Studies on the Reproductive Performance of *Moniliformis moniliformis* (Acanthocephala)

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I hereby declare that apart from the acknowledgements made above, this dissertation describes research carried out by myself. It is from my own composition and has not, in whole or part, been presented for any other degree.

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CONTENTS

GENERAL SUMMARY

1. Aspects of the reproductive performance of the archiacanthocephalan endoparasitic helminth *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 have been investigated under experimental conditions using laboratory rats (*Rattus norvegicus*) as the definitive host.

2. Current knowledge of the reproductive biology of the Acanthocephala has been reviewed with special reference to the results published by other workers on *M.moniliformis*. In the context of this dissertation, reproductive performance is considered to be a convenient concept covering the environmental, behavioural and genetic factors that serve to enhance the reproductive fitness and success of individual worms.

3. An analysis of the course of *M.moniliformis* infections from mixed and single sex populations was undertaken. Female worms, on average, showed a greater rate of survival than male worms from mixed and single sex infections. Mated female worms were observed eventually to grow at a greater rate than unmated females of the same age. Rats harbouring primary infections with *M.moniliformis* did not show any evidence of acquired immunity when challenged with secondary infections.

4. In *M.moniliformis* insemination was observed to occur between male and female worms as young as 17-day-old, and an individual male worm was observed to be able to inseminate at least 22 female worms of the same age during the first 5 weeks of a primary infection. The results showed evidence that the copulatory caps on male worms are mounted by other male/s present in the population, which might result from sperm competition.

5. During the course of primary infections of *M.moniliformis* in rats, with an initial dose of 10 male and 10 female cystacanths, the fecundity of female worms appeared to be influenced by the male worm age. Female worms inseminated by younger male worms produced more eggs than the female worms inseminated by the male worms of same age.

6. In the experiments deliberately designed to expose male *M.moniliformis* to

the females of different ages and sizes, active mate choice by the males was considered to occur on the basis that male worms "choose" female mates having monitored the female age (where they switched mating with from older females to the younger ones), female size, in terms of body length, and female worm location in the small intestine. The results also indicated, for the first time as far is known, that male worms mature before the female worms of the same age.

7. The fecundity of female *M.moniliformis* appeared to be affected when cystacanths were exposed to various doses of X-irradiation prior to infection of rats. There are possibilities that either oogenesis in female worms had been affected by X-irradiation or the male worms might have been recognizing female worms fertility. Further research is, however, needed in this field.

8. Interactions between *Moniliformis moniliformis*, *Trichinella spiralis*, and *Nippostrongylus brasiliensis* in rats were investigated. The concurrent nematode infections appeared to affect the positions of attachment, growth and mating success of *M.moniliformis*. An earlier loss of *T.spiralis* and *N.brasiliensis* from the hosts was observed in concurrent infections with *M.moniliformis*.

9. Different anthelmintic drugs were used in trials against *M.moniliformis* infections in rats. Of the anthelmintics used levamisole was found to be the most effective against primary and challenged infections. The anthelmintic drug was found to have no long term effect on the parasite. Also, no effect on the fecundity of *M.moniliformis* of the anthelmintic drug was observed.

These results demonstrate that there are many factors that affect the mating behaviour of *M.moniliformis* in its natural host.

CHAPTER 1. ASPECTS OF REPRODUCTION IN ACANTHOCEPHALA : A REVIEW

1.1 INTRODUCTION

Acanthocephalans have been described in the literature as "hooked worms", since the late seventeenth century, but the existence of distinct genera and species was not recognised until late eighteenth century. Rudolphi (1802, 1809) named these worms as "Acanthocephala" (Greek: akantho= spiny; kephala= head). Studies on the systematics of the Acanthocephala have been evolving steadily since Hamann's studies laid a working foundation (1891,1892). Following a comprehensive review of known species by Meyer (1932, 1933), Van Cleave (1948), Hyman (1951) and Bullock (1969) have ensured that the Acanthocephala should be treated as a separate phylum consisting of three classes, Palaeacanthocephala, Archiacanthocephala and Eoacanthocephala. Until recently, a rough total of about 1150 species (Conway Morris and Crompton, 1982) for the phylum have been described. All known species are endoparasites which attain sexual maturity in the alimentary tract of terrestrial and aquatic vertebrates ranging from fish to man. Their life cycles, where known, have been found to involve an arthropod intermediate host.

Adult acanthocephalans are dioecious, usually white to cream in colour with a cylindrical or slightly flattened body. Worms of most species are not more than 10 mm long, but some measure up to 700 mm (Miller and Dunagan, 1985). Acanthocephalans have several prominent distinguishing characters. They possess a retractile proboscis with rows of recurved hooks, the patterns of which have considerable taxonomic significance, a muscular proboscis sheath, a pair of lemnisci, a typical body wall, no alimentary tract, a copulatory bursa in the male and a gonopore in the female. Functionally, the body is divided into two major regions, the praesoma and the trunk. The praesoma, which comprises the armed proboscis, proboscis receptacle, cerebral ganglion, lemnisci and various muscles, and the unarmed neck is primarily responsible for the attachment of the worm to the intestinal mucosa. Nutrients from the lumen of the host gut are absorbed across the syncytial body wall, the surface area of which is enormously expanded by an

extensive array of tegumentary pore canals. The male and female acanthocephalans possess a highly characteristic reproductive organs which are suspended in the body cavity. The body wall of the trunk encloses the pseudocoele in which the ligament sacs, sex organs, excretory organs, and genital ganglia in the male, are present.

The acanthocephalan body contains two separate hydraulic systems which can act independently of one another. The first is the fluid- filled cavity of the proboscis and proboscis receptacle and the second, body cavity of the trunk. These two separate systems permit trunk movements without loosing the hold of the proboscis on the intestinal wall.

In this chapter, aspects of acanthocephalan reproduction will be discussed and features of acanthocephalan reproduction will be compared briefly, in general terms, with the features of reproduction in other helminth groups including Monogenea, Digenea, Cestoda and Nematoda. The aspects of the biology of *Moniliformis moniliformis* will also be discussed in some detail.

1.2 REPRODUCTION IN ACANTHOCEPHALA

Parshad and Crompton (1981), have reviewed aspects of acanthocephalan reproduction in great detail. Reproduction according to Cohen (1977), is more than multiplication or breeding and occurs when a population of parents is replaced by another parent population. This view draws attention to ideas about germ plasm and soma and implies that reproduction depends not only on the properties of nucleic acids and behaviour of gametes, but also on numerous somatic and environmental factors. Acanthocephalan reproduction depends on heterosexuality followed by the active transfer of the male gamete by the male to the female. Hermaphroditism, parthenogenesis and any other form of asexual reproduction are unknown in Acanthocephala (Van Cleave, 1953). Acanthocephalans appear to be polygamous (Jewell, 1976) and following gametogenesis, copulation, insemination and internal fertilization occur while the worms are inhabiting the vertebrate alimentary tract. A most intriguing feature in reproductive activity is the population of free-floating ovaries (Crompton and Whitfield, 1974; Atkinson and Byram, 1976; Parshad and

Crompton, 1981) from which the zygotes are released into the body cavity after fertilization.

1.2.1 SEXUAL DIMORPHISM

In their review of acanthocephalan reproduction, Parshad and Crompton (1981) described differences in various external features which exist between the sexes of adult acanthocephalan worms in their definitive hosts. The difference in body shape and size, the distribution of body spines, the size, shape and number of proboscis hooks, the occurrence of papillae and the position of genital openings are frequently observed in acanthocephalan worms (Van Cleave, 1920; Ward and Nelson, 1967; Yamaguti, 1963). The most obvious difference between the sexes is that of body size; with the females usually being larger than the males. *Moniliformis moniliformis*, *Mediorhynchus grandis* (Archiacanthocephala) and *Hexaspiron nigericum* (Eoacanthocephala) are three species in which mature females are known to be five times as long as the males (Yamaguti, 1963). The Palaeacanthocephalans *Echinorhynchus lageniformis* (see Olson and Pratt, 1971) and *Corynosoma hamanni* (see Holloway and Nickol, 1970), are exceptional in that the males have been observed to be bigger than the females. Variations in the body sizes of some acanthocephalan species are known to be influenced by the age of the worms (Crompton, 1972), their reproductive state (Crompton, 1974), their population structure (Graff and Allen, 1963, Nesheim *et al*, 1978), host species and distribution (Bullock, 1962; Amin, 1975b; Buckner and Nickol, 1979), host sex (Graff and Allen, 1963), host diet (Nesheim *et al*, 1977, 1978; Parshad *et al*, 1980, Crompton and Keymer *et al*, 1988) and host environment (Walkey, 1967).

Experimental studies on *Moniliformis moniliformis* have shown that both male and female worms grow at a greater rate in male than female rats (Crompton, 1972) and that the males grow bigger in absence of female worms (Graff and Allen, 1963). Crompton (1972) observed that until a time which probably coincides with the onset of copulation, male and female *M.moniliformis* grew at similar rate and contained about the same amount of protein. After the time of copulation, the female worms

grew more than the males regardless of whether the insemination has occurred or not (Crompton, 1974). Similar results have been show for *Polymorphus minutus* by Crompton and Whitfield (1968). Van Cleave (1920) assumed that somatic differences between the sexes might be involved in sexual selection. It is difficult, however, to relate the differences in body size to reproductive behaviour and successful copulation in the Acanthocephala. Morphological differences also occur at the posterior ends of individuals of the same species; these may facilitate sexual recognition and copulation in the Acanthocephala. According to Van Cleave (1940), genital spines of female *Gorgorhynchus clavatus* become embedded in the bursal tissue and so strengthen the copulatory union. At present, however, it remains unresolved as to whether acanthocephalans produce or detect chemical factors which may be involved in sex recognition (Bone, 1976)

1.2.2 SEX DETERMINATION AND SEX RATIO

The sex of acanthocephalan worms, like that of many other dioecious organisms (Sinnott *et al*, 1958), is established during fertilization by a process involving sex chromosomes. Two types of sex determination have been identified in which males are heterogametic (XO or XY) and the females are homogametic (XX). Mechanisms of sex determination which depend on one heterogametic and one homogametic parent are supposed to give rise to a sex ratio of 1:1 at the time of fertilization unless one of the two types of gamete from the heterogametic sex is in some unusual way more favoured or more active during fertilization (Parshad and Crompton, 1981). Observations on natural and experimental worm populations of various species of Acanthocephala generally reveal either a sex ratio of 1:1 or a ratio in which female worms predominate. Females, however, live longer than males (Crompton, 1970). The observed sex ratio reflects the fact that male and female acanthocephalans develop in equal numbers so that cystacanths occur with a sex ratio of 1:1 in the intermediate hosts (Muzzall and Rabalias, 1975; Brattey, 1980) and young worms similarly in the definitive hosts.

1.2.3 DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Acanthocephalan reproductive organs have long been of interest to taxonomists. At present our knowledge of acanthocephalan reproduction and of the functional morphology of the reproductive system of various species is very limited. Some of the aspects of the development of acanthocephalan reproductive system will be discussed here.

The development of all the organs of an acanthocephalan worm takes place in the body of the intermediate host (Van Cleave, 1953; Nicholas, 1967; Crompton, 1970). Organogenesis of some acanthocephalan species in their intermediate hosts has been described by Awachie (1966), King and Robinson (1967), Cable and Dill (1967) and Robinson and Jones (1971).

Upon ingestion by a susceptible intermediate host, the acanthor larvae emerges from the egg, penetrates through the gut wall, enters the body cavity and undergoes a series of developmental changes (acanthellae) to give rise to a juvenile or cystacanth stage which is infective to the definitive host (Plate 1.1). The rate of development, however, varies between species and is related to the ambient temperature.

Parshad and Crompton (1981) have tabulated the approximate timing (days) of when the genital primordia give rise to genital rudiments. The sexes can be identified in the developing acanthella stages. Hynes and Nicholas (1957) described the development of *Polymorphus minutus* in its *Gammarus* intermediate host. The sexes become distinguishable at about day 35, when the testes appear as two dense masses of cells. Development of *M.moniliformis* in its cockroach intermediate host has been described by Moore (1946), King and Robinson (1967) and Lackie (1972). Two acanthor stages, 6 acanthella stages and finally a cystacanth stage for *M.moniliformis* have been identified by these authors. Depending on the environmental temperature at which the intermediate host is, development of *M.moniliformis* from pre-acanthella to cystacanth stage takes about 8 weeks (27^0C). On approximately the 22 nd day after the escape of the acanthor from the egg, the first rudiments of the internal organs are seen. Thereafter, the pre-acanthella stage

begins which is characterised by the process of organ formation. By day 29, at the posterior end of the body cavity there appears a solid aggregation of nuclei from which the genital primordia arise. By the end of 38 days of development, the genital ligament extending from the posterior end of the larva to the base of the proboscis receptacle is evident. The primordia of the genital organs are more distinct at this stage and the sexes are distinguishable. In male specimens the immature testes can be seen arranged in tandem in the posterior region of the body cavity and the primordia of the rest of the male genital structures located in the posterior tip of the body cavity. From 44th to 51st day of development, the pre-acanthella begins to change into acanthella. Development takes place rapidly at this point and in a few days the cystacanth or the infective stage is reached.

In most acanthocephalan species, the male and female reproductive systems develop along comparatively similar lines. It appears, however, that the development of acanthocephalan testes begins somewhat before that of ovaries. In *Prosthorhynchus formosus*, the genital primordia of the female are visible in a 30-day-old acanthella and ovaries can clearly be seen in the cystacanth (Schmidt and Olson, 1964). The ovaries are one of the most interesting features of female acanthocephalan worms. The relatively large number of ovaries in the fluid of the body cavity of females is assumed to originate from the ovarian tissue. Van Cleave (1953) considered that the ovary becomes fragmented to form free-floating ovaries. The occurrence of ovarian fragmentation and the presence of ovaries in the body cavities of female cystacanths of various species of Acanthocephala in their intermediate hosts is described by many workers.

There are several reports in the literature which suggest that the ovarian rudiment in *M.moniliformis* breaks up to form immature ovaries while the development is still in progress in the intermediate host. Moore (1946), studied the development of *M. moniliformis* in its intermediate host and observed that by the end of 38th day of development, the ovaries in the female larvae develop from cells contained in the genital ligament. He further stated that in female acanthella,

"No compact ovary is present, but small masses of cells, which are immature egg balls, may be seen free in the body cavity or in the genital ligament." This observation has been confirmed by Asaolu (1976). In contrast, Atkinson and Byram (1976), have reported that seven to nine days after the infection of the definitive rat host, the genital primordium of female *M.moniliformis* is transformed from fragmented mass of cells into discrete ovarian balls. On the basis of the observations made by Moore and others, it seems unlikely that the ovarian rudiment would still be present in *M.moniliformis* which had been developing in the definitive host for seven days. Further more, the establishment of single-sex infections of *M.moniliformis* in rats using surgical techniques by Crompton (1974) and Crompton *et al* (1976) depended on recognising the presence of either testes or ovaries in 2 to 6-day-old worms from donor rats. During the present study these observations have confirmed those described by these workers; eight immature ovaries in 1-week-old female *M.moniliformis* were counted by direct observation of intact worms (Plate 2.1). Also, it was possible to recognise the sex of *M.moniliformis* cystacanth as male or female prior to infection of the definitive (rat) host (see Chapter 2 section 2.5).

1.2.4 FUNCTIONAL MORPHOLOGY IN THE DEFINITIVE HOST

Once acanthocephalan worms have become established in their definitive hosts, further differentiation and maturation occur in the gonads and also the growth and normal functioning of various organs and structures starts in the somatic part of the tract.

The reproductive tract of male acanthocephalans, as in females, is located in the body cavity in association with the ligament sacs. The tract of the male consists of a pair of testes and associated ducts, a series of cement glands, Saefftigen's pouch, pyriform glands, penis and copulatory apparatus. In some species, the reproductive tract extends for most of the length of the body, whereas in others it appears to be concentrated near the posterior end. The reproductive organs are usually arranged with the testes being located anterior to the cement glands which in turn are anterior to the copulatory bursa.

The cement glands are of much taxonomic interest in acanthocephalan reproductive systems. Van Cleave (1949) investigated the morphology and phylogenetic significance of the cement glands and recognised three general types. These are the eoacanthocephalan type which consists of a single syncytial gland and a reservoir, the archiacanthocephalan type in which eight uninucleate discrete glands are usually present, and palaeacanthocephalan type (Fig.1.1) in which the number of glands may vary from two to eight and in each gland the nuclear fragments are found. As Parshad and Crompton (1981) pointed out, there are exceptions which Van Cleaves's descriptions do not cover. For example, there is structural continuity between the four cement glands of *Polymorphus minutus* (Whitfield, 1969). Also cement glands vary frequently in number as seen in *Acanthocephalus dirus* where the range is from none to 12 (Amin, 1975).

It is evident that the cement glands and their products have considerable significance in the reproductive process and at the same time they are important elements in the morphology as well as the taxonomy of all acanthocephalans. The secretion from the cement glands functions in cementing the bodies of the two sexes together during copulation and is believed to contribute to the copulation caps which are often observed around the posterior end of the females (Van Cleave, 1949) and some times on the males (Abele and Gilchrist, 1977) (Plate 1.2. a-b). The cement gland secretion is reported in literature to contain protein. The cement glands and the material in the cement ducts stain deep blue-black with iron haematoxylin (Parshad and Crompton, 1981). The presence of copulatory caps on the gonopore of the females is a useful indicator to the observer that copulation and probably insemination have occurred (sometimes the female worms with copulatory caps were observed to be uninseminated, see Chapter 4). The significance of the copulatory caps is not straightforward. Several worker have suggested that the copulatory caps on female worms prevent (i) the loss of spermatozoa from the female (ii) the females from subsequent inseminations and (iii) provide advantages for the genes of the individual male that secreted the cement by assuring that at least some of the spermatozoa would encounter mature oocytes without competition

from spermatozoa from other males. As has been described by Van Cleave (1949), the caps become hardened around the genital spines of the females of the genus *Corynosoma*, which may prevent further insemination for a time, but at the same time the egg release may also be delayed. An interesting observation by Fisher (1960), who found both eggs and spermatozoa in the chamber of the copulatory cap on female *N.emyditoides*, suggests that some times the cap may possibly serve as a storage vessel for spermatozoa.

Some observers have described the appearance of copulatory caps over the posterior ends of the male worms. Abele and Gilchrist (1977) interpreted the capping of male *M.moniliformis* in terms of sexual selection (or homosexual rape) which would effectively and temporarily remove some of the males from the reproductive population. The caps on the males could result from poor sex recognition, but the evidence that only cement and not the spermatozoa were transferred to the male *M.moniliformis* clearly suggests that male *M.moniliformis* can distinguish between the sexes. Experimental results on single and mixed sexes of *M.moniliformis* in rats during the present study show an increase (not significant) in the number of capped males from mixed sex populations suggesting that sex discrimination applies to *M.moniliformis* (see Chapter 4).

In addition to the cement glands, a pair of glands is observed which Whitfield (1969) described and named as pyriform glands in *P.minutus*. Asaolu (1977) has observed a somewhat similar pair of glands in association of the cement glands of *M.moniliformis*. Dunagan and Miller (1973) found two types of gland-like cells located at the margin of the bursa of *F.fessus*, and suggested that these cells, located in a convenient position, may be releasing some catalyst involved in cap formation.

The role of the male in copulation is achieved by means of bursa which becomes fully extruded before the female can be grasped and spermatozoa transferred. The bursa is equipped for its role with complex musculature which has been described for *M.moniliformis* by Dunagan and Miller (1978). Together with complex bursal musculature, the bursal ganglion has also been described from male

M.moniliformis and *Macracanthorhynchus hirudinaceus*. This more extensive nervous system of the male Acanthocephala and their possession of greater neural capacity suggests that they may be more active during sexual congress than the females.

The female reproductive system appears to be unique in the Animal Kingdom. The efferent duct system consists of a uterine bell, uterus and vagina, while the ovarian tissue is organised into hundreds of ovaries which are contained in the fluid of the body cavity, floating freely or loosely constrained in the ligament sacs (Bullock, 1969; Crompton and Whitfield, 1974). The efferent duct of the female acanthocephalan provides a route for the entry of spermatozoa and a means for the discharge of mature eggs (= infective shelled acanthors) from the body cavity into the lumen of the definitive host's alimentary tract. The uterine bell is a complex organ and differences exist in the literature for the organisation of the bell structure and its function (Yamaguti and Miyata, 1942; Whitfield, 1968; Asaolu, 1980). Since mainly mature eggs are found in the infected host's faeces, some form of egg-sorting function is assumed to occur inside the acanthocephalan body and the uterine bell is considered to be involved. Whitfield (1968) studied the histology and anatomy of the uterine bell of *P.minutus*. By estimating the population structure of the eggs from the body cavity and the uterus, in terms of stage of embryonic development, he observed that no eggs entered the uterus until about the time that the worms were beginning to release eggs and that all the eggs in the uterus were fully developed. He confirmed this observation by the use of an *in vitro* preparation of the efferent duct of *P.minutus*, and concluded that this egg-sorting function of the bell depends on the bell distinguishing between eggs of different sizes. The efferent duct system of *F.fessus* and *M.moniliformis* examined by Dunagan and Miller (1973) and Asaolu (1980) respectively, also favour the function of egg-sorting for the uterine bell.

1.2.5 COPULATION AND INSEMINATION

The association of males and females for reproductive purposes (Cohen, 1977) is difficult to observe in the case of Acanthocephala. Information about mating

behaviour in the *M.moniliformis* may be deduced from the results of experimental infections in laboratory rats (Burlingame and Chandler, 1941; Holmes, 1961; Crompton *et al.*, 1972; Crompton, 1974, 1975; Atkinson and Byram, 1976; Abele and Gilchrist, 1977; Nesheim *et al.*, 1977, 1978; Parshad *et al.*, 1980). The distribution of the parasites in the definitive host's gut plays an important role in sexual congress of the worms. The anterior emigration of *M.moniliformis* from the posterior part of the host's alimentary tract have been described for various species of Acanthocephala by Crompton (1973). The anterior emigration and sexual development of the parasites is dependent on the composition of the host's diet (Nesheim *et al.*, 1977; Parshad *et al.*, 1980). Studies have shown (Crompton, 1975) that in well-nourished rats, when the worms are aged 4-weeks, during their occupation of the anterior part of the intestine, each female worm on average is in contact with the maximum number of males and that the highest proportion of females are experiencing contact with at least one male worm. At this period of infection, when the sex ratio is 1:1 (Muzzall and Rabalias, 1975) and when fertilization can be shown to have begun (Crompton, 1974; Atkinson and Byram, 1976) copulation would be expected to be occurring frequently.

The duration of the association between male and female worm appears to be more important rather than mere association of the two in acanthocephalan reproduction. The experimental studies on *M.moniliformis* by Crompton (1974), have revealed that the average patent period, which is 106 $\pm$ 16 days (Crompton *et al.*, 1972) is not achieved unless the males and females have been together for at least 5 weeks after becoming established in rats. This period has been suggested by Crompton (1985) to be necessary for the worms to have maximum physical contact with each other and to allow females to acquire spermatozoa from males for the fertilization of the average production of mature oocytes during the lifespan.

Little is known about the insemination in Acanthocephala. In most animals, spermatozoa are transferred to the female in semen (Cohen, 1977). It is presumed, however, that acanthocephalan spermatozoa are also transferred in some form of fluid. Atkinson and Byram (1976) have suggested the presence of spermatophores in

the body cavity of male *M.moniliformis* but no obvious organ for the production of spermatophores in male *M.moniliformis* has been described.

1.3 REPRODUCTION IN OTHER HELMINTH GROUPS

1.3.1 TREMATODA

1.3.1.1 GENERAL FEATURES

Platyhelminths, flukes and tapeworms, are an interesting group of parasites for the great variety and complexity of their organisation, especially of the reproductive system. In addition, parasitic helminths, including acanthocephalans and nematodes exhibit different strategies of reproduction owing to the variabilities of their life histories (see Table.1.1). Most species of digenean fluke are endoparasites and most are hermaphrodites, in which the male organs mature before those of the female. Many have complex life cycles which involve stages that reproduce asexually in one host and sexually in another. Asexual reproduction is typical of all digeneans and all strobilate cestodes. The asexual phase of the digenean life cycle occurs in the molluscan intermediate host (usually a snail) and involves sporocyst and redia stages. As many as six recognizably different body forms may develop during the life cycle of a single species of a digenean trematode. The basic pattern of the digenean life history, with its implications for reproduction, is as follows.

Adult ------ egg ---- miracidium ----- sporocyst ----

redia ----- cercaria ------- metacercaria

Some variations on this basic pattern are shown in Fig.1.2.

Since most digenean flukes are hermaphrodites, cross- and self- fertilization are known to take place. The members of Schistosomatidae are exceptional in being dioecious and in having only one intermediate host. The adult worms mature in the blood vascular system of their definitive hosts. *Schistosoma hematobium, Schistosoma mansoni* and *Schistosoma japonicum* are the most commonly known parasites of humans. The female worm is often found in the gynecophoral canal of the male worm, where copulation takes place (Schmidt and Roberts, 1981). The

female worm may leave the gynecophoral canal to reach small venules to deposit eggs. The mechanism of expulsion of the eggs from the host is less clear, however, about two thirds of the eggs are never expelled from the host but are swept away by the blood from the venules, eventually to lodge in the viscerae. Apart from their having separate sexes, there are no obvious similarities between schistosomes and acanthocephalans.

Monogenean flukes are mostly ectoparasitic on the gills and skin of fishes. Although they possess a common opening for the male and female reproductive systems and are hermaphrodites, no asexual reproduction has been described. Cross-fertilization between separate individuals is usual but self-fertilization occasionally occurs. Most adult monogeneans produce eggs which hatch in water releasing oncomiracidia. Species of Gyrodactylidae are unusual in their method of reproduction by being viviparous. There can be up to 3 generations of embryos, in various stages of development, within a single fluke (Scott, 1982). Egg production in monogeneans may be prolific, for example, *Polystoma integerrium* lays upto 2500 eggs (see Chappell, 1980).

1.3.1.2 COPULATION AND INSEMINATION

Very little is known about the manner in which cross- inseminating hermaphrodites find their partners. The monogenean fluke *Diplozoon paradoxum* becomes permanently attached to its partner. The digenean *Paragonimus kellicotti* usually lives with a hermaphroditic partner in a cyst in the lungs of cats. Experimental infections of cats with single *P.kellicotti* has shown no egg production, however, eggs were produced when cats were infected with two flukes. Copulation has rarely been observed, but flukes appear to adopt particular positions and sperm are assumed to be transferred by means of cirrus or other intromittent apparatus. In some flukes cross- insemination appears to be the usual practice, but, self-insemination may occur in the absence of other partners and occasionally in the presence of partners. The monogenean fluke *Diplectanum aequans*, which lives on the gills of bass, is interesting and unusual in that it is a species in which the sperm

are transferred by means of a spermatophore.

1.3.1.3 FERTILIZATION

After insemination of the females, fertilization in parasitic flatworms is believed to occur in the part of the reproductive tract known as the ootype. The membranes of the sperm and egg fuse to become one. The eggs of digenean flukes can develop parthenogenetically without the intervention of sperm, but the miracidia of this origin have been found to be less infective to the intermediate hosts than the miracidia from fertile eggs.

1.3.2 CESTODA

1.3.2.1 GENERAL FEATURES

Virtually all adult cestodes are intestinal parasites of vertebrates, and are almost always hermaphrodites. The adults usually release proglottids containing eggs, that are passed to the exterior in the host's faeces. The egg, however, is not an ovum but contains a fertilized oocyte or even a fully developed larva.

1.3.2.2 COPULATION AND INSEMINATION

Information on various aspects of cestode reproductive biology is limited to only a few families. Tapeworms contain reproductive systems of both sexes in a single proglottid (see Davis and Roberts, 1983). Various theories have been advanced to explain how insemination is carried out by tapeworms. Insemination may occur either by direct transfer of sperm via the cirrus into the vagina or indirectly by hypodermic injection of sperm via the cirrus through the tegument of a proglottid. The sperm then may find their way into the female reproductive system. Insemination usually occurs while the proglottids are still attached to the strobila. Indirect evidence for the occurrence of self-insemination has been shown from the production of viable eggs in single worm infections with *Hymenolepis microstoma* (Jones *et al.*, 1971) and from cultivation *in vitro* in isolation (Roberts and Mong, 1969; Berntzen and Mueller, 1972). Nollen (1975), using radioactive labelling of spermatozoa, found evidence for self-insemination in *Hymenolepis diminuta*. Cross-

insemination has been observed in cyclophyllideans and tetraphyllideans. Nollen's (1975) labelling studies provide evidence that there is transfer of sperm. It should be noted, however, that insemination or transfer of sperm does not mean that fertilization of the oocytes necessarily follows.

1.3.2.3 FERTILIZATION

During fertilization, in most species studied, sperm surround and penetrate the primary oocyte. In *H.diminuta*, the mode of penetration has been described as involving a lateral fusion of sperm and oocyte plasma membranes (Swiderski, 1976).

1.3.3 NEMATODA

1.3.3.1 GENERAL FEATURES

Nematodes, which parasitise animals, are dioecious organisms in which sexes show well defined differences in morphological and anatomical characters. Males are usually smaller than females and are equipped with various copulatory organs such as bursae, papillae, and spicules.

Both hermaphroditism and parthenogenesis have been shown to occur in plant parasitic and free-living species. Animal parasitic species, however, reproduce sexually and cross-fertilization takes place. Mating activities are rarely observed in parasitic nematodes. The common nematode parasite of rats, *Nippostrongylus brasiliensis*, has never been observed *in copula*, in laboratory investigations (Weinstein and Jones, 1959; Phillipson, 1969).

1.3.3.2 COPULATION AND INSEMINATION

In nematodes, the phenomena of sexual attraction and copulatory behaviour of adult worms are of much interest. Attractants, usually released by the females, play an important role in bringing the sexes together (Greet, 1964; Chin and Taylor, 1969; Cheng and Samoiloff, 1972). Copulation involves the use of spicules, caudal papillae associated with the bursa and the genital cone. Initial contact between the sexes involves a searching behaviour on the part of the male. Sudden coiling of

male's tail around the female is common and has been described for various species. The male worm may brush the ventral surface by moving the tail backwards and forwards against the female and if cloaca and gonopore coincide the spicules enter the gonopore and the male ceases to move (Fisher, 1972). Presumably location of the gonopore and correct alignment of the worms involve both the sensory papillae on the tail of the male and the spicules which are also sensory structures (Somers *et al.*, 1977; Lee, 1973).

After copulation the females of many species have been found to have a copulatory plug in the gonopore (Somers *et al.*, 1977). The substance of this plug has been described as 'cement' (Chitwood, 1930; Fisher, 1972) and is believed to come from the males.

The duration of copulation varies between species as well as between individuals of the same species (Duggal, 1978). In some species the number of spermatozoa passed to the female is very small and in others it is greater. In *Nippostrongylus brasiliensis* it has been described that between 1,000 to 1,500 spermatozoa reach the uterus at each insemination (Phillipson, 1969). Probably all spermatozoa are used specially in the species in which there are few spermatozoa (Ward and Carrell, 1979), but not all eggs are fertilized.

Frequency of copulation in nematodes has been described to vary with the age of the male (Somers *et al.*, 1977). Phillipson (1969, 1970, 1973) suggested that potential production of spermatozoa exceeds the number of fertile eggs actually produced by about 15 times in *N.brasiliensis*.

1.3.3.3 FERTILIZATION

The nematode egg is usually a fully-grown oocyte at the time of fertilization (see Foor, 1970). Fertilization in nematodes, as in other groups of helminths, occurs by the fusion of the plasma membranes of spermatozoa and oocyte.

1.3.3.4 SEX DETERMINATION AND SEX RATIO

In most dioecious organisms, the mechanisms underlying sex determination and differentiation are well known. The genetic material of the zygote is assumed to

possess two sets of genes, each of which promotes the differentiation of the developing organism into either male or female. Sex determination in most animal parasitic species of nematodes studied so far appears to be of the XX female - XO male type and the operation of the genetic mechanism usually ensures 1:1 sex ratio in the population (Anya, 1976).

1.4 ASPECTS OF BIOLOGY OF *MONILIFORMIS MONILIFORMIS*

The life history and development of *M.moniliformis* in the rat *Rattus norvegicus*, which is known to be a natural definitive host (Holland, 1983) and in the intermediate host, *Periplaneta americana*, has been described by Moore (1946) and development in the cockroach further described by King and Robinson (1967) and Lackie (1972). Shelled acanthors are released by the adult female worms which live in the small intestine of the rat, and are infective to the cockroaches. After ingestion by the cockroach, hatching occurs in the lower midgut of the insect where the larvae burrow through the intestinal tissue and come to lie in the haemocoel. Further development occurs here leading to the cystacanth stage which is then infective to the rat (Plate 1.1). Development of *M.moniliformis* in the cockroach appears to be temperature dependent (Lackie, 1972). After ingestion by the rat host, the parasite attaches itself to the lining of the gut with the aid of the hooked proboscis, grows to sexual maturity and copulates in the small intestine of the host (Crompton, 1972).

Moniliformis moniliformis feeds by absorbing nutrients across its body surface from the anterior of the rat small intestine (Crompton, 1973; Edmonds, 1965). Crompton (1973), described that adult acanthocephalans (*Moniliformis*) are confined to the regions of their host's alimentary tracts which are specialised for the absorption of the nutrients and that acanthocephalans appear to emigrate from one part of the intestine to another during the establishment of the adults. As larvae, *M.moniliformis* begin to grow in the posterior part of the intestine and migrate forward. Female worms appear to migrate first and become sexually mature during the process; males move later and in general are more mobile than the females

which move only occasionally. The concept that developmental migration is a response to intestinal gradients is supported by experimental evidence which suggests that emigration is positive response to physiological conditions correlated with glucose concentration and other signals associated with host feeding (Mettrick & Podesta, 1974).

Moniliformis moniliformis has an age-dependent survival rate with a maen expected lifespan of 8.2 weeks and a maximum lifespan of about 22 weeks in rats given a primary infection of 12 cystacanths. Under laboratory conditions, adult female worms can grow up to 150- 200 mm., and large males tend to be 70-80 mm in length. Female worms, from both male and female rats, have been found on average, to survive longer than males and male rats are found to support, on average, more worms of both sexes than female rats (Crompton and Walters, 1972). Miremad-Gassmann (1981), considered that male *M.moniliformis* lived longer than females in male rats with an expulsion of female worms beginning in the 4th week of a primary infection of 30 cystacanths per rat. Density-dependent mechanisms, are considered to be of central importance in stabilizing the population growth of parasitic organisms (Anderson and May, 1978). There is some evidence to indicate that the establishment and survival of *M.moniliformis* may be regulated in a density-dependent manner. Recently, Crompton *et al.*, (1988a), investigating the reproductive performance of *M.moniliformis* found that, survival of worms was severely density-dependent when rats were infected with doses from 10 to 80 cystacanths.

Egg release under laboratory conditions, is first detected when *M.moniliformis* are approximately 38 day-old and ends on average when they are 144 day-old. Considerable daily variation in egg production occurs from the worms in a given rat and in the numbers released by similar infections in other rats. On average, a female *M.moniliformis* releases about 5500 eggs per day and about 600,000 eggs during an average patent period (Crompton *et al*, 1972).

1.5 FACTORS AFFECTING FECUNDITY IN *MONILIFORMIS MONILIFORMIS*

The number of infective eggs produced by a female worm is a measure of fecundity of the species which Cohen (1977) defines as the actual number of offspring produced. Thus fecundity depends directly upon the number of viable oocytes which are formed in the females and on the successful transfer of sufficient quantity of competent spermatozoa from the males to the females. It is to be expected that these events may be affected by many environmental factors including the nutrition and digestive physiology of the host, the immune responses of the host, competition between the worms in the population present and interspecific interactions (Crompton, 1974). The longevity, growth and sexual development of *M.moniliformis* have been shown to be highly sensitive to the quality and quantity of carbohydrate consumed by the host (Crompton, 1987) and, since a rat's food intake is expected to be related to its energy needs (Morgenson and Calaresu, 1978), it follows that competition for carbohydrate will be likely to occur between worms in a rat as the number of worms is increased. Competition between worms for a limited resource is one possible explanation for the density-dependent effects on fecundity that have been demonstrated (Anderson and May, 1978; Keymer, 1982). Effects of host's dietary composition on fecundity of *M.moniliformis* have been investigated by many workers. Recently, Crompton *et al.*, (1988a) described that with an initial dose of 10 cystacanths per rat, dietary fructose concentrations of 3 and 6% (w/w) supported better reproductive performance by the parasite than concentrations of 1 and 12%. On the other hand, density-dependent effects showed declined reproductive performance as parasite dose increased from 10 to 80 cystacanths per rat. Some degree of delay in the attainment of sexual maturity, growth, reduction in average survival time of the worms and some delay in time of production of mature eggs was observed.

1.6 OVERVIEW

This review of the literature has demonstrated that a reasonably detailed knowledge exists about factors affecting the course of infection and fecundity of

M.moniliformis in rats. It has also indicated the need for further research in this field. The experimental work described in this dissertation was carried out to extend knowledge of the reproductive performance of *M.moniliformis*. In this context reproductive performance is a convenient if arbitrary concept covering the environmental, behavioural and genetic factors that serve to enhance the reproductive fitness and success of individual worms. Much attention has been paid to experiments designed to investigate female fecundity, male and female mating behaviour and the possibility of mate choice. The more efficient an individual *M.moniliformis* is within these categories of events, the greater will be its reproductive performance.

Plate 1.1 Photographs of the stages of *Moniliformis moniliformis* in the intermediate host *Periplaneta americana.*

a. Mature egg containing acanthor.
b. Acanthor in process of escaping from egg shells.
c. Acanthor from the gut wall tissue of the cockroach.
d. Acanthella stage.
e. A fully developed cystacanth.

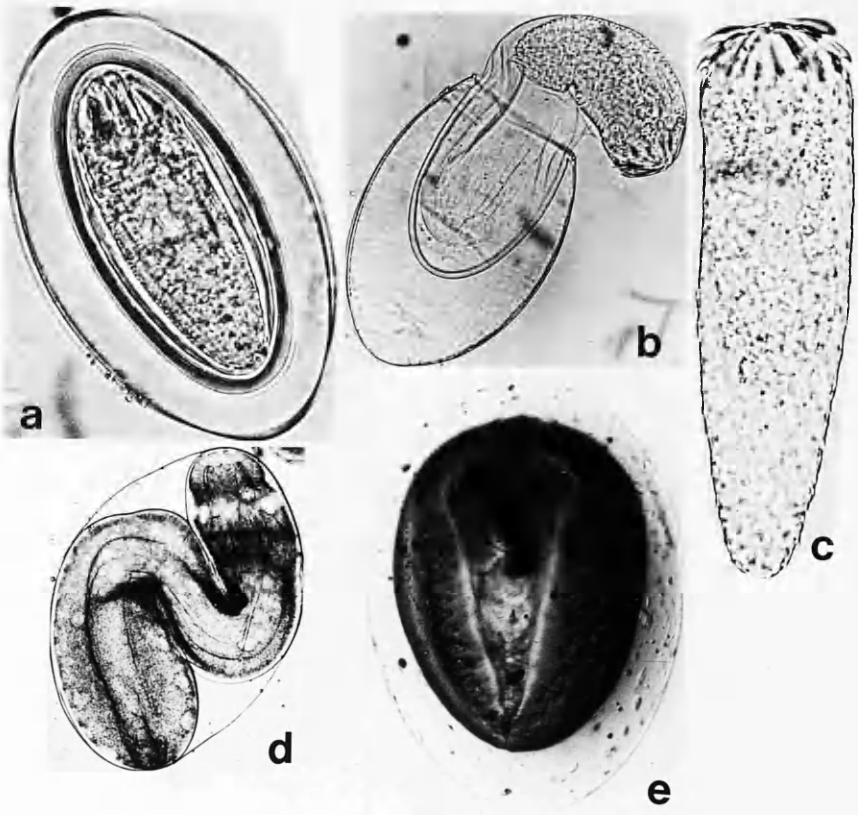
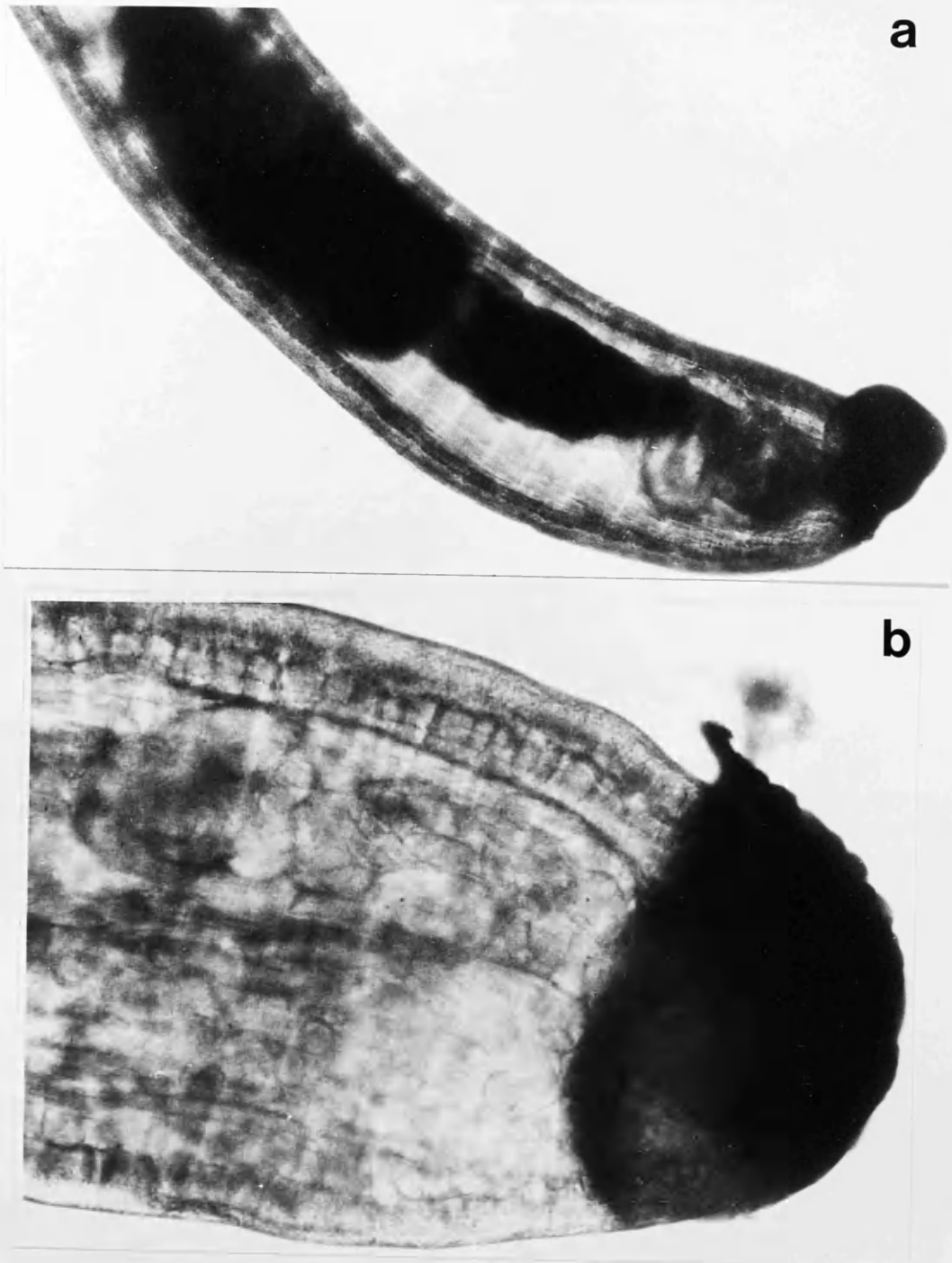
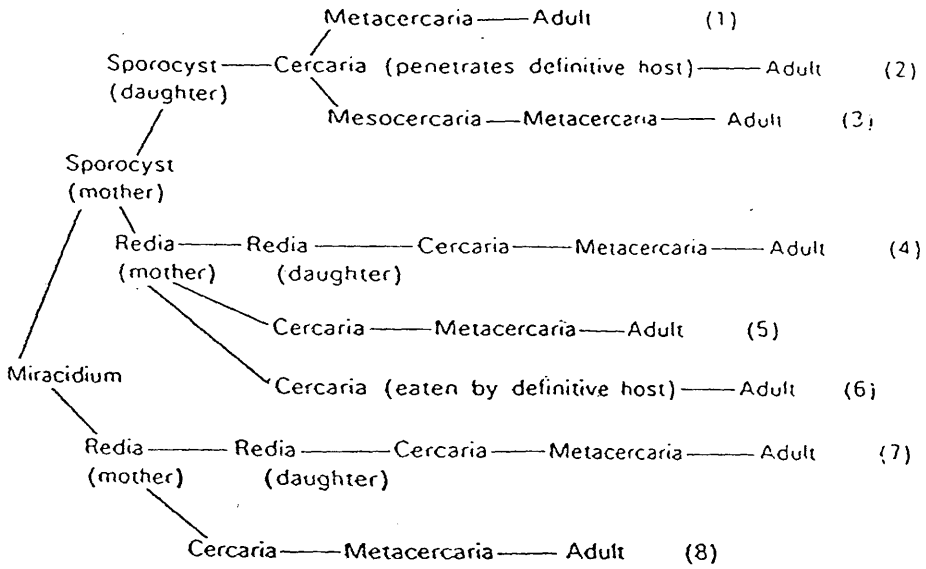

a
b
c
d
e

Plate 1.2 Photographs of the posterior parts of 21-day-old *Moniliformis moniliformis* with copulatory cap.
a) male worm b) female worm


a
b



(1) *Diplostomum flexicaudum* (Cort and Brooks, 1928)
(2) *Trichobilharzia physellae* (Talbot, 1936)
(3) *Alaria mustelae* Bosma, 1931
(4) *Fasciola hepatica* Linnaeus, 1758
(5) *Metorchis conjunctus* (Cobbold, 1860)
(6) *Proterometra dickermani* Anderson, 1962
(7) *Stichorchis subtriquetrus* (Rudolphi, 1814)
(8) *Caecincola parvulus* Marshall and Gilbert, 1905

Fig. 1.2 Some possible life cycles of digenetic trematodes. (From Schell, S.C., 1970. How to know the trematodes, William C. Brown Co., Publishers, Dubuque, Iowa).

Table 1.1 Helminth reproductive biology

Strategy		Monogenea	Digenea	Eucestoda	Acanthocephala	Nematoda
Mode	Ectoparasitic	a	c	-	-	-
	Endoparasitic	c	a	a	a	a
Life history	Direct	a	-	-	-	a
	Indirect	-	a	a	a	a
Asexual reproduction	Polyembryony	-	a	-	-	-
	Budding	-	-	b	-	-
	Fission	-	-	b	-	-
	Strobilization	-	-	a	-	-
Sexual reproduction	Dioecious	-	b	c	a	a
	Hermaphrodite	a	a	a	-	-
	Self-fertili-zation	c	c	b	-	-
	Cross-fertili-zation	a	a	a	a	a
	Copulation	a	a	a	a	a
	Hyperdermic	b	-	a	-	-
	Spermatophore	b	-	-	-	-

a= general pattern
b= common
c= occasional

CHAPTER 2. MATERIALS AND METHODS

2.1 INTRODUCTION

The description of materials and methods in this chapter provides a summary of the procedures employed during the experimental investigations. All procedures were carried under the conditions laid down by The Animals (Scientific Procedures) Act 1986, and were covered by both a Home Office Project Licence (PPL 60/00370), and a Home Office Personal Licence (PIL 60/01301).

2.2 CARE AND MAINTENANCE OF HOSTS AND PARASITES

The hosts and procedures used for the maintenance of life cycles and for experimental studies on *Moniliformis moniliformis*, *Trichinella spiralis* and *Nippostrongylus brasiliensis* are described below.

2.2.1 HOSTS

2.2.1.1 RATS

Rattus norvegicus is the natural definitive host of *M.moniliformis* (Holland, 1983). For life cycle maintenance and experiments, outbred Wistar rats of both sexes were used. The rats were either bred in the laboratory or purchased from Bantin and Kingman. All animals were housed in the animal unit in the Department of Zoology.

Rats used for experiments were known to be free from Acanthocephala and, at the time of infection, were between 6 and 8 weeks old and weighed 250-300 g. The rats were kept in plastic cages measuring 9.2 x 15.2 x 7.6 inches and covered with stainless steel lids. The rats were fed *ad libitum* on CRM rodent diet and water unless stated otherwise. The temperature of the animal unit was kept between 20-22^{0}C under a regime set to provide 12 h of artificial light and 12 h of darkness.

2.2.1.2 COCKROACHES

Throughout this study, cockroaches (*Periplaneta americana*) were used as intermediate hosts for *M.moniliformis* in the laboratory. Stock cultures of cockroaches were maintained in glass tanks at an internal cage temperature of 27^{0}C,

which is within the optimum range for larval development of *M.moniliformis* (Lackie, 1972). Large pieces of cardboard were added to the tanks to provide shelter and breeding areas as well as to trap moisture. The tanks were sealed and covered with a fine mesh screen. Cockroaches were fed *ad libitum* on CRM rodent diet. Water was supplied *ad libitum* by an inverted wick-feed method, a Petri dish being lined with moistened cotton wool placed on the top of a glass beaker filled with water and the entire apparatus inverted. A timing device was set to give 12 h of artificial light and 12 h of darkness and / or natural light.

2.2.2 PARASITES

2.2.2.1 *MONILIFORMIS MONILIFORMIS*

The *M.moniliformis* studied in this work was of either Molteno or Texas strain. The Molteno strain has been maintained in the laboratory for at least 20 years. The parasite was found originally in Queensland and was given to D.W.T. Crompton by S.J.Edmonds, Department of Zoology, University of Adelaide in 1969. The life cycle was then maintained in the laboratory at the Molteno Institute, University of Cambridge, in cockroaches *Periplaneta americana* and an outbred strain of Wistar rats (CFHB). The same strain was transferred to Glasgow University in 1985.

In December 1983, D.W.T. Crompton was given an isolate of *M.moniliformis* by B.B.Nickol, University of Nebraska. At this time, worms of this isolate had undergone four generations in the laboratory since being collected by J. Moore, Colorado State University, from wild rats (*Rattus norvegicus*) living by the Houston ship canal, Texas, U.S.A., in January 1982. The Texan isolate has passed through 17 complete generations in the laboratory at Cambridge and Glasgow.

2.2.2.2 *TRICHINELLA SPIRALIS*

The strain of *Trichinella spiralis* used in this study was originally derived from a stock at the London School of Hygiene and Tropical Medicine and has been maintained in various strains of both rats and mice at the Department of Zoology, University of Glasgow, by M.W.Kennedy.

2.2.2.3 *NIPPOSTRONGYLUS BRASILIENSIS*

The strain used in this study may be designated the Glasgow strain, since it has been established and maintained at the Department of Zoology, University of Glasgow, by M.W.Kennedy. Outbred Wistar rats were used as hosts.

2.3 PROCEDURE FOR THE INFECTION OF HOSTS

2.3.1 INFECTION OF RATS WITH *MONILIFORMIS MONILIFORMIS*

Cystacanths were recovered from cockroaches which had been offered shelled acanthors at least 10 weeks before. The cockroaches were dissected in aqueous 0.6% NaCl solution and the cystacanths were flushed out of the haemocoel. Cystacanths were then carefully separated and held in 0.6% NaCl solution for use. For each cockroach the number of cystacanths found was recorded. Five hundred and forty three was the highest number of cystacanths observed during this study from an individual cockroach given rat faeces containing shelled acanthors. Only phenotypically normal cystacanths were used for the experiments.

Rats selected for the experiments and for life cycle maintenance procedures were lightly anaesthetized with diethyl ether before receiving infective doses. Rats gained consciousness within 10-15 sec, the time required for the inoculation of the dose.

Rats were given 15-40 cystacanths each in 0.6% NaCl solution via stomach tube. Cystacanths were sexed prior to infection as described in section 2.5. for the experiments on reproduction of the parasite. For maintenance infections, cystacanths were not sexed individually, it being assumed that the sex ratio of cystacanths would be approximately 1:1 (Crompton & Walters, 1972).

2.3.2 INFECTION OF COCKROACHES WITH *MONILIFORMIS MONILIFORMIS*

Shelled acanthors of *M.moniliformis* were obtained from gravid female worms freshly obtained from rats. Female worms were macerated in 0.6% NaCl solution and sieved through a 40 μm metal sieve that retained most of the mature acanthors. The acanthors were washed in 60% sucrose solution and were then stored in fresh 60% sucrose solution in a refrigerator (Lackie, 1972). Shelled acanthors were

detected in faeces from rats harbouring *M.moniliformis* infections for at least 36 days. Faeces were then stored at either room temperature or in the fridge at 7°C, until they were fed to cockroaches.

A known number of Acanthocephala-free cockroaches were either individually isolated in glass jars covered with ventilated lids or were kept in plastic tanks and denied food and water for 24 h prior to infection. The infection was achieved by the following methods:

a) Acanthors were administered to the cockroaches by placing a few drops of acanthor sucrose suspension on the bottom of the jars. Drops were swallowed within few minutes. The roaches were starved for further 24 h and then returned to normal maintenance conditions.

b) Faecal pellets from infected rats harbouring sexually active female worms were collected. A few pellets were soaked in water, sieved through 1 mm mesh and then diluted with a little water. A drop of this mixture was placed on a glass slide, covered with a cover slip and checked for the presence of acanthors under the microscope. The infected pellets were then given to the roaches and were eaten within few days. After assuming that the roaches had been infected, they were allowed access to food and water again.

2.3.3 INFECTION OF RATS WITH *TRICHINELLA SPIRALIS*

Infective larvae were recovered from stock animals which had been infected for at least two months. The infected animals were killed, skinned, cut into pieces and then minced in a blender. The material was digested for 2.5 h in 0.5 % pepsin in 0.5 % HCl at 37°C. Undigested sediment was filtered out with a coarse sieve and the larvae collected by successive washings and sedimentations in 0.9 % NaCl. The larvae were finally suspended in dilute agar to give a concentration of 300/ 0.1 ml (Wakelin & Lloyd, 1976). For calculation of the larval doses of *T.spiralis* the stock larval suspension was diluted to give 300 L_2/0.1 ml and each rat received 0.5 ml of the suspension (= 1500 L_2). These second-stage larvae were given to rats as oral doses of 1500 in 0.5 ml of water per rat with a syringe and blunted cannula

(Howard *et al.* 1978).

2.3.4 INFECTION OF RATS WITH *NIPPOSTRONGYLUS BRASILIENSIS*

Faeces from infected rats were collected between days 6 and 9 p.i. Pellets were soaked in water, ground to a paste and mixed with equal volume of charcoal. The mixture was spread on a moist hard filter paper leaving a 1 cm margin clear which was then placed in a Petri dish with a piece of wet cotton wool to support the filter paper. The dish was covered and incubated at 25 ^{0}C for about 6 days.

The infective third-stage larvae were collected by cutting off the edges of the filter paper with the larvae attached and immersing these in a glass funnel lined with a nylon gauze and a rubber tube attached to the top of the funnel and sealed. The funnel was then filled with water and inserted into a conical flask and left for a few hours to release the larvae. The larval suspension was then transferred to the centrifuge tubes and spun at approximately 2, 000 r.p.m. for 3 min. The larvae were stored in glass Petri dishes in a few ml of tap water in darkness until required, for not more than 4 weeks.

For infection with *N.brasiliensis*, the larvae were suspended in 10 ml of water. After thorough mixing, the larvae were counted in 0.1 ml samples under the dissecting microscope. Larval concentration was then adjusted to give 4, 000 L_3/0.2 ml for each rat. Rats were infected by subcutaneous inoculation into lower abdomen.

2.4 *POST MORTEM* PROCEDURES

2.4.1 *MONILIFORMIS MONILIFORMIS*

Rats were sacrificed as needed between 15- 63 days p.i. by extended inhalation of diethyl ether followed by cervical dislocation. The small intestine was removed and placed in aqueous 0.9% NaCl. In order to determine the attachment position of individual worms, the small intestine was tacked down with dissecting pins on a wooden scale measuring 0-100 cm. The small intestine was measured, from pylorus to caecum and was slit then with care using surgical scissors directed around the

worms to avoid cutting them; occasionally an unattached worm was damaged. The point of attachment for each worm along the length of the small intestine was recorded.The worms were gently removed with pair of forceps and preserved in 5 % formaldehyde solution. The data collected on termination of each experiment included the number of worms established, attachment position, sex and length (mm) of individual worm in the gut. Later, the percentage of the small intestine anterior to the attachment point was used as a measure of each worms' position. For growth, dry weights were measured by placing worms individually on small pre-weighed aluminium foil dishes for 24 h in an oven set at 100 ^{0}C. For the experimental investigation of fecundity, the body cavity contents of each female worm were collected and fixed in aqueous 5% formaldehyde solution for further investigation (see section 2.6.2).

2.4.2 *TRICHINELLA SPIRALIS* AND *NIPPOSTRONGYLUS BRASILIENSIS*

Rats were killed as described earlier and adult worms were recovered from the small intestine by means of the routine Bearmann technique. The small intestine was slit open and cut into 2- 3 cm pieces and placed on a layer of nylon gauze tied across a 50 ml glass beaker filled with 0.9% NaCl solution. The material was then incubated in a water bath at 37^{o}C for 3 h during which time, the majority of worms left the intestine and were collected in the beaker. The worms were transferred to a Petri dish and counted under the dissecting microscope.

2.5 DETERMINATION OF SEX OF *MONILIFORMIS MONILIFORMIS*

Sex of *M. moniliformis* can be recognised at the later acanthella and certainly by the cystacanth stage. The male and female reproductive system develop along comparatively similar lines, but the testes can usually be identified earlier than the ovarian tissue. Studies on the development of *M. moniliformis* in its intermediate host, by Moore (1946) and Asaolu *et al* (1981), have shown that there is no compact ovary present in the female, but small masses of compact cells, which are immature ovaries, and can be seen free in the body cavity. On average, eight spherical ovaries have been observed in 7-day-old female *M. moniliformis* from rats (Plate 2.1)

(Crompton *et al* 1976).

The male reproductive organs are usually arranged with the testes being located anterior to the cement glands which are in turn anterior to the copulatory bursa. The two testes arranged in tandem are the most anteriorly placed organs in the reproductive system. They are cylindrical in shape and slightly ventrally curved. In the cystacanth and in young male worms, usually the posterior end of the anterior testis and the anterior end of the posterior testis overlap slightly.

Experiments on fecundity of *M. moniliformis* were dependent on accurate determination of the sexes of cystacanths prior to infection. The sexes were identified as males mostly by the presence of two testes in the posterior half of the body and occasionally by the presence of cement glands when testes were not clearly visible. Females were identified by the presence of small free ovaries scattered in the ligament sac. Sexing was done in a cavity slide preparation using Wild M 11 compound microscope at a total magnification of X6 (Plate 2.2 a-b). Male and female cystacanths were held separately in aqueous 0.6% NaCl solution. The cystacanths were used within 3 h of recovery from cockroaches; this time was needed to determine the sex of the cystacanths when many were required.

2.6 ASSESSMENT OF THE REPRODUCTIVE STATUS OF *MONILIFORMIS MONILIFORMIS*

2.6.1 INSEMINATION

Experiments were undertaken to investigate evidence of insemination, to detect the earliest occurrence of insemination, to study polygyny and to examine the behaviour of male worms to those of the same and opposite sexes. Rats were infected with known ratios of male and female cystacanths on day 0 and sacrificed on day 21 post infection. At the termination of experiment, all female worms recovered were examined for the evidence of insemination by dissecting them and observing the contents of their body cavities for zygotes and free immature or mature shelled acanthors. The female worms showing the presence of zygotes or developing acanthors were known to have been successfully inseminated (Plate 2.3).

2.6.2 FECUNDITY

The body cavity contents of female *M.moniliformis* were obtained as described in section 2.3.1, and fixed in aqueous 5% formaldehyde solution. Fixed body cavity contents were then centrifuged using Pyrex 10 ml centrifuge tubes, for 2 min. at 2,000 r.p.m. The supernatant fluid was then discarded and pellet resuspended in a known volume, usually 1-2 ml, of 0.6% NaCl. After thorough mixing, samples of 20 ul were withdrawn with a Gilson pipette and placed on microscopic slides, covered with cover slips and all free ovaries and shelled acanthors (immature/mature) were counted. At least five samples were examined for each female worm.

2.7 DIETARY FORMULATION

For the experimental investigation on the effects of host dietary fructose on fecundity of *M.moniliformis*, rats were fed *ad libitum* on 6% fructose diet. The diet was prepared from maize oil and other essential nutrients, as described by Crompton *et al* (1983). The diet contained 13% casein (w/w) and 6% fructose (w/w). The theoretical composition of the diet is shown below.

Ingredients	Composition (g/kg)
Casein	130.00
Maize oil	386.40
Cellulose	394.10
Mineral mixture	45.00
Vitamin mixture	10.00
Choline chloride	1.50
L-threonine	1.50
DL-methionine	1.50
Fructose	60.00

Rats were fed on this diet at least 15 days prior to infection and then throughout the course of infection.

2.8 X-IRRADIATION PROCEDURE

Effects of X-irradiation on the establishment, growth and fecundity of *M.moniliformis* were investigated (see Chapter 7). On recovery from the body cavities of cockroaches, cystacanths of *M.moniliformis* were counted and sealed in glass vials containing 5 ml of 0.6% NaCl solution. Each vial contained 105

cystacanths, so that 15 cystacanths could be given to each rat in a group of 7. Cystacanths were X-irradiated at the Belvidere Hospital, Glasgow, using an X-ray machine (Siemens unit). The X-ray machine was operated at 300 kV with an external filtration of 1 mm. Cu. The machine had an integral flat incubator (Pancake chamber) which was calibrated daily. For irradiation, a series of doses ranging from 500 to 2500 centigrades (CG) was used. For each set of experiments, 3 vials containing cystacanths were placed under the window of the X-ray machine at a point where the dose rate to the surface was approximately 100 CG/min. The X-ray machine was switched off for a while after each 500 CG dose and one of these vials was removed (depending on X-ray dose) during this time interval and the procedure resumed. Cystacanths in the fourth vial remained unirradiated and were used as control group, to check for infectivity and for the effects of environmental conditions under which the cystacanths were kept before they were fed to the rats.

For the first trial, the cystacanths were kept at room temperature until they were used for the infection. This led to the evagination of proboscides. To avoid this situation, cystacanths for the rest of the experiments were kept at $7^{o}C$ until they were used except for the time when they were irradiated.

2.9 ANTHELMINTIC TREATMENT

Different anthelmintics were used in trials against *M.moniliformis* so that one could be selected for routine termination of the infection when required.

2.9.1 DOSE AND ADMINISTRATION

1) Valbazen---------- albendazole

For albendazole the manufacturer's recommended dose is 5 mg/kg of body weight. Each rat was given 0.25 ml (250 g body weight) of diluted solution on consecutive days after 24 h of fasting.

2) Ketrax---------- levamisole

For levamisole the manufacturer's recommended dose is 30 mg/kg of body weight. To give the concentration of 7.5 mg of drug/ 250 g body weight, 30 mg of

drug was dissolved in 1 ml of water and each rat was given 0.25 ml on two occasions.

3) Ovitelmin---------- mebendazole

Mebendazole solution contained 50 mg of drug per ml and the manufacturer's recommended dose is 10 mg/kg of body weight, by mouth. One ml of Mebendazole solution was diluted (1+ 4) to give 10 mg/ml and each rat was given 0.25 ml (250 g body weight) orally, for 5 days.

4) Droncit---------- praziquantel

Tablets each contained 50 mg of drug and the manufacturer's recommended dose is 5-10 mg/kg of body weight, by mouth. Each tablet was crushed and suspended in 2 ml of water and each rat was given 0.25 ml (250 g body weight) on two occasions with an interval of 24 h.

2.9.2 MODE OF ACTION

1) Valbazen

The mode of action of the benzimidazoles (BZs) can be separated into three categories: (1) fumarate reductase inhibition, (2) inhibition of glucose transport, (3) interruption of microtubular function. Most of the recent work suggests that category 3 represents the primary underlying mechanism. The observation of cross-resistance among all BZs in controlled efficacy trials (Colglazier *et al*, 1975) makes it likely that BZs have a common mode of action.

2) Ketrax

Levamisole acts by selective inhibition of succinate dehydrogenase in nematode muscle whereby the conversion of succinate into fumarate is suppressed. The production of muscular energy is markedly decreased and the drug paralyses the worm within a few minutes of contact.

3) Ovitelmin

Its mode of action is to inhibit the glucose uptake in worms resulting in glycogen depletion and decreased formation of ATP which is essential for energy supply.

4) Droncit

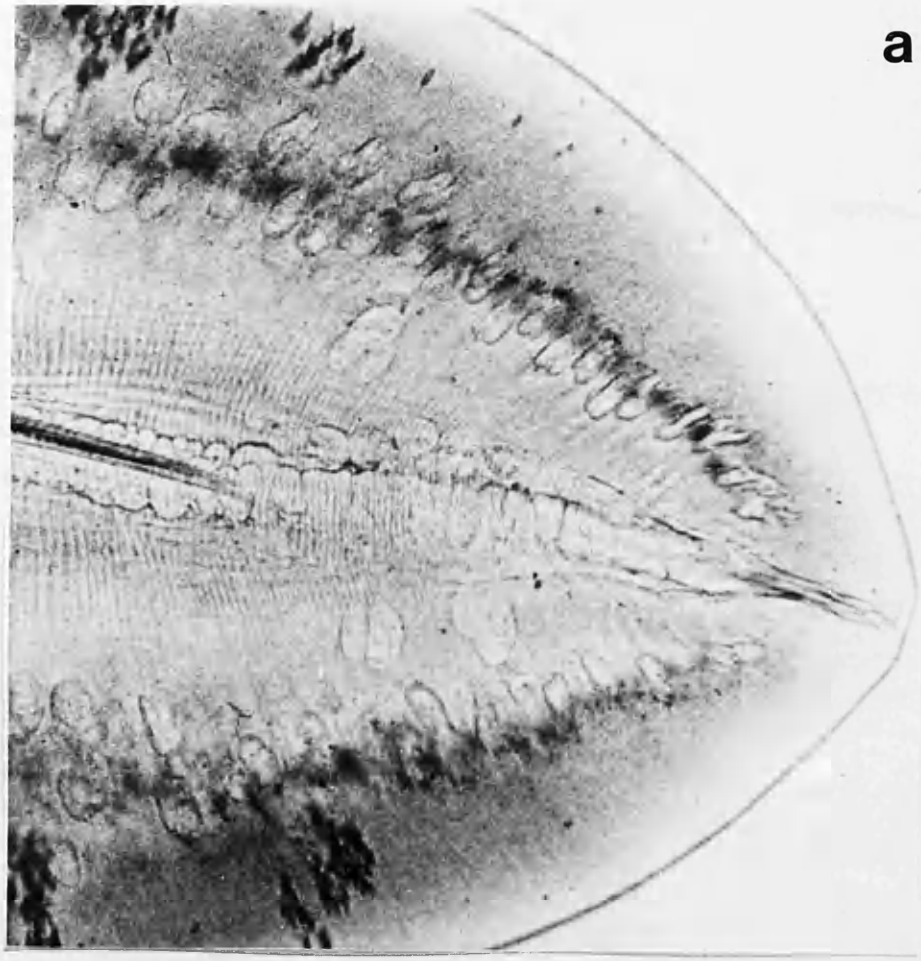
Praziquantel affects the mobility of cestodes and the function of their suckers. At concentrations of 10-100 mg/ml and above, it causes a strong contraction of the entire strobila. When concentrations exceed 1 g/ml, this contraction occurs almost instantaneously. The effectiveness of the drug *in vivo* appears in part to be due to dislocation of the worm in the small intestine (Schild *et al*, 1975).

2.10 STATISTICAL INVESTIGATION

The analysis of variance (ANOVA), Chi-square test, and Mann- Whitney U tests were routinely used to compare the values of parameters measured between the rat groups, unless otherwise stated. In the test, a difference is referred to as significant when $P \leq 0.05$.

Plate 2.1 Photograph of a 7-day-old female *Moniliformis moniliformis*. The arrow indicates the presence of eight spherical ovaries contained in the ligament sac.

Plate 2.2 Photographs of the posterior parts of cystacanths of *Moniliformis moniliformis.*
a) female b) male
The arrow indicates the presence of two testes arranged in tandem in male cystacanth.


a

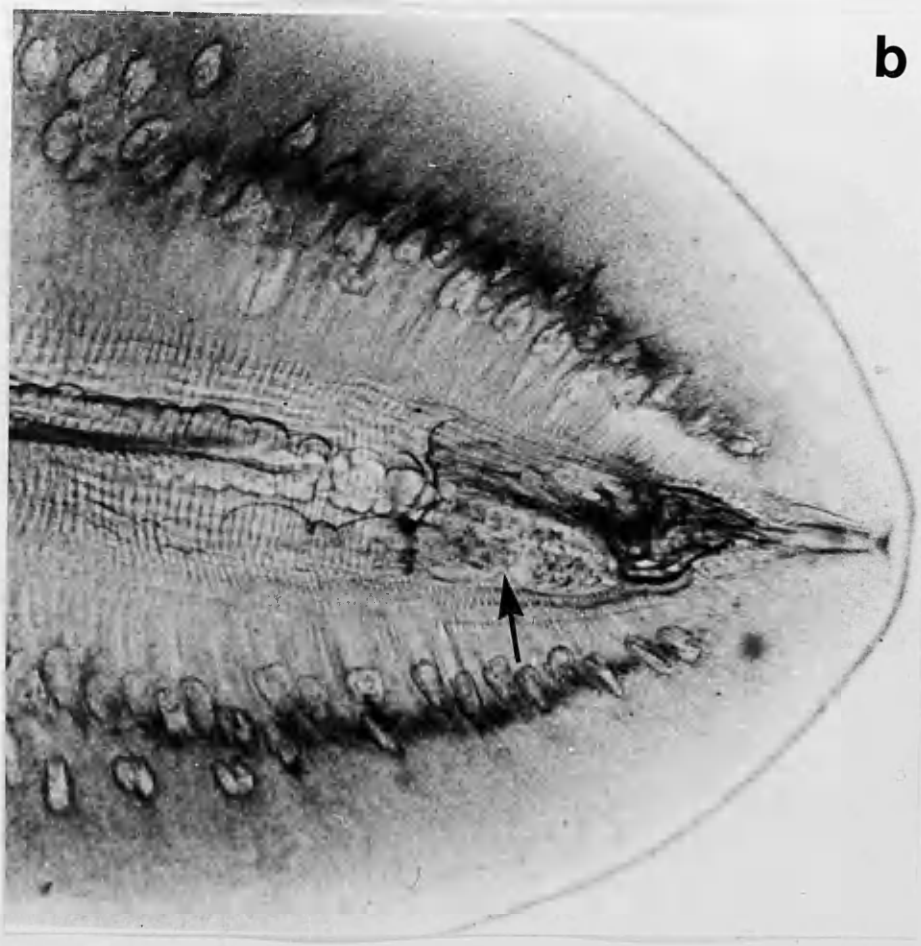

b

Plate 2.3 Photograph of fertilised ovaries containing eggs at various stages of development, from a 21-day-old female *Moniliformis moniliformis*. The arrow indicates the presence of early developing eggs on the surface of the ovary.

CHAPTER 3. THE COURSE OF INFECTION OF *MONILIFORMIS MONILIFORMIS* IN RATS.

3.1 INTRODUCTION

The many factors affecting controlled laboratory infections of acanthocephalans in their intermediate and definitive hosts are varied and incompletely understood (see Burlingame and Chandler, 1941; Kates, 1944; Crompton and Whitfield, 1968; Crofton, 1971; Crompton and Walters, 1972; Lackie, 1972; Kennedy, 1972, 1974; Harris, 1972). The course of infection in nature is even more complex and difficult to analyse because of the effects on the parasite of (1) intra- and interspecific reactions between parasites, (2) the responses of the host's digestive physiology to seasonal, dietary and other environmental changes and (3) the natural ecological interactions between the intermediate, definitive and paratenic hosts.

Studies on the course of infection of *Moniliformis moniliformis* in immuno-competent, well-nourished rats with primary infections of 12-20 cystacanths each (Crompton and Walters, 1972), have shown that male and female worms become established in equal numbers at the start of infection. A gradual and equal loss of worms of both sexes follows until around 10 weeks p.i. However, Burlingame and Chandler (1941) reported that male worms are lost after the end of seventh week. Little work has been done on the host's immune response to *M.moniliformis.* The susceptibility of rats to secondary infections was, according to Burlingame and Chandler (1941), proportional to the number of worms that had been present during the primary infection. Andreassen (1975 a, b) described that at infection levels above 100 cystacanths per rat, the rate of recovery of *M.moniliformis* was very low at 8 weeks p.i. The loss of parasites occurred between 4 and 8 weeks when establishment fell from 85% to 15%. Rats given 100 cystacanths in a primary infection, dosed with anthelmintic drugs and then challenged with a secondary infection, showed a lower recovery in worm numbers and retarded growth of secondary parasites. He also demonstrated the presence of reaginic antibodies in association with the host rejection, and concluded that there was some evidence for

a host immune response to *M.moniliformis*.

The aims of the work described in this chapter were to further investigate the rate of growth and survival of both sexes of *M.moniliformis* in mixed and single sex infections and to determine the fate of secondary infections in rats.

3.2 EXPERIMENTAL DESIGN

Two experiments were carried out. The first experiment (experiment 1) was carried out to determine the rate of growth and survival of *M.moniliformis*. Four time points (days 1, 21, 56, and 84 p.i.) were chosen for the *post mortem* examination of the rats. For each time point, a group of 5 female rats were infected, on day 0, with the intended dose of 10 cystacanths per rat as either male and female (1:1 sex ratio), males only, or females only.

The second experiment (experiment 2) was undertaken to determine the fate of a secondary infection of *M.moniliformis*. Fourteen female rats were each infected with 15 cystacanths of *M.moniliformis* on day 0. After 112 days, 7 rats were killed and the worms recovered. On day 115 p.i. the other 7 rats were challenged with secondary infections based on the same dose per rat. At the same time, 7 previously uninfected rats were each given a primary infection of 15 cystacanths to serve as controls. All these rats were killed 5 weeks later. In the primary infections, daily faecal collection and analysis was carried out during week 7-16 p.i.

3.3 RESULTS

At *post mortem* examination the number of worms recovered, their sexes and attachment positions along the length of the small intestine, their lengths and wet weights (dry weights for experiment 2) were recorded.

Experiment 1

3.3.1 ESTABLISHMENT AND SURVIVAL OF *MONILIFORMIS MONILIFORMIS*

On average, 40%, 74%, 72%, and 58% of the infection dose of 10 cystacanths per rat, regardless of sex, was retrieved from the small intestine at days 1, 21, 56, and 84 p.i. respectively. It must be assumed that not all the worms present, which

were extremely small and villus-like in shape at 24 h p.i., were found. This would explain how more worms were found at day 21 than at day 1 p.i. It was also found to be difficult to record the exact attachment position of these very small worms and so the results from day 1 p.i. were not included in the analysis. The mean values (±SE) of the number of worms recovered, their sexes and attachment positions in the gut, lengths and wet weights are given in Table 3.1. The mean numbers of *M.moniliformis* found in the groups of rats examined at different infection levels and times during the course of infection are shown in Fig. 3.1. From mixed sex populations a gradual and significant ($P < 0.05$) loss of male worms was observed during the course of infection, with the majority of worms being expelled between days 56 and 84 p.i. Female worms, however, showed no difference in worm recovery (i.e. numbers present) until day 56 p.i., but thereafter, there was a significant decrease in the number of worms recovered ($P < 0.05$) (see Fig.3.1). Worms recoveries of both sexes from single sex infections showed no differences at any time point during the course of infection. The numbers of male and female worms recovered from mixed sex infections were compared with those recovered from single sex infections. The analysis of the data revealed significant differences ($P < 0.05$) in worm recoveries; more male and female worms were recovered from single sex populations than from mixed sex populations. A decline in the number of female worms, however, was observed after 8 weeks of infection from both mixed and single sex infections, suggesting that female *M.moniliformis* live longer than males in either the presence or absence of male worms in the host's gut.

3.3.2 LOCATION OF *MONILIFORMIS MONILIFORMIS* IN THE SMALL INTESTINE

The range in the distribution of attachment positions of *M.moniliformis*, at days 21, 56, and 84 p.i., in rats given different types of infection is shown in Fig. 3.2 a-c, and the differences in the range of attachment positions of males and females from mixed and single sex infections are compared in Fig. 3.3 a-d. At day 21 p.i., 84% of the worms from mixed sex infections were found to be attached in the

region ranging from 16-35% of the distance along the length of the small intestine, with female worms being attached more anteriorly than male worms. In single sex infections, however, male and female worms were observed to be located in the region ranging from 20-60% and from 11-60% in the intestine respectively (Fig.3.2 a-c). Worms from all infection levels showed a posterior shift in attachment positions with time. Additionally, worms of both sexes from single sex infections were attached significantly ($P < 0.05$) more posteriorly than the worms from mixed sex infections (Fig.3.3 a- d).

3.3.3 GROWTH OF *MONILIFORMIS MONILIFORMIS*

The length and wet weight of each worm collected at the *post mortem* examination was recorded to give a rough estimate of growth. The mean lengths and wet weights of male and female *M.moniliformis*, recovered at different times during the course of infection, are illustrated in Figs. 3.4. and 3.5. respectively. Although worms showed a rapid increase in growth after 21 days of infection, no difference was observed, in either the mean lengths or mean wet weights between male worms from mixed and single sex populations at any time point. Female worms from day 21 p.i. from mixed sex populations were found to be significantly ($P < 0.05$) smaller and lighter than the females recovered from single sex infections. However, a significant increase ($P < 0.05$) both in length and wet weight of these females was observed thereafter. No significant difference in growth between the female worms from single sex infections was detected.

Experiment 2

3.3.4 FATE OF SECONDARY INFECTIONS

Faeces from rats harbouring primary infections with 15 cystacanths each, were collected daily, after 49 days of infection, and examined for any expelled worms. The loss of worms was first observed at 63-70 days p.i. with the majority of worms being expelled around day 110 p.i. At *post mortem* examination (day 112 p.i.), a mean of 2.3 $\pm$1.2 worms per rat was recovered from the rats harbouring primary infections (Table 3.2). The faecal examination at day 129 p.i., from rats challenged

with secondary infections of *M.moniliformis* did not reveal any expelled worms. At *post mortem* examination (on day 150 p.i., for the primary worms and 35 for the secondary worms, 74.2% of the secondary infection dose of 15 cystacanths per rat was found in the small intestine. It was assumed that some of the secondary worms might have been lost from the hosts during the first week of secondary infection. NO worms, however, as expected were recovered from the primary infections of these rats. No significant difference between mean worm recoveries per rat was observed between control primary (11.1 $\pm$2.3) and challenged (10.7 $\pm$ 3.7) infections (Table 3.2). Also, no significant difference was observed between mean dry weights of the worms from challenged and control primary infections. The mean dry weight per male and female worms from challenged infections was 5.4 $\pm$1.1 and 21.4 $\pm$1.6 and those from control primary infections was 6.1 $\pm$1.0 and 23.1$\pm$0.8 per worm respectively (Table 3.2).

3.4 DISCUSSION

The course of infection of *M.moniliformis* maintained in the laboratory was observed to follow the general pattern described by Burlingame and Chandler (1941) and Crompton and Walters (1972). Male and female worms from mixed sex infections initially became established in equal numbers followed by a gradual loss of worms, with males being lost earlier than females. In single sex infections, on the other hand, the number of worms of both sexes that had survived until day 21 p.i. remained the same until the end of the experiment, suggesting their greater rate of survival. The distribution of *M.moniliformis* along the distance of small intestine of the host, suggested that the degree of anteriorly directed migration by the worms was greater in mixed sex rather than in single sex populations. Similar results were observed by Miller (1980) for *M.moniliformis*. The more posterior attachment positions of worms, after day 21 p.i., may represent a posteriorly directed migration. Since the number of worms also declined (particularly in mixed sex infections) with time, there is a possibility that the anteriorly attached worms were lost more rapidly after day 21 p.i. than those posteriorly attached. Throughout the infection, worms

showed an increase in growth with females growing more rapidly after day 21 p.i. than male worms, and in mixed than in single sex infections (see Fig. 3.4, 3.5).

The patterns of survival, distribution and growth of *M.moniliformis* in rats, observed in this study, might suggest a possible mating effect. The loss of male worms (from mixed sex infections) that have been with females long enough to ensure the maximum egg production by the females (Crompton, 1974) would maximise the reproductive success of both sexes, as the females will be left in the intestine of the host having all available nutrients to nourish the offspring. A significantly more rapid growth of these females was observed as compared with unmated females from single sex infections (Fig. 3.4, 3.5) also there was no difference between growth of male worms from mixed and single sex infections, suggesting that the females that have mated and started producing eggs may utilize more nutrients than males and unmated females and thus grow bigger. Crompton (1972) reported that the fertilized female *M.moniliformis* contained significantly more nitrogen (protein) in their bodies than the males, which probably represents (1) the early phase of egg production and (2) growth of worm's body to accommodate the developing eggs.

Burlingame and Chandler (1941), reported that the rate of establishment and survival of *M.moniliformis* from secondary infections was affected only when large and mature primary worms were present in the optimum site in the small intestine, as a result of which secondary worms were established outside the optimum site and were lost. They further concluded that, the secondary worms that managed to migrate and established themselves in the optimum site, survived and grew at the same rate as the worms in simple primary infections. In contrast, Miremad-Gassmann (1981), reported that *M.moniliformis* that survived from secondary infections were always fewer in number and smaller suggesting a possible effect of immunity acquired by the rats due to primary infections of *M.moniliformis*. In both of these studies, rats were challenged with secondary infections while still harbouring worms from primary infections. In the present study, rats were challenged with secondary infections, when almost all worms from primary

infections had been naturally expelled from their hosts. The successful establishment and growth of secondary worms at the same rate as primary worms, suggests that the secondary worms were established in the optimum site in the small intestine of the hosts and so having enough space and nutrients, grew normally. The results, however, do not suggest any direct influence of acquired immunity on the establishment and survival of secondary infections in terms of growth and worm numbers present in the small intestine of the host.

3.5 SUMMARY

The analysis of the course of infection of mixed and single sex oral infections of 10 cystacanths of *Moniliformis moniliformis* in female Wistar rats were undertaken. There was a decline in the average recovery rate of worms of both sexes from mixed sex populations during the course of infection. Female worms showed, on average, a greater rate of survival than male worms from day 21 p.i. onwards. Mated female worms were observed to grow at a greater rate (after day 21 p.i.) than unmated female worms of the same age. No evidence of any direct influence of acquired immunity on the establishment and survival of worms from challenge infections could be detected.

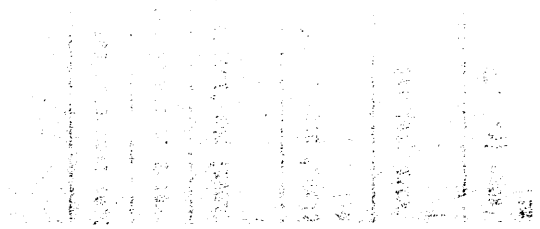


Table 3.1. Observations on the establishment and growth of Moniliformis moniliformis during the course of primary infections in rats.

	Cystacanths given (male : female sex ratio)/rat								
	5 : 5			10 : 0			0 : 10		
	Days p.i.			Days p.i.			Dyas p.i.		
	21	56	84	21	56	84	21	56	84
Mean male worms recovered/rat ±SE	[a] 4.2 ±0.8	3.0* ±1.5	[b] 2.4* ±1.1	6.0 ±3.6	6.2 ±2.7	6.0 ±3.6	--	--	--
Mean female worms recovered/rat ±SE	[c] 4.4 ±0.8	4.4 ±0.5	[d] 2.6* ±1.1	--	--	--	7.6 ±2.3	8.0 ±1.5	6.4 ±2.0
Mean % attachment position/worms ±SE (male worms)	28 ±5.0	29 ±10.3	34 ±16.9	32 ±7.2	34 ±10.0	46 ±14.0	--	--	--
Mean % attachment position/worms ±SE (female worms)	25 ±4.2	31.9 ±12.4	31 ±14.0	--	--	--	29.3 ±12.0	30.5 ±11.0	38 ±16.6
Mean length/worm (mm) ±SE (male worms)	38.5 ±6.3	63.2 ±7.5	79.8 ±10.4	34.2 ±4.3	59.8 ±5.4	72 ±6.4	--	--	--
Mean length/worm (mm) ±SE (female worms)	72.1 ±5.9	165 ±23.7	188 ±30.6	--	--	--	139 ±9.0	126.6 ±19.9	152 ±9.7
Mean wet weight/worm (mg) ±SE (male worms)	15.9 ±5.1	49.2 ±12.3	73.0 ±9.0	14.2 ±4.7	48.6 ±6.3	70 ±8.1	--	--	--
Mean wet weight/worm (mg) ±SE (female worms)	26.5 ±5.7	183.7 ±45.7	327.3 ±62.2	--	--	--	167.9 ±21.4	104 ±17.1	175 ±20.3

Mann-Whitney U test between a and b = 0.0472; $P < 0.05$, between c and d = 0.0283; $P < 0.05$

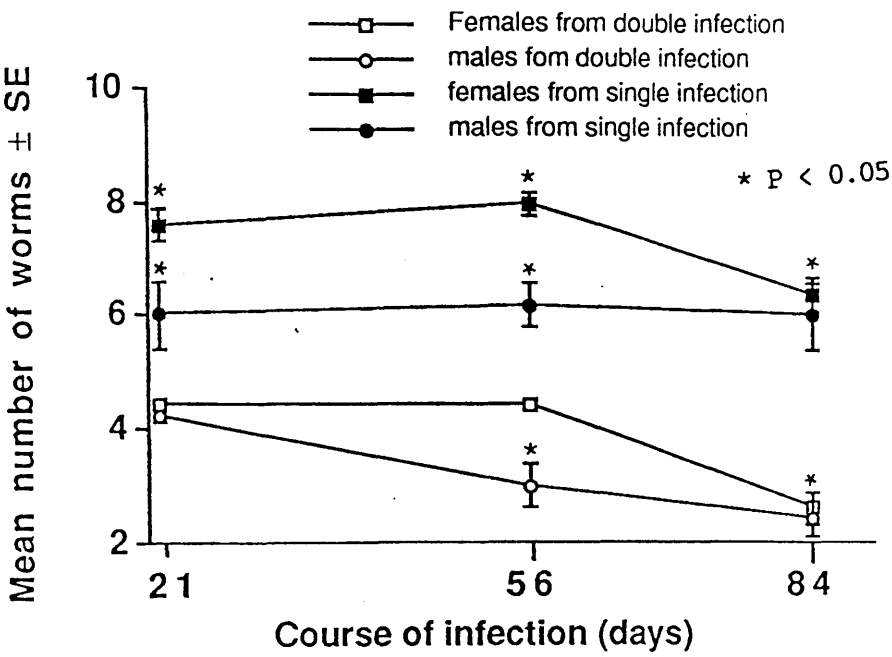


Fig. 3.1 Mean numbers of male and female *Moniliformis moniliformis* recovered from rats at days 21, 56, and 84 p.i., given mixed and single sex infections.

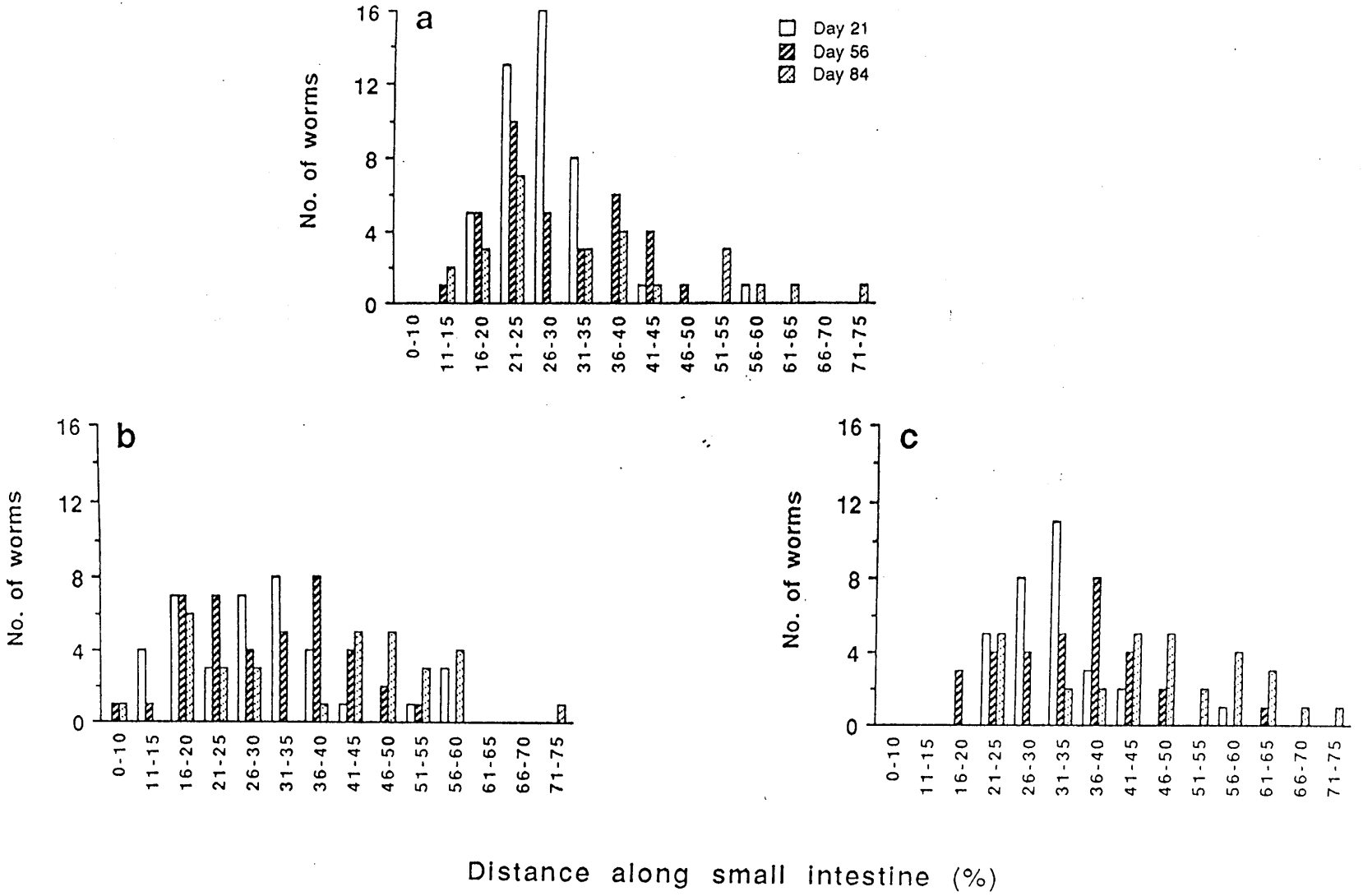


Fig. 3.2 Distribution of *Monilliformis moniliformis* along the length of small intestine in rats infected with a) worms of both sexes, b) females only, c) males only.

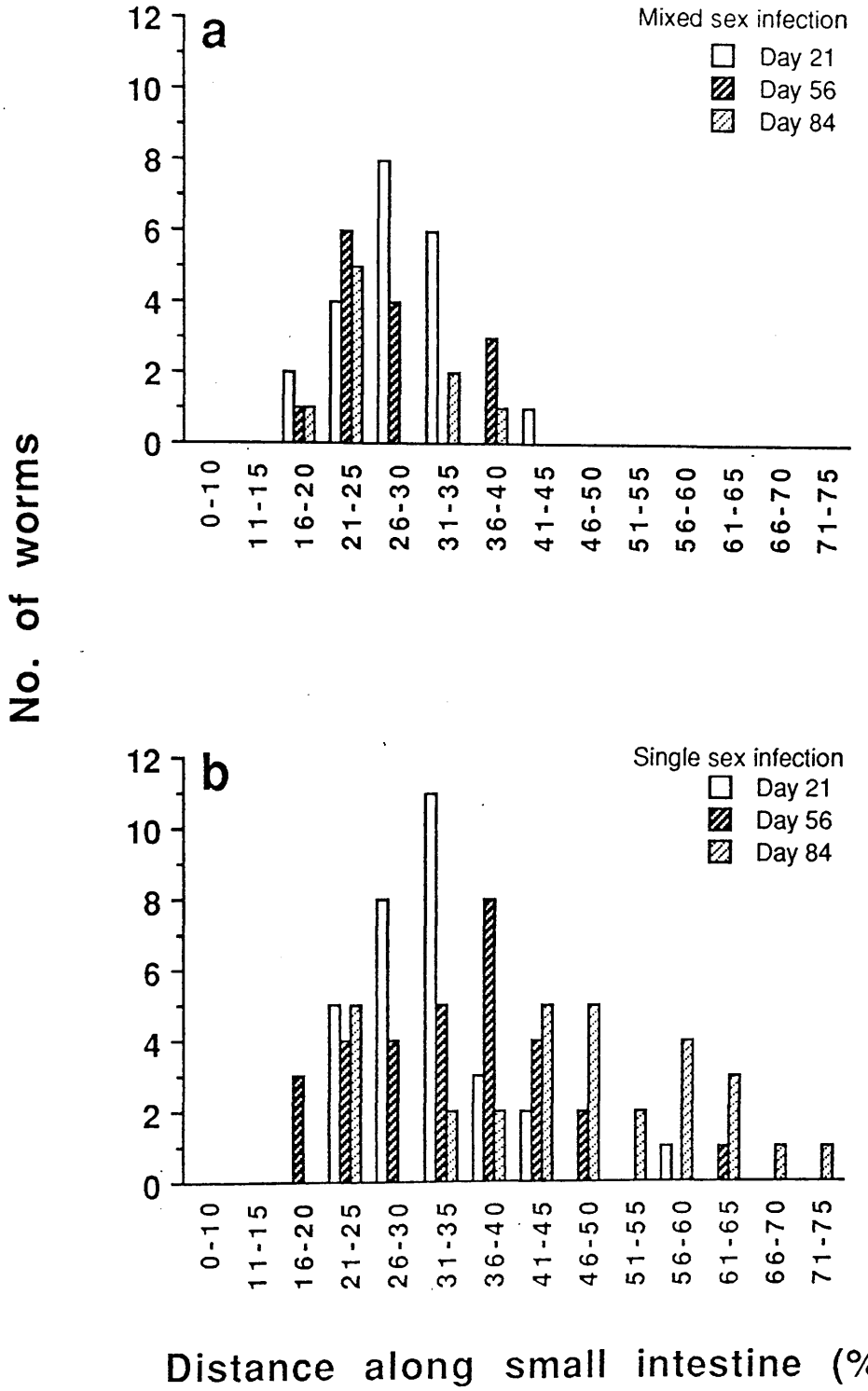
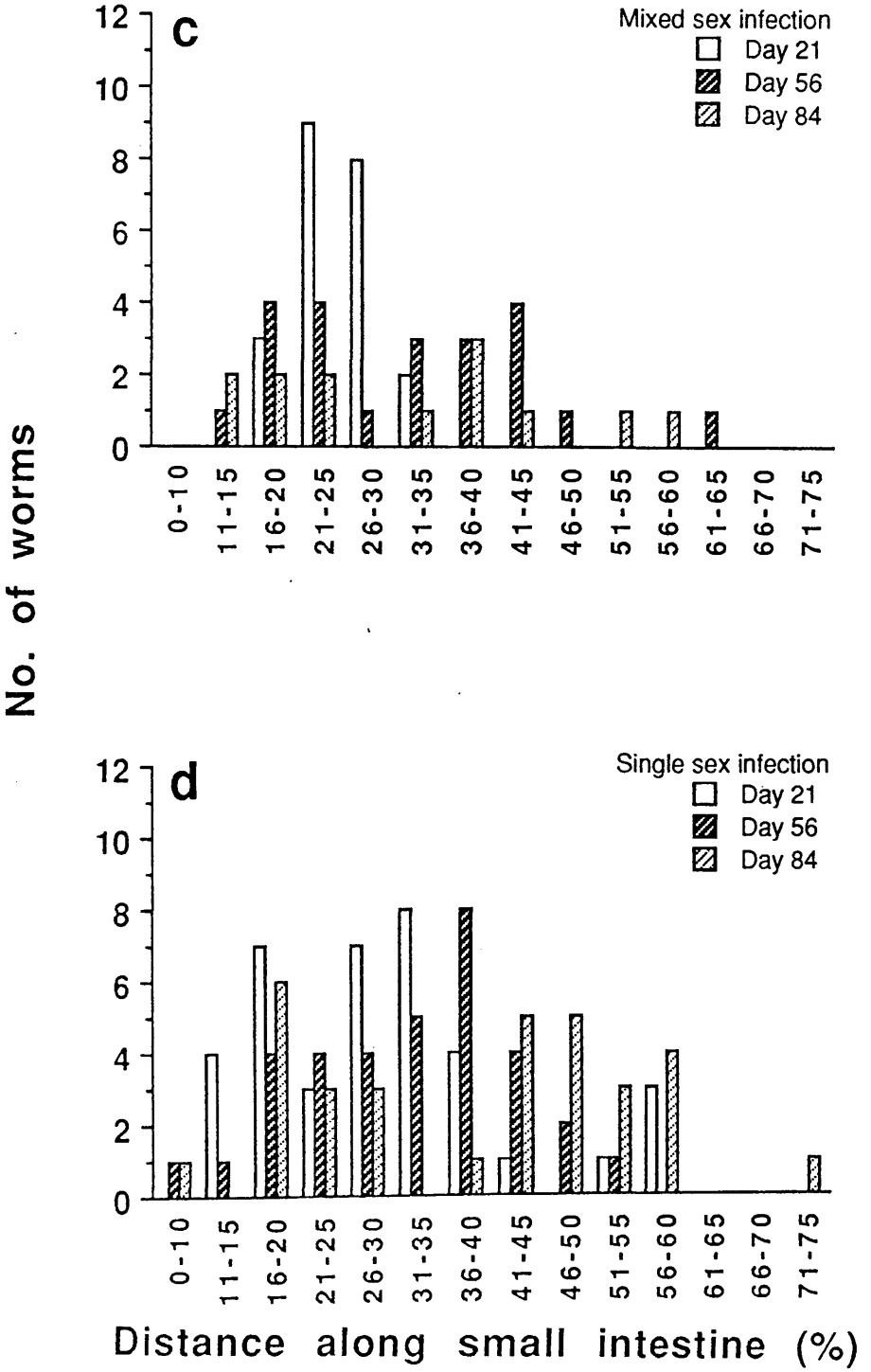


Fig. 3.3 A comparison between the distribution of *Moniliformis moniliformis* along the length of small intestine, recovered from mixed and single sex populations. (a-b) male worms (c-d) female worms


c
Mixed sex infection
Day 21
Day 56
Day 84
12
10
8
6
4
2
0
0-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
66-70
71-75
d
Single sex infection
Day 21
Day 56
Day 84
No. of worms
Distance along small intestine (%)

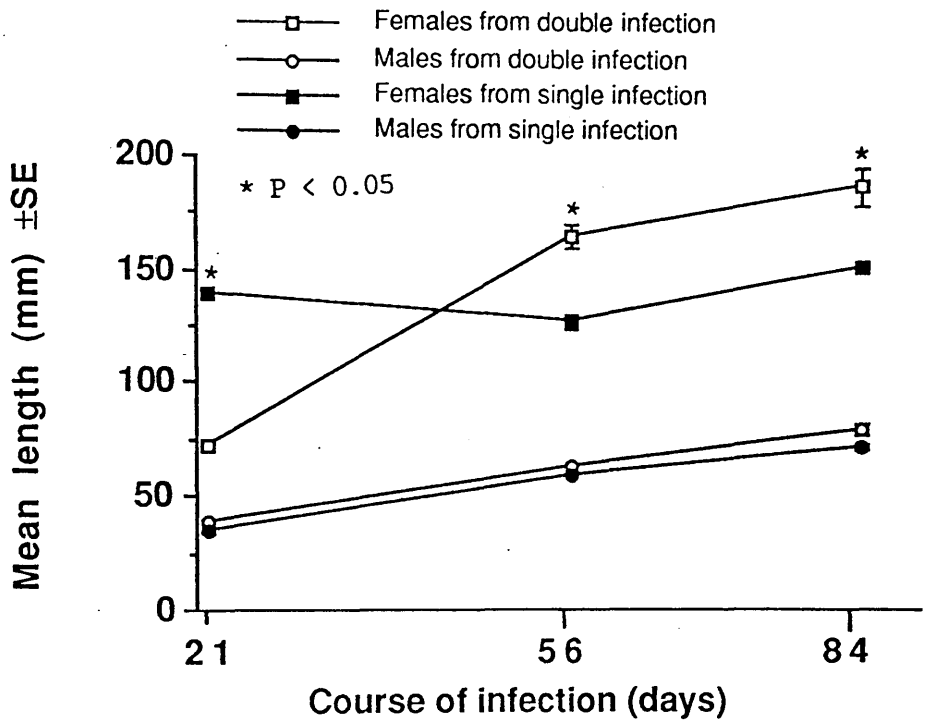


Fig. 3.4 Mean length ±SE of male and female *Moniliformis moniliformis* recovered from rats given mixed and single sex infections.

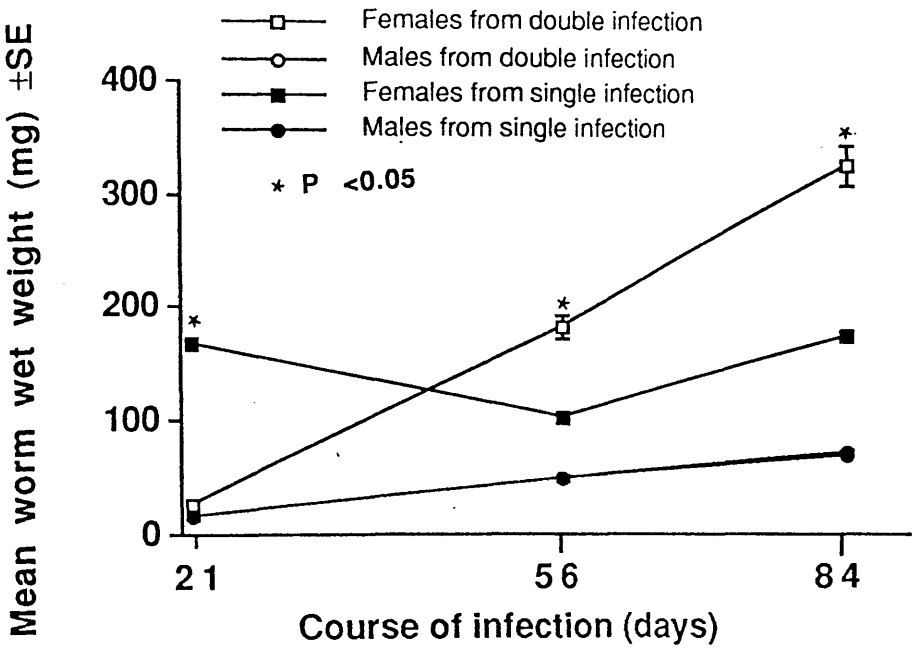


Fig. 3.5 Mean wet weights ±SE of male and female *Moniliformis moniliformis* recovered from mixed and single sex infections.

Table 3.2 Summary of the results from an experiment to investigate fate of secondary infections of Moniliformis moniliformis in rats.

No. rats infected	Duration of infection (days)	No. worms recovered mean ±SE	Dry weight male worms mean ±SE(mg)	Dry weight female worms mean ±SE (mg)
7	112	2.3 ±1.2	nd	nd
7	150 (primary) 35 (secondary)	0 10.7 ±3.7	- 5.4 ±1.1	- 21.4 ±1.6
7	35	11.1 ±2.3	6.1 ±1.0	23.1 ±0.8

nd= not determined

CHAPTER 4. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION : MATING BEHAVIOUR

4.1 INTRODUCTION

Accounts of aspects of reproduction of *Moniliformis moniliformis* and its course of infection in rats were described in chapters 1 and 3. Some observations on the mating behaviour of *M.moniliformis* in laboratory rats have been made by Abele and Gilchrist (1977) and by Crompton (1974) and in the absence of any evidence in the literature, to suggest that worms may be monogamous, it is generally assumed that polygamous mating occurs. Presumably individual worms mate with more than one partner of the opposite sex in the definitive host. Mating probability can be defined as the chance that an adult female worm will copulate successfully (i.e. become inseminated) with a male worm and produce fertile eggs. Many factors are expected to affect this chance in population of worms present in the small intestine, including physical and chemical factors and the structure of the worm burden. In the mating behaviour of dioecious animals there are 3 ways in which insemination between male and female worms occurs to produce zygotes; these are, polygamy, polygyny, and polyandry. In polygamy, individual worms may mate with more than one partner of the opposite sex. In polygyny, a male worm may consort with and mate with more than one female in the population, and in polyandry, a female may consort with and mate with more than one male (Jewell, 1976).

In the present study, experiments were undertaken (1) to detect the earliest occurrence of insemination in *M.moniliformis*, (2) to investigate polygyny and to estimate the number of female worms that would possibly be inseminated by an individual male worm, (3) to investigate male to male capping behaviour, and (4) to estimate the number of female worms inseminated in populations of varied sex ratios.

4.2 EXPERIMENTAL DESIGN

The sex of cystacanths of *M.moniliformis* was noted prior to infection of rats (see chapter 2 section 2.5) to set up structured populations of worms in the host's

intestine so that the mating behaviour of the parasite could be studied with some degree of control. Four experiments were undertaken. In experiment 1, for the detection of the earliest occurrence of insemination, 4 groups of female rats (n=7 per group) were infected with 1 male and 5 female cystacanths per rat on day 0. *Post mortem* examination of each group of rats took place after 15, 16, 17, and 18 days of infection. Experiment 2 was undertaken to investigate polygyny and to estimate the number of female worms inseminated by an individual male. Forty two female rats were infected, in groups of 7 or 8 as shown below.

Protocol for Experiment 2

No. rats per group	No. female cysta-canths given/rat with 1 male	Experiment ended (days p.i.)
7	9	21
7	9	28
8	5	35
8	10	35
8	20	35
4	40	35

In experiment 3, to investigate male to male capping behaviour in *M.moniliformis* from mixed and single sex populations, 14 female rats were used. On day 0, 7 rats were infected with 20 male cystacanths each, and the other 7 were given 15 unsexed cystacanths each. All the rats were killed on day 35 p.i. Experiment 4 was carried out to investigate the number of female worms inseminated in populations where male and female worms were in varied sex ratios. Forty-two rats, in groups of 7, were infected with 5 male and 5, 10, 15, 20, 25, and 30 female cystacanths per rat in their respective groups, and 14 rats in 2 groups of 7, were infected with 10, and 15 male and 5 female cystacanths per rat. *Post mortem* examination of all the rats took place on day 21 p.i.

4.3 RESULTS

At the *post mortem* examinations of all the rats, the numbers and sexes of the worms recovered per rat were recorded. Later, the body cavity contents of individual female worms were collected and examined for the evidence of insemination (see Chapter 2, section 2.5 for details). Worms of both sexes were examined for the presence of copulatory caps.

Experiment 1

4.3.1 DETERMINATION OF THE EARLIEST OCCURRENCE OF INSEMINATION IN *MONILIFORMIS MONILIFORMIS*

The total number of worms recovered and the numbers of female worms found to have been inseminated per rat group at days 15, 16, 17, and 18 p.i. are given in Table 4.1. In female worms aged 15 and 16 days, no evidence of insemination could be detected. However, a total of 15 out of 21, and 26 out of 30 female worms recovered on days 17 and 18 p.i. respectively, were found to have been inseminated. At this time, no free eggs in the body cavity contents of the inseminated females were observed, but zygotes (=early developing eggs) were found on the surface of the ovaries (see Plate 2.3).

Experiment 2

4.3.2 DETERMINATION OF POLYGYNY AND THE NUMBERS OF FEMALE *MONILIFORMIS MONILIFORMIS* INSEMINATED BY A SINGLE MALE WORM

The results of these experiments are also summarized in Table 4.1, but data from 6 rats in which none of the female worms, recovered at *post mortem* examination, were inseminated, are not included in the table. Out of 4 rats infected with 1 male and 40 female cystacanths, inseminated females were found from 1 rat. Only female worms were recovered from the other 3 rats and none of them was found to have been inseminated; male worms might have been lost from the rats before they had reached to maturity or might never have became established. The male worm from the rat given 40 female cystacanths, had inseminated at least 22 female worms (Table 4.1), but, on average, an individual male *M.moniliformis* was

observed to have inseminated 7 female worms of the same age. The presence of female worms that did not show any evidence of insemination, may be interpreted as suggesting that (1) copulation may have occurred very recently in these females with the result that zygotes could not be detected, (2) the males might have had reached the limit of their reproductive capacity and (3) they might have failed to locate all the females present in the small intestine. The results clearly indicate polygyny in *M.moniliformis*; one male worms mating successfully with more than one female worm in the population.

Experiment 3

4.3.3 MALE TO MALE CAPPING BEHAVIOUR IN *MONILIFORMIS MONILIFORMIS*

The results are shown in Table 4.2 and relevant data from experiment 4 are also included in the analysis. At day 21 p.i., from single sex infections (20 male cystacanths/rat) 4 out of 110 male worms recovered at *post mortem* examination of the rats, were observed with copulatory caps (see Plate 1.2). From mixed sex infections, 4 out of 38 (15 unsexed cystacanths/rat), 7 out 231 (20 male, 5 female cystacanths/rat) and 2 out of 88 (10 male, 5 female cystacanths/rat) male worms were observed to possess copulatory caps. No significant difference between the numbers of capped male worms could be detected when the numbers of worms from the 4 rat groups were compared.

Experiment 4

4.3.4 NUMBER OF FEMALE *MONILIFORMIS MONILIFORMIS* INSEMINATED IN POPULATIONS OF VARIED SEX RATIO

The results from these experiments are summarised in Table 4.3. It was observed that the proportion of inseminated females increased when the number of male worms in the small intestine increased. The number of female worms inseminated from the rat groups in which male *M.moniliformis* were present in sex ratios of 1:4 was significantly higher ($P < 0.001$) than the other groups. In rats, given 5 male and either 5, 10, 15, 20, 25 and 30 female cystacanths each no significant

difference was observed between the number of inseminated and uninseminated female worms.

4.4 DISCUSSION

The results of the experiments described in this chapter, revealed that, under the conditions imposed by the observer in laboratory, insemination between male and female *Moniliformis moniliformis* occurs at day 17 p.i.; Crompton (1974), also demonstrated similar results when he found that, some of the female worms that were in contact with males for 16 or 17 days of infection, and transplanted in donor rats, contained eggs in their body cavities on day 35 p.i. Depending on the number of worms present in the small intestine, an individual male worm was found to be capable of inseminating as many as 22 female worms of the same age.

The results from experiments 3 and 4 are of interest when considering the possibility of competition between male worms for female mates. Abele and Gilchrist (1977) studied the sexual selection, which they called homosexual rape, among acanthocephalan males. They proposed that "victim" had it's genital region sealed off by a copulatory cap made with cement by a "rival" worm. This action would temporarily remove the raped individual from the reproductive population. Acanthocephalans conform to the model where the female invests considerably more in reproduction than the male and, therefore, males would be expected to compete among themselves for female mates. Acanthocephalan sperm appear to be long-lived and so the potential exists for multiple inseminations resulting in progeny of mixed parentage indicating that sperm competition can occur (Abele and Gilchrist, 1977). This would result in evolutionary processes favouring males that either (1) displace stored sperm from previous inseminations or (2) reduce the probability of subsequent inseminations (Parker, 1970). Cement gland secretions and capping behaviour are considered to have the function of preventing the escape of sperm (described in Chapter 1 section 1.2.4), whereas Abele and Gilchrist (1977) emphasized the possibility that cement glands and capping behaviour evolved in response to sexual selection and function in preventing in the short term subsequent

Table 4.1 Observations on the results from the experiments to determine the earliest occurrence of insemination and the reproductive capacity of male Moniliformis moniliformis.

No. rats infected	No. rats examined	No. female cystacanths given/rat with 1 male	Age of all worms on recovery (days)	No. worms recovered male	female	No. females inseminated	No. females not insemi-nated	No. worms with copula-tory caps male	female
7	7	5	15	3	19	0	19	-	-
7	7	5	16	6	31	0	31	-	-
7	7	5	17	5	21	15	6	-	1
7	7	5	18	5	30	26	4	-	3
7	7	9	21	5	34	21	13	-	-
7	7	9	28	4	35	32	3	-	3
8	7	5	35	5	34	29	5	-	-
8	6	10	35	6	50	40	10	-	-
8	6	20	35	6	92	63	29	-	2
4	1	40	35	1	30	22	8	-	1

Table 4.2 Observations on male-to-male capping behaviour in Moniliformis moniliformis

Intended dose (cystacanths given/rat) male	female	Duration of infection (days)	No. of worms recovered male	female	No. of worms with copulatory caps male	female
20	--	21	110	--	4	--
10	5	21	88	49	2	4
20	5	21	231	57	7	15
15 unsexed		35	38	39	4	3

Chisquare = 5.851, ns

Table 4.3. Observations on the determination of number of female Moniliformis moniliformis inseminated in populations of varied sex ratios

No. rats infected	Intended dose/rat male	Intended dose/rat female	No. worms recovered male	No. worms recovered female	No. females inseminated	(%)	No. females not inseminated	No. worms with copulatory caps male	No. worms with copulatory caps female
7	5	5	19	13	7	(54)	6	-	-
7	5	10	25	23	15	(65)	8	-	2
7	5	15	23	51	42	(82)	9	-	3
7	5	20	21	63	44	(70)	19	-	2
7	5	25	19	75	42	(56)	33	-	3
7	5	30	12	80	34	(43)	46	-	3
7	10	5	88	49	44	(90)	5	2	4
7	20	5	231	57	* 53	(93)	4	7	15

* Chisquare = 62.481; P <0.001

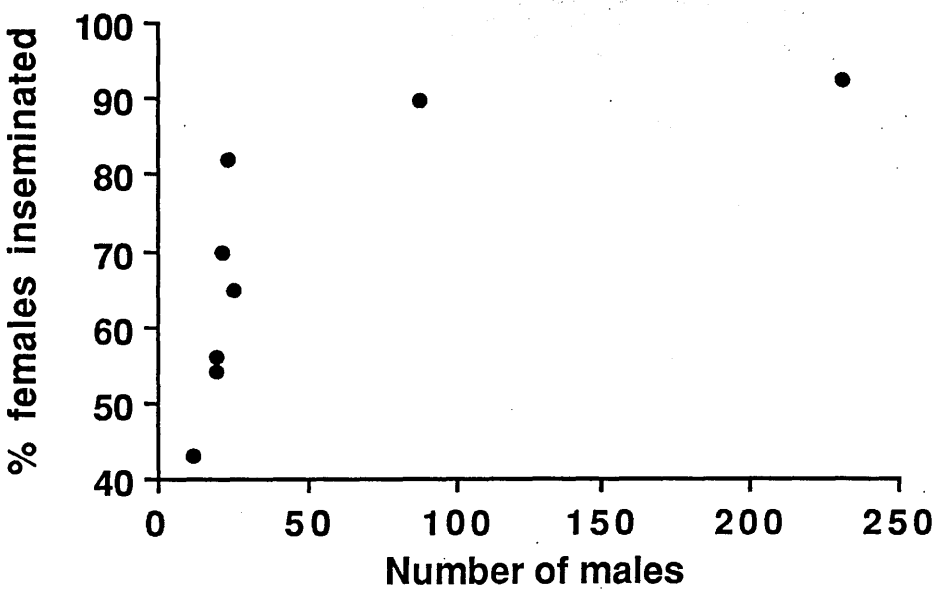


Fig. 4.1 Percentage of female *Moniliformis moniliformis* inseminated in rats given varied number of male worms. Spearman's rank correlation co-efficient = 0.922; P <0.01

inseminations by other males. Ball (1930), suggested that the caps prevent sperm from entering immature females but this idea seems to have been was based on the misconception that the females themselves secrete the caps. The results from the present study also provide evidence that male *M.moniliformis* do not cap themselves because the results from the rats deliberately infected with only one male cystacanth showed that capped males were never present. The distribution in time and space of reproductive females also affects sexual selection among males (Trivers, 1972; see Chapter 6). In *M.moniliformis* female worms are distributed in the intestine, and all attain maturity from a single infection at about the same time. Thus the availability and location of females may result in severe interactions between the worms in the population present.

4.5 SUMMARY

Under the experimental conditions used, insemination was observed to occur between male and female *Moniliformis moniliformis* as early as 17 days p.i., and an individual male worm was observed to be capable of insemination at least 22 female worms of the same age. The results provide the evidence that the copulatory caps on male worms are mounted by other male/s in the population which might result from a possible sperm competition. The results also suggest that the numbers of inseminated female *M.moniliformis* increases with the increase in the numbers of worms of the opposite sex.

CHAPTER 5. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION : MALE WORM AGE

5.1 INTRODUCTION

The number of infective eggs produced by a female worm is a measure of fecundity which according to Cohen (1977), depends directly upon the number of viable oocytes which are formed in a female and on the successful transfer of sufficient quantity of competent spermatozoa from a male to a female. These events are expected to be affected by a number of environmental factors. Recently, experimental work has indicated that size and location of female *Moniliformis moniliformis* in the host gut may be two of the factors that influence either fecundity or the opportunities for insemination (Crompton *et al.*, 1988 a,b).

Under natural conditions, hosts are unlikely to harbour primary infections of *M.moniliformis* based on the simultaneous ingestion of a number of cystacanths. The aims of the work described in this chapter were to investigate, under laboratory conditions, the relationship between age and mating behaviour of *M.moniliformis* and to detect the effects of male worm age on female worm fecundity.

5.2 EXPERIMENTAL DESIGN

The research strategy was to provide female worms with males of either the same or different age, so that effects of male worm age on mating preference and fecundity could be examined.

Twenty one female rats were used. Rats were divided into three groups (A, B, and C) of 7 rats each. Each rat received 10 male and 10 female cystacanths (Australian/ Molteno isolate). In the experimental design, rats of group A received male and female cystacanths on day 0, those of group B received males on day 0 and females on day 7 p.i., whereas rats of group C received females on day 0 and males on day 7 p.i. The experiment ended when female worms were 35 days old in each group. Thus, male worms at *post mortem* examination of the rats were aged 35, 42, and 28 days in groups A, B, and C respectively.

5.3 RESULTS

At post mortem examination, the numbers of *M.moniliformis* recovered, their attachment positions in the small intestine and their lengths were recorded. Later the insemination status and fecundity of individual female worms was assessed (see Chapter 2 Section 2.6.1-2).

5.3.1 ESTABLISHMENT OF *MONILIFORMIS MONILIFORMIS*

All the results are summarized in Table 5.1. The total number of worms recovered from the rats of 3 groups revealed no significant difference between them. On average, the number of female worms recovered from group B and those of males recovered from group C, was higher revealing a significant difference ($P < 0.05$) between the 2 groups. From group A, however, worms of both sexes were recovered with a sex ratio of 1:1 (see Fig. 5.1).

5.3.2 LOCATION AND GROWTH OF *MONILIFORMIS MONILIFORMIS*

The mean percentage attachment positions in the host's small intestine and lengths of male and female worms are given in Table 5.1. No statistically significant difference in the mean attachment positions of the worms along the distance of small intestine was observed between the 3 groups of rats. The length of each worm collected from the small intestine was recorded to give an estimate of growth. Worms from group C were observed to be smaller than those recovered from groups A and B and a significant difference ($P < 0.01$) in mean lengths was observed between the 3 groups (see Fig. 5.2).

No significant correlation between the worms of 3 groups was detected as regards female length and attachment position.

5.3.3 ASSESSMENT OF FECUNDITY

Individual female worm body cavity contents were collected and the numbers of free ovaries, immature and mature eggs were estimated.

5.3.3.1 NUMBER OF FREE OVARIES PER FEMALE WORM

Estimates of the mean number of ovaries (±SE) per female *M.moniliformis*

from the 3 groups of rats are given in Table 5.1 and illustrated in Fig. 5.3. The analysis of variance of the data revealed a significant difference ($P < 0.05$) between the mean number of ovaries per female worm from the 3 groups. Although a considerable variation in the actual number of ovaries per female worm from an individual rat was observed, female worms from group A were found to contain consistently fewer ovaries than female worms from groups B and C.

5.3.3.2 NUMBER OF IMMATURE EGGS PER FEMALE WORM

At *post mortem* examination, all female worms recovered from the 3 groups of rats were found to have been inseminated and contained eggs at various stages of development in their body cavities. Estimates of the mean number of immature eggs (±SE) per female worm are shown graphically in Fig. 5.4. The graph indicates that the lowest number of immature eggs were found in worms from group A. The numbers of immature eggs in worms recovered from each rat within a group was extremely variable. The analysis of variance of the data revealed a significant difference ($P < 0.05$) between the mean number of immature eggs per female worm from the 3 groups. A linear regression analysis revealed a relationship between the number of ovaries and the number of eggs per female worm from the 3 rat groups. (Fig. 5.6).

5.3.3.3 NUMBER OF MATURE EGGS PER FEMALE WORM

Although the number of female worms containing mature eggs was observed to be higher in group C than in groups A and B (Table 5.1), the difference was not significantly so. These female worms, however, were found to contain a significantly lower number of mature eggs when compared with the female worms from groups A and B ($P < 0.05$). The mean estimated number (±SE) of mature eggs per female worm, illustrated in Fig 5.5, was 126 ±15, 99 ±12, and 66 ±10 in worms from groups A, B, and C respectively.

5.3.4 RELATIONSHIP BETWEEN FEMALE WORM ATTACHMENT POSITION, GROWTH AND FECUNDITY

No evidence was found to indicate that the attachment position of female

worms in the small intestine had any effect on either the growth or fecundity of female worms. A significant negative correlation ($P < 0.05$) between the length and the number of eggs per female worm (i.e smaller worms produced more eggs), was detected, suggesting that, under the experimental conditions used, female worm fecundity does not directly depend upon the length of the female worms.

5.3.5 RELATIONSHIP BETWEEN MALE WORM AGE AND FEMALE WORM FECUNDITY

It was observed from the results that the female worms from group C contained more eggs in their body cavities than the female worms from groups A and B. This could be related either to the number of male worms present in the rats of the respective group or to the differing age of the worms. No evidence could be detected for the relationship between the number of male worms present and the number of eggs produced per female worm. However, a significant correlation ($P < 0.01$) was observed when the number of eggs per female worm and the age of the male worms were compared suggesting that different ages of *M.moniliformis* favour fecundity.

5.4 DISCUSSION

The present investigation has described a relationship between age and fecundity of *Moniliformis moniliformis*. Reproduction in the Acanthocephala depends on the successful establishment of male and female worms in the small intestine, insemination, fertilization, mature egg production and longevity (Crompton *et al.*, 1988 a,b). In the present investigation there is no evidence of intraspecific competition for establishment sites or resources such as nutrients (Keymer *et al.*, 1983). There is also no evidence of competition for mates or sexual selection (Abele and Gilchrist, 1977).

The results suggest a possible effect of differing age of male and female worms on fecundity of *M.moniliformis*. The significantly lower value associated with 35-day-old male worms may be related to the cycles in insemination by the males or

to the number of ovaries per female worm. The significantly higher number of mature eggs in female worms from this group suggests that the females might have been inseminated earlier than the females from groups B and C. Crompton *et al.*, (1976) observed that the number of ovaries in female *M.moniliformis* increased with the age of the worm and that uninseminated females, on average, contained more ovaries than the inseminated females which might be due to less synthetic activity and slower tissue turnover rate when the zygotes are not being produced. Assuming that insemination of most of the females in group A, where both male and female worms were of same age, occurred when they were 16 days old (Crompton, 1974) and those of group C, when they were 23 days old (by which time male worms would be 16 days old), then the number of ovaries in female worms from the latter group would be greater than those from group A. Because fertilization in the Acanthocephala involves a reaction between spermatozoa and mature oocyte at the surface of free ovaries, the greater the surface area relative to the mass of ovarian tissue and the greater the rate of mature oocyte production, the better will be an individual female worms's chances to pass on its genes to the next generation (Crompton, 1985). The higher number of ovaries in female worms from group C appears to favour male worms as these females were also found to contain higher number of eggs in their body cavities indicating that these females were more fecund than the females from other groups. Assuming that the passage of an individual's genes to the next generation is the driving force in mating behaviour, then from this investigation it would appear that this is served more efficiently when the male and female worms are of different ages.

Under natural conditions it is unlikely that definitive hosts harbour infections of *M.moniliformis* based on all the cystacanths being ingested simultaneously. By varying the age of the males an attempt was made to simulate a more natural situation. The fecundity pattern observed in this study appears to be closer to what may happen in a naturally occurring infection where worms are lost from and recruited by the definitive host over a period of time. It is important to note that this was a horizontal study where fecundity was measured only when female worms

were 35 days old.

5.5 SUMMARY

During the course of a primary infection of *Moniliformis moniliformis* in rats, involving an experimentally structured population of worms derived from infective doses of 10 male and 10 female cystacanths per rat, the fecundity of the female worms appeared to be influenced by the age of males. On average, the estimated mean number of eggs per female worm recovered from rats containing 28-day-old males was 20,930 $\pm$2,143, that from rats containing 35-day-old males was 7,434 $\pm$937 and that from rats containing 42-day-old males was 14,396 $\pm$1,704; these values were found to be significantly different (Least significant difference, $P \leq 0.01$). The results suggest that the variable age of the worms would be likely to favour fecundity in naturally occurring infections.

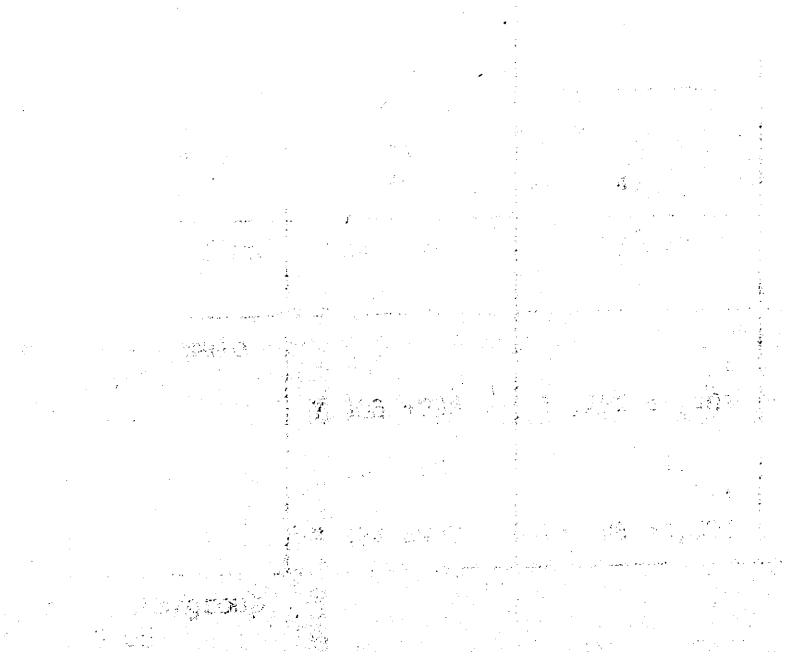


Table 5.1 Observations on the reproductive status of 35-day-old female Moniliformis moniliformis in rats harbouring male worms of differing age.

Rat group [a]	A	B	C
Male worm age on recovery (days)	35	42	28
No. worms recove -red	99	90	97
Male worms	49	34	59*
Female worms	50	56*	38
Attachment posit -ion mean ±SE			
Male worms	35.5 ±1.1	32.6 ±1.3	33.4 ±0.8
Female worms	29.2 ±1.4	30.0 ±1.1	31.1 ±1.4
Length/worm mean ±SE			
Male	57.9 ±0.5	59.3 ±0.6	54.0 ±0.6
Female	162.9 ±1.1**	151.7 ±2.3	129.1 ±2.0
No. females inse- minated	50	56	38
No. females with			
Immature eggs	11	9	5
Mature eggs	39	47	33
No. ovaries/fema -le mean ±SE	246 ±28	393* ±27	508* ±37
No. eggs/female mean ±SE			
Immature	7,308 ±935*	14,296 ±1,701*	20,863 ±2.141*
Mature	126 ±15	99 ±12	66 ±10
All	7,434 ±937	14,396 ±1,704*	20,930 ±2,143*

a, n=7 rats/group
* $P < 0.05$
** $p < 0.01$

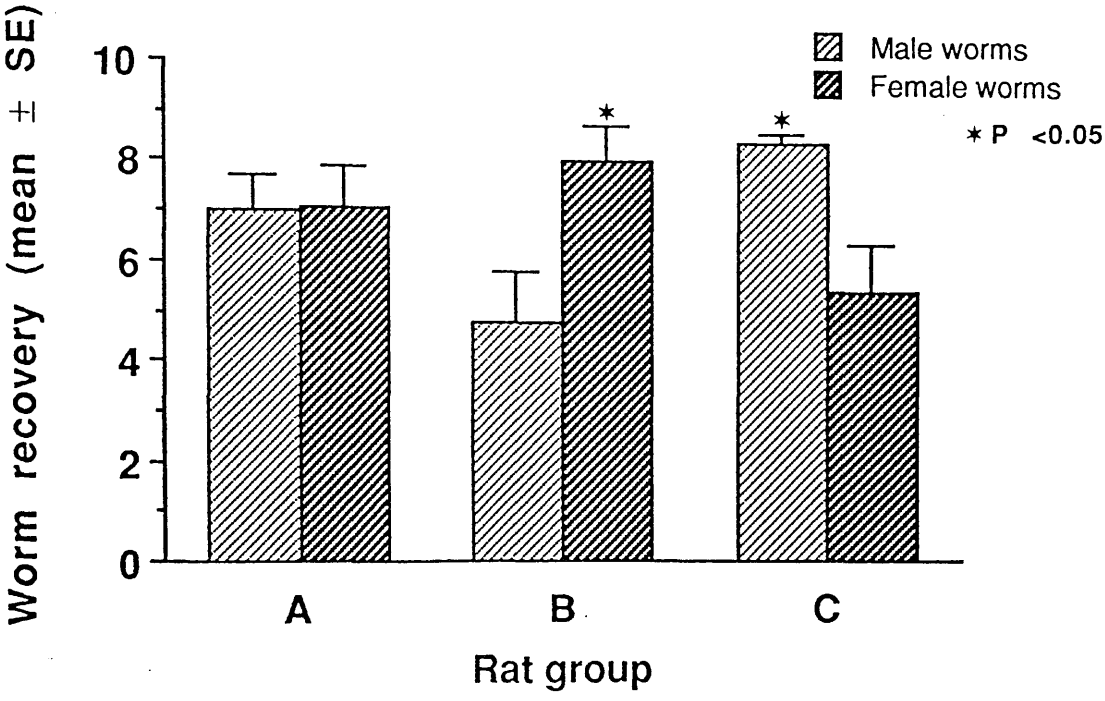


Fig. 5.1 Mean ±SE recoveries of male and female *Moniliformis moniliformis* from rat groups where male worms were (A) 35, (B) 42 and (C) 28 days old respectively with 35-day-old female worms.

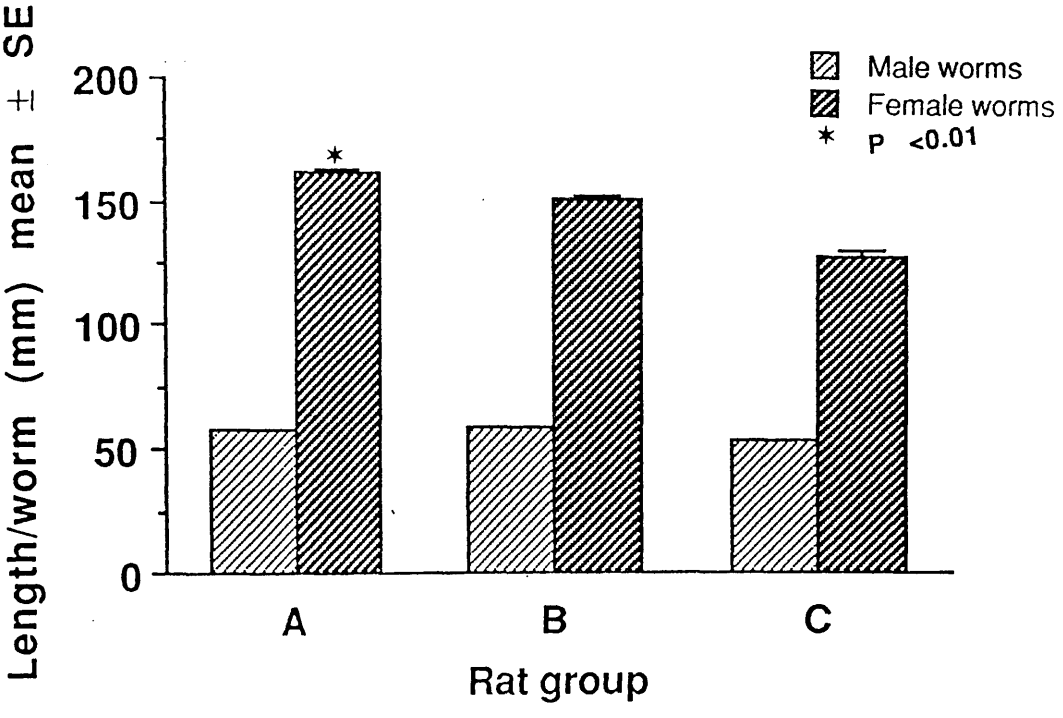


Fig. 5.2 Estimated mean ±SE length (mm) of male and female *Moniliformis moniliformis* from rat groups where male worms were (A) 35, (B) 42, and (C) 28 days old respectively with 35-day-old female worms.

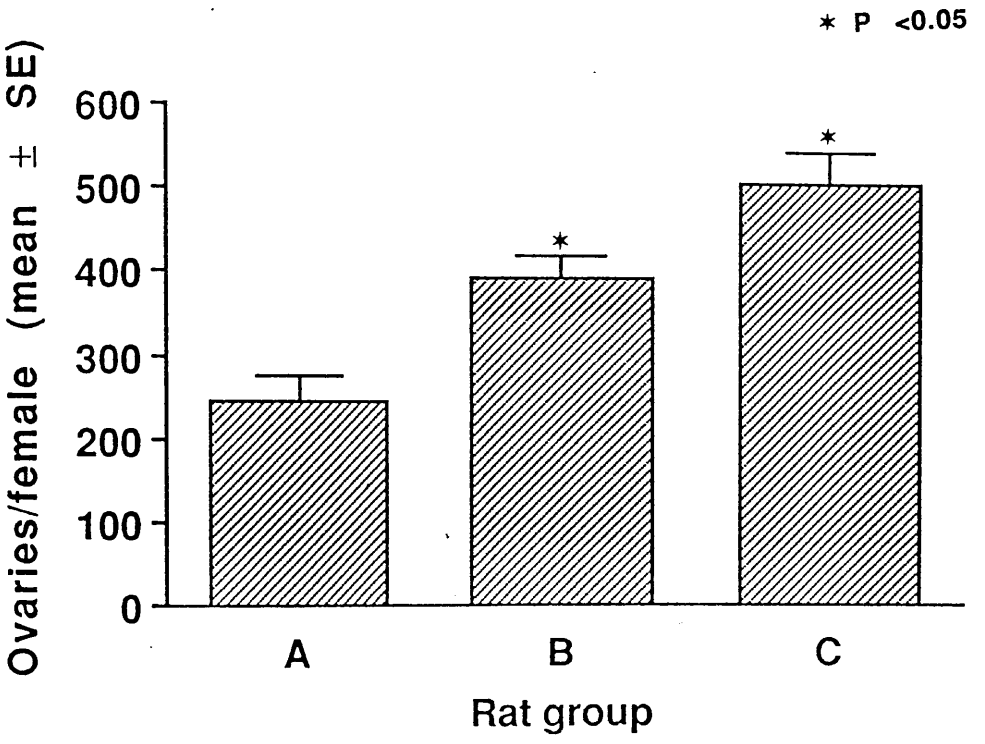


Fig. 5.3 Estimated mean ±SE number of ovaries per 35-day-old female *Moniliformis moniliformis* from rat groups where male worms were (A) 35, (B) 42 and (C) 28 days old respectively.

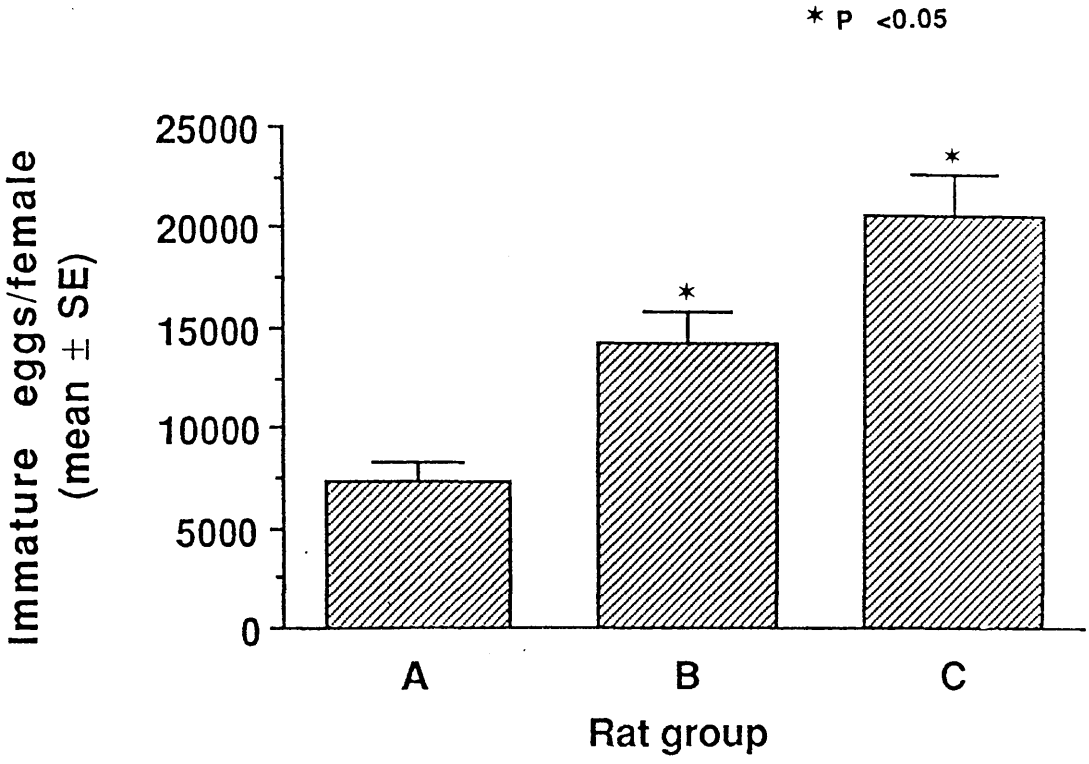


Fig. 5.4 Estimated mean ±SE of immature eggs per 35-day-old female *Moniliformis moniliformis* from rat groups where male worms were (A) 35, (B) 42 and (C) 28 days old respectively.

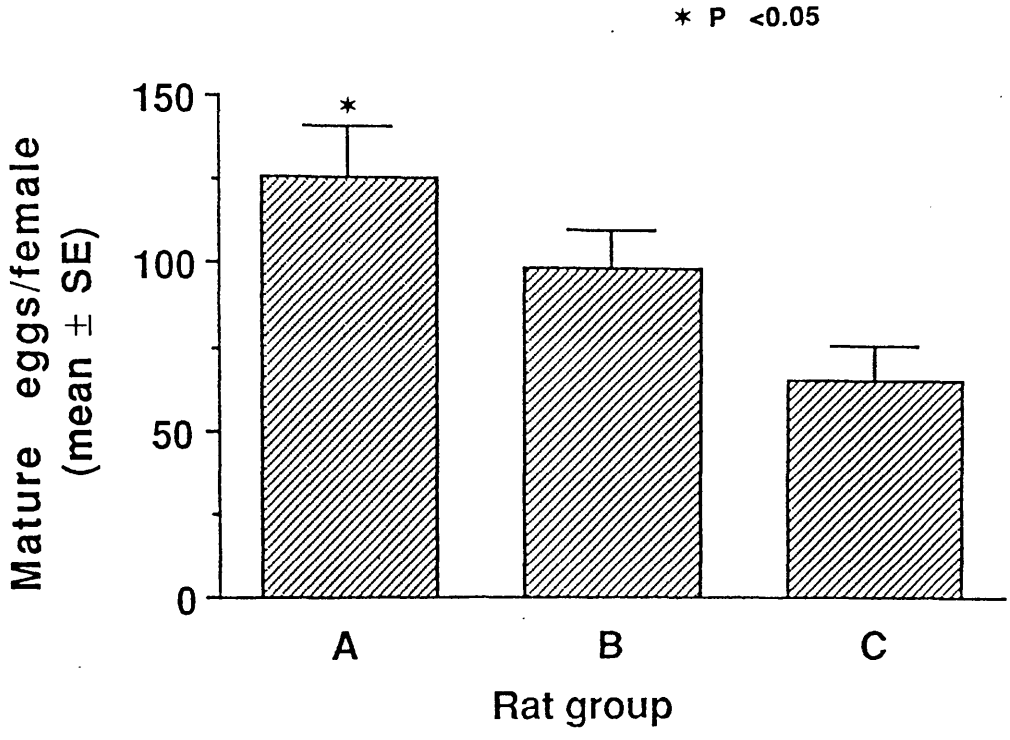


Fig. 5.5 Estimated mean ±SE number of mature eggs per 35-day-old female *Moniliformis moniliformis* from rat groups where male worms were (A) 35, (B) 42 and (C) 28 days old respectively.

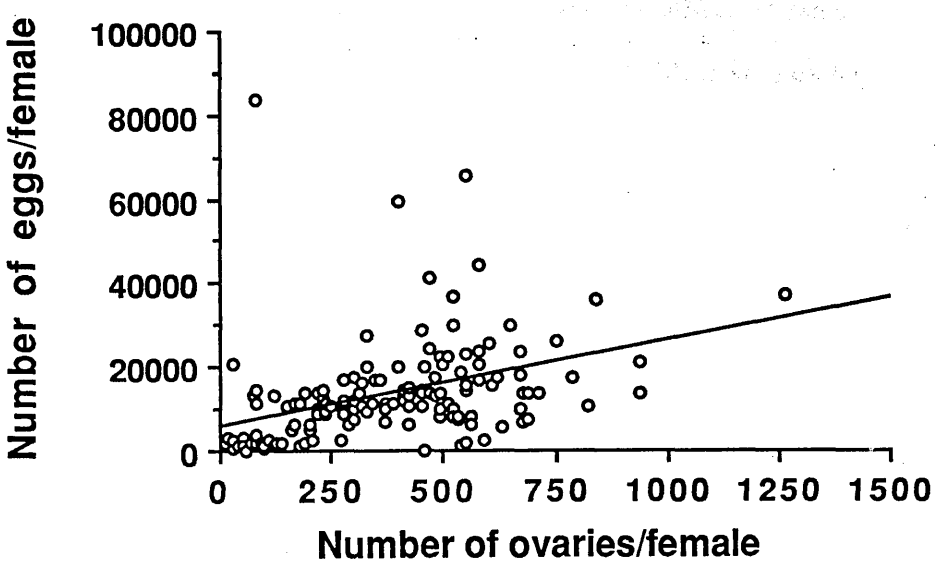


Fig. 5.6 Relationship between the number of ovaries and eggs per female *Moniliformis moniliformis* recovered from the 3 rat groups. One-way regression analysis, $P < 0.001$.

CHAPTER 6. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION : MATE- CHOICE BY MALES?

6.1 INTRODUCTION

In a strictly dioecious species, mating preferences and competition between the sexes would be expected to lead to the evolutionary process which was called sexual selection by Darwin (1871). Sexual selection involves a preference for characters giving certain individuals an advantage over the others of same sex in obtaining successful mates (Partridge and Halliday, 1984). Most studies have revealed that females tend to be the "choosy" sex, where as males tend to compete for access to females and are expected to be less choosy. This is thought to be because the reproductive investment, and therefore the cost of misallocating the resources to a particular mating, is smaller for males than for females. Under certain circumstances, males should maximize their reproductive success by mating with the most fecund females. Thus, if a situation should occur in a species in which male investment in mating is high, choice by males of fecund females should be favoured. Fecundity in many invertebrates, is correlated with female body size (see Greenspan, 1980; Coadwell and Ward, 1982; Elgar and Pierce, 1988) and males of some free-living invertebrate species have been shown to mate preferentially with large females (Ridley, 1983).

There is marked sexual dimorphism amongst the Acanthocephala (Parshad and Crompton, 1981). Female *Moniliformis moniliformis* can grow to over 200 mm in length and males to about 80 mm given favourable conditions in the host's intestine; and female fecundity varies accordingly with large females producing more eggs (= offspring) than small ones. It may be expected, therefore, that males might be choosy about which females to mate with. Previous work by Crompton (1974) has determined an upper limit to the total number of females each male can inseminate. After insemination, the male temporarily seals the female gonopore with a copulatory cap made from the secretions of the cement glands. Although the copulatory cap will function to prevent the loss of sperm from the female, Abele

and Gilchrist (1977) believed that the copulatory cap could also function as an adaptation for sexual selection because it would prevent, for a time, subsequent inseminations by other males and so might stimulate competition between males for access to the best females (Crompton, 1985). Male *M.moniliformis* have been observed to place copulatory caps on other males (Abele and Gilchrist, 1977) possibly to remove temporarily competitors from the breeding population. These observations also suggest that male *M.moniliformis* might be expected to exert mate choice. In addition, since one male can inseminate several females and since males have more nervous tissue than females (Miller and Dunagan, 1985), it seems plausible to expect males to seek out females.

The aims of the present study were to investigate the mating behaviour of *M.moniliformis* with special regard to the possible existence of male choice of female mates. This type of mate choice could be investigated by exposing males to a variety of females of different age and size and then comparing the attributes of the females that were inseminated with those that were not. Host dietary carbohydrate content was manipulated in one experiment because this is known to influence the growth of *M.moniliformis* in rats in a precise manner (Parshad *et al.*, 1980). Thus the host's diet can be used to promote differing degrees of growth among females that would be of the same age.

6.2 EXPERIMENTAL DESIGN

6.2.1 FEMALE WORM AGE AS AN INFLUENCE ON MALE MATE CHOICE

In order to discover whether male worms choose to mate preferentially with females in terms of age, two experiments were carried out. Ten female rats were used in each experiment and were infected as shown in Table 6.1 and in the diagrams below.

Experiment 1

10 rats

Day 0	Day 35	Day 63	
15 female cystacanths	15 female + 2 male cystacanths	*post mortem* examination of all rats	females aged 63 days females aged 28 days males aged 28 days

Experiment 2

10 rats

Day 0	Day 14	Day 21	Day 49	
15 female cystacanths	15 female cystacanths	2 male cystacanths	*post mortem* examination of all rats	females aged 49 days females aged 35 days males aged 28 days

At the *post mortem* examination of all the rats, the numbers and sexes of worms recovered were noted, their attachment positions along the length of the small intestine and their lengths, to the nearest mm, were recorded. Attention was given to the location of female position in the small intestine because Burlingame and Chandler (1941) considered it to be of significance to the course of infection and Crompton (1975) showed that maximum body contact between male and female *M.moniliformis*, possibly associated with mating, occurs in a particular region of the intestine. Female worm body cavity contents were collected, as described in Chapter 2 section 2.5, and were subsequently examined for evidence of insemination. Individual female fecundity was assessed by sampling and counting the numbers of immature and mature eggs. A criticism of this experimental design is that older females, aged 63 days and 49 days (experiments 1 and 2 respectively), will be larger than younger females, aged 28 and 35 days, so that the observer might not be able to separate age and size as influential factors on male mate choice. While it is accepted that this point is valid the experiments nevertheless yielded some interesting new results (see below).

6.2.2 FEMALE WORM SIZE AS AN INFLUENCE ON MALE MATE CHOICE

To examine whether male *M.moniliformis* choose to mate with larger rather than smaller females when both are available, two further experiments were undertaken.

In the first experiment, now redesignated as experiment 3, data from experiment 2 (female age) were used and analysed after arbitrarily classifying females into 2 groups according to their lengths. Group 1 females measured $\geq$ 100 mm in length and group 2 females measured $<$ 100 mm in length.

Rats in experiments 1,2, and 3 were allowed to feed *ad libitum* on the standard commercial diet, but in the experiment, identified as experiment 4, 2 weeks prior to infection and during the course of infection, the rats were allowed to feed *ad libitum* on an experimental diet containing 6% fructose (w/w). During the initial period of 2 weeks before infection, the rats were fed on this diet to give them adequate opportunity to become adjusted to it. The diet was prepared from fructose, maize oil, casein, celluse powder and essential nutrients, as described by Crompton *et al.*, (1983) (see Chapter 2 section 2.8). Forty five male rats were used as hosts. Each rat was infected with 30 female and either 1 or 2 male cystacanths of *M.moniliformis* on day 0. At day 35 p.i. all rats were killed and the worms were recovered.

This work (experiment 4) was carried out by me in collaboration with colleagues (B. Lawlor, A.F. Read and A.E. Keymer) at the Department of Zoology, University of Oxford. Ten rats were based in Glasgow and 35 in Oxford and the results have now been accepted for publication as a paper entitled "Non-random mating in a parasitic worm: mate choice by males?" in *Animal Behaviour*. My contribution to this set of experiments carried out in collaboration with Oxford was to calculate the lengths of the worms and their attachment positions in the small intestine of rats at *post mortem* examination. In addition, I then examined the body cavity contents of each of the 456 female *M.moniliformis* recovered, for evidence of insemination and in order to assess quantitatively their fecundity by counting immature and mature eggs (see Chapter 2 section 2.6.2). Analysis of the data from the Glasgow and Oxford rats produced no evidence to suggest that the data should not be pooled and treated as one set.

6.3 RESULTS

The overall results from the 4 experiments are summarized in Tables 6.1. and 6.2. In 22 rats, none of the female worms recovered at the *post mortem* examination was found to have been inseminated. The data from them is not included in the tables because these rats were also found not to contain male worms. At *post mortem* examination of the rats females worms were classed as older or younger according to their appearance; older females being judged to be more robust and larger in body length.

6.3.1 OBSERVATIONS ON FEMALE AGE AND MALE MATE CHOICE

Experiment 1

Male and female *Moniliformis moniliformis* were recovered from 7 out of 10 rats (Table 6.1). On average, 12.8 female and 1.2 male worms were recovered from each rat. A total of 12 out of 35 (34%) older females and 35 out of 55 (63%) younger females were found to have been inseminated and contained eggs in their body cavities. The number of female worms inseminated from the younger population was found to be significantly higher ($P < 0.05$) than the number of females from the older population. An analysis of variance revealed a significant difference between the lengths of female worms from the 2 populations ($P < 0.05$). Older female worms, on average, measured 136 mm in length and younger females measured 82 mm in length. The interesting point observed here was that the older inseminated females were significantly smaller ($P < 0.05$) than the older uninseminated female worms (mean length= 126 mm and 140 mm respectively). However, inseminated younger female worms were found to be significantly larger ($P < 0.05$) than the uninseminated younger females (mean length= 85 mm and 73 mm respectively). For some reason, the male worms switched from mating with the older to the younger female worms.

Observations on the attachment positions of worms in the small intestine were also interesting. On average, female worms (inseminated and uninseminated) from older and younger populations were found to be attached in the zone ranging from

15-45% of the distance along the small intestine. No significant difference between the attachment positions of the older inseminated and uninseminated females could be detected. However, inseminated younger females were found to be attached significantly more anteriorly ($P < 0.05$) than the uninseminated female worms of both populations. The range in attachment positions of inseminated and uninseminated female worms from both populations is illustrated diagrammatically in Fig 6.1 a-b.

The numbers of eggs (immature and mature) per female worm were estimated to investigate the reproductive status and fecundity of female worms (Table 6.2). Analysis of variance revealed significant differences between the estimated mean numbers of immature eggs per older and younger female worm ($P < 0.05$). Older females were also found to contain significantly more ($P < 0.05$) mature eggs (mean $\pm$SE= 1892 ± 233) in their body cavities than the younger female worms (mean $\pm$SE= 457 ± 62) (Table 6.2), indicating conclusively that the older female worms must have been inseminated earlier than the younger ones, despite the fact that the males and younger females were of the same age. Clearly, significant difference between the total numbers of eggs per older and younger female was also observed ($P < 0.05$) (Table 6.2).

Experiment 2

Moniliformis moniliformis of both sexes were recovered from each of the 10 infected rats, but inseminated female worms (older and younger) were found in 9 out of 10 rats; the male worm in the rat which harboured no inseminated females, was found to be very small (15 mm in length) and was considered to be sterile. On average, 19.6 female and 1.5 male worms were recovered from each rat. A total of 35 out of 84 (42%) older females and 45 out of 93 (48%) younger female worms were found to have been inseminated (Table 6.1). No significant difference between the numbers of inseminated and uninseminated females from the 2 populations could be detected. These females were closer in age (14 days difference) than the female worms involved in experiment 1 (35 days difference). Approximately 80% of the worms were found to be confined in the region ranging from 15-45% of the

distance along the small intestine. As was found with the female worms from experiment 1, no significant difference between the attachment positions of older inseminated and uninseminated females could be detected, but inseminated younger females were found to be attached significantly more anteriorly than the uninseminated females from both populations ($P < 0.05$) (Fig. 6.2 a-b).

Female worms from the older population measured, on average, 114 mm in length, and those from the younger population measured, on average, 83 mm in length. A significant difference between the lengths of older and younger females was observed ($P < 0.05$). Unlike worms from experiment 1, no significant difference between the lengths of inseminated and uninseminated female worms of a given age could be detected. Investigation of the reproductive status of females revealed no significant difference between the numbers of immature eggs from the older and younger females, but, again the older females were found to contain significantly more mature eggs (mean 582 $\pm$116) than the younger females (mean 260 $\pm$53) ($P < 0.05$), indicating that older females must have been inseminated before the younger ones (Table 6.2). No significant difference between the total numbers of eggs per older and younger female worm could be detected.

6.3.2 OBSERVATIONS ON FEMALE SIZE AND MALE MATE CHOICE

Experiment 3

Data collected from experiment 2 were analysed after classifying female worms according to their size, based on body length. Group 1 females measured $\geq$ 100 mm in length and group 2 females measured < 100 mm in length. On average, group 1 females measured 114 mm in length and those from group 2, measured 77.5 mm in length. Forty four out of 98 (44.9%) larger females and 36 out of 79 (45%) smaller females were found to have been inseminated and contained eggs in their body cavities. Clearly no significant difference between the numbers of females inseminated from the 2 groups was expected or observed. When sizes of inseminated and uninseminated females were compared, no significant difference between the lengths of inseminated and uninseminated females from the two groups could be

detected. However, inseminated smaller females were found to be attached significantly more anteriorly than both uninseminated large and small females ($P < 0.05$). In addition, larger females were found to contain significantly more eggs than the smaller females ($P < 0.05$). Again on average, larger females contained significantly more mature eggs (mean 544 $\pm$95) than the smaller females (mean 237 $\pm$59) (Table 6.2), indicating once again that larger females have been inseminated before the smaller ones.

Experiment 4

The main findings from the collaborative experiment with the Oxford group can be summarized as follows. Inseminated female worms were recovered from 27 of the 45 rats. From the rats that harboured no inseminated females, male worms were not recovered and the data from them is not included in the analysis. On average, 20.4 $\pm$1.1 female *M.moniliformis* were recovered from each rat which had received an infective dose of 30 female together with either 1 or 2 male cystacanths 35 days previously. Again, on average, larger female worms were found to be attached much more anteriorly in the small intestine than the smaller ones (regression analysis $P < 0.0001$). In 19 out of 27 rats found to harbour female *M.moniliformis* at *post mortem* examination on day 35, inseminated females were also found to be attached significantly more anteriorly than uninseminated females ($P < 0.05$). In fact, large and inseminated were the characteristics of the same female worms. In only one rat out of 27, were any of the uninseminated female worms found to be larger than inseminated females. It should be remembered that a strength of these experiments was the "double-blind" element in that the Oxford observers did not know any thing about insemination state or fecundity assessment until my part of the work had been completed. A significant difference between the numbers of eggs was observed in females that were larger and more anteriorly attached ($P < 0.05$). Although these females were found to contain fewer mature eggs (mean 12 $\pm$115) compared with the female worms from experiments 1,2, and 3, the results indicate that these were inseminated earlier than the posteriorly attached females which did not contain any

mature eggs in their body cavities (Table 6.2).

6.4 DISCUSSION

The results presented here can be interpreted to give a picture of the mating behaviour of *Moniliformis moniliformis* in which males can be considered to be taking the more active role. Male worms in all the 4 experiments were found to inseminate females that were either larger or smaller and older or younger. From the populations of older and younger females, generally similar numbers of female worms were found to have been inseminated on most occasions, but older (larger) females were found to contain significantly more mature eggs than younger (smaller) females indicating that the older females must have been inseminated before the younger females. Clearly males can recognise when a female *M.moniliformis* is mature or ready for mating. This role must be assigned to males because they mature first (see below).

The intriguing question here is why did the male worms not inseminate all the older females or why did they switch to younger females, particularly when older females must have been larger than the younger females? Male worms in these populations were hardly likely to be in competition with each other since there was often one and never more than two per rat. A possible explanation for this problem might be the location of the younger females in the small intestine. Were the male worms "choosing" females from the different age groups largely on the basis of where these female worms were located in the small intestine? If Burlingame and Chandler (1941) are correct in their concept of the "zone of viability" the worms in that zone might experience the best feeding station and be best placed to provide for the development of zygotes, embryos and acanthors.

Looking back at the results obtained by Crompton (1974) and those described in Chapter 4, it is observed that insemination can take place as early as days 16 or 17 p.i. and that 35-day-old female *M.moniliformis* are mature and capable of producing mature eggs (= shelled acanthors) that are infective to cockroaches *Periplaneta americana* when insemination has taken place at this time. In the present

study, when male and female worms from the younger population were of the same age, the fact that the older females contained more mature eggs must mean that the male worms had matured earlier than the females of the same age because these worms must not have been inseminated until later. Therefore, even in a primary infection, when male and female worms of the same age show evidence of insemination from day 16 onwards, male worms must have matured first.

In the first experiment, length or size of the female worms seems to be the determinant of mating pattern, where as, in the second experiment, location of females is important. Insemination of female *M.moniliformis* takes place at day 16 p.i., and the examination of the worms from these experiments was undertaken at least 29 days after (experiment 4). If active choice is involved, the exact mechanism could only be determined by measuring female attributes at the time mate choice takes place. Data from Crompton *et al.*, (1988) was collected, in which 8 rats were fed on the same 6% fructose diet, as used for experiment 4, throughout the course of infection and were infected with 10 cystacanths of *M.moniliformis*. *Post mortem* examination of these rats, in groups of 4, took place at days 21 and 35 p.i. Analysis of the data revealed no significant difference in the mean attachment positions of male and female worms recovered at day 21 p.i. On average, male and female worms were found to be attached in the zone ranging from 23-37% and 21-37% respectively, along the length of the small intestine. At day 21 p.i., female worms measure 43 $\pm$10 mm in length and male worms measured 26 $\pm$5 mm in length, revealing an expected significant difference ($P < 0.05$). At day 35 p.i. female worms were observed to be attached significantly more anteriorly (range 18-33%) than male worms of the same age as well as 21-day-old female worms ($P < 0.05$). These results suggest that at the time of insemination male and female worms are confined in the same region of the small intestine, and female worms may move more anteriorly after insemination to the region where they may access to more nutrients. This might also suggest that once the females are mature (on the basis that males mature before females) they may produce a signal used by the males for mate location and that signal strength might be proportional to female size. This hypothetical signal is

not entirely without foundation. Miller (1980) in a series of elegant surgical experiments, in which 13-day-old female *M.moniliformis* were positioned anteriorly in the rat's intestine while males were positioned posteriorly, showed that males migrate to the females. Also the males migrated further in relation to the number of females present and even towards sachets of lyophilized female tissue. Miller interpreted these observations as indicative of pheromone production by female *M.moniliformis*. Earlier, Bone (1976) had not been able to detect any evidence *in vitro* of the production of sex attractant chemicals by female *M.moniliformis*.

In conclusion, the results from this study, for the first time, as far as is known, have indicated that male *M.moniliformis* mature before female worms of the same age, and provide evidence to show that male worms "choose" female mates on the basis of their monitoring female features that include age, size and intestinal location. These three properties also appear to be highly correlated to female potential fecundity, a characteristic which clearly favours the male worms as regards passing on their genes to the next generation. The proposed ability of male *M.moniliformis* to choose female mates on the basis of these three properties, in addition to recognising when females have matured or contain ripe oocytes, also has biological significance. It has been established that male *M.moniliformis* live for a shorter time than females. Therefore, on gaining adulthood it makes sense for a male to mate first with the larger and usually older females (these will contain at that time more oocytes to accept male genes), secondly, once they become available, for a male to mate with the larger of the younger females (these can store sperm and so should be able to provide for male genes over a longer a period) and thirdly, to mate with females in Burlingame and Chandler's (1941) viable zone (these will acquire most nutrients to nurture the male genes).

6.5 SUMMARY

An investigation of the mating behaviour of *Moniliformis moniliformis*, was conducted using rats which were each infected with 30 female together with either 1 or 2 male cystacanths. The experiments were designed to expose male worms to

the females of different age and size in the small intestine. After an experimental period when female worms were 28, 35, 49 and 63-day-old, some (but not all) of the female worms had been inseminated. The pattern was for roughly equal numbers of female *M.moniliformis* to have been inseminated from the older and the younger populations. Insemination was non-random with respect to female age, size and location in the small intestine. It appears that the pattern of mating adopted by male worms is consistent with the maximization of reproductive success. The results also indicate that male *M.moniliformis* mature before the females of the same age. Although several mechanisms could account for the patterns observed in the experiments, the results presented here remain consistent with the hypothesis of active mate choice by males.

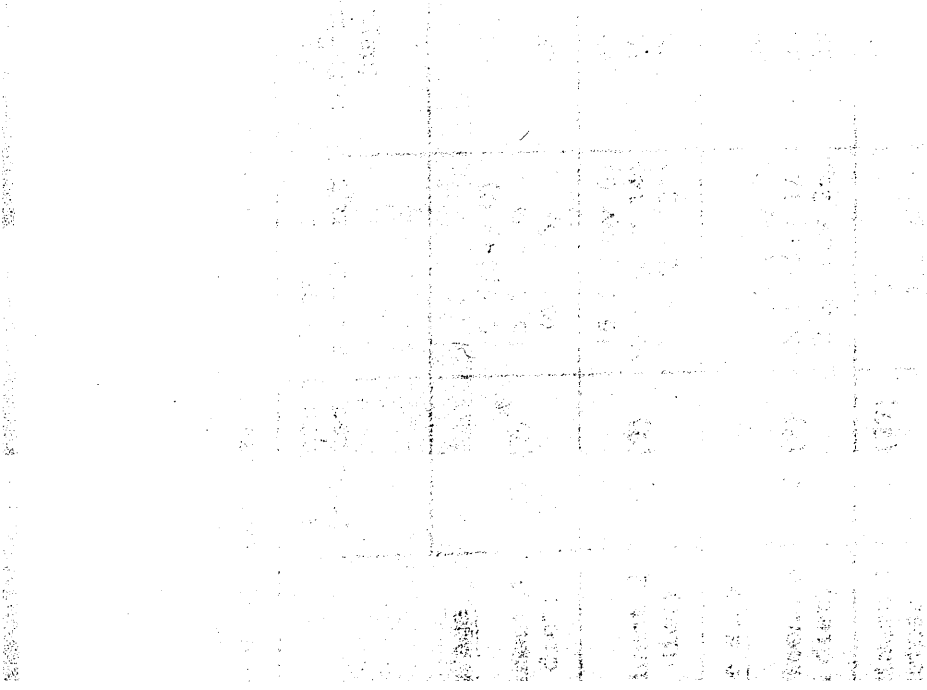


Table 6.1 Observations on the numbers of female Moniliformis moniliformis inseminated/uninseminated in terms of age and size in rats fed either on standard or experimental diet.

	No. rats infected	Dose cyst-acanths/rat	Duration of infection (days)	Total male worms recovered (mean /rat)	Total female worms recovered (mean/ rat)	No. female worms inseminated mean/rat	No. female worms not inseminated mean/rat
Female age							
Experiment 1 (CRM diet)	10 (7)[a]	15 f(day 0) 15 f+ 2 m (day 35)	63 28	9 (1.2)	35 (5) 55 (7.8)	12 (1.7) * 35 (5)	23 (3.3) 20 (2.8)
Experiment 2 (CRM diet)	10 (9)	15 f (day 0) 15 f (day 14) 2 m (day 21)	49 35 28	14 (1.5)	84 (9.3) 93 (10.3)	35 (3.9) 45 (5)	49 (5.4) 48 (5.3)
Female size							
Experiment 3 (CRM diet)	10 (9)	15 f (day 0) 15 f (day 14) 2 m (day 21)	49 35 28	14 (1.5)	98 (10.9) 79 (8.7)	44 (4.9) 36 (4)	54 (6) 43 (4.7
Experiment 4 (Fructose diet	45 (27)	30 f 1,2 m	35	20 (1.2)	341 (20.0)	147 (8.6)	194 (11.4)

a= No. of rats that harboured inseminated females, b= f, female, m, male,

*Chisquare = 7.385, $P < 0.01$

Table 6.2 Estimates of the fecundity of female Moniliformis moniliformis during the experiments designed to investigate male mate choice.

Female worms	Fecundity estimates			
	Immature eggs mean ±SE/female	Mature eggs mean ±SE /female	Total eggs mean ±SE /female	Mature:immature egg ratio
Experiment 1[a]				
Older (12) (63-day-old)	* 64467 ±12833	* 1892 ±233	* 66358 ±12858	1:34*
Younger (35) (28-day-old)	38849 ±4308	457 ±62	39306 ±4303	1:85
Experiment 2[a]				
Older (35) (49-day-old)	6621 ±548	* 582 ±116	7203 ±539	1:11*
Younger (45) (35-day-old)	10084 ±2123	260 ±53	10300 ±2163	1:38
Experiment 3[a]				
Larger (44)[c] (35,49-day-old)	* 9419 ±1691	* 544 ±95	* 9963 ±1707	1:17*
Smaller (36)[d] (35,49-day-old)	6125 ±1307	237 ±59	6363 ±1310	1:26
Experiment 4[b,e]				
Anteriorly attached (150) (15-30%)	* 23955 ±12833	* 12 ±115	* 23967 ±12858	1:199
Posteriorly attached (40) (31-45%)	18226 ±15431	0	18226 ±15431	0

a= rats fed on standard commercial diet, b= rats fed on 6% fructose diet, c= female worms measuring ≥ 100 mm im length, d= female worms measuring < 100 mm in length, e= 35-day-old female worms, * $P < 0.05$

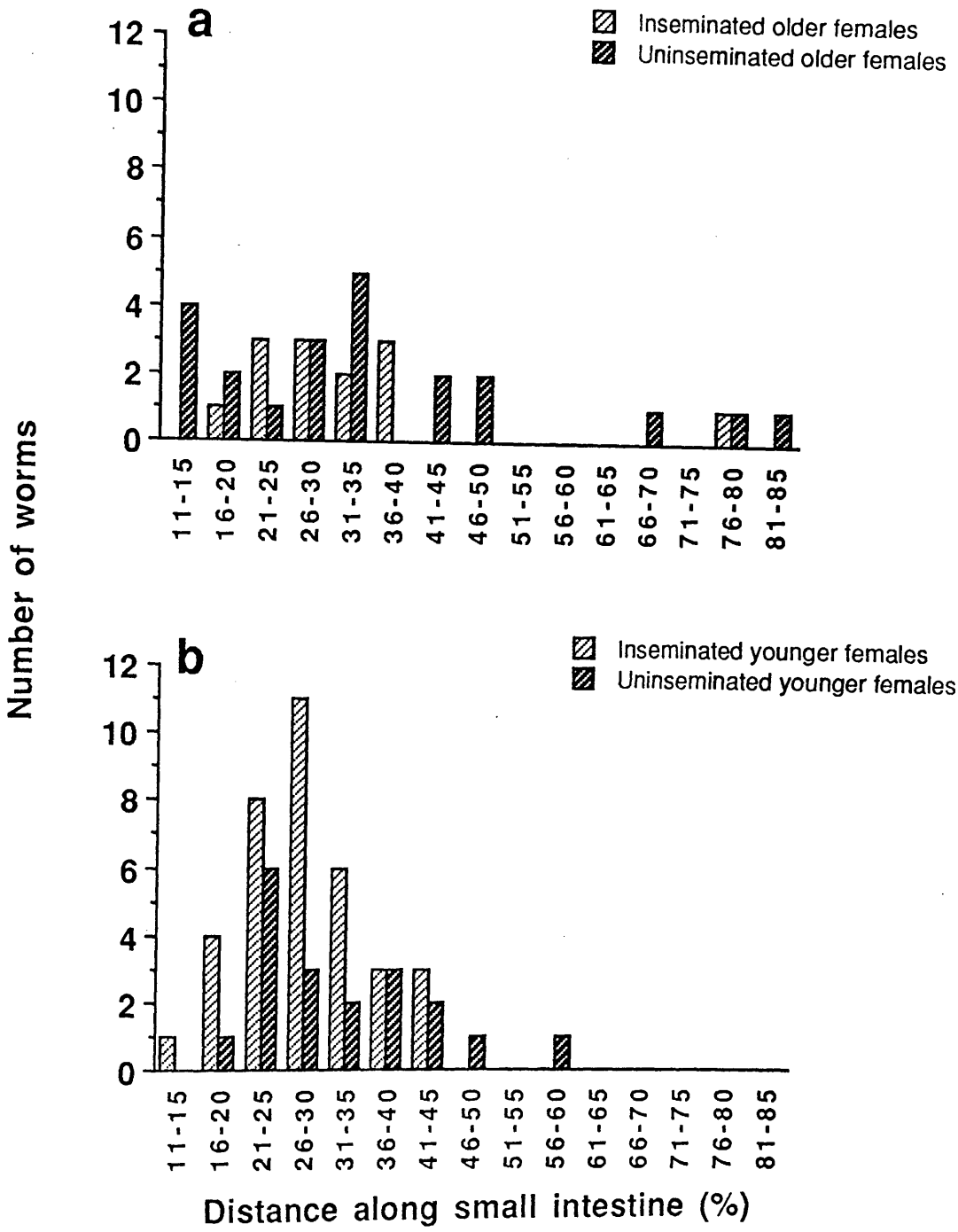


Fig. 6.1 Histograms showing the range of attachment positions of inseminated and uninseminated female *Moniliformis moniliformis* along the distance of the small intestine. a) 63-day-old female worms, b) 28-day-old female worms.

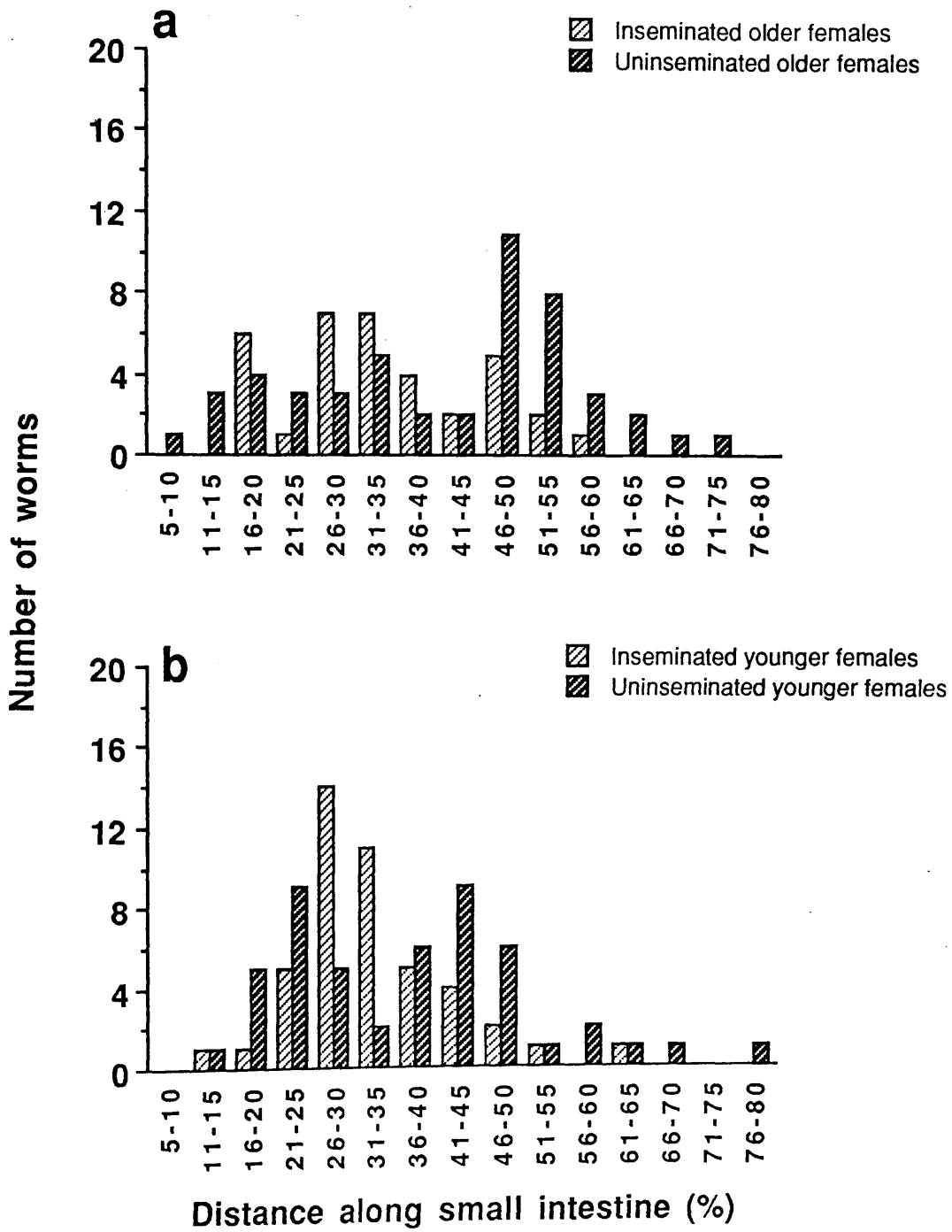


Fig. 6.2 Histograms showing the range of attachment positions of inseminated and uninseminated female *Moniliformis moniliformis* along the distance of the small intestine. a) 49-day-old female worms, b) 35-day-old female worms.

CHAPTER 7. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION: AN EXPERIMENTAL APPROACH TO STUDYING MATE CHOICE USING X-IRRADIATED FEMALE WORMS

7.1 INTRODUCTION

It has long been known that X-rays have a deleterious effect on the reproduction of certain species of nematode. Many workers in the earlier part of the 20th century demonstrated that radium or roentgen irradiation of *Trichinella spiralis* larvae, before feeding to a suitable host, would either destroy the larvae before they grew to maturity in the intestine of the host or prevent the formation of young in the adult female; the gonads did not develop. The extent of the injury to the parasite was apparently proportional to the dose of irradiation. Levin and Evans (1942) and Gould *et al.* (1955) showed that irradiated *T.spiralis* larvae given to rats orally survived and adult infection resulted, but these adults were sterile.

According to Schwartz (1921), the sex cells of the adult *T.spiralis* following X-irradiation of the larvae, appeared to atrophy, no spermatozoa were found in the receptaculum seminalis of the females and no evidence of successful copulation could be detected. This was due to the fact that the X-rays exerted a selective action on the sex cells of *T.spiralis* without necessarily affecting other vital organs. In 1960, Jarrett *et al.*, after using X- irradiation on the infective larvae of *Dictyocaulus viviparus*, described a high degree of immunity to the parasite in cattle, and showed that if the larvae were irradiated with high doses of X-rays normal adult development did not occur. X-irradiation has also been described to affect the germ cells in miracidia of *Schistosomatum douthitti* (Loker, 1978), but no effects on the swimming speed, behaviour, longevity or penetrating ability of *S.douthitti* were noticed.

Robinson and Jones (1971), described the effects of X- irradiation on the morphogenesis of *Moniliformis moniliformis* in its cockroach intermediate host. They exposed the eggs, shelled acanthors and early acanthellae to low doses of radiation before infecting cockroaches. A high frequency of abnormalities was

found in the reproductive and other organs of the resulting cystacanths and such cystacanths were not infective to rats. It was decided therefore, to further investigate the radiation-induced effects on the growth and development of *M.moniliformis* in the definitive host. Fully developed cystacanths were used to detect whether radiation could influence the gonadal development in the surviving adults as described for *T.spiralis*. If this was the case, having full-sized, sterile, females available for experiments would facilitate investigation of the mating behaviour of *M.moniliformis* as described in Chapter 6. Male worms appear to mate with females in terms of size,location and fertility. For example, if large sterile female worms are present anteriorly how would male worms react to small, posteriorly attached fertile females?

7.2 EXPERIMENTAL DESIGN

The method of X-irradiation used in these experiments is described elsewhere (Chapter 2 section 2.8). The experiment was divided into two parts. In part I, 500 - 1,500 CG and in part II, 1,500 -2,500 CG of radiation were used as doses with an increment of 500 CG. A total of 105 cystacanths was exposed to each radiation dose. Rats in groups of seven were then each given 15 cystacanths exposed to the corresponding radiation dose. Two groups of 7 rats each received a similar number of unirradiated cystacanths and served as controls for part I and II of the experiment respectively. All animals were killed 35 days p.i.

7.3 RESULTS

At *post mortem* examination, the numbers of *M.moniliformis* recovered, their attachment positions in the small intestine and their lengths were recorded. Later, the insemination status and fecundity of individual female worms were assessed (as described in Chapter 2 section 2.6.1-2).

Part 1

7.3.1 OBSERVATIONS ON THE EFFECTS OF LOW DOSES OF X-IRRADIATION ON THE ESTABLISHMENT, GROWTH AND SURVIVAL OF *MONILIFORMIS MONILIFORMIS*

Moniliformis moniliformis were recovered from all rat groups. The mean worm recovery (±SE) from control and X-irradiated groups were 2.4 ±0.6, 3.4 ±0.5, 6.2 ±0.8 and 1.4 ±0.7 respectively, revealing a significant increase ($P < 0.05$) from rats given cystacanths exposed to 1,000 CG (Fig 7.1). The mean percentage attachment position of the worms in the small intestine was not found to show any significant difference between the 4 groups of rats (Table 7.1). Although female worms from rats receiving cystacanths exposed to 1,500 CG appeared smaller in length as compared with the females from other groups the difference was not statistically significant (Fig. 7.2). Male worms also showed no significant difference in mean length between the 4 groups.

7.3.2 OBSERVATIONS ON EFFECTS ON INSEMINATION AND FECUNDITY

The results are summarized in Table 7.1. All female worms recovered from control and irradiated groups were found to have been inseminated and contained developing eggs in their body cavities. There appeared to be no difference in the quality of the developing eggs between control and irradiated groups. A significant difference in the mean number of ovaries per female worm was observed between the 4 groups of rats. Between X-irradiated groups the mean number of ovaries per female worm was observed to be significantly higher in the group exposed to 1,000 CG dose than the groups exposed to 500 and 1,500 CG respectively ($P < 0.05$) (Fig 7.3). However, a significant decrease in the mean number of eggs per female worm was observed ($P < 0.05$) between the 4 groups of rats with increasing X-irradiation dose (Table 7.1) (Fig 7.4).

Part II

7.3.3 OBSERVATIONS ON THE EFFECTS OF HIGH DOSES OF X-IRRADIATION ON THE ESTABLISHMENT, GROWTH AND SURVIVAL OF *MONILIFORMIS MONILIFORMIS*

The establishment and survival of *M.moniliformis* were not found to be affected by higher X-irradiation dose used in this part of the experiment. The mean recoveries, lengths and attachment positions of the worms from the 4 groups are given in Table 7.2. Analysis of the data revealed no significant difference in worm recoveries between the 4 groups. However, female worms from the irradiated groups were observed to be significantly smaller when compared with controls ($P < 0.05$) (Table 7.2). This reduction in length was not found to be correlated to the radiation dose to which the cystacanths were exposed. Female worms recovered from the group receiving cystacanths exposed to 2,000 CG, were observed to be attached significantly anterior than those recovered from other groups ($P < 0.05$) (Table 7.2).

7.3.4 OBSERVATIONS ON EFFECTS ON INSEMINATION AND FECUNDITY

As in part I of the experiment, all female *M.moniliformis* recovered from the 4 groups in part II, were found to have been inseminated and contained developing eggs in their body cavities. There was no difference observed in the quality of developing eggs between the 4 groups. A significant increase ($P < 0.05$) in the mean number of ovaries per female worm was observed from the group given cystacanths exposed to 1,500 CG (Table 7.2) (Fig. 7.5). However, unlike the worms from part I, there was no significant difference in the mean number of eggs per female worm between the 4 groups (Table 7.2) (Fig. 7.6). A significant linear correlation was observed when the number of eggs and length per female worm was compared ($P < 0.05$).

7.4 OBSERVATIONS ON THE EFFECTS OF X-IRRADIATION ON *NIPPOSTRONGYLUS BRASILIENSIS*

To confirm that the X-irradiation method used for *M.moniliformis* during this study was reliable, an experiment was undertaken to X- irradiate third-stage larvae of *N.brasiliensis*. Larvae were recovered from faecal cultures (see Chapter 2 section 2.3.4), kept in 0.6% aqueous NaCl solution at room temperature and were then exposed to 2,500 CG dose of radiation. Seven rats were each infected with 1,000 irradiated larvae by subcutaneous injections. Another group of 7 rats, each receiving 1,000 unirradiated larvae, served as controls. The course of infection was followed and faeces were collected from both groups of rats after days 8 and 9 of infection. Eggs per gram of faeces were determined by the McMaster method. Rats were killed at day 10 p.i. for the worm recovery.

7.4.1 OBSERVATIONS ON EFFECTS ON FECUNDITY OF *NIPPOSTRONGYLUS BRASILIENSIS*

The method used for the recovery of adult *N.brasiliensis* is described elsewhere (Chapter 2 section 2.4.2). On day 10 p.i. no worms were recovered either from control or X-irradiated groups. However, faecal analysis on days 8 and 9 p.i. revealed some expelled worms from both groups.

The analysis of data on faecal egg counts on day 8 p.i. revealed a significant difference ($P < 0.05$) in mean e.p.g between control (8400 e.p.g) and X-irradiated (400 e.p.g) groups. On day 9 p.i., however, no eggs were recovered from the X-irradiated group as compared to 2000 e.p.g. from control group, indicating the reliability of the method of X-irradiation used in this study.

7.5 DISCUSSION

It is difficult to reach any firm conclusions from these experiments. However, the results so far suggest some effects of X- irradiation on the fecundity of *Moniliformis moniliformis*. It was observed that none of the radiation doses used in this study was sufficient to sterilize *M.moniliformis* cystacanths. Successful establishment, growth of worms and insemination of female worms showed that X-

irradiation doses as high as 2,500 CG, did not interfere either with the infectivity of the cystacanths to the definitive host or with the mating behaviour of the parasite.

In part I of the experiment, low worm recoveries obtained from the control and X-irradiated groups could be attributed to the fact that, before the infection of rats the proboscides of some of the cystacanths (irradiated and controls) were observed to be evaginated; this might have affected the establishment of the parasite. The evagination was considered to be due to the room temperature at which the cystacanths were kept. Care was taken to avoid this problem during part II of the experiment (see chapter 2 section 2.8).

Although a significantly higher number of ovaries per female worm was observed from the groups given cystacanths exposed to 1,000 and 1,500 CG, in part I and II of the experiments respectively, the female worms appeared to produce approximately similar number of eggs as those of the controls and other X-irradiated groups. The number of male worms present in these two groups of rats was also found to be significantly higher when compared with other groups, but no evidence for a relationship between the number of male worms present and mean number of eggs produced per female worm could be detected. It may be suggested that X-irradiation has affected, to some degree, the oogenesis in the female worms as a considerable number of ovaries was present, but the egg production was reduced.

Robinson and Jones (1971) suggested that the abnormalities seen in the early embryonic development of *M.moniliformis* in cockroaches were due to radiation-induced mitotic blockage. These workers have described that the normal growth and development of *M.moniliformis* was altered in three main ways; (1) inhibition of nuclear division; (2) failure of the cells to differentiate; (3) interference with structural organisation.

Schiller (1959) cited the same major changes after examination of developing larvae derived from X-irradiated eggs of the cestode *Hymenolepis nana*. He also observed that the cells destined to divide and differentiate into special organs were

the most seriously affected, where as morphological structures, which had already attained complete development in the cysticercoid stage before X- irradiation, exhibited no observable morphological alterations.

At the cystacanth stage, the male and female reproductive organs in*M.moniliformis* are well developed containing immature gonads, and in the definitive host, although differentiation of the gonads continues, there is also much growth. The ovarian fragmentation in *M.moniliformis* has been described to occur at the cystacanth stage (Yamaguti and Miyata, 1942; Moore, 1946; Asaolu *et al.*, 1981) and in young worms in the definitive host, Parshad *et al.*, (1980) considered that the number of free ovaries increases with age through some form of division process, from about 8 in 1-week-old female *M.moniliformis* to about 6,000 in 14-week-old female (Crompton *et al.*, 1976).

Robinson (1965) described the ovarian development of *M.moniliformis* in the definitive host. He observed that in the ovary, oogonia divide mitotically to produce primary oocytes which grow then to become mature oocytes. Meiotic divisions occur in the mature oocytes, but only after these cells have been activated by fusion with a spermatozoon.

The radiation-induced effects on lower egg production in female worms exposed to 1,000 and 1,500 CG as cystacanths, could therefore, be explained by assuming that, the X-irradiation has either affected oogonia from undergoing mitotic division to produce oocytes, or it might have, to some degree, inhibited the mature oocytes from undergoing meiosis, consequently delaying the process of fertilization. Also it seems reasonable to suggest, from the results so far, that X-irradiation of *M.moniliformis* cystacanths at 1,000 CG has a threshold effect on fecundity.

No obvious effects of X-irradiation on the morphology of first generation eggs was observed. It is important to note that this was a horizontal study where the infection was followed only at one time point (i.e 35 days p.i.) and no observations were made on the development of eggs into acanthors, neither was their infectivity to the intermediate hosts measured. Therefore, long term effects of X- irradiation on *M.moniliformis* require further investigation.

7.6 SUMMARY

Experiments were undertaken using various doses of X-irradiation (500-2,500 CG) to investigate mate choice. None of the X-irradiation doses used was found to affect the course of infection of *Moniliformis moniliformis* in rats. However, radiation-induced effects on the mating behaviour of the parasite were observed. Female worms exposed to 1,000 and 1,500 CG as cystacanths were found to contain significantly fewer numbers of eggs as compared to the female worms from exposed to 500, 2,000 and 2,500 CG. The results suggest that X- irradiation might have some degree of effect on either the oogenesis or the process of fertilization. Further research is needed in this field.

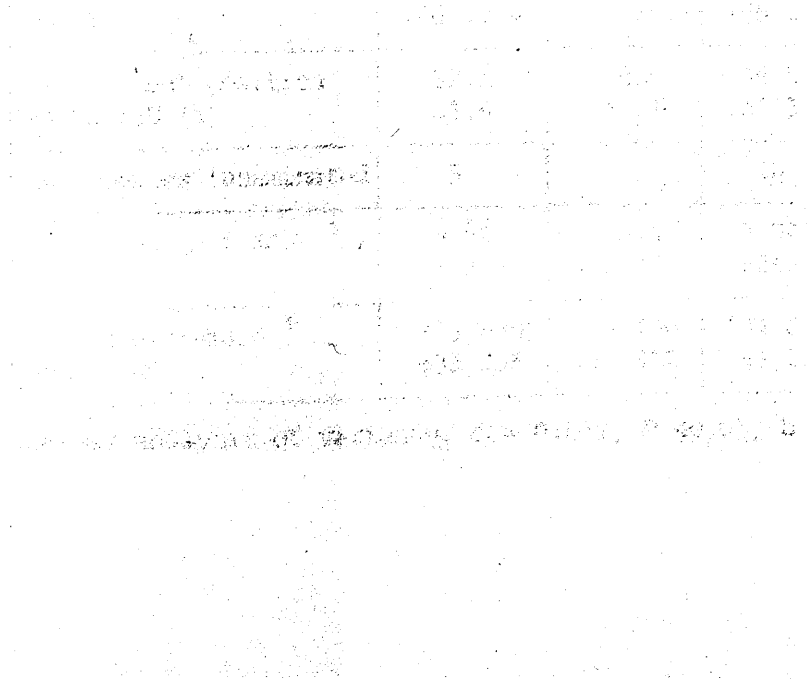


Table 7.1. Observations on the establishment, growth and fecundity of Moniliformis moniliformis in rats given cystacanths exposed to various X-irradiation doses (Part I).

	X-irradiation dose (CG)			
	control	500	1,000	1,500
Worm recovery No. male worms No. female worms Mean ±SE/rat	 12 5 2.4 ±0.6	 17 7 3.4 ±0.5	 23 21 6.2 ±0.8	 6 4 1.4 ±0.7
Length/worm mean ±SE male female	 62 ±2.9 146 ±7.4	 61 ±2.9 138 ±6.7	 64 ±1.6 135 ±4.5	 64 ±3.5 122 ±8.5
Attachment position mean ±SE (%)	32.5 ±1.6	28.0 ±1.1	34.5 ±1.3	29.6 ±1.9
No.females inseminated	5	7	20	3
No. ovaries/female [a]	5,580 ±1,367	2,614 ±426	3,548 ±246	2,950 ±470
No. eggs/female [b] mean ±SE	173,820 ±33,235	137,400 ±17,312	134,095 ±1,229	46,650 ±33,546

One-way analysis of variance, a = 0.012; P <0.05, b = 0.020; P <0.05

Table 7.2. Observations on the establishment, growth and fecundity of Moniliformis moniliformis in rats given cystacanths exposed to various X-irradiation doses (Part II).

	X-irradiation dose (CG)			
	control	1,500	2,000	2,500
Worm recovery No. male worms No. female worms Mean ±SE/rat	 32 38 10 ±0.6	 42 39 11.5 ±1.7	 35 43 11.1 ±0.8	 39 43 11.7 ±1.1
Length/worm mean ±SE male female	 61 ±1.1 122 ±2.6	 60 ±1.2 110 ±3.6	 59 ±1.3 113 ±2.5	 65 ±2.2 111 ±3.4
Attachment position mean ±SE (%)	35.0 ±1.1	35.5 ±1.1	26.3 ±0.8	34.4 ±1.1
No.females inseminated	32	31	43	43
No. ovaries/female [a]	2,200 ±198	2,868* ±249	2,064 ±185	2,138 ±195
No. eggs/female mean ±SE	65,774 ±3,841	57,271 ±4,863	49,405 ±4,540	58,752 ±5,390

One-way analysis of variance, a = 0.039; P <0.05

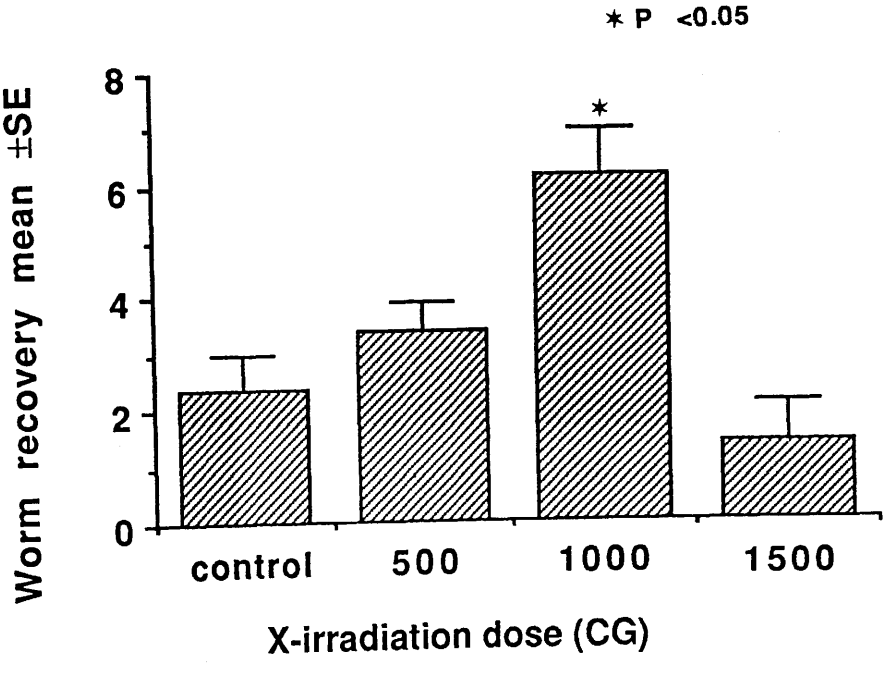


Fig. 7.1 Mean worm recovery ±SE of *Moniliformis moniliformis* from rats given cystacanths (orally) exposed to various doses of X-irradiation.

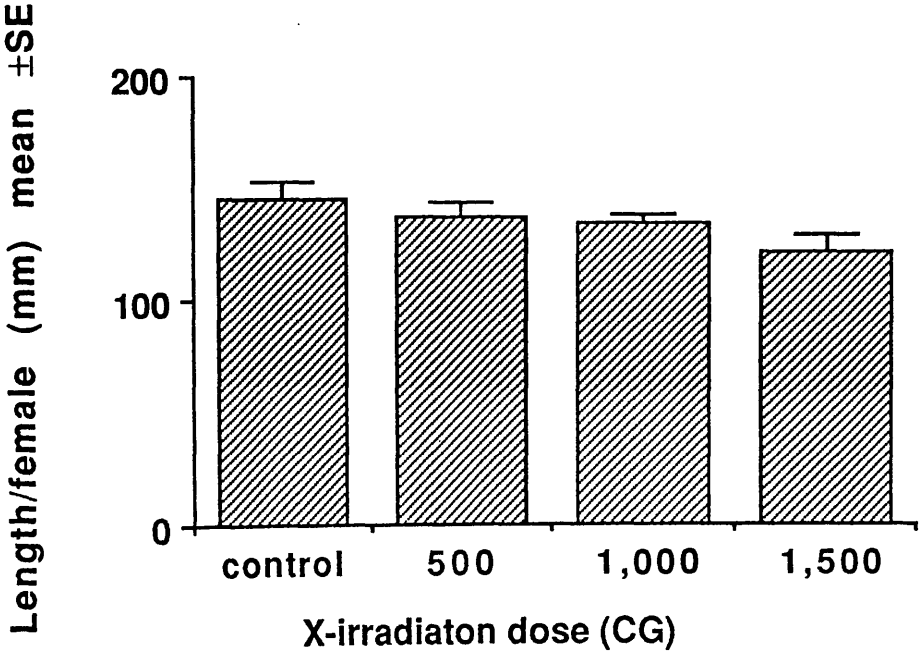


Fig. 7.2 Mean length (mm) ±SE per 35-day-old female *Moniliformis moniliformis* from rats given cystacanths (orally) exposed to various doses of X-irradiation.

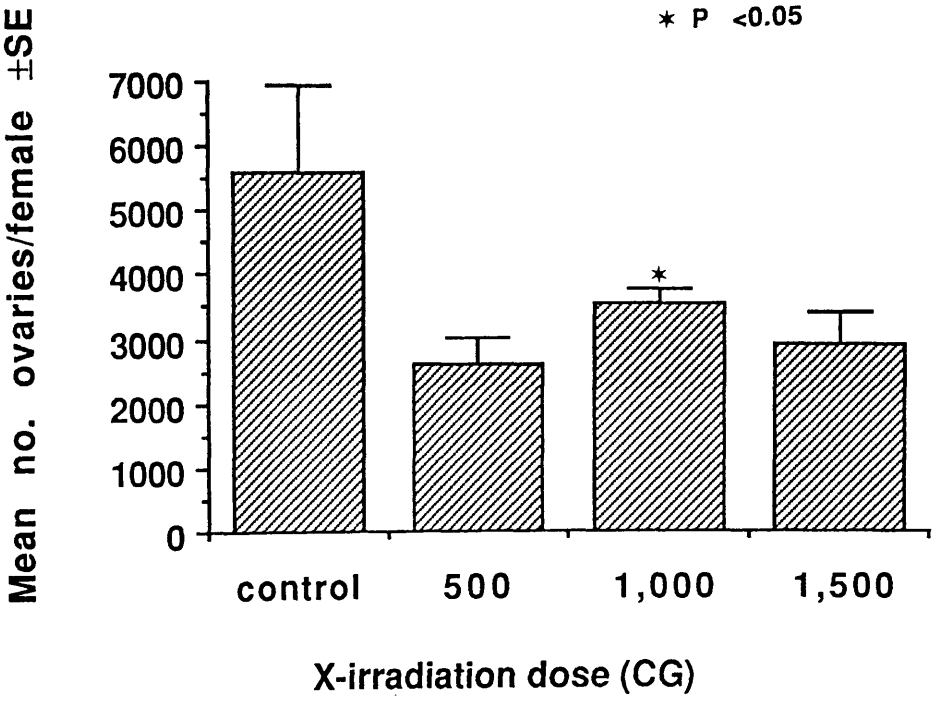


Fig. 7.3 Mean number of ovaries ±SE per 35-day-old female *Moniliformis moniliformis* from control and X-irradiated (500-1,500 CG) groups.

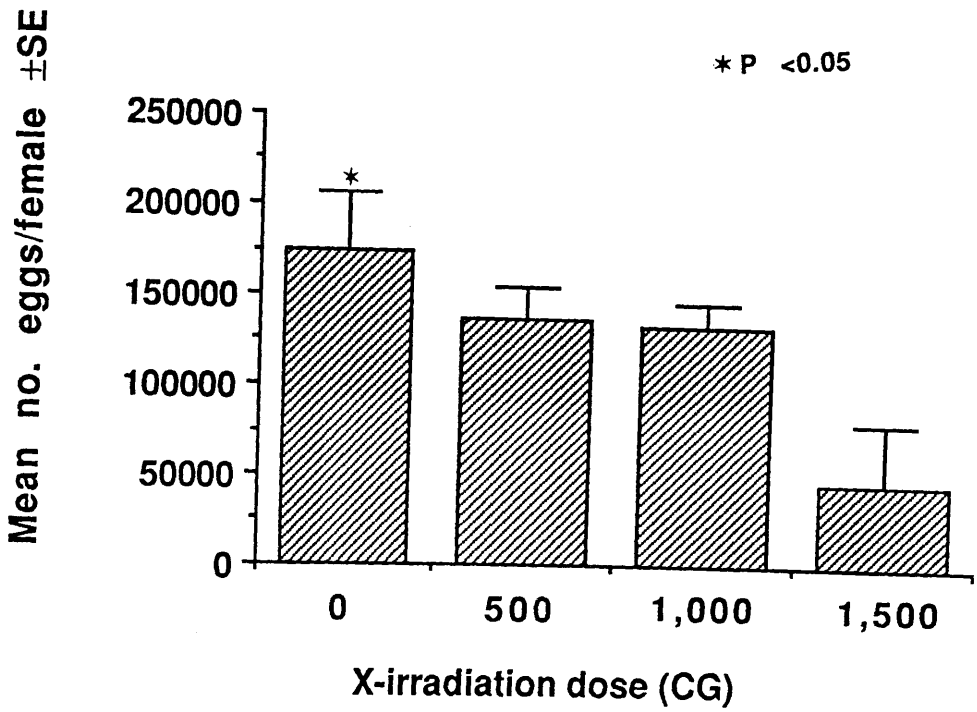


Fig. 7.4 Mean number of eggs ±SE per 35-day-old female *Moniliformis moniliformis* from control and X-irradiated (500-1,500 CG) groups.

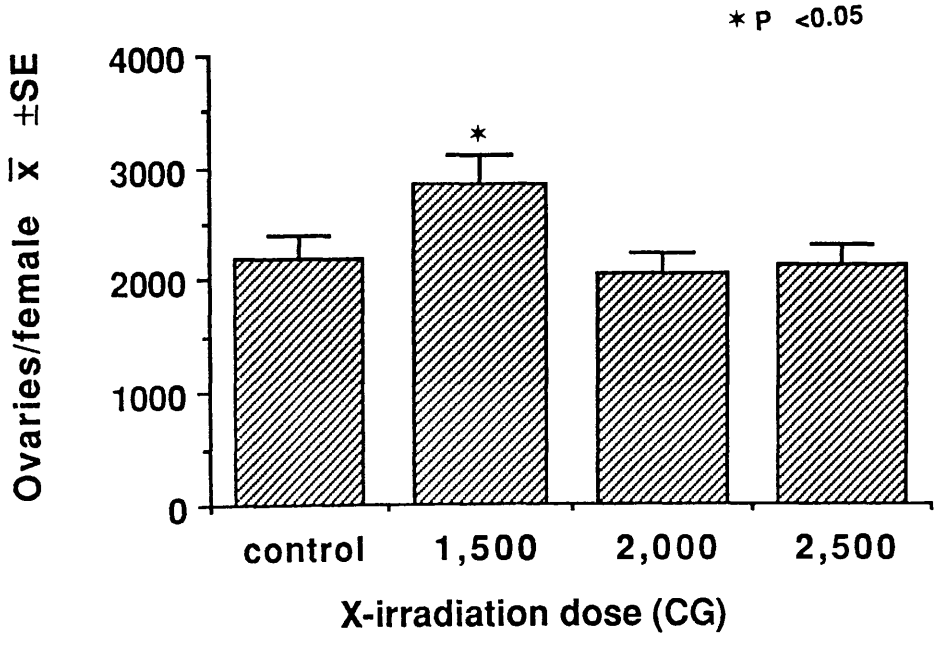


Fig. 7.5 Mean number of ovaries ±SE per 35-day-old female *Moniliformis moniliformis* from control and X-irradiated (1500-2,500 CG) groups.

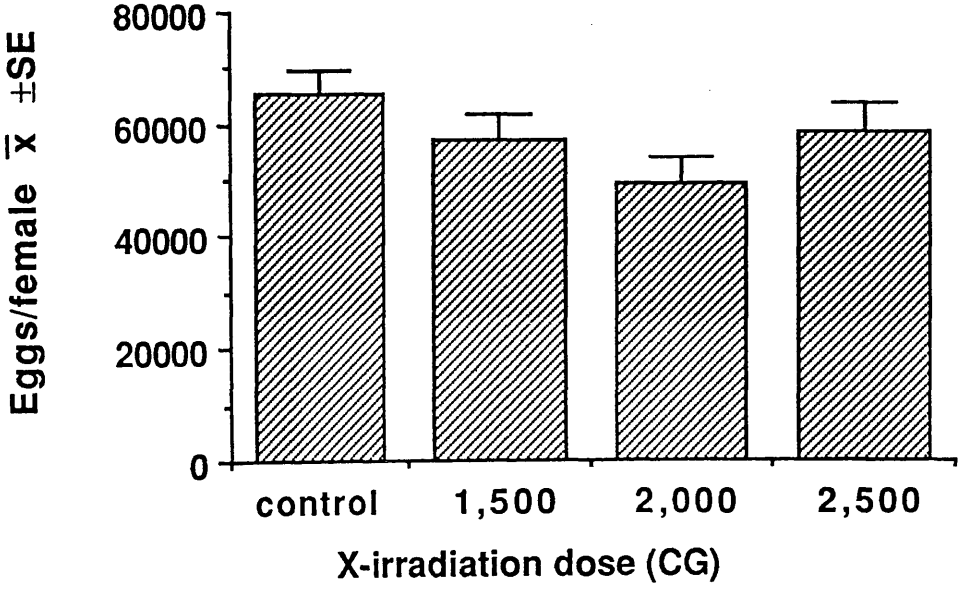


Fig. 7.6 Mean number of eggs ±SE per 35-day-old female *Moniliformis moniliformis* from control and X-irradiated (1500-2,500 CG) groups.

CHAPTER 8. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION : INTERACTIONS BETWEEN HELMINTH SPECIES

8.1 INTRODUCTION

The interaction between species of gut-dwelling helminths in the same species of host have been widely studied for some time. Single- species infections are often uncommon in nature and multiple infections provide opportunities for interactions between parasites, possibly influenced by the defence mechanisms of the host (Kazacos, 1976). The importance of these studies is because of the relevance of the parasites to natural infections of man and animals and also to elucidate the mechanisms by which mammalian hosts can expel intestinal helminths, in particular through the involvement of non- specific factors (Kennedy, 1976). In a number of species combinations, previous or concurrent infection with one species has been shown to affect deleteriously a second species (reviewed by Kazacos, 1975). Parasites with complex life cycles, present a special problem for the vertebrate host insofar as their differentiation is often associated with conspicuous changes in the expression of antigens that can excite a protective response (Despommier and Muller, 1976). *Trichinella spiralis* can induce a complex array of host immune responses while it is exhibited in host gut. These responses affect several facets of the parasite's development and ultimately result in the failure of its reproductive process and worm expulsion (Bell *et al.*, 1979).

Another form of interaction between species of helminths is likely to involve competition for resources, such as nutrients and space. This might be particularly true for species which have similar distributions in the intestine of the host and have similar nutritional requirements.

Some interactions between *Moniliformis moniliformis* and other helminth species have been studied in the laboratory (Holmes, 1961, 1962; Holland, 1984). The present study was aimed at extending the observations on interspecific interactions by determining the effects of *Trichinella spiralis* and *Nippostrongylus brasiliensis* infections upon a concurrent *Moniliformis moniliformis* infection in relation to the

growth, survival and fecundity of *M.moniliformis*.

8.2 EXPERIMENTAL DESIGN

Four experiments were conducted. In the first of these, the aim was to investigate the effects of recent establishment of *T.spiralis* on the growth and survival of previously established *M.moniliformis* infection and *vice versa*. Twenty eight rats out of 35 infected 5 weeks previously with 15 cystacanths of *M.moniliformis*, plus 28 previously uninfected rats were each given 1,500 L_2 larvae of *T.spiralis*. Seven rats from each group were killed humanely at 4,10,12 and 17 days p.i. This procedure meant that both species of parasites were mature and patent. Seven rats infected only with *M.moniliformis* served as controls.

The second experiment, investigated the reaction of *M.moniliformis* in rats known to have had *T.spiralis*. Fourteen rats were each infected with 1,500 L_2 larvae of *T.spiralis*. The infection was allowed to terminate naturally. Four weeks later half of these rats, plus 7 previously uninfected rats, were each given 15 cystacanths of *M.moniliformis*. Post mortem examination of these rats took place at day 35 p.i. The remaining rats were each reinfected with 1,500 L_2 larvae of *T.spiralis* and were killed 7 days p.i.

In the third experiment, 7 out of 14 rats, infected 5 weeks previously with 15 cystacanths of *M.moniliformis* each, and 7 uninfected rats were treated with the anthelmintic drug levamisole for two days (see chapter 2 sections 2.9.1,2 for details). These, plus 7 uninfected rats, were each infected with 1500 L_2 larvae of *T.spiralis* and were killed a week later.

The effects of concurrent *T.spiralis* and *N.brasiliensis* infections on mating probability and consequently on fecundity of *M.moniliformis* were investigated. Twenty rats were each infected with 15 cystacanths of *M.moniliformis*. On day 13 p.i., half of these rats were each given 1,500 L_2 larvae of *T.spiralis*, and the other half were each infected on day 14 p.i. with 4,000 L_3 of *N.brasiliensis*. Five rats from each group were killed on day 21 and 35 p.i. respectively. Twenty rats served as controls by harbouring single *M.moniliformis* infections, with post mortem

examination taking placed on the same day as for the concurrent infections.

8.3 RESULTS

8.3.1 OBSERVATIONS ON THE ESTABLISHMENT AND GROWTH OF *MONILIFORMIS MONILIFORMIS* IN SINGLE AND CONCURRENT INFECTIONS WITH *TRICHINELLA SPIRALIS*

Experiment 1

All measurements on *M.moniliformis* recovered from single and concurrent infections are shown in Table 8.1. Statistical analysis of the data using Mann-Whitney U tests revealed no significant difference in the number of *M.moniliformis* recovered that could be ascribed to the presence of other helminth species or to the duration of infection. The mean percentage attachment position of worms along the distance of the small intestine in single and concurrent infections is illustrated in Fig. 8.1. An anterior shift in position of attachment of *M.moniliformis* was observed in concurrent infection with 4-day-old *T.spiralis* revealing a significant difference ($P < 0.05$). *Moniliformis moniliformis* growth, in terms of dry weight, showed a linear relationship with time in single infections. However, in concurrent infections, with 4-day-old *T.spiralis*, both male and female *M.moniliformis* showed significant differences ($P < 0.05$) in dry weight when compared with worms from single infections (Table.8.1). The mean male worm dry weight measured was 6.5 ± 0.2 and 5.4 ± 0.2, and those of female worms was 20.8 ± 0.7 and 17.2 ± 0.4 from single and concurrent infections respectively. Worms from concurrent infections were significantly lighter (Fig. 8.2). No significant difference between the mean dry weight of either sexes was observed from concurrent 14- day-old *T.spiralis* infection.

8.3.2 OBSERVATIONS ON THE ESTABLISHMENT OF *TRICHINELLA SPIRALIS* IN SINGLE AND CONCURRENT INFECTIONS WITH *MONILIFORMIS MONILIFORMIS*

Mean worm recoveries of *T.spiralis* from single infections and from concurrent infections with *M.moniliformis* are given in Table 8.1. A recovery of about 22% and 4% of the inoculum was achieved on day 4 p.i. from single and concurrent

infections with *M.moniliformis* respectively, revealing a significant difference ($P < 0.01$). Loss of *T.spiralis* was observed thereafter and on days 10 and 12 of infection the numbers of worms recovered were reduced in both single and concurrent infections, but there was no significant difference between them. This reduction in the single infection corresponds to the normal immune-mediated expulsion of a single primary infection taking place on approximately day 8 of infection (Christie et al., 1979). Complete elimination of the worms was observed on day 17 p.i. (Fig. 8.3).

Experiment 2

8.3.3 OBSERVATIONS ON CROSS IMMUNITY IN RATS AGAINST *MONILIFORMIS MONILIFORMIS* KNOWN TO HAVE HAD *TRICHINELLA SPIRALIS* INFECTIONS

Infections of *M.moniliformis* became established successfully in rats that had been infected with *T.spiralis* 4 weeks earlier. The mean number of worms (±SE) recovered from these and control rats were 12.2 ± 1.6 and 13.3 ± 0.5 respectively, and no significant difference was observed between them. In addition, no difference in either the percentage attachment position of *M.moniliformis* in the small intestine, or in the mean dry weights of male worms (7.2 ± 0.8, 6.0 ± 0.9) was observed. However, the female worms from these concurrent infections showed a significant difference ($P < 0.05$) in mean dry weight (10.7 ± 0.6) by being lighter than those from single infections (13.0 ± 0.7). The mean dry weights of male and female worms from the two groups of rats are illustrated in Fig. 8.4.

8.3.4 OBSERVATIONS ON CROSS IMMUNITY IN RATS AGAINST *TRICHINELLA SPIRALIS* KNOWN TO HAVE HAD *MONILIFORMIS MONILIFORMIS* INFECTIONS

Four weeks after the primary infection with *T.spiralis*, rats were challenged with 1500 L_2 larvae and assayed for the expulsion 7 days later. No worms were recovered from these rats indicating that rats had developed the expected immunity

induced by and expressed against the parasite.

Experiment 3

8.3.5 OBSERVATIONS ON THE EFFECTS OF ANTHELMINTIC DRUG ON THE COURSE OF INFECTION OF *MONILIFORMIS MONILIFORMIS*

Rats harbouring 35-day-old *M.moniliformis* infections were treated with levamisole (0.25 mg/kg body weight) for two days. The numbers of worms recovered at *post mortem* examination of these rats were significantly lower when compared with the controls ($P < 0.01$). The mean worm recoveries ($\pm$SE) from treated and control animals were 3.4 $\pm$ 1.5 and 9.7 $\pm$ 2.6 respectively (Fig. 8.5).

8.3.6 OBSERVATIONS ON THE ESTABLISHMENT OF *TRICHINELLA SPIRALIS* IN RATS PRETREATED WITH ANTHELMINTIC DRUG

The mean worm recoveries ($\pm$SE) from control, pretreated single, and treated concurrent infections with *T.spiralis*, were 105 $\pm$16, 129 $\pm$ 26, and 7.4 $\pm$ 3.1 respectively, revealing a significant difference between single and concurrent infections ($P < 0.01$) (Fig. 8.6). No difference was observed between mean worm recoveries from control and pretreated animals indicating no effects of levamisole on protective immunity in rats against *T.spiralis* infections. A significant correlation ($P < 0.01$) was found between the lower numbers of *T.spiralis* and the presence of *M.moniliformis*.

Experiment 4

8.3.7 OBSERVATIONS ON THE EFFECTS ON MATING BEHAVIOUR AND FECUNDITY OF *MONILIFORMIS MONILIFORMIS* OF CONCURRENT INFECTIONS WITH *TRICHINELLA SPIRALIS* AND *NIPPOSTRONGYLUS BRASILIENSIS*

Measurements on worm recovery, individual worm length and on attachment position in the small intestine were made at *post mortem* examination. Individual female *M.moniliformis* reproductive status was determined and number of eggs (=shelled acanthors) per female worm was estimated (as described in Chapter 2

section 2.6.1, 2). The results are summarised in Table 8.5.

In concurrent infections at days 21 and 35 p.i. when *T.spiralis* infections were 8 and 22-day-old, and those with *N.brasiliensis* were 7 and 21-day-old rspectively, the numbers of *M.moniliformis* recovered showed no statistically significant difference when compared with single infections. An interestingeffect on female worm length was observed in that the 21-day-old female *M.moniliformis* from concurrent infections with *T.spiralis* and *N.brasiliensis* were significantly bigger (56.3 ±2.8 and 55.6 ±5.1 respectively) than the controls (34.7 ±2.7) and 35-day-old females from concurrent infections were significantly smaller ($P < 0.05$) (Table 8.2). Correlation coefficient analysis revealed significant relationship betwee the lengths of *M.moniliformis* and the presence of concurrent *T.spiralis* and *N.brasiliensis* infections ($P < 0.01$). No difference in the percentage attachment position and length of male worms from concurrent infections at days 21 and 35 p.i. was observed when comapred with single infections.

Measurements on the reproductive status of *M.moniliformis* from single and concurrent infections are shown in Table 8.3. Not all female worms recovered on day 21 p.i. from the 3 groups of rats were inseminated, and no significant difference in the numbers of female *M.moniliformis* inseminated could be detected when the samples from single and concurrent infections with *T.spiralis* and *N.brasilensis* were compared. However, the number of females that were not inseminated was found to be significantly lower from the concurrent infections with *N.brasiliensis* when compared with single infections. These uninseminated females from concurrent infections showed a significant correlation with the presence of *T.spiralis* and *N.brasiliensis* infections ($P < 0.05$). The mean numbers of inseminated and uninseminated females in single and concurrent infections are illustrated in Fig.8.7. On day 21 p.i. not all inseminated female *M.moniliformis* from single and concurrent infections with *T.spiralis*, were found to contain free eggs in their body cavities but none of the female worms recovered from concurrent *N.brasiliensis* infections contained free eggs in their body cavities (Table 8.3).

On day 21 p.i. no significant difference was observed betwee mean numbers of

of eggs per female worm when samples from single and concurrent infections with *T.spiralis* were compared (Fig 8.8). However, on day 35 p.i. the mean number of eggs per female worm from single infections was found significantly lower ($P < 0.01$) than those from concurrent infections with *T.spiralis* and *N.brasiliensis* (Fig. 8.8).

The multiple comparisons of the reproductive status of female *M.moniliformis* indicated that the lower numbers of inseminated females was mostly correlated to the presence of concurrent *N.brasiliensis* infection.

8.4 DISCUSSION

The observations made on concurrent infections of *Moniliformis moniliformis*, *Trichinella spiralis* and *Nippostrongylus brasiliensis* in rats reveal a significant reduction in numbers of the nematodes when *M.moniliformis* was present. However, *M.moniliformis* was affected in a number of ways. Growth was found to be retarded when *T.spiralis* was present in the small intestine of the host, where with concurrent *N.brasiliensis* infections, female *M.moniliformis* grew at a greater rate than in single infections. In addition, *M.moniliformis* was observed to exhibit an anterior shift in attachment position along the length of the small intestine when present with concurrent nematode infections. Finally mating success of *M.moniliformis* appeared to be disrupted.

The reduction in numbers of *T.spiralis* was found to have occurred by day 4 of a concurrent infection, at least 4 days prior to the usual immune-mediated expulsion of this nematode in a single primary infection (Christie *et al.*, 1979). The central role of the immune response in the expulsion of intestinal helminths is well known, and has been demonstrated (Wakelin and Wilson, 1977; Ferretti *et al.*, 1984) by the effects of immuno-suppressive treatments. Holland (1984), describing the role of *M.moniliformis* in the interaction between *N.brasiliensis* and the latter, suggested that reduction in the numbers of the nematode from concurrent infections could result from (i) a direct immune-mediated response on the part of the host enhanced by the presence of acanthocephalan, (ii) a direct inflammatory response mounted by the host and (iii) the secretion by *M.moniliformis* of some toxic substance that

deleteriously affected the establishment of *N.brasiliensis*.

Holmes (1961) concluded similar sort of effects of concurrent *M.moniliformis* infections on *Hymenolepis diminuta* in that the acanthocephalans might excrete some toxic substance and hence modify the physiology of the host. This suggestion could prove to be possible in this case, as supported by the data collected from experiment 3, where the reduction in numbers of *T.spiralis* was observed to correlate with the presence of *M.moniliformis* and not to the levamisole treatment of the rats. Earlier loss of *T.spiralis* from concurrent infections, may indicate the involvement of some sort of immune response towards *M.moniliformis*. Little is known about the immune response of the rat host against *M.moniliformis*. Burlingame and Chandler (1941), Andreassen (1975) and Miremad-Gassmann (1981) reported that rats develop some level of immunity against secondary infections in terms of retarded growth of worms from secondary infections. Dineen (1963 a,b) proposed that many antigenic aspects of a parasite species stimulate immune responses which do not have a negative effect on the survival of the parasite. In addition, these characters may be available for eliciting a response effective against the co-occurring species of the parasite. Observations made by Ferretti *et al.*, (1984), suggest that the consequences of the initiation of inflammation in the host gut is that the intestinal environment becomes unsuitable for the normal development and survival of the initiating species and then for the co-occurring ones.

Interactions between *T.spiralis* and other helminth species have been observed during the time of expulsion of *T.spiralis*. Apparently, the inflammatory responses associated with the expulsion of *T.spiralis*, induce premature loss of other species; *N.brasiliensis* (Kennedy, 1980); *Hymenolepis nana* (Ferretti *et al.*, 1984); *H.diminuta* (Christie *et al.*, 1979); *S.ratti* (Moqbel and Wakelin, 1979) and *H.microstoma* (Howard *et al.*, 1978). This premature loss has not been observed in the present study, which may indicate the superiority of *M.moniliformis* in situations of competition.

The major observable effect of concurrent infection on *M.moniliformis* was slowing down of its growth and an anterior shift in attachment position, during the time of expulsion of *T.spiralis* from the host. The anteriorly directed migration for *M.moniliformis* has previously been reported by many workers, as a consequence of intraspecific (Burlingame and Chandler, 1941) and interspecific (Holmes, 1961) interactions and of crowding and competition for nutrients (Nesheim et al., 1978; Crompton *et al.*, 1983). The inflammatory response of the host towards the expulsion of concurrent *T.spiralis* infection could be attributed to the more anterior position of *M.moniliformis* in the small intestine in a less inflamed habitat. It seems not unlikely that the worms are selecting optimum sites along one or more of the many gradients known to exist along the length of the small intestine.

Insemination in female *M.moniliformis* has been found to have occurred as early as day 16 or 17 p.i. (Crompton, 1974; see chapter 4), in a single primary infection based on 15 cystacanths per rat. It was proposed, during this study, to investigate the effects of concurrent infections with *T.spiralis* and *N.brasiliensis* on the opportunities of mate-finding in *M.moniliformis*. The results from experiment 4, reveal a slight anterior shift of female worms with concurrent *N.brasiliensis* infection, but the difference was not significant when compared with single infection. The more interesting result observed was a significant increase in worm wet weight and length of female worms from concurrent infections. When the reproductive state of female worms was determined, significantly lower number of females were found to have been inseminated in concurrent *N.brasiliensis* as compared to single and concurrent *T.spiralis* infections. In addition, none of those females was found to contain free eggs in their body cavities suggesting that insemination had occurred lately in this group. Many possibilities could contribute to these findings. The presence of *N.brasiliensis*, which is found to be correlated to the poor insemination status of female *M.moniliformis*, might have altered the physiology of the host gut in such a way that, it would favoured the growth of *Moniliformis* (competition) on the expense of reproduction. The release of pheromones by female *N.brasiliensis* might possibly have interfered with mate-

finding mechanisms of *M.moniliformis* (see chapter 6) and as a consequence would have delayed the copulation/ insemination of females.

The results revealed a higher fecundity index in female *M.moniliformis* from concurrent infections suggesting that, co- occurring helminth species, although delaying the time of insemination in *M.moniliformis*, may favour its fecundity. As concurrent infections with helminths are likely to occur in natural populations of hosts, it may be that these experiments mimic the natural situation. If this is the case then it would make evolutionary sense for *M.moniliformis* to produce larger number of eggs in shorter time.

8.5 SUMMARY

Experiments were undertaken to investigate the interactions between *Moniliformis moniliformis, Trichinella spiralis* and *Nippostrongylus brasiliensis* in the laboratory rats. Cross-immunity in rats against the helminths and the effects of anthelmintic drug, levamisole, were also investigated. Rats harbouring a 35-day-old primary infection of *M.moniliformis* were challenged with *T.spiralis* and after 4 and 10 days of the nematode infections, rats harboured significantly fewer *T.spiralis* than rats with single infections. *T.spiralis* was naturally eliminated after 10 days of infection, from the rats harbouring either single-species or concurrent infections. *M.moniliformis* population was not significantly affected, in terms of numbers, but it did show an anterior shift in site and female worms' growth was retarded. In the presence of *N.brasiliensis*, mating success of *M.moniliformis* was disrupted and significantly fewer worms were inseminated than from the single infections. Nevertheless, female *M.moniliformis* from concurrent infections produced significantly higher number of eggs than the females from single infections.

Rats primarily infected with *M.moniliformis* and then treated with levamisole for two days were challenged with *T.spiralis*. At *post mortem* examination these rats harboured significantly fewer *T.spiralis* than the untreated and pretreated controls, suggesting that loss of the nematode species might be due to the inflammatory response of *M.moniliformis*.

Table 8.1 Observations on the establishment and growth of Moniliformis moniliformis and Trichinella spiralis from single and concurrent infections of rats.

Infection	Worm age on recovery (days)	No. worms recovered/rat mean ±SE male	female	Mean dry weight/worm ±SE male	female	Attachment position (%) mean ±SE
Moniliformis moniliformis						
Single	38	5.2 ±0.3	6.5 ±0.3	6.5 ±0.2	20.8 ±0.7	36.6 ±1.4
	52	4.2 ±0.3	5.4 ±0.1	8.5 ±0.5	34.5 ±0.9	35.7 ±1.7
Concurrent	38	4.5 ±0.3	3.8 ±0.4	5.4 ±0.2	17.2 ±0.4	31.6 ±0.9*
	52	5.2 ±0.2	3.2 ±0.4	9.5 ±0.5	35.0 ±1.8	35.8 ±1.7
Trichinella spiralis						
Single	4 [a]	335.0 ±44.1		--		--
	10	53.9 ±31.9		--		--
	12	2.4 ±1.8		--		--
	17	0		--		--
Concurrent	4 [b]	55.3 ±12.4		--		--
	10	11.0 ±6.9		--		--
	12	2.0 ±0.9		--		--
	17	0		--		--

Mann-Whitney U test between a and b = 0.002; $P < 0.01$

Table 8.2 Observations on the establishment and growth of *Moniliformis moniliformis* recovered from rats harbouring single and concurrent infections with *Trichinella spiralis* and *Nippostrongylus brasiliensis* during the course of infection.

Infection	Worm age on recovery (days)	No. worms recovered/rat mean ±SE	Length/female worm mean ±SE	Attachment position (%) mean ±SE
Moniliformis moniliformis from single infection	21	5.2 ±2.2	34.7 ±2.7*	30.2 ±3.3
	35	10.4 ±1.3	134.0 ±3.0	30.8 ±2.1
from concurrent infection with *Trichinella spiralis*	21	6.6 ±1.9	56.3 ±2.8	37.4 ±1.2
	35	7.2 ±0.8	124.5 ±3.3	35.3 ±3.4
From concurrent infection with *Nippostrongylus brasiliensis*	21	8.8 ±0.8	55.6 ±5.1	26.4 ±1.6
	35	7.2 ±0.7	118.5 ±4.1	33.9 ±2.2

* One-way analysis of variance = 0.026; P <0.05

Table 8.3. Observations of an investigation on the effects of concurrent infections with Trichinella spiralis and Nippostrongylus brasiliensis on mating success and fecundity of Moniliformis moniliformis in rats.

Infection	Worm age on recovery (days)	No. female worms inseminated	No. female worms not inseminated	No. female worms with eggs	No. female worms without eggs	No. ovaries /female worm mean ±SE	No. eggs/female worm mean ±SE
Moniliformis moniliformis from single infection	21	8	3	6	2	862 ±195	2,816 ± 545
	35	24	0	24	0	2,271 ±222	64,721 ± 5,898
from concurrent infection with Trichinella spiralis	21	11	6	8	3	2,755 ±257	3,082 ± 704 *
	35	19	0	18	1	4,029 ±355	2,95,400 ±27,082
from concurrent infection with Nippostrongylus brasiliensis	21	6	17	0	6	2,150 ±226	-- *
	35	12	3	11	1	5,008 ±663	2,05,350 ±24,522

* One-way analysis of variance = P <0.05

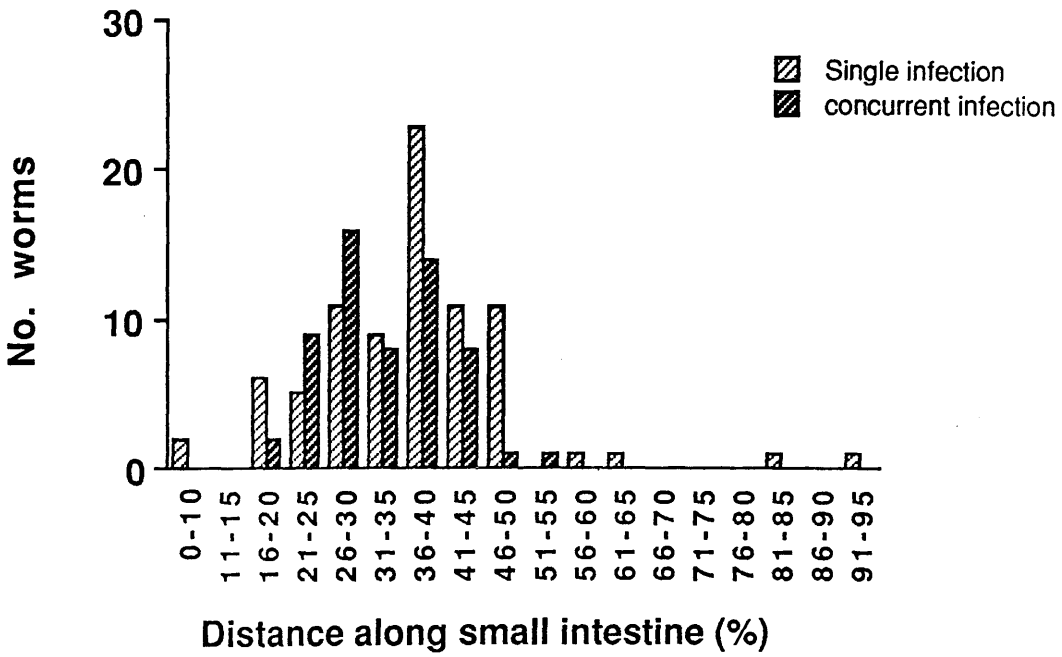


Fig. 8.1 Histograms showing the range (%) in distribution of attachment positions of *Moniliformis moniliformis* along the length of small intestine. (▨), 38-day old from single and (▨) concurrent infections with *Trichinella spiralis*.

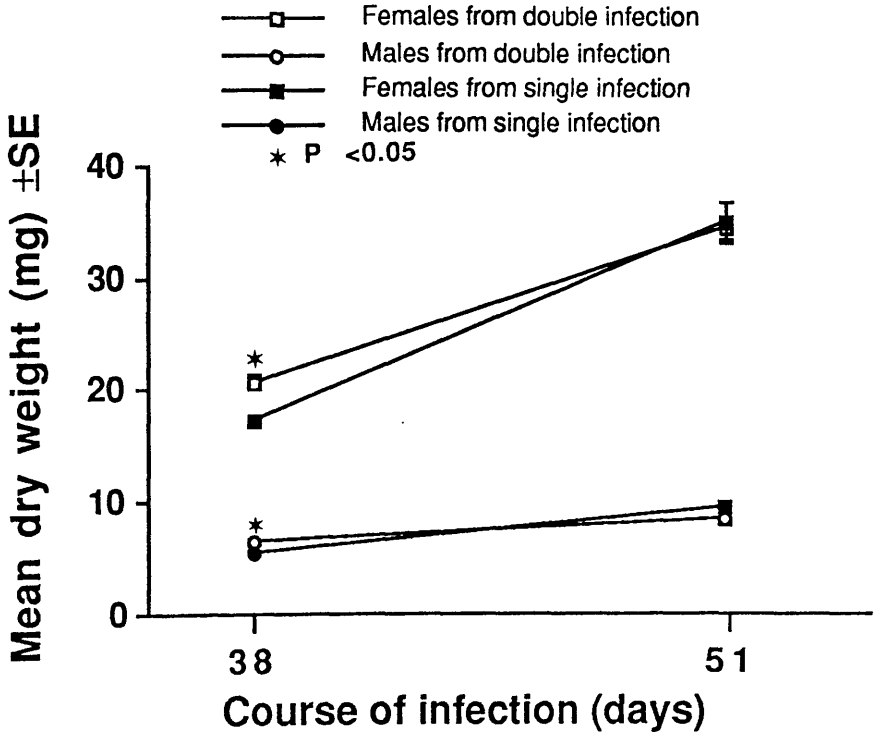


Fig. 8.2. Mean dry weight (±SE) of male and female Moniliformis moniliformis recovered at days 38 and 51 p.i. from rats harbouring single (■—■, ●—●) and concurrent (□—□, ○—○) infections with *Trichinella spiralis*.

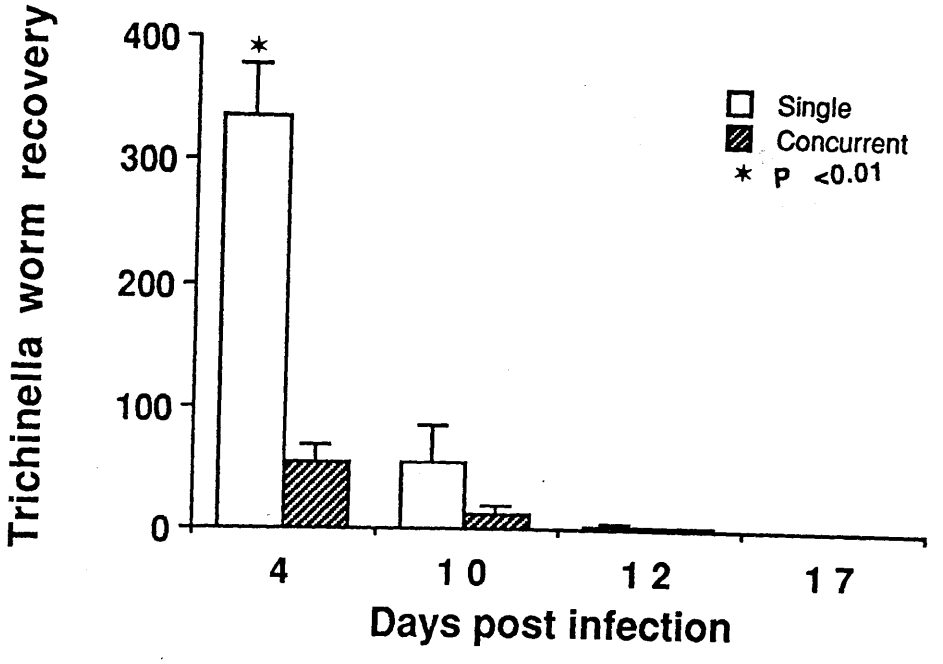


Fig. 8.3 Mean worms recovery (±SE) of *Trichinella spiralis* from rats harbouring single (□) and concurrent (▨) infections with *Moniliformis moniliformis* during the course of infection.

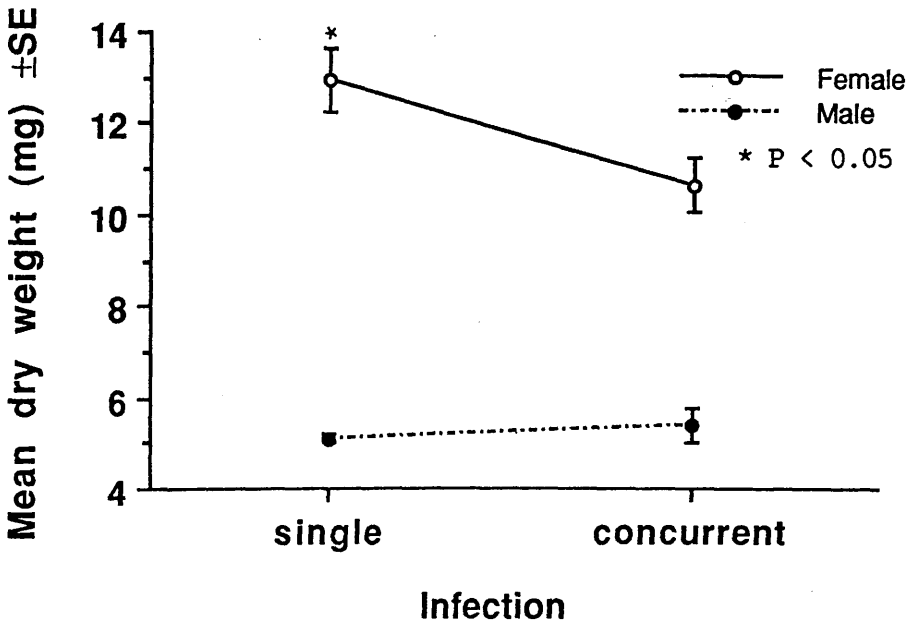


Fig. 8.4 Mean dry weights of 38-day-old female Moniliformis moniliformis recovered from rats harbouring single and concurrent infections with *Trichinella spiralis*.

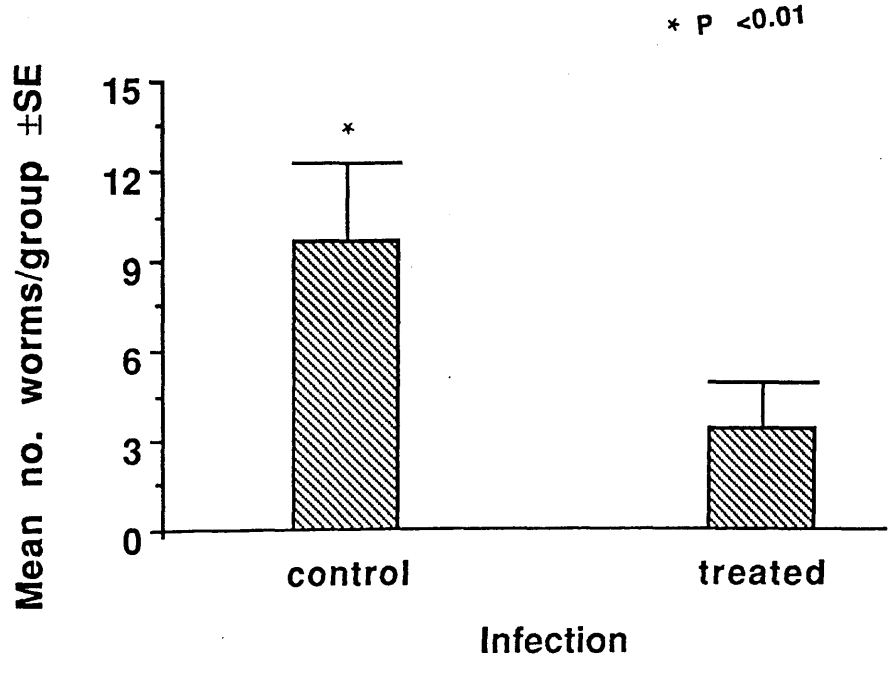


Fig. 8.5 Mean numbers (±SE) of *Moniliformis moniliformis* recovered from rats treated with levamisole at day 35 p.i. and controls.

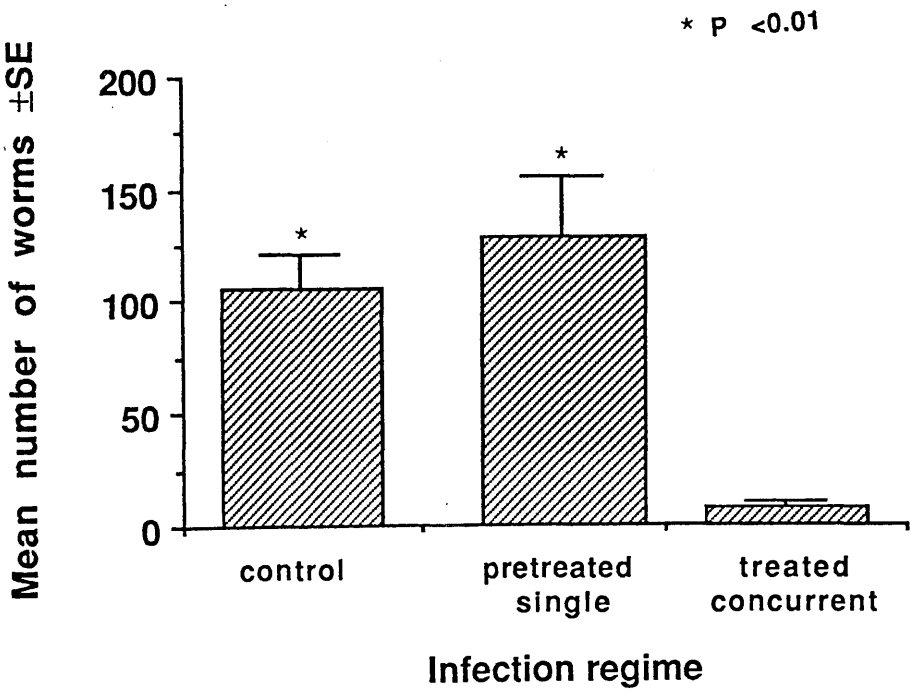


Fig. 8.6 Mean worm recovery (±SE) of *Trichinella spiralis* from pretreated rats harbouring single infections, treated rats with concurrent infections and the controls.

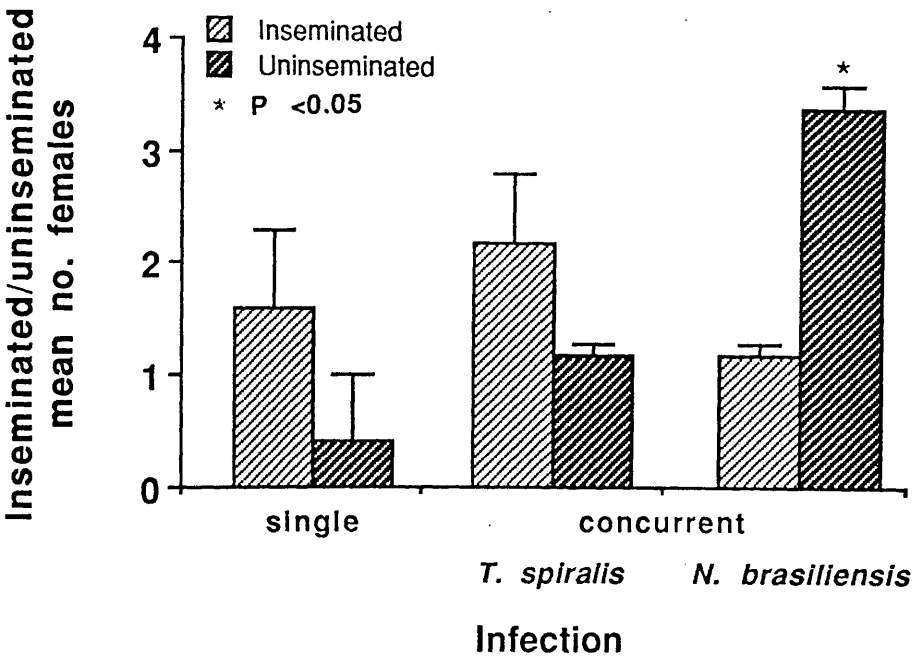


Fig. 8.7 Mean number (±SE) of 21-day-old inseminated (▨) and uninseminated (▨) female Moniliformis moniliformis recovered from rats harbouring single and concurrent infections with *Trichinella spiralis* and *Nippostrongylus brasiliensis*.

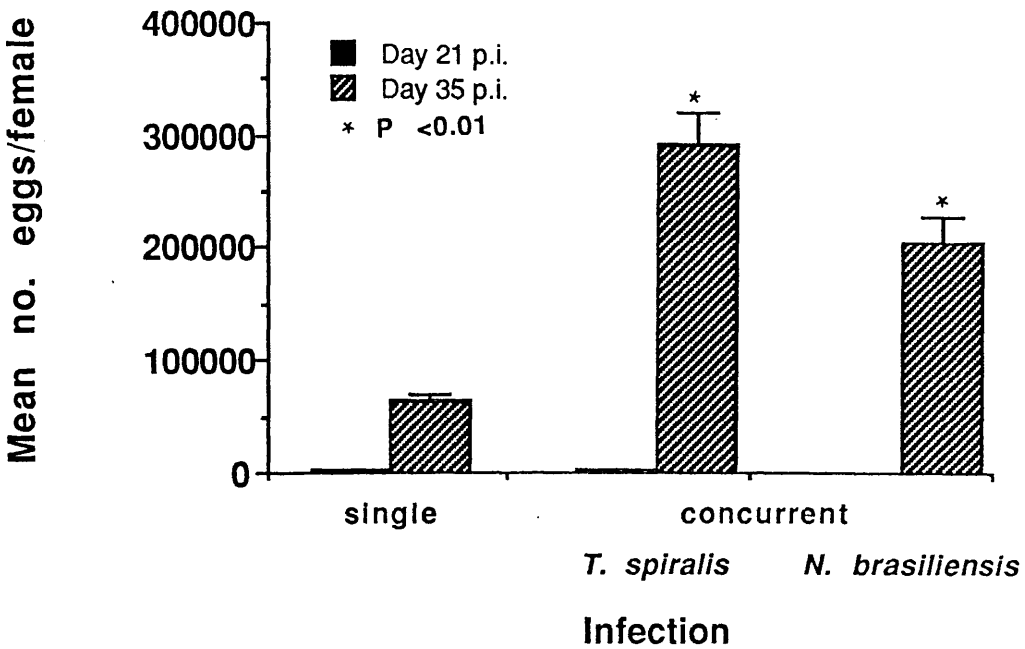


Fig. 8.8 Estimated mean number (±SE) of eggs per 21 and 35-day-old female Moniliformis moniliformis recovered from rats harbouring single and concurrent infections with *Trichinella spiralis* and *Nippostrongylus brasiliensis*.

CHAPTER 9. INFLUENCE ON *MONILIFORMIS MONILIFORMIS* REPRODUCTION: ANTHELMINTIC DRUGS AND THEIR USE AS AN INVESTIGATIVE TOOL

9.1 INTRODUCTION

The function of anthelmintic drugs is to remove helminth parasites from their hosts without causing any ill effects on the host. A large number of drugs have been shown to have effects on intestinal helminths. An anthelmintic drug may act by killing or paralysing worms, or it may injure the cuticle leading to partial digestion of the worm. Anthelmintic drugs may also interfere with the metabolism of the worm and since the metabolic activities of parasitic worms vary from one species to another, this may be the reason why drugs which are highly effective against one species are ineffective against the others. In the present study different anthelmintic drugs were used in trials against *Moniliformis moniliformis* so that one could be selected for routine termination of experimental infections when required. The ability to remove worms from the difinitive host would be a most useful asset for certain types of experiment upto certain trials, levamisole was used in the experiments undertaken to (1) investigate the role of immune response of rats as an influence on the reproduction of *M.moniliformis*, and (2) study the effects of the drugs on the fecundity of *M.moniliformis*.

9.2 EXPERIMENTAL DESIGN

Three experiments were undertaken. In the first experiment (experiment 1), 4 anthelmintic drugs albendazole (Valbazen), levamisole (Ketrax) , mebendazole (Ovitelmin) and praziquantel (Droncit) were used against according to the manufacturer's instructions *Moniliformis moniliformis*. A total of 69 rats were used as hosts. For each drug 7 or 8 rats were selected randomly for anthelmintic treatment and the remaining served as controls. The experimental protocol is summarized in Table 9.1. In the second experiment (experiment 2), 16 out of 37 rats, infected with 15 cystacanths of *M.moniliformis* each, were treated with levamisole in groups, and then were either killed after a given period of time or were challenged with secondary infections. The remaining 21 rats served as controls

and were killed accordingly. The experimental protocol is shown in Table 9.2. In the third experiment (experiment 3), 15 rats were each infected with 15 cystacanths of *M.moniliformis* on day 0. After 35 days, 7 rats were killed and the worms were recovered. On days 33, 34 p.i., the remaining 8 rats, plus 8 previously uninfected rats were treated with levamisole. On day 35 p.i. rats were each infected with either 15 or 30 cystacanths of *M.moniliformis* respectively. The experimental protocol is given in Table 9.3.

9.3 RESULTS

At *post mortem* examination, for each experiment, the numbers of worms and their sexes were noted. For experiment 3, the attachment positions of all the worms recovered, along the distance of the small intestine and their lengths were recorded. Later, the body cavity contents of individual female worms were collected and their fecundity was assessed by counting the numbers of ovaries, immature and mature eggs.

Experiment 1

9.3.1 OBSERVATIONS ON THE GENERAL EFFECTS OF DIFFERENT ANTHELMINTIC DRUGS ON THE COURSE OF INFECTION OF *MONILIFORMIS MONILIFORMIS* IN RATS

At *post mortem* examination of the rats, the numbers and sexes of the worms recovered were recorded and the results are summarized in Table 9.4. The numbers of worms recovered from each rat group are shown in Fig 9.1 as percentages of the total dose given. Worms of both sexes were recovered from all the control and the rats treated with albendazole. A total of 118 (65.5%) and 107 (59.4%) of the worms were recovered from the control and treated groups respectively, and no significant difference was observed between them (Table 9.4). Again from all the control rats and from those treated with levamisole, worms of both sexes were recovered (Table 9.4). Treated rats were found to contain significantly fewer worms than the control rats ($P < 0.001$). A total of 6 (5.7%) and 52 (49.5%) worms were recovered from

treated and control rats respectively (Fig. 9.1). Although from the results of rat group treated with mebendazole, it seemed that the drug had a significant effect on *M.moniliformis*, but at the *post mortem* examination, worms were recovered from 4 out of 6 control rats and 16 out of 20 treated rats. On average, 1.0 ±0.3 and 0.8 ±0.1 worms were recovered from control and treated rats respectively (Table 9.4). As the numbers of worms recovered from the control rats were significantly fewer than the normal recovery rate of *M.moniliformis* observed in other experimental studies (see Chapter 3), it might be possible that the cystacanths might have not been established in these rats at all. Worms of both sexes were recovered from all the rats treated with praziquantel and from the untreated controls. A total of 35 (58%) and 19 (47%) of the worms were recovered from treated and control groups respectively (Table 9.4). The analysis of the data revealed no significant difference between the numbers of worms recovered from the two groups (Fig 9.1).

From the results of this experiment it was observed that, of the anthelmintic drugs tried against *M.moniliformis*, levamisole was the most effective.

Experiment 2

9.3.2 OBSERVATIONS ON THE IMMUNE RESPONSE OF RATS TREATED WITH LEVAMISOLE

To find out whether levamisole has any long term effects, an experiment was undertaken in which rats were treated with levamisole and then challenged with secondary infections of *M.moniliformis* see below. The results are summarized in Table 9.5. At the *post mortem* examination, worms were recovered from all the rats (control and treated) in each group. At day 49 p.i., on average, 7.1 ±0.9 and 0.87 ±0.3 worms were recovered from control and treated rats respectively revealing a significant difference between them ($P < 0.001$). A significant difference was observed between the numbers of worms recovered, at day 63 p.i., from primary and challenged infections of treated rats ($P < 0.001$). On average, 1.0 ±0.4 and 9.5 ±1.0 worms were recovered from primary and challenged infections respectively. No significant difference could be detected when the numbers of worms recovered, at

day 63 p.i., from control rats were compared with those recovered from the primary infections of treated rats (Table 9.5). The results indicate that previous treatment of rats with the drug has no long term effects on subsequent infections of *M.moniliformis*. When the numbers of worms recovered from challenged infections in treated rats were compared with the numbers of worms recovered from control rats at day 14 p.i., no significant difference could be detected (Table 9.5). The worm recoveries, expressed as percentages of the total dose given, are illustrated in Fig. 9.2.

Experiment 3

9.3.3 OBSERVATIONS ON THE COURSE OF INFECTION AND FECUNDITY OF *MONILIFORMIS MONILIFORMIS* IN RATS TREATED WITH LEVAMISOLE

The results are summarized in Table 9.6. Worms of both sexes were recovered from all the rats. A total of 91 (86.7%), 56 (26.6%) and 198 (94.2%) worms were recovered from untreated control rats, treated rats and pretreated control rats respectively. Significant differences were observed when the numbers of worms recovered from control infections (untreated and pretreated) were compared with those of treated primary and challenged infections ($P < 0.001$). Significantly more worms were recovered from pretreated control rats compared with the untreated control and treated challenged infections might be due to the fact that rats from former group were given 30 cystacanths each compared to 15 cystacanths per rat in the latter groups. The mean numbers of worms ($\pm$SE) recovered from each rat group are shown in Fig 9.3.

Worms from all rat groups were, on average, found to be confined in the zone ranging from 15-50% along the distance of the small intestine except for the worms from treated primary infections which were confined in the zone ranging from 30-74%. No significant difference between the attachment positions of female worms recovered from untreated and pretreated control groups could be detected (Table 9.6). However, female worms from rats treated during primary infections were observed to be attached significantly more posteriorly in the small intestine than

the female worms from the challenged infection ($P < 0.05$). The range of attachment positions of female worms from the 3 rat groups is illustrated in Fig. 9.4 a-c. No correlation between the female worm attachment position and the anthelmintic treatment given, could be detected. The mean lengths $\pm$SE per female worm recovered from untreated control rats, treated rats and pretreated control rats are given in Table 9.6 and are illustrated in Fig. 9.5. No significant difference between the lengths of female worms recovered from untreated control and pretreated control rats was observed. However, female worms of challenged infections recovered from treated rats were found to be significantly smaller than the worms recovered from all other rat groups ($P < 0.05$). No correlation between the lengths of female worms and the anthelmintic treatment given, could be detected.

Forty one, 30 and 112 female worms were recovered from control, treated and pretreated rat groups respectively. All female worms were found to have been inseminated and contained eggs at various stages of development, in their body cavities. An analysis of variance revealed significant differences between the numbers of eggs per female worm recovered from treated primary infection and the female worms from all other groups ($P < 0.01$). Female *M.moniliformis* from treated primary infection were found to contain, significantly more eggs than the female worms recovered from all other groups ($P < 0.01$). On average, female worms recovered from primary and challenged infections of treated rats, untreated and pretreated control rats contained 173478 $\pm$19432, 64992 $\pm$11367, 61615 $\pm$4825 and 71196 $\pm$ 2685 eggs in their body cavities respectively (Table 9.6) These values are also illustrated in Fig 9.6. No correlation between the fecundity of female *M.moniliformis* and the treatment given, was detected. However, as expected, female worm fecundity was observed to be significantly correlated with the length of the female worms ($P < 0.05$), i.e. larger females produced more eggs.

9.4 DISCUSSION

The results described in this chapter have indicated the superiority of levamisole compared with albendazole, mebendazole and praziquantel as an anthelmintic drug

against *Moniliformis moniliformis*. Although mebendazole seemed to have an anthelmintic effect on *M.moniliformis*, but very few worms were recovered from the control rats as compared to the normal recovery rate of *M.moniliformis* under the experimental conditions, suggesting that the cystacanths, for some reason, might have not been established in these rats at all. From the second experiment, it was observed that levamisole was highly efficacious against primary infection of *M.moniliformis* as well as against the challenged infection given 2 days post treatment. But the effect was observed to be decreased thereafter and when the rats were challenged with secondary infections 15 days post treatment, no significant difference between worm recovery from the treated and untreated control rats could be detected, indicating that previous treatment of rats with the drug did not have long term effects on *M.moniliformis*. From the results of experiment 3, no evidence of the effect on the attachment positions and growth of female worms could be detected. At the *post mortem* examination of rats, the female worms from control (untreated and pretreated) and challenged infections (treated) were 35-day-old and those from primary infections (treated) were 70-day- old. The female worms from the latter group were found to be significantly larger and attached significantly more posteriorly in the small intestine, than the female worms from all other groups. This might be due to the fact that by week 7 p.i., under the experimental conditions, *M.moniliformis* are lost from the hosts (see Chapter 3).

When the estimates of fecundity were compared between the female worms recovered from treated and control rats, it was observed that female *M.moniliformis* from recovered primary infection of treated rats contained significantly more eggs in their body cavities than the female worms recovered from all other groups, indicating the fact that the former being much older (70-day-old) than the latter (35- day-old) are expected to produce larger numbers of eggs.

These results suggest that the anthelmintic drug, levamisole, which is efficacious against *M.moniliformis*, does not have any obvious effect on the fecundity of the parasite.

9.5 SUMMARY

Experiments were undertaken to investigate the efficacy of different anthelmintic drugs against *Moniliformis moniliformis* in rats. Of the anthelmintics used (albendazole, levamisole, mebendazole and praziquantel) in these experiments, levamisole was found to be the most effective against primary and challenged infections (given 2 days post treatment) of rats with *M.moniliformis*. But, levamisole was found to have no long term effect against the parasite when rats were given subsequent infections 15 days after the treatment. Also, no effect of the anthelmintic drug on the fecundity of *M.moniliformis* was observed.

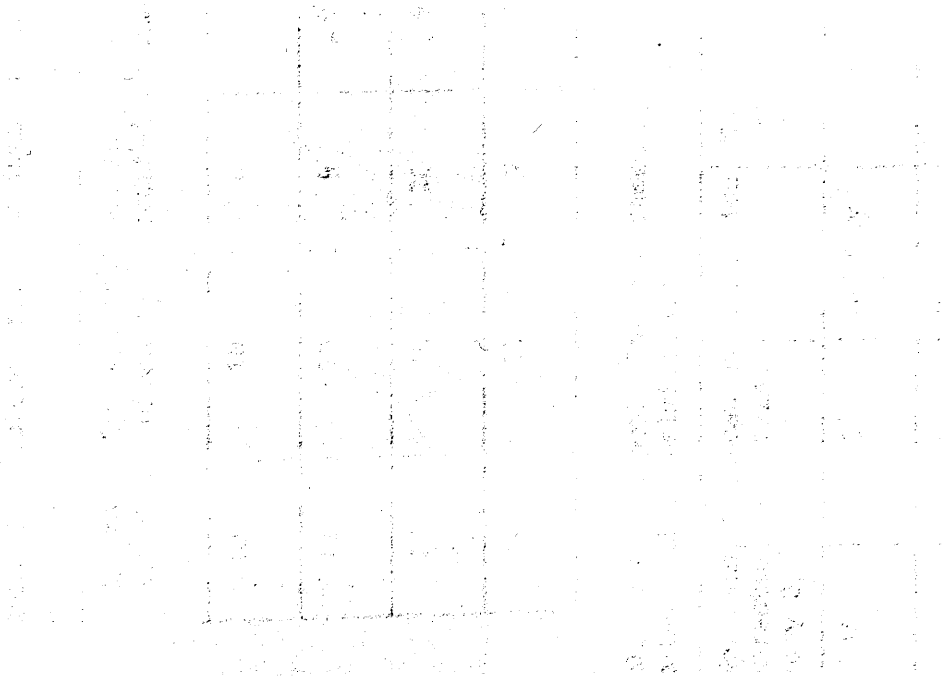


Table 9.1 Protocol for experiment 1, to investigate the effects of different anthelmintic drugs

No. rats infected	Cystacanths given/rat	No. rats treated	No. rats untreated	Treatment (dose)	Treatment given (days p.i)	Experiment ended (days p.i)
10	10	6	4	Droncit (10 mg/kg)	35-36	37
18	20	9	9	Ovitelmin (10 mg/kg)	34-38	39
27	15	20	6	Valbazen (5 mg/kg)	32-37	37
14	15	7	7	Levamisole (30 mg/kg)	33-34	35

Table 9.2 Protocol for experiment 2, to investigate the role of immune response in rats treated with anthelmitic drug

No. rats infected (day 0)	Cystacanths given/rat	Infection	No. rats treated	No. rats untreated	Treatment (dose)	Treatment given (days p.i)	Experiment ended (days p.i)
15	15	primary	8	7	Levamisole (30 mg/kg)	35-36	49
8	15 + 15	Primary + Challenge (day 49 p.i.)	8 --	--	" " " "	35-36	63
7	15	Primary	--	7	--	--	63
7	15	Primary	--	7	--	--	14

Table 9.3 Protocol for experiment 3, to estimate fecundity in rats treated with anthelmintic drug

No. rats infected (day 0)	Cystacanths given/rat	Infection	No. rats treated	No. rats untreated	Treatment (dose)	Treatment given (days p.i)	Experiment ended (days p.i)
7	15	primary		7			35
8	15 + 15	Primary + Challenge (day 35 p.i.)	8 --	--	Levamisole (30 mg/kg)	33-34	70
8	30	Primary	8		Levamisole (30 mg/kg)	Twice prior to infection	35

Table 9.4 Observations on the effects of treatment with different anthelmintic drugs on the course of infection of Moniliformis moniliformis in rats

Anthelmintic drug (n rats)	Duration of infection (days)	No. of worms recovered (total) male	female	Mean no. of worms/rat male	female	% worm recovery
Valbazen						
Treated rats (9)	39	65	42 (107)	7.2	4.6	59.4%
Control rats (9)	" "	58	60 (118)	6.4	6.6	65.5%
Ketrax						*
Treated rats (7)	35	4	2 (6)	0.5	0.2	5.7%
Control rats (7)	" "	30	22 (52)	4.3	3.1	49.5%
Ovitelmin						
Treated rats (20)	37	12	4 (16)	0.6	0.2	5.3%
Control rats (6)	" "	4	2 (6)	4.3	6.8	6.6%
Droncit						
Treated rats (6)	37	17	18 (35)	2.8	3.0	58.3%
Control rats (4)	" "	11	8 (19)	4.0	3.5	47.5%

* Chisquare = 50.404; P <0.001

Table 9.5 Observations on the effects of treatment with ketrax on the course of infection of Moniliformis moniliformis in rats

Rat group (n)	Infection	Duration of infection (days)	No. of worms recovered (total) male	female	Mean no. of worms/rat	% worm recovery
Control (7)	Primary	49	26	24 (50)	7.1 ± 0.9	47.6%
Treated (8)	" "	""	6	1 (7)	0.8 ± 0.3	5.8%
Control (7)	Primary	63	15	5 (20)	2.8 ± 0.8	19.0%
Treated (8)	Primary	63	8	- (8)	1.0* ± 0.4	6.6%
	Challenged	14	38	38 (76)	9.5 ± 1.0	63.3%
Control (7)	Primary	14	20	37 (57)	8.1 ± 1.9	54.2%

* Chisquare = 91.746; P <0.001

Table 9.6 Observations on the course of infection and fecundity of Moniliformis moniliformis in rats treated with Ketrax

Rat group (n)	Infection	Duration of infection (days)	No. of worms recovered (total) male	female	Mean no. of worms/rat male	female	Mean length /female worm ±SE (mm)	Mean attachment position/female worm ±SE (%)	Estimates of fecundity: No.immature eggs/female mean ±SE	No. mature eggs/female mean ±SE	Total number of eggs/female mean ±SE	Number ovaries /female worm mean ±SE
Control (7)	Primary	35	50	41 (91)	7.1	5.8	134 ±2.3	31 ±1.5	61368 ±4758	247 ±134	61615 ±4825	2340 ±223
Treated (8)	Primary	70	9	16 (25)	1.1	2.0	176.8 ±4.4	47.8 * ±3.5	158635 ±18431	14844 ±3416	173478 ±19432	3394 ±310
	Challenged	35	17	14 (31)	2.1	1.7	111.0 * ±7.5	30.5 ±4.7	64483 ±11264	508 ±482	64992 ±11367	1667 ±196
Pretreated (8)	Primary	35	86	112 ** (198)	10.7	14.0	131.0 ±1.4	30.5 ±1.0	71108 ±2683	89 ±34	71196 ±2685	2603 ±118

* $P < 0.05$

** $P < 0.001$

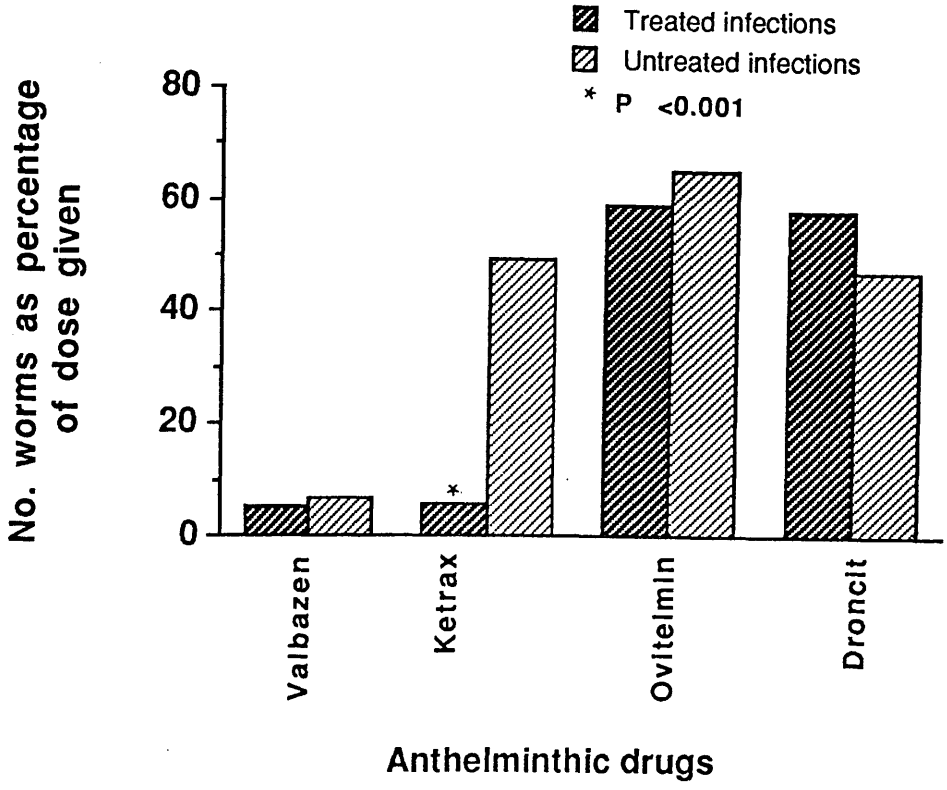


Fig. 9.1 Histograms showing the numbers of *Moniliformis moniliformis* recovered, as percentages of doses given to rats treated with different anthelminthic drugs and from untreated controls.

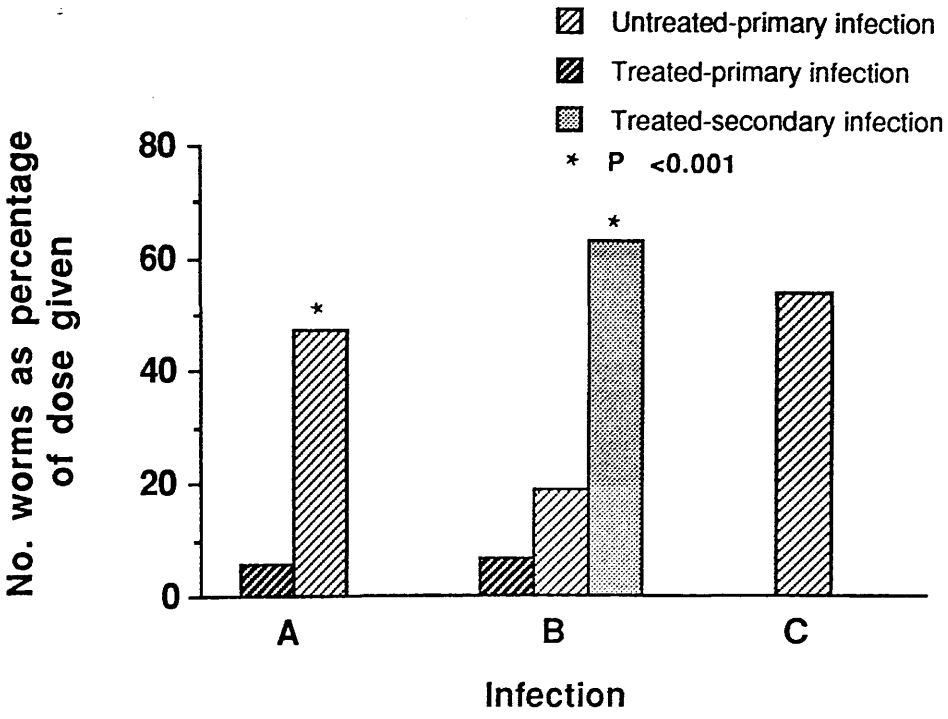


Fig. 9.2 Histograms showing the numbers of *Moniliformis moniliformis* recovered, as percentages of doses given to rats treated with levamisole and from untreated controls. A) 49 days old infection (▨ treated rats, ▨ untreated rats). B) 63 day old primary infection (▨), 14 day old secondary infection, (▩), C) 14 day old infection untreated rats (▨).

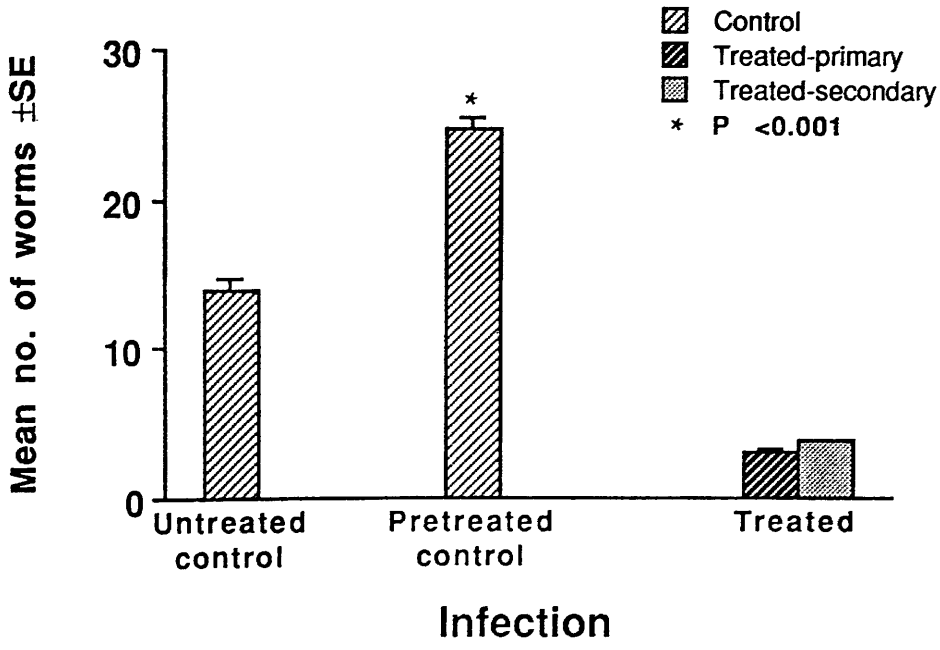


Fig. 9.3 Mean numbers ±SE of *Moniliformis moniliformis* recovered from rats treated with levamisole (primary infection, secondary infection), (untreated and pretreated controls.

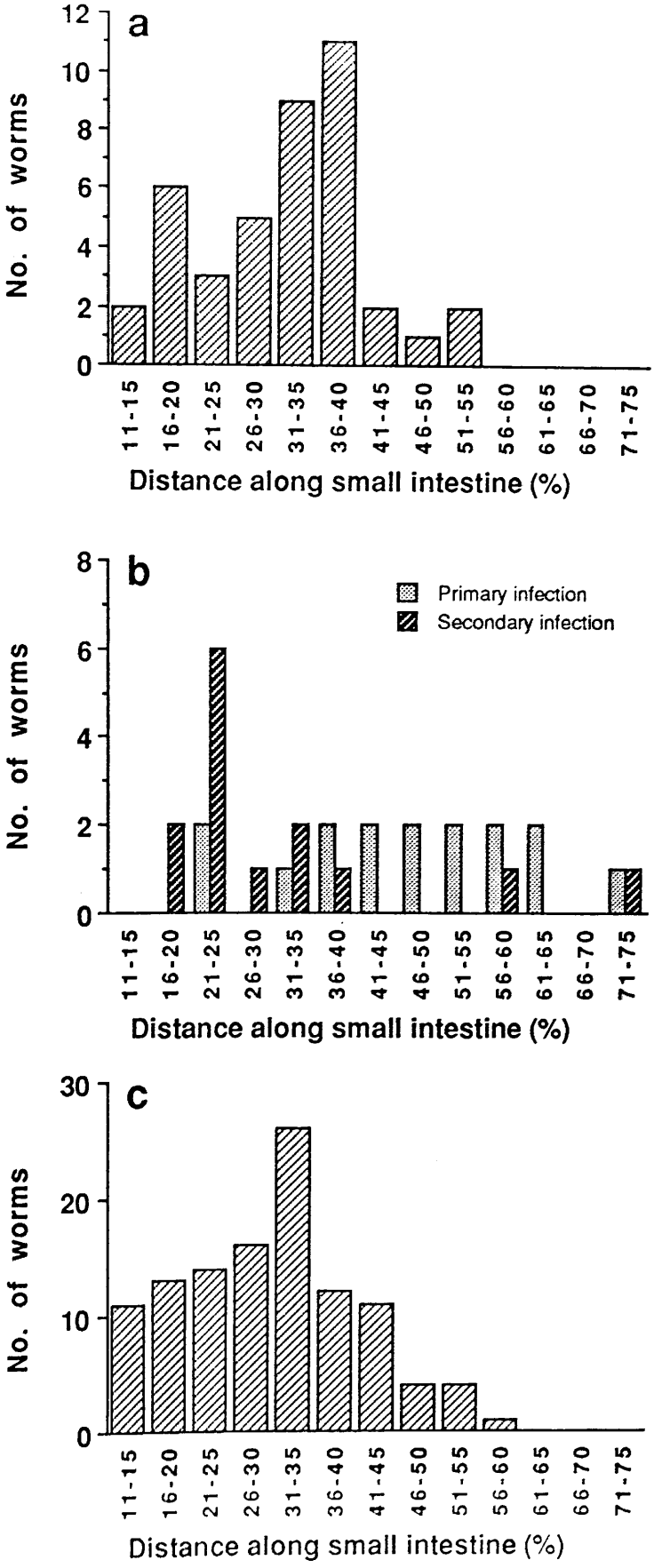


Fig. 9.4 Histograms showing the range of attachment positions of female *Moniliformis moniliformis* recovered from untreated and pretreated controls ( ), treated (primary, secondary infections).

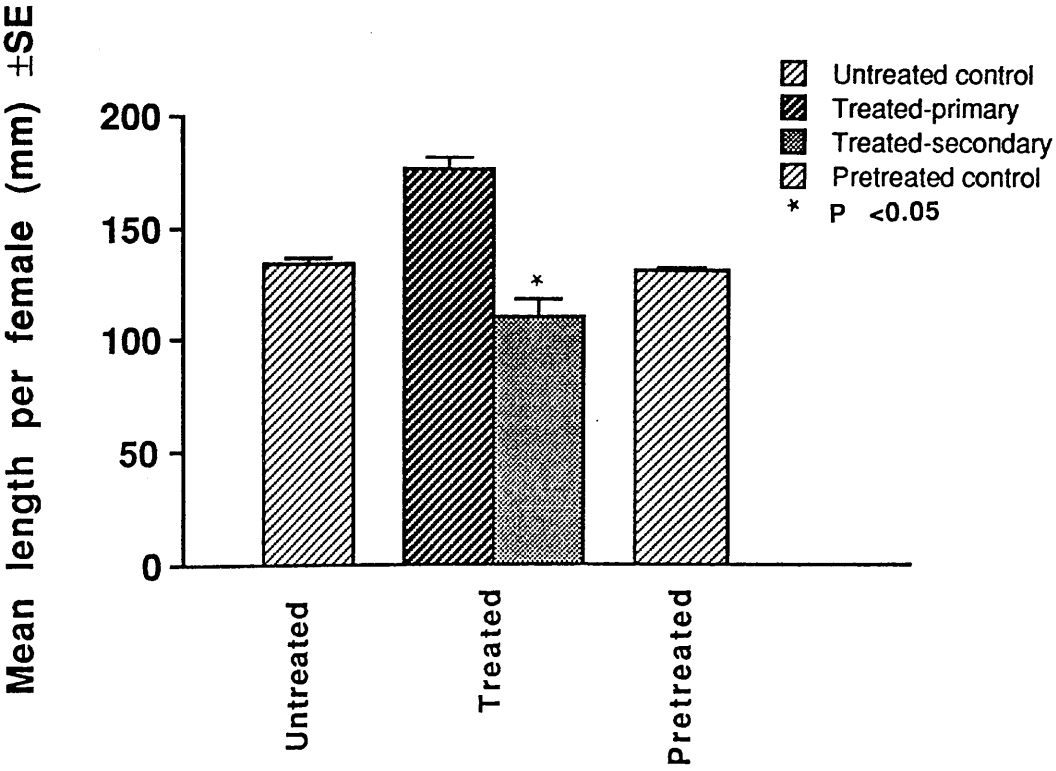


Fig. 9.5 Mean ±SE length (mm) of female *Moniliformis moniliformis* recovered from untreated and pretreated controls (▨), treated (▨ primary, ▩ secondary infections).

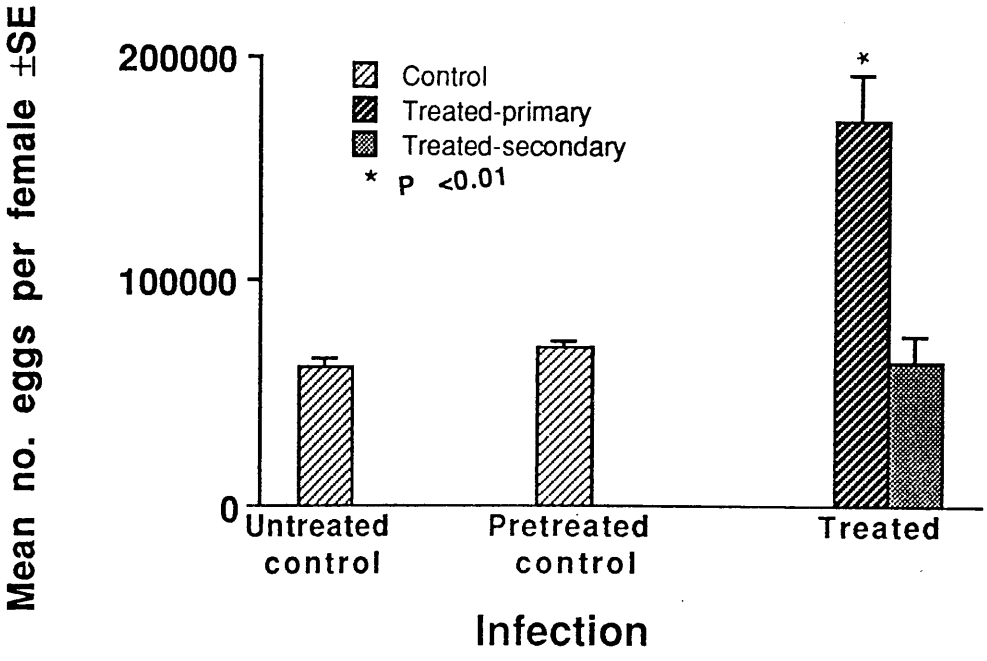


Fig. 9.6 Estimated mean numbers of eggs ±SE per female *Moniliformis moniliformis* recovered from untreated and pretreated controls (▨), treated (▨ primary, ▩ secondary infections).

GENERAL DISCUSSION

The results of experiments designed to characterize and elucidate the reproductive performance of *Moniliformis moniliformis* and described in previous chapters, have indicated that there are a variety of factors that affect the course of infection, the mating behaviour and fecundity of this parasite in its natural definitive host (*Rattus norvegicus*). According to Holland (1983) there is much evidence to show that wild *R.norvegicus* in many countries are infected with *M.moniliformis* and strains of laboratory rats are derived from *R.norvegicus* (Lindsey, 1979).

Analysis of the course of primary infection of *M.moniliformis* indicated that female worms survive longer than male worms in the experimentally structured populations. Results published on *M.moniliformis* by Burlingame and Chandler (1941), and by Crompton and Walters (1972) and on other species of Acanthocephala, for example, *Polymorphus minutus*, by Nicholas and Hynes (1958), Hynes and Nicholas (1963), and Crompton and Whitfield (1968) have also indicated somewhat similar patterns (see also Crompton, 1985).

Crompton (1972, 1974) reported that fertilized female *M.moniliformis* contained significantly more protein, measured as nitrogen, in their bodies than male and unfertilized female worms. The results I obtained (Chapter 3) were in agreement with these observations in that the mated female worms eventually grew at a greater rate than unmated females of the same age. This probably represents firstly, the early phase of egg production and secondly, the growth of female worms' body to accommodate the developing eggs.

In the literature, few observations have been reported about the mating behaviour of the acanthocephalan worms despite studies carried out by Bone (1976) and Miller (1980) and the information reviewed by Parshad and Crompton (1981) and Crompton (1985). Most information relevant to sexual congress, copulation and insemination in the Acanthocephala that has been published is based on the results of experimental infections of *M.moniliformis* in the laboratory rats. But still there lies a gap in the knowledge and understanding the effects of different factors on the

reproduction of this parasite under experimental and natural conditions.

In an attempt to improve the basis for understanding this problem, research was directed to investigate, under laboratory conditions, the influence of different factors on the mating behaviour and fecundity of *M.moniliformis* and to find out whether these factors might also affect the parasite in a similar way under the natural conditions. The parameters studied in this context included population structure of *M.moniliformis* in the host, the ages of male and female worms and interactions with other helminth species (*Trichinella spiralis* and *Nippostrongylus brasiliensis*). Many of the experiments depended on my being able to determine, in advance of infection, the sex of the cystacanths of the parasite. Also the use of X-irradiated worms and the selection of an effective anthelmintic drug for termination of infection were studied; the availability of such "tools" would greatly improve methods for studying the reproductive performance of this and other species of helminth parasites. As far as is known, these have been the first studies on the effects of X-irradiation and anthelmintic drugs on acanthocephalan worms.

The results obtained in this study showed that insemination between male and female *M.moniliformis* takes place as early as 17 days p.i. which is close to Atkinson and Byram's (1976) and Crompton's (1974) results showing that insemination in *M.moniliformis* could take place as early as 16 days after infection of the rat host. The results from the present study have also indicated that during the first 5 weeks of a primary infection, an individual male worm is capable of inseminating as many as 22 female worms of the same age. Previously, an upper limit of 17 females being inseminated by a single male worm has been observed for *M.moniliformis* by Crompton (1974).

The results obtained on the mating behaviour of the parasite in this study are of much interest. In Chapter 6, the hypothesis of active male mate choice influenced by female worm age, size and location in the small intestine was put forward. The explanation for this hypothesis is that, male *M.moniliformis* "choose" female mates on the basis that in a population of worms of different ages and sizes, male

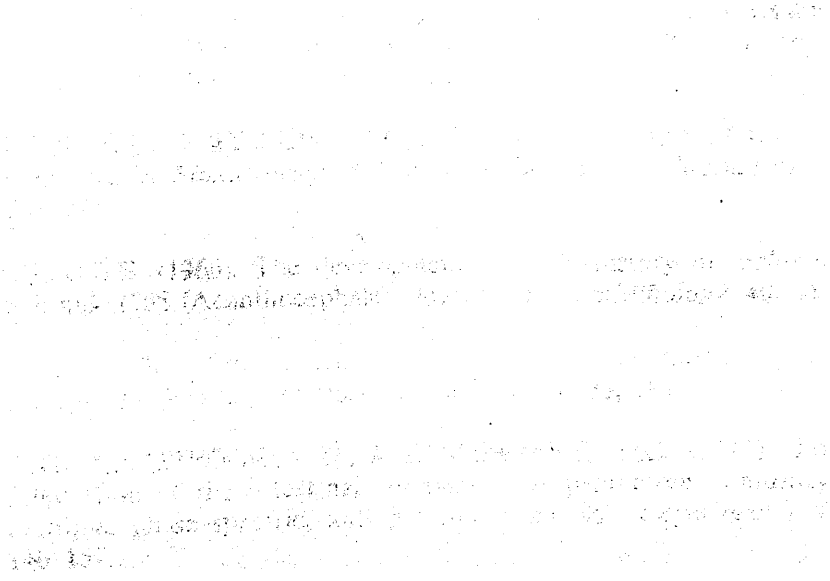
M.moniliformis mature before the females of the same age. Therefore, when female worms that are older but contain large numbers of mature oocytes in their body cavities are chosen by the males for mating so that sperm will be utilized more efficiently i.e have the best chances of meeting up with the oocytes. Switching mating from these older females to the younger ones (which have matured by this time) gives the males another opportunity to increase the chances of passing on their genes to the next generation, because these young female worms are likely to live longer than the older females. It is known that male worms do not live as long as females worms and also that acanthocephalan sperm are long lived or are stored (Meyer, 1933; Crompton, 1985). Younger females may be assumed to produce eggs for a longer period of time than the older females would, therefore choice of the most fecund females and those which are likely to live longer would from an evolutionary point favour the reproduction of the parasite.

Results from the experiments using X-irradiated worms are still preliminary in nature, but it appears that *M.moniliformis* is much more tolerant of X-irradiation than other species of helminths representing other phyla (Levin and Evans, 1942; Gould *et al.*, 1955; Jarrett *et al.*, 1960; Schiller, 1959). Further research in this field would eventually improve understanding of male mate choice on the basis of female worm size and fertility.

The view that the presence of a concurrent infection with *N.brasiliensis* in the rat might affect the mating behaviour of *M.moniliformis* was discussed in Chapter 8. The possibility that pheromones released female *N.brasiliensis*, might have interfered with mate finding mechanisms of *M.moniliformis* could be of interest. If male *M.moniliformis* choose female mates on the basis of where they are located in the small intestine and are attracted to them through some sort of chemical signal (Miller, 1980), interference from *N.brasiliensis* pheromones with this signal might cause confusion. Under natural conditions, hosts not infrequently harbour multiple infections of intestinal helminth species (Holmes, 1973), so these results may mimic the natural situation. Much of this discussion, despite Miller's (1980) views, is speculative because Miller only proposed that *M.moniliformis* produced pheromones

and Bone (1976) could not detect any form of attractant chemical although his experiments were carried out *in vitro* in apparatus that did not any way resemble the gut of a rat.

The results presented in this dissertation have given a glimpse into the complex process of mating and reproductive behaviour in *Moniliformis moniliformis*, an intestinal endoparasitic helminth. There exists in the literature the notion that parasitic worms are degenerate, simple, lowly and primitive animals, a view that has perhaps arisen because statements have been made about worms before knowledge of their biology had been obtained. In the case of individual *M.moniliformis*, and perhaps for individual worms of all acanthocephalan species, the picture is emerging of complex organisms adapted to thrive and reproduce in a specialized environment in a continuum of recruitment and loss to their populations.



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