis that maturation time is facultatively adjusted by parasitic nematodes was examined using a tissue-migratory species, *Strongyloides ratti*. Initial rate of larval production was inversely related to the strength of host immunity. Tissue migration times were significantly longer in the absence of thymus-dependent (T-dependent) immune responses and the onset of egg production (maturity) was delayed. However, T-dependent immunity had no appreciable effect on parasite body length suggesting either a non-adaptive mechanism or an unidentified benefit underlying delayed maturity in immune-suppressed hosts. An extensive literature survey found that slowed development is a common feature of studies on parasitic nematodes concerned with immune-suppression. Results are discussed in relation to models of maturation time, nematode biology and phenotypic plasticity.

6.2 Introduction

For most newly born animals, life begins with a period of pre-maturational growth. The duration of this period can have profound effects on major fitness components such as generation time, mortality and body size. Maturity is therefore a pivotal event in most life histories and its timing a central target of natural selection in

wild populations (Roff 1992; Stearns 1992). For parasitic nematodes, a simple optimality model based on the allometry of fecundity with size at maturity explains much of the cross-taxa variation in maturation time (Chapter 5). According to this model, as pre-maturational mortality rate increases, natural selection favours individuals that mature rapidly if the resulting increase in survival to maturity outweighs size-related reductions in per unit time fecundity. Conversely, as pre-maturational mortality rate declines, the size-related fecundity benefit of longer maturation tips the balance in favour of delayed maturity.

In some animal species, individuals can optimise their own maturation time in response to shifts in the prevailing mortality schedule. These phenotypic adjustments can be induced by abiotic factors such as photoperiod, temperature and habitat stability (Nylin *et al.* 1996; Nunney and Cheung 1997; Denver 1997) and by the action of other organisms such as predators or parasites (Minchella and LoVerde 1981; Crowl and Covich 1990; Lafferty 1993). For example, the larval stages (tadpoles) of some desert-dwelling anuran species show facultative acceleration of metamorphosis in response to desiccation of aquatic larval habitats (Denver *et al.* 1998). This plastic response is mediated via early activation of particular neuro-hormonal pathways, is specific to water level as a proximate cue, and is reversible in the event that water levels are replenished (Denver 1997; Denver *et al.* 1998).

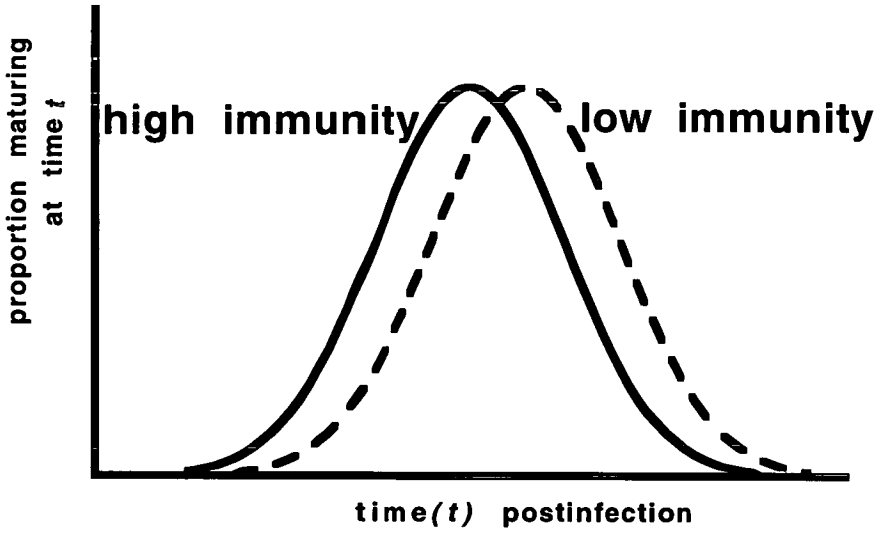
Theoretical studies commonly find that adaptive plasticity is favoured where environmental variability has large effects on fitness and where individuals have reliable cues indicating the nature of local environmental change (e.g. Moran 1992; Scheiner 1993). In parasitic nematode infections, host immune responses have severe effects on parasite mortality and body size. Common features of infections in previously exposed (immunised) hosts are relative brevity and lower parasite establishment rates compared to primary infections. Nematodes from immune hosts are frequently described as small and their fecundity as being reduced (e.g. Stear *et al.*

1999). Further, genetic-, gender- and age-related variation among hosts in response to nematode infection suggest that these parasites inhabit a highly variable environment with regard to immune-imposed mortality rates (e.g. Wakelin & Blackwell 1988; Stear and Wakelin 1998). Individual parasitic nematodes may therefore stand to gain sizeable fitness benefits by adjusting maturation time towards local optima determined by prevailing levels of immune-imposed mortality. Such a flexible life history schedule would maximise lifetime reproductive success across a spectrum of host immunity, promoting small, rapidly maturing worms where high mortality rate makes the eventual fecundity benefit of large size less likely to be reaped. This is the logic of the model developed in Chapter 5 and I assume here that fecundity increases more than linearly with maturation time within, as well as across, species of parasitic nematode.

If individual parasitic nematodes adjust maturation time facultatively as predicted by the theory outlined above, how would the kinetics of such infections be affected? In most *in vivo* parasitological studies with nematodes, large numbers of infective stages are administered to host animals. Figure 1 shows a hypothetical distribution of parasite maturation times under high and low immune-imposed mortality. If host immune-suppression induces a delay in parasite maturation then, if infections are sampled at an early enough point, there will be less reproductive activity in immune-suppressed hosts.

Facultative adjustment of maturation time, if it occurs, should be most apparent in studies of immune-suppression and least apparent in studies of immunisation for the following reasons. First, acquired immune responses generally result in the destruction of a large percentage of nematode infective stages prior to establishment and maturation. If establishment is substantially reduced, a decrease in average maturation time among parasites in immunised hosts need not result in a larger initial population of mature parasites compared to that in non-immunised hosts.

Figure 1. Hypothetical distributions of parasite maturation times under high (solid line) and low (broken line) immune-imposed mortality. If host immune-suppression induces a facultative delay in the average maturation time of parasites, fewer mature parasites should be found in immune-suppressed hosts if infections are sampled sufficiently early. Scale and units arbitrary. A normal distribution is assumed.



Second, in species with a tissue-migratory phase, acquired immune responses can physically impede migrating parasites - nematode larvae in immunised hosts are often surrounded and attacked by immune effector cells *en route* to their breeding sites (Maizels and Holland 1998).

Here I investigate the possible adaptive significance of immune-dependent maturation time in *Strongyloides ratti*, an intestinal parasite of rats. In four previous studies of T-dependent immunity in *S. ratti* infections, initial parasite reproductive output was lowest in congenitally athymic (nude) host animals (Gemmill *et al* 1997, Appendix; Dawkins *et al* 1982; Chapter 3, Fig. 3a). The published data of Dawkins *et al.* (1982) show lower larval output on day 5 PI in nude mice infected with *S. ratti.* Furthermore, these authors found significantly lower gut burdens on day 5 PI in nude

compared to normal mice, though no subsequent timepoints were sampled. In the case of the data on nude mice in Chapter 3, this represented an absolute delay in the prepatent period of infection.

In this chapter, two approaches were taken to studying immune-dependent variation in maturation time. First, I both manipulated host immunity and made use of congenitally athymic (nude) rats. I then quantified the resulting variation in parasite establishment, maturation time, body size and reproductive output. If *S. ratti* adjusts maturation time adaptively in response to varying host immunity, parasites should mature later and at a larger size when immunity is suppressed. Second, a large body of relevant published data concerning measurements of parasite burdens or reproductive output in normal and immune-suppressed hosts was reviewed in order to assess whether slowing of development in immune-suppressed hosts is at all common among gastrointestinal nematodes. Slowing of parasite development in immune-suppressed hosts is a documented phenomenon in other helminth taxa (e.g. Cheever and Duvall 1987) The hypothesis tested here offers an adaptive, functional explanation for much of this type of variation in parasitic nematodes.

Methods

(i) Parasitology

The isofemale *S. ratti* line ED5 was used throughout and was maintained by serial passage in Wistar rats (Banton and Kingman, U.K.). Food and water were provided to animals *ad libitum*. Unless otherwise stated, experimental animals were male Wistar rats, six to seven weeks old (Banton and Kingman, U.K.) and infections were initiated by subcutaneous injection of inocula containing infective third stage larvae (iL3s) suspended in physiological saline (0.8% w/v NaCl solution). Inocula containing > 100 iL3s were prepared by dilution. Inocula of ≤ 100 iL3s were prepared and administered as follows. Under a binocular microscope, 100 iL3s were counted

and collected into a 1ml plastic tube filled with physiological saline. Each inoculum was agitated, taken up in a 1ml syringe and administered. Then, the syringe and hypodermic needle used were flushed through with fresh physiological saline and the washings collected into the tube which held the inoculum originally. The number of worms remaining in the tube was counted. This allowed inocula sizes to be recorded precisely. For assessment of parasite reproductive output (worm output) faeces from infected animals were collected overnight and cultures prepared as described in Viney *et al.* (1992). After two days incubation at 25 °C mature worms were washed from culture plates, collected and counted under a binocular microscope as described elsewhere (Gemmill *et al.* 1997). Intestinal parasite numbers (gut burdens) were determined as described in Chapter 3. The order in which experimental animals were infected, the position of collecting cages, the order in which samples were processed and counted, as well as the positions of cultures in incubators, were randomised anew at each sampling point.

(ii) Host immunity and worm output

Protective immunity to *S. ratti* infection can be elicited by immunisation with dead iL3s (Conder and Williams 1983). Host immune responses can be substantially suppressed by administration of corticosteroid drugs (Gemmill *et al.* 1997). In this experiment immunisation and immune-suppression were used to generate variation in immune-imposed parasite mortality. Eighteen animals were infected with 500 iL3s. Seven days prior to infection, six of these were immunised with 100 heat-killed iL3s suspended in sterile physiological saline administered by subcutaneous injection. Beginning 3 days prior to infection, a further six rats received 10 mg/kg mean body weight of betamethasone (Betsolan, Pitman Moore) by subcutaneous injection daily until day 5 postinfection (PI). The remaining six rats were not manipulated immunologically and served as a control group. Whenever animals in the immunised

or steroid-treated groups received injections, all other animals in the experiment were injected with an equal volume of sterile physiological saline. Worm output was monitored from day 3 PI onward.

(iii) T-dependent immunity and parasite maturation

To quantify the effects of immunity on parasite maturation more directly, an experiment was conducted using congenitally athymic (nude) rats. Nude rats are incapable of mounting effective T-dependent immune responses. Animals were females six to ten weeks old (Harlan, U.K.). Fifteen nude rats (genotype Hsd:RH-*rnu/rnu*) and fifteen normal rats (genotype Hsd:RH-*rnu*/+) were infected with around 100 iL3s of ED5, precise inocula sizes being determined as described above. All animals (nude and normal rats) received a wide-spectrum antibiotic (Baytril, Bayer) at a concentration of 0.01% w/v in drinking water. Groups of five nude and five normal rats were sacrificed each day, on days 4, 5 and 6 PI, and gut burdens determined. The numbers of egg-bearing and non egg-bearing worms were recorded. Faeces from the animals sacrificed on day 6 PI were collected overnight on days 3 and 4 PI, cultured and worm outputs determined. Recovered parasites were killed in boiling, 10% formal saline (3:1 v/v physiological saline: 40% Formalin) transferred to a droplet of glycerol on a plain glass microscope slide and encircled with fine ground glass. Mounted specimens were then covered with a glass cover slip sealed at the edges with a polyurethane-based sealant. Parasite body length was measured using the PC_IMAGE software package (version 2.2.01: Foster Finlay Associates, U.K.) and a JVC video camera module (model TK 1270) linked to an Olympus SZ60 light microscope.

(iv) Statistics

Total numbers of parasite offspring produced throughout infection (total worm output) were estimated by numerical integration of the area under the nightly worm

output by time curves. Analysis-of-variance (ANOVA), ordinary-least-squares (OLS) regression, Chi-squared analyses and non-parametric tests were carried out in the Statview program (Statview release 4.5). Ordered heterogeneity (OH) tests were used to test directional hypotheses in experiments involving more than two treatments (Rice and Gaines 1994). Where necessary, count data were $\log_{10}$ transformed and proportion data angular (arcsin [square-root]) transformed prior to analysis.

(v) Literature survey

Seven major journals dealing with parasitology and parasite immunology were searched for experiments in which parasite burdens or parasite reproductive output had been compared in normal and immune-suppressed host animals. The journals searched were: *American Journal of Tropical Medicine and Hygiene*; *Experimental Parasitology*; *Infection and Immunity*; *Journal of Parasitology*; *Parasitology*; *Parasite Immunology* and *Parasitology Research.* All studies involving gastrointestinal nematodes measured in "permissive" hosts were considered as potentially useful, a permissive host being defined as one in which the parasite can complete its life cycle. The aim was to collect an unbiased sample of published data comparing parasite establishment and reproduction in immune-suppressed and normal hosts. Each issue of each journal published between January 1972 and September 1997 [1], was examined and potentially useful data identified. Separate experiments within the same study were treated as separate datapoints. The criteria for inclusion in the dataset and their justifications are listed in Table 1. For each comparison found, the following variables were collated: *initial rank order* (in every comparison mean parasite counts in immune-suppressed groups were ranked as higher or lower than control groups), *relative*

[1] *Parasite Immunology*, which commenced publication in January 1979, was searched from that date onwards. *Zeitschrieft fur Parasitenkunde* changed title to *Parasitology Research* in June 1984 and is referred to here as a single journal.

Table 1. The criteria for inclusion in literature survey. Each criterion is listed alongside its justification.

Criterion	Justification
(1) Control and treatment groups of the same age, gender and genetic background.	Gender, age and genotype affect susceptibility to parasitic infection.
(2) Host immune-suppression led to elevation of parasite counts.	Evidence that treatment affected parasite mortality required.
(3) Numbers of established parasites or parasite offspring assessed by direct counts.	Other measures (e.g. biomass) not amenable to analysis.
(4) A minimum of two measurements taken at sequential time points.	Initial and subsequent parasite counts are required for this analysis.
(5) Comparisons of treatment groups with immunologically reconstituted or surgically modified 'controls' excluded.	No "baseline" reference value of parasite counts in intact, naive control animals.
(6) Manipulation of host immune status begun at least 24 hours prior to infection.	If manipulation is initiated mid-infection, predictions concerning maturation time are redundant.

timing of initial count, (the day postinfection when measurement began, divided by the average prepatent period of the nematode species involved), *relative timing of peak count* (parasite counts in immune-suppressed groups were scored as peaking before, at or after the peak counts in control groups), *delay in peak* (day of peak in immune-suppressed groups minus day of peak in control groups, divided by day of peak in control groups), *extension of infection* (the last day of positive parasite counts in immune-suppressed groups minus that in control groups, divided by that in control group). In the case of *initial rank order*, a 50:50 ratio is expected in the absence of facultative adjustment, if immune-suppression has no early effect on parasite counts (in either direction) and if measurement is begun at a sufficiently early time postinfection.

Average values of prepatent period (the time between infection and the shedding of parasite propagules from the host) were those compiled from the literature by Skorping *et al.* (1991). One published study (Behnke 1975), provided original data on prepatent period and this value was used in the analysis.

6.3 Results

(i) Immunity and parasite reproductive output

Parasite offspring were first detected in the faeces of all rats on day 4 PI. Figure 2 shows initial and total worm outputs across the three treatment groups. Immune manipulation produced significant variation in initial worm output in the expected direction, being highest in immunised rats and lowest in steroid-treated rats (OH test: $r_s P_c = 0.85$, $p < 0.05$). Similarly, total worm output was greatest in steroid-treated rats and lowest in immunised rats ($r_s P_c = 0.98$, $p < 0.05$). Thus, immune-suppression lowered initial reproductive activity and increased total reproductive success, while immunisation produced the opposite pattern of worm output.

(ii) T-dependent immunity and parasite maturation time

Inocula sizes ranged between 88 and 100 (mean = 94.8, 95% confidence intervals ± 1.1) and did not differ between treatment groups ($F_{1,27} = 0.21$, NS) One of the nude rats scheduled for gut burden counts on day 6 PI died of an unknown cause prior to sacrifice. Parasite offspring were first recovered from faecal cultures on day 4 PI. Worm output on day 4 PI was lowest in nude rats, ($F_{1,7} = 7.6$, $p < 0.05$). Gut burdens in nude and normal rats on days 4, 5 and 6 PI are plotted in Figure 3.

Figure 2. The effect of immunisation and steroid treatment on initial and total worm output. Worm output on day 4 PI (open circles) and total worm output (closed circles) are plotted across three experimental treatments. Plotted values are means of six rats per treatment. For both Y-variables, a line of best fit (OLS regression) through the means is shown for illustration. Errors are ± 1 S.E.M.

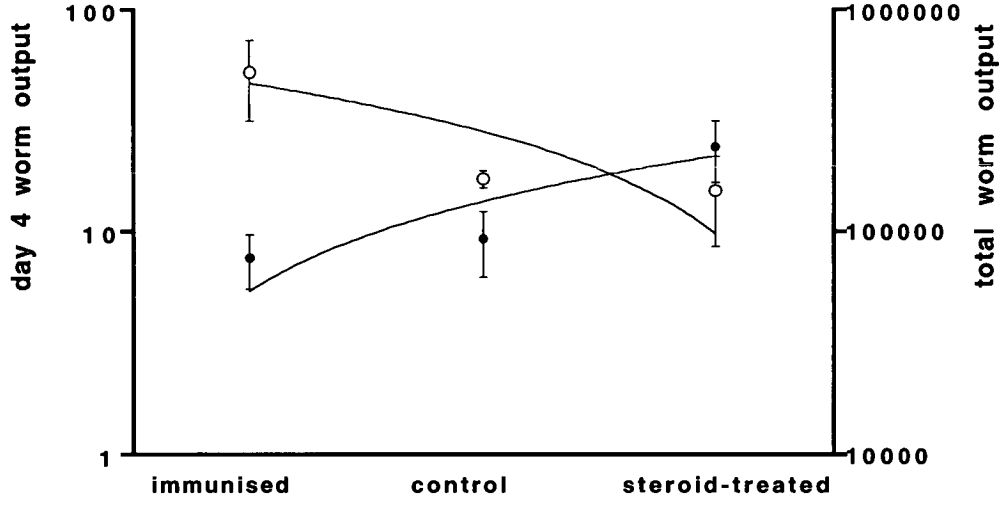
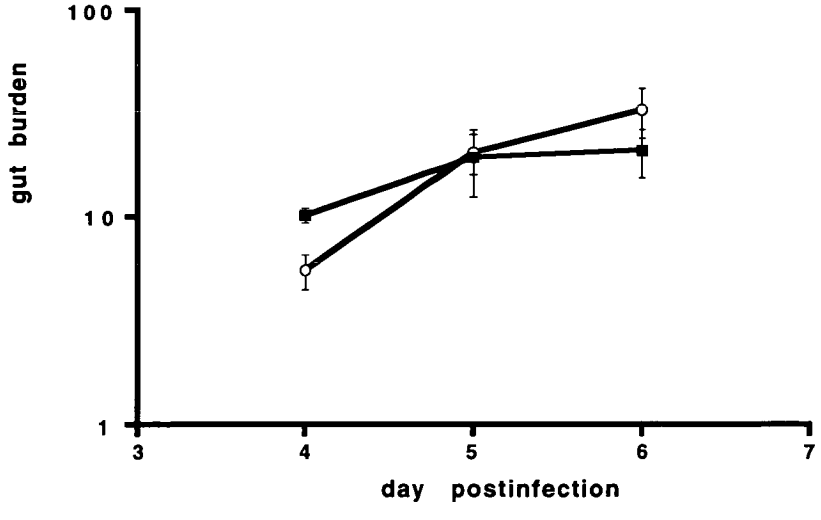


Figure 3. The early kinetics of gut burden in nude (open circles) and normal (closed squares) rats. Plotted values are the means of 5 animals per group, except at day 6 PI where only 4 nude rats are represented (see text). Errors are ± 1 S.E.M.



Significantly fewer worms were recovered (as a proportion of inoculum) from the small intestines of nude rats on day 4 PI ($F_{1,8} = 10.4, p < 0.01$) indicating that a smaller proportion of worms had completed tissue-migration and established in nude rats by this time.

Figure 4 shows the proportion of established parasites in nude and normal rats which were egg-bearing on day 4 PI. The transformed proportion of egg-bearing worms was significantly smaller than that in normal rats, indicating a delay in the onset of reproductive maturity in the absence of T-dependent immune responses ($F_{1,8} = 6.99, p < 0.05$). Nearly all worms recovered on days 5 and 6 PI had eggs *in utero*.

The body lengths of parasites from nude and normal rats are shown in Figure 5. Host immune status did not affect mean parasite body size on day 4, day 5 or day 6 PI. A two-way ANOVA found a significant main effect of day ($F_{2,21} = 22.2, p < 0.0001$), but no effect of host thymic status on mean parasite body length per rat (main effect of thymic status, $F_{1,27} = 0.46$, NS; day by thymic status interaction, $F_{2,21} = 0.03$, NS).

(iii) Published experiments

In all, 20 papers containing 31 comparisons of parasite burdens or parasite reproductive output in normal and immune-suppressed hosts were found (Table 2). In 12 of the 31 comparisons (39 per cent of those found) parasite counts (adult burden or output) were lowest in immune-suppressed animals at the first time point measured. This is not significantly different from the 50:50 ratio expected if immune-suppression has no effect on early parasite mortality. However, early measurement is highly uncommon in these published data, being significantly later than the average maturation time of the nematode species involved (Sign test using *relative timing of initial count*, $p = 0.006$).

Figure 4. The effect of host thymic status on the proportion of established parasites with eggs *in utero* on day 4 PI. Plotted values are the mean proportions in nude (n = 5) and normal (n = 5) rats. Errors are ± 1 S.E.M.

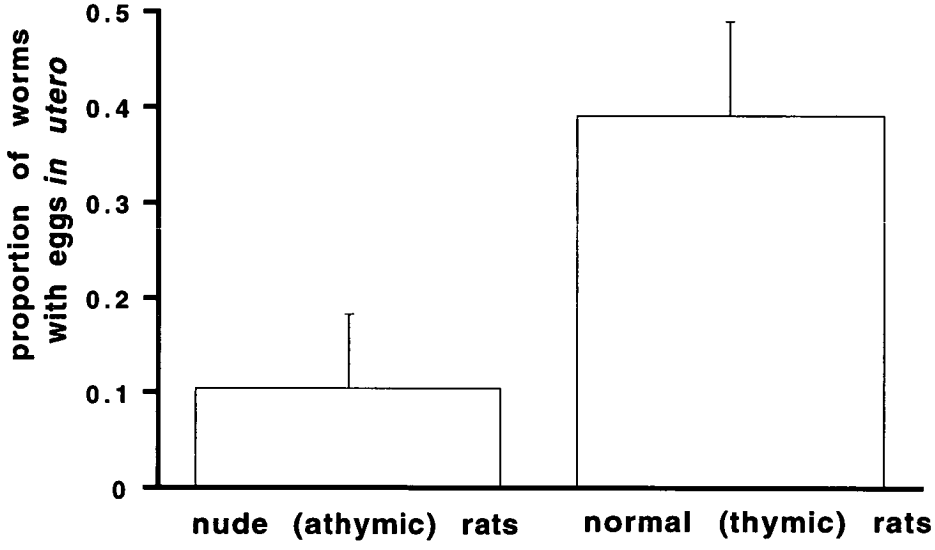
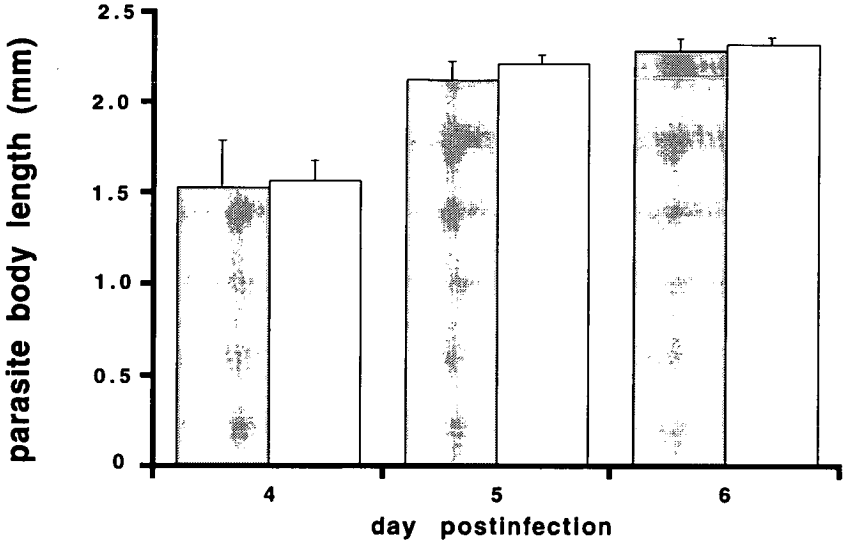


Figure 5. Mean body lengths of parasites recovered from nude rats (stippled columns) and normal rats (open columns) on days 4, 5 and 6 PI. The mean number of worms measured from each rat was 11.6 (95% confidence intervals ± 3.9). Errors are ± 1 S.E.M.



Of the 31 comparisons, parasite counts in immune-suppressed animals peaked later than control groups in 16 cases, there being no difference in the remaining 15 comparisons. Consequently, parasite counts in immune-suppressed groups *per se* peaked significantly later than those in control groups (Sign test using *delay in peak*, $p < 0.0001$). This result could be because worm expulsion occurs earlier in control groups, and can not therefore be viewed as incontrovertible evidence of slower parasite development in immune-suppressed hosts. However, later peak counts in immune-suppressed groups relative to controls was most common in the 12 cases where initial parasite counts were lowest in immune-suppressed animals (9 of these 12 cases) although not significantly so (G-test of independence, $p = 0.066$). Furthermore, in these 12 cases, the peak parasite count in immune-suppressed hosts was delayed beyond that in normal hosts for a significantly longer time (Mann-Whitney U-test using *delay in peak*, $p = 0.014$). Thus, the lower parasite counts were in immune-suppressed hosts at initial measurement, the later they peaked, suggesting a slowing of development. Similarly, prolongation of infection in immune-suppressed hosts was significantly longer in these 12 cases (Mann-Whitney U-test, $p = 0.049$). Thus, there is some evidence in the existing literature that low initial parasite reproductive activity is associated with a slowing of infection dynamics and with increased parasite life expectancy.

Table 2. Results of literature survey. The number of separate experiments in each published study is indicated in the sixth column. Whether parasite counts in immune-suppressed hosts were initially below those in controls, is indicated in the last column by *initial rank order*.

Original paper*	Nematode species	Experimental host species	Cause of host immune-suppression	Stage of life cycle counted	Number of experiments in study	*Initial rank order* (immune-suppressed group)
1	Aspiculuris sp	mice (*Mus musculus*)	oral hydrocortisone	established parasites	1	above control
2	*Haemonchus contortus*	sheep (*Ovis aries*)	oral dexamethasone	parasite offspring	2	above control
3	*Heterakis spumosa*	rats (*Rattus norvegicus*)	testosterone injections	parasite offspring	1	above control
4	*Nippostrongylus brasiliensis*	mice	congenital mast cell deficiency (*W/W^v* mutation)	established parasites	1	below control
4	*N. brasiliensis*	mice	*W/W^v* mutation	parasite offspring	1	below control
4	*N. brasiliensis*	mice	congenital thymic deficiency (*Nu/Nu* mutation)	established parasites	1	above control
5	*N. brasiliensis*	mice	*W/W^v* mutation	established parasites	1	above control
6	*N. brasiliensis*	mice	steroid	established parasites	1	above control

7	*N. brasiliensis*	mice	*W/W*^{v} mutation	established parasites	1	below control
8	*N. brasiliensis*	Indian soft furred rats (*Millardia meltada*)	testosterone injections	established parasites	1	below control
8	*N. brasiliensis*	*M. meltada*	testosterone injections	parasite offspring	1	above control
9	*Strongyloides ratti*	mice	steroid	parasite offspring	1	above control
10	*S. ratti*	mice	*W/W*^{v} mutation	parasite offspring	1	above control
10	*S. ratti*	mice	*W/W*^{v} mutation	parasite offspring	1	above control
11	*S. ratti*	mice	'beige' mice (*bg/bg* mutation)	parasite offspring	1	above control
12	*S. ratti*	rats	steroid	parasite offspring	2	below control
13	*Trichostrongylus affinis*	rabbits	steroid	parasite offspring	1	above control
14	*Trichuris muris*	mice	anti-CD4+ antibody	established parasites	1	below control
14	*T. muris*	mice	anti-CD4+ and anti-CD8+ antibody	established parasites	1	below control
15	*Trichinella spiralis*	mice	steroid	established parasites	1	above control
16	*T. spiralis*	mice	*Nu/Nu* mutation	parasite offspring	1	below control
17	*T. spiralis*	mice	*W/W*^{v} mutation	established parasites	2	below control

18	*T. spiralis*	mice	anti-lymphocyte serum	established parasites	1	above control
18	*T. spiralis*	mice	anti-lymphocyte serum	established parasites	1	above control
18	*T. spiralis*	mice	anti-lymphocyte serum	parasite offspring	1	above control
19	*T. spiralis*	mice	*Nu/Nu* mutation	established parasites	1	below control
20	*T. spiralis*	rats	*Nu/Nu* mutation	established parasites	1	above control
20	*T. spiralis*	rats	*Nu/Nu* mutation	parasite offspring	1	above control

* Key to Original Papers.

1= Behnke 1975
2 = Presson *et al.* 1988
3 = Harder 1994
4 = Crowle and Reed 1981
5 = Ishikawa 1994
6 = Khan *et al.* 1995
7 = Mitchell *et al.* 1983
8 = Tiura *et al.* 1995
9 = Grove and Dawkins 1981
10 = Nawa *et al.* 1985
11 = Nawa *et al.* 1988
12 = Olson and Schiller 1978
13 = Knight 1977
14 = Koyama *et al.* 1995
15 = Bolas-Fernandez *et al.* 1989
16 = Gustowska *et al.* 1980
17 = Ha *et al.* 1983
18 = Machinka 1972
19 = Ruitenberg and Steerenberg 1974
20 = Vos *et al.* 1983.

6.5 Discussion

The experiments described here confirm that T-dependent host immune responses affect the speed of maturation in *S. ratti*. The reproductive behaviour of this parasite in the presence and absence of T-dependent immunity has now been studied on five occasions (Dawkins *et al.* 1982; Gemmill *et al.* 1997; Chapter 3, and this chapter). On each occasion, initial reproductive activity was lowest and overall reproductive success highest when T-dependent immunity was suppressed. The literature survey conducted here suggests that this is a common result in experimental studies with other gastointestinal nematodes. Nevertheless, only 39% of published studies show any evidence of phenotypic adjustment of maturation time and there may be several reasons for this. Most experimental measurement is begun after the typical prepatent period has elapsed, and so relevant data may be rare. For example, in the case of *S. ratti* an absolute delay in maturity of 24 hours would represent a 25% extension of the prepatent period (prepatent period four days) but would remain undetected if sampling was delayed until a week postinfection. Some species may simply be incapable of modulating growth rates or some other cost of doing so may outweigh the potential benefits. Parasitic nematodes exhibit a great deal of life history and life cycle diversity (Anderson 1992; Poinar and Hansen 1983). The simple theoretical model developed in Chapter 5 may be too simple to usefully predict the optimal phenotypic response of all these species to variation in immune-imposed mortality. In particular, if stage- or size-dependent mortality are themselves important functions of maturation time, this might be expected to affect the nature of the optimal phenotypic response (Skorping and Read 1998).

Indeed, in the second experiment reported here, the measurable delay in maturation time among worms in nude rats was not accompanied by any significant increase in parasite body size, contrary to the prediction of the hypothesis under test. It is possible that there was a real but very small difference in body size at maturity

which the experiment failed to detect and we can try to assess the likelihood of this as follows. Power calculations allow an estimate of the probability of detecting a specific difference, at a specified significance level, using information about the sample size and variance of the data. Calculations using values from the data gathered here show that there was an 80% chance of detecting (as significant at the 5% level) a difference of as little as a 2% in parasite body length. The average length of an iL3 of the line ED5 is around 0.55 mm (A. Graham personal communication) and the average length on day 4 PI measured here around 1.5 mm (Fig. 5). Using these values and assuming an exponential rate of growth, body size shows a roughly cubic relationship with time across this period (slope of regression of log length on log time = 3.065). Based on this relationship, it follows that a 2% increase in body length at maturity would be achieved after a delay of only 36 minutes. The difference in the proportion of mature worms in nude and normal rats (Fig. 4) would appear to provide evidence of a far more substantial difference in mean maturation time. If this delay represents an adaptive plastic response in line with the theoretical predictions tested here, a difference in body size is therefore likely to have been detectable in this experiment.

Could immune-dependent maturation time in *S. ratti* have a plausible, non-adaptive explanation? One possibility is that parasites use components of the host immune system as developmental cues in moving from one larval stage to the next. Under this hypothesis, parasites would develop more slowly in immune-suppressed hosts not because of any size-associated fitness benefit, but because immune cues are lacking or slow to appear. In many helminths, changes in a range of intra-host factors such as pH and enzyme levels affect the timing of specific developmental events and in some trematodes host cytokines cue parasite reproduction in an all-or-nothing manner (Amiri *et al.* 1992). However, this explanation is not entirely satisfactory for at least two reasons.

First, in several parasitic nematode species, including some represented in the

literature survey, complete parasite life cycles can be maintained *in vitro*. (e.g. Weinstein and Jones 1956; Berntzen 1965). To my knowledge, in none of these culture systems is it necessary to introduce host immune components, though variations in temperature, pH and enzyme levels figure in most protocols. Second, if there is no fitness benefit to delaying maturity in immune-suppressed hosts, worms that tie their developmental rate to such cues will forego a substantial amount of early reproduction in a relatively benign environment. Congenitally athymic rats are not, of course, common in nature but loss of host immune function due to pregnancy, lactation, stress etc. may well be common. If so, natural selection should favour parasites that are able to adjust reproductive behaviour to maximise their fitness across a range of immune environments. Last, this particular non-adaptive explanation begs the question it sets out to answer. Why should a tying of development time to specific immune cues be favoured by natural selection in the first place? Indeed, the hypothesis that parasitic nematodes adjust maturation time facultatively presupposes that parasites have reliable cues indicating host immune status (and hence parasite mortality rates) and that parasites, not hosts, have control over parasite life history decisions. Such cues could be components of the host immune system but in principle, worms might utilise internal information on their own physiological condition.

In the present study, while maturation time in *S. ratti* varied with host immune status in the direction predicted from theory, the weight of evidence was that delayed maturity led to no increase in adult female body size. Thus, a tangible fitness advantage to delayed maturity in immune-suppressed hosts remains undemonstrated for this and other species of parasitic nematodes. At least one non-adaptive explanation for this phenomenon in *S. ratti* is non-immunological in nature. It is possible that some unknown physiological consequence of host thymus-deficiency means that nude hosts offer a poor habitat for developing larvae, so depressing immature growth rates even while enhancing survivorship.

Chapter 7

General Discussion

"It seems as if Mother Nature has invested
this parasite with a cloak of manifold mysteries
which parasitologists, the world over, have
failed so far to penetrate."

A. L. Levin

"I think I spy a kind of hope."

William Shakespeare

7.1 **SUMMARY:** The conclusions to be drawn from the main findings of each chapter are discussed separately in the relevant sections. In this chapter I discuss the main findings in relation to the general aims of the thesis and prospects for future work. I aimed to investigate the nature of plasticity in three major life history traits in a parasitic nematode, *S. ratti*. In light of these aims, what have the last five chapters revealed? First, in terms of the dictionary definition which heads Chapter 1, the phenotype of this parasite is clearly "mouldable" and "modifiable" given the differences in fitness (Chapters 2 and 3), sexuality (Chapter 4) and maturation time (Chapter 6) which it displays across a range of environments. Second, the dividing lines between the adaptive and non-adaptive components of this broad-sense plasticity are now better defined. Last, by using plasticity as a framework to investigate parasite life histories, empirical data were garnered which motivated theoretical work, the results of which were successfully applied to explain life history variation in parasitic nematodes at a cross-taxa level (Chapter 5).

7.2 Main Findings

The results reported in Chapter 2 show that in terms of the pattern of reproductive output displayed by parasites, host genotype can have a measurable impact on parasite phenotypes, but that this need not affect parasite fitness. When measurement was confined to only one or a few time points, this led to erroneous conclusions regarding the power of the host immune response to limit infection. At the very least, these results should serve as a reminder that variation which appears large on one scale may, in reality, be having very little impact on fitness. Why the shape of the *S. ratti* larval output curve differs between PVG and Wistar rats is not known but two broad explanations are possible - an adaptive time-shift in reproductive effort by parasites, and a non-adaptive shift imposed by the host immune response. Whether the actual shift represents evidence of plasticity as an adaptation or not, it does demonstrate the capacity of the *S. ratti* life history schedule for "permanent deformation without giving way". Lifetime reproductive output in hosts of either genotype was very similar in these experiments.

In Chapter 3, it was argued that the plasticity in fitness represented by host-specificity is not necessarily the best indicator of specialisation. This viewpoint may seem highly semantic. However, habitat-specific models of specialisation clearly imply the potential for a restricted ecological range to evolve solely as a result of mutation and drift at non-selected, habitat-specific loci (e.g. Kawecki 1998). Thus, habitat restriction might evolve via a loss of adaptation whereas, under any reasonable definition of the term, specialisation can not. I believe this is potentially an important practical distinction in ecology and worthy of continued attention.

In the work on specialisation reported here I attempted to alter host-specificity by exposing worm populations to natural selection in a novel host (mice) in order to uncover the genetic basis of this trait. In the event, no response to selection was obtained and so it was not possible to do so. Of course, it remains possible that

artificial selection or other controlled breeding regimes might reveal useful information about the evolution of host-specific adaptation in this or other parasitic species. An improved understanding of these issues may be of more than academic interest. For example, if "animal reservoirs" harbouring particular zoonotic diseases can be greatly reduced in size but not eliminated altogether, what is the best course of action to minimise disease levels in human populations? The answer may depend partly on whether or not parasite fitness is governed by loci with host-specific effects. If so, large reductions in the size of animal reservoirs could increase the strength of selection on loci with human-specific effects, facilitating closer parasite adaptation to the human host. Thus, studying the genetic architecture underlying host-specificity is potentially of value in controlling parasitic diseases successfully.

Chapter 4 confirmed previous findings concerning the immune-dependent nature of conditional sexuality in *S. ratti* (Gemmill *et al.* 1997, see Appendix). Further, these results suggest that conditional sex does not occur as a response to general environmental stresses, but that it is specific to immune responses acquired against *S. ratti.* This may suggest that sex in this nematode is indeed maintained as some kind of immune-evasion mechanism. If true of *S. ratti* and other species in the genus *Strongyloides*, this may have wider implications for vaccine development and other parasite control measures (Gemmill *et al.* 1997).

In Chapter 5 a theoretical model was developed aimed at explaining variation in maturation time in parasitic nematodes. The model is simple and revolves around a trade-off between the costs and benefits of attaining a large body size prior to reproductive maturity. The model makes the clear prediction that optimal maturation time is inversely proportional to pre-maturational mortality rate, the constant of proportionality being the allometric slope linking fecundity to maturation time. In the cross-species case, considerable support for this prediction is found. This may point to the fact that the trade-off at the centre of the model is a fundamental one in the life

histories of most gastrointestinal parasitic nematodes. However, nematode species show a great breadth of life history variation and the model presented in this thesis represents only a starting point for further investigation. Within species it seems reasonable to expect that other constraints, perhaps other trade-offs, specific to the particular ecology may be equally or even more important in determining the optimal life history schedule.

Chapter 6 addressed the possibility of adaptive control of maturation time at the phenotypic level by *S. ratti*. While the simple model in Chapter 5 had some success in predicting maturation time across taxa, little support was found for the hypothesis of adaptive plasticity in this trait. Delayed parasite maturity in T-deficient (nude) rats was clearly demonstrated. However, it now seems unlikely that the reason for this delay is an adaptive plasticity aimed at optimising the trade-off between growth and survival to maturity. Even a small (2%) difference in body length should have been detectable in this experiment and given the apparent size of the observed delay, there should have been far more than a 2% difference between worms from nude and normal rats if the hypothesis was correct. Thus, the phenomenon of delayed parasite maturity in immune-suppressed hosts is currently confirmed in *S. ratti* but its adaptive basis, if any, defies explanation. The actual reason may ultimately be found to be non-adaptive but this does not necessarily mean that it is of no interest. That a parasite should fail to exploit any weakness in its host's immune defences is surely a puzzling state of affairs and may well point to some fundamental aspect of the host-parasite relationship.

7.3 Future Directions

Arising from the work on specialisation (Chapter 3) are a number of possibilities for future work. First, the selection experiment may have suffered from an inherent weakness in that genetic variation in the starting population may have been too low. Ideally, future experiments would use large, freshly isolated parasite

populations to maximise the chances of obtaining a response to selection. Second, it should be possible to move beyond the use of natural selection in such an experiment. Artificial selection and controlled breeding regimes, if successful, would allow more detailed information about the genetic basis of host-specific adaptation to be collected.

The theoretical work in Chapter 5 can be built upon to make more detailed predictions in the context of particular parasite ecologies. For example, tissue-dwelling (filarial) nematodes and those intestinal species which have tissue-migratory phases may face a quite different set of constraints than those which dwell solely in the host alimentary tract. Specifically, future models should focus on the effects of post-maturational and stage- or tissue-specific mortality rates on optimal maturation time by explicitly making them functions of maturation time. Direct estimates of in-host parasite mortality rates would of course make testing any resulting predictions much easier. The implications of the current optimality model for medical intervention strategies are largely positive. For example, even drugs or vaccines which were only partially effective in preventing infection or clearing established parasites, may nonetheless select for smaller, less fecund worms by increasing the in-host mortality rate.

It has recently been argued that the best hope for advancing the science of parasite life history biology lies with a more concerted application of comparative analyses and related techniques (Poulin 1998). While the potential of comparative work should not be underestimated, there is undoubtedly much to be learned through a direct empirical, intra-specific approach. The power of experiment to further understanding of parasite life histories by directly testing intuitive assumptions and the predictions of evolutionary theory are demonstrated by a number of recent studies (e.g. Buckling *et al.* 1997; Mackinnon and Read in press; Tompkins and Clayton in press).

The problems inherent in measuring trait values directly in endoparasitic species, while considerable, are surely not insurmountable. It is to be hoped that the

challenge posed by these difficulties will encourage rather than discourage work that will (hopefully) see them overcome in the not too distant future.

Perhaps if there is anything at all unique about the biology of internal parasites it is the unusual degree of care and forethought required merely to allow reliable measurement of traits, access to which is more or less taken for granted when working with free living organisms.

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Appendix

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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 hypothymic 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-

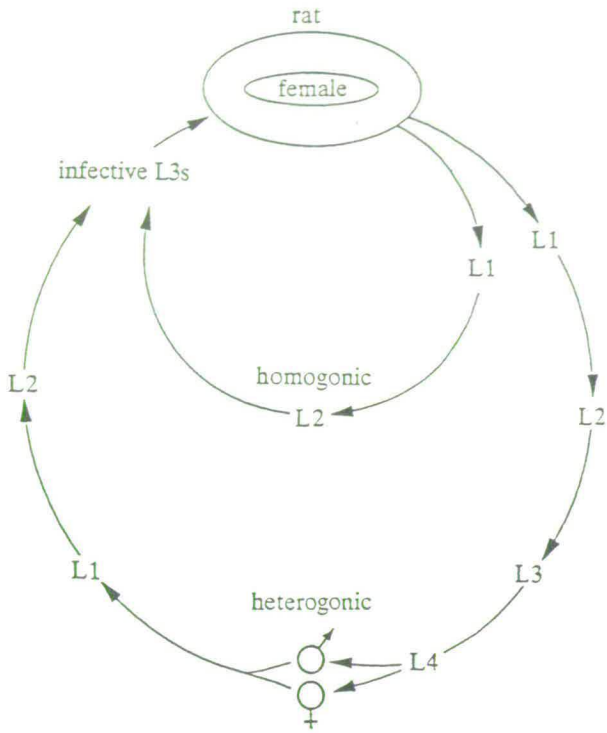

FIG. 1. Life cycle of *Strongyloides ratti*, showing the heterogonic (sexual) and homogonic (clonal) routes. Adult worms in the rat are always parthenogenetic females. Larval stages are denoted by L. Reproduced from Viney et al. (1993).

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 resiliant 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 dessication, 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 hemagluttinating 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 hypothymic (nude) rats. Rats and mice homozygous for the hypothymic condition have only a remnant thymic stump and are dysfunctional with respect to T-cell maturation. Heterozygotes do not express the hypothymic 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 hypothymic 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 pentabarbitone (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 repeatedly at time points throughout the infections as follows. Faeces were collected overnight onto damp paper, weighed and a maximum of five 1.5 (± 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}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 Hypothymic (Nude) Rats.—Infections in four homozygous (hypothymic, 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 0.11ml of 17.6% w/v thiabendazole suspension (Thibenzole, 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 × 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 × 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 prolonged 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.

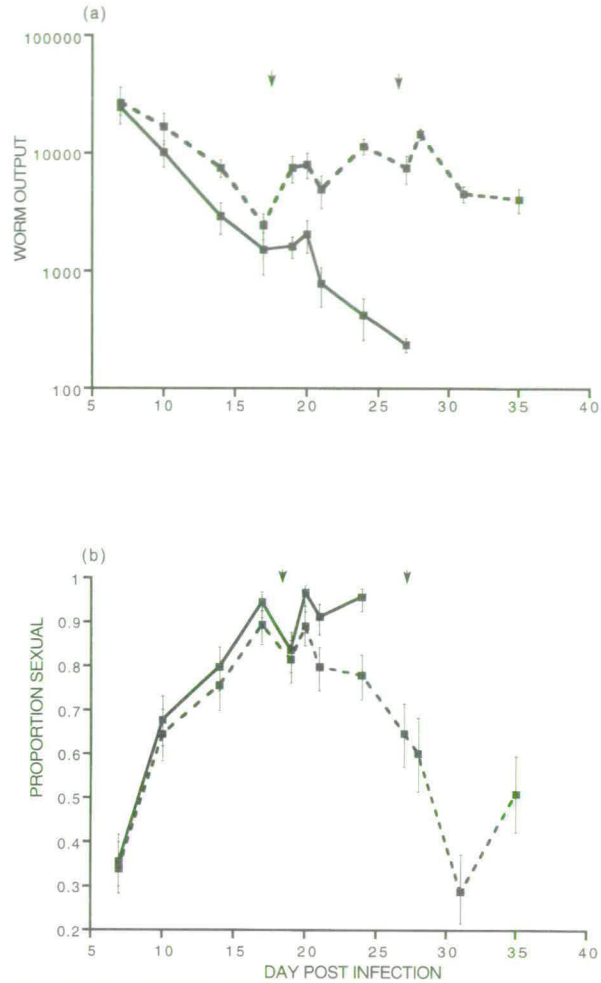

FIG. 2. The effect of corticosteroid treatment on (a) worm output (larvae per night), and (b) proportion of larvae developing into sexual morphs. Broken lines = rats treated with corticosteroid; solid lines = rats treated with saline. Arrows indicate first and last treatment days. Plotted values are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

Congenitally Hypothymic (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 (hypothymic) rats until day 214 PI and all hypothymic 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 hypothymic 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 hypothymic rats had a dramatically smaller impact on parasite fecundity than those mounted by thymic rats. The proportion

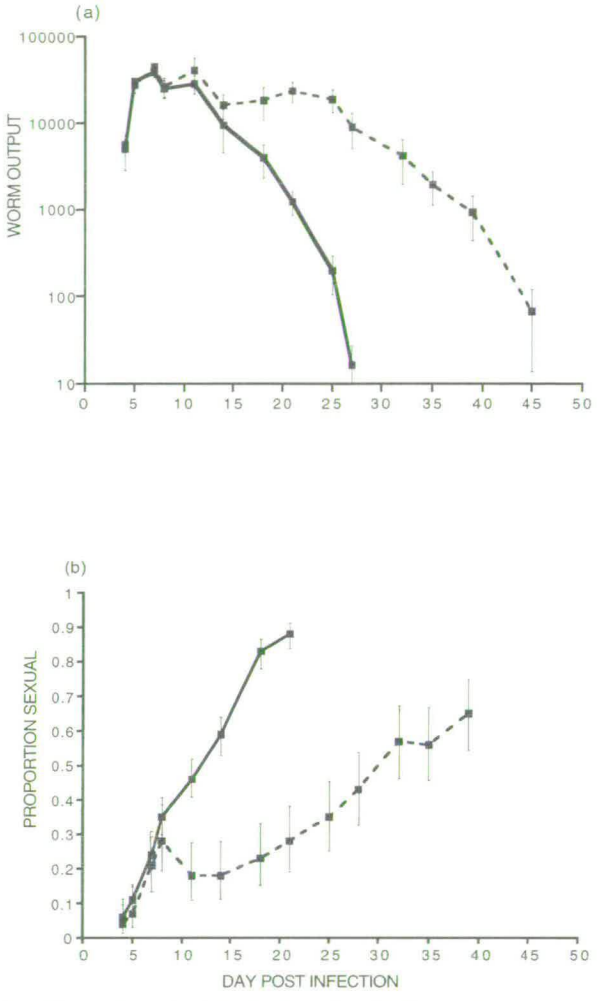

FIG. 3. The effect of whole-body γ-irradiation on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = irradiated rats; solid lines = nonirradiated rats. Plotted values are means of three rats (± 1 SE); in some cases, error bars are smaller than symbol.

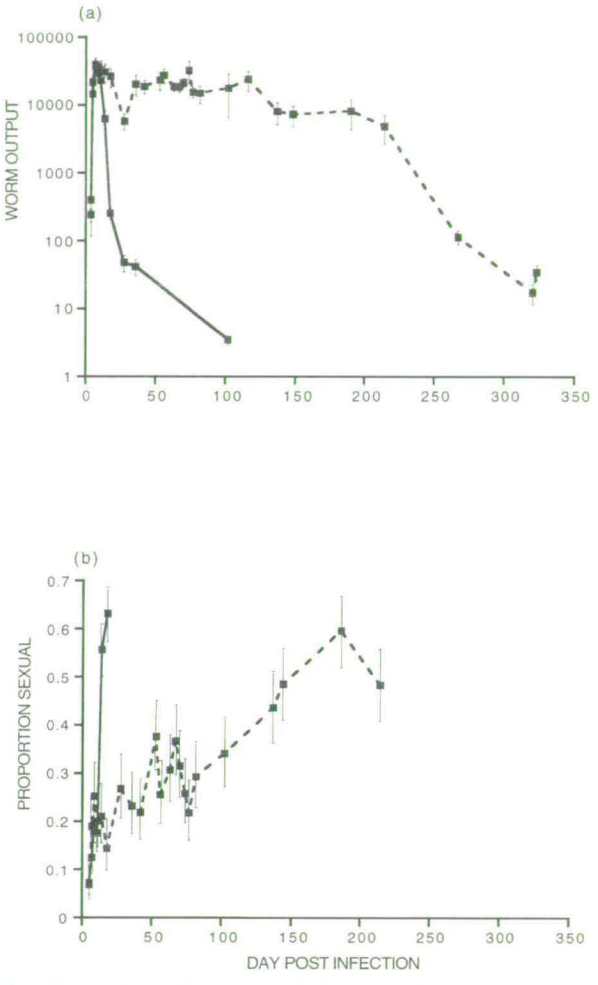

FIG. 4. The effect of congenital thymic deficiency on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = hypothymic (homozygous) rats; solid lines = thymic (heterozygous) rats. Plotted values are means of four rats (± 1 SE); in some cases, error bars are smaller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

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 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 × 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

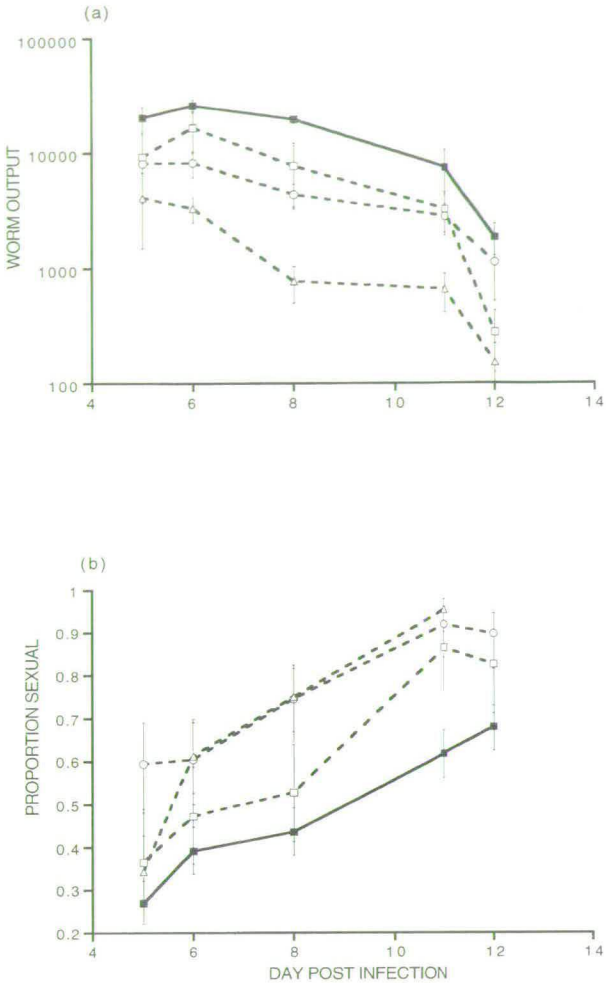

FIG. 5. The effects of varying levels of previous exposure to *Strongyloides ratti* on (a) worm output, and (b) proportion of larvae developing into sexual morphs. Broken lines = rats previously exposed to 1 (open squares), 10 (open circles), and 50 (open triangles) iL3s respectively; solid lines = previously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

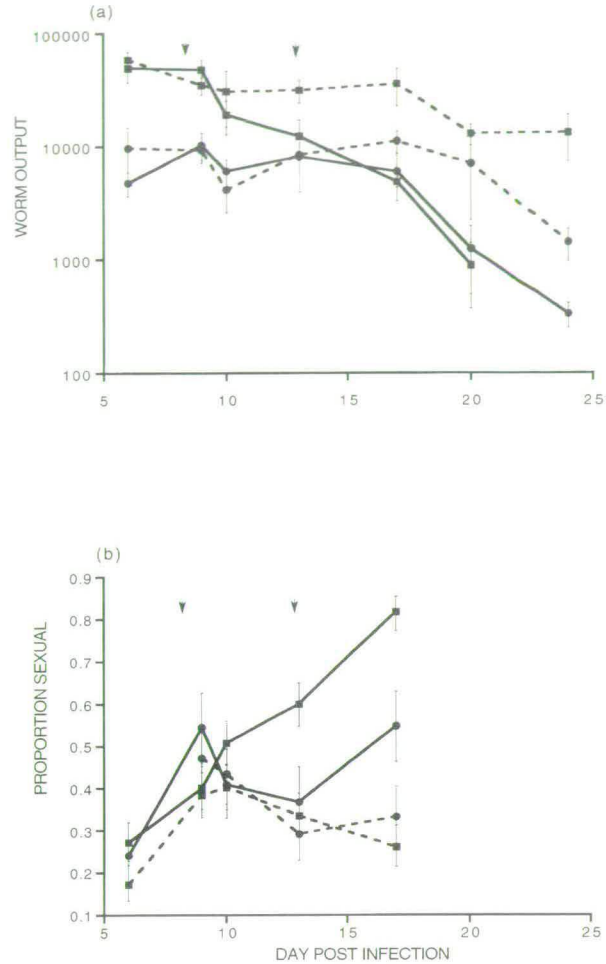

FIG. 6. The effects of corticosteroid treatment and number of iL3s in inocula on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = rats treated with corticosteroid; solid lines = rats treated with saline; circles = rats exposed to 100 iL3s; squares = rats exposed 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.

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 × 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 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

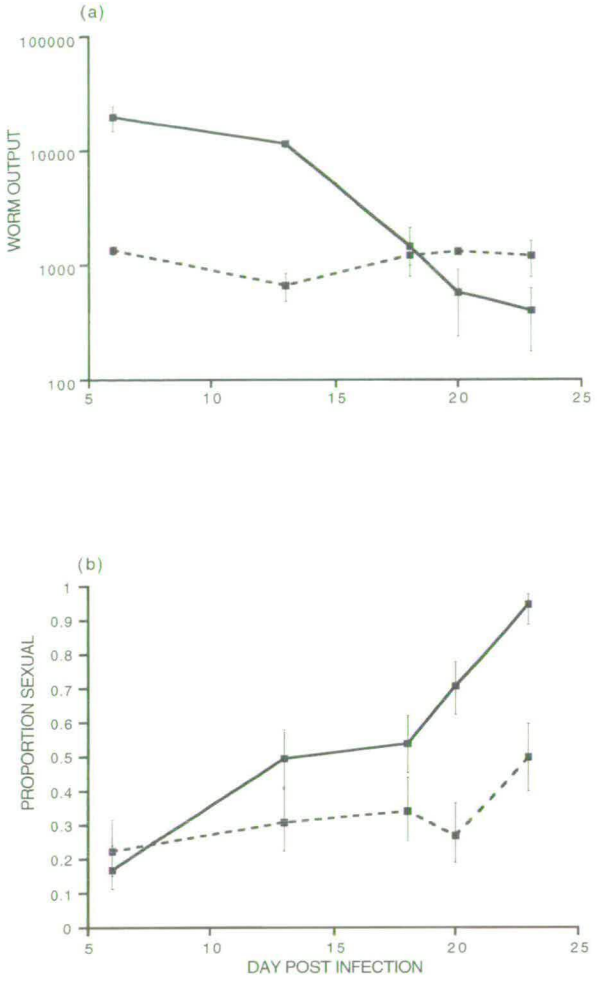

FIG. 7. The effects of whole-body γ-irradiation and number of iL3s in inocula on (a) worm output and (b) proportion of larvae developing into sexual morphs. Broken lines = irradiated rats given 50 iL3s; solid lines = nonirradiated rats given 500 iL3s. Plotted values are means of three rats (± 1 SE); 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 (Riddle 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 homogonic 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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Counting the cost of disease resistance

A well-known fictitious race of predatory alien would always admonish the species they intended to subjugate with the unforgettable phrase 'RESISTANCE IS USELESS'. A recent paper in *Nature*[1] shows that an animal's ability to mount a robust immunological response to incoming pathogens, while being far from useless, may indeed be less than useful in certain circumstances. Evolutionary biologists have suspected this for some time. The central observation fuelling these suspicions is that genetic variation exists among conspecifics in the capacity to repel or control infection successfully. If resistance is useful, in the sense that it contributes positively towards an individual's fitness, then why are some genotypes refractory to disease and others congenitally defenceless? Why does natural selection not fix genes conferring resistance throughout animal populations?

There are numerous explanations[2]: for example, large asymmetries between host and parasite generation times may leave hosts 'lagging' behind pathogens in coevolutionary arms races. Alternatively, substantial genetic variance, for instance due to the effects of dominance, can remain at the limits of artificial selection, and similar constraints might obtain in nature. Nonetheless, a major possibility is that resistance correlates negatively with other important fitness components (a so-called 'cost' of resistance). Consequently, so the idea goes, resistance genes are subject to antagonistic selective forces which conspire to impose an equilibrium frequency somewhere short of complete fixation.

So far, so good. The great problem with the cost of resistance model, however, has been a continuing lack of direct evidence identifying the all-important costs themselves. Recent years have seen a quickening of interest in this problem, and now in an elegant experiment with an insect host–parasitoid system, Kraaijeveld and Godfray[1] add convincingly to a small but expanding body of empirical data in support of the 'cost' hypothesis.

Using the parasitoid wasp *Asobara tabida*, a common biological enemy of several European *Drosophila* species, the authors selected replicate lines of *D. melanogaster* for increased resistance to parasitoid attack. Ovipositing females of *A. tabida* lay their eggs in the body cavity of larval flies. The young wasp then develops within its host, ultimately causing its death. Occasionally, however, a larval fly successfully contains the intruder within multiple layers of immune cells and deposits a dark pigment upon its surface. If this process, known as melanotic encapsulation, is successful, the invading parasitoid is destroyed and the larval fly can develop to adulthood. The dark melanotic capsule remains visible through the fly's abdominal wall so that as an adult, a larva that survives parasitization displays the little black spot like a badge of honour.

Kraaijeveld and Godfray used these spots as the phenotypic marker in their selection regime, choosing only those flies with a melanotic capsule to parent subsequent generations. The response to selection was rapid and substantial. In the original field isolate, *c.* 5% larval flies encapsulated wasp eggs, a figure typical of northern European *D. melanogaster* populations. After eight generations, encapsulation rates in the selected lines exceeded 50%. Aside from confirming the genetic basis of encapsulation ability, the magnitude of this response suggests that in wild populations there may be considerable constraints on the evolution of resistance.

The authors then turned their attention to locating possible costs associated with the resistant phenotype. Comparison of a battery of traits between selected and control lines revealed that at high population densities resistant larvae suffer a significant decline in ability to compete for a limited food supply when measured against a genetically marked 'tester' strain of *D. melanogaster*. According to Kraaijeveld and Godfray, the population densities imposed in these competition assays are frequently encountered by developing larvae in the field. By demonstrating a negative genetic correlation between larval encapsulation ability and competitive performance, these experiments provide hard evidence of a trade-off between resistance to parasitoids and other components of fitness.

Data pointing to a cost of resistance are now accruing in a diverse assemblage of host–pathogen systems. The conditional inferiority of resistant phenotypes has been demonstrated in the interactions of bacteria with bacteriophages[3] and moths with viruses[4] as well as mosquitoes with protozoan[5] and nematode parasites[6]. These latter two results have implications for the successful control of debilitating human diseases – specifically malaria and the tropical filariases, in which mosquitoes act as vector. Eradication programmes based on the release of pathogen-resistant vectors to the field could ultimately prove futile if resistant mosquitoes pay too high a fitness cost in the absence of parasitism[5].

In plant[7] and vertebrate[8] biology, the genetics of resistance mechanisms have been intensively studied. Our understanding of the genetics underlying host resistance mechanisms in invertebrates is less impressive, but progress is being made. In the case of mosquito refractoriness to *Plasmodium* spp., both susceptibility and resistance respond to selection in the laboratory[9] and, more recently, QTL mapping suggests a complex basis to the mode of inheritance of resistance[10]. For some parasitoid systems at least, the genetic basis of resistance may be much simpler. For example, melanotic encapsulation ability of *D. melanogaster* larvae

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infected by eggs of the wasp *Leptopilina boulardi* may be largely dependent on a single autosomal locus with complete dominance of resistance-conferring alleles[11]. Such a situation apparently does not pertain in the experiment reported in the recent *Nature* article[1], where a cross of selected and control lines produced an F_1 showing intermediate levels of encapsulation capacity.

A fitness 'price tag' attached to disease resistance has repercussions in several areas of evolutionary biology, including the study of virulence[12], ecological immunology[13], genetic diversity and coevolution[12] (the rates of which are crucial for parasite models of sex and sexual selection). But Kraaijeveld and Godfray's experiment[1] may indicate the sort of data that will allow progress in a challenging area so far avoided by most evolutionary biologists.

Optimal immunology

Cellular and molecular biologists have accumulated an enormous collection of facts about the diversity of host responses to infection. NeoDarwinism successfully makes sense of a number of equally disparate facts from other branches of natural history. Yet there is currently no evolutionary synthesis underpinning immunology. What generates quantitative variation in responsiveness? When does natural selection favour qualitatively different responses (e.g. behavioural or physiological; specific or non-specific)? It is possible that much of this variation can be understood in terms of the relative costs and benefits of particular resistance mechanisms. Immunological effectors in the vertebrate gastrointestinal tract, for example, are frequently non-specific. They include widespread inflammatory responses and result in drastic changes in gut motility and mucosal structures. In contrast, tissue responses (e.g. in the eye or testes) are typically more specific, localized and often very muted. Is this because the fitness consequences of major trauma in the gastrointestinal tract are smaller than for other organs? Temporarily impaired digestion is probably less harmful than impaired vision or reproductive function[14].

These kinds of answers lie outside the traditional interests (or training) of immunologists, but may have considerable medical and veterinary relevance. To our knowledge, the first coherent case for an optimality approach to immunology was put in a seminal paper by Behnke, Barnard and Wakelin[15]. Amongst other things, they argued that it may not be desirable (or even possible) to produce vaccines capable of eliciting sterilizing immunity against parasites of domestic animals: the fitness costs associated with responses of sufficient efficacy may simply exact too high a price. This reasoning holds for other immunoprophylactic attempts at disease control. Selective breeding to enhance resistance offers a potential solution to increasing levels of drug resistance in the helminth populations of domestic animals. But the economic viability of selective breeding depends crucially on the direction of correlated responses in production traits and, so far, what little evidence we have is mixed[16].

All of this points to the need for comprehensive data of the sort obtained by Kraaijeveld and Godfray on *Drosophila*[1]. In the context of vertebrate immunology, obtaining analogous data may seem a tall order. But the availability of antigen- and germ-free environments, and the existence of pharmacological and genetic technology designed to disable particular components of resistance, may actually make vertebrate work more tractable. And the questions are important: just when resistance is useful is of interest to more than just the fans of Dr Who.

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Influence of Rat Strain on Larval Production by the Parasitic Nematode *Strongyloides ratti*

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ABSTRACT: The course of infection with *Strongyloides ratti* in a range of rat strains was assessed by monitoring the production of larvae. To our knowledge, this is the first such study of *S. ratti* using its natural host *Rattus norvegicus*. Host strain influenced the pattern of larval production. The results were qualitatively the same for 2 *S. ratti* lines of North American and Japanese origin.

Nematodes in the genus *Strongyloides* are important gastrointestinal parasites of humans and domestic livestock (Dawkins, 1989). *Strongyloides ratti* is a parasite of rats and is used extensively as a convenient laboratory model of strongyloidiasis. The influence of host genetics on the course and kinetics of infection has received little attention in *S. ratti*. This is despite its possible significance in various aspects of parasitic disease in general (Grenfell and Dobson, 1995). With *S. ratti*, the majority of work in this area has been conducted using mice (*Mus musculus*) in which a number of inbred and mutant strains have been shown to differ in susceptibility to infection with this nematode (e.g., Dawkins et al., 1980; Nawa et al., 1985, 1988). Studies with the natural host (*Rattus norvegicus*) have been confined to examination of the effects of gender (e.g., Katz, 1967) and a single immunologic mutation (Gemmill et al., 1997). Our aim here was to quantify the pattern of larval production by *S. ratti* in various inbred and random bred strains of the natural host *R. norvegicus*. The rat strains used were chosen on the basis of differing profiles of antibody production in response to another gastrointestinal nematode, *Nippostrongylus brasiliensis* (Kennedy et al., 1990), and differ in haplotype at a major histocompatibility complex (MHC) (RT1) locus.

We carried out 2 experiments. In the first we compared larval production by a single *S. ratti* line (ED5 Heterogonic; Viney, 1996; referred to here as ED5) in a random bred rat strain (Wistar) and 4 inbred strains. The second was designed to provide greater detail on patterns of larval production in 2 rat strains that differed consistently in the first experiment. Specifically, we sampled infections more frequently and used larger numbers of rats. In addition, in this second experiment, we examined whether the same patterns were observed for a second *S. ratti* line (ED279) of different geographic origin. ED5 and ED279 are isofemale lines descended from North American and Japanese isolates, respectively (Viney, 1996: ED279 derives from isofemale line 132).

Methodology was the same in both experiments. Six-week-old male rats (Harlan UK) were used with food and water provided ad libitum. Parasite lines were maintained by serial passage in laboratory rats. Experimental animals were infected by subcutaneous injection with 500 infective third-stage larvae

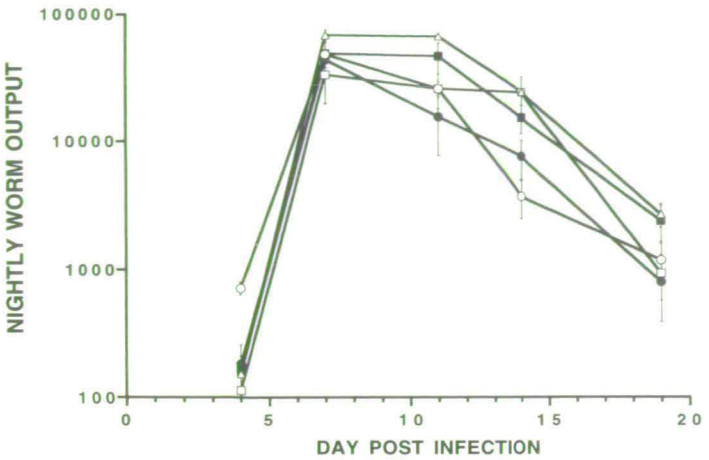

FIGURE 1. Nightly worm output from 5 strains of rat infected with 500 iL3 of the *Strongyloides ratti* line ED5. For each rat strain, haplotype at the MHC RT1 locus is given in parentheses following the sample size. Open triangles = Wistar (n = 2, variable); open circles = PVG (n = 4, $RT1^c$); open squares = LOU (n = 4, $RT1^u$); closed circles = F344 (n = 4, $RT1^{lv1}$); closed squares = LEW (n = 4, $RT1^l$). Errors are ±1 SE. In some cases, error bars are smaller than the symbol.

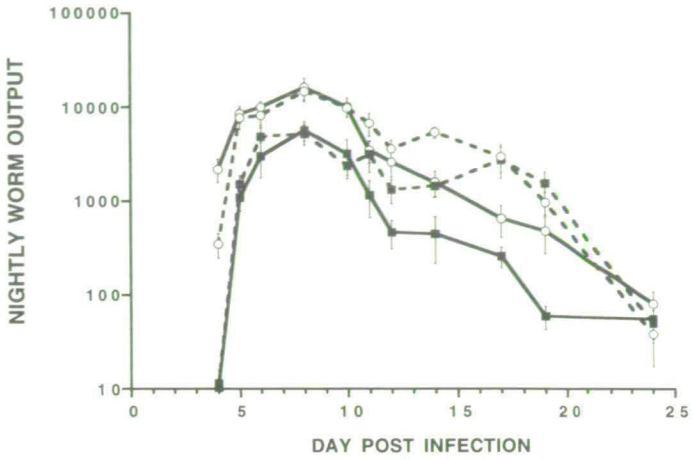

FIGURE 2. Nightly worm output from PVG and Wistar rats infected with 500 iL3 of the *Strongyloides ratti* lines ED5 or ED279. Open circles, broken line = Wistars infected with ED5 (n = 5); closed squares, broken line = Wistars infected with ED279 (n = 5); open circles, solid line = PVGs infected with ED5 (n = 6); closed squares, solid line = PVGs infected with ED279 (n = 6). Errors are ±1 SE. In some cases, error bars are smaller than the symbol.

(iL3) and the numbers of parasite offspring emerging from feces (nightly worm output) monitored throughout infection. Feces were collected onto damp paper overnight, cultured in glass petri dishes, and incubated for 2 days at 25 C or 3 days at 19 C. Cultures were then washed extensively in distilled water to collect worms into a known volume and the numbers present determined by counting worms in repeated samples under a binocular microscope. The positions of animals in the collecting apparatus, the order in which cultures were made and subsequently processed, as well as the positions of cultures in incubators were randomized anew at each sampling point. The total number of parasite offspring produced over the course of infection (total worm output) was estimated by numerical integration under the worm output by time curves. Data were analyzed by conventional analysis of variance (ANOVA).

Nightly worm outputs from the the first experiment are shown in Figure 1. Total worm output was highest in Wistar rats and lowest in F344 rats. One-way ANOVA using Bonferroni/Dunn multiple means comparison revealed that total worm output was higher in Wistar rats than in PVG and F344 rats (Wistar × PVG, $P = 0.049$; Wistar × F344, $P = 0.03$). The same comparison yielded P-values of 0.07 and 0.22 for the LOU and LEW strains, respectively.

In our second experiment, we examined patterns of larval production in Wistar and PVG rats with 2 lines of *S. ratti*. Nightly worm outputs from the 4 groups are shown in Figure 2. Two-way ANOVA on total worm ouput revealed the following. Total worm ouput was not significantly greater in Wistar rats than in PVG rats ($F_{1,18} = 3.1$, NS). Infection with ED279 led to lower total worm output regardless of rat strain ($F_{1,18} = 42.2$, $P < 0.0001$; worm line × rat strain interaction $F_{1,18} = 0.047$, NS).

At first sight, the results of the 2 experiments appear contradictory. In the first experiment, there was a significant difference in total worm output between PVG and Wistar rats infected with ED5. In the second, there was not. By analyzing the early and late parts of our second experiment separately, the reason for this inconsistency becomes clearer. Nightly worm output from rats infected with ED5 was highest in PVG rats prior to day 11 postinfection (PI) and highest in Wistar rats thereafter. Total worm output until and including day 10 PI did not differ significantly between rat strains ($F_{1,9} = 0.3$, NS), but total worm output from day 11 PI onward did ($F_{1,9} = 12.6$, $P < 0.01$). This difference is given undue weight in the first experiment. In the first experiment, only 2 time points were sampled prior to day 11 PI, the period when nightly worm output was at its highest. The difference in the timing of sampling leads to an underestimate of total worm output prior to day 11 PI and to the (false) conclusion that total worm output differs significantly between the PVG and Wistar strains. Furthermore, if only those sampling points common to both experiments are used to calculate total worm output in the second experiment, the effect of rat strain becomes significant for infections with both ED5 and ED279 ($F_{1,18} = 12.17$, $P < 0.01$; worm line × rat strain interaction $F_{1,18} = 0.51$, NS).

In the second experiment, there is also a difference in the pattern of worm ouput in the latter period. This is apparent as an interaction of rat strain × day PI in a repeated-measures ANOVA ($F_{5,45} = 2.55$, $P = 0.041$). Thus, from day 11 PI on, ED5 behaved differently in PVG and Wistar rats without significantly affecting total worm output across the experiment as a whole.

Our results illustrate 2 points. First, rat strain had no overall effect on total worm output. Second, rat strain did have subtle effects on the pattern of larval production. The observed magnitude of these effects depends crucially on when and how often infections are sampled. This observation is not surprising but is nevertheless important in quantitative studies of host–parasite interactions. Much work in this area relies on measurements taken at a fraction of possible sampling points (e.g., examine relevant figures in Wakelin and Blackwell [1988], chapters 4 and 5).

The fact that patterns of larval production by *S. ratti* differ between rat strains is a novel finding in this host–parasite sys-

tem. These differences may stem from divergent aspects of immunology and parasite reproductive strategies in different host strains. The overall difference in total worm output between ED5 and ED279 could be the result of environmental variance or it may have a genetic cause.

In summary, we report that genetic variation in the host (*R. norvegicus)* leads to subtle alteration in patterns of larval production by *S. ratti.* In addition, our results caution that experiments employing incomplete sampling regimes can fail to determine accurately the outcome of host–parasite interactions. Certainly, much remains to be clarified concerning the host–parasite relationship in the system studied here.

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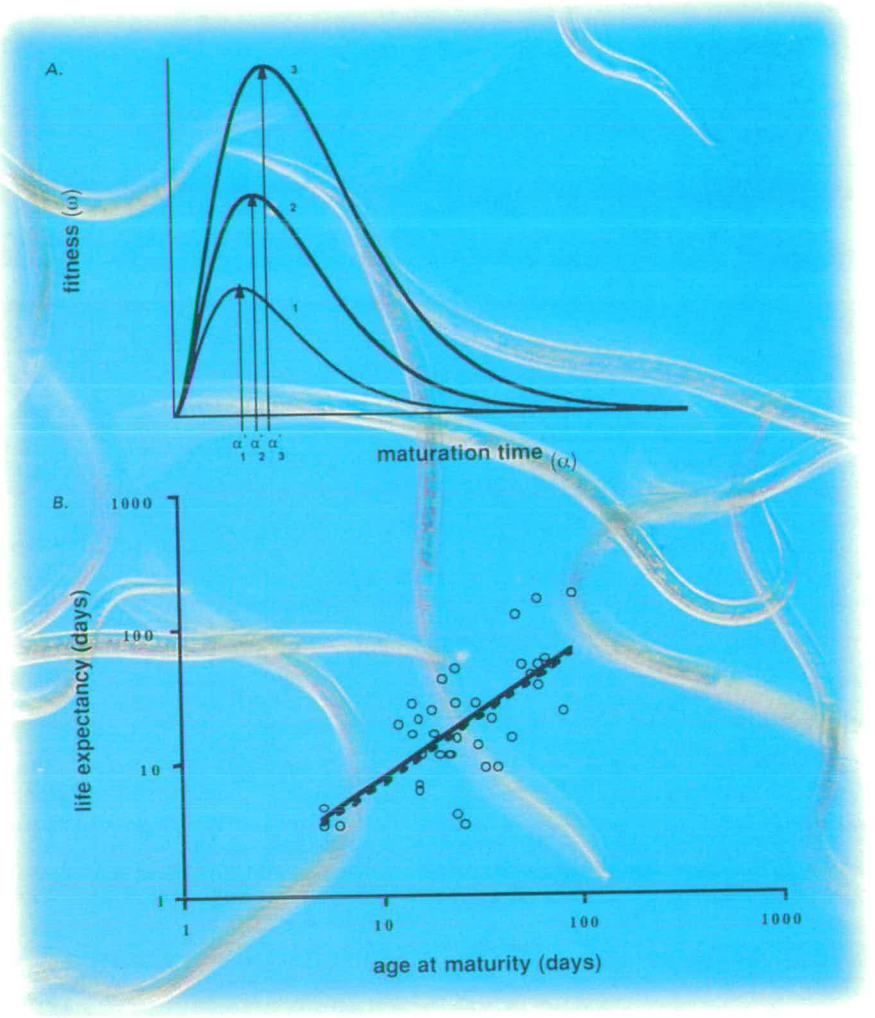
Elvis on worm size

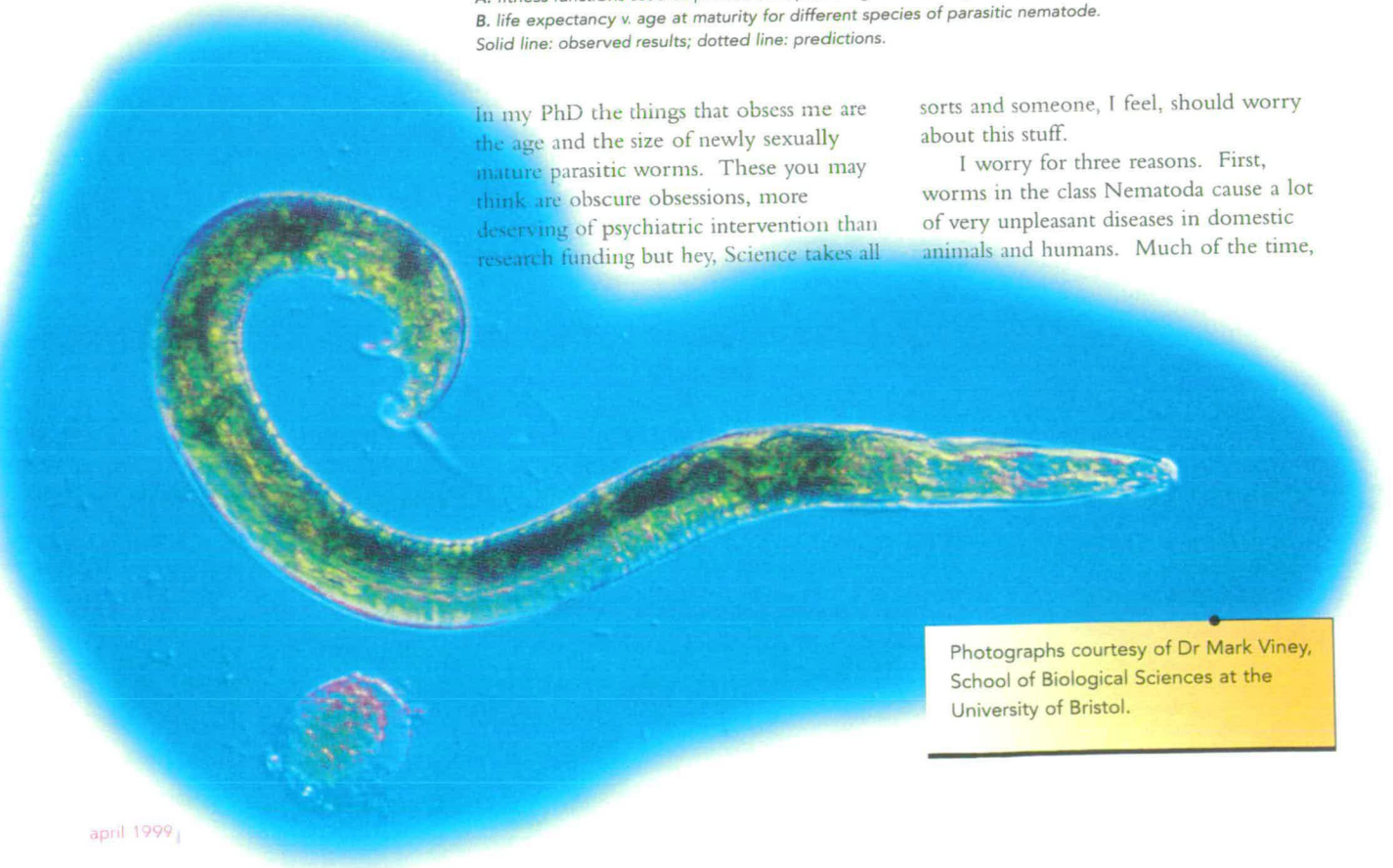
In an early screen role the young Elvis Presley snarled a defiant promise to "Live fast, die young and leave a good looking corpse". The real Elvis pursued a different course, living to middle age, attaining a famously impressive body size and leaving at least one surviving offspring. This is on my mind and Elvis is on the CD player as I goggle down a microscope, my counter tallying the reproductive success of the parasitic worm I work on.



A. fitness functions used to predict the optimal age of maturity in parasitic nematodes
B. life expectancy v. age at maturity for different species of parasitic nematode.
Solid line: observed results; dotted line: predictions.

In my PhD the things that obsess me are the age and the size of newly sexually mature parasitic worms. These you may think are obscure obsessions, more deserving of psychiatric intervention than research funding but hey, Science takes all sorts and someone, I feel, should worry about this stuff.

I worry for three reasons. First, worms in the class Nematoda cause a lot of very unpleasant diseases in domestic animals and humans. Much of the time,



Photographs courtesy of Dr Mark Viney, School of Biological Sciences at the University of Bristol.

This article by Alan Gemmill was his "highly commended" entry to the Wellcome Trust/New Scientist Millennial Science Essay Competition 1998. Reproduced here with permission.

the nastiest damage is caused by worms undergoing migratory growth phases through the tissues of their host prior to settling down as adult parasites. Big worms and long growth phases add up to more severe disease. Next, the age at which worms start reproducing determines how quickly epidemics spread. Third, and trickiest to phrase, my worms are behaving very strangely.

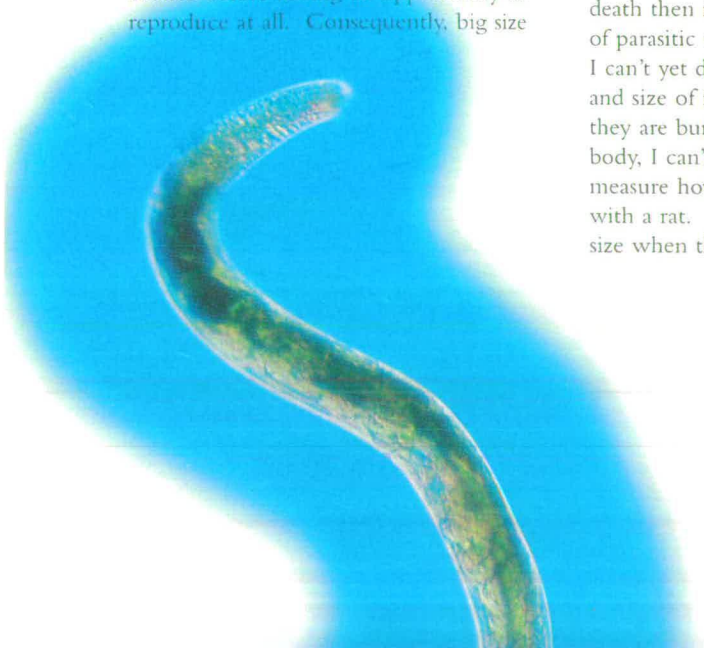
In the intestines of a normal rat they live and reproduce for a month, loading the rat's faeces with eggs. These scatter through the world in their thousands before the rat's immune system finally clears the infection. If on the other hand, I prevent the immune system from working, worms live for a year and leave very many more offspring.

The queer thing is, there is then no rush to begin the business of making babies. With the host immune system disabled and a clear run at reproduction, the worms take their time about it, sometimes beginning a day later, always producing fewer eggs at first. Basically, when I lower their risk of death, worms take longer to get going.

This seems strange. From the perspective of a worm though, it might just make a great deal of sense. In females generally, large size endows high reproductive success by allowing big mothers to produce bigger, better or more numerous offspring. Large size however also has its downsides. For a start, larger bodies take longer to build. Growing up to be large at adulthood usually means spending longer as a juvenile. This ups the danger of dying by chance before having an opportunity to reproduce at all. Consequently, big size pays off only when the prize of leaving more descendants outweighs the risk of dying unexpectedly.

Among nematode worms that live in the guts of mammals, longer lived species spend longer growing and are bigger, making more offspring than species with shorter lifespans. I can use this relationship to make a mathematical model of age and size at maturity in parasitic worms. I can show that the less effective the host immune system is at killing them, the longer worms should spend on growth before reproducing. I can show that stronger host immunity means worms would do well to live fast because, unlike Elvis, they will indeed die young.

If these worms are really capable of shifting their own schedule of life and death then it is possible that other species of parasitic worm are doing it too. What I can't yet do easily is measure the age and size of individual worms. Because they are buried away in another animal's body, I can't watch them grow up or measure how long it takes, as I could with a rat. Nor can I measure their body size when they first give birth.



What I can do is vary their chances of dying and record the occurrence of their offspring. That's why I'm goggling down the microscope, listening to Elvis and worrying about age and size in worms. Because I think it might be important in understanding and controlling disease. Because the idea that a microscopic creature might be capable of such a thing fills me with straightforward amazement. And yes, maybe just because I have a suspicious mind.

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