Patterns of *Trichostrongylus tenuis* infection in individual Red Grouse (*Lagopus lagopus scoticus*)

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To Mum and Dad

Declaration

I declare that the work contained in this thesis is entirely my own, unless otherwise stated, and it is of my own composition. Collaborative authorship is acknowledged where appropriate. In Chapter 6, L.S. made significant contributions to the data collection and to laboratory parasite sampling. No part of this work has been submitted for a degree at the University of Stirling or any other University. All sources of information have been fully acknowledged.

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Abstract

Parasite infections of individual hosts are determined largely by their exposure, resistance and susceptibility to parasites. Heterogeneity in patterns of infections between individual hosts reflects the outcome of a variety of host-parasite interactions, which will be influenced by factors such as host physiology, behaviour, and habitat, as well as parasite-related factors. Establishing patterns of parasite infection among individual hosts, and identifying the complex interactions that create them, is of interest not only to epidemiologists, but also has relevance within the wider field of ecology. Using a variety of statistical and experimental techniques, this study examined particular patterns and associated processes acting to create heterogeneities in parasite infection in individual red grouse (*Lagopus lagopus scoticus*), a species of ecological and socio-economic importance. This study began by calibrating the use of faecal egg counts to estimate intensities of infection of the gastrointestinal nematode *Trichostrongylus tenuis*, an important parasite of red grouse. Analyses of patterns of infection within and between breeding pairs and broods of chicks showed that there was no relationship between infection intensities of paired males and females or between genetically similar individuals. This suggested that both exposure and host resistance may be important in determining an individual's parasite infection. A cross-fostering experiment was attempted to tease apart the relative contribution of exposure and resistance, but was not successful due to extraneous events. Within breeding pairs, red grouse were found to exhibit positive assortative mating with respect to body condition and, in both sexes, bigger ornamental combs were associated with better body condition, suggesting that comb size might function as a signal of individual quality. Comb size is testosterone dependent and in males, further experimental work showed that experimentally elevated testosterone in male red grouse imposed a cost through reduced immunocompetence and higher intensities of *T. tenuis*. These interactions have important implications for our understanding of sexual selection and cyclic population dynamics.

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And so, as one journey comes to an end, another begins.................

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Chapter 1.

General introduction

General introduction

1.1 Host-parasite interactions

Parasites have the ability to impact considerably on their hosts, by reducing their survival, fecundity and thus general fitness (Gulland, 1995; Goater & Holmes, 1997). As such, much quantitative research has focussed on understanding the epidemiology of host-parasites interactions. The impact of parasites on their hosts is of particular interest when hosts are of conservational importance (Daszak *et al.*, 2000) and socio-economic importance, such as those affecting human health (Crompton, 1994; Woolhouse, 1994) or commercial activities such as livestock production (Grenfell *et al.*, 1995) and the harvesting of fish and game (Hudson *et al.*, 1985; Burt, 1994; Tompkins *et al.*, 2000). Furthermore, host-parasite interactions are also of considerable ecological interest (Grenfell & Dobson, 1995). Recent theoretical advances suggest that parasites may play an important role within natural animal populations in areas such as life history evolution (Møller, 1997), sexual selection (Hamilton & Zuk, 1982; Read, 1988; Maynard-Smith, 1991; Møller, *et al.*, 1999), the maintenance of genetic variation (Anderson & May, 1982; Hamilton, 1982) and the evolution of sex (Jaenike, 1978; Hamilton, 1980; Hamilton *et al.*, 1990). A further area of interest to ecologists is the role of parasites in population dynamics (Anderson & May, 1978, 1979; May & Anderson, 1978; Roberts *et al.*, 1995).

1.2 The effect of parasites on host population dynamics

Several northern species of vertebrates exhibit recurrent fluctuations in population abundance, commonly referred to as population cycles (Watson, 1979; Keith, 1990; Korpimaki & Norrdahl, 1991; Small *et al.*, 1993; Krebs, 1996; Stenseth *et al.*, 1996; Hanski & Henttonen, 2002).

Since the work of Elton, who first described regular oscillations in numbers of the snowshoe hare (*Lepus americanus*) and its associated predator the Canadian lynx (*Lynx Canadensis*) (Elton, 1924), identifying the mechanisms driving such fluctuations in numbers, has been the subject of considerable theoretical and empirical research programs over the past 6 decades (Batzli, 1992; Oli & Dobson, 2000; Bjornstad & Grenfell, 2001; Moss & Watson, 2001) and the source of much controversy (Chitty, 1960 ; Krebs, 1996 ; Lidicker, 1988 ; Seldal *et al.*, 1994).

A variety of mechanisms have been identified as capable of driving regular oscillations in population dynamics: those involving extrinsic factors such as food, parasites or predators (Krebs *et al.*,1995; Hudson *et al.*, 1998; Korpimaki *et al.*, 2002), and those involving intrinsic factors, such as changes in male aggressiveness or kin-facilitation (Chitty, 1967; Lambin & Yoccoz,1998; Moss & Watson, 2001; Mougeot *et al.*, 2003 a, b). Much work to date has focussed on identifying the relative importance of extrinsic and intrinsic processes in driving these cyclic fluctuations and the topic remains widely debated (Lance & Lawton, 1990; Stenseth *et al.*, 1996). However, the interaction between these two types of mechanism has received comparatively little attention (but see Grenfell *et al.*, 1998; Coulson *et al.*, 2001).

Theoretical models suggest that parasites may have a regulatory effect on their host populations if they reduce host survival and/or fecundity in a density-dependent manner (Anderson & May, 1978; May & Anderson, 1978). However, given the difficulty in collecting long-term data necessary to explain the dynamics of natural populations, there are few studies providing empirical data to support these theoretical models. Evidence for a regulatory effect of parasites on population dynamics has so far been limited to studies of red grouse (*Lagopus lagopus scoticus:* Hudson *et al.*, 1985, 1992, 1998; Dobson & Hudson, 1992), Svalbard reindeer (*Rangifer* *tarandus plathyrynchus*: Albon *et al.*, 2002), and more recently field voles (*Microtus agrestis:* Cavanagh *et al.*, 2004).

Parasites are divided into microparasites and macroparasites (Anderson & May, 1979; May & Anderson, 1979). Microparasites (viruses, bacteria, and fungi) are characterized by their rapid reproduction directly within individual hosts, and their tendency to induce lasting immunity in recovered individuals (Dobson & Hudson, 1995). Macroparasites (helminths and arthropods), on the other hand, are usually visible to the naked eye and tend to have much longer generation times than microparasites. They do not usually reproduce directly within their definitive hosts and instead produce infective stages that usually pass out of the host before transmission to another host. Immune responses elicited by macroparasites depend on the number of parasites present in a given host, and tend to be of relatively short duration. Infections therefore tend to be of a persistent nature, with hosts being continually reinfected (Hudson & Dobson, 1995).

Within a host population, the overall impact of a macroparasite will depend on the interaction between both parasite pathogenicity and the frequency distribution of parasites within the host population (see Figure 1.1; Anderson, 1979; Anderson & Gordon, 1982; Hudson & Dobson, 1991). Highly pathogenic parasites that kill their host at low intensities will have an insignificant effect on the host population dynamics, as transmission rates will be low. On the other hand, moderately pathogenic parasites found at higher intensities, will have higher rate of transmission resulting in a greater impact on the population. Where parasites will have the greatest impact on host population equilibrium size, however, is where parasite pathogenicity is low enough not to impact on host mortality but where host fecundity is reduced (Hudson & Dobson, 1991). Thus, parasite pathogenicity can have contrasting effects on host population dynamics, either stabilising the host populations, where the parasite increases host mortality, or de-stabilising, where the effect of parasites reduces reproductive rate of their host (Anderson & May, 1978; May & Anderson, 1978).

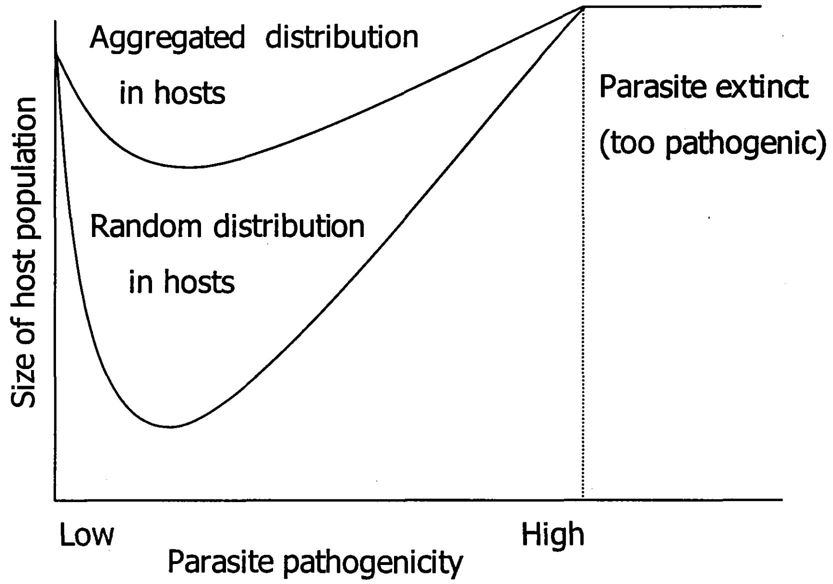

Figure 1.1: The effect of macroparasite pathogenicity and pattern of distribution on the host population (from Hudson & Dobson, 1991). Moderately pathogenic species tend to have the greatest effect on the host population size.

A characteristic of macroparasitic infections is that parasites tend to be unevenly distributed throughout the host population. In the majority of cases parasites are clumped or aggregated within a few individual hosts (Crofton, 1971; Anderson & May, 1978; Anderson & Gordon, 1982; Grenfell *et al.*, 1995; Shaw & Dobson, 1995; Wilson *et al.*, 1996; Shaw *et al.*, 1998). A measure of the degree of dispersion is the variance to mean ratio (Anderson & Gordon, 1982). Where the variance in parasite numbers exceeds the mean, parasites are said to be over-dispersed within the host population. This pattern of over-dispersion or aggregation, is commonly described empirically by the negative binomial, which is governed by two parameters; the mean and *k* which is an inverse measure of the aggregation (Crofton, 1971; Anderson, 1979; Anderson & Gordon, 1982; Hudson & Dobson, 1991; Poulin, 1993; Grenfell *et al.*, 1995; Shaw & Dobson, 1995; Wilson *et al.*, 1996, 2002).

The degree to which parasites are aggregated within the host population has important implications for the dynamics of the parasite-host system (Dobson & Grenfell, 1995). Where a large proportion of the parasite population is concentrated into a small proportion of the hosts, host factors that regulate parasites will affect large numbers of parasites and aggregated distributions will tend to stabilise host-parasite interactions. Impacts of parasites on host survival and fecundity will then affect only a small number of individuals. On the other hand, where the distribution of parasites within the host population is less aggregated, a greater proportion of the host population will be affected by the impact of parasites and host-parasite interactions will tend to be de-stabilised (Anderson & May, 1978; May & Anderson, 1978; Hudson & Dobson, 1995; Jaenike, 1996).

1.3 Factors affecting patterns of parasite infection

As the degree of aggregation plays a significant role in determining the degree of stability of host-parasite interactions, knowledge of causes of aggregated distributions has important implications for our understanding of host population dynamics, (Anderson & May, 1978; Boulinier *et al.*, 1996; Grenfell, 1988, 1992). Two main mechanisms may act on individuals to influence patterns of aggregated parasite distributions observed at the population level: 1) those creating variation in host exposure to parasite infective stages and 2) those relating to differences between individuals in their resistance or susceptibility to parasites (Anderson & Gordon, 1982;

Wilson, 1983; Anderson, 1993; Boulinier *et al.,* 1996; Holmstad & Skorping, 1998; Wilson *et al*., 2002).

The words 'susceptible' and 'resistant' are used in a variety of contexts in parasitology. A susceptible individual is defined as being either naïve (previously uninfected) or having lost immunity and the ability to control the infection (Watt *et al.,* 1995). The immune system is the main defense mechanism with which to fight or control parasite infections and vertebrates have evolved the ability to produce a sophisticated immune response that usually involves both antigen-specific cell-mediated responses and humoral responses (Sheldon & Verhulst, 1996; Saino *et al*., 1997). Immunity involves the presence of antibodies or activated cells as a means of counteracting disease or infection and may have a heritable component (innate immunity) or may be acquired by an individual following recovery from a disease or infection (Watt *et al*., 1995).

Resistance is generally defined as the ability of the host to resist infection by a parasite. Differences in resistance may be either innate or dependent on the genetic, physiological or morphological characteristics of individual hosts (Wakelin, 1984), or may be influenced by varying host immune responses as a result of past experience of infection (Anderson & Gordon, 1982; Stear *et al*., 1999). Natural or non-specific resistance to disease has been defined as "a physiological incompatibility between parasite and host environment, which prevents invasion or establishment without the intervention of immunologically-based protective responses" (Wakelin, 1984). Acquired resistance occurs when a host exhibits some degree of regulation of parasite infection through activity of the immune system (Wakelin, 1984). That parasite resistance, is at least partially genetically determined, is a key assumption of certain theoretical evolutionary models (Anderson & May, 1982; Hamilton & Zuk, 1982; Folstad & Karter, 1992;

Brinkhof *et al.*, 1999). However, evidence for genetic variability in parasite resistance in natural vertebrate hosts is limited to a handful of studies (Møller, 1990; Bouliner *et al.,* 1997; Brinkhof *et al.*, 1999; Smith *et al.*, 1999; Appleby *et al.,* 1999; Coltman *et al.*,2001).

Within a host population, intensities of parasite infection generally vary considerably between individuals. A wide variety of both extrinsic and intrinsic factors, may influence an individual's exposure or susceptible to parasite infection, which may accentuate heterogeneities in patterns of parasite infection between individuals within a population (Wilson *et al.*, 2002). If all individuals within a population are equally susceptible to parasites, then parasite infections will be determined simply as a function of the exposure rate. Exposure rate may be affected by a number of factors, such as habitat, temporal or spatial changes in climate, and differences in host physiology or behaviour (Crofton, 1971; Anderson & Gordon, 1982; Apanius & Schad, 1994; Hudson & Dobson, 1995; Shaw & Dobson, 1995). Hosts engage in a variety of behaviours, including foraging, reproductive and social behaviours, which parasites can exploit for their transmission (Apanius & Schad, 1994). Heterogeneity in exposure may be further influenced by host age and length of exposure to parasite infection, continued self-infection of hosts and by other infected individuals in the immediate area (Hudson & Dobson, 1995).

Evidence from an extensive number of vertebrate studies, across a wide variety of taxa, however, has shown that individuals may vary widely in their resistance to infection (Scott, 1988; Chevassus & Dorson, 1990; Møller, 1990; Wakelin, 1994; Stear & Murray, 1994; Douch *et al.,* 1995; Holmstad & Skorping, 1998; Lysne & Skorping, 2002). Moreover, a number of host-related factors may modulate the development and/or expression of the immune system reducing the ability of a host to resist parasite infection, and thus increasing host susceptibility to parasites

(Anderson & Gordon, 1982; Lloyd, 1995). Factors which have been hypothesized to increase individual susceptibility to parasites of factors including toxic chemicals in pollution (Khan, 1990), thermal stress from climate change (Harvell *et al.,* 1999) and host diet and nutritional status (Solomons & Scott, 1994; Beck & Levander, 2000; Ezenwa, 2003). Host malnutrition may lead to impaired immunity, and as such may facilitate the establishment and survival of parasites (Solomons & Scott, 1994). Studies of domestic ruminants have also highlighted the fact that a synergistic interaction may occur between malnutrition and infection, such that negative effects of gastrointestinal parasite infections may aggravate nutritional deficiencies, and dietary deficiencies may exacerbate the effect of infection on host metabolism (Scrimshaw *et al.*, 1959; Van Houtert & Sykes, 1996; Coop & Kyriazakis, 2001).

1.4 Parasites, life-history and sexual selection

In the last decade, ecologists and evolutionary biologists have become increasingly interested in parasites, and in particular in how they may shape a host's life-history (Loye & Zuk, 1991; Grenfell & Dobson, 1995; Clayton & Moore, 1997). Traditionally, life-history trade-offs have been regarded as those between life-history components such as breeding vs survival, or the number and quality of offspring in current vs future reproduction (Stearns, 1992). However, growing interest in the field of evolutionary ecology, has increasingly focussed on how parasite defence may shape a host's life history (Zuk & Stoehr, 2002).

As animals have only a limited amount of resources, individuals must allocate these resources optimally between competing life history activities such as growth, reproduction and parasite defence (Stearns, 1989, 1992; Roff, 1992; Owens & Wilson, 1999). The main physiological means by which animals resist or fight parasitic or pathogenic infections is through the immune system. Mounting an immune defence is regarded as an investment, as it is likely to be costly (Sheldon & Verhulst, 1996; Owens & Wilson, 1999; Lochmiller & Deerenberg, 2000; Tella *et al.,* 2002). Thus, an individual may allocate resources to parasite defence rather than in other life-history activities such as reproduction (Hakkarainen *et al.,* 1998; Owens & Wilson, 1999; Tella *et al.*, 2002; Lozano & Lank, 2003). The trade-off between current and future reproduction is the central concept to life-history theory and a cost of current reproduction is regarded as a reduction in reproductive success later in life (Norris *et al.*, 1994; Sheldon & Verhulst, 1996). One way that parasites may play a role in shaping life-history traits, is where increased investment in activities associated with reproduction may lead to reduced parasite defence and ultimately to increased susceptibility to deleterious parasite infections (Zuk & Stoehr, 2002 ; Møller, 1997; Norris *et al.,* 1994; Sheldon & Verhulst, 1996; McKean & Nunney, 2001).

Trade-offs may be particularly significant in species where individuals require elevated hormone levels to ensure reproductive success. In many species the hormone testosterone (T) is necessary for the expression of sexual behaviour, or secondary sexual ornaments that function in intra- and inter-sexual selection (Folstad & Karter, 1992; Eens *et al.*, 2000; Peters, 2000; Stoehr & Hill, 2000). In vertebrate species males often have higher parasite intensities relative to females (Zuk, 1990; Poulin, 1996; Schalk & Forbes, 1997; Sheridan *et al.,* 2000; Reimchen, 2001; Moore & Wilson, 2002; Wilson *et al.*, 2002). One possible mechanism causing sex biased parasitism may be immune suppression by androgens (Folstad & Karter, 1992; Grossman, 1985, 1989; Zuk, 1992; Sheridan *et al.*, 2000; but see Hillgarth & Wingfield, 1997; Hasselquist *et al.,* 1999). Immune function is regulated by the gonadal steroids oestrogens, androgens and progesterone (Grossman, 1984). Whilst female oestrogens have a dichotomous effect on the immune system, male androgens, in particular (T), are thought to suppress the immune system (Folstad & Karter, 1982; Grossman, 1984; Marsh, 1992).

Many animals display bright colours or elaborate ornaments that appear unlikely to enhance survival. Darwin (1871) suggested such ornaments may assist members of one sex to acquire mates and it is now widely established that ornamental traits, as expressed in males, are a product of sexual selection, and evolved through female mate preference. Models of sexual selection argue that ornamental traits in males function as indicators of male quality (Zahavi, 1975; Hamilton and Zuk, 1982; Andersson, 1994). Females may thus be able to assess the variation in male quality and discriminate between potential mates, by basing their preference on the size of sexual ornaments or intensity of sexual displays (Andersson, 1994). One hypothesis regarding the role of these ornaments, is that they may function as indicators of health and resistance to parasites. First proposed by Hamilton and Zuk (1982), this hypothesis has attracted increasing interest in the role that parasites might play in sexual selection (reviewed in Møller, 1990; Zuk, 1992). This hypothesis proposes that secondary sexual characteristics function to provide an honest signal to females, of a male's gene-based ability to resist parasites. By choosing a healthy male, a female may accrue indirect advantages of resistant offspring (Hamilton and Zuk, 1982). A female may also gain direct fitness benefits through decreased risk of exposure to contagious parasites (the parasite avoidance hypothesis) (Borgia, 1986; Borgia & Collis, 1989).

Following the landmark paper by Hamilton & Zuk (1982) on the possible role of parasites in sexual selection, many studies have focussed on the dilemma faced by males. If sexual signalling is to be reliable, then there should be a cost associated with ornament or display exaggeration, in order to prevent cheating ('Handicap hypothesis'; Zahavi, 1975; Grafen, 1990). With the 'immunocompetence handicap hypothesis', Folstad and Karter (1992) proposed that although males may benefit from sexual attractiveness or increased competitive ability derived from elevated T levels, a physiological cost would be imposed from T-related suppression of immune function. According to this hypothesis, the costs that males pay for elevated T depends on the detrimental effects of parasites and on their ability to cope with levels of parasite infection. Males with comparatively low susceptibility to parasites or high resistance to parasite should pay a lower cost, other things being equal, for high levels of T (Folstad & Karter, 1992). Thus, males that exhibit exaggerated T dependent ornaments or behaviour, should be reliably signalling to potential mates, their ability to cope with the potentially detrimental consequences of high T (Saino *et al.*, 1995). The interaction between testosterone and parasites is of interest not only to furthering our understanding of the selective pressure of parasites on their hosts, but may also play an important role in advancing our understanding of host population dynamics (Sheldon & Verhulst, 1996; Holmstad & Skorping, 1998; Lysne & Skorping, 2002).

1.5 Outline of the Thesis

The red grouse (*Lagopus lagopus scoticus*) is a game-bird species specific to upland moorland areas of the UK, and the tendency of this species to exhibit regular population fluctuations is of considerable socio-economic, conservation and ecological interest (Jenkins *et al.*, 1967; Potts *et al.*, 1984; Hudson, 1986; Dobson & Hudson, 1994; Moss *et al.*, 1996; Hudson *et al.*, 1998; Moss & Watson, 2001). The red grouse-*Trichostrongylus tenuis* host-parasite system is believed to play a significant role in the cyclic population dynamics of the host, and has been widely studied both at individual host and at host population levels. However, factors that may contribute to an individual's parasite infection remain poorly understood within this system.

This thesis aims to describe and explore, using experimental manipulations, factors influencing patterns of *T. tenuis* infection in red grouse. The red grouse is a good model species in which to examine patterns of parasite infection within individuals for several reasons. The relative ease with which free-living individuals can be caught at night, relocated through radio-telemetry techniques and most importantly recaptured, allows us to examine temporal changes in parasite infection. It also allows us to experiment on individuals in their natural environment. In addition, the gastrointestinal nematode *T. tenuis* is the main parasite of red grouse. It is host specific and has a simple, direct life-cycle.

Chapter 2 describes the host study species and introduces the parasite species examined in this thesis. Background information on red-grouse host-parasite interactions is given and general methods relevant to various chapters are also described. Chapter 3 focuses on the importance of being able to accurately measure parasite infections in living hosts of natural populations. Among infected hosts of a population, macroparasite infection levels may vary greatly. For epidemiologists studying macroparasite infections, describing a host as infected is not enough, and knowledge of the degree of infection is required to model host-parasite interactions (McCallum & Scott, 1994). An important epidemiological aspect of studies of host-parasite interactions, is the requirement to assess the distribution of parasites within the host population. One of the main parasite sampling techniques essential to this project and to research into red grouse in general, is the use of faecal egg counts as an indicator of worm burden. The main aim of Chapter 3 is to examine the reliability of this sampling method as an indicator of worm burden in red grouse. In Chapter 4, I examine patterns of infection between paired adult grouse and between chicks of the same brood, to assess naturally occurring patterns of infection. I then go on to examine which factors may be important in determining the intensity of an individual's parasite infection. In order to understand why patterns of parasite aggregation in host populations might occur, experiments are required to separate the effects of heterogeneities in exposure rate from heterogeneities in host susceptibility (Lysne & Skorping, 2002). This was attempted through a cross-fostering experiment, which aimed to identify a heritable component to parasite infection. In Chapter 5, I investigate the possible role of parasites in sexual selection. I examine whether potential mates may use sexual ornament size as a signal of an individual quality and health, reflecting a better condition or fewer parasites. I also examine whether there is any evidence of positive assortative mating in red grouse. Chapters 6 & 7 examine the potential interaction between the male hormone testosterone and parasite infection. Testosterone may act upon the immune system to alter an individual's resistance to parasites, increasing their susceptibility to infection. Chapter 6, examines the effect of experimentally elevated testosterone levels on sexual ornament size and immunocompetence. Specifically, this chapter sets out to test key predictions of the Immunocompetence Handicap Hypothesis in red grouse. Also using experimentally manipulated levels of testosterone, Chapter 7 investigates more specifically the effect of elevated circulating testosterone levels on *T. tenuis* infection intensity in males. Finally, Chapter 8 contains a general discussion and examines the wider implications of these findings.

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Chapter 2.

General methodology

General methodology

2.1 Introduction

Detailed methods relevant to each results chapter are contained therein. However, a number of materials and methods are relevant to all chapters. These are set out below.

2.2 The study species

The red grouse *Lagopus lagopus scoticus* (Figures 2.1 a, b), a subspecies of the willow grouse (*Lagopus lagopus*), is a medium sized game bird (wing-span 55-66cm), which exhibits slight sexual dimorphism, males being c. 5% larger than females (Cramp & Simmons, 1980). Red grouse feed predominantly on highly fibrous, but protein poor heather *(Calluna vulgaris)* and thus are restricted in their distribution throughout the British Isles to heather-dominant moorland (Husdon & Watson, 1985). Males are generally socially monogamous but polygyny sometimes occurs (<5% of pairs). The normal pair-bond may last 2 or more years, and depends on the success of the male in securing and maintaining a territory in the autumn (Cramp & Simmons, 1980).

In August and September, old males compete first to maintain or enlarge their territory from the previous autumn, and later young males attempt to establish new territories in unoccupied areas or between existing ones (Jenkins *et al.*, 1963). The reproductive success of male depends on his ability to establish and maintain a territory and to attract a mate, and both aggressive behaviour and the expression of secondary sexual ornamental combs during the autumn and spring require the hormone testosterone (Moss *et al.,* 1979; Mougeot *et al.*, 2004).

Most males failing to establish a territory in the autumn will disperse or will die from starvation, predation or disease over winter and spring, as a result of being forced into marginal habitats (Jenkins *et al.*, 1963). Thus, territorial behaviour determines not only whether an individual will breed, but also largely determines survival (Cramp & Simmons, 1980). Having loosely secured a female in the autumn, males will generally remain with that female throughout the winter. From February through to May, sexual activity is at its most intensive; males defend their territories all day long and both males and females are aggressive towards birds of their own sex (Jenkins *et al.*, 1963). Males advertise to females through courtship displays on their territories and females will visit and stay with the most vigorous males (Watson & Jenkins, 1964).

Many red grouse populations are harvested annually and as such, bag-records have provided long-term data from which to examine population dynamics. Using a combination of bag records and breeding density data, it is apparent that red grouse populations regularly exhibit cyclic fluctuations in abundance which vary in periodicity from 4-10 years depending on latitude (Potts *et al.*, 1984; Williams, 1985; Hudson, 1986a).

Figures 2.1 (a) Male red grouse (above) and **(b)** female red grouse (below)

(J. Renet, 2003)

2.3 The parasites

Trichostrongylus tenuis

One of the main parasites of red grouse is the nematode *Trichostrongylus tenuis* which inhabits the relatively large caeca of the red grouse and, like most other gastrointestinal nematodes, has a simple, direct life cycle (Figure 2.2). Eggs laid by adult worms are voided onto the moor once daily in caecal droppings, and when the temperature exceeds 5°C, embryos develop and the first two larval stages complete their development in the faeces. Under conditions of adequate temperature and moisture, during May to November, they develop into infective larvae, which ascend the heather *Calluna vulgaris* (the main plant food of red grouse) and are ingested (Hudson, 1986b; Shaw *et al.*, 1989). During the summer the ingested larvae soon develop into adults, usually within 2 weeks. However, in autumn, ingested larvae may become hypobiotic and delay development until spring in March and April when many may mature simultaneously and begin to produce eggs (Shaw, 1988). In spring, the numbers of eggs can increase suddenly even though no larvae have been ingested for months.

Coccidia

Red grouse are also frequently infected by coccidia. Coccidia are single celled protozoan parasites that infect the intestine and caecum. Oocysts, are passed in faeces where they begin to mature or sporulate, after which they become infective to any host. Once ingested, the oocyst breaks open and releases motile sporozoites, each of which finds an epithelial cell and begins to reproduce inside it to form merozoites. Ultimately, the cell becomes full that it bursts, releasing the merozoites which seek out their own intestinal cells and the process begins again. As the intestinal cells are destroyed in larger and larger numbers, denudation of the epithelium may occur and intestinal function may be disrupted resulting in bloody, watery diarrhoea which may be dangerously dehydrating to very young hosts (Boden, 1998).

2.4 Red grouse-*T. tenuis* host-parasite interactions

Adult worm survival in the red grouse has been estimated at 34%, nematodes surviving for up to 2 years (Hudson & Dobson, 1997), a lifespan similar to that of a grouse. Described as a moderately pathogenic parasite (Hudson 1986a,b), *T. tenuis* has proved lethal at high intensities, however, as with most infections of macro-parasites, infections of *T. tenuis* tend to be chronic (Shaw & Moss, 1989), leading to sub-lethal effects.

The red grouse has an unusually long caecum up to 75 cm in length, compared with much shorter caeca found in other herbivorous grouse, presumably to cope with the nitrogen poor, highly fibrous diet. Degeneration of the caecal mucosa is associated with the presence of nematodes and the effect of large infections of *T. tenuis* may be to cause pathological changes to the caeca such as a thickening of caecal mucus and desquamation of the mucosal wall (Watson *et al.* 1987). By causing a decrease in the absorptive area of affected parts of the caecum, *T. tenuis* may interfere with the normal digestion of heather and thus may affect the survival, competitive ability and fitness of red grouse, particularly when stressed (Figure 2.2; Watson *et al*, 1987; Dobson & Hudson, 1994).

Hudson (1986a) showed that within a population of red grouse, prevalence of infection is high, with 100% of old grouse and 99% of birds more than 3 months old found to be infected with *T. tenuis*. Such high prevalence of this nematode in populations suggests that transmission between individual hosts is extremely effective (Shaw, 1988). The gastrointestinal nematode *Trichostrongylus tenuis,* is known to have important detrimental effects on red grouse physiology and energetics (Wilson & Wilson, 1978; Watson *et al.*, 1987; Delahay, 1995; Delahay *et al.*, 1995) as well as on life history traits such as survival and reproduction (Hudson, 1986b; Shaw & Moss, 1990; Hudson *et al.*, 1992). Coccidia, is also an important parasite of gamebirds and infections may cause enteritis, decreases in body weight and mortality (Fantham, 1911). However, little is currently known about the effects of this parasite in grouse.

2.5 Study sites

The study was carried out at nine study sites (Figure 2.3). Data were collected from populations from six moors in Scotland. These were located in Aberdeenshire on Edinglassie estate (57° 12'N, 3° 07'W), Glas Choille (Invercauld estate) (57° 07'N, 3° 19'W), Glen Dye estate (56° 58'N, 2° 34'W), Glen Muick estate (56° 59'N, 3° 08'W) and Invercauld estate (57° 04'N, 3° 04'W), and in Angus, on Invermark estate (56° 55'N, 2° 56'W). Data were also collected from birds from three study moors in England, one at Moorhouse Nature Reserve, Alston (54° 46'N, 2° 24'W), and at Wathgill Army Training Camp (Catterick), Bellerby (54° 20'N, 1° 51'W) and Feldom Army Range, Richmond (54° 27'N, 1° 47'W).

2.6 Data collection and analyses

Finding and Catching Birds at Night

During autumn (August-November) and spring (March-May) (2000-2002) roosting birds were dazzled at night and caught in hand-held nets (Hudson & Newborn, 1995). For safety and efficiency, night work was carried out in pairs. A 1000-watt halogen lamp powered by a 12V motorbike battery was used to scan the moor, searching for eye reflections of the grouse. Walking straight towards the bird, the beam on the light was held steady on the bird thus dazzling it. A tape recording of a land-rover engine was played as "white noise" to muffle any noise of movement through the heather, enabling the person with the lamp to approach and bring the net down over the grouse. The grouse was removed immediately from under the net by an assistant, ringed with an individually numbered metal patagial wing tag or aluminium leg ring and placed in a well-ventilated rucksack. Group size was noted and birds were taken back to a central location on the moor to enable measurements and parasite samples to be taken. Birds were placed in a small cotton handling bag to enable several biometric measurements to be taken for each bird (see Table 2.1). Handling time was kept as brief and birds were monitored throughout handling for symptoms of stress such as a gaping behaviour with an open beak. Any birds exhibiting this behaviour were placed in a holding box and left to settle, before continuing.

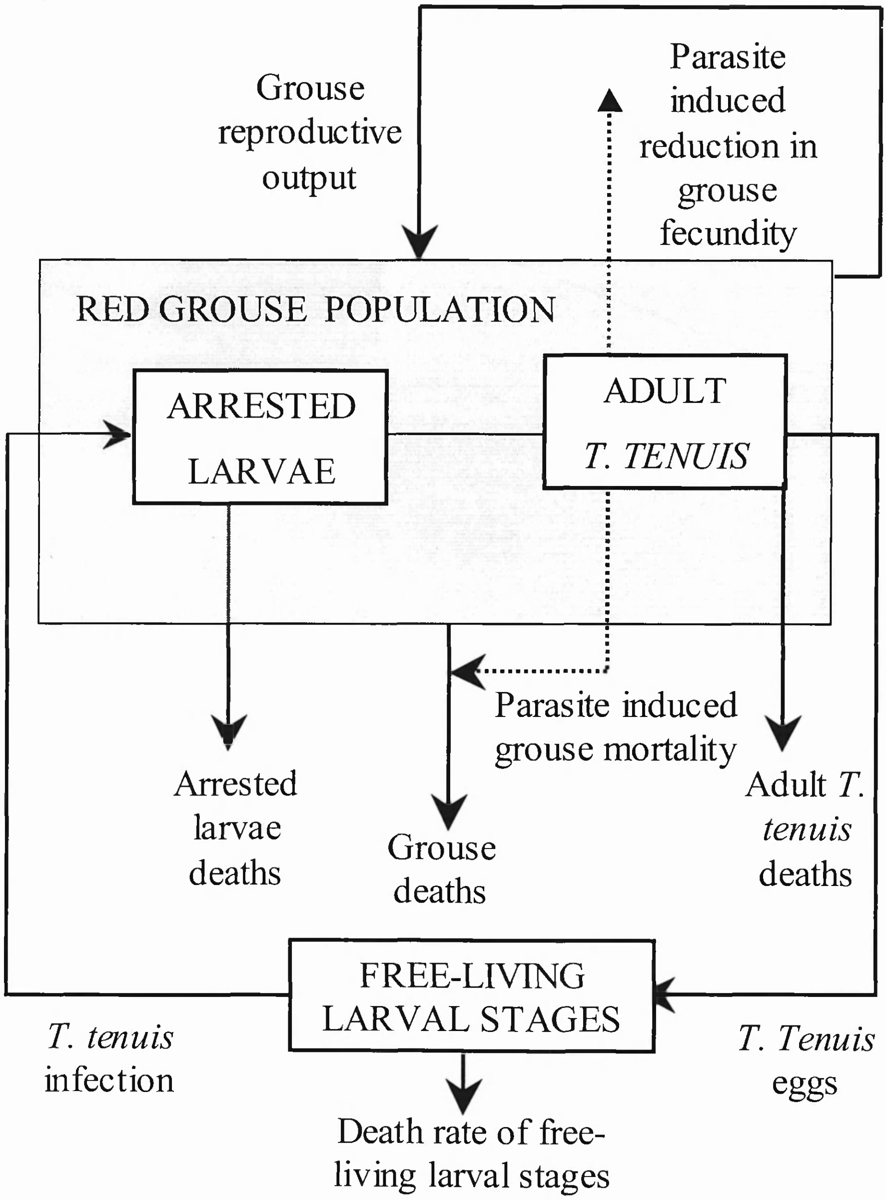
Parasite sampling

After each bird had been measured, they were placed in an individual wooden holding box (Figure 2.4). Each box was approximately 30' x 30'x 30' in size, and was constructed with a wire netting floor. A layer of freshly cut heather was placed on the floor of box. Birds were kept in boxes overnight and released early the next morning, allowing enough time for the birds to produce a caecal faecal dropping for parasite sampling. The following morning, birds were released and their caecal faeces containing the parasite eggs and oocytes collected and taken to the lab for examination. Details of all laboratory methods used to carry out parasite sampling are contained in Chapter 3.

Relocating Individuals

Where captured birds were required to be relocated and recaptured, individuals were fitted with a c.15g necklace type radio-tag (Figure 2.5) (TW3-necklace radio-tag Biotrack Ltd, Dorset, UK) and tracked using a YAGI 3-element field hand-held antennae (Wildlife Materials Inc, Illinois, USA) and a TR4 radio-receiver (Telonics Inc., Arizona, USA)

Figure 2.2: Flow chart representing the direct life-cycle of *Trichostrongylus tenuis* in red grouse, illustrating the various life, birth, death and transmission processes occurring in both the host and the parasite (Dobson & Hudson, 1994).



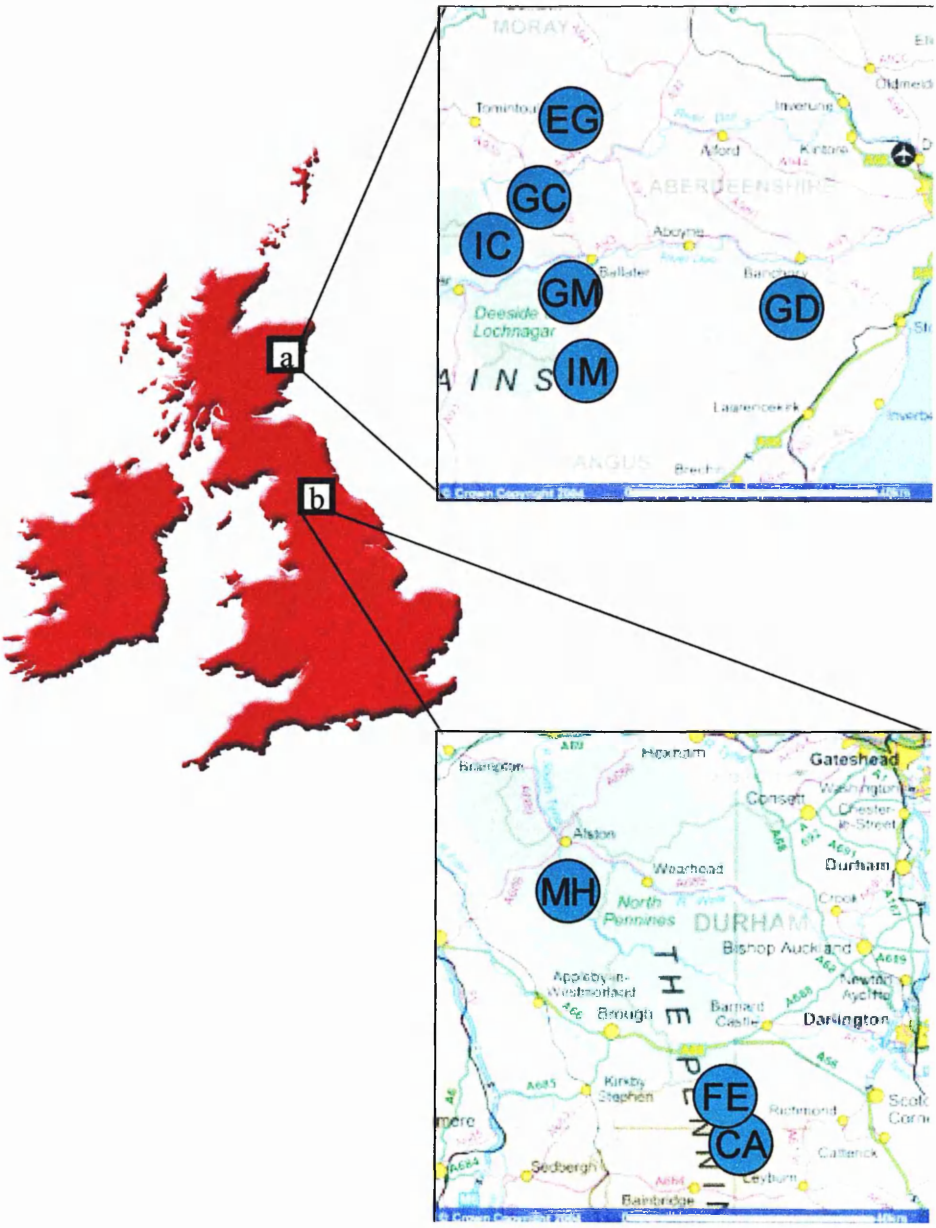


Figure 2.3: Map of the study sites in Scotland (enlarged section (a): EG-Edinglassie, GC-Glas Choille, IC-Invercauld, GM-Glen Muick, IM-Invermark and GD-Glen Dye) and north of England (enlarged section (b): MH-Moorhouse, FE- Feldom, CA-Catterick).

Figure 2.4 Boxes used for holding grouse overnight (F. Mougeot, 2003)

Figure 2.5: Male red grouse with necklace-type radio and wing tag (J. Renet, 2003)

Table 2. 1 Biometric measurements

Variables	**Description**
Date	Date was recorded as julian date.
Sex	Birds were sexed according to both plumage characteristics and comb size and colour. Males tend to have dark rich reddish plumage, and larger deeper reddish combs, whilst the plumage of females is usually more golden in colour and speckled (Hudson & Newborn, 1995).
Age	Birds were classified as "old" or "young" (hatched the previous summer) based on both the pigmentation of their primaries, the shape & colour of their 2nd and 3d primaries (pointed and dotted in young, round and plain in old birds) and from the texture of their claws (smooth in young, with a growth ridge in old birds; Parr, 1975).
Comb Size	Supra-orbital combs were spread out and flattened and comb length measured as distance from left fringe edge to right fringe edge, and comb height recorded as the distance from a central position on the eye-lid to the fringe edge perpendicular to this point. Length and height of the comb (measured to the nearest 1 mm) were multiplied to give an index of comb size.
Weight	Measured to the nearest gram with a Pesola spring scale (weight of the handling bag deducted).
Wing	The length from the carpal to the tip of the 10th primary was measured to the nearest 1 mm using a wing ruler.
Tarsus	The length of the tarsometatarsus was measured to the nearest 0.1mm using dial vernier callipers and also used as a measure of body size.

2.7 Experimental procedures

All procedures were performed under Home Office project licence PPL 80/1437.

Hormone implants

Implanting procedures were carried out by F. Mougeot, F. Leckie & S. Redpath. Male grouse were randomly assigned one of two treatments: testosterone treated (T-males) and control (C-males). Birds were each implanted with two silastic tubes (each one 20 mm long, 0.62 mm of inner and 0.95 mm of outer diameter; sealed with silastic glue (Polmer Systems Technology Ltd, Buckinghamshire) (see Figure 2.6). Implants were inserted between skin and breast muscles on the flank, under local anaesthesia . Control-males were given two empty implants, Testosterone-males two implants filled with crystalline testosterone proprionate (Sigma-Aldrich Co Ltd, Dorset). The length of the tubing was previously determined during trials on captive grouse so that implants would last for 2-3 months.

Blood Sampling

Blood samples were taken by myself, F. Mougeot, F. Leckie & S. Redpath to enable haematological analysis and testosterone assays to be carried out. Birds were held securely by an assistant and the wing opened to allow the underside of the wing to be exposed. The brachial vein was located at the base of the patagium, between the humerus and radius. The area around the vein was swabbed with a sterile antiseptic wipe (Isopropyl Alcohol BP 70%) and a 25G sterilized needle used to prick the vein. Blood was removed using sterile glass capillary tubes (1mm x 75mm) and transferred into heparinized epindorf vials for the plasma collection. The vein was wiped clean with cotton wool, compression applied to stop the bleeding and dusted with antibiotic Aureomycin topical powder. Blood was collected tubes and centrifuged immediately for 3 minutes at 12 000 rpm. Plasma was separated from the packed cells stored in a cold box, taken to the lab within 3 hours after collection and kept frozen at –70°C for subsequent analyses. Testosterone assays were carried out by A. Dawson at the Centre for Ecology and Hydrology, Monkswood. For full details of this method see Chapter 7).

Anthelmintic dosing & T. tenuis larvae challenge

Where an experimental protocol required an individual's infection of *T. tenuis* to be removed, birds were orally dosed with 1ml of anthelmintic (Nilverm Gold, Schering-Plough Ltd., Welwyn Garden City, UK) using a 10ml syringe with 10cm (2mm diameter) tube attached (see Figure 2.7). Individuals were challenged with 1-3 ml of distilled water containing the 2000 infective larvae using the same method. The exact volume was calculated so that each dose would contain c. 2000 larvae. The tube was carefully inserted over the tongue, down the throat and into the crop, and the anthemintic or larvae administered. The anthelmintic treatment acts simply to remove an existing parasite infection. Thus, the effects of the anthelmintic are expected to be relatively short-lived.

Laboratory culture of T. tenuis infective larvae. In order to experimentally challenge individual red grouse with a standardised level of parasite infection, *T. tenuis* infective larvae were cultured in the laboratory. Fresh caecal faeces were washed thoroughly over a 125μm sieve (Endecotts Ltd, Moreden, London) with a jet of tap water, to remove any coarse fibrous material. The faecal residue containing the parasite eggs was collected in a 25 μm sieve (Endecotts Ltd, Moreden, London), rinsed thoroughly with distilled water and poured into plastic petri-dishes (88mm diameter, 12mm depth) to a depth of about 5mm. The use of distilled water rather than tap-water is thought to improve yields of larvae (pers. comm. D. Delahay) and poor hatching and mortality has been observed at depths above 5mm (Delahay, 1995). Approximately 10g of unsieved caecal material was added to the contents of each petri-dish to create a culture with a consistency similar to single cream. Dishes were covered and incubated at 20°C for 7 –10 days, when the highest proportions of infective larvae have been found be present (Delahay, 1995; Shaw, 1988). Cultures were stirred daily to aerate lower layers and sprayed with distilled water to prevent cultures from drying out. To remove infective *T. tenuis* infective larvae, the culture medium was washed through a 125μm sieve with a 25 μm below to collect the residue containing the infective larvae. This mixture was poured onto a filter paper (grade 113, 12.5 cm diameter, from Whatman Scientific Ltd, Maidstone, Kent) and the filter paper suspended in a Baerman apparatus containing distilled water. Larvae actively migrated out of the faecal residue into the water and collected at the bottom of the funnel over a period of 2-3 days, where they could be collected daily by drawing off a small amount of water using the screw clamp.

Figure 2.6: Male red grouse with testosterone implants (F. Mougeot, 2002)

Figure 2.7: Technique used to administer anthelmintic or infective larvae

(F. Mougeot, 2002)

2.8 Cross-fostering experiment

Catching of pairs in the spring

In order to try to tease apart the relative contribution of environmental and genetic factors to variations in individual *T. tenuis* intensities, I carried out a partial cross-fostering experiment on Moorhouse in 2002. A total of 63 pairs were caught at night using lamping techniques detailed in this chapter and sampled for parasites. As both parents are involved in caring for the chicks, it was necessary to record biometric and parasite details for both parents. As grouse chicks are precocial and nidifugous, leaving the nest shortly after hatching it was necessary for this experiment not only to be able to locate females whilst they were nesting, but also to be able to locate both females and their broods after hatching. In order to achieve this, females were fitted with a necklace-type radio collar at time of capture in early spring.

Nest location and egg measuring

Females were radio-tracked at frequent intervals during April and May until the nest was found. Females lay a clutch of between 2-15 eggs (average 7.5 eggs) from mid-April to mid-May (Jenkins *et al.*, 1963) and incubation lasts on average 22 days. The nest was then marked using a 4' green garden cane, located at 15 paces due north. The nest was not marked at its exact location, because it might increase predation risk. In order to swap chicks of a similar age between broods, it was necessary to accurately predict when the eggs would hatch. To do this, when females had laid a full clutch of eggs (or a minimum of five eggs), I took egg measurements of length and breadth, measured to the nearest 0.1mm using dial-callipers and the weight of eggs taken to the nearest 0.1g using a small pocket balance. These measurements were repeated for a total of five eggs picked randomly from the nest and were then used to predict an average hatch date for the nest. This was done using the steps in Table 2.2 (A. Smith, unpublished data).

Table 2.2: Procedure for calculating hatch date from egg measurements

Step	Parameter	Calculation
1.	Initial weight of egg	0.53 X (egg length X egg breadth^2) + 0.07
2.	Days incubated	((100 – ((weight/initial weight)X 100)) / 13) X 21
3.	Setting Date	Date collected - Days incubated
4.	Hatch date.	Set date + 21

Although the predicting hatch date formulas were only deemed accurate to within 1-2 days, this still enable the nest monitoring effort to be concentrated only on those nests close to hatching and thus minimised the disturbance to other nests.

Identifying hatched nests

With the aid of a hand-held Global Positioning System (GPS) and the signal from the hen's radio-collar, nests were located daily around the time of the predicted hatch date. In order to minimise disturbance to the incubating hen, the position of the nest was identifiable from the position of the cane, and hens were identified as still incubating if the radio-signal could be triangulated to the approximate nest point, whilst remaining a reasonable distance from the nest. Hens may brood chicks while they are still small, therefore, a hen may have on occasion, been falsely identified as still incubating when in fact she may have been brooding chicks close to the nest site.

Chick hatching

Red grouse eggs hatch synchronously. Although chicks are precocial and leave the nest shortly after hatching, broods tend to remain within 100 metres of the nest during the first two days (Hudson, 1986). If the hen had moved away from the nest site, the nest was located and examined, and details of the state of the eggs noted. If whole eggs were found in the nest, the nest would be relocated on a different day to check if the hen had resumed incubation or to determine whether the eggs had been abandoned. Often, the discovery of cold eggs in a nest, was enough to determine that it had been abandoned. If the eggs had been destroyed, either the nest would be completely empty or there would be remains of small pieces of eggshell. The state of destroyed eggshells was distinguishable from eggshells left after chicks had hatched. For each nest deemed to have hatched, the potential number of chicks was estimated from the number of eggs that had hatched successfully and the number of any whole eggs that remained unhatched.

Chick location

For the purpose of this experiment, it was necessary to locate chicks when 5-10 days old. Chicks of this age, however, are very small and as they are only capable of precocial flight from about 12-13 days (Cramp & Simmons, 1980), they tend to try to freeze and in response to the parent's call warning and alarm calls. Searching for chicks therefore can be extremely difficult. Even with the benefit of being able locate and flush the hen with the aid of radio-telemetry, the chicks may be scattered some distance from the hen. Searching for chicks in vegetation can therefore be tricky as chicks are extremely well camouflaged, and in heather especially, this may prove almost impossible, as chicks will tend to hide as close to the ground as possible, in amongst the woody stems and roots. Attempting to find hidden chicks also comes with risk of trampling them underfoot. In order to find chicks for this experiment, we used specially trained pointing dogs (Figure 2.8). The use of pointing dogs makes the task of chick finding much easier and less time consuming and greatly increases the chance of being able to locate a full brood of chicks.

Figure 2.8: Trained pointing dog locating a chick (F. Mougeot, 2003)

Partial cross-fostering of chicks

Chicks grow rapidly during the first 10 days of life, but mortality is also highest during this period, particularly from 0 to 5 days old (Hudson, 1986). The optimum opportunity for cross-fostering was when two similarly aged broods both over 5 days but less than 14 days, could be located and both containing a suitable number of chicks (i.e. a minimum of 4). Although broods remain close to the nest site for the first couple of days, thereafter they tend to move longer distances, and in some cases have been found to more than a quarter of a mile (400 metres) from one roost site to the next (Hudson, 1986).

Due to the poor breeding success of the hens in the year this experiment was conducted, only 8 broods could be used for cross-fostering. Whilst such a small sample size of broods rendered the original experiment worthless in terms of data collection, three partial cross-fosterings were nevertheless attempted, in order to assess feasibility and to measure the success of the actual methodology, in order to develop the protocol for future experiments.

Two teams of experienced grouse researchers, each with a pointing dog, simultaneously radio-tracked and flushed the hens of two broods of similar age, and attempted to find as many chicks as possible from the brood. Pointing dogs searched the area close to where the female flushed for a maximum of 20 minutes. Each chick upon detection, was placed in a well ventilated cardboard box filled with heather. After 20 minutes, once either the whole brood had been located or a minimum of 4 chicks found, the wing length and weight of each chick was recorded and each chick was individually wing-tagged with a size 1 Monel metal tag (National Band & Tag Co., KY, USA). Half the brood, or a minimum of two chicks, picked at random, were kept in the box to be cross-fostered and the remaining chicks were placed together back into the heather, close to where the female had originally flushed.

With time as the critical factor, the two teams met to exchange chicks and each team returned as quickly as possible to where the brood had been originally found. As the non-fostered chicks were very young and unlikely to move very far in a short space of time, and as the time taken by each team to return to the original brood location was relatively short (10-15 minutes), fostered chicks were placed in the spot where the non-fostered chicks had been left. The team retreated to a safe distance, to a point from which the hen's radio-tag signal could be checked, to ensure that she was in the area where the chicks had been left. When a hen wishes to locate her chicks and to muster them, she will give a soft cooing "assembly call" and similarly, chicks in distress will give a shrill cheep, repeated frequently, summoning the parent (Cramp & Simmons, 1980). We returned to the hen after two days to check that she was with the chicks and to check that the fostered chicks had survived.

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Chapter 3

Faecal egg counts provide a reliable measure of *Trichostrongylus tenuis* intensities in free-living red grouse *Lagopus lagopus scoticus.*

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Faecal egg counts provide a reliable measure of *Trichostrongylus tenuis* intensities in free-living red grouse *Lagopus lagopus scoticus.*

3.1 Abstract

The reliability of different egg counting methods for estimating the intensity of *Trichostrongylus tenuis* infections in red grouse, *Lagopus lagopus scoticus*, was investigated in the autumn, when grouse may harbour high parasite intensities, and possible limitations to the use of these methods were also examined. Faecal egg counts were found to accurately estimate *T. tenuis* worm intensities, at least up to an observed maximum of c. 8000 worms. Two egg counting methods (smear and McMaster) gave consistent results, although the exact relationship with worm intensity differed according to the method used. Faecal egg counts significantly decreased with increasing length of sample storage time, but egg counts were reliable for estimating worm intensity for three weeks. The concentration of eggs in the caecum was also found to reliably estimate worm intensity. However, egg counts from frozen gut samples cannot be used to estimate worm intensities. These results conclude that, despite some limitations, faecal and caecum egg counts provide useful and reliable ways of measuring *T. tenuis* intensities in red grouse.

3.2 Introduction

Host-parasite interactions have long been of interest to ecologists, especially when they are thought to play an important role in host population dynamics (Anderson, 1978; May & Anderson, 1978; Hudson *et al.*, 1985; Albon *et al.*, 2002). Red grouse populations exhibit cyclic fluctuations, and *T.tenuis* parasites are suspected to play a major role in causing the population cycles (Hudson *et al.*, 1992, 1998).

A quantitative understanding of the population dynamics of parasites and of their impact on individual hosts and populations requires a good estimation of the intensity of infection in each host and how the parasites are distributed through the host population. In some systems, faecal egg counts are often the only measure available to estimate parasite intensity in free-living animals, and are routinely used in a range of species (Gordon & Whitlock, 1939; Shaw & Moss, 1989b; Gulland & Fox, 1992; Guyatt & Bundy, 1993; Irvine *et al.*, 2001). The validity of using faecal egg counts to estimate host worm intensity has nevertheless been widely questioned and needs to be addressed in any host-parasite system (Michael & Bundy, 1989; Sithithaworn *et al.,* 1991; Guyatt & Bundy, 1993). The primary concern is that egg counting might be an unreliable index of worm intensity if it is influenced by a number of factors such as density-dependent constraints on worm fecundity (Anderson & Schad, 1985; Smith *et al.*, 1987; Tompkins & Hudson, 1999) or seasonal variation in worm egg production rate (Hudson, 1986a; Shaw & Moss, 1989a).

In the *T. tenuis*- red grouse system, Moss *et al.,* (1990) showed that egg counts are a reliable measure of worm burdens. However, that work was limited to spring and used only one egg counting method. Moreover, worm intensities in that study were relatively low (only two of the forty birds were found to have a worm intensity greater than 3000 worms). Since grouse are routinely sampled for parasites during autumn and may carry worm intensities of up to 24

000 worms (Hudson, 1986b), it is important for the validity of ecological studies involving red grouse-*T. tenuis* interactions, to establish whether the relationship between *T. tenuis* egg counts and adult worm intensity holds for different seasons and for higher worms intensities.

In this paper, we assess the reliability of various egg counting methods for estimating worm intensities of red grouse, and possible limitations to the use of these methods. We examine the relationship between egg counts and *T. tenuis* worm intensities in red grouse harbouring up to 7992 *T. tenuis* worms, during the autumn. Egg count reliability may depend on the method used to count eggs, as well as variability in the concentration of eggs in faecal material (Sinniah, 1982). We examine three different ways of counting parasite eggs (smear and McMaster egg counts carried out on faecal samples, and McMaster egg counts carried out on material from the caecum) and assess the comparative reliability of each for estimating worm intensity. Egg counts on gut material from the caecum have already been used previously for estimating *T. tenuis* intensities (Hudson & Dobson, 1997) and can provide a quicker and easier method of estimating parasite infection levels than a straight worm count. However, *T. tenuis* worms are not evenly distributed within the caecum (Shaw, 1988b), so we examine in more detail, spatial patterns in egg concentrations, and the applicability of using egg counts from different sections of the gut to estimate worm intensities. Lastly, we examine potential limitations to the use of egg counts, such as conditions for the storage of samples and storage time.

3.3 Materials and methods

Life cycle of Trichostrongylus tenuis

Trichostrongylus tenuis inhabits the relatively large caeca of the red grouse, and like most other gastrointestinal nematodes, has a simple, direct life cycle. Eggs laid by adult worms are voided onto the moor once daily in caecal droppings, and when the temperature exceeds 5°C, embryos develop and the first two larval stages complete their development in the faeces. Under conditions of adequate temperature and moisture, during May to November, they develop into infective larvae, which ascend the heather *Calluna vulgaris* (the main plant food of red grouse) and are ingested (Hudson, 1986b; Shaw *et al.*, 1989). During the summer the ingested larvae soon develop into adults, usually within 2 weeks. However, in autumn, ingested larvae may become hypobiotic and delay development until spring in March and April when many may mature simultaneously and begin to produce eggs (Shaw, 1988a). In spring, the numbers of eggs can increase suddenly even though no larvae have been ingested for months.

Study Areas, Material Collection and Storage

Parasite counts were conducted on 36 cocks from three study sites located in northeast Scotland. Previous studies have found no evidence for sex-biased parasitism in grouse, as males have been shown to have approximately the same number of worms as females (Wilson, 1983; Hudson, 1986b). Birds were caught at night using lamping techniques (Hudson & Newborn, 1995) in September and October 2001. Each bird was held in an individual holding box overnight and caecal faeces collected the following morning. Birds were then euthanased and their caeca removed for parasite and parasite egg estimation. Post-mortem parasite estimations were also conducted (using only the caeca) on 12 grouse shot on grouse moors in Aberdeenshire and Berwickshire in October 2001. All samples collected in the field were brought immediately to the lab and stored in a cold room at a constant temperature of 5°C to inhibit the hatching of eggs. The second caecum from each of 10 shot birds was stored in a freezer at a temperature of –40 °C for two weeks for studies on the effect of freezing on caecum egg counts. In addition, the original data from 44 cocks from Moss *et al.*, (1990) were used.

Laboratory analysis of intensity of T. tenuis worm infection and egg counts

Estimating worm intensity. Direct worm counts were carried out on one caecum from each grouse. Each caecum was opened lengthways and all the gut material flushed with water over 150μm gauze to collect the worms. The contents of the sieve were washed into 300ml of water, mixed thoroughly and adult worms counted with the aid of a binocular dissecting microscope with 25x magnification (an objective lens 2.5x used in conjunction with a 10x ocular). Where possible, all worms in the sample were counted and this number doubled to obtain the total number of worms per bird, as worm numbers do not significantly differ between the two caeca (Wilson, 1979, 1983). Otherwise, worms were counted from a minimum of three 10 ml sub-samples. The average of these counts was multiplied by 30 to obtain an estimate of the total number of worms in a single caecum, and this number was doubled to give the total number of worms per bird.

Estimating egg intensity. The intensity of parasite eggs per gram of caecal faeces was estimated using two different counting methods. First, a measure of eggs per gram (EPG) was carried out using the modified McMaster egg counting technique (MAFF, 1986). For this method, approximately 0.2g (range 0.19 – 0.21g) of well-mixed faecal material was put into a shaker tube with approximately 10 glass balls and 5 ml of saturated NaCl solution. The tube was shaken until the faecal matter was suspended. Using a Pasteur pipette, a sample of the faecal suspension was extracted and carefully run into one chamber of a McMaster counting slide. The tube was shaken again and another sample extracted and run into the second section of the chamber. The saline suspension was left to settle for 2-3 min, allowing the eggs to float to the top of each chamber. Eggs were then counted beneath a marked grid on each chamber using a compound microscope with 100x magnification (an objective of 10x used in conjunction with a 10x ocular). The number of eggs per gram of faeces was calculated by multiplying the total number of eggs counted under both grids by the total volume of faecal suspension contained in both chambers and then dividing this by the quantity of faeces used in the suspension.

A second egg counting method (smear) was carried out on the same faecal sample, after Moss *et al.,* (1990). These were carried out on the same day the McMaster egg count was conducted, to allow a direct comparison between the two methods. In the smear method, caecal faeces were thoroughly mixed, and a 5-10 mg sub-sample was weighed onto a cover slip, which was then pressed onto a glass microscope slide. All the eggs in the sub-sample were counted using a compound microscope with 100x magnification. Successive sub-samples from the same sample of faeces were carried out until two counts varied by no more than 10%. The number of eggs, either per 10 mg (to allow comparison with original data from Moss *et al.,* (1990)) or per gram of faeces, was calculated according to the average of the two counts and the average weight of the sub-samples.

Having carried out a direct worm count on one caecum, the second caecum was used to investigate spatial variability in *T. tenuis* egg concentration in different sections of the gut. The weight and length of each caecum was recorded, then the caecum was divided into three sections of approximately equal length. These were, namely, the proximal section, which is the end nearest the opening into the intestine, the mid section, and the distal section, that nearest the blind end of the gut. The gut contents from each section were squeezed out, weighed and an egg count carried out on each section. The remaining material was combined, thoroughly mixed and also sampled for worm eggs. For gut material, only the modified Mcmaster egg counting technique was used.

Effect of freezing caeca on the relationship between egg counts and worm intensity.

The second caeca of 10 birds which had been shot in September were frozen and used to compare egg counts from the frozen caecum with direct worm counts carried out on the first caecum prior to freezing. The frozen caeca were kept in the freezer for 14 days, then thawed at room temperature and a McMaster egg count was carried out on thoroughly mixed caecal material from the whole gut.

Effect of sample storage time on the relationship between egg counts and worm intensity.

Faecal samples from 13 birds were stored in a cold room at a constant temperature of $5^{o}C$ (to inhibit the hatching of eggs) for a period of 14 weeks. For each sample, a first egg count was conducted on the day of sample collection, and thereafter on the same day weekly for a period of 10 weeks, with a final egg count 3.5 months after collection. The McMaster faecal egg count technique was used throughout this section of the study.

Statistical analyses

Statistical analyses were conducted using Minitab (version 13) and SAS (version 8.01, SAS, 2001). Where necessary, data were transformed to approximate normal distribution. All relationships between egg counts and worm intensity were tested using regression analysis. ANCOVAs were used to compare the relationships between worm intensity and egg counts obtained in different seasons; spring (original data used from Moss *et al.,* (1990)) vs. autumn (this study), or obtained using different techniques (Smear and McMaster). As the original data from Moss *et al.,* (1990) included low values of eggs and worm intensities outside the range of the autumn data from this study, in order to directly compare the two data sets, these low values were excluded from the spring data in the ANCOVA. An ANCOVA was also used to compare the relationships between worm intensity and EPG counts obtained from each gut section.

Generalized Linear Mixed Models (GLMMs) were used to test for differences in caecal egg counts between gut sections, and to test for an effect of storage time on faecal egg counts. Models were fitted to the data using a Poisson error distribution and a log link function. Denominator degrees of freedom were estimated using Satterthwaite's formula (Littell *et al.*, 1996). The total number of eggs counted in both chambers of the McMaster slide was used as the response variable, with the exact amount of faeces or caecal material sampled as an offset in the model. GLMMs were implemented in SAS using the GLIMMIX macro (Littell *et al.*, 1996). For differences in egg counts between gut sections, the model included 'individual gut' as a random effect, in order to identify counts within a given gut. For the effect of storage time of egg counts, the model included 'individual sample' and the 'individual sample*time' interaction as random effects, in order to account for the repeat sampling of individual faecal samples at different time periods. The data were unbalanced, as some faecal samples ran out before the end of the experiment. Time period (week number; 0-14) was included as a class variable. Difference of Least Square Means (DLSM) was used to compare egg counts from different time periods and to determine when they differed significantly from the initial count (week 0).

3.4 Results

Relationship between faecal egg and worm counts

The number of adult *T. tenuis* worms found in the caeca of individual birds was significantly correlated with corresponding counts of eggs in caecal droppings (Fig. 3.1; $F_{1,29} = 294.24$, $P < 0.001$). T*richostrongylus tenuis* worm intensities in individual grouse ranged from 103 worms to 7992, with at least 7 birds carrying intensities of greater than 4000 worms. The relationship found between numbers of adult worms (W worms bird^{-1}) and counts of eggs (E eggs. 10 mg^{-1}) in faecal droppings in autumn, using the smear egg counting technique, was very similar to that found in spring, using data from Moss *et al.,* (1990). Shown below are the regression equations for both studies. The regression equation from this study is also given with standard errors for the slope and intercept.

Autumn : $\log_{10} (W+1) = 0.917 (\pm 0.053) \log_{10} (E+1) + 1.20 (\pm 0.116)$

$(r^2 = 0.91, n = 31, P < 0.001)$.

Spring : $\log_{10} (W+1) = 0.915 (\pm 0.076) \log_{10} (E+1) + 1.28 (\pm 0.121)$

$(r^2 = 0.77, n = 44, P < 0.001)$.

There was no significant effect of season on the relationship between egg counts and worm intensity ($F_{1,63} = 1.95$, $P = 0.168$). 95% Confidence intervals for the slopes of both the autumn (0.917 ± 0.109) and spring (0.919 ± 0.153) regression equations include a slope of unity, further suggesting that there is no density-dependent reduction in worm fecundity.

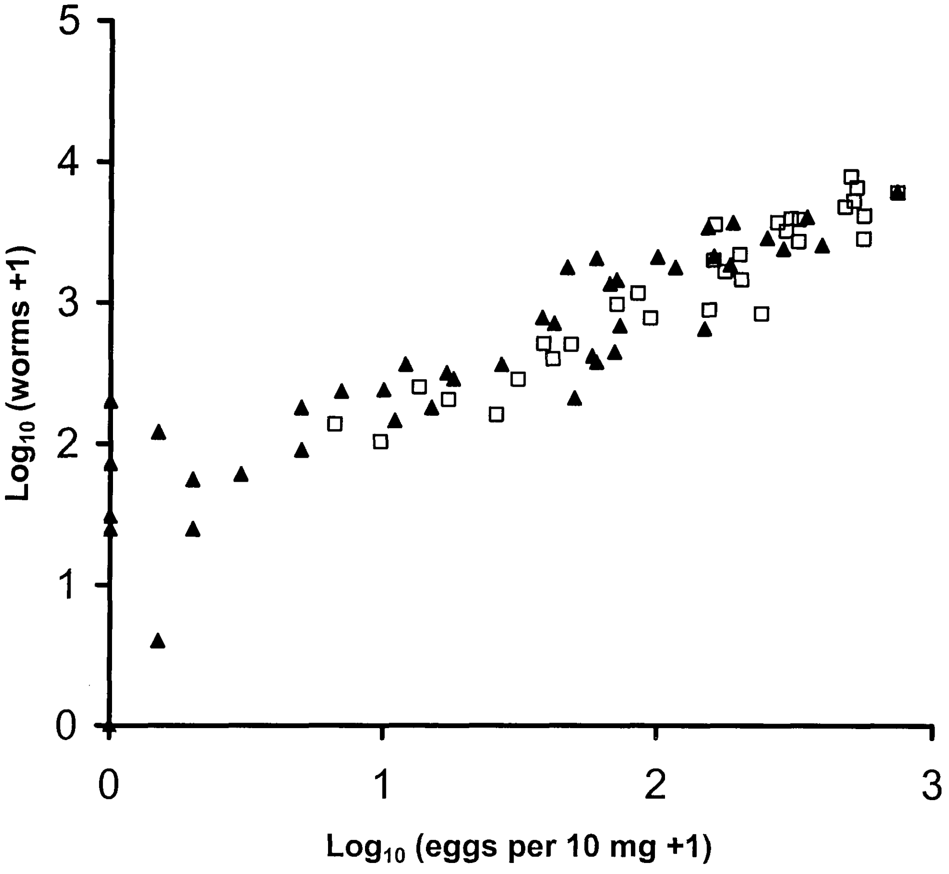

Figure 3.1: Relationship between *Trichostrongylus tenuis* intensity (W, worms. bird $^{-1}$) and egg concentration (E, eggs. 10 mg $^{-1}$) using smear egg counting method) in the caecal droppings of cock red grouse in relation to season. Symbols represent data for autumn from this study (□) and data for spring, from Moss *et al.*, (1990) (▲).

Comparison of egg counting methods

Regression analysis carried out on the two egg counting methods (smear and Mcmaster) showed a highly significant relationship between egg concentration and worm intensity for both methods (Figure 3.2, Table 3.1; smear $r^2 = 0.90$, $F_{1,29} = 287.73$, $P < 0.001$; McMaster $r^2 = 0.88$, $F_{1,29} = 218.28$, $P < 0.001$ respectively). However, there were significant differences both in the slopes of the regression lines (smear slope = 0.895 (± 0.053), McMaster slope = 0.651 (± 0.044), $F_{1,58} = 12.20$, $P = 0.001$) and in the intercepts of the regression lines (smear intercept = -0.541 (± 0.218), McMaster intercept = 0.664 (± 0.17), $F_{1,58} = 18.15$, $P < 0.001$).

Spatial distribution of eggs in the caecum

EPG counts significantly differed between each of the three sections, with the proximal end carrying significantly more eggs (Figure 3.3; Glimmix; $F_{2,42} = 6.8$, $P = 0.003$). Results of a pairwise comparison showed a significant difference between the proximal and mid sections ($t = -2.511$, $P < 0.05$) and between the proximal and blind sections ($t = -3.595$, $P < 0.05$).

Relationship between caecum EPG counts and worm intensity

There were significant linear relationships between worm intensity and the EPG counts from each section of the gut (Table 3.2; proximal $r^2 = 0.76$, $n = 15$, $P < 0.001$; mid $r^2 = 0.88$ and blind $n = 15$, $P < 0.001$; $r^2 = 0.87$, $n = 15$, $P < 0.001$ respectively). There was no significant difference between the slopes of the three regression lines ($F_{2,39} = 0.15$, $P = 0.858$) but the intercepts differed significantly ($F_{2,41} = 28.19$, $P < 0.001$).

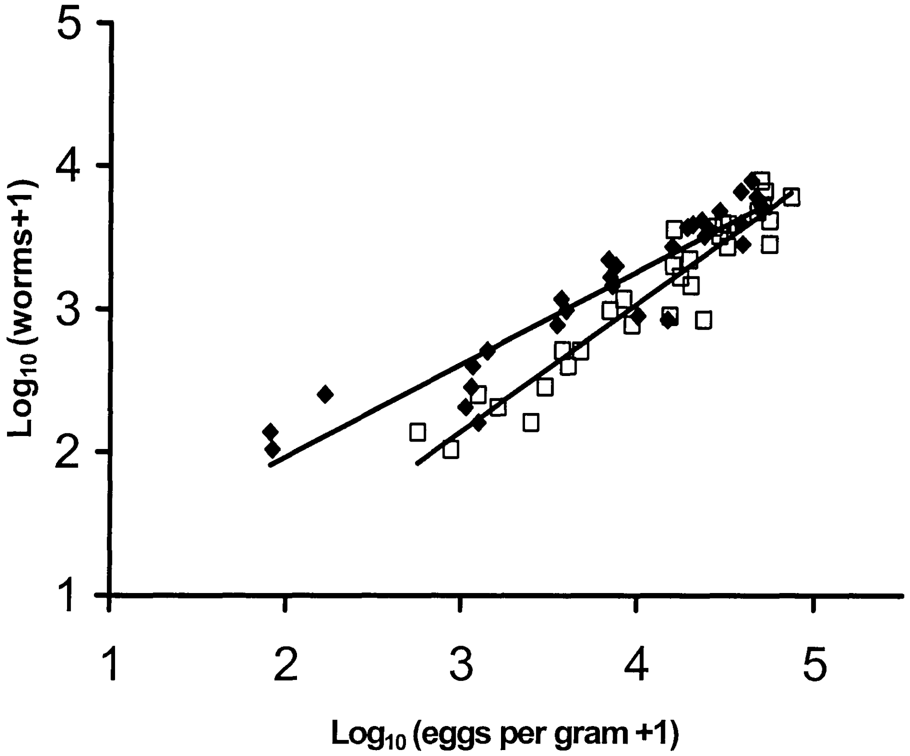

Figure 3.2 Relationship between *Trichostrongylus tenuis* worm intensity (W, worms. bird $^{-1}$) and egg concentration (E, eggs. g $^{-1}$) in the caecal droppings according to egg counting method (□ Smear technique; ◆McMaster technique).

Egg counting method	Regression equation	r^2	N, P
Faecal sample			
McMaster technique (FM)	$\log_{10}(W+1) = 0.651\ (\pm\ 0.044)\ \log_{10}(FM+1) + 0.664\ (\pm\ 0.17)$	0.88	n=31, P<0.001
Faecal sample			
Smear technique (S)	$\log_{10}(W+1) = 0.895\ (\pm\ 0.053)\ \log_{10}(S+1) - 0.541\ (\pm\ 0.218)$	0.90	n=31, P<0.001
Caecum mixed contents			
McMaster technique (T)	$\log_{10}(W+1) = +\ 0.646\ (\pm\ 0.047)\ \log_{10}(T+1) + 0.547\ (\pm\ 0.194)$	0.86	n=32, P<0.001

Table 3.1: Relationships between *Trichostrongylus tenuis* worm intensity (W, worms. bird $^{-1}$) and egg concentrations (eggs. g $^{-1}$) obtained from various egg counting methods. Regression equations are given with standard errors for the slope and intercept.

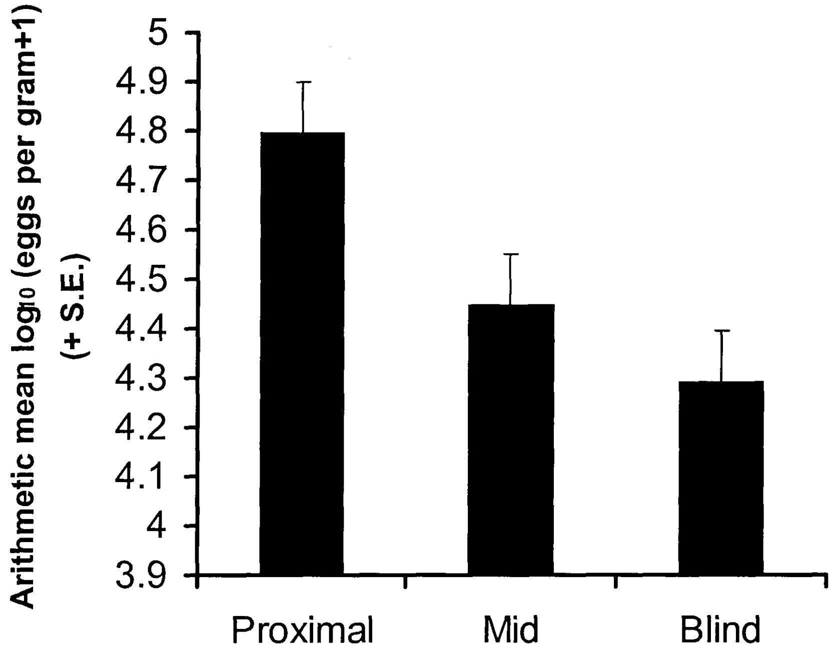

Figure 3.3: Arithmetic mean (+ S.E.M.) of *Trichostrongylus tenuis* egg concentration ($\log_{10}$ eggs. g $^{-1}$) in the proximal, mid & blind sections of the caecum (N = 15 guts), using the Mcmaster egg counting technique.

Section	Regression equation	r^2	N, P
Proximal	$\text{Log}_{10}(W+1) = 0.977 (\pm\ 0.143) \log_{10}(E+1) - 1.23 (\pm\ 0.689)$	0.76	$N=15, P<0.001$
Mid	$\text{Log}_{10}(W+1) = 1.07 (\pm\ 0.104) \log_{10}(E+1) - 1.28 (\pm\ 0.464)$	0.88	$N=15, P<0.001$
Blind	$\text{Log}_{10}(W+1) = 1.01 (\pm\ 0.104) \log_{10}(E+1) - 0.85 (\pm\ 0.446)$	0.87	$N=15, P<0.001$

Table 3.2: Relationships between *Trichostrongylus tenuis* intensity (W, worms. bird $^{-1}$) and egg concentrations (E eggs. g $^{-1}$) measured from three sections (proximal, mid and blind) of the gut. Regression equations are given with standard errors for the slope and intercept.

Effect of freezing on the relationship between caecum egg counts and worm intensity

Eggs per gram counts using the McMaster method from mixed caecum contents, were strongly correlated with worm intensity (Table 3.1; $r^2 = 0.86$, $F_{1,30} = 188.72$, $P < 0.001$). However, it would appear that the freezing process resulted in the deterioration of eggs, since this relationship was no longer significant ($F_{1,8} = 0.05$, $P = 0.829$) when using egg counts carried out on frozen samples.

Effect of sample storage time on egg counts

Analysis of egg counts from 13 individual faecal samples, repeated over a period of 14 weeks, showed a significant decrease in egg counts with increased storage time ($F_{11,82} = 4.55$, $P < 0.001$). Pairwise comparisons (Table 3.3) indicated that egg counts conducted at week 1-3 do not differ from the initial egg count (week 0), but from week 4 onwards, egg counts were significantly lower than those of the initial count.

Pair-wise comparisons	DF	t value	Pr > |t|
Week 0 – Week 1	58.4	-0.02	NS
Week 0 – Week 2	68.3	1.48	NS
Week 0 – Week 3	61.2	0.31	NS
Week 0 – Week 4	77.4	2.72	**
Week 0 – Week 5	79.1	3.05	**
Week 0 – Week 6	83.1	3.56	***
Week 0 – Week 7	83.6	3.5	***
Week 0 – Week 8	75.2	3.24	**
Week 0 – Week 9	68	2.81	**
Week 0 – Week 10	78.4	3.6	***
Week 0 – Week 14	77.1	3.89	***

** **$P < 0.01$, *** $P < 0.001$.**

Table 3.3: Results of an analysis testing for differences between initial week (week 0) and subsequent weekly egg counts (weeks 1 to 14) repeated on the same faecal samples. Differences of Least Square Means analyses were used for the pair-wise comparisons.

3.5 Discussion

Previous studies have shown that the reliability of using worm egg counts to indirectly estimate the worm intensity within a living host may be influenced by both seasonal variation in worm egg production (Shaw & Moss, 1989a; Moss *et al.*, 1993) and by density-dependent constraints in worm fecundity (Anderson & Schad, 1985; Smith *et al.*, 1987; Tompkins & Hudson, 1999). In this study however, we found that the strong relationship between faecal worm egg count and host worm intensity derived from samples collected in the autumn, is similar to that derived from samples collected during spring (Moss *et al.*, 1990). Further, we found no evidence for a decline in egg count reliability with increasing worm intensity, up to an observed maximum worm intensity in this study, of c. 8000. Although worm intensities in grouse may occasionally reach numbers as high as 24,000 worms (Hudson, 1986b), the maximum observed value in this study represents a level of parasite intensity more routinely observed in the field.

These findings agree with a number of other studies of helminth infections, which have similarly demonstrated a significant relationship between parasite intensities and faecal egg counts. Faecal egg counts are strongly correlated with liver fluke *(Opisthorchis viverrini)* intensities in humans (Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991). In sheep, there is a good correlation between egg counts and intensities of *Haemonchus contortus* (Le Jambre *et al.*, 1971; Coadwell & Ward, 1982; Roberts & Swan, 1981) and intensities of *Trichostrongylus colubriformis* (Chiejina & Sewell, 1974; Gibson & Parfitt, 1973). However, in contrast, there are also many studies of nematode infections where there is little or no correlation between the number of eggs that a host produces and worm intensity. This is particularly evident for *Ostertagia* species, important parasites of cattle and sheep (Michel, 1963, 1969; Callinan & Arundel, 1982; Coop *et al.*, 1985).

Such variation between parasitic helminth populations may be due to variations in the rate of worm egg production, a regulatory mechanism considered to be important in the regulation of many gastrointestinal parasite populations (Barger, 1987). In the red grouse-*T. tenuis* host-parasite system, our findings suggest that density-dependent suppression of egg production is weak or non-existent, at least up to the observed worm intensity of c. 8000 worms. A number of other factors may influence egg production, but there are two main mechanisms thought to dominate. The first of these mechanisms is the host's immunological response to infection, which may cause density-dependent suppression of egg production (Hudson & Dobson, 1997). However, evidence suggests that there is little or no effective acquired immunity in red grouse to infections of *T. tenuis* (Shaw & Moss, 1989b; Wilson, 1979). Unlike artificially induced *T. tenuis* infections in domestic chickens, where young domestic chickens develop a resistance to the nematodes resulting in worms being actively expelled and the infection rejected (Watson *et al.*, 1988), the number of worms present in the caeca of red grouse increases throughout the life of the bird (Wilson, 1983).

Secondly, competition between worms for resources, for example space, may also be an important mechanism influencing egg production. Adult *T. tenuis* worms are found only in the caeca of red grouse, which is thought to play an important role in the absorption of water and proteins and the digestion of cellulose (Gasaway *et al.,* 1976; Moss & Parkinson, 1972). The length of the caeca is known to vary amongst herbivorous birds, probably in relation to food digestibility, the length of the caeca increasing as the diet becomes more fibrous (Leopold, 1953). The diet of red grouse is composed mainly of heather (*Calluna vulgaris*), a high-fibre, low-protein food, and accordingly, the caecum of this bird is relatively long (Moss & Parkinson, 1972). Although colonizing *T. tenuis* favour the proximal regions of the caeca (Shaw & Moss, 1988b), worms are distributed throughout the whole gut. Thus, at the level of worm intensities observed in this study, it may be that space was not a limited resource.

Egg counts derived from two different methods were both highly correlated with worm intensity, although the smear method consistently gave a higher eggs per gram concentration than the McMaster method. This is a similar finding to that of Sinniah (1982), who compared direct smear methods with a dilution egg count technique. As the exact form of the relationship between egg count and worm intensity differed significantly between the two methods, care should be taken when using regression equations to estimate worm intensity, to ensure that the relevant equation is used. The decision as to which method to use, may be influenced by factors such as time and the relative ease of each carrying out different techniques. In our case, the smear count was the more time consuming of the two methods. This was especially true where faecal samples contained bits of grit and heather from the collection process, which ultimately affected contact between the slide and coverslip, making the actual counting of eggs under the microscope more difficult.

Previous studies have demonstrated that egg counts may be carried out on caecum material (Hudson & Dobson, 1997) and have only used caecal material from the proximal end of the gut. The research conducted here showed that the spatial concentration of worm eggs varied significantly throughout the gut, with the concentration of eggs falling as the distance from the opening of the caecum (the proximal end) increased. This is consistent with the finding that worms are unevenly distributed throughout the gut, and tends to inhabit the proximal section (Shaw & Moss, 1988b). Despite this, egg counts from distinct sections of the gut were significantly related to worm intensity. However, caution should again be applied if using gut section EPG counts to estimate worm intensity, as the exact form of the relationship to worm intensity differed between the egg counts from different sections of the gut. If using caeca to estimate worm intensity, it would therefore be more reliable to use egg counts from thoroughly mixed caecum contents, which were also found to strongly correlate with worm intensity.

Where this technique may be of particular use, is where time is a limiting factor. Direct worm count method, can be very time consuming, whereas McMaster egg counts are relatively quick. Depending on the concentration of eggs in the sample, each direct worm count may take between 40 to 60 minutes to complete, whereas McMaster egg counts routinely take only a third of that time. Using egg counts from the caecum may also be of use where birds are shot or have been killed by predators and their guts have been broken. In this case a direct worm count is inaccurate due to loss of caecal material. By removing some material from an unaffected section of the caecum and using the EPG count in conjunction with the correct regression equation instead, it may be possible derive a reliable worm intensity estimate from an otherwise unusable sample.

Frozen guts are regularly used to carry out direct worm counts in red grouse. However, in this study freezing at a temperature of $-40\ ^{o}$C appeared to cause a deterioration of eggs within the gut material. Thus, the possibilities for using caecum egg counts as an estimate of worm intensity, are strictly limited to unfrozen gut samples. Further investigation is required to examine whether eggs may be preserved at freezing temperatures closer to 0 ^{o}C.

Finally, even when storing faecal samples at an optimum temperature of 5^{o}C to inhibit the hatching of eggs, we have acquired evidence to suggest that egg concentration in faecal samples decreases with increased storage time. In the fourth week of repeated sampling, this egg loss resulted in a significant difference between the estimate of worm intensity and actual worm intensity. We conclude therefore that, in order to maximise the accuracy of our worm intensity estimates, faecal egg counts should be conducted as soon as possible after collection and within a period of three weeks maximum.

3.6 Acknowledgements

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Chapter 4.

Variation in *T. tenuis* intensity between sexes, and within and between pairs and broods in red grouse

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Variation in *T. tenuis* intensity between sexes, and within and between pairs and broods in red grouse

4.1 Abstract

Macroparasite infections tend to be aggregated within their host populations, with relatively few individuals harbouring the majority of parasites. Identifying the relative contributions of the main mechanisms thought to generate such patterns of infection, namely exposure to parasite infective stages and individual susceptibility or resistance to parasites, may have important consequences for studies of host-parasite dynamics and host population dynamics. After controlling for factors such as the study moor, date of sampling and age of the bird (which were all found to significantly explain variation in an individual's level of *T. tenuis* infection), patterns of infection between related and non-related individuals were examined. There was no relationship in *T.tenuis* intensity between pairs of male and female grouse in the spring, although a significant difference between male and female parasite intensities was detected on one study moor, with males having more parasites than females. No relationship was found between parasite intensities of related individuals within broods. Sixty-one females were radio-tagged in the spring 2002, to carry out a cross-fostering experiment to examine the relative contributions of exposure and susceptibility. Of a potential of 61 broods only 8 survived; it was not possible thus to conduct the experiment. There was a significant effect of *T. tenuis* intensity on the breeding success outcomes of the female red grouse in this experiment. This study documents the first attempt at cross-fostering red grouse chicks.

4.2 Introduction

Macroparasites tend to be unevenly distributed throughout their host population, with the majority of parasites being aggregated within a few individual hosts (Crofton, 1971; Anderson & May, 1978; Anderson & Gordon, 1982; Wilson *et al.*, 1983; Grenfell *et al.*, 1995; Shaw & Dobson, 1995; Shaw *et al.*, 1998). Two main mechanisms are thought to influence patterns of aggregated parasite distributions observed at the population level: those creating variation in host exposure to parasite infective stages and those relating to differences between individuals in their resistance or susceptibility to parasites (Anderson & Gordon, 1982; Wilson, 1983; Anderson, 1993; Boulinier *et al.*, 1996; Holmstad & Skorping, 1998; Wilson *et al.*, 2002).

The gastrointestinal nematode *Trichostrongylus tenuis* is typically over-dispersed within red grouse populations (*Lagopus lagpus scoticus*) (Wilson, 1983). As this moderately pathogenic parasite is known to have negative effects on the energetics, breeding success and even survival of its host, understanding the factors contributing to variations in individual intensities may have important consequences for studies of the population dynamics of red grouse (Hudson 1986a,b; Watson *et al.*, 1987; Shaw & Moss, 1989a; Hudson *et al.*, 1992, 1998; Delahay *et al.*,1995).

In red grouse, both exposure to infective stages and susceptibility may be contributing to determining an individual's level of *T. tenuis* intensity. Within a population of red grouse, prevalence of infection is high, with 100% of grouse greater than one year old and 99% of birds more than 3 months old found to be infected (Hudson, 1986b). This suggests that the transmission between individual hosts must be extremely effective (Shaw & Moss, 1989a). Since grouse are highly territorial, it seems likely that a large proportion of the *T. tenuis* infection that a grouse carries may be derived from self-infection or from close relatives or mates (Saunders *et al.*, 1999). Unlike domestic chickens, which can develop a form of resistance to experimental infections of *T.tenuis*, red grouse show a relatively poor acquired immune response to *T. tenuis* infection (Wilson, 1979; Shaw & Moss, 1989b).

This study had two main aims, the first being to investigate patterns of infection in pairs of grouse. If all individuals within a population are equally susceptible and exposure is a dominant mechanism in determining an individuals parasite infection, then given that pairs of grouse spend a significant proportion of the year together feeding and roosting in close proximity to one another, individuals should share similar levels of infection, with the variation within pairs of adult grouse being smaller than the variation observed between pairs. The second aim of this study was to look for evidence of a shared genetic component in resistance to parasites in red grouse chicks. I began by examining naturally occurring patterns of infection between chicks within families of chicks (broods) to explore whether genetically similar individuals shared similar levels of resistance to parasite infection. Whilst correlative studies are useful in identifying patterns of infection in natural populations, they tell us little about relative importance of the mechanisms involved in creating those pattern. Consequently, I set up a cross fostering experiment, which aimed to tease apart variation due to exposure and to susceptibility, and thus address the question of heritability of parasite resistance in red grouse.

Prior to addressing these questions, I examined a number of factors that may have explained some of the heterogeneity in patterns of *T. tenuis* in red grouse observed in this study. As data came from a number of geographically distinct moors from both Northern England and Scotland,

and because birds were sampled on different nights over a period of two months, I first investigated the influence these factors might have on parasite intensities. As red grouse have been found to have little effective acquired immune response to re-infection (Wilson, 1983), the number of worms present in the caeca of red grouse increases throughout the life of the bird, so I examined the effect of age on parasite intensities. I also investigated the effect of sex, as it is often observed in vertebrate species, that males are more heavily parasitized than females (Moore & Wilson, 2002; Wilson *et al.*, 2002) perhaps due to differences in hormones affecting immune function (Grossman, 1984,1985; Schuurs & Verheul, 1990; Poulin, 1996; Hillgarth & Wingfield, 1997).

Specifically, this study set out to test the following predictions: if exposure is a dominant factor, individuals within a pair and within a brood will have similar levels of infection, however if natural resistance is a dominant factor, genetically similar chicks from the same brood exposed to different levels parasite infection, will have similar levels of infection.

4.3 Methods

Study Areas & Capture

Data from 83 pairs of grouse were collected from 6 grouse populations, two located in northern England (Catterick and Moorhouse in 2002) and four in northeast Scotland (Edinglassie, Invercauld, Invermark, Glas Choille in 2001). Pairs of male and female adult grouse were captured at night during spring (from 28th February to 2nd May) using standard lamping techniques (Hudson & Newborn, 1995). Paired grouse roost together in close proximity and, where possible, both males and females were caught together on the same evening. Where only one of a pair was caught, a necklace-type radio-collar was fitted to the bird and that individual relocated on a separate night to capture the second individual. Pairs were checked subsequently to confirm the pair bond. Data from 13 broods were collected from one site in Scotland (Glas Choille) in the autumn of 2001 and 2002. Families of grouse (chicks and adults) roost together and were located at night using lamping techniques during August, before the broods split up and dispersed. Although the actual hatch date of the chicks was not known, they were approximately 2-3 months of age. At this age, chicks were considered old enough to have picked up an infection of *T. tenuis*. The number of individuals caught in each brood ranged from 2 to 6 and the date on which chicks were sampled also varied between broods. Plumage and morphological characteristics were used to sex and age birds. For birds caught in the spring, those hatched the previous summer, were classed as "young" and those greater than 1 year old classed as "old". The sexing and aging of birds, are described in greater detail in Chapter 2. All individual birds (chicks and adults) were marked either with a numbered metal leg ring or with a numbered metal wing tag.

Parasite Data Collection

For a detailed description of the life-cycle of *Trichostrongylus tenuis* refer to Chapter 2. Parasite data were collected from pairs of grouse caught in the spring in order to examine patterns of *T. tenuis* infection within and between breeding pairs of individuals. Parasite data were also collected from broods caught during the autumn (a total of 41 individuals, ranging from 2-6 individuals from each brood), in order to compare patterns of *T. tenuis* infection within and between broods. Although invertebrates (a vital source of protein) are an important part of a young grouse chick's diet (Savory, 1977), chicks also start to feed on heather at an early age.

Therefore, *T. tenuis* infections may begin when grouse are less than a month old and increase throughout the life of the bird (Hudson, 1986b). Upon capture, each bird was kept in an individual holding box overnight and caecal faeces collected the following morning for parasite sampling. The following morning, birds were released and their caecal faeces containing the parasite eggs collected and taken to the lab for examination.

Laboratory analysis of T. tenuis egg intensity.

The intensity of *T. tenuis* eggs per gram (EPG) of caecal faeces were estimated from approximately 0.2g of faecal material using the modified McMaster egg counting technique (MAFF, 1986). For a detailed description of this method, refer to Chapter 3. Previous studies on *T. tenuis* faecal egg counts in red grouse indicate that there is a strong positive correlation between *T. tenuis* worm burden and EPG (Moss *et al.,*1990; Seivwright *et al.*, 2004; see also Chapter 3). Worm burdens were calculated from average EPG counts (Seivwright *et al.*, 2004; see also Chapter 3)

Partial Cross-Fostering Experiment

To try to tease apart the relative contribution of environmental and genetic factors to variations in individual *T. tenuis* intensities, I attempted to carry out a partial cross-fostering experiment on Moorhouse, N. England, in 2002. For full details of this experiment see Chapter 2. A total of 61 pairs were caught at night on Moorhouse during early spring, sampled for parasites using the techniques outlined above and females fitted with a necklace-type radio to allow future relocation. Females were radio-tracked at frequent intervals during April and May until their nests was found. After clutch completion, egg measurements (length, breadth to the nearest 0.1mm and weight to the nearest 0.1g) were taken and used to predict an average hatch date for the nest (Smith, unpublished data).

Statistical analyses

Factors influencing variation in parasite intensities

In order to explore the influence of moor, sampling date, age and sex as sources of variation in faecal egg counts (FEC) between individuals, parasite count data were analysed using generalized linear models (SAS GENMOD procedures). Models were fitted to the data using a negative binomial distribution and a log link function (Wilson & Grenfell, 1997), with the logarithm of the mass of faecal material sampled as an offset. The models were constructed using a backwards step-wise selection procedure, starting with all the terms in the model, then dropping the least significant term in the subsequent model until only terms significant at the 5% level remained, using SAS type I tests (SAS, 2001). As it is known that development of infective larval stages of the parasite is subject to seasonal effects (Shaw, 1988) and that parasite intensities increase with age, it was considered to be biologically meaningful to include parasite sampling date in all models, despite the fact that it was found to be no longer statistically significant in the full model.

Parasite intensities within and between pairs and broods.

The relationship between male and female parasite burdens within pairs was analysed initially using Spearman Rank Correlation and male and female indices of *T. tenuis* intensity. These indices were the residuals of *T. tenuis* count data after controlling for sampling date, study moor and age as fixed effects in a generalized linear model, as above. To compare parasite variation within and between pairs of grouse, *T. tenuis* count data were analysed using Generalized Linear Mixed Models. The method is based on the Poisson-lognormal model, and aims at partitioning the observed variance among sources of heterogeneity as described in Elston *et al.* (2001). In order to implement the Poisson-lognormal model, I made the following assumptions; that conditional on their respective means μ_{ijk} the number of *T. tenuis* eggs counted from individual *i* of pair *j* from study site *k* followed a Poisson distribution. I then modelled the mean egg counts μ_{ijk} with respect to the following dependent variables; sampling date, study site, sex, age and individual nested within pair. Models were implemented in SAS using the GLIMMIX macro (Littell *et al.*, 1996) and were fitted to the data using a Poisson error distribution with a log link function and included the logarithm of the mass of faecal material sampled as an offset. Denominator degrees of freedom were estimated using Satterthwaite's formula (Littell *et al.*, 1996). As I was interested in variance as a measure of non-randomness, the dispersion parameter was fixed at 1.0. Using *T. tenuis* egg counts as the response variable, a series of models were run, initially with no fixed effects and then with the addition of a number of explanatory variables including categorical fixed effects of study moor (a pair-specific effect), sex and age, and a continuous fixed effect of sample date (all individual-specific effects). Random effects of pair and individual nested within pair were included in each model. If the variance of the pair means (σ^2_e) is greater than zero, this implies that there is additional variation among pairs mean that cannot be accounted for by Poisson variation or by the pair-specific fixed effects in the model. In the same way, if the variance of the individual nested within pair means (σ^2_ϵ) is greater than zero, this implies that that there is additional variation in individual egg counts within pairs that cannot be attributed to pair means, Poisson variation about the means or by the individual-specific effects. Comparing values of the two variances components of the random effects (σ^2_e and σ^2_ϵ) from models with 1, 2, 3 or no fixed effects, not only gave an indication of the variance explained by the fixed effects but also made it possible to quantify the amount of variance occurring between individuals within pairs, relative to that occurring between pairs in the population.

Data on *T. tenuis* intensities collected from individual chicks within broods were also analysed using this method. The explanatory variables then included a categorical fixed effect of year, a continuous fixed effect of sample date and random effects of brood, and individual nested within brood. The parameters to be estimated were the effects of moor, sex, age and date, together with the variances of the random effects.

Breeding attempt outcome of cross fostering experiment

Due to a parasite outbreak at Moorhouse during 2002, the reproductive success of individuals was particularly low. By the end of the breeding season, 16 pairs had failed to lay eggs, died or were lost, 21 pairs had laid eggs but failed to hatch chicks, and only 16 pairs successfully hatched chicks. I tested if the overall breeding success of a pair was explained by *T. tenuis* intensities in females or males, after controlling for age and parasite sampling date. To do so, I used generalised linear models, with the probability that an individual produced chicks (or not) as a binary measure, analysed with a binomial error structure and a logit link function (SAS GENMOD procedures).

Statistical analyses were conducted using Minitab (version 13) and SAS (version 8.01, SAS, 2001). Where necessary, data were $\log_{10}$-transformed to approximate normal distribution.

4.4 Results

Variation in individual <u>T. Tenuis</u> infections according to study moor, sampling date and age.

I used generalized linear models to explore the influence of moor, sampling date, age and sex as sources of variation in faecal egg counts (FEC) between individuals. Intensities of *T. tenuis* increased significantly over the sampling period across moors (GENMOD; $\chi^2 = 13.90$, 1 d.f., $P < 0.001$). After controlling for sampling date, intensities of *T. tenuis* varied significantly between study moors (Figure 4.1; Table 4.1) (GENMOD; $\chi^2 = 52.76$, 5 d.f., $P < 0.0001$) but a significant interaction between sampling date and study moor (GENMOD; $\chi^2 = 43.70$, 4 d.f., $P < 0.0001$), indicated that increases in *T. tenuis* intensities over sampling period were not consistent across all moors. After controlling for sampling date, study moor and a date*moor interaction, *T. tenuis* intensities varied significantly between age groups, with old birds having significantly higher intensities of *T. tenuis* than young birds (GENMOD; $\chi^2 = 14.60$, 1 d.f., $P = 0.0001$) but a significant interaction between age and moor effect indicated that differences in *T. tenuis* intensities between age groups were not consistent across moors (GENMOD; $\chi^2 = 21.80$, 5 d.f., $P < 0.001$).

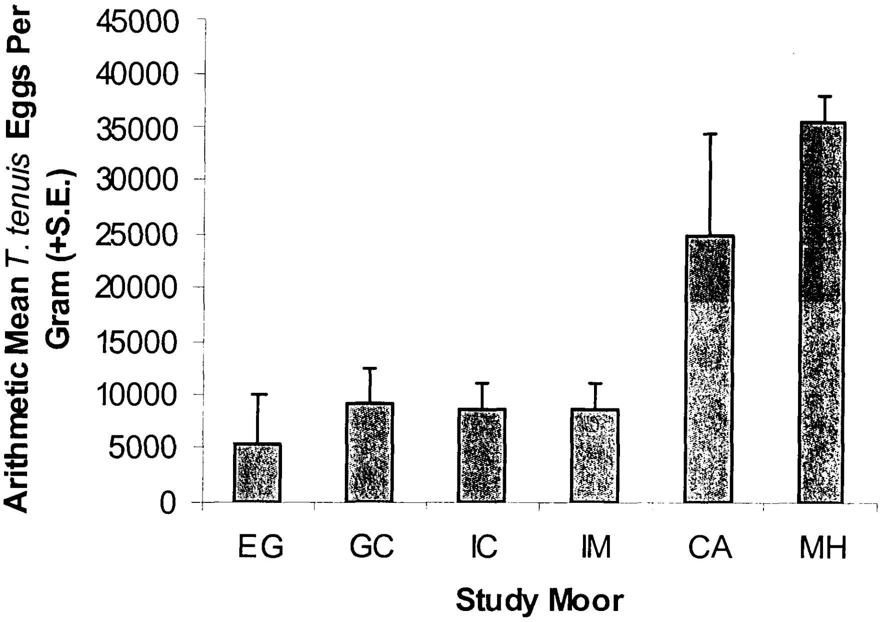

Figure 4.1: Arithmetic mean of *T. tenuis* egg concentrations (eggs. g^{-1}) in the six study moors; CA (Catterick, N = 17), EG (Edinglassie, N = 4), GC (Glas Choille, N = 15), IC (Invercauld, N = 12), IM (Invermark, N = 6) and MH (Moorhouse, N = 114).

	EG	*GC*	*IC*	*IM*	CA
GC	*0.98* *(NS)*				
IC	*0.76* *(NS)*	*0.02* *(NS)*			
IM	*0.55* *(NS)*	*0.03* *(NS)*	*0.00* *(NS)*		
CA	*0.89* *(***)*	*8.40* *(***)*	*8.22* *(***)*	*5.40* *(NS)*	
MH	*14.49* *(***)*	*25.41* *(***)*	*22.49* *(***)*	*12.17* *(**)*	*1.92* *(NS)*

P-values: NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table 4.1: Comparison of *T.tenuis* parasite intensities between study moors (see Fig. 4.1). Chi-square values (P values below) for Difference of Least Square Means (DLSM). (GENMOD procedure, SAS, 2001).

Sex differences in T.tenuis intensities.

Intensities of *T. tenuis* from birds sampled in this study ranged between 116 and 111279 eggs per gram in females (equivalent to approximately 100 to 8895 worms) and 165 to 141379 in males (approximately 128 to 10,396 worms). Using data from all sites, after controlling for sampling date, study moor, age and date*moor and moor*age interactions, *T. tenuis* intensities did not vary between male and female grouse (Figure 4.2). However, within age classes, although there was no difference between infection intensities between the sexes in old birds, young males had significantly more *T. tenuis* than in young females (Figure 4.3).

In order to further examine sex differences in individual *T. tenuis* intensities, data were examined from a single study site with the greatest sample size of individuals (Moorhouse, N = 114). The geometric mean worm burdens for males and females at Moorhouse were 4411(95% C.I: 3483 – 5548) and 2732 (95% C.I: 2251- 3300) worms respectively. Males had significantly higher intensities of *T. tenuis* than females, after controlling for sampling date and age. Intensities of infection of *T. tenuis* increased significantly over time and (Figure 4.4). Increases in male and female *T. tenuis* intensities over the sampling period were also consistent between the sexes (Figure 4.4).

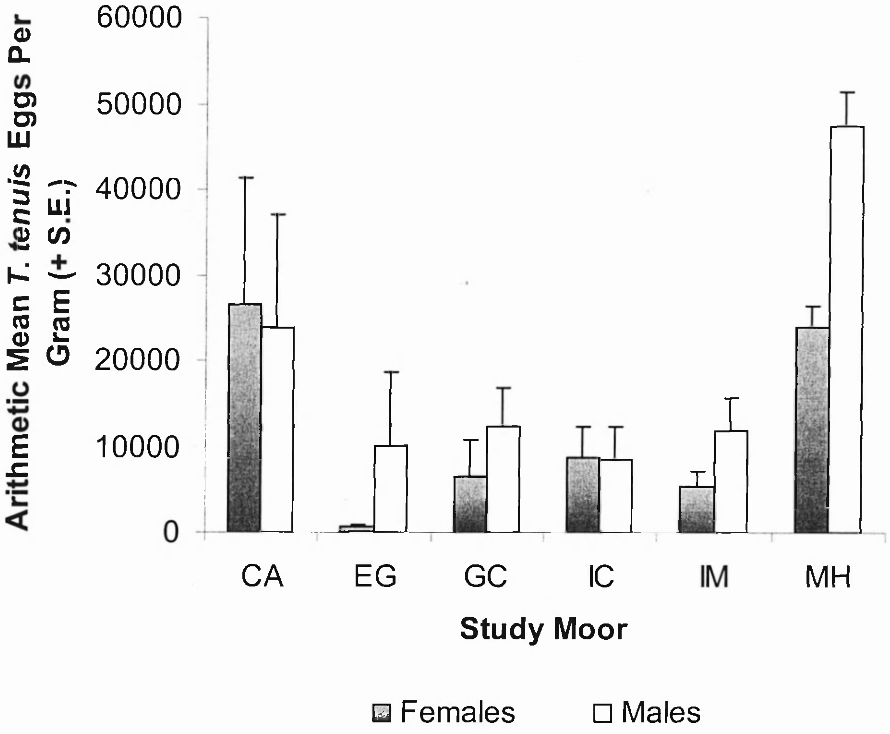

Figure 4.2: Arithmetic mean of female and male *T. tenuis* egg concentrations (eggs. g^{-1}) in the six study moors; CA (Catterick; Females N = 7, Males N = 10), EG (Edinglassie; Females N = 2, Males N = 2), GC (Glas Choille; Females N = 18, Males = 7), IC (Invercauld; Females N = 6, males = 6), IM (Invermark; Females N = 3, Males = 3) and MH (Moorhouse; Females N = 58, Males =56). For all study sites, after controlling for sampling date, study moor, age and date*moor and moor*age interactions, there was no difference between male and female *T. tenuis* intensities (GENMOD; χ^2 = 1.03, 1 d.f., P = 0.3107).

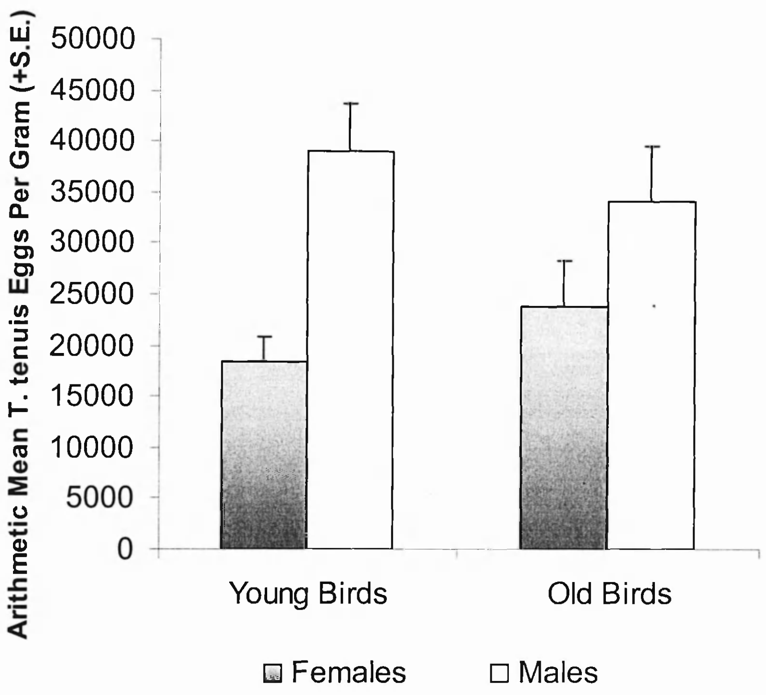

Figure 4.3: Arithmetic mean of *T. tenuis* egg concentration (eggs. g^{-1}) in old and young male and female birds. In a general linear model with date of sampling as a fixed effect, *T. tenuis* intensities varied significantly between the sexes in young birds. Young males had significantly higher *T. tenuis* intensities than young females (GENMOD; χ^2 = 14.60, 1 d.f., P < 0.001). There was no difference in *T. tenuis* intensities between old males and females.

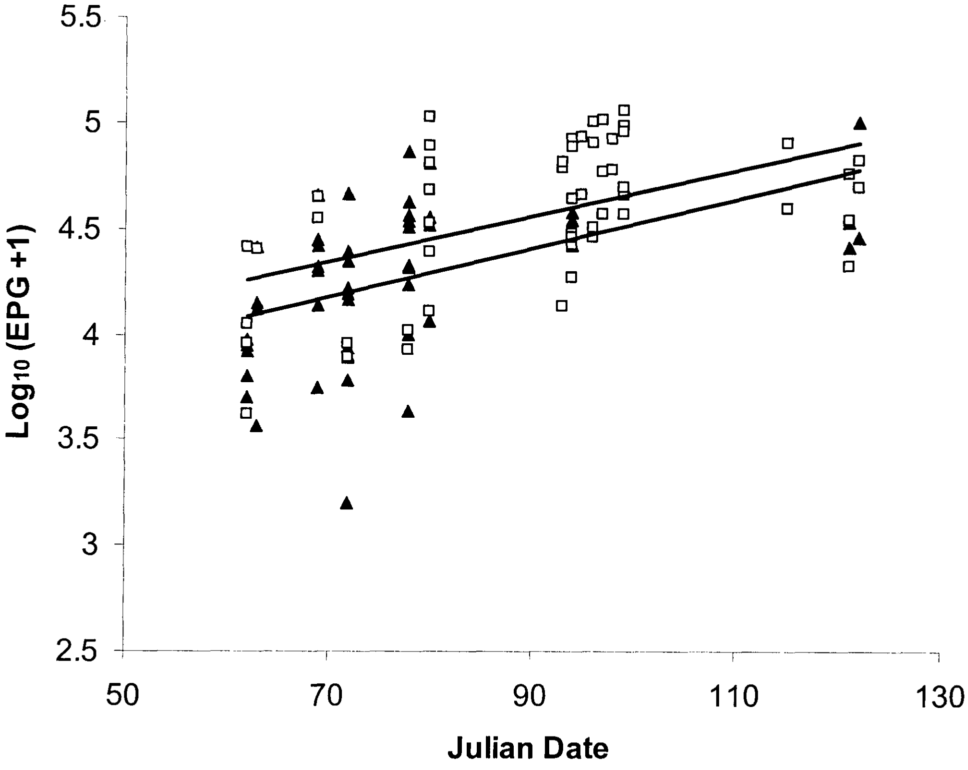

Figure 4.4: The relationship between *T. tenuis* egg concentration (E, eggs. g $^{-1}$) and sampling date (Julian day) for Moorhouse data only. Symbols represent females (▲)and males (□) Intensities of *T. tenuis* increased significantly over time (Figure 4.1: GENMOD; χ^2 = 43.99, 1 d.f., P < 0.0001). After controlling for sampling date and age, there was a significant different difference between male and female *T. tenuis* intensities (GENMOD; χ^2 = 4.22, 1 d.f., P < 0.05). No significant interaction between sampling date and sex (GENMOD; χ^2 = 0.18, 1 d.f., P = 0.67), indicated that increases in male and female *T. tenuis* intensities over the sampling period were consistent between the sexes.

Variation in parasite intensities within and between pairs of grouse.

To investigate patterns of *T. tenuis* infection in pairs of red grouse, FEC data were collected in the spring from pairs from all six study sites. Once the effects of site, sampling date and individual age were corrected for, residual *T. tenuis* FECs between males and females within pairs were not correlated, (Figure 4.5: Spearman rank correlation $r_s = 0.241$, $n = 80$, $p = 0.13$). Although there was no direct relationship between *T. tenuis* intensities of males and females within pairs, I investigated whether the variation between individuals within pairs was less than variation among pairs using a method based on the Poisson-lognormal model, which enables the observed variance to be partitioned among sources of heterogeneity. Due to the significantly higher levels of parasites found on Moorhouse (Figure 4.1; Table 4.1), initial attempts to examine the data from all the moors combined, showed that the "moor" effect was so strong, no variance was explained between pairs, over and above that explained by "moor". The data were therefore analysed, firstly using only Moorhouse data (site effect not included as a fixed effect) and secondly, using data from the remaining five moors (CA, EG, GC, IC& IM) combined.

Data from Moorhouse

After controlling for the effects of sampling date, sex and age, *T. tenuis* egg counts varied more within pairs than between pairs: the estimated variance component for individuals nested within pairs (0.391) was estimated to be almost 7 times as great as the variance component for pairs (0.056) (Table 4.2, model 5). Comparing the variance component for mean *T. tenuis* counts for individuals nested within pairs in model 1 without fixed effects (0.848), with that of model 5 (0.391), the difference between these variance components indicates that half the variation attributable to individuals, was due to the effects of sample date, age and sex (Table 4.2). As all these factors were specific to individuals nested within pairs, they explain only variation among individuals and not variation among pairs. The variance component associated with pairs in all the models was extremely small, and in the case of model 1, was negative. A negative variance component indicates that there was less variation between pairs than was to be expected, given the level of variation between individuals within pairs. Such a small effect found between pairs as compared to between individuals within pairs indicates that there was no evidence that males and females within a pair had similar parasite burdens.

Data from All moors (excluding Moorhouse)

The results from all the other moors combined, gave similar results to those from Moorhouse (Table 4.3). The estimated variance component after controlling for the effects of study moor, sampling date, age and sex, was 2.626 for individuals nested within pairs, compared with a relatively small negative variance component of –0.177 for pairs. Again, this negative variance component indicated that there was less variation between pairs than was to be expected, given the level of variation between individuals within pairs. In contrast to the Moorhouse analysis, the addition of individual-specific effects of sampling date, age and sex in the models, explained only a small amount of the variation among individuals and the addition of the pair-specific effect of study moor explained none of the variation among pairs. There was thus no evidence that males and females within a pair have similar parasite burdens.

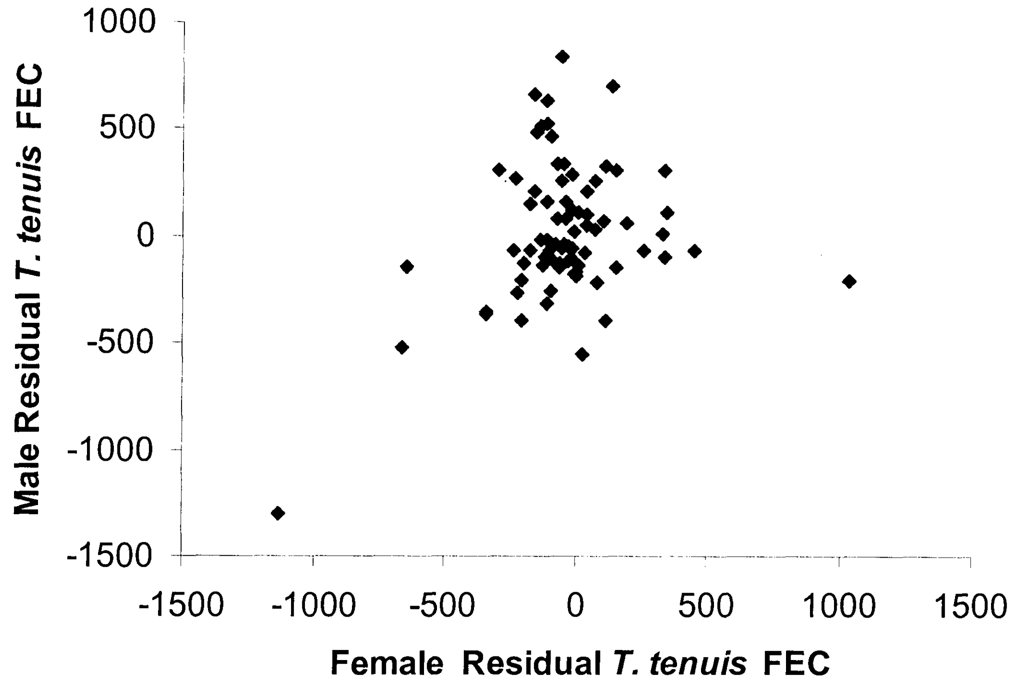

Figure 4.5: Relationship between male and female residual *T. tenuis* FEC within pairs of grouse. There was non-significant relationship between FEC between paired males and females (Spearman rank correlation coefficient r = 0.241, n = 80, p = 0.13)

Table 4.2: Variance components of random effects in different models. Data from Moorhouse site alone. The Poisson dispersion parameter was fixed at 1 throughout.

Model	**Fixed Effects**	**Random Effects**	**Variance Component**	**95% C.I.**
1	Mean	Pair	-0.060	-0.168 to 0.048
		Individual	0.848	0.687 to 1.01
2	Mean & Date	Pair	0.0714	-0.007 to 0.150
		Individual	0.454	0.363 to 0.546
3	Mean, Date & Sex	Pair	0.062	-0.009 to 0.133
		Individual	0.438	0.352 to 0.524
4	Mean, Date & Age	Pair	0.067	-0.004 to 0.138
		Individual	0.396	0.315 to 0.477
5	Mean, Date, Sex & Age	Pair	0.056	-0.009 to 0.121
		Individual	0.391	0.312 to 0.470

Table 4.3: Variance components of random effects in different models. Data from all sites excluding Moorhouse. The Poisson dispersion parameter was fixed at 1 throughout.

Model	**Fixed Effects**	**Random Effects**	**Variance Component**	**95% C.I.**
1	Mean	Pair	-0.04	-0.63 to 0.55
		Individual	2.80	1.98 to 3.62
2	Mean & Date	Pair	-0.21	-0.79 to 0.37
		Individual	2.85	2.01 to 3.68
3	Mean, Date & Site	Pair	-0.04	-0.64 to 0.56
		Individual	2.81	1.99 to 3.63
4	Mean, Date & Age	Pair	0.04	-0.61 to 0.68
		Individual	2.60	1.78 to 3.43
5	Mean, Date & Sex	Pair	-0.18	-0.75 to 0.39
		Individual	2.603	1.95 to 3.60
6	Mean, Date, Site, Sex & Age	Pair	-0.18	-0.58 to 0.81
		Individual	2.63	1.78 to 3.47

Variation in parasite intensities within and between broods of chicks.

Initial attempts to examine the brood data from Glas Choille in the autumn for years 2001 and 2002 combined, using the Poisson-lognormal model, showed that the "year" effect was so strong, no variance was explained between pairs, over and above that explained by "year" (Figure 4.6; Table 4.4). The data were therefore re-analysed, using 2002 data alone, as the sample size of broods from 2001 was too small (N= 4 broods). Using only brood parasite data from 2002 (9 broods), with sampling date as a fixed effect, the estimated variance component for individuals nested within broods was 1.041 compared with a relatively small negative variance component for broods of –0.157 (Table 4.4, model 3). The addition of sex in this model, explained no further variation among broods or individuals nested within broods (Table 4.4, model 4). The negative variance component for broods indicates that there is less variation between broods than is to be expected, given the level of variation between individuals within broods. There was thus no evidence that chicks within broods had similar parasite burdens.

When *T.tenuis* infections were compared between male and female chicks, in a general linear model with year and date of sampling as a fixed effects, *T. tenuis* intensities were significantly higher in male chicks than in female chicks (Figure 4.7: GENMOD; $\chi^2 = 6.08$, 1 d.f., $P < 0.05$).

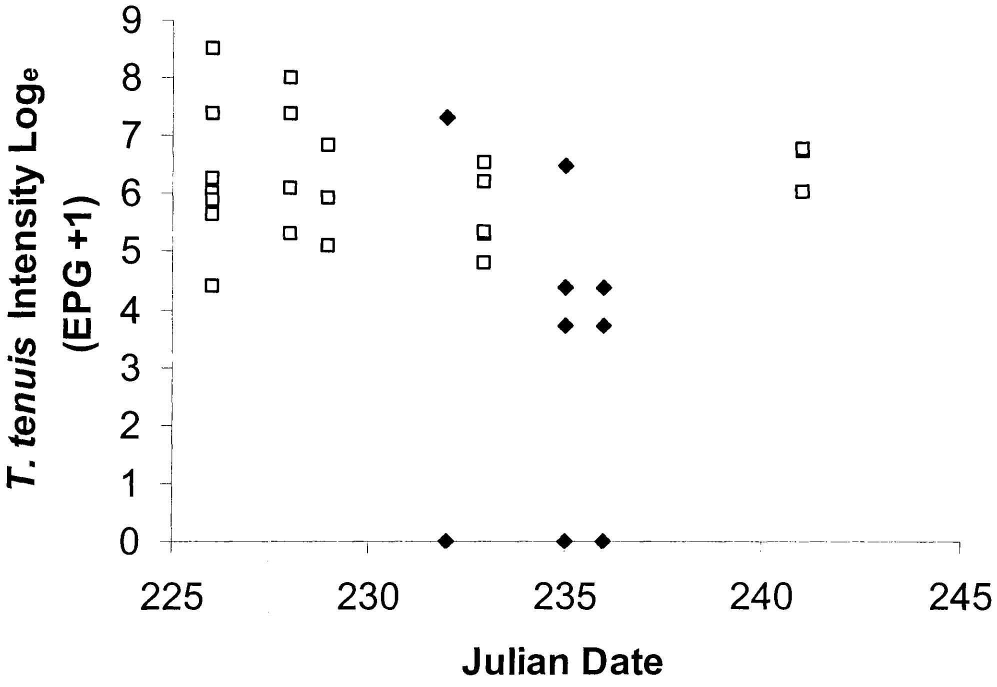

Figure 4.6: *T.tenuis* intensities of chicks from Glas Choille in 2001 (♦) and 2002 (□)

Table 4.4: Variance components (VC) of random effects in different models. Data from broods of chicks from Glas Choille. The Poisson dispersion parameter was fixed at 1 throughout).

			Glas Choille 2001 & 2002		**Glas Choille 2002 Only**	
Model	**Fixed Effects**	**Random Effects**	**VC**	**95% C.I.**	**VC**	**95% C.I.**
1	Mean	Brood	1.10	0.32 to 1.87	-0.18	-0.36 to -0.01
		Individual	1.01	0.65 to 1.37	1.05	0.61 to 1.48
2	Mean & Year	Brood	-0.25	-0.45 to -0.06		
		Individual	1.26	0.74 to 1.77		
3	Mean & Date	Brood	1.06	0.86 to 1.26	-0.16	-0.38 to 0.06
		Individual	1.01	0.65 to 1.37	1.04	0.60 to 1.48
4	Mean, Date Sex	Brood	1.18	0.30 to 2.08	-0.16	-0.38 to -0.06
		Individual	0.94	0.59 to 1.29	1.04	0.60 to 1.48
5	Mean, Date, Sex & Year	Brood	<0.000	<0.000		
		Individual	1.56	1.56		

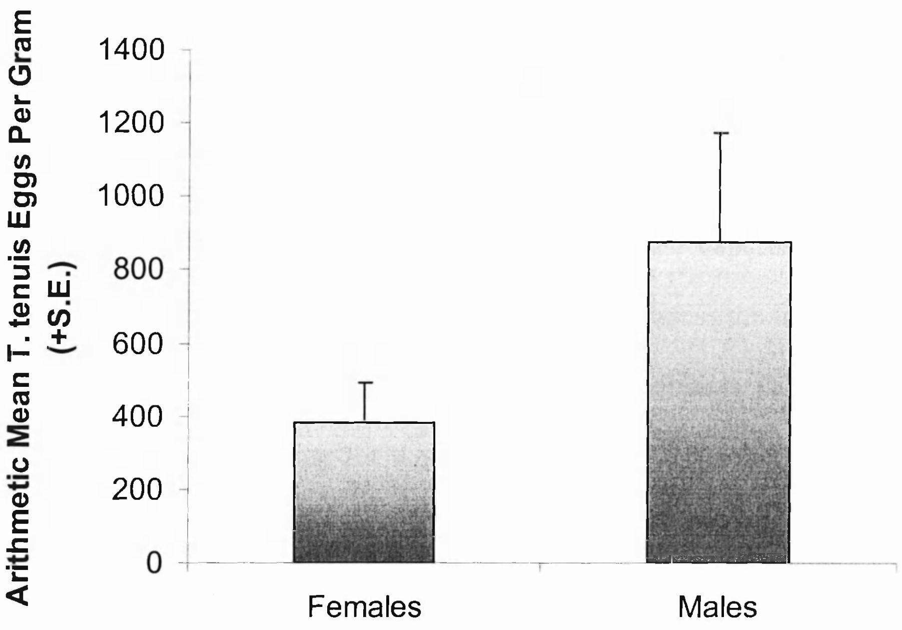

Figure 4.7: Arithmetic mean of *T. tenuis* egg concentration (eggs. g^{-1}) in female (N=15) and male (N=18) chicks from Glas Choille In a generalised linear model with year and date of sampling as a fixed effects, *T. tenuis* intensities were significantly higher than male chicks than in female chicks (GENMOD; $\chi^2 = 6.08$, 1 d.f., $P < 0.05$).

Cross-Fostering Experiment

Crash of Experimental Population

During 2002, densities of grouse on the Moorhouse study population fell from 193 birds per km^2 in the spring to 40 birds per km^2 in the following spring. The poor breeding success of the hens in this experiment, resulted in only 8 out of a potential of 61 broods surviving. Whilst such a small sample size of broods rendered the usefulness of data from the experiment redundant, three partial cross-fosterings were nevertheless attempted, in order to assess the feasibility of the actual method used and in order to develop the protocol for future experiments. Out of a total of 61 hens caught and radio-tagged in the spring for this experiment, 16 (26%) failed to lay eggs and 21 nests (34%) were lost at the egg stage either through predation or desertion. Of the 16 nests that hatched out (26%), only 8 broods survived to greater than 5 days old. In the early stages of the experiment, 8 radio-tagged hens were lost (13%) (possibly due to either the radio battery failing or the hens moving a far enough distance from the study site for the radio signal not to be detected). Of the 8 surviving broods, 3 cross-fostering attempts were made between 6 broods. A total of 28 chicks were tagged and 13 chicks were placed into foster broods. Only 3 tagged chicks (1 fostered chick) were located the following autumn when the hens were relocated in order to remove the radio-tag.

Survival of Radio-tagged Hens

Throughout the course of the experiment, of the 61 radio-tagged hens, a total of 14 hens were found dead. Predation by either mustelids or raptors was identified in 8 cases (13%) (Hudson & Newborn, 1995) and 6 cases (10%) were found with no obvious signs of predation. External examination of these carcasses in the field revealed these hens to be in extremely poor condition, highly emaciated with very little breast muscle tissue and the sternum generally protruding very obviously beneath the skin. These characteristics are consistent with the clinical symptoms of grouse suffering from the pathophysiological effects of a *T.tenuis* infection (Hudson *et al.*, 1997). The FECs of these females in the spring corresponded to worm burdens in these females ranging from 2049 to 5292.

The effect of T. tenuis on breeding success

Of the 53 pairs followed throughout the breeding season, only 16 pairs successfully produced chicks. Overall breeding success was negatively significantly related to hatch date (GENMOD; $\chi^2 = 3.97$, 1 d.f., $P = 0.046$), and marginally significantly related to female age ($\chi^2 = 3.12$, 1 d.f., $P = 0.078$), but not to male age ($\chi^2 = 0.64$, 1 d.f., $P = 0.424$). After controlling for age and date of parasite sampling, a pair's overall breeding success was significantly negatively related to intensity of infection of *T. tenuis* in females (Figure 4.8; $\chi^2 = 6.15$, 1 d.f., $P = 0.013$) but not in males ($\chi^2 = 1.57$, 1 d.f., $P = 0.211$).

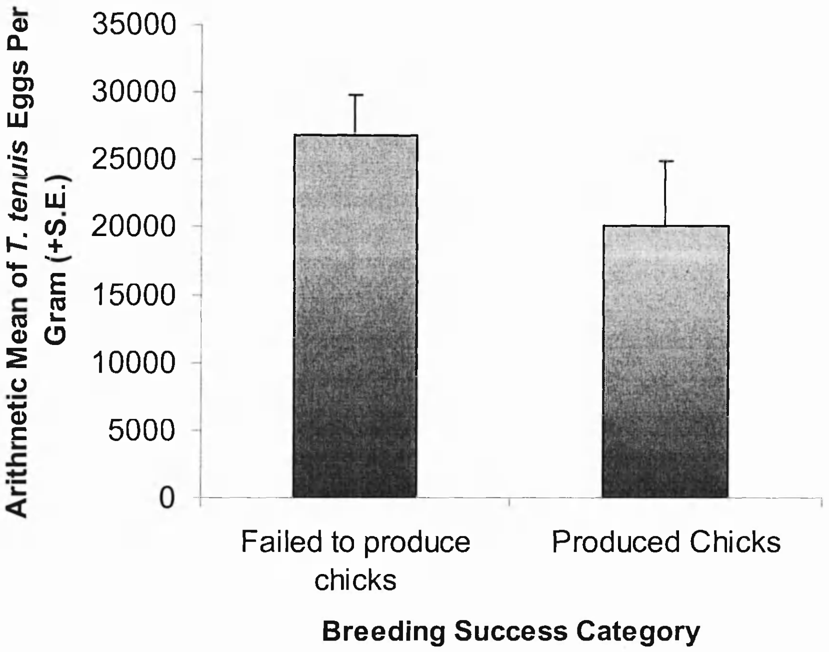

Figure 4.8: Arithmetic mean of *T. tenuis* intensity of females that failed to produce chicks (N = 37) and females that succeeded (N = 16) in producing chicks.

4.5 Discussion

Infection intensities of the caecal threadworm *T. tenuis* vary greatly between individual red grouse (Wilson, 1983). In the present study, I showed that spatial, temporal as well as biological factors may explain some of this variation.

Data for this study came from a number of moors from both England and Scotland and *T. tenuis* intensities varied significantly between study moors, with Moorhouse in northern England showing highest parasite intensities. Previous studies have attributed geographic variability in parasite intensities of red grouse to heterogeneities in climatic conditions and host density between moors, both of which may affect transmission rates between hosts (Wilson, 1983; Hudson, 1986b; Hudson & Dobson, 1995). Hudson (1986b) found greater worm burdens in grouse from moors in wetter regions with high densities of grouse. On a geographical scale, moors in the east of the country tend to be drier than in the west and desiccation and extreme low temperatures may significantly reduce the survival of free-living developing larval stages of *T. tenuis* (Shaw *et al.,* 1989). To establish whether this may account for lower parasite intensities in the four moors located in north east Scotland, data on climate variables are required. Another explanation for the relatively higher intensities of infection observed on the two study sites in England, is that higher grouse densities may lead to greater concentration of parasite eggs being deposited onto the moor, thus increasing transmission of the parasite within and between hosts (Hudson, 1986b). At Moorhouse and Catterick, densities of grouse in the previous autumn and in the spring reached 193 and 111 birds per km^2 respectively. This is high in comparison to densities of grouse found at Glas Choille (36.5 birds per km^2,Autumn 2000) and Invercauld (35 birds per km^2, Autumn 2000) in Northeast Scotland.

High grouse density may also explain the significant difference between male and female parasite intensities found on Moorhouse, the main study site. Males had significantly higher infections of *T. tenuis* than females, with over 70% of males having higher levels of infection than females within pairs. Sex differences may result from physiological differences between the sexes such as seasonal differences in levels of circulating steroid hormones, such as testosterone (Wilson *et al.,* 2002). Elevated testosterone levels are often required during breeding periods for the expression of sexual traits and behaviour involved in male-male competition and female choice, but might also be associated with physiological costs, such as impaired immune function (Folstad & Karter, 1992; Grosmann, 1985; Hillgarth & Wingfield, 1997).

Grouse populations tend to exhibit regular cyclic fluctuations. Male aggressiveness and ornamental comb size, which are both testosterone dependent, have been shown to increase with population density (Moss *et al*., 1979; Watson *et al*., 1994; Moss *et al*., 1996). Previous studies conducted on red grouse found that males artificially implanted with testosterone in the autumn had lower cell-mediated immunity (Mougeot *et al*., 2004; see also Chapter 6). Thus males, particularly those in high density populations as in the case of Moorhouse, may be increasing their susceptibility to parasites by elevating their hormone levels during the spring breeding period.

In chicks from Glas Choille, and in young birds from all study sites, males had significantly higher *T. tenuis* intensities than females. Again, these differences may be due to differences in levels of circulating steroid hormones. In order to secure a territory in their first breeding season, young males may have to compete not only against other young males, but also against old males that have already established a territory. This may require young males to elevate their testosterone levels to facilitate aggressiveness at the expense of making themselves more susceptible to parasites. The relationship between testosterone and *T. tenuis* is examined in more detail in Chapter 7.

In this study, birds were sampled for parasites over a two-month period from early to late spring (28th February to 2nd May) and *T. tenuis* intensities increased significantly over this period. Developmental stages of *T. tenuis* infective larvae may account for this increase in the spring as larvae ingested in late autumn and winter may arrest their development, resuming maturation synchronously during late March and early April, causing an observed rise in *T.tenuis* egg output (Shaw, 1988; Moss *et al.*, 1993). *T. tenuis* intensities also increased significantly with age as previously found in other studies, with old birds having more parasites than young (Wilson, 1979; Hudson, 1986b). Unlike artificially induced *T. tenuis* infections in domestic chickens, where young domestic chickens develop a resistance to the nematodes resulting in worms being actively expelled and the infection rejected (Watson *et al.*, 1988), the number of worms present in the caeca of red grouse increases throughout the life of the bird (Wilson, 1983).

This study went on to examine patterns of infection in pairs of male and female grouse in the spring and broods of grouse chicks in the autumn. Given that pairs spend a significant proportion of the year together, feeding and roosting in close proximity to one another, it was predicted that if exposure is a strong mechanism in determining an individual's parasite burden, then the variation between individuals within pairs and broods, should be smaller than variation between random individuals. There was however, no evidence to suggest that pairs of grouse or broods of grouse chicks had similar parasite burdens. This was in contrast to the work of Elston *et al.*

(2001) who examined patterns of infection of the sheep tick ectoparasites *Ixodes ricinus* in grouse chicks and found a greater similarity in levels of infection between chicks with the same brood than the variation in chicks from different broods. It may be however, that not enough broods were sampled to allow variation between and within broods to be detected. Similarly, it may also be that the sampling method (Mcmaster), which can be reliably used to count parasite eggs in faecal samples from adult grouse (Seivwright *et al.*, 2004; see also Chapter 3), may not have been sensitive enough to detect variation between chicks of the age used in this study. A different sampling method, such as the flotation method (MAFF, 1986), where all the eggs present in faeces are separated from faecal debris and counted may have provided more accurate counts.

Unfortunately, correlative studies of this nature merely describe rather than explain patterns of infection. Thus, there may be a number of explanations to account for the variation in infection levels between individuals in pairs and broods. First, although pairs and broods feed and roost together, mostly within the male's territory, it could be that heterogeneities in exposure to infective stages, may be operating on much smaller spatial scale, at the level of the heather plant and not at a territory level, as was predicted. Indeed previous studies have shown that not only is caecal faeces containing the parasites eggs generally aggregated on the moor, the resultant infective larval stages of *T. tenuis* may be aggregated on the tips of heather (Saunders *et al.*, 2000; Hudson, 1986b). Second, patterns of infection between pairs may be explained by variation in individual resistance to infection similar to that shown in captive grouse (Wilson, 1979; Shaw & Moss, 1989b; Moss *et al.*, 1993). It has been shown previously in captive birds, however, that grouse show a wide variation in innate susceptibility to the same dose of *T. tenuis* larvae (Wilson, 1979; Shaw & Moss, 1989b) and a study by Moss *et al.* (1993) found that relative differences in parasite egg counts among individuals within years, tend to persist across years, so that relatively high or low egg counts were found to be characteristics of individual birds. Thirdly, as already mentioned, differences between male and female infection levels associated with differences in circulating hormones, may also explain the observed patterns of infection between pairs and broods.

In order to formally assess the contribution of environmental exposure versus innate resistance as determinants of an individual's level of infection, experimental manipulations are required. I attempted to tease apart the environmental and genetic components of an individual's parasite infection by carrying out a cross-fostering experiment. Unfortunately, poor breeding success resulted in only 8 broods from a total of 61 radio-tagged hens survived to 5 days of age. Whilst hens were being radio-tracked throughout the breeding season, every effort was made to minimise the disturbance to the hen and the nest and poor breeding success affected the whole moor in 2002. Breeding success on the control area of the moor was only 1.2 chicks per hen (pers. comm. F. Mougeot), which is below values observed in other studies of 1.8 chicks per hen (Hudson & Dobson, 1997).

There was a significant negative effect of female's *T. tenuis* intensity on her overall breeding success. The average worm burden for females at Moorhouse was estimated to be approximately 2700, ranging from 2049 to 5292 worms. A decline in body condition has been shown in adult grouse with more than 4000 worms (Hudson, 1986b) however many of the females sampled for parasites during early spring may have been harbouring high numbers of arrested infective larvae which have been shown to be more pathogenic than adult worms (Shaw & Moss, 1990; Delahay *et al.*, 1995). Other evidence also suggests that the poor breeding success observed was due to relatively high intensities of *T. tenuis* infection across the population, commonly referred to as an outbreak of "grouse disease" (Lovat, 1911). Not only were 8 radio-collared females found dead with clinical symptoms of grouse suffering from the pathophysiological effects of a *T.tenuis* infection (Hudson *et al.*, 1997), throughout the course of this experiment, another 11 dead untagged birds with similar symptoms were found by chance. Furthermore, the breeding success of 1.2 chicks per hen observed on the 1 Km^2 control area of the moor was in contrast to 3.1 chicks per hen on a 1 Km^2 treatment area of the moor where all birds had been caught and experimentally dosed with anthelmintic to remove parasites in the spring of 2002.

Parasites may play an important role in host life history by impacting on host reproductive effort (Minchella, 1985). A reduction in reproductive effort may be either as a result of the host reducing the negative impacts of parasitism or may be due to parasites impacting negatively on host physiology (Møller, 1997). Previous studies have demonstrated that *T. tenuis* can have a detrimental effect on female host reproduction. Hens treated with levimisole (an anthelmintic) to reduce worm burden produced more young; through improved clutch size and chick survival (Hudson, 1986a) and in the same study, heavily infected hens were shown to leave the nest more frequently during incubation than treated hens resulting in greater vulnerability of these nests to predation.

In conclusion, the poor breeding success on Moorhouse in 2002, was indicative of the start of a population crash. Grouse population are known to follow regular cyclic fluctuations in density, and moors in the north of England have been shown to peak on average every 4.8 years (Hudson, 1986b). Although data on the densities of grouse on Moorhouse are unavailable for 2001 when counts were prohibited due to the Foot and Mouth epidemic, there was an estimated density of 80 birds per km2 for 2001 and densities of grouse in the spring of 2002 on Moorhouse reached 193 per square kilometre. The following year, spring densities had fallen to 40 birds per km^2 across the moor (pers. comm. F. Mougeot).

To my knowledge this study documents the first attempt at cross-fostering red grouse chicks and has shown, in principle, that the partial cross fostering of grouse chicks is possible. Although the question of whether resistance to parasite infection may be a heritable component remains unanswered, the experimental methodology to go about thoroughly testing this question has been developed as a result of this study, and it is hoped that future attempts to examine this question will prove successful.

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Chapter 5

Ornamental combs as indicators of individual quality and assortative mating in red grouse

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Ornamental combs as indicators of individual quality and assortative mating in red grouse

5.1 Abstract

Studies of the function of elaborate animal ornaments have largely focussed on the males of sexually dimorphic species. In contrast, in spite of the fact that many females also exhibit ornaments, comparatively little empirical information exists on the relationship between ornament size and individual quality in females. Using red grouse (*Lagopus lagopus scoticus*), a species in which both males and females display ornamental supra-orbital combs, I first investigated whether the size of this main ornament may advertise aspects of individual quality, such as parasite resistance or a better body condition, in both sexes. Secondly, I looked for evidence of non-random assortative mating in pairs of red grouse, with respect to age, ornament size, body size, body condition and measures of parasite infection. Thirdly, I explored whether certain phenotypic traits could be used to predict breeding success in either of the sexes, in order to investigate the possibility of direct benefits from mate choice based on these traits. Ornamental combs in both males and females were identified as potential signals of individual quality, reflecting a better body condition, and their relevance as sexually selected traits is discussed. Adult red grouse were found to mate assortatively with respect to body condition and body size. Overall, breeding performance during the year of study was poor. The probability that a female would succeed in hatching a clutch was significantly greater for larger females with bigger combs and in better condition in early spring. In light of the findings, I discuss the possibility and potential benefits of mutual mate choice in red grouse.

5.2 Introduction

An important determinant of an individual's fitness is its choice of mate (Burley, 1981). Within a breeding population, individuals vary in qualities, leading to differential survival and reproduction. For the majority of animal species, an individual's choice of mate is rarely random (Bateson, 1983; Real, 1990). The fitness benefits of mating with an individual of high quality may be both direct, leading to short-term material benefits such as avoidance of contagious pathogens, territorial resources or parental care (reviewed in Halliday, 1983; Møller, 1994; Kirkpatrick & Ryan, 1991), or may be indirect, whereby some advantage is accrued through increased attractiveness or viability of offspring (Fisher, 1930; Zahavi, 1975; Hamilton & Zuk, 1982; Møller, 1994).

So, how do individuals choose a mate? It is now widely established that ornamental traits, as expressed in males, are a product of sexual selection, and have evolved through female mate preference and male competitive advantage (Darwin, 1871). Models of sexual selection argue that ornamental traits in males function as cues of male quality (Zahavi, 1975; Hamilton and Zuk, 1982; Andersson, 1994). Females may thus be able to assess the variation in male qualities and discriminate between potential mates, by basing their preference on the size or brightness of sexual ornaments or intensity of sexual displays (Andersson, 1994). One hypothesis regarding the role of these ornaments, is that they may function as indicators of health and resistance to parasites. By choosing a healthy male, a female may accrue indirect advantages by acquiring for her offspring resistance important against prevailing parasites (Hamilton and Zuk, 1982). Alternatively, a female may also gain direct fitness benefits through decreased risk of exposure to contagious parasites (the "parasite avoidance" hypothesis) (Borgia, 1986; Borgia & Collis, 1989).

When it comes to choosing a mate, females are generally the sex that chooses as they produce relatively few nutrient-rich energy-costly eggs compared to males who produce an enormous number of low-investment sperms (Halliday, 1983). Females therefore tend to be selective of males, as they as they want their few progeny to be of the highest quality (Bateman, 1948; Manning, 1985). However, when sexes contribute to parental investment or where the mating system is monogamous and males mate only once, both are predicted to show selectivity in mate choice and mutual mate preferences may occur (Burley, 1977; Trivers, 1972; Parker, 1983).

Whilst males of many animal species display elaborate ornaments, females often show reduced expression of the same ornaments as males. Two main hypotheses have been proposed to explain female ornamentation (reviewed in Amundsen, 2000). The "correlated response" hypothesis suggests that female ornaments are themselves selectively neutral or even detrimental, but have occurred through non-adaptive genetic correlation between the sexes (Lande, 1980; Lande & Arnold, 1985) and several studies have demonstrated support for this hypothesis (Muma & Weatherhead, 1989; Hill, 1993; Cuervo *et al.*, 1996; Tella *et al.,* 1997). The "direct selection" hypothesis first proposed by Darwin (1871), suggests that the processes driving sexual selection for male ornaments i.e. intra-sexual competition and mate preference may also be acting upon female traits. Where resources such as territories or mates are limited, female ornaments may function in female-female contests to signal social dominance (West-Eberhard, 1983; Johnstone & Norris, 1993; Johnstone *et al.*, 1996). Female ornaments may also function in mate choice by males and in a number of bird species, males have been shown to choose females on the basis of their ornaments (Jones & Hunter, 1993,1999; Møller, 1993; Amundsen *et al.*, 1997; Roulin, 1999). It is thought that these ornaments may advertise reproductive or genetic quality and hence may accrue fitness benefits to choosy males (Jones and Montgomerie, 1992; Møller, 1993; Johnsen *et al.*, 1996; Potti and Merino, 1996; Amundsen *et al.,* 1997, Linville *et al.,* 1998; Roulin *et al.*, 1998; Roulin *et al.*, 2000).

Since both male and female secondary sexual characteristics may be simultaneously subjected to inter-sexual selection, similar preferences among individuals of the two sexes may lead to a tendency for females and males with the most elaborate secondary sex traits to pair with each other, resulting in positive assortative mating (Bortolotti & Iko, 1992; Møller, 1993, Wiebe, 2000; Forero *et al.*, 2001). Assortative mating describes a non-random population pattern of mating in relation to phenotypic characteristics and may be positive or negative (Burley, 1983). Positive assortative mating is the most widely described mating pattern and occurs when individuals of a similar phenotype mate with each other more often than expected by chance (Burley, 1983). Assortative mating has been described in a number of bird species with respect to age (Coulson *et al.*, 1981; Reid, 1988; Cézilly *et al.*, 1997; Jouventin *et al.*, 1999), body or morphological size (Boag & Grant, 1978; Cooke & Davies, 1983; Masello & Quillfeldt, 2003), plumage (O' Donald, 1983) and ornaments (Jones & Hunter, 1993, 1999; Daunt *et al.,*2000).

In this study, I examined phenotypic trait variation, including sexual ornament size, in pairs of red grouse (*Lagopus lagopus scoticus*), a socially monogamous species, in which both sexes participate in guarding and protecting their precocial young. Red grouse exhibit only slight sexual size dimorphism in morphology, males being c. 5% larger than females, and both sexes possess red supra-orbital combs. The combs of male red grouse are larger and redder than those of females (Cramp & Simmons, 1980). Combs may be partially or completely hidden below the feathers during feeding, resting or preening activities, but are erected and shown during periods of sexual and agonistic activity (Watson & Jenkins, 1964). In male red grouse, it is well established that comb size plays a major role in intra-sexual competition: males with bigger combs are more aggressive, hold bigger territories and are more likely to establish a territory in autumn (Moss *et al.*, 1979; MacColl *et al.*, 2000).

Although it is unclear for red grouse whether male combs function in mate choice, a link between comb size and mating success has been demonstrated in a number of other, closely related grouse and gallinaceous species (Ligon *et al.*, 1990; Zuk *et al.*, 1990; Brodsky, 1988; Hannon & Eason, 1995; Hanon & Dobush, 1997; Rintamaki *et al,* 2000). In contrast to numerous studies investigating the role of ornamental combs in males, the function of female combs in red grouse has not been investigated. Female red grouse can also erect their combs and appear to show them off in inter-sexual head-wagging displays, as well as during hostile encounters with other females associated with territorial defence (Watson & Jenkins, 1964). Female combs might therefore function in sexual selection in a similar way to male ornamental combs. Therefore, we hypothesise that rather than being a genetically-linked redundant feature, female combs in red grouse may function in sexual selection as indicators of quality.

Using data collected from 114 pairs of grouse caught in the spring, the first objective of this study, was to investigate which aspect of quality underline comb size in male and in female red grouse. Given that sexual ornament size is expected to be condition dependent, I analysed comb size variation in both sexes in relation to age, phenotypic quality (size, condition) and parasite intensities, and predicted that individuals with the biggest combs would be those in better condition and possibly also those with fewer parasites. Second, I looked at the level of variation in combs size in males and in females to examine what factors may influence comb size, and to look for evidence that ornamental combs in female red grouse may be sexually selected traits, as opposed to being selectively neutral. The third objective was to test the prediction that red grouse will exhibit some form of mutual mate preference and will mate non-randomly with respect to age or to some other phenotypic trait, such as comb size or condition. Finally, I investigated whether the quality of pairs was correlated with breeding success, to test the prediction that if assortative mating occurs, then individuals should benefit from pairing with a mate of high quality.

5.3 Methods

Study Areas & Capture

Data on pairs of grouse were collected in 2002 from five grouse populations, two located in northern England (Catterick and Moorhouse) and three in northeast Scotland (Edinglassie, Invercauld and Invermark). Pairs of male and female adult grouse (N=114) were captured at night during spring (from 28th February to 2nd May) using standard lamping techniques (Hudson & Newborn, 1995). Paired grouse roost together in close proximity and, where possible, both the male and female were caught together on the same evening. Where only one of a pair was caught, a necklace-type radio-collar was fitted to the bird and that individual was relocated on a subsequent night in an attempt to capture the second individual. All individual birds were marked either with a numbered metal leg ring or with a numbered metal patagial wing tag

Biometric & Parasite Data Collection

Plumage and morphological characteristics were used to sex and age birds (Cramp & Simmons, 1980). Birds hatched the previous summer, were classed as "young" and those greater than 1 year old were classed as "old", and four males were not aged in error. For full details on the sexing and aging of birds, as well as all of the measurements, see Chapter 2. For each individual caught, the following data were recorded: date of capture (recorded as Julian date), weight (to the nearest 1g), wing length (to the nearest 1 mm), tarsus (length of tarsometatarsus to the nearest 0.1mm) and comb size (length and height of the comb were measured to the nearest 1mm and multiplied to give an index of comb size).

The parasites: Trichostrongylus tenuis and Coccidia

For a detailed description of the life-cycle of both parasites refer to Chapter 2. A main parasite of red grouse is the gastrointestinal nematode *Trichostrongylus tenuis* which inhabits the caeca of the red grouse but red grouse are also frequently infected by coccidia, single celled parasites that also infects the intestine. Faecal samples were collected for parasite sampling by placing captured birds in individual holding boxes overnight. The following morning, birds were released and their caecal faeces containing the parasite eggs and oocytes collected and taken to the lab for examination.

Laboratory analysis of T. tenuis egg and coccidia oocyte intensity.

The intensity of *T. tenuis* eggs per gram (EPG) and coccidia oocytes per gram (OPG) of caecal faeces were estimated determined from approximately 0.2g of faecal material using the modified McMaster egg counting technique (MAFF, 1986). For a detailed description of this method, refer to Chapter 3. Previous studies on *T. tenuis* faecal egg counts in red grouse indicate that there is a strong positive correlation between *T. tenuis* worm burden and EPG (Moss *et al.,*1990; Seivwright *et al.*, 2004). Worm burdens were calculated from average eggs per gram counts (Seivwright *et al.,* 2004). Indices of *T. tenuis* and coccidia infection intensities, were calculated as the residuals of count data after controlling for sampling date, study moor and age as fixed effects in a generalized linear model (SAS GENMOD procedures), with a negative binomial distribution and a log link function and were fitted with $\log_{10}$ faecal sample quantity as an offset.

Breeding success in relation to phenotypic traits

Breeding success was determined for 53 pairs captured at night during early spring on the Moorhouse study site. Individuals were sampled for parasites using the techniques outlined above and the females fitted with a necklace-type radio-tag to allow future relocation. Radio-collared females were relocated frequently during April and May until their nests were found, in order to determine the outcome of their breeding attempt (for details see Chapter 2). After a minimum of 5 eggs had been laid, egg measurements (length, breadth and weight) were taken and used to predict an average hatch date for the nest using a nomogram (Smith, unpublished data).

Statistical analyses

Derivation of residual measurements

For body condition, I used an index, calculated as the residuals of linear regression of body mass on wing length (used as an indicator of body size (Brittas & Marcström, 1982) for each sex separately. These data were derived using a General Linear Mixed Model (SAS PROC MIXED procedures) with study moor and a study moor*wing interaction as random effects. Hence this index is a measure of relative body mass controlling for differences in structural body mass, which may vary between study moors, as indicated by wing length. Other phenotypic indices were also calculated using residuals; comb area index, condition index and size index (tarsus length). Residuals were calculated after controlling for the effects of date of sampling, study moor, measurer and age included as fixed effects in a General Linear Model (SAS GLM procedures). All residuals were produced for each sex separately, as males are larger than females (Cramp & Simmons, 1980). Measurer was also included as a fixed effect, as data were collected by four different observers. Coefficients of variation (CV) for comb size were derived to allow the extent of variation in this trait to be compared with the variation of morphometric traits under sexual selection in other avian species. In order to control for the observer effect in measuring comb size, data from only one observer (80% of comb measurements) were used to calculate the CV.

Relationships between comb, condition and parasites

To explore what measures of quality might be advertised by ornamental combs in male and female red grouse, I examined the relationship between comb size, body condition, body size (tarsus length) and parasites. I controlled for any additional variation that might be present due to the influence of moor, sampling date and age by analyzing the data using General Linear Mixed Models with study site and measurer as random effects and controlling for age and sex as fixed effects, where necessary (SAS PROC MIXED procedures). The models were constructed using a backward step-wise selection procedure, starting with all the terms in the model, and dropping the least significant term in the model until only terms significant at the 5% level remained, using SAS type III tests (SAS, 2001).

Examining the evidence for assortative mating

To explore whether grouse mate assortatively according to age, I used data only from Moorhouse (the greatest sample size of 53 pairs). I tested whether the frequency with which old birds mate with old and young birds with young differed from that expected from random pairing using chi-square analysis. Evidence for assortative mating based on phenotypic or parasite variables was examined by exploring the relationship between phenotypic and parasite indices of the male and female of a pair using correlation analyses of data from all sites. In addition, Principle Component Analyses (PCA) scores were also computed to simultaneously explore variation in multiple traits between individuals in order to investigate which phenotypic traits provided the greatest contribution to variation between individuals. PCA were performed on males and females using residual values for four biometric traits; comb size, condition, tarsus and wing lengths, and one measures of parasite intensity (*T.tenuis*). Individual scores for PC1 and PC2 were stored and used to investigate whether there was a correlation between individual scores within pairs.

Relationship between breeding attempt outcome and phenotypic traits

Due to a population crash at Moorhouse in 2002, data on the reproductive success of individuals was particularly low. By the end of the breeding season, 16 pairs had failed to lay eggs, 21 pairs had laid eggs but failed to hatch chicks, and only 16 pairs successfully hatched chicks. As reproductive success may be influenced by hatch date, which in turn may be related to the condition or age of a female, I began by investigating the relationship between predicted hatch date and a female's age or condition. Data were analysed in a General Linear Model with female age, and capture date and condition index as fixed effects (SAS GLM procedures).

I then tested whether breeding attempt outcome could be predicted either from an individual's age or from phenotypic traits measured in the spring. Data were analyzed using Generalised Linear Models with breeding attempt outcome determined as overall breeding success (hens that produced chicks), laying success (eggs produced versus no eggs produced) or hatching success (pairs laying eggs hatching versus pairs laying failing to hatch), all as binary measures, analyzed with a binomial error structure and a logit link function (SAS GENMOD procedures). Breeding attempt outcome was examined initially with respect to individual age, and then with respect to comb size, condition index and tarsus (after controlling for age and capture date); included as fixed effects in the model.

Statistical analyses were conducted using Minitab (version 13) and SAS (version 8.01, SAS, 2001).

5.4 Results

Male comb size, condition, size and parasites

There was considerable variation in male comb size in spring, the largest combs being twice the size of the smallest (Figure 5.1; mean comb size of 354.88 ± 52.83 mm^2, range 240-480mm^2, N = 112, CV = 14.7%). Comb size variation was not explained by sampling date, wing length or coccidia intensity (Table 5.1). Variation in comb size was, however, significantly explained by age (older birds had smaller combs than younger birds; Table 5.1), condition index (males in better condition had bigger combs; Table 5.1, Figure 5.2) and *T.tenuis* intensity (males with the biggest combs had more parasites; Table 5.1). There was also a significant interaction between condition index and age, indicating that the relationship between comb size and condition index differed between young and old birds (Table 5.1). In order to clarify this interaction, I considered old and young males separately. Comb size variation was significantly explained by condition index in young males, but not in old males (Figure 5.2). Thus, male red grouse with bigger combs appeared to be those in better condition, but not necessarily those with fewer parasites. A better condition may underline fewer parasites. However, condition index was not significantly explained by *T.tenuis* intensity ($F_{1,63}$ = 0.02, P = 0.890) or coccidia intensity ($F_{1,63}$ = 0.07, P = 0.80). Male condition was also not related to sampling date ($F_{1,67}$ = 1.82, P = 0.182) or age ($F_{1,63}$ = 0.58, P= 0.45).

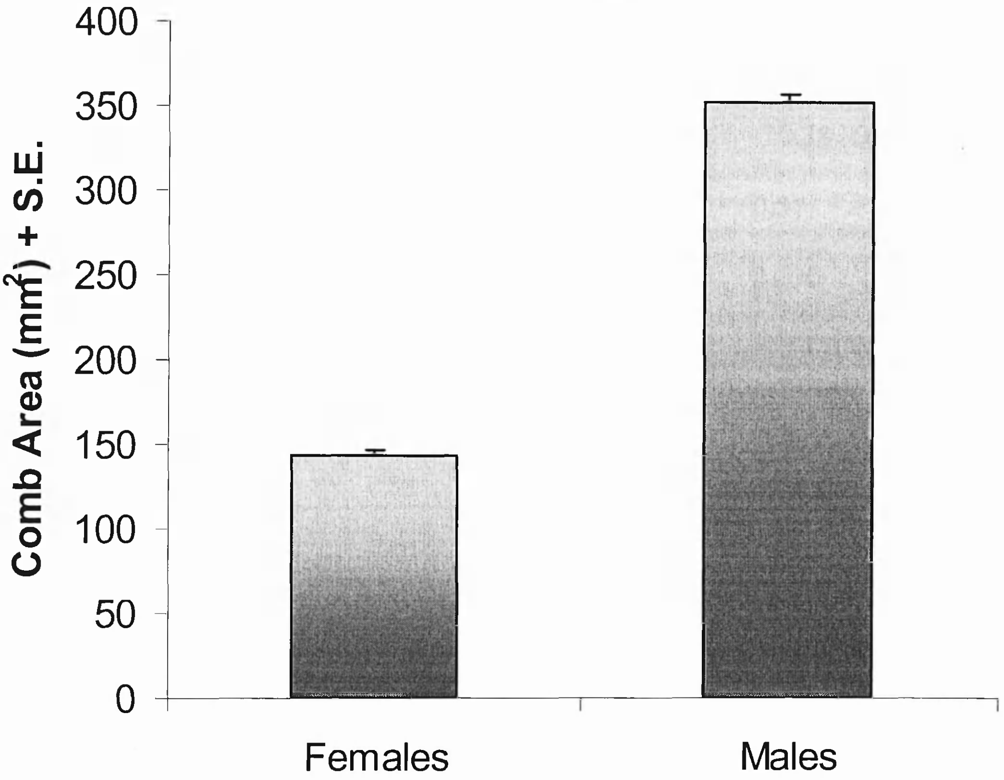

Figure 5.1: Arithmetic mean (+1 S.E.) for females (*N* = 99) and males (*N* = 112) comb size (mm^2). After controlling for sampling date, study moor, age and measurer effects, males had significantly larger combs than females (GLM; $F_{1,200} = 1290.38$, $P < 0.0001$)

Variable	**Num DF**	**Den DF**	**F Value**	**Estimate**	**Pr > F**
Age	1	59	4.38	-20.38 (Old)	0.041
Condition	1	59	5.41	0.54	0.023
T. tenuis	1	59	4.00	21.30	0.050
Condition*Age	1	59	5.46	-0.54 (Old)	0.023
Non-significant terms					
Date	1	58	2.49	0.27	0.460
Age**T. tenuis*	1	57	0.51	12.49 (Old)	0.476
Tarsus	1	53	0.16	1.70	0.689
Coccidia	1	52	0.02	-1.08	0.876

Table 5.1: Results of the GLMM for male comb size. GLMM included site and measurer as random effects. F values and probabilities for non-significant terms are those obtained when the terms were added alone to the model containing all significant terms.

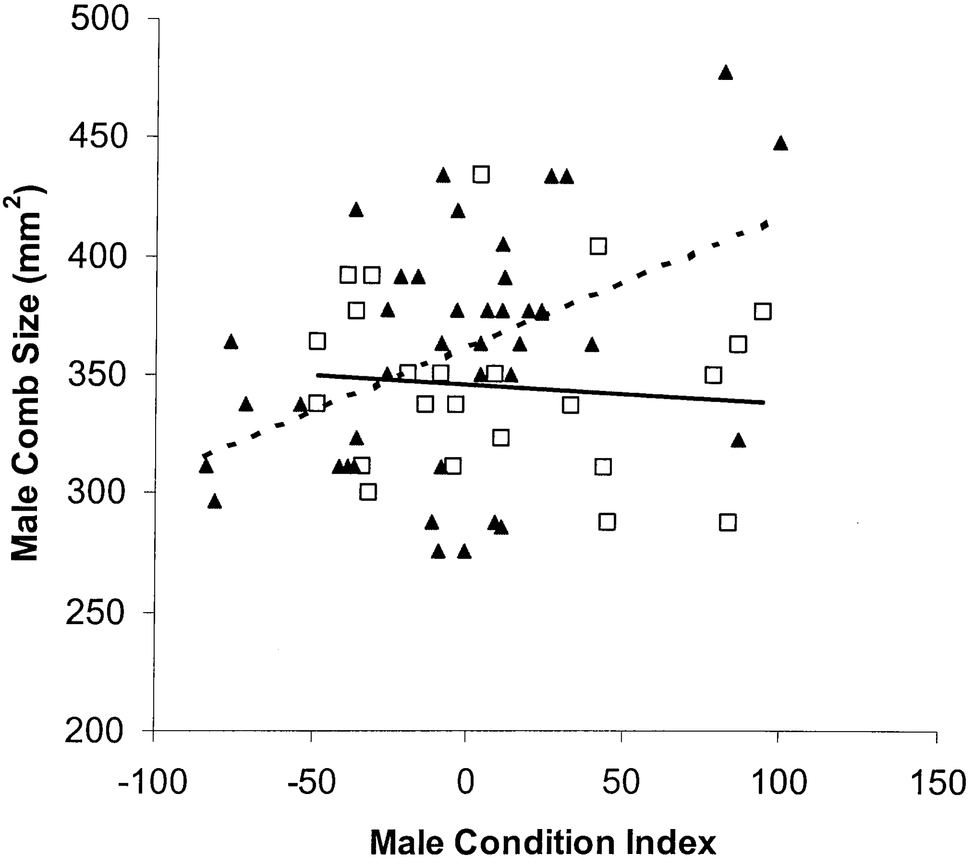

Figure 5.2: Relationship between condition index and comb size in males. Symbols represent young males (▲) and old males (☐). Condition index significantly explained comb size variation in young males ($F_{1,36}$ = 9.65, $P < 0.01$), but not however, in old males ($F_{1,27}$ = 0.14, $P = 0.71$). The solid trend line is a simple linear regression through the points for old males and the broken trend line that for young males.

Female comb size, condition, size and parasites

Comb size of females sampled in spring also varied two-fold and had a CV = 19.4 % (Figure 5.1; mean 143.43 ± 27.83 mm^2, range 84 - 210mm^2, $N = 99$). Comb size variation was not explained by sampling date, wing length, *T. tenuis* or coccidia intensity (Table 5.2). However, comb size variation was significantly explained by age (older females had larger combs than young females; Table 5.2), and condition index (female grouse with bigger combs were in better condition; Table 5.2, Figure 5.3; this was true for both young and old females: non-significant age*condition interaction).

Female condition index increased during spring ($F_{1,67} = 7.68$, $P < 0.01$) and differed between age groups, older females being in better condition than young females ($F_{1,67} = 6.98$, $P = 0.01$). However, female condition was not influenced by intensities of either parasite (*T.tenuis*: $F_{1,66} = 1.91$, $P = 0.172$; coccidia; $F_{1,66} = 0.27$, $P = 0.607$).

Variable	**Num DF**	**Den DF**	**F Value**	**Estimate**	**Pr > F**
Age	1	62	6.39	15.9 (Old)	0.014
Condition	1	62	8.17	0.18	0.006
Non-significant terms					
Age*Condition	1	61	2.57	0.27	0.114
Coccidia	1	60	1.67	-6.71	0.201
Date	1	59	2.79	-0.36	0.100
T. tenuis	1	58	0.96	-5.98	0.330
Age**T.tenuis*	1	57	0.01	-1.92 (Old)	0.905
Tarsus	1	56	0.06	0.54	0.807

Table 5.2: Results of the GLMM for female comb size. GLMM included site and measurer as random effects. F values and probabilities for non-significant terms are those obtained when the terms were added alone to the model containing all the significant terms.

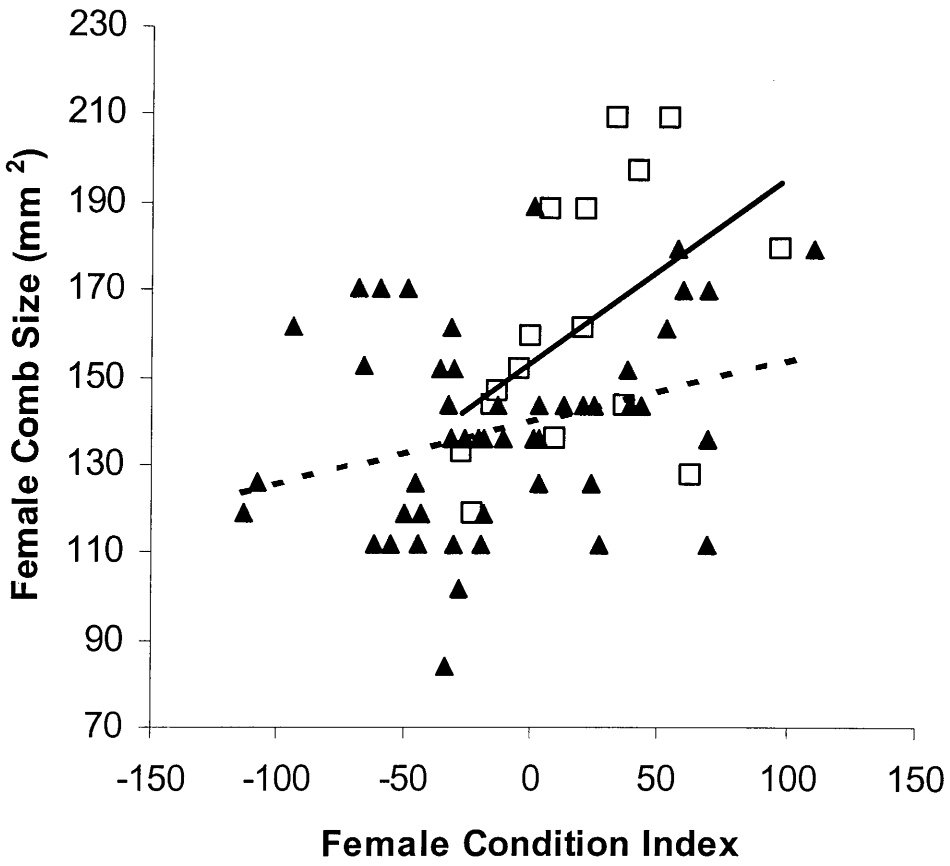

Figure 5.3: Relationship between female condition index and female comb size. Symbols represent young females (▲) and old females (□). Condition index significantly explained comb size variation in young ($F_{1,49}$ = 4.88, P < 0.05) and old ($F_{1,17}$ = 6.18, P < 0.05). The solid trend line is a simple linear regression through the points for old females and the broken trend line that for young females.

Assortative mating

Assortative mating in relation to age.

In 109 pairs of grouse, 12 old males were paired with an old female, 25 old males paired with a young female; 17 young males paired with an old female and, 55 young males were paired with young females. Using pairs from the Moorhouse study site only where sample size was greatest (N = 53 pairs), I compared the observed frequency of pairing age-combinations with that expected if individuals mate randomly in relation to age (according to the potential number of young and old mates available in the population). A chi-square test for goodness of fit test showed that the observed frequencies by which old and young birds select mates were not significantly different from those expected by random mating according to age ($\chi^2 = 0.199$, 3 d.f., $P = 0.978$) (Figure 5.4). I thus had no evidence of assortative mating according to age.

Assortative mating in relation to comb size, body condition, body size and parasite infection.

I used correlation analyses between residuals of male and female traits of pairs to further look for evidence of assortative mating. Female condition index was significantly positively correlated with male condition index (*Pearson correlation,* $r_p = 0.38$, $n = 69$, $P = 0.001$) and male tarsus was significantly positively correlated with female tarsus ($r_p = 0.30$, $N = 69$, $P < 0.05$). There was no relationship between male and female comb size ($r_p = 0.14$, $N = 98$, $P = 0.174$), and as found previously (Chapter 4), no relationship between the parasite intensities of paired males and female ($r_p = 0.19$, $N = 73$, $P = 0.102$). There was however, a significant positive relationship between female condition index and male comb size ($r_p = 0.23$, $N = 72$, $P = 0.047$).

I further conducted a PCA analysis using residual measures of: a sexual characteristic (comb size), condition (condition index), size (tarsus) and parasite intensity (*T. tenuis)*. This analysis was conducted for each sex separately, but produced similar results in males and females with regards to the first principle component axis (Table 5.3). The first PCA axis represented 42.2% of the variance in females and 41.0% of the variance in males. For both males and females the first axes were heavily influenced by tarsus, condition and comb. Analysis of the relationship between male and female scores generated from PC1 & PC2 showed that the PC1 scores of paired males and females were significantly positively correlated (Figure 5.5: Pearson correlation, $r_p = 0.344$, N = 63, P = 0.006) but PC2 scores were not correlated ($r_p = 0.144$, N = 63, P = 0.265). These results suggest that red grouse may pair assortatively according to condition and size and provide further evidence for a positive, significant relationship between comb size and condition in both males and females.

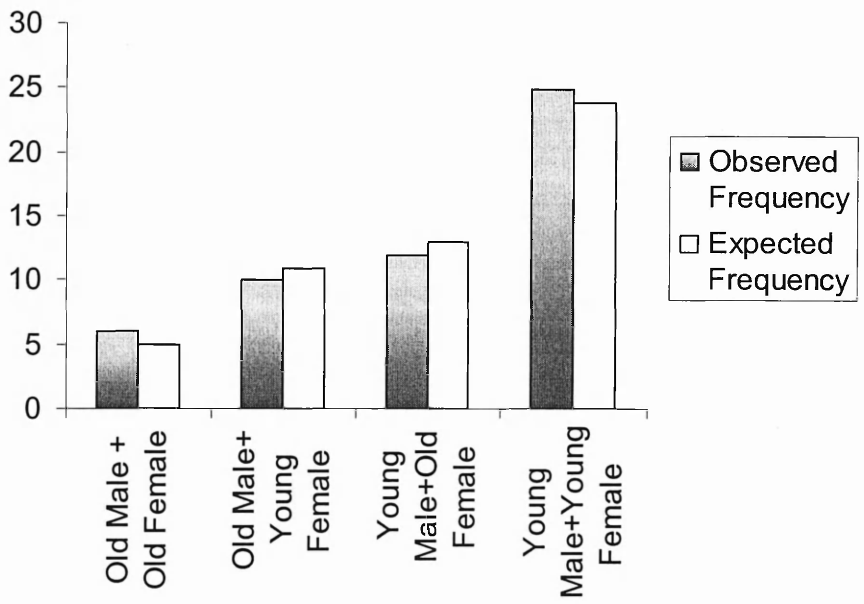

Figure 5.4: Expected and observed frequency of pairing between old and young males and females at Moorhouse.

Sex	**Variable**	**PCA 1**	**PCA 2**
Females	Comb Size	0.477	-0.394
	Condition Index	0.596	-0.285
	Tarsus	0.572	0.173
	T. tenuis	-0.302	-0.857
	% variance	41.0%	27.0%
Males	Comb Size	0.533	-0.352
	Condition Index	0.652	0.065
	Tarsus	0.537	0.357
	T. tenuis	0.054	-0.863
	% variance	42.2%	24.5%

Table 5.3: Weightings of eigenvectors by variable on PCA axes 1 and 2 for males and for females.

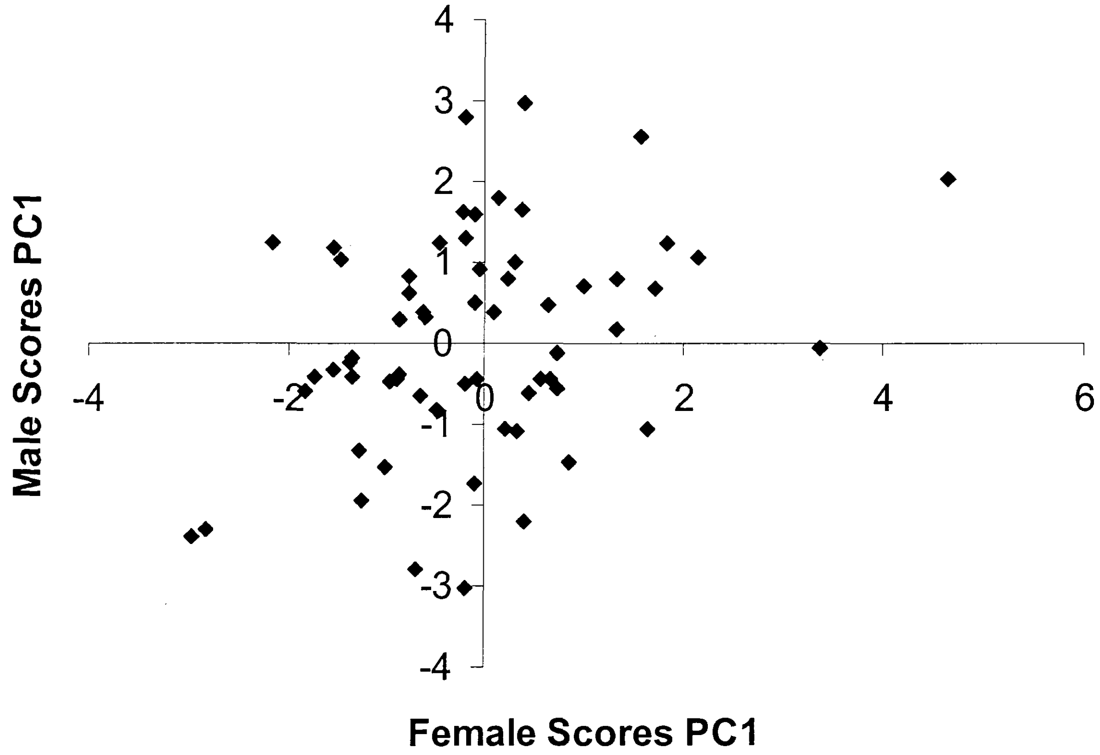

Figure 5.5: Relationship between paired male and female PC1 scores for PCA analysis of comb size, condition index, tarsus and *T. tenuis* intensity.

Breeding success in relation to residual condition index, comb size and tarsus length

Of the 53 pairs on Moorhouse followed throughout the breeding season, only 16 paired females produced chicks (30%; N = 16). For the 16 paired females that failed to lay eggs (30%; N = 16), the probability of laying a clutch was not related to female age or body condition index, residual comb size or residual tarsus length (after controlling for age and date of capture) (Table 5.4). Nor was it related to any of these same variables in males (Table 5.5).

For the 37 pairs (70%) that laid a clutch, I firstly examined whether there was an effect of female or male age or condition on the predicted hatch date for the clutch. Predicted hatch date was marginally significantly explained by female age and was significantly related to male age (Figure 5.6). Older males and females were predicted to hatch later than young males and females. After controlling for age, there was no relationship between female ($F_{1,24} = 0.05$, P = 0.826) or male ($F_{1,23} = 1.24$, P = 0.277) residual condition index and predicted hatch date

I then examined what factors might affect the probability of a clutch hatching (29.6% of clutches successfully hatched; N = 16 pairs). The probability that a clutch of eggs would successfully hatch was significantly negatively related to predicted hatch date (GENMOD; $\chi^2 = 3.97$, 1 d.f., $P < 0.05$), indicating that earlier laid clutches were more likely to hatch. Hatching probability differed between age groups, with younger females having a higher probability of hatching (Table 5.4). As hatch date differed between age groups, I controlled for only one of these terms (age), in subsequent analyses examining whether comb size, condition index or tarsus could predict hatching success. After controlling for both age and date of capture, the probability of a clutch hatching was significantly positively related to female comb size and condition index and female tarsus length (Table 5.4). Hatching probability was not significantly explained by male age, male condition index, male comb size or male tarsus length (Table 5.5).

The probability of a pair breeding successfully was marginally significantly related to female age (Table 5.4), with younger females being more likely to produce chicks and was also significantly positively related to female condition, comb size and marginally significantly positively related to body size (Table 5.4). Overall breeding success of pairs was not related to any of these same variables in males (Table 5.5).

		Probability of Breeding Outcome		
Breeding outcome	**Trait**	**df**	**X^2 Value**	**P**
Breeding Success	*Age*	*1*	*3.12*	*0.078*
Laying Success	*Age*	*1*	*1.08*	*0.298*
Hatching Probability	*Age*	*1*	*5.51*	***0.019***
Breeding Success	*Condition Index*	*1*	*4.26*	***0.039***
(after controlling for	*Comb Size*	*1*	*4.65*	***0.031***
age & capture date).	*Tarsus Length*	*1*	*3.50*	*0.061*
Laying Success	*Condition Index*	*1*	*0.16*	*0.485*
(after controlling for	*Comb Size*	*1*	*0.49*	*0.255*
age & capture date)	*Tarsus Length*	*1*	*0.00*	*0.957*
Hatching Probability	*Condition Index*	*1*	*4.47*	***0.034***
(after controlling for	*Comb Size*	*1*	*4.01*	***0.045***
age & capture date)	*Tarsus Length*	*1*	*7.10*	***0.008***

Table 5.4: Effects of female age and phenotypic traits: condition index, comb size and tarsus length (after controlling for age and capture date) on the probability of a pair producing chicks (breeding success), probability of laying a clutch, and the probability of clutch laid, hatching.

		Probability of Breeding Outcome		
Breeding outcome	**Trait**	**df**	**X^2 Value**	**P**
Breeding success	*Age*	*1*	*0.64*	*0.424*
Laying success	*Age*	*1*	*1.26*	*0.263*
Hatching success	*Age*	*1*	*0.11*	*0.739*
Breeding success	*Condition index*	*1*	*0.36*	*0.557*
(after controlling for	*Comb size*	*1*	*0.02*	*0.895*
age & capture date)	*Tarsus length*	*1*	*0.52*	*0.469*
Laying success	*Condition index*	*1*	*0.83*	*0.363*
(after controlling for	*Comb size*	*1*	*0.81*	*0.367*
age & capture date)	*Tarsus length*	*1*	*0.03*	*0.872*
Hatching probability	*Condition index*	*1*	*0.07*	*0.790*
(after controlling for	*Comb size*	*1*	*0.07*	*0.785*
age & capture date)	*Tarsus length*	*1*	*0.99*	*0.319*

Table 5.5: Effects of male age and phenotypic traits: condition index, comb size and tarsus length (after controlling for age and capture date) on the probability of a pair producing chicks (breeding success), probability of laying a clutch, and the probability of clutch laid, hatching.

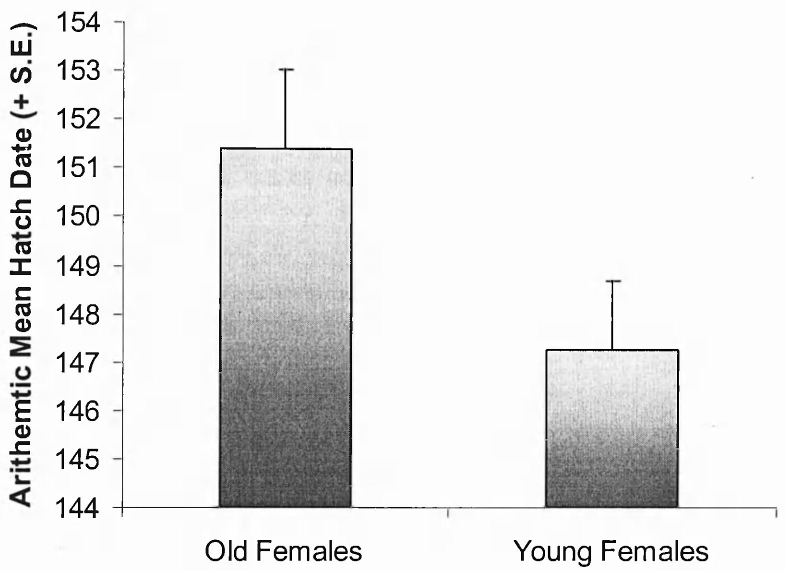

Figure 5.6: Arithmetic mean of average hatch date for old (N= 10) and young (N= 18) females. Predicted hatch date was significantly explained by male age (GLM: $F_{1,26} = 7.26$, $P < 0.05$) and marginally significantly explained by female age (GLM: $F_{1,26} = 3.26$, $P = 0.082$).

5.5 Discussion

Data from this study were consistent with the following predictions: 1) that individuals with the bigger combs would be those in better condition (although they were not those with fewer parasites), 2) that red grouse paired non-randomly with respect to phenotypic condition and 3) that males benefited from pairing with a female of high quality in terms of better breeding success. Data also supported the hypothesis that ornamental combs in female red grouse may function in sexual selection.

Ornamental combs as signals of individual quality

In this study, I found that the size of supra-orbital combs of red grouse provided an indicator an individual's phenotypic condition in young males and in both young and old females. A similar relationship between condition and comb size in red grouse males was also observed by Mougeot *et al.* (2004).

Models of sexual selection predict that as sexual ornaments are considered to be costly, they should covary positively with condition (Andersson, 1994; Anderson & Iwasa, 1996). Male and female grouse show off their combs in inter-sexual head-wagging sexual displays (Watson & Jenkins, 1964). Both male and female red grouse may thus be able to assess variation in current mate condition and discriminate between potential mates, by basing their preference on the size of their combs (Andersson, 1994; Anderson & Iwasa, 1996). Evidence that supra-orbital combs of males function in sexual selection comes from a number of closely related tetranoid species. In rock ptarmigan (*Lagopus mutus*), males use combs in both courtship and male-male interactions (Brodsky, 1988; Holder & Montogomerie, 1993) and male pairing success was positively correlated with comb size and comb condition (Brodsky, 1988; Holder &

Montogomerie, 1993, Bart & Earnst, 1999). Similarly, in Willow Ptarmigan (*L. lagopus*), males with the smallest combs had the lowest pairing success (Hannon & Eason, 1995; Hannon & Dobush, 1997) and Rintamäki *et al.* (2000) observed a positive relationship between comb size and copulatory success in lekking black grouse (*Tetao tetrix*) males, further suggesting that combs may provide sampling females honest cues to individual quality.

In female red grouse, however, no other studies have looked at the function of combs. Whilst female ornamental traits may occur through non-adaptive genetic correlation between the sexes (Lande, 1980; Lande & Arnold, 1985; Muma & Weatherhead, 1989), processes driving sexual selection for male ornaments i.e. intra-sexual competition and mate preference may also act directly upon female traits (West-Eberhard, 1983; Jones & Hunter, 1993,1999; Johnstone *et al.*, 1996; Roulin, 1999; Velando *et al.*, 2001). Results from this study, support the hypothesis that female comb size may be under sexual selection. Firstly, the extent of variation in the size of female combs (CV = 19.4%) was greater than that of male combs (CV = 14.7%). Secondly the CV for comb size for both males and females was similar to the variation in traits assumed to be sexually selected for in other species (mean CV = 11.7%, range 6.0 – 25.6%; Alatalo *et al.*, 1988). Experimental work is now required to explicitly test the sexual function hypothesis in female red grouse.

Ornamental combs and parasite infection

Hamilton & Zuk (1982) suggested that ornaments may function to reliably signal an individual's state of health, and in particular, their resistance to parasites. Contrary to this hypothesis, however, there was a significant positive relationship between comb size and *T. tenuis* infection in red grouse males. In their study, Mougeot *et al.* (2004), (also see Chapter 6), observed no relationship between male red grouse comb size and parasites, and correlative studies of secondary sexual traits and parasite loads in other species, have also been inconsistent, providing a mix of support and negative evidence for reliable signal theory (review in Møller *et al.*, 1999).

Thus, it seems unlikely that females are choosing males with bigger combs in order to avoid contamination of parasites. It may be however, that males with largest combs are advertising their ability to cope with parasites instead or that some other comb trait signal such as iridescence, in both the visible and ultraviolet (UV) spectra which were not measured in this study, may be important in signal parasite resistance (Mougeot, unpublished data). For ornaments to act as an honest signal of quality, their expression should be costly to prevent poor quality individuals from cheating ('Handicap principle'; Zahavi, 1975, 1977). One such cost may be greater conspicuousness and greater risk of predation (Andersson & Iwasa, 1996). Another cost, however, may be impaired immune function, the main defence against parasites. In male red grouse, comb size is testosterone dependent (Moss *et al.,* 1979) a hormone associated with immune suppression (Grossman, 1985, 1989; Folstad & Karter, 1992; Poulin, 1996). Red grouse males implanted with testosterone in the autumn have been found to show lower cell-mediated immunity (Mougeot *et al.*, 2004). Thus the positive relationship between red grouse comb size and *T. tenuis* infection intensity may represent a cost of elevated testosterone levels, through impaired immune function. In keeping with the "Immunocompetence" hypothesis (Folstad & Karter, 1992), male comb size may thus provide an honest signal to females, of a male's gene-based ability to cope with parasites. The relationship between testosterone and parasite infection is explored in further detail in Chapter 7.

Until recently, studies on the effect of testosterone in the expression of ornaments and on the possible immunosuppressant effects of androgens have focused on males. However, recent work on moorhens (*Gallinula chloropus*) demonstrated the involvement of testosterone in the expression of female ornaments (Eens *et al.*, 2000). Little is known of the role of androgens in female red grouse, although previous work found a positive correlation between female dominance and parasite burden in captive red grouse (Shaw, 1988). Further work is therefore required to examine the physiological mechanism and potential associated costs of comb expression in female red grouse.

Assortative mating in red grouse

In breeding pairs of red grouse sampled in the spring, we found as predicted, evidence for positive assortative mating according to body condition and according to size (tarsus length). Positive assortative mating occurs when individuals of similar phenotype mate more often than expected by chance (Burley, 1983) but inferring how assortative mating patterns may arise is generally difficult as different processes can lead to similar patterns (*equifinality*; Burley, 1983). In monogamous bird species with bi-parental care, assortative mating is thought to arise from adaptive mutual mate choice by males and females for characters linked to individual quality that will increase fitness of the other mate (Trivers, 1972; Andersson, 1994).

Positive assortative mating according to size traits and condition has been previously observed in a number of species (Coulter, 1986; Hedenström, 1987; Stern & Jarvis, 1990; Bortolotti & Iko, 1992; Heitmeyer, 1995; Olsen *et al.*, 1998; Rosenfield & Bielefeldt, 1999). Assortative mating according to size or condition may be a by-product of mating preferences based on age, where condition or size increases with age. Unlike a number of other bird studies in which age of mates influences mate choice (Marzluff & Balda, 1988; Cezilly *et al*, 1997; Reid, 1988, Jouventin *et al.*, 1999; Potti, 2000), this study found no evidence of age assortative mating in red grouse. This could be explained by low mate fidelity and/or high mortality rate (survival of grouse over winter varies with density but may be as low as 37%; Hudson, 1986). If a mate dies in spring, females will associate with another male on a nearby territory (Cramp & Simmons, 1980). Although normal pair bonds may last 2 or more years, if a male fails to gain or to hold a territory in the autumn, the pair bond will be broken.

It has been argued that patterns of non-random pairing based on body condition may be attributable not to mate choice but simply as a consequence of other external complementary factors such as territory quality (Rosenfield & Bielefeldt, 1999). Indeed, both male and female grouse forage almost entirely in the male's territory from January until the chicks have hatched (Watson & Miller, 1971). Males and females from the same pair might thus have a similar condition because of similar feeding opportunities on the shared territory. However, in my radio-tracking study, I also noticed that in early spring females readily shift between male territories. A previous study on red grouse reports similar observations (Moss *et al.,* 1987). Females may range across a number of territories until mate choice and pairing is definitive, which can be in late spring. It is likely therefore, that male and female foraging ranges differ. Moreover, although previous work showed that male territory size correlated both with the survival of cock grouse over winter and summer and the chance of acquiring a mate, there was no difference between territories in the overall productivity of the heather (*Calluna vulgaris*) (Watson & Miller, 1971; Miller & Watson, 1978). Although larger territories had more green shoots of heather overall, weight of green shoots per bird was approximately the same (Miller & Watson, 1978). Although it cannot be ruled out, it seems unlikely that condition in both males and females was related simply to territory quality.

In this study, although we did not find assortative mating with respect to ornaments size, results of PCA analysis for a number of characteristics including phenotypic traits and parasite intensities, revealed high positive weightings for condition index, tarsus length and comb size in the first component, for both males and females. There was also a significant positive correlation between ranked first component scores of paired males and females, i.e. high-ranking males were paired with high-ranking females. Thus, it is difficult to tease apart which of these cues may be used in mate choice. Indeed, in order to decrease the chances of choosing a poor quality mate, selection may favour individuals choosing mates on the basis of many characteristics that indicate fitness. In red jungle fowl (*Gallus gallus*), several male traits are important to females including tail length, hackle feather colour, comb length and colour and courtship display (Zuk *et al.,* 1992), whilst rock doves (*Columba livia*) use a combination of plumage characteristics age, experience and dominance to select mate (Burley, 1981).

Ornaments in females and potential benefits of mate choice

The potential benefits associated with mating preferences depend on the variation in quality among mating partners (Parker, 1983). I found female comb size and condition index to be a predictor of future breeding success and I found that the largest females with the largest combs and in better condition, were more likely to produce chicks and successfully hatch out eggs. Where differences in female quality relates to differences in fecundity, this is thought to lead to the evolution of male mating preferences (Andersson, 1994). Darwin (1871) proposed a specific hypothesis, which was later expanded by Fisher (1958), for monogamous birds, that males with the most exaggerated traits should receive a reproductive advantage through mating with the most fecund females. In this study I found a significant correlation between male comb size and female condition, indicating that red grouse males mating with the best quality females might benefit due from greater breeding success. This has also been observed in a number of other avian species including barn swallows (*Hirundo rustica*; Møller, 1991), blackbirds (*Turdus merula*; Faivre *et al.*, 2001), Inca terns (*Larosterna inca*; Velando *et al.*, 2001) and European shags (*Phalacrocorax aristotelis*; Daunt *et al.*, 2003).

Ornaments in males and potential benefits of mate choice

Whilst female red grouse "quality" may have influenced reproductive success, male red grouse "quality" did not. Consistent with observations in a study of willow ptarmigan (Hannon & Eason, 1995), there was no relationship between the male red grouse traits and breeding outcome measured in this study. It may have been, however, that male red grouse quality could have influenced some other aspect of breeding success that we did not measure. In burrowing parrots (*Cyanoliseus patagonus*), whilst adult female phenotypes influenced parameters of breeding success and parameters of nestling body condition, male phenotypes strongly influenced structural characters of the nestlings such as bill, tarsus and wing length (Masello & Quillfeldt, 2003). On the other hand, combs may function in males to indicate some other measure of quality such as territory size or quality (Moss *et al.*, 1987), or ability to cope with parasites (Mougeot *et al.*, 2004; also see Chapter 6)

Overall these results indicate that ornament size and phenotypic quality are linked in both sexes in red grouse and results suggest that comb size may have an important signalling function not just in males, but also in females. However, as I did not specifically test whether female combs are used as signals, I cannot refute the correlated response hypothesis. I also concentrated solely on measures of comb size in this study, and it may be that other qualities of combs such as colour and ultra-violet reflectance may also be important. This study also documents for the first time, non-random assortative mating in pairs of red grouse: male and female red grouse mated assortatively with respect to condition and body size. Furthermore, results also suggest that a potential benefit of mate choice in red grouse may be improved breeding success, as females in better condition and with bigger combs, have better breeding success. Experimental studies are now required to test the role that red grouse ornaments play in mate choice and intra-sexual interactions and in signalling parental quality, particularly with respect to females.

5.6 References

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Chapter 6

Testosterone, immunocompetence and honest sexual signaling in male red grouse

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Testosterone, immunocompetence and honest sexual signaling in male red grouse

6.1 Abstract

The expression of sexual ornaments has been suggested to reliably indicate individual quality, such as the ability to cope with parasites and diseases. The Immunocompetence Handicap Hypothesis (IHH) states that testosterone-dependent ornaments honestly signal such quality because of physiological costs associated with testosterone, such as impaired immune function. We tested predictions of the IHH both correlatively and experimentally in red grouse *Lagopus lagopus scoticus*. Male grouse exhibit supra-orbital red combs whose size is testosterone-dependent. We found that comb size was not correlated to infection intensity by two parasites (coccidia and the nematode *Trichostrongylus tenuis*), but significantly positively correlated with condition and T-cell-mediated immunity (the ability to mount a primary inflammatory response). We manipulated testosterone by the means of implants and re-caught males after a month to investigate the effects on comb size, condition, immunity and parasite load. Males implanted with testosterone had increased comb size, lost more condition and had lower T-cell-mediated immunity than control males. Increased testosterone also resulted in a significant increase in coccidia infection intensity, but had no effect on *T. tenuis* burden. The results are consistent with predictions of the IHH and suggest that comb size honestly indicates immunocompetence and males' ability to cope with certain parasites. Females could thus benefit from choosing mates based on the expression of this sexual trait.

6.2 Introduction

In many species, males exhibit ornaments that probably evolved through intra- or inter-sexual selection. These sexual traits assume the dual function of dissuading other males competing for the same resources and facilitating mate choice, allowing females to identify the fittest males on the basis of their ornaments (Andersson, 1994). Hamilton & Zuk (1982) suggested that female preferences for males with the most exaggerated sexual traits evolved because ornament expression signals the genetic ability of males to resist parasite infections. Females could benefit by choosing a mate with fewer parasites, or whose ability to cope with parasites will be passed onto offspring (Andersson, 1994; Hamilton & Zuk, 1982; Møller, 1990; Møller *et al.*, 1999; Zuk, 1992).

The role of parasites in sexual selection has been particularly well studied amongst birds. Many studies have looked for negative relationships between sexual signals and parasite loads. However, the results are inconsistent, with studies reporting either negative relationships, no relationship, or even positive relationships between signals and parasites (see Folstad & Karter, 1992; Getty, 2002; Møller *et al.*, 1999). Moreover, the lack of relationship between sexual signal and infection intensity by a particular parasite does not, however, exclude the possibility that either the ornament expression relates to the ability to cope with the detrimental effects of the parasite rather than its abundance itself, or that other parasites act as a constraint on the level of signaling.

Measures of host immune responses may therefore provide more accurate indications of the role of parasite-mediated sexual selection than estimates of the intensity of infection (Møller *et al.*, 1999; Møller *et al.*, 1998b; Møller & Saino, 1994; Zuk, 1996). Indeed, several studies have shown that sexual signaling relates to immunocompetence (the ability of an individual to produce an immune response to pathogens), providing evidence that sexual signals can be used by females as cues to this aspect of male quality (Duffy & Ball, 2002; Folstad & Karter, 1992; Møller *et al.,* 1999; Møller & Saino, 1994; Saino *et al.,* 1997; Zuk, 1996; Zuk *et al.*, 1995). The relationship between signals and immunocompetence also appears to be stronger than that between signal and parasites (Møller *et al.*, 1999).

Signaling theory predicts that signals of individual quality should be costly in order to ensure the honesty of the signaling system and prevent poor quality individuals from cheating (Zahavi & Zahavi, 1997). Elevated testosterone levels are usually required for the expression of sexual traits involved in male-male competition and female choice, but might also be associated with physiological costs, such as impaired immune function (Folstad & Karter, 1992; Grosmann, 1985; Hillgarth & Wingfield, 1997). According to the Immunocompetence Handicap Hypothesis (IHH), testosterone dependent traits or behaviors would be particularly useful as honest signals of health, because of the immunosuppressive effects of elevated testosterone (Folstad & Karter, 1992). On the one hand, elevated testosterone levels increase the expression of ornaments relevant in an intra-sexual or a mate choice context, but on the other hand, they could reduce immune function and increase susceptibility to parasite infections (Folstad & Karter, 1992). It has been suggested that this 'double-edged sword' effect of testosterone would prevent cheating, as males have to trade the benefits against the costs of elevated testosterone and optimize their level of signaling accordingly. Although correlative studies may provide insights about the individual qualities underlined by sexual traits, they do not establish causality. Moreover, optimal signaling theory is unable to predict the relationship between signal intensity and parasite loads (Getty, 2002). The IHH is based on trade-offs within individuals (between testosterone, levels of signaling, immunocompetence and parasites), and experimental studies are therefore the most appropriate to investigate these trade-offs. Testosterone levels can be manipulated by the means of implants, allowing workers to modify the level of signaling of males and investigate effects on immunocompetence and parasite infections (e.g. Casto *et al.*, 2001; Duckworth *et al.*, 2001; Saino *et al.*, 1994) or on fitness components such as viability, mating and breeding success (e.g. Alatalo *et al.*, 1996; Moss *et al.,* 1994).

In this study, we investigated whether sexual ornamentation relates to condition, parasite burdens and / or immunocompetence, and we used an experimental approach to test predictions of the IHH in male red grouse *Lagopus lagopus scoticus.* Grouse combs are brightly colored ornaments that function in both male-male competition and female choice (Alatalo *et al.,* 1996; Bart & Earnst, 1999; Moss *et al.,* 1979; Rintamaki *et al.*, 2000). Their size is testosterone dependent (Moss *et al.*, 1979; Rintamaki *et al.*, 2000), so these ornaments might therefore be particularly useful as honest signals of health. We first looked at the relationships between comb size, condition, intensity of infection by two parasites (a gut nematode, *Trichostrongylus tenuis* and coccidia *Isospora ssp.*) and immunocompetence (T-cell-mediated immunity, the ability to mount a primary inflammatory immune response). If comb size signals male's quality, we expected males with bigger combs (and more testosterone) to be those in better condition, with a greater T-cell-mediated immunity and fewer parasites. Second, we manipulated testosterone levels of males to test two main assumptions of the IHH, namely that testosterone enhances sexual ornamentation and impairs immune function. A previous study highlighted that males with more testosterone can be more immunocompetent despite the immunosuppressive effects of testosterone (Peters, 2000). Predictions in the experimental situation were thus different from those in the natural situation. If signaling is honest, changing the optimal level of sexual signaling by increasing testosterone should be associated with immunosuppressive effects and should therefore be costly. Thus, we expected increased testosterone levels to result in a reduction of T-cell-mediated immunity, a loss of condition and an increase in parasite intensity.

6.3 Methods

Study species

The red grouse is a medium sized *Tetraonid* bird characteristic of the heather moorland habitats of the UK (Cramp & Simmons, 1980). This species is territorial and mostly monogamous (Cramp & Simmons, 1980). Male territorial behavior is particularly important in the autumn, during territory establishment, and in early spring. Pairing starts in autumn and continues throughout the winter until early spring, when breeding starts (Cramp & Simmons, 1980; Watson, 1985). Like other grouse species, red grouse combs are larger and brighter in males, and their size is testosterone-dependent (Moss *et al.,* 1979). These sexual ornaments are known to play an important role in male-male competition: male red grouse with big combs are dominant and more aggressive (Moss *et al*., 1979; Moss *et al*., 1994) and are more likely to obtain a breeding territory than others (MacColl *et al*., 2000). Comb size also functions in mate choice, females preferring males with the biggest combs (Bart & Earnst, 1999; Brodsky, 1988; Rintamaki *et al*., 2000). In willow grouse *Lagopus lagopus lagopus*, both territory size and comb size were found to be equally important for mate choice (Bart & Earnst, 1999).

Captures, measurements and testosterone manipulations

In September-November 2001, we caught male grouse at night by dazzling and netting them (Hudson, 1986b) on two sites located in the northeast of Scotland (Glen Dye and Edinglassie estates, Aberdeenshire). We aged males (young vs. old, i.e. > 1 year old) from the shape and colour of their 2nd and 3d primaries (tips pointed and mottled in young, round and plain in old birds) and the texture of their claws (smooth in young, with a growth ridge in old birds; Cramp & Simmons 1980). At the time of first capture, we ringed all males, weighed them with a 1-kg balance, to the nearest 5g, and measured the following morphological features: length and width of flattened combs, wing length (with a ruler, to the nearest 1 mm) and pectoral angle (with a protractor, to the nearest degree), as a measure of the extent of breast muscles on either sides of the breast bone. The same person (FM) did all the measurements, and each was taken twice on a sample of 30 males to assess measurement errors. Repeatability values were calculated according to Lessells (1987), and were high and significant for all the above morphological features (all repeatability values > 0.93; all $P < 0.0001$). We calculated a condition index (weight corrected for size) as the residuals from the relationship between $\log_{10}$-transformed body weight and $\log_{10}$-transformed wing length ($F_{1,108} = 52.31$; $P < 0.0001$).

We randomly assigned males to one of three treatments: no implant, sham implants or testosterone implants. Implanted males were each given two silastic tubes (each 20 mm long, 0.62 mm inner and 0.95 mm outer diameter) that were either empty (sham implanted males) or filled with testosterone proprionate (testosterone treated males) and sealed with glue at both ends. Implants were inserted in the chest, between the skin and breast muscles, under local anesthesia. The length of the tubing was determined previously during laboratory trials on captive grouse so that the testosterone implants lasted for 2-3 months. We fitted each male with a necklace radio-tag (TW3-necklace radio-tags, Biotrack) to facilitate subsequent re-capture, kept them overnight in an individual pen to collect faecal samples for parasite counts and released them the following morning.

We re-captured most males about a month (32 ± 8 days) after the first capture. However, some males were found killed by predators or could not be re-located, possibly because the radio failed, and were not re-sampled. Thus, sample size differed between capture and re-capture. At re-capture, we re-measured comb size, pectoral angle and body weight, collected another faecal sample for parasite load estimates and measured T-cell-mediated immunity.

When analyzing the data, we found no significant differences between un-manipulated males and sham-implanted males in any of the study parameters or relationships. Therefore, in order to simplify the presentation of the results, we pooled the data from these two groups, hereafter referred to as control males (as opposed to testosterone implanted males).

Assessment of T-cell-mediated immunity

We measured T-cell-mediated immunity (CMI) by challenging the immune system through the subcutaneous injection of an innocuous plant lectin (phytohaemagglutinin, or PHA). The PHA test follows a long-established protocol developed in poultry science (Goto *et al.* 1978) and is routinely applied in avian studies (e.g. Smiths *et al.* 1999). The injection of PHA produces a prominent perivascular accumulation of T-lymphocytes followed by macrophage infiltration (Goto *et al.* 1978) and the intensity of the CMI response (swelling) indicates an individual's ability to mount a primary inflammatory response. Each male was injected with 0.1 ml of physiological saline solution (PBS) at a marked site on the right wing web (control) and with 0.2 mg of PHA in 0.1 ml of PBS (challenge) on the left wing. We measured the web thickness at each injection site four times prior to injection and 24 hr (± 10 min.) after injection, with a pressure-sensitive dial thickness gauge (to the nearest 0.01 mm). Wing web thickness measures were highly and significantly repeatable ($R = 0.83$; $P < 0.01$). CMI response was calculated as the change at 24 h in average thickness of the left wing web (PHA test) minus that of the right wing web (control). For this procedure, males were kept in pens provided with food (heather) and water. After 24h, we removed the radio tags and released all males. Sample size for immune challenges included all the males that we re-caught one month after first capture. At the same time, we also caught other males (un-manipulated) on which we conducted measurement, parasite counts and immune challenges, in order to increase sample size.

Parasite counts

Red grouse are host to a relatively small number of parasites. One of their main parasites is a gut nematode, the caecal threadworm *T. tenuis*, which has a direct life style with no alternative hosts within the same habitat (Hudson, 1986a). It is known to have important negative effects on the energetics, breeding success and survival of grouse (Delahay *et al.*, 1995; Hudson, 1986a; Hudson *et al.*, 1992; Shaw & Moss, 1990). Red grouse are also frequently infected by coccidia *Isospora ssp.* (Fantham, 1911). Patterns of infections and effects on red grouse are little known, but this parasite can have important adverse effects on avian host, in terms of reduced growth and condition (Fehlberg & Pohlmeyer, 1991). For this study, we focused on these two parasites for which infection intensity is relatively easy to measure on live birds.

We used faecal egg counts to measure intensity of infection by *T. tenuis* worms and coccidia. Samples collected in the field were kept in the lab at 4 degrees Celsius and were analyzed within two weeks of collection. For each, a sub-sample of 0.2 g was diluted into 5 ml of saline solution, mixed and placed in a MacMaster slide under a x 100 microscope where *T. tenuis* and coccidia eggs were counted. Parasite infection intensity was estimated as number of eggs per g. Faecal egg counts were shown to reliable estimate the number of *T. tenuis* worms in grouse both in spring (Moss *et al.*, 1990) and autumn (Seivwright *et al.*, in press). Infection rates by coccidia and the rate of oocyst shedding can show important diurnal and seasonal variations (Brawner & Hill, 1999; Hudman *et al.*, 2000; Svoboda, 1992). Diurnal variation was minimized by collecting all faecal samples early morning and seasonal variations in parasite infections over the study period were taken into account by controlling for sampling date.

Statistical analyses

We used SAS 8.01 for the statistical analyses (SAS, 2001). We used Generalized Linear Models to investigate natural correlates of comb size, condition or cell-mediated immunity. Dependent variables were fitted with a normal distribution and identity link function (comb size, condition index and pectoral angle) or with a Poisson distribution and log link function (coccidia and *T. tenuis* egg counts). For the correlation analyses, Coccidia and *T. tenuis* loads were $\log_{10}$-transformed as explanatory variables. We first tested for differences between study sites, age groups and variation according to sampling date, and controlled for these effects when necessary.

For the experiment, we used Generalized Linear Models to test for differences in study parameters between treatment groups prior to and (one month) after implanting with testosterone. Because most parameters varied between capture and recapture, we also tested whether within individual changes over time in parameters differed between treatment groups (testing for a time*treatment interaction). The data set was unbalanced (i.e. not all individuals were measured before and after treatment and not all parameters were measured at a given time, especially parasite loads). For these analyses, we used Generalized Linear Mixed Models (Glimmix, SAS) with individual males included as random effects (in order to account for the repeated measures on individual males and the unbalanced data set). Models included site, age, time (first capture vs. re-capture), treatment (control vs testosterone treated males) and the time*treatment interaction as fixed effects. All data are expressed as mean ± S.D and all tests are two-tailed.

6.4 Results

Comb size, condition and parasites burdens

We investigated the relationships between comb size, condition and parasites, or between condition and parasites, using the measures made at the time of first capture (i.e. prior to treatment).

Comb size variation was not significantly explained by site ($F_{1,52} = 0.37$; $P = 0.54$), age ($F_{1,52} = 0.79$; $P = 0.34$), sampling date ($F_{1,51} = 1.55$; $P = 0.22$), *T. tenuis* load ($F_{1,40} = 0.35$; $P = 0.56$) or coccida load ($F_{1,40} = 0.86$; $P = 0.36$), but was significantly explained by both measures of condition: males with bigger combs had more breast muscles (pectoral angle: $F_{1,52} = 4.78$; $P < 0.05$) and were heavier relative to their size (condition index: $F_{1,52} = 7.06$; $P < 0.05$). These relationships between comb size and condition were still significant after controlling for site, age and date (pectoral angle: $F_{1,49} = 7.09$; $P < 0.05$; condition index: $F_{1,49} = 6.75$; $P < 0.05$).

Pectoral angle did not differ between study sites ($F_{1,52} = 0.32$; $P = 0.57$), but significantly decreased during autumn ($F_{1,52} = 9.94$; $P < 0.01$) and differed between age groups after controlling for date ($F_{1,51} = 6.31$; $P < 0.05$), with old males having a greater pectoral angle (more breast muscles) than young males. However, both old and young males were similarly losing breast muscles during early autumn (non significant date*age interaction: $F_{1,50} = 2.44$; $P = 0.13$). After controlling for sampling date and age, pectoral angle was not related to *T. tenuis* load (Partial $F_{1,38} = 0.62$; $P = 0.44$) or coccidia load (Partial $F_{1,38} = 0.16$; $P = 0.69$). Condition index did not differ between study sites ($F_{1,52} = 0.24$; $P = 0.63$) or age groups ($F_{1,52} = 2.39$; $P = 0.13$) and was not significantly related to sampling date ($F_{1,51} = 0.09$; $P = 0.76$), *T. tenuis* load ($F_{1,39} = 1.62$; $P = 0.21$) or coccidia load ($F_{1,39} = 1.43$; $P = 0.24$).

Effects of testosterone on comb size, condition and parasites

Before treatment, none of study parameters (comb size, pectoral angle, condition index, T. tenuis and coccidia load) differed between treatment groups (control vs testosterone males; Generalised Linear Models controlling for site, sampling date and age; all F-values < 2.01; all $P > 0.05$; data in Table 6.1). One month after treatment, we found significant differences between T-implanted and control males in comb size ($F_{1,51} = 43.45$; $P < 0.001$), pectoral angle ($F_{1,51} = 17.95$; $P < 0.001$), condition index ($F_{1,51} = 8.57$; $P < 0.01$) and coccidia load ($F_{1,39} = 7.32$; $P < 0.01$), but no difference in *T. tenuis* load ($F_{1,39} = 0.75$; $P = 0.39$; data in Table 6.1).

We further tested whether changes over time in study parameters (between first capture and re-capture) differed between treatment groups. Comb size increased in both groups, but increased significantly more in testosterone implanted than in control males (Table 6.2; significant time*treatment interaction; Figure 6.1). Both measures of condition (pectoral angle and condition index) also changed over time, and in relation to treatment: males lost condition between capture and re-capture, and testosterone treated males lost significantly more breast muscles (pectoral angle) and body weight (condition index) than control males (Table 6.1 & 6.2). Experimentally increased testosterone levels thus resulted in enhanced sexual ornamentation but also in a greater loss of condition.

T. tenuis burdens decreased between capture and re-capture (Table 6.1 & 6.2), this decrease being similar in testosterone treated and control males (non significant time*treatment interaction; Table 6.2). In contrast, coccidia load increased between capture and re-capture (Table 6.2). This increase was greater in testosterone treated than in control males (Table 6.1 & 6.2; Figure 6.1). Increased testosterone levels thus had no significant effect on changes in *T. tenuis* load but resulted in increased coccidia infection intensity.

Table 6.1: Changes over time (between capture and re-capture, c. 1 month after treatment) in study parameters according to treatment. Sample size, in brackets, refers to number of individual males. Comb area, pectoral angle and condition index are expressed as arithmetic means ± SD. Coccidia and *T. tenuis* egg counts are expressed as geometric means $\overset{\times}{\div}$ SD.

		Treatment	
Parameters	Time*	Control	Testosterone treated
Comb area (mm^2)	t_0	273.3 ± 46.9 (35)	286.8 ± 38.6 (21)
	t_1	280.4 ± 47.8 (39)	401.5 ± 60.9 (18)
Pectoral angle (°)	t_0	43.4 ± 2.5 (35)	45.5 ± 3.8 (21)
	t_1	42.8 ± 4.0 (39)	39.7 ± 2.7 (18)
Condition index	t_0	-0.001 ± 0.043 (35)	0.039 ± 0.053 (21)
	t_1	-0.012 ± 0.044 (39)	-0.005 ± 0.055 (18)
coccidia (egg / g)	t_0	1180 $\overset{\times}{\div}$ 4.8 (26)	1524 $\overset{\times}{\div}$ 2.9 (16)
	t_1	1254 $\overset{\times}{\div}$ 4.6 (30)	4486 $\overset{\times}{\div}$ 3.7 (14)
T. tenuis (egg / g)	t_0	2342 $\overset{\times}{\div}$ 3.3 (26)	1808 $\overset{\times}{\div}$ 6.1 (16)
	t_1	1527 $\overset{\times}{\div}$ 11.0 (30)	1669 $\overset{\times}{\div}$ 8.7 (14)

* t_0: initial capture; t_1: re-capture, c. 1 month later.

Table 6.2: Results of the Generalized Mixed Models (Glimmix procedure, SAS 2001) testing for an effect of treatment (testosterone treated vs control) on changes over time in study parameters. Models included study site and age as a fixed effect and individual males as random effects. Dependent variables were fitted with a normal distribution and an identity link function, except for coccidia and *T. tenuis* egg counts, which were fitted with a Poisson distribution and a log link function. (ns: P > 0.05; **: P < 0.01; ***: P < 0.001).

		Time		Treatment		Time*Treatment	
	df	F	P	F	P	F	P
Comb size (mm^2)	1,47	95.81	***	16.21	***	99.30	***
Pectoral angle (^{o})	1,47	24.87	***	4.48	*	31.37	***
Condition index	1,47	32.32	***	2.85	ns	13.41	***
Coccidia ($egg.g^{-1}$)	1,39	42.8	***	1.28	ns	7.65	**
T. tenuis ($egg.g^{-1}$)	1,39	19.81	***	0.08	ns	0.20	ns

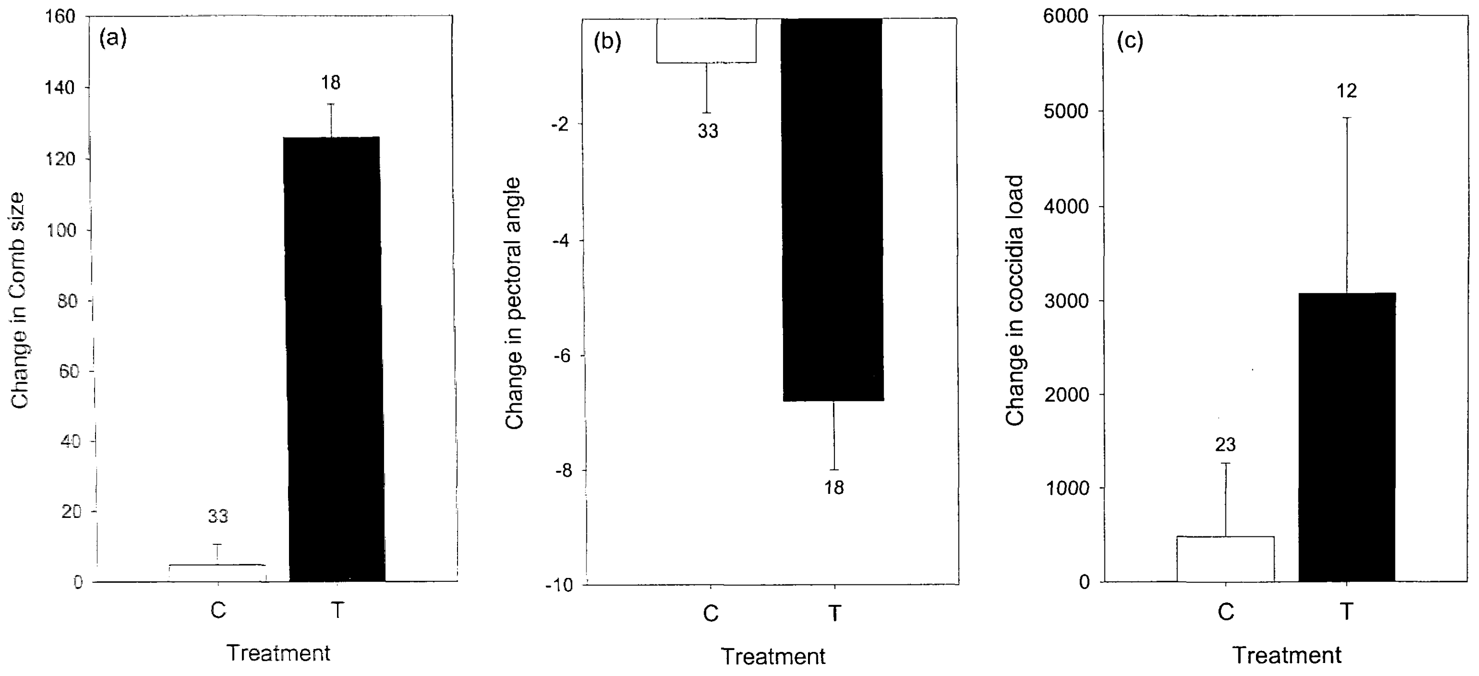

Figure 6.1: Mean (± SED) changes over time (between capture and re-capture, c. 1month later) in a) comb size, b) pectoral angle (extent of breast muscles) and c) coccidia load according to treatment (C: control males; T: Testosterone-implanted males). Sample size on each bar refers to number of individuals in each group.

T-cell-mediated immunity, comb size, condition and parasite burdens

We investigated the natural correlates of T-cell-mediated immunity in the control males only (we excluded testosterone treated males in these analyses, whose responses were affected by the treatment; see below). Variation in wing web swelling was not significantly explained by study site (GLM: $F_{1,33} = 2.45$; $P = 0.13$), sampling date ($F_{1,33} = 0.30$; $P = 0.59$), age ($F_{1,33} = 3.03$; $P = 0.10$) or *T. tenuis* load ($F_{1,28} = 0.01$; $P = 0.93$), but was significantly explained by coccidia load ($F_{1,28} = 5.38$; $P < 0.05$), pectoral angle ($F_{1,33} = 29.53$; $P < 0.001$), condition index ($F_{1,33} = 4.62$; $P < 0.05$) and comb size ($F_{1,32} = 20.08$; $P < 0.001$). Males with greater T-cell-mediated immunity had fewer coccidia, more breast muscles, were heavier relative to their size and had bigger combs (Figure 6.3).

Effect of testosterone on T-cell-mediated immunity

In males caught one month after treatment, wing web swelling 24h post-challenge was significantly lower in testosterone treated than in control males (Figure 6.2; $F_{1,45} = 6.59$; $P < 0.05$). Differences in wing web swelling between treatment groups were significant after controlling for site, age, comb size, pectoral angle and condition index ($F_{1,40} = 5.57$; $P < 0.05$). Experimentally increased testosterone levels thus resulted in reduced T-cell-mediated immunity.

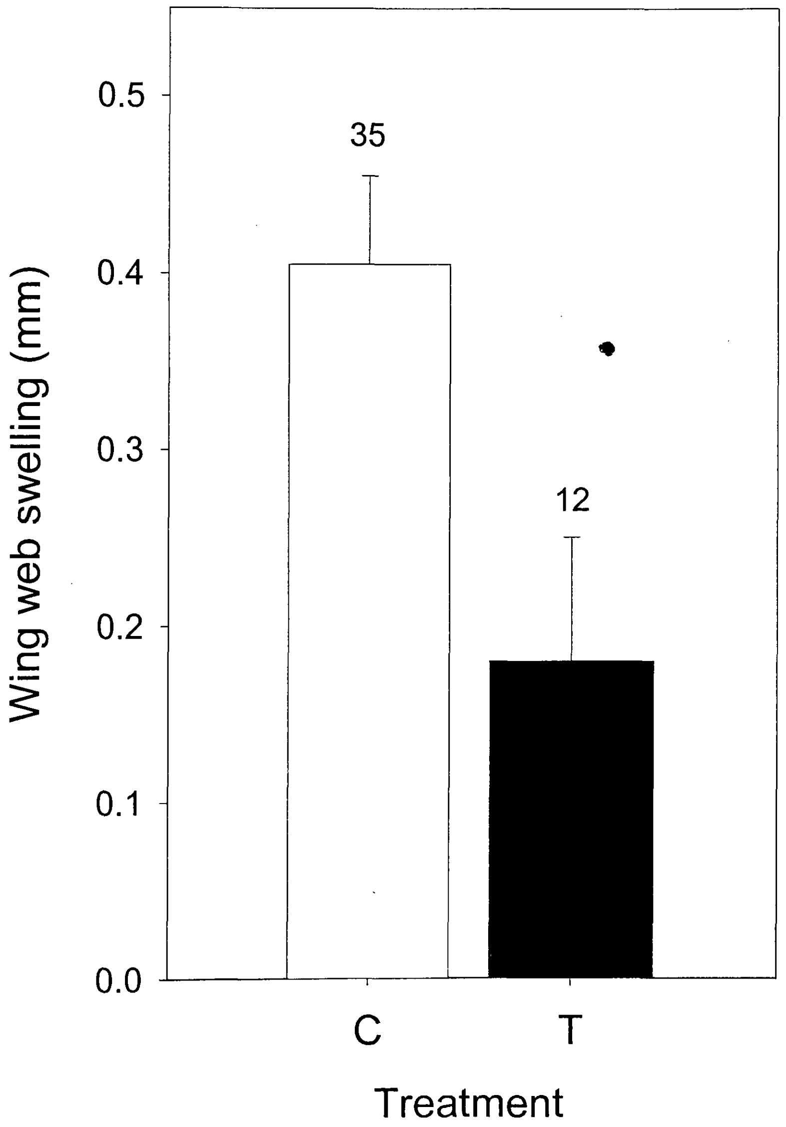

Figure 6.2: Mean (± SED) wing web swelling according to treatment (C: control males; T: testosterone implanted males). Sample size, above bars, refers to number of individuals in each group.

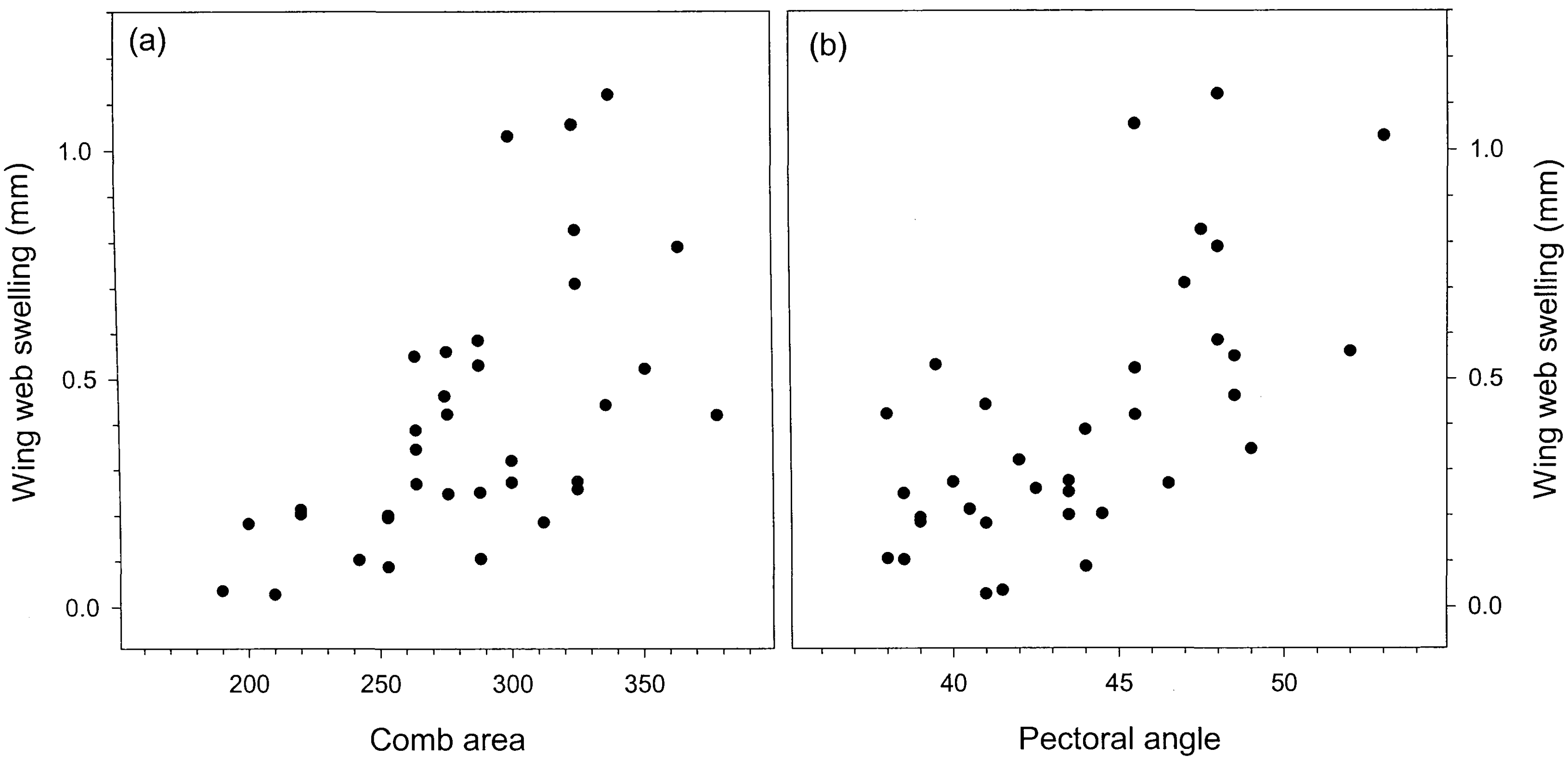

Figure 6.3: Relationships a) between wing web swelling and comb area, and b) between wing web swelling and pectoral angle (extent of breast muscles) in control male

6.5 Discussion

In red grouse, we found that males with the biggest combs were in better condition, had greater cell-mediated immunity but did not have fewer *T. tenuis* and coccidia parasites. We also found that experimentally increased testosterone enhanced comb size but reduced condition and cell-mediated immunity, and increased coccidia infection intensity. Below, we discuss these findings in line with predictions from the Immunocompetence Handicap Hypothesis.

Relationships between comb size, condition, immunity and parasite loads

We found that comb size during autumn was positively correlated with condition (males with bigger combs had more breast muscles). Levels of sexual signaling are usually expected to be condition-dependent, so that only individuals in prime condition are able to produce the most exaggerated ornaments without compromising their ability to cope with the costs imposed by the production and maintenance of the signal (Andersson, 1994). Parasites and diseases usually negatively affect condition (Møller *et al.,* 1998a; Wilson *et al.*, in press), so males with big combs might also be expected to have fewer parasites. However, we did not find that bigger combs were associated with fewer *T. tenuis* worms or fewer coccidia. Other studies have nevertheless showed that infection by intestinal nematodes and coccidia can reduce levels of sexual signaling, such as comb size (Zuk *et al.*, 1990a; Zuk *et al.*, 1990b) or carotenoid-based color intensity (Brawner *et al.*, 2000; Hill & Brawner, 1998).

We also found little evidence for a good condition to be associated with fewer of these parasites. *T. tenuis* is a main parasite of grouse, but previous work has shown that red grouse infected with *T. tenuis* worms lose more weight and condition than uninfected controls (Shaw & Moss, 1990; Wilson & Wilson, 1978). The *T. tenuis* infection levels found in this study were relatively low and decreasing, and might not have been sufficient to have noticeable detrimental effects on male condition or immune function. It is also possible that parasite transmission, which is low for *T. tenuis* in autumn, is more important than the parasite burden itself, as most damage is caused when ingested larvae develop into worms and bury in the caecal guts (Delahay, 1995). Similarly, coccidia infections can significantly reduce weight gain (Conway *et al.*, 1995), although such an effect was not detected in this study. These results nevertheless suggested that comb size was unlikely to reliably indicate *T. tenuis* or coccidia infection levels, and thus that females were unlikely to prefer males with bigger combs because they have fewer of these parasites.

Our study shows that cell-mediated immunity, as measured by the response to the PHA skin test, was positively correlated with comb size and condition. Various aspects of the immune system are condition-dependent, because of the costs of raising an immune response, and poor condition might thus result in a weak immune responsiveness (Saino & Møller 1996, Sheldon & Verhulst 1996, Møller *et al.* 1998a). Accordingly, we found that cell-mediated immunity was positively correlated with condition, with responses to the PHA test being positively correlated to both the relative weight and the amount of breast muscles of males. The relationship between sexual ornamentation and immunocompetence might thus be mediated by condition. Male red grouse in better condition and with greater cell-mediated immunity were those with bigger combs, and were likely to be those of higher phenotypic / genetic quality. Females might therefore prefer males with bigger combs for their higher immunocompetence, which might underline a better ability to cope with parasite infections.

Effects of testosterone on comb size, cell-mediated immunity and condition

Previous studies conducted on red grouse in spring showed that increased testosterone caused an increase in comb size (Moss *et al.*, 1979), so we expected a similar effect in autumn. Indeed, we found that the testosterone implants caused a significant increase in comb size, up to the size that is usually exhibited by males in spring (and therefore within the range of natural variation). We also found that males implanted with testosterone had lower cell-mediated immunity (lower responsiveness to the PHA skin test) and lost more condition (body weight and breast muscles) than control and un-manipulated males.

Male red grouse implanted with testosterone typically call and display more often, are more aggressive and expand their territory to the detriment of others (Moss *et al.*, 1979; Watson & Parr, 1981). All these activities are energetically costly, which might explain why testosterone implanted males lost condition. Because cell-mediated immunity was found to be condition dependent, the testosterone treatment might have indirectly affected immunity, via its effects on condition. However, differences in immune response between treatment groups were still significant after controlling for condition, suggesting a more direct, physiological, effect of testosterone on immunity. While many studies have showed that testosterone enhances the size or intensity of sexual ornaments (e.g. Rintamaki *et al.*, 2000; Zuk *et al.*, 1995; Hillgarth & Wingfield, 1997), direct experimental evidence for immunosuppressive effects of testosterone is still limited. Some studies found evidence for testosterone to be immunosuppressive (Casto *et al.*, 2001; Peters, 2000), but others found little (Ros *et al.*, 1997) or no support (Hasselquist *et al.*, 1999) for this prediction, which is central to the IHH. Our study provides experimental evidence for elevated testosterone levels to be associated with both enhanced sexual ornamentation and reduced immunocompetence. The experimental results were also consistent with the correlative results in showing that testosterone, comb size, condition and immunocompetence are all closely inter-related in male red grouse.

Males with bigger combs therefore had greater cell-mediated immunity despite having more testosterone and concomitant immunosuppression. These findings are similar to those from another study: in superb fairy-wrens *Malurus cyaneus*, testosterone treatment decreased immune responsiveness but wild males with more testosterone were found to be more immunocompetent (Peters, 2000). This association between immunosuppressive testosterone and immune responsiveness may arise if males with big combs enhance their immunity to compensate for and counter the impact of immunosuppression by testosterone, or if both testosterone and immunocompetence correlate with male quality and condition. In autumn, male red grouse extensively engage in territorial displays to establish territories, and in courtships, as females start assessing potential mates. Autumn territorial behavior is crucial for male red grouse, as most males failing to maintain or establish a territory usually die over-winter or will not reproduce (Watson, 1985). These activities require elevated testosterone, are energetically costly and potentially stressful, as they usually involve fights and associated risks of injuries. Autumn territorial and display behaviors might thus require enhanced immune responsiveness, as a compensatory measure. Our results suggest males with the biggest combs are those better able to do so and cope with immunosuppressive testosterone, possibly because they are also in better condition and of higher phenotypic / genetic quality than others.

Testosterone and parasite infections

Because increased testosterone reduced immunocompetence, it might also have had indirect effects on parasite infection levels. A number of studies have shown that increased testosterone levels can cause significant increase in parasite infection intensities (Duckworth *et al.*, 2001; Hughes & Randolph, 2001; Saino *et al.*, 1995; Zuk *et al.*, 1995). We found that the testosterone treatment affected levels of coccidia infection of males. Infection intensities increased during the study, but the increase in coccidia load was significantly greater in testosterone implanted than in other males. The increased infection levels in testosterone males might reflect a greater exposure or a reduced ability to control infection by coccidia. In control birds, cell-mediated immunity was found to correlate negatively with coccidia infection levels. The greater coccidia infection levels observed in testosterone males might thus have been caused by physiological effects associated with the testosterone treatment, which may have impaired the ability of males to control the establishment success or the reproductive rate of coccidia. In another study conducted on male house finches *Carpodocus mexicanus*, experimentally increased testosterone was found to accelerate the rate of coccicia infection, also suggesting that testosterone may have direct effects on resistance to this parasite (Duckworth *et al.*, 2001). Elevated testosterone, however, does not always result in increased coccidia infection (e.g.Hudman *et al.,* 2000). In contrast, we found no effect of testosterone on *T. tenuis* intensity. In this case, a possible explanation lies in the life history of *T. tenuis*. In autumn, ingested larvae become hypo biotic and delay development until the next spring when they mature into adult worms and begin to produce eggs (Shaw, 1988). The adult worms that die in autumn are not replaced, as shown in this study by a decrease in worm numbers throughout the autumn. It is thus possible that over the short time-scale of our experiment, the testosterone treatment had no effect on the adult worms because transmission was low, but affected the arrestment of parasite larvae, which would affect parasite loads only in the following spring, when these larvae de-arrest. Further work is therefore needed to fully assess the effect that testosterone might have on this parasite.

Comb size as an honest signal of male quality

Males red grouse with bigger combs, and more testosterone, benefit in terms of increased recruitment probability during autumn (MacColl *et al.*, 2000) and increased territory size (Moss *et al.*, 1994). They may also benefit from pairing earlier, attracting more than one female or a better quality female, and were shown to achieve a higher breeding success (Moss *et al.*, 1994). Our results show that high testosterone levels are also costly, in terms of reduced immunocompetence, condition and ability to control coccidia infection. Male red grouse therefore have to trade the benefits of elevated testosterone against the associated costs. Our experimental results, supported by correlative data, provide conclusive evidence to support the Immunocompetence Handicap Hypothesis (Folstad & Karter 1992). In male red grouse, the ability to mount an immune response is condition dependent and testosterone dependent, and is signaled by comb size. Our findings suggest that males with the biggest combs are better able to cope with immunosuppressive testosterone and to control infection by certain parasites. Comb size is thus likely to reliably indicate the phenotypic and/or genetic quality of males, and females could benefit from choosing a mate based on this sexual trait.

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CHAPTER 7

Interactions between hormones and parasites: The effects of testosterone on *Trichostrongylus tenuis* infections

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Interactions between hormones and parasites:

The effects of testosterone on *Trichostrongylus tenuis* infections

7.1 Abstract

Recent interest in the role parasites play in sexual selection and in shaping the life history of their hosts, and in the role of parasites in host population dynamics has led to growing interest in the trade-offs involved in parasite defence. In the red grouse (*Lagopus lagopus scoticus*) males exhibit behaviour and sexual ornaments that are testosterone dependent. However, testosterone is thought to impair immune function, the main defence against parasites. This study experimentally investigated the effect of testosterone on parasite infections of *Trichostrongylus tenuis* in free-living male red grouse. There was no relationship between plasma testosterone levels and parasite infection level in males in autumn. However, male red grouse implanted with testosterone had significantly higher intensities of infection of *T. tenuis* one year after implanting than did control males that were implanted with sham implants. This study shows a cost of elevated testosterone, in terms of increased *T. tenuis* intensity, possibly because of immunosuppressive effects associated with elevated testosterone. The results suggest that parasites may be involved in a complex trade-off between current reproductive fitness, and future reproductive success and even survival, due to the potentially detrimental physiological effects of increased *T.tenuis* infections.

7.2 Introduction

Growing interest in the field of evolutionary ecology has increasingly focussed on how parasite defence may shape a host's life history (Zuk & Stoehr, 2002). As animals have only a limited amount of resources, individuals must allocate these resources optimally between competing life history activities such as such as growth, reproduction and parasite defence (Stearns, 1989, 1992; Roff, 1992; Owens & Wilson, 1999). Thus, increased investment in activities associated with reproduction may lead to reduced parasite defence and ultimately to increased susceptibility to deleterious parasite infections (Zuk & Stoehr, 2002 ; Møller & Saino, 1994; Møller, 1997; Norris *et al.,* 1994; Sheldon & Verhulst, 1996; McKean & Nunney, 2001).

Such trade-offs may be particularly significant for species in which individuals require elevated hormone levels to ensure reproductive success. Immune function, the main physiological means by which animals resist or fight parasitic or pathogenic infections, is regulated by the gonadal steroids oestrogens, androgens and progesterone (Grossman, 1984). The male androgen, testosterone (T), is required for the expression of many of the behaviours and morphological characters necessary to ensure reproductive success (Folstad & Karter, 1992; Eens *et al.*, 2000; Peters, 2000; Stoehr & Hill, 2000). T is also thought to suppress the immune system (Folstad & Karter, 1982; Grossman, 1984; Marsh, 1992). As mounting an immune defence is likely to be costly (Sheldon & Verhulst, 1996; Owens & Wilson, 1999; Lochmiller & Deerenberg, 2000, Tella *et al.,* 2002), individuals may trade off allocating resources to parasite defence against the cost of investing energy available for other life history activities such as survival or reproduction (Hakkarainen *et al.*, 1998; Owens & Wilson, 1999; Tella *et al.*, 2002; Lozano & Lank, 2003). Assessing the interaction between testosterone and parasites is important, not only to advancing our knowledge of the selective pressure of parasites on their hosts, but also to better understand host-parasite interactions and the role that interactions play in host population dynamics (Sheldon & Verhulst, 1996; Holmstad & Skorping, 1998; Lysne & Skorping, 2002). Much work to date has focussed on identifying the relative importance of extrinsic and intrinsic processes in regulating populations and the topic remains widely debated (Lance & Lawton, 1990; Stenseth *et al.*, 1996). However, the interaction between these two mechanisms has received comparatively little attention.

Where interactions might be particularly important is in red grouse population dynamics. Red grouse populations commonly exhibit cyclic dynamics (Jenkins *et al.*, 1967; Potts *et al.*, 1984; Williams, 1985; Hudson, 1986a; Dobson & Hudson, 1994; Moss & Watson, 2001). Parasites, in particular the gastrointestinal nematode *Trichostrongylus tenuis,* play a major role in the unstable population dynamics of red grouse (Hudson *et al.*, 1985,1992,1998). Moreover, there is increasing evidence that behavioural factors, such as changes in aggressiveness also play a significant role in the unstable population dynamics (Watson *et al.,* 1984; Mountford *et al.*, 1990; Watson *et al.*, 1994, Moss *et al.*, 1994; Moss *et al.*, 1996; Matthiopoulos *et al.,* 2000; Mougeot *et al.*, 2003a,b). This aggressive behaviour is dependent on the hormone testosterone (Moss *et al.*, 1979). While previous studies have tended to focus on the impact of intrinsic and extrinsic processes separately, this study considers how these processes may interact.

In this study, the effect of testosterone on parasite infections in individual male red grouse was investigated by experimentally manipulating T while controlling for parasite exposure. It was predicted that, if testosterone has immunosuppressive effects, it should reduce the ability of males to control the establishment of parasite infective larvae, and should result in increased intensities of parasite infection.

7.3 Methods

Study Areas

This experiment was conducted on six study sites located in the northeast of Scotland (N = 62 males; Edinglassie, Invermark and Invercauld estates, Aberdeenshire; 2000-2001) and in Northern England (N= 62 males; Feldom and Catterick, North Yorkshire and Moorhouse, Cumbria; 2002-2003).

Capture, measurements and samples

Adult male grouse (N = 124 birds) were captured at night using standard lamping techniques (Hudson & Newborn, 1995). Upon first capture (4th September- 16th October), males were marked either with a numbered metal leg ring or with a numbered metal patagial wing tag and were fitted with a radio-collar (TW3-necklace radio-tags, Biotrack) to facilitate future re-location and re-capture. Males were aged from the shape & colour of primaries and from the texture of their claws (Parr, 1975). Birds less than one year old, were classed as "young" and those greater than 1 year old classed as "old". At the time of first capture the following data were recorded: date of capture (recorded as Julian date), weight (to the nearest 1g), wing length (to the nearest 1 mm) and comb size (length and height of the comb were measured to the nearest 1mm and multiplied to give an index of comb size). A body condition index (weight corrected for wing length) was calculated as the residuals from a General Linear Mixed Model (Proc Mixed procedures, SAS 2001), with weight as the response variable, wing length as the fixed effect and site and a site*wing length interaction as random effects. This index is a measure of relative body mass controlling for differences in structural body mass, which may vary between study moors, as indicated by wing length. At first capture, and at each re-capture a minimum of one and six months post-implanting treatment, a blood sample (*ca.*200μl in heparinized haematocrit capillary tubes) was taken from the brachial vein to measure plasma T levels. Faecal samples were collected at first capture, six and twelve months post-implanting treatment for parasite sampling. Birds were placed in individual holding boxes overnight and their caecal faeces containing the parasite eggs collected the following morning. In autumn 2001, 36 birds were euthanased and their caeca removed for parasite and parasite egg calibration (see Chapter 3). All faecal samples collected in the field were brought immediately to the lab and stored in a cold room at a constant temperature of 5°C to inhibit the hatching of parasite eggs

Hormone Implants

Male grouse were randomly assigned one of two treatments: testosterone treated (T-males) and control (C-males). Birds were each implanted with two silastic tubes (each one 20 mm long, 0.62 mm of inner and 0.95 mm of outer diameter) sealed with silastic glue. Implants were inserted between skin and breast muscles on the flank, under local anaesthesia and were either empty (C-males) or filled with crystalline testosterone proprionate (Sigma Aldrich Co Ltd, Poole, Dorset, UK) (T-Males). The length of the tubing was previously determined during trials on captive grouse so that implants would last for 2-3 months.

Testosterone assays

Blood samples were taken to enable testosterone assays to be carried out. Blood was collected in heparinized capillary tubes and centrifuged immediately for 3 minutes at 12 000 rpm. Plasma was separated from the packed cells stored in a cold box, taken to the lab within 3 hours after collection and kept frozen at –70°C for subsequent analyses Within two months of collection, plasma testosterone concentrations were measured using a direct double antibody radio-immuno-assay. Testosterone assays were carried out by A. Dawson at the Centre for Ecology and Hydrology, Monkswood. Duplicate 20 (µl) microlitre plasma samples were assayed. The standards, serially diluted in charcoal-stripped chicken serum, were assayed in triplicate. Both unknown samples and standards were heated to 80°C for 2 min to denature binding proteins. The primary antibody (8680-1419 Biogenesis, Poole, U.K.) was used at a dilution of 1:3500 and the tracer was [1,2,6,7-3H] testosterone (Amersham Pharmacia Biotech, Bucks. U.K.). After 24h incubation the second antibody (donkey anti-rabbit) was added and bound and free hormone were separated after a further 24h by centrifugation at 5 000g. The sensitivity of the assay was 0.2 nmol.l^{-1}. Intra- and inter-assay coefficients of variance were 8.2% and 12.4% respectively.

Parasite manipulations

At first capture, all males were dosed with an anthelminthic to clear them of their nematode parasites. One month after implanting (October-November), males were recaught and challenged with c.2000 *T. tenuis* infective larvae before releasing them, to standardise the initial parasite infection between males at the beginning of the experiment.

Parasite Counts

For a detailed description of the life-cycle of both parasites refer to Chapter 2. The main parasite of red grouse is the gastrointestinal nematode *Trichostrongylus tenuis* which inhabits the caeca. *T. tenuis* burdens were measured using faecal egg counts (live males) carried out using the McMaster technique (MAFF, 1986), or direct worm counts (dead males). For full details of these methods see Chapter 3. Worm burdens were calculated from average eggs per gram counts (Seivwright *et al.,* 2004; see also Chapter 3). Previous studies on *T. tenuis* faecal egg counts in red grouse indicate that there is a strong positive correlation between *T. tenuis* worm burden and EPG (Moss *et al.,*1990; Seivwright *et al*., 2004).

Statistical analyses

Where necessary, parasite measures were log (x+1) transformed to approach normality. The data were unbalanced as some individuals died throughout the course of the experiment and not all individual's or parameters were measured at a given time (see Table 7.1). Testosterone assays were carried out only in Scotland. All available data were used in analyses conducted using SAS (version 8.01, SAS, 2001). General Linear Mixed Models (Mixed procedures), with study site as a random effect were used to check that time intervals between sampling events did not vary between treatment groups. Generalised Linear Mixed Models (Glimmix procedures, Littell *et al.*, 1996) were used to test for an effect of sampling date, age, condition index and *T. tenuis* worm intensity on plasma testosterone levels prior to treatment. Models were fitted to the data using a Poisson error distribution and a log-link function. Denominator degrees of freedom were estimated using Satterthwaite's formula (Littell *et al.*, 1996).

To investigate whether changes in testosterone over time differed according to treatment all available data were used in General Linear Mixed Models which included study site and individual nested within site as random effects, and hormone treatment, time and a time*treament interaction as a fixed effect. Data on parasite infections of *T. tenuis* were highly over-dispersed and could not be analysed in mixed models. Parasite count data were therefore analysed by season using Generalised Linear Models (Genmod procedures) with a negative binomial distribution and a log link function. Models controlled for significant effects of study site, date of sampling and age of male where necessary by including these terms as fixed effects in the model.

Table 7.1: Timetable of Experimental Protocols

	Year 1		**Year 2**	
	Autumn 0	**Autumn 1**	**Spring**	**Autumn 2**
	(4 Sept -16 Oct)	(23 Sept - 5 Dec)	(5 Mar - 29 May)	(31 Aug- 25 Sept)
Total No. of Birds	**(N = 124)**	**(N = 114)**	**(N = 82)**	**(N = 50)**
Testosterone Implanted	• (N = 124)			
Anthelmintic Treatment	• (N = 124)			
Larvae Challenge		• (N= 114)		
Testosterone Sampled	• (N= 36)	• (N = 26)	• (N = 19)	
***T. tenuis* Count**	• (N = 110)		• (N = 73)	• (N = 48)

7.4 Results

The date of first capture (sampling period 'Autumn 0'; 4th September to 16th October) did not differ between treatment groups (Mixed model including site as a random effect: $F_{1,117} < 0.001$, $P = 0.954$). Time intervals (number of days) between sampling events thereafter, also did not vary between treatment groups (Autumn 0 to Autumn 1, $F_{1,107} = 0.23$, $P = 0.635$; Autumn 1 to Spring , $F_{1,71} = 0.26$, $P = 0.609$; Spring to Autumn 2, $F_{1,40} = 2.28$, $P = 0.139$).

At the time of initial capture (i.e. prior to implant treatment) plasma testosterone levels varied from 0.3 $\mathrm{nmol.l^{-1}}$ to 2.6 $\mathrm{nmol.l^{-1}}$ ($N = 35$). Variation in T plasma level was not significantly explained by sampling date (Mixed model including site as a random effect; $F_{1,32} = 0.28$, $P = 0.60$), age ($F_{1,32} = 1.37$, $P = 0.25$), condition index ($F_{1,32} = 1.18$, $P = 0.29$) or *T. tenuis* worm load ($F_{1,29} = 1.25$, $P = 0.27$).

Effects of testosterone implant treatment on plasma testosterone levels

Generalised Linear Mixed Models were used to test for an effect of treatment (testosterone treated vs control) on changes over time in plasma T. At the time of initial capture, T plasma levels of T-males were significantly higher than those of C-males (Mixed model including site as a random effect (Figure 7.1; $F_{1,34} = 6.04$, $P = 0.019$). There was a significant natural increase in plasma T levels over time of C-males caught one month after treatment (Autumn 1), but T -males had significantly higher circulating levels of T plasma than C-males one month after implanting due to treatment (Table 7.2). A significant time*treatment interaction indicated that T-levels increased more in T-males than in C-males following treatment (Table 7.2; Figure 7.1). When males were challenged with larvae (Autumn 1), T-males had c. 4 times more circulating testosterone than C-males. Six months after hormone treatment (Spring), T-levels in both treatments groups had significantly increased over time compared with T-levels in autumn prior to treatment (Table 7.2: Figure 7.1). However, plasma T levels then did not differ between T-males and C-males (Figure 7.1; $F_{1,15} = 0.10$, $P = 0.761$) indicating that after a period of six months, there was no longer a significant effect on T concentration.

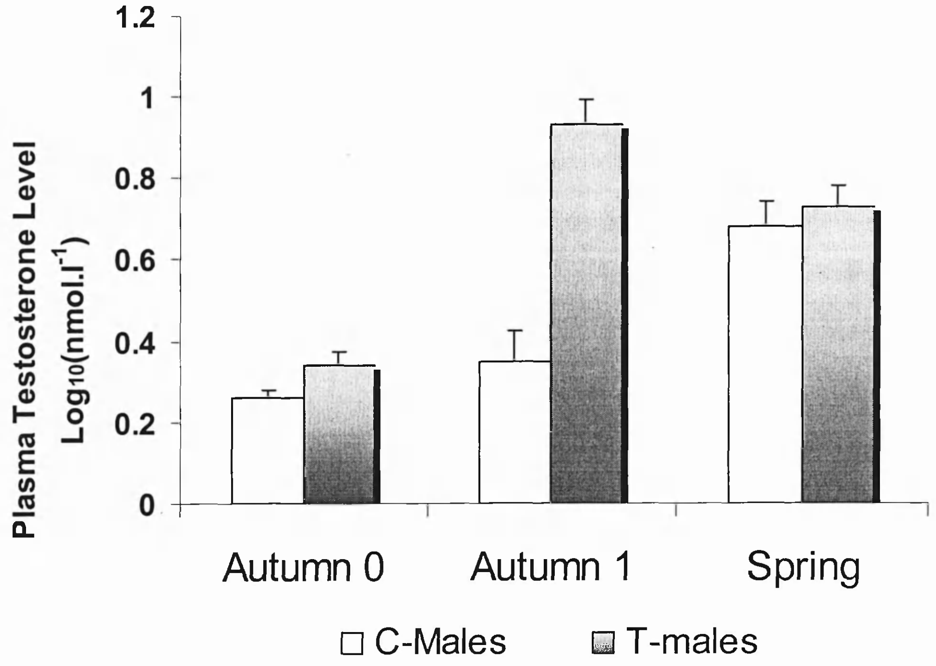

Figure 7.1: Arithmetic mean (+S.E.) for plasma testosterone levels ($\log_{10}$nmol.l^{-1}) for inert and testosterone treated males for Autumn 0 (N = 35), Autumn 1 (N= 26) and Spring (N= 19).

Table 7.2: Results of the Generalised Linear Mixed Models (Glimmix procedures, SAS 2001) testing for an effect of treatment (testosterone treated vs control) on changes over time in plasma T levels. Models included study site and individual males nested within site as random effects.

Parameter	**Fixed Effects**	**df**	**F**	**P**
Plasma T ($nmol.l^{-1}$) Autumn 0 to Autumn 1	Time	1,39.5	78.24	< 0.0001
	Treatment	1,50.5	62.21	< 0.0001
	Time*Treatment	1,39.5	48.55	< 0.0001
Plasma T ($nmol.l^{-1}$) Autumn 1 to Spring	Time	1,21.1	4.11	0.056
	Treatment	1,34.5	30.29	< 0.0001
	Time*Treatment	1,21.1	88.33	< 0.0001
Plasma T ($nmol.l^{-1}$) Autumn 0 to Spring	Time	1,31.9	177.71	< 0.0001
	Treatment	1,37.9	2.43	0.1277
	Time*Treatment	1,32.6	3.16	0.085

Effects of testosterone implant treatment on parasite infections

At the time of initial capture (i.e. prior to implant treatment) *T. tenuis* levels varied from 4 to 12225 worms per bird. Prior to treatment, differences between study site significantly explained variation in *T. tenuis* (Genmod; $X^2 = 45.38$, d.f. = 5, $P < 0.0001$). There was a significant effect of male age on parasite intensities, with old males having significantly more *T. tenuis* ($X^2 = 16.87$, d.f. = 1, $P < 0.0001$) than young males. There was also a significant study site*age interaction for *T. tenuis* infections ($X^2 = 11.88$, d.f. = 5, $P < 0.05$) which indicated that differences in infection levels of *T. tenuis* between old and young birds were not consistent across sites. There was no effect of sampling date on *T. tenuis* intensity ($X^2 = 0.70$, d.f. = 1, $P = 0.40$).

Generalised Linear Models were used to test for differences between treatments groups in *T. tenuis* worm intensities 1) prior to treatment (Autumn 0), 2) six months later (Spring) and 12 months later (Autumn2). Where necessary, models controlled for site and age effects. At the time of initial capture, there was no difference between C-Males and T-Males in infection levels of *T. tenuis* ($X^2 = 1.02$, d.f. = 1, $P = 0.31$) (Figure 7.2, Table 7.3). Following anthelmintic treatment and all birds receiving a dose of c. 2000 infective larval, in the spring, there were still no differences between C-Males and T-Males in intensities of *T. tenuis* (Figure 7.2, Table 7.3; $X^2 = 0.21$, d.f. = 1, $P = 0.65$). One year on from the hormone treatment (c. 11 months following the *T. tenuis* larval challenge), a significant difference was found in intensities of *T. tenuis* infection between C-Males and T-Males in the second autumn (Autumn 2), ($X^2 = 5.11$, d.f. = 1, $P < 0.05$). T-Males had significantly more *T. tenuis* worms than C-Males (Figures 7.2, Table 7.3).

Table 7.3: Results of effect of treatment on plasma testosterone and parasite infection levels and mean values prior to treatment and at points of re-capture (c. 1 month and 6 months after treatment). Sample size, in brackets, refers to number of individual males. Plasma T and *T. tenuis* egg counts are expressed as geometric means $\overset{\times}{\underset{\div}{-}}$ SD.

		Treatment	
Parameters	**Time***	**Control**	**Testosterone treated**
Plasma T (nmol.l^{-1})	Autumn 0	1.61 $\overset{\times}{\underset{\div}{-}}$ 0.07 (18)	2.18 $\overset{\times}{\underset{\div}{-}}$ 0.15 (17)
	Autumn 1	2.25 $\overset{\times}{\underset{\div}{-}}$ 0.27 (13)	8.61 $\overset{\times}{\underset{\div}{-}}$ 0.22 (13)
	Spring	4.82 $\overset{\times}{\underset{\div}{-}}$ 0.18 (9)	5.36 $\overset{\times}{\underset{\div}{-}}$ 0.17 (10)
T. tenuis (worm.bird^{-1})	Autumn 0	1319 $\overset{\times}{\underset{\div}{-}}$ 0.74 (57)	1151.6 $\overset{\times}{\underset{\div}{-}}$ 0.89 (58)
	Spring	349.5 $\overset{\times}{\underset{\div}{-}}$ 0.74 (38)	278.7 $\overset{\times}{\underset{\div}{-}}$ 0.83 (34)
	Autumn 2	1069.7 $\overset{\times}{\underset{\div}{-}}$ 0.39 (23)	2013.5 $\overset{\times}{\underset{\div}{-}}$ 0.40 (25)

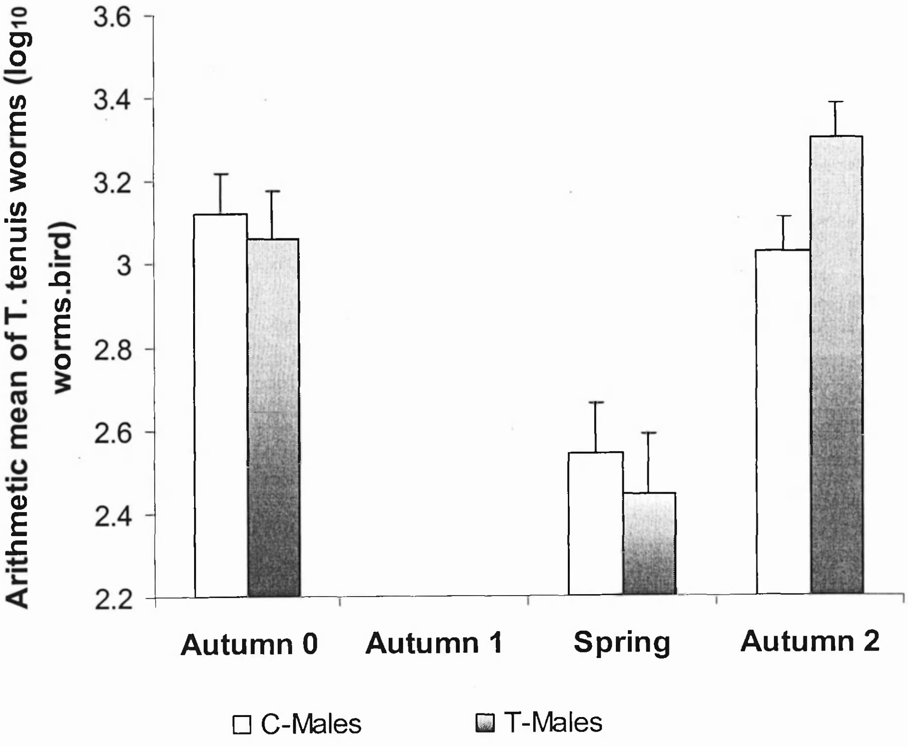

Figure 7.2: Arithmetic mean (+S.E.) for *T. tenuis* infections (log worms.bird^{-1}) for inert and testosterone treated males for Autumn 0 (N = 115), Spring (N= 72) and Autumn 1 (N= 48).

7.5 Discussion

This study provides experimental evidence for a cost associated with elevated T levels in male red grouse. Males with elevated T had higher *T. tenuis* parasite infections one year later despite the fact that the effect of testosterone treatment on circulating T levels was no longer detectable after 6 months. Below I discuss these findings and their implications for our understanding of the role that parasites might play in shaping aspects of red grouse life-history traits and host population dynamics.

Relationships between testosterone and parasites

I found that parasite infections of males with artificially elevated levels of plasma T were higher than those of control males, 1 year after treatment. These results add to the growing body of evidence that increased levels of testosterone lead to increases in parasite infection intensities (Saino *et al.,* 1995; Zuk *et al.*, 1995; Eens *et al.,* 2000; Duckworth *et al.,* 2001; Hughes & Randolph, 2001a).

I found no difference in parasite levels either 1 month or 6 months after implanting (see also Mougeot *et al*, 2004; Chapter 6). A possible explanation to account for the difference between studies may be due to the length of time taken for *T. tenuis* infections to increase. Ingested *T.tenuis* infective larvae have been shown to cease development and enter into a state of hypobiosis during late autumn/winter, resuming development in the spring (Shaw, 1988), which may explain why no difference in intensity of parasite infection between hormone treatment groups was observed in this study until the following autumn ca 12 months after T-treatment and the larval challenge.

So, what mechanisms lead to red grouse males with experimentally increased steroid levels having higher parasite infections? In free-ranging animals, there are two potential pathways. First, testosterone may increase susceptibility to parasites by directly or indirectly, lowering an individual's immunocompetence (Hillgarth & Wingfield, 1997). In previous studies, male red grouse with experimentally elevated T had reduced T-cell-mediated immunity (Mougeot *et al.*, 2004; see Chapter 6). Immunosuppression by T has also been shown in a number of other bird species (Saino *et al.,* 1995; Zuk *et al.*, 1995; Al-Afaleq & Homeida, 1998; Verhulst *et al.*, 1999; Duffy *et al.*, 2000; Peters, 2000 but see Hillgarth & Wingfield, 1997; Hasselquist *et al.,* 1999). Little is currently known about the types of mechanisms of resistance associated with *T. tenuis* infections in red grouse. However, recent advances in the field of immunoparasitology have significantly enhanced our knowledge and understanding of the numerous and highly complex effector mechanisms involved in host protective immune defence against gastrointestinal nematodes (reviewed in Onah & Nawa, 2000). Although care must be taken in extrapolating from one host-parasite system to another, laboratory studies of rodents have been widely used as models of livestock and human gastrointestinal infections (Onah & Nawa, 2000), and may help to define the types of mechanisms involved in immune defence against *T. tenuis* in red grouse.

Second, testosterone, through its effect on behavioural traits such as increasing levels of aggression, movement and display rates, may also increase an individual's exposure to, and contact with parasite infective stages (Randolph, 1977; Klein, 2000; Hughes & Randolph, 2001b). In another gallinaceous species, the ring-necked pheasant (*Phasianus colchicus)*, wattle size reliably indicates male testosterone levels at the beginning of the breeding season (Papeschi *et al.*, 2003) and males with well-developed wattles attracted more females and established territories early in spring, prior to the onset of feeding season of the ecto-parasitic ticks (*Ixodes* *ricinus*), whereas less attractive males were forced to roam greater distances in search of females (Hoodless *et al.*, 2001). However, in red grouse, testosterone increased levels of aggression which in turn leads to increased territory size and a decline in population density (Watson & Parr, 1981; Moss *et al.*, 1994; Mougeot *et al.* 2003a). Male mountain spiny lizards (*Sceloporus jarrovi*) given T have also been observed to feed less (Klukowski *et al.*, 2001). Both these factors would be likely to reduce rather than increase an individuals exposure to infective stages (Hughes & Randolph, 2001b). Thus, it seems most likely in the case of red grouse, that the mechanism increasing susceptibility to parasite infection is physiological rather than behavioural.

The role of parasites and the immune system in host life history and population dynamics.

The role of parasites in life-history theory has gathered increasing attention (Forbes, 1993; Møller, 1997; Owens & Wilson, 1999; Dawson & Bortolotti, 2001) and evidence from this study, suggests that the parasite *T. tenuis* and parasite defence may play an important role in life-history trait trade-offs faced by male red grouse. Testosterone levels of T-implanted males in this study were elevated to levels significantly higher levels than in control birds, which allowed the costs of testosterone to become apparent. However, the absence of a relationship in this study between testosterone and parasites in males prior to treatment in this study indicates the self-regulatory aspect of testosterone levels in individuals. It is also consistent with the immunocompetence handicap hypothesis, in that males with higher levels of T did not pay higher costs in terms of parasites than males with lower levels of T (Folstad & Karter, 1992). Other studies have also reported a lack of association between testosterone levels and parasite resistance in natural populations (Weatherhead *et al.*, 1993; Saino & Møller, 1994, Duckworth *et al.*, 2001). From a female mate choice perspective, male red grouse that exhibit exaggerated T dependent ornaments appear to be reliably signalling their ability to cope with the detrimental effects of high T on the immune system (see also Mougeot *et al.*, 2004). A number of studies also support this theory (Grossman, 1985; Saino *et al*, 1995; Hughes and Randolph, 2001 a,b).

Results from this study suggest that periods of high aggressiveness requiring higher T levels, could contribute to increasing parasite intensities in the next year. A key concept of life history theory is that individuals respond to environmental stresses and perceived risks through behavioural, physiological and anatomical adaptations (Ricklefs & Wikelski, 2002). As such, individuals must adapt to a constantly evolving social environment, and in order to survive and reproduce, must be able to account for the costs through a trade-off in the allocation of resources to fitness components (Stearns, 1989). In red grouse agonistic interactions and the expression of secondary sexual ornamental combs during the autumn and spring, necessary for reproductive success, are T dependent (Moss *et al.,* 1979; Mougeot *et al.*, 2004). Results suggest that, if a male red grouse increases investment in reproduction through elevating levels of T necessary for the expression of sexual ornamental combs and aggressive territorial defence, this may elicit a trade-off in resources available for immune-defence and will result in increased susceptibility to parasitism. Parasites are costly because they have the potential to extract nutrients from their host (Price, 1980; Delahay *et al.*, 1995), so increases in intensity of parasite infection have the potential to negatively impact on the availability of resources available for future reproduction (Møller, 1997).

A central premise of life-history theory is that investment in current reproduction is made at the expense of investment in future reproduction (Norris *et al.*, 1994; Sheldon & Verhulst, 1996). This study showed that elevated levels of T increases *T. tenuis* infections, so males increasing investment in current T-dependent reproductive activities may have to bear the subsequent cost of not only potentially detrimental pathophysiological effects of increased intensities of parasites (Wilson & Wilson, 1978; Wilson, 1983), but also the effect of parasites on future reproductive success. Folstad & Karter (1992) suggested that the development of testosterone-dependent signals is a plastic response in that individuals can only exhibit such signals by producing high levels of testosterone and thus can do this only if they have a low parasite infection. Fox and Hudson (2001) showed that when parasites were removed from male grouse, aggressive behaviour was enhanced, suggesting that parasites at some level, may limit aggression and thus reproductive activity. Similarly, Duckworth *et al.* (2001) observed that there was a tendency for male house finches (*Carpodacus mexicanus*) infected with coccidia to have lower levels of testosterone than disease-free males. Thus, the relationship between testosterone and parasites is a complex one and identifying the circumstances in which aggression or parasites may dominate, requires further experimental work.

This study has also highlighted a number of other areas for future work. This experiment showed that elevated levels of the hormone T led to greater parasite infection in male red grouse. Further experimental work is required to support the assumption that the mechanism influencing susceptibility is the impact of testosterone on the immune system. Similarly, little is currently known about the mechanism through which T may affect the immune system. Recent studies have highlighted the potentially important role of corticosteroids as a mechanism through which T may be acting (Duffy *et al*, 2000; Evans *et al.*, 2000). The possible role that corticosteroids play in suppressing the immune, requires further investigation.

Overall, the results from this study have important implications for understanding the population dynamics of red grouse and, in particular, for furthering our understanding of which factors may be important in driving cyclic population fluctuations. To date, studies have focussed on identifying the relative importance of extrinsic and intrinsic mechanisms, both of which may function in a density-dependent manner, to influence recruitment of young grouse into the population (Watson *et al.*, 1984, 1994; Mountford *et al.*, 1990; Moss *et al*, 1994, 1996; Hudson *et al.*, 1992, 1998; Matthiopoulos *et al.,* 2003; Mougeot *et al.,* 2003). Whilst intrinsic factors such as density-dependent changes in male aggressiveness involving T and kin-facilitation have been recognised as being important (Moss & Watson, 2001, MacColl *et al.*, 2000; Mougeot *et al.*, 2003), the role of parasites as an extrinsic mechanism driving cycles has focussed on the direct impact of parasites on females reproductive output (Hudson, 1986b; Hudson *et al.*, 1992; Hudson *et al.*, 1998). One way that the two mechanisms may interact is through a trade-off between reproductive behaviour and parasite defence via the immune system in males (Deerenberg *et al.*, 1997).

Previous studies have demonstrated that changes in population densities of red grouse throughout the cycle are accompanied by density-dependent changes, both in levels of aggression (Watson *et al.*, 1984, 1994; Moss *et al.*, 1979,1994, 1996; Mougeot *et al.,* 2003) and in levels of infection intensities of parasites (Hudson *et al.*, 1992; 1998). By increasing the susceptibility of individual males to parasite infection, the overall impact of elevated T. on the host population may be to reduce aggregation of *T. tenuis*. The degree to which *T. tenuis* are aggregated within red grouse populations has implications for the dynamics of this parasite-host system. Instead of just a few individuals harbouring the majority of the parasite population, *T. tenuis* will be distributed amongst a greater number of individuals. Where distribution of parasites within the host population is less aggregated and is more random or regular, host-parasite interactions tend to be de-stabilised, and a greater proportion of the host population will be affected by the impact of parasites (Anderson & May, 1978; May & Anderson, 1978; Hudson & Dobson, 1995; Jaenike, 1996).

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Chapter 8

General discussion

General discussion

8.1 Introduction

The relationship between a macroparasite and its host is a highly complex, constantly evolving one. As such, the intensity of an individual host's parasite infection will reflect the outcome of a variety of interactions, involving both host-related factors such as host physiology, behaviour, and habitat, as well as parasite-related factors, such as parasite density, pathogenicity and life-cycle (Anderson & Gordon, 1982; Wakelin, 1984; Barnard *et al.*, 1993; Grenfell & Dobson, 1995; Boulinier *et al.*, 1996; Poulin, 1996; Nordling *et al.*, 1998; Gandon *et al.*, 2002; Wilson *et al.*, 2002). Given the heterogeneities that exist between individual hosts, not all hosts within a population will be infected by parasites to the same degree. Establishing patterns of parasite infection that occur within individual hosts, and identifying and isolating the complex interactions that may create them, is not only of interest to epidemiologists, ecologists and evolutionary biologists, but may also have wider social and economic implications (Dobson & Grenfell, 1995). This thesis represents an attempt to understand some of the patterns and associated processes acting to create heterogeneities in parasite infection in individual red grouse (*Lagopus lagopus scoticus*), a species of ecological and socio-economic importance.

Experimental manipulation of parasites provides the best test of host-parasite interactions and this was successfully accomplished through the use of both larvae culturing and challenging techniques (to increase parasite numbers) and the use of an anthelmintic drug (to remove parasites), developed for use in domestic livestock (in Chapter 7).

Furthermore, advances in the use of parasitological statistical analyses (in Chapter 4), hormone implanting techniques (in Chapters 6 & 7) and methods to measure aspects of immunocompetence (in Chapter 6), provided the necessary tools with which to further our understanding of the intriguingly complex interactions between red grouse and *T. tenuis*. In addition, the findings from this thesis have relevance in general for the fields of parasitological methodology, population biology and evolutionary ecology, and as such, the aim of this chapter is to discuss the wider implications of this work, and to highlight areas for future research.

8.2 Implications of thesis findings

Parasitological methodology

Central to this thesis, and indeed parasitological studies in general, is the need to make accurate quantitative measurements of parasite infection intensities in living hosts. This was achieved through calibration of the methodology used to estimate intensities of *T. tenuis* worm infection of red grouse from faecal egg counts (Seivwright *et al.*, 2004; Chapter 3). For methods of parasite sampling that rely on faecal egg counts, a primary concern is that factors such as density-dependent constraints on worm fecundity (Anderson & Schad, 1985; Smith *et al.*, 1987; Tompkins & Hudson, 1999) or seasonal variation in worm egg production rate (Hudson, 1986a; Shaw & Moss, 1989a) may create unreliable estimates of worm infection intensity. Findings from Chapter 3 (Seivwright *et al.*, 2004), however, were in agreement with earlier findings of Moss *et al.* (1990), and provided evidence that faecal and caecum egg counts can be used to reliably estimate worn infection intensity in red grouse at least up to an observed maximum of c. 8000 worms. Furthermore, this method can be reliably used both during the spring and autumn, the seasons most commonly used for parasite sampling in red grouse.

These findings are important not just for ecological studies of this host parasite system, but have practical implications for managers of commercial grouse moors. Traditionally, managers have used direct post-mortem counts from birds shot in the autumn, which are costly and time-consuming to perform. The use of faecal egg counts provides an opportunity to reliably monitor parasite infection intensities from living hosts on a regular basis, and may thus provide important information regarding the overall health of the population. In particular, the use of faecal eggs counts may enable managers to make informed decisions on whether to undertake costly anthelmintic treatment programmes.

Patterns of infection in red grouse

Identifying underlying patterns of individual parasite infection, may provide important clues to unravelling and understanding complex host-parasite interactions (Sheldon & Velhurst, 1996; Hughes and Randolph, a, b, 2001). Since red grouse are highly territorial, transmission of *T. tenuis* between individual hosts is thought to be extremely effective (Shaw & Moss, 1989a). Breeding pairs of male and female red grouse spend a significant proportion of the year feeding and roosting in close proximity to one another. It was therefore predicted that individuals within pairs would be exposed to similar levels of infective larval stages, and would thus share similar levels of infection. This was not, however, reflected in the heterogeneities of infection observed between individuals within pairs (Chapters 4 & 5). Whilst heterogeneities in parasite exposure may have been operating at a microhabitat scale, a combination of both exposure and genetically determined resistance, as previously identified from experimentally manipulated parasite infections of captive grouse (Wilson, 1979; Shaw & Moss, 1989b; Moss *et al.*, 1993), seems likely to have contributed to heterogeneities of infection between pairs. Similar analyses, investigating patterns of infection between individual chicks from within the same family, however, also showed that infection intensities of genetically similar chicks varied considerably. Whilst it is likely that both exposure and host resistance are important in determining an individual's parasite infection, it is also likely that a range of other factors such as differences in host nutrition, physiology or behaviour may also be acting to influence an individual's parasite infection (Crofton, 1971; Anderson & Gordon, 1982; Apanius & Schad, 1994; Solomons & Scott, 1994; Hudson & Dobson, 1995; Shaw & Dobson, 1995; Beck & Levander, 2000; Ezenwa, 2003). The information we can extrapolate from "noisy" patterns of infection, such as those described above, is clearly limited. Experimental manipulations, such as the cross-fostering experiment attempted in 2002 (Chapter5), however, provide a more powerful tool to tease apart and evaluate the influence of exposure and parasite resistance.

One aspect of parasite infection examined in this thesis that highlighted new and interesting results a difference in infection between sexes (Chapter 4). Contrary to previous studies, which found no overall sex differences in parasite infection levels between males and females (Hudson, 1996a), young male red grouse had higher infection intensities than young females. This pattern between the sexes was also found for all age classes of males and females at one particular study site in northern England (Moorhouse). One explanation to account for the differences in findings between this study and that of Hudson, (1996a) may be related to the time of year that parasite sampling took place. Parasite sampling in this study took place in the spring, whereas parasite sampling in Hudson's study was carried out on shot birds in the autumn. Differences between the sexes at different times of the year, may reflect seasonal physiological differences between the sexes. For example, sex differences in parasite infections have been suggested to arise from physiological differences between the sexes in levels of circulating steroid hormones, such as testosterone (Wilson *et al.*, 2002). Elevated testosterone levels are often required during breeding periods for the expression of sexual traits and behaviour, but might also be associated with physiological costs, such as impaired immune function, an individual's main defence against parasites (Folstad & Karter, 1992; Grosmann, 1985; Hillgarth & Wingfield, 1997).

In red grouse, agonistic interactions and the expression of secondary sexual ornamental combs during the autumn and spring are necessary for reproductive success. The expression of aggressive behaviour and combs are also testosterone dependent (Moss *et al.,* 1979; Mougeot *et al*., 2004). By experimentally elevating levels of testosterone in male red grouse, Chapters 6 and 7 provided evidence, that elevated testosterone reduced aspects of immune function (Chapter 6), and also increased *T. tenuis* intensities in males, one year after implanting (Chapter 7). The question arises, however, that if elevated levels of testosterone lead to increased levels of parasite infection, why did males not have higher intensities of parasite infection than females across all age classes and at all sites? (Chapter 4). A possible explanation may lie in life-history theory.

An individual must allocate limited resources optimally between competing life history activities such as such as growth, reproduction and parasite defence (Stearns, 1989,1992; Roff, 1992; Owens & Wilson, 1999). Trade-offs made in the allocation of resources, will be dependent on, and will reflect, changes in environmental conditions. Territory establishment and defence is an essential requirement for male red grouse, not only for reproductive success but also for survival. At low population densities, territories are not a limited resource and aggression levels between males are relatively low. Males, therefore, may not be required to invest so heavily in reproductive activities and may allocate more resources to maintaining parasite defence. As population density increases, territory sizes become smaller and therefore, more limiting, and male aggressiveness and ornamental comb size, which are both testosterone dependent, increase

(Moss *et al.*, 1979; Watson *et al.*, 1994; Moss *et al.*, 1996). Males may be increasingly forced to invest in reproduction by elevating their testosterone levels to the extent that susceptibility to parasites becomes an inevitable cost. Such a cost may be particularly apparent in males where population densities reach peak levels, as in the case of Moorhouse. Similarly, young males may have higher *T. tenuis* infections than young females, as young red grouse males may need to elevate their testosterone levels to facilitate levels of aggressiveness in order to secure a territory in their first breeding season. Not only will they have to compete against other young males, but they will also have to compete against old males that have already established a territory, either earlier that autumn or in the previous year. Again, young males may be forced to bear the cost of susceptibility to parasites through investing heavily initially, to increase their chance of gaining a territory and reproducing. Thus, given the deleterious and potentially lethal effects of increased parasite intensities on their hosts, red grouse males may be forced to invest in current reproduction at the expense of investment in future reproduction; a central premise of life-history theory (Norris *et al.*, 1994; Sheldon & Verhulst, 1996).

Parasites and sexual selection

Since the seminal work of Hamilton and Zuk (1982), the role that parasites might play in sexual selection has become increasing interesting to evolutionary ecologists (Zuk, 1992). In the process of examining patterns of parasite infection between male and female red grouse and the role that *T.tenuis* might play in sexual selection and mate choice (Chapter 5), several interesting findings were revealed. First, red grouse were found for the first time, to exhibit positive assortative mating with respect to condition. Second, in old and young females and in young males, the size of an individual's combs was an indicator of an individual's condition. Third, males can benefit from pairing with females with the largest combs (and in better condition), through increased reproductive success.

One hypothesis regarding the role of ornamental traits in males is that they may function as indicators of health and resistance to parasites. By mating with a male with elaborate ornaments, a female may gain direct fitness benefits through decreased risk of exposure to contagious parasites (the 'parasite avoidance' hypothesis) (Borgia, 1986; Borgia & Collis, 1989). Different chapters in this thesis however, gave varying results about the relationship between combs size and parasite infection in male red grouse. Whilst results from Chapter 5, found that parasite infections were positively correlated with comb size in male red grouse, Chapter 6 found no relationship between *T. tenuis* and comb size. Whilst both these findings rule out the parasite avoidance hypothesis, an alternative explanation is that ornamental combs in males may have been signalling a male's ability to cope with or resist parasites, rather than their parasite burden (see also Mougeot & Redpath, in press)

Models of sexual selection argue that for ornaments to function as cues of male quality they must be honest signals and must be costly, to prevent males from cheating (Zahavi, 1975; Grafen, 1990). The 'Immunocompetence Handicap Hypothesis' (Folstad and Karter, 1992) proposes that, although males benefit from sexual attractiveness or increased competitive ability derived from elevated testosterone levels, they are also exposed to a physiological cost, from testosterone-related suppression of the immune system. This cost would prevent cheating and males who exhibit exaggerated testosterone dependent ornaments or behaviour, should be reliably signalling to potential mates, their ability to cope with the potentially detrimental consequences of high testosterone (Saino *et al.*, 1995).

By experimentally manipulating testosterone levels of male red grouse, Chapter 6, provided conclusive evidence to support several key assumptions of the Immunocompetence Handicap Hypothesis in red grouse. This chapter demonstrated, that the ability to mount an immune response is both condition and testosterone dependent, and is signalled by comb size. A key finding was that males with bigger combs (and thus more testosterone) had greater cell-mediated immunity, despite immunosuppression by testosterone. Males with artificially elevated testosterone had larger combs, but lost more condition and had lower T-cell-mediated immunity, than their comparative controls. In both chapter 6 and 7, males with elevated testosterone also had higher infections of both coccidia (Chapter 6) and *T. tenuis* (Chapter 7). Thus, it was demonstrated that males benefit from high testosterone in terms of increased expression of their sexual ornaments, but also suffer a cost in immunosuppression and increased parasites.

According to the Immunocompetence Handicap Hypothesis, the costs that males pay for elevated testosterone depends on the detrimental effects of parasites or the ability to cope with levels of parasite infection. Males with comparatively low susceptibility to parasites or high resistance to parasite should pay a lower cost, other things being equal, for higher levels of T (Folstad & Karter, 1992). Results from Chapters 5 and 6 provided contrasting evidence for this hypothesis. In support, no relationship was found between *T. tenuis* and comb size (Chapter 6). Whereas, results from Chapter 5 were contradictory, with results showing a positive correlation between male comb size and parasite infection. Other studies have also found positive correlations between sexual traits and parasites loads (reviews in Folstad & Karter, 1992; Møller *et al.*, 1999). A recent paper by Getty (2002) argues that although signals may be positively, negatively or non-related to parasites, the Folstad-Karter (1992) immunocompetence signalling or the Hamilton-

Zuk (1982) coevolution models should not be automatically refuted. Instead, relationships should be ideally tested through experiments.

The suggestion that the development of testosterone-dependent signals is a plastic response merits further investigation in red grouse. Folstad & Karter (1992) proposed that individuals can only exhibit testosterone signals by producing high levels of testosterone and thus can only do this if they have a low parasite infection, whilst Fox and Hudson (2001) showed that when parasites were removed from male grouse, aggressive behaviour was enhanced, suggesting that parasites at some level, may limit aggression and therefore comb size.

Implications of thesis findings for host populations dynamics

The extent to which an individual is exposed, resistant and susceptible to parasites, is of particular interest to understanding host population dynamics (Anderson & Gordon, 1982; Wilson, 1983; Anderson, 1993; Boulinier *et al.*, 1996; Holmstad & Skorping, 1988; Wilson *et al.*, 2002). These mechanisms will all influence the extent to which parasites will be aggregated within the population, and therefore the extent to which parasite aggregation will have stabilising or de-stabilising effects on the host population. Identifying the mechanisms important in driving regular oscillations in population dynamics, such as those typically exhibited by many grouse populations, has been the central premise of considerable theoretical and empirical research programs over the past 6 decades (Batzli, 1992; Oli & Dobson, 2000; Bjornstad & Grenfell, 2001; Moss & Watson, 2001). Studies of red grouse population dynamics have, to date, identified two distinct mechanisms: changes in male aggressiveness involving testosterone and kin-facilitation (Moss & Watson, 2001, MacColl *et al.*, 2000; Mougeot *et al.*, 2003a,b) and the direct impact of parasites on a female's reproductive output (Hudson, 1986b; Hudson *et al.*, 1992; Hudson *et al.*, 1998). Both these mechanisms have been shown independently, to function in a delayed density-dependent manner and to influence recruitment of young grouse into the population (Watson *et al.*, 1984, 1994; Mountford *et al.*, 1990; Moss *et al*, 1994, 1996; Hudson *et al.*, 1992, 1998; Matthiopoulous *et al.,* 2003; Mougeot *et al.,* 2003). Until now, the interaction between these two mechanisms had received little attention. The key finding from Chapter 7, that experimental elevation of testosterone causes increased parasite infection of implanted male red grouse, provides a new perspective on how these two mechanisms, testosterone dependent aggressive behaviour and parasites, may interact.

Previous studies have demonstrated, using mathematical models, how the effect of *T. tenuis* on female red grouse fecundity (Hudson, 1986b; Hudson, 1992), could generate cyclic fluctuations (Hudson *et al.*, 1992; Dobson & Hudson, 1992). It is recognised that the basic mechanism required to generate population cycles in any species is a time delay (Moss & Watson, 2001). The time taken for the development and infection of the parasite within its host creates the delayed impact necessary to cause cyclic dynamics. An alternative hypothesis proposes that changes in aggressiveness lagging behind density, and affecting recruitment into the population, causes the cycles. Findings from Chapter 7 showed that the effect of hormone treatment on parasite infections in males was not immediate and that there was a time lag of a year before the increases in *T. tenuis* infection became apparent. The lack of a correlation between parasite infections of paired males and females (Chapters 4 and 5), is suggestive of two scenarios; either that innate resistance in females defends against the effects of increased male parasite infection, or, more importantly for cyclic dynamics, that a further time lag may exists until increases in male infection intensities result in increased parasite infection intensities in females. Another theoretical possibility is that the interaction between testosterone and parasites may reduce the level of aggregation of *T. tenuis* at high population densities. Where aggression and testosterone levels increase with population density, a greater proportion of the male population may have lowered resistance to parasite infection. Instead of just a few individuals harbouring the majority of the parasite population, *T. tenuis* will be distributed amongst a greater number of individuals. Where distribution of parasites within the host population is less aggregated, host-parasite interactions tend to be de-stabilised (Anderson & May, 1978; May & Anderson, 1978; Hudson & Dobson, 1995; Jaenike, 1996). If levels of parasite aggregation were to change according to population density, quantification of this rate of change may be an important parameter to incorporate in future mathematical demographic models. The ability to quantify the contribution of individual-related processes to a population scale dynamics is not only of mathematical and ecological interest, but may also have practical implications in the fields of conservation and wildlife management.

Red grouse, are a species of high importance to upland areas of Scotland and the north of England not only due to their economic status as commercially harvestable game bird, but also because the habitat upon which they depend, heather (*Calluna vulgaris*) moorland, is also of considerable conservation importance. The active management of heather moorland benefits not only the red grouse but also provides an important breeding or feeding habitat for 57 bird species, of which 12 are of European importance (Thomson *et al.*, 1995). From the 1870s onwards sporting estates thrived, but a decline in their economic value has resulted in fewer gamekeepers to manage the habitat, and rising sheep numbers coupled with falling standards of muirburn have contributed to a decline in the heather cover upon which grouse are dependent (Watson & Lance, 1984). A contributing factor to the economic fragility of sporting estates, is that red grouse populations fluctuate over periods of many years. Many estates rely on income derived from driven grouse shooting which requires a minimum population density of 60 birds per km^2 to be successful. By identifying the key individual-related processes involved in driving cyclic population dynamics, it may be possible to intervene at a management level to manipulate and alter red grouse population dynamics. A more predictable harvest from a greater stability in population density could lead to improved economic sustainability, which would benefit not only the currently fragile rural economy but would also ensure the continued management of a conservationally important, biodiverse habitat.

From the scientific evidence we have now, it seems that a traditional gamekeeping practice of removing old territorial males in the autumn at the end of the shooting season, may hold some validity in its intention to prevent population crashes. In addition to the experimental evidence from Moss *et al.* (1996) who successfully prevented a population crash by removing old territorial males experimentally, a recent study has highlighted the importance of sexually mature males in the transmission of parasites. This quantitative study on transmission patterns of tick (*Ixodes ricinus*) infection in the yellow-necked *mouse (Apodemus flavicollis*) highlighted that within a population, removal of a cohort of sexually mature males of high body mass representing 26% of the population, dramatically reduced the transmission potential of ticks by 79%. Thus a relatively small proportion of the population is responsible for the greatest proportion of the transmission. If the same holds true for red grouse populations, sexually mature males could be the key driving force in the transmission of *T. tenuis*, particularly when aggression levels within the population are high and testosterone levels are also high. Removal of these cocks may result in reducing impact of *T. tenuis* on the population.

8.3 Future Work

The findings of this thesis have highlighted a number of important areas for future work. Whilst correlative studies are useful in identifying patterns of infection in natural populations, they tell us little about relative importance of the mechanisms involved in creating those patterns. Despite best efforts to experimentally tease apart the variation in an individual's parasite infection attributable to exposure and genetic resistance (Chapter 4), an attempt to carry out a large-scale cross-fostering experiment using red grouse chicks proved unsuccessful due to extraneous factors. The value of this type of experiment for furthering our understanding of what determines an individual's level of infection and tackling the question of whether parasite resistance in red grouse is heritable, however, remains important and should remain a priority for future research. Studies on laboratory and domestic populations, where conditions can be standardized and sources of non-genetic variation controlled for, have provided evidence for heritability of resistance to endoparasites (Wagland *et al.*, 1996; Douch *et al.,* 1995). However, in wild populations, identifying genetic processes responsible for generating patterns of infection distribution has proved very difficult, due to confounding environmental and maternal effects (Anderson & Gordon, 1982; Sorci *et al.*, 1997; Smith *et al.*, 1999). Thus, despite the implications not only for studies of host population dynamics (Hudson & Dobson, 1995) but also for furthering our understanding of the selective pressure of parasites on their hosts (Lysne & Skorping, 2002; Holmstad & Skorping, 1998), evidence for genetic variability in parasite resistance in natural vertebrate hosts is limited to a handful of studies (e.g. Møller, 1990; Bouliner *et al.* 1997; Brinkhof *et al*, 1999, Smith *et al.*, 1999; Appleby *et al.,* 1999; Coltman *et al.*, 2001).

Hopefully experience from this attempt will help this to be achieved. A key assumption of certain theoretical evolutionary models, is that resistance to parasites, is at least partially, genetically determined (Anderson & May, 1982; Hamilton & Zuk, 1982; Folstad & Karter, 1992; Brinkhof *et al.*, 1999). Within the *red grouse-T. tenuis* system, the presence of a heritable component to parasite resistance may have implications not only for expanding our knowledge of how mechanisms of sexual selection may operate in this species, but may also assist in furthering our understanding of factors that may influence red grouse populations dynamics. For example, an important finding in grouse behaviour, confirmed recently through advances in molecular DNA techniques, is the existence of differential male aggressive behaviour towards kin and non-kin (Moss & Watson, 1991; MaColl *et al.*, 2000; Lock, 2004). Through kin-recognition, spatial clusters of genetically related territorial males appear within the population, and this is thought to be an important driving mechanism in population dynamics, through a corresponding decline in aggression with a resulting increase in density. An interesting question to address, therefore, would be whether these clusters of males share similar levels of resistance to parasites (Lewis, 1998).

Other avenues for future work in red grouse lie in exploring in more detail, with the use of experimental tests, aspects of sexual selection in this species. This study described for the first time assortative mating in red grouse, and other findings from Chapter 5 highlighted the novel possibility that ornamental combs in female red grouse, rather than being residual appendages, may actually function as signals of quality, and could be used in mutual mate choice. Furthermore, whilst size of ornamental ornaments was the parameter measured and used in analyses, it may be that other measurable aspects of ornamental comb are also of importance in sexual signalling, such as comb colour and ultra-violet reflectance (Bright & Waas, 2002; Mougeot *et al.*, MS).

Lastly, Chapter 6 demonstrated the suppressive effect of testosterone on cell-mediated-immunity, and Chapter 7 showed that testosterone treatment resulted in higher infection intensities of *T. tenuis*. However, little is currently known about which specific aspects of immune function may be important in resisting or defending against parasite infection in red grouse. Furthermore, recent work has highlighted the role of corticosterone, a hormone typically secreted in response to stress, as a possible alternative mechanism causing immune suppression (Evans *et al.,* 2000). Elevated testosterone may be associated with increased aggression and courtship behaviors which are energetically costly and which may be physiologically stressful (Dufty, 1989).

8.4 Conclusions

It is hoped that this thesis has increased our knowledge of patterns of *T. tenuis* infection in individual red grouse and that through a combination of correlative and experimental techniques, has contributed to furthering our understanding of the factors which may influence those patterns. It is also hoped, that whilst the findings from this thesis are specific to the red grouse-*T. tenuis* host parasite system, they may also be recognised as having relevance in the wider fields of life-history theory, sexual selection theory and population dynamics.

Within the field of population dynamics, there has been growing interest in use of 'bottom-up' individual-based models rather than population based ones, to examine patterns of population dynamics (DeAngelis & Goss, 1992; Goss-Custard & Sutherland, 1997; Pettifor *et al.*, 2000; Stillman, *et al.*, 2001; Johansson & Sumpter, 2003; Stillman, 2003; Persson & De Roos, 2003).

The important interplay of the various factors discussed and revealed throughout this thesis it is hoped, will be integral for developing such models, which would aim to link individual patterns to population processes in red grouse and in other species. Studies of red grouse population dynamics are reaching a fascinating and pivotal point in their long history. Recent advances in fields such as immunology and molecular genetics have enabled exciting breakthroughs to be made in studies of red grouse cyclic dynamics by paving the way to test empirically the many theoretical models and ideas that have developed over the decades since grouse research began at the beginning of the 1900's. Whilst long-term studies, such as those on red grouse, provide us with an invaluable opportunity to identify long-term processes and anomalous events, their virtue also lies in giving models and short-term studies, such as those presented in this thesis, better meaning and context within the real world (Moss & Watson, 1991; Grenfell *et al.*, 1995).

8.5 References

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