SELECTION AND GENETIC VARIATION OF WEAPONRY IN A LARGE MAMMAL

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ABSTRACT

Understanding the maintenance of the variation that is typically observed in natural populations has been a central aim of evolutionary biology. In a feral population of Soay sheep on the island of Hirta, St.Kilda there is a phenotypic polymorphism for horns with males growing either normal or reduced (scurred) horns, and females growing either normal, scurred or no (polled) horns, with further variation in horn size within each of the horn types. This thesis examines the potential factors which maintain these polymorphisms. I first present an overview of the literature relating to the factors that potentially maintain variance in traits in natural populations. In chapter two I present an analysis that suggests that polymorphisms in both horn type and horn size may be maintained by trade-offs between allocation to reproductive success and survival in males, and by sexually antagonistic selection between males and females. In chapter three I test the hypothesis that female weaponry may convey an advantage in intrasexual conflicts over resources, rather than just being expressed as a consequence of genetic associations with the male phenotype. Chapter four examines the environmental factors which create variation between individuals in their horn length, revealing that individuals vary in response to the environment. In chapter five I investigate whether the temporally fluctuating environmental conditions of St.Kilda generate fluctuating selection on the horn length of normal-horned males, revealing that this mechanism constrains the evolution of horn length potentially maintaining variance. In chapter six I examine the genetic relationships between morphological traits, revealing that these relationships are dependent upon the environmental conditions experienced during the first year of life. Finally, I discuss the wider implications of these findings for our understanding of the maintenance of trait variation in the wild.

DECLARATION

The research described in this thesis is only possible through collaboration, details of which are provided below.

Data
All of the data used in Chapters 2, 4, 5, and 6 has been collected by others as part of a wider project which has been running since 1985. The data used in Chapter 3 was collected by me, with assistance from two volunteers.

Analysis and Presentation of Results
All of the analyses and words contained within this thesis are my own.

Signed:

Matthew R. Robinson

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INTRODUCTION

1.1 GENERAL OVERVIEW

Individuals in natural populations often differ in their survival and fertility, and therefore contribute different numbers of reproductive offspring to the next generation. This contribution of offspring is known as the fitness of an individual. Many traits that individuals display may be associated with an individual's fitness and if so, then it is said that selection operates on those traits. The evolution of any trait depends upon the action of selection, the inheritance of the trait from parents to offspring, and the relationship between that trait and others. If selection acts on a trait in a directional way, for example always favouring larger forms, then we would expect that trait to evolve in the direction of selection (i.e. become larger). As a result, differences between individuals (variation) for this trait should reduce as individuals with larger trait forms will contribute more offspring to the next generation. In wild populations however, we often find high levels of variation in traits that we know are heritable and that we would expect to be under directional selection. Understanding the maintenance of the variation that we typically observe all around us has been a central aim of evolutionary biology.

This thesis attempts to better understand how variation in horn form and size is maintained within a natural population of Soay sheep on the Island of Hirta, St. Kilda, Scotland. I will examine how selection pressures act on horn form and size within males and females (Chapter two), describing how sexually antagonistic selection pressures and trade-offs between components of fitness may shape the variation that we observe. I will demonstrate that female horns are used in

intrasexual competition for resources (Chapter three), suggesting that their expression may not just be a consequence of selection acting in males. I will then concentrate on determining the factors that maintain variation between individuals in horn length by examining the environmental factors which influence horn length (Chapter four), examining the effects of age and the environment on genetic expression and selection pressures (Chapter five), and then by exploring the effects of the environment on genetic relationships between traits (Chapter six). First, I will discuss the genetic and environmental factors which create variation in natural populations and then describe how selection can act to shape that variation. This chapter ends with the aims of this thesis.

1.2 INDIVIDUAL VARIATION

Individuals in natural populations often differ in countless ways. These differences can be discreet, with individuals classified into groups or classes (e.g. male or female), or individuals can differ by such small degrees that there is a continuous scale (e.g. height, size, number of offspring). We can measure continuous traits in order to quantify individual-level differences, and record an individual's value as the phenotypic value for that trait (Falconer and Mackay 1996). Most of the evolutionary changes which occur as natural populations respond to changing environmental conditions are changes in continuous traits, such as the timing of events or changes in phenotypic value. Furthermore, most traits of economic value to plant and animal breeders are continuous traits, such as milk production or body size. Therefore, understanding individual differences has a direct bearing on species conservation and an important application to food production.

So what creates the biological diversity commonly observed in natural populations? Phenotypic differences between individuals can be attributed to the genotype that an individual has, the environment it has experienced, or a combination of both (Falconer and Mackay 1996; Lynch and Walsh 1998). In this chapter, I will concentrate on studies of natural populations and provide an overview of the factors

that generate differences in phenotype, outline how their effects can be determined, and discuss the outcomes of previous work.

1.2.1 Genetic variance and resemblance between relatives

Genetic components of variance are of particular interest because they determine the evolutionary potential of a given character (Falconer and Mackay 1996). DNA sequences that encode for particular products (e.g. RNA and proteins) are referred to as genes, and their chromosomal locations are called loci. Diploid organisms have two 'copies' of each gene and because DNA replication is an imperfect process, mutations arise, and these copies may not be identical. Different forms of a gene are called alleles and gene loci that exhibit more than one allele are said to be polymorphic. At a given locus, different alleles may have different effects on the phenotypic value of a trait and this can be calculated as the deviance of individuals with a particular allele from the population mean. For continuous traits, it is expected that the simultaneous effects of many loci, each with small effects on a given trait, can create a continuous distribution, if individuals differ in the combinations of alleles which they posses. An individual's breeding value or 'additive genetic merit' for a given trait is the total additive effect of its genes on that trait (Falconer and Mackay 1996).

Determining the genetic basis of a continuous trait in wild populations therefore requires examining variation between individuals in that trait. For example, the phenotypic value *y* of individual *i* can be described as:

Equation 1.1 $$y_i = \mu + \alpha_i + e_i$$

where μ is the population mean, α_i is the additive genetic merit of individual *i*, and e_i is the random residual error (environmental effect). This relationship describes the relative effects of genotype and the environment on the deviance of an individual from the population mean. Within a population, variation between individuals in breeding values for a given trait is equivalent to the additive genetic variance of that trait (σ^2_A) and residual errors will have population level variance (σ^2_R). The relative magnitude of these variance components determines the degree of resemblance

between relatives, with the ratio of σ^2_A to the phenotypic variance σ^2_P defined as the heritability (narrow-sense) of a given trait (Falconer an Mackay 1996).
The genetic component of variation can be estimated in many ways, such as parent-offspring regression, full or half-sib designs, or through the use of an 'animal model' (Falconer and Mackay 1996; Lynch and Walsh 1998; Kruuk 2004). For each of these methods, the resemblance between relatives in key to determining the genetic basis of a trait. Relatives inherit copies of the same genes as parents donate one allele per locus to each of their offspring. The average effects of those combined alleles determine the genotypic value of the offspring at a given locus. Here, I will concentrate on describing an animal model, where the genetic variance for a given trait *y* can be estimated as:

Equation. 1.2 $y = \mathbf{X}\beta + \mathbf{Z_a}\alpha + e$

where *y* is a vector of all phenotypic observations across individuals, **X** is a design matrix relating the values of *y* to one or more fixed effect parameters in the vector β, α is a vector describing the additive genetic effects, $\mathbf{Z_a}$ is an incidence matrix relating each of the additive genetic effects to an individuals phenotype and e is a vector of residual effects. This model is a form of mixed model which is typically implemented using restricted maximum likelihood (REML; Thompson and Shaw 1990).

The animal model estimates σ^2_A by comparing phenotypic deviations from the population mean between all pairs of relatives within a population, scaled by their relatedness (Lynch and Walsh 1998). This requires knowledge of the relatedness between individuals and thus of the parentage of each individual within the population. All members of a population are related to each other to some extent as they are all descended from a remote ancestor, but we can define a population's starting point as when we began measuring them (base population) and then determine a pedigree or family tree from that point (Kruuk 2004). If we sample a gene from two randomly selected individuals (*i* and *j*) within a population, the additive genetic relationship (covariance) between them is $2\Theta_{ij}\sigma^2_A$ where Θ_{ij} is the

coefficient of ancestry, the probability that an allele from individual from individual *i* will be identical by descent (inherited directly from the same mutation event) to an allele drawn at random from individual *j*. For example, between parent and offspring $\Theta_{ij} = 0.25$ and therefore the additive genetic covariance is $1/2\sigma^2_A$. We can then gain the matrix **G** (variance-covariance matrix in breeding values) defined as $\mathbf{G} = \mathbf{A}\sigma^2_A$ where **A** is a matrix of relatedness between all measured individuals (with the elements $2\Theta_{ij}$) which is multiplied by the additive genetic variance in the base population.

Phenotypic characters such as morphological traits (e.g. body weight) or life history traits (e.g. reproductive success) are likely to be affected by large numbers of loci (Falconer and Macay 1996; Lynch and Walsh 1998). Mutation creates genetic variance and the higher the mutational target size (number of loci), the greater the expected additive genetic variance (Houle 1992). Because differences between individuals in life-history traits are influenced by numerous heritable morphological and behavioural traits they are expected to show high genetic variance (Price and Schluter 1991). The genetic basis of these characters has been very successful quantified for many years (Falconer and Mackay 1996) and this has success has been increased by the recent application of the animal model in natural populations (Kruuk 2004). Studies have shown significant levels of additive genetic variance within natural populations in traits associated with attracting mates (Merilä and Sheldon 2001; Kruuk et al 2002; Garant et al. 2004; Hadfield et al. 2006), in life-history traits (Kruuk et al. 2000; Pettay et al 2005; Charmantier et al 2006), in morphological traits (Merilä et al 2001; Charmantier et al. 2004; Wilson et al 2005), in behaviour (MacColl and Hatchwell 2003; Duckworth and Badyaev 2007), and in resistance to parasite infection (Coltman et al. 2001). Below, I will outline how many different factors can create variation in phenotype, create resemblance between relatives, and influence genetic expression.

1.2.2 Environmental effects

Changes in environmental conditions experienced by natural population are frequently accompanied by changes in life history and morphological traits,

expressed by individuals within those populations (Stearns 1992; Stenseth and Mysterud 2002). Differences in the environmental conditions experienced by individuals can therefore contribute to phenotypic diversity. Climatic conditions can affect reproduction, survival, and hence population dynamics (Lindström and Kokko 2002; Clutton-Brock and Pemberton 2004). Various environmental factors such as weather conditions, toxins, or parasites may also alter the phenotypic value of morphological traits. Environmental stress may trigger an adaptive diversion of limited resources to somatic maintenance, away from traits not critical to immediate survival (Hoffman and Parsons 1991). For example in natural populations, individual differences in body weight are often associated with climatic conditions (Clutton-Brock and Albon 1989; Pettorelli et al. 2001; Garant et al. 2000) and allocation to antler or horn growth in ungulates may also determined by environmental conditions (Kruuk et al. 2002; Festa-Bianchet et al. 2004; Mysterud et al. 2005). Population dynamics such as the density of individuals may also influence trait value via an effect on, for example, food availability (Schmidt et al. 2001; Kruuk et al. 2002; Herfindal et al. 2006).

Individuals may differ in their susceptibility to climatic variation depending upon their age, sex or the population dynamics they experience and thus environmental effects on phenotypic value may not be constant (Stearns 1992; Roff 1992). Individuals may expend different amounts of energy at different times of the year, may elicit different behaviours, may differ in the costs and timing of reproductive effort, differ in rate of growth, or differ in levels of maintenance required (Stearns 1992). Unfavourable ecological conditions are expected to create greater environmental variance. For example, variation in food abundance may be a greater determinant of an individual's phenotype when food is scarce (Merilä and Sheldon 2001). Individuals may be particularly sensitive to environmental variation at the beginning of life, as there are often large differences between individuals in life-history traits and morphology, which can be attributed to the early environment (e.g. Albon et al. 1987; Kruuk et al. 1999; Lumma and Clutton-Brock 2002).

Individual plasticity is described as variation in the phenotype expressed by a given individual across changing environmental conditions (Pigliucci 2001; Nussey et al. 2007) and individuals may differ in their environmental sensitivity as a result of the environmental conditions that they have previously experienced. Continuous characters may be expressed repeatedly over an individual's lifetime (e.g. reproduction each year, growth, immune response) and if we measure individuals throughout their life, repeated measures will often be gained over a range of environmental conditions that individuals experience. We can then examine changes between individuals in their phenotypic value as a function of the environmental conditions which they experience (Nussey et al. 2007).

Environmental components of variance are therefore unlikely to be static and will vary with all of the factors listed above. The environmental component of trait variation for life-history traits is often a greater proportion of the variance than genetic effects (e.g. Kruuk et al. 2000; Foerster et al 2007). Most of the variation in morphological traits also results from environmental factors (e.g. Milner et al 2000; Coltman et al 2001; Kruuk et al 2002; Jensen et al. 2003; Parker and Garant 2004) and most variation in behaviour may be context dependent (Duckworth and Badyaev 2007). Therefore, determining the effects of the environment on differences between individuals in phenotypic value and how these effects vary is an important component of understanding the biological diversity in natural populations.

1.2.3 Environmental effects and genetic variance

Individuals of a particular genotype may differ in their susceptibility to environmental effects and thus phenotypic differences between individuals attributed to genetic effects may vary across environments (Lynch and Walsh 1998; Hoffman and Merilä 1999). Two situations that may arise from this:

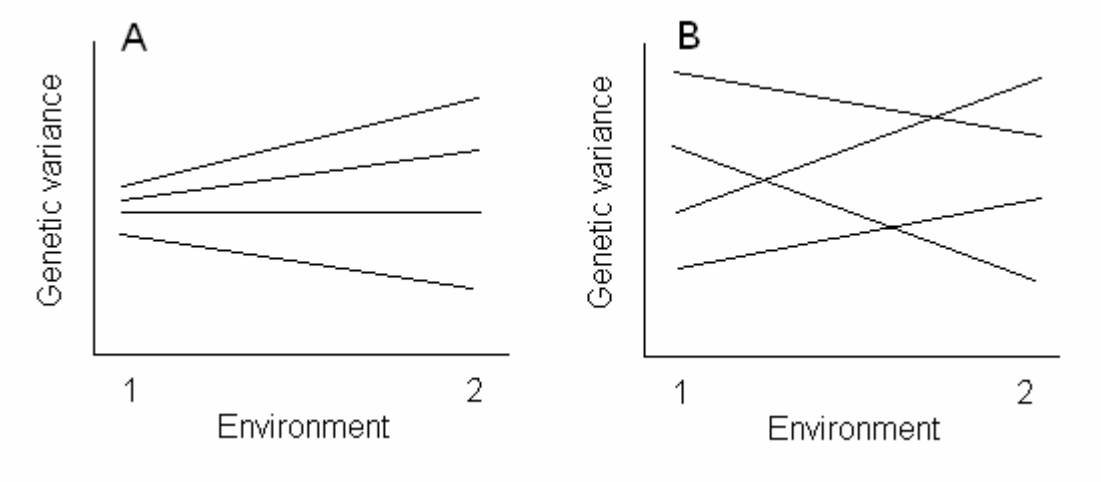

Figure 1.1. Changes in differences between genotypes (lines) between two environments

where either (A) genetic variance is higher in one environment than another and the rankings of different genotypes remain the same; or (B) genetic variance remains constant but the ranking of different genotypes changes between environments (Schlichting and Pigliucci 1993; Hoffmann and Merilä 1999). In the first scenario (A), it is likely that the same loci influence the trait in both environments (genetic correlation of one) but their effects depend upon the environment. For example, the effects of alleles associated with body growth may increase when environmental conditions do not constrain the growth of individuals, which will increase genetic variance in favourable environmental conditions (Merilä and Sheldon 2001). In the second scenario (B), different loci may be associated with a given trait in different environments and thus unlike the first scenario, the genetic relationship between the trait expressed in different environments will be less than one. For example, different loci may contribute to an individual's ability to survive or reproduce as different morphological traits may important in different environments. These scenarios are by no way exclusive and a combination of both may be likely (Hoffmann and Merilä 1999). Both A and B of Figure 1.1 are examples of genotype-by-environment interaction as variation between genotypes changes as a function of the environment. However, only in B will genetic variance be maintained as different loci will influence phenotypic value in different environments

When related individuals are reared in different environments then it is possible to estimate differences in genetic effects between environments (genotype-by-environment interaction: GEI). There is limited evidence of changes in genetic variance across environments for many traits expressed in wild populations (for a review see Charmantier and Garant 2005), but there does appear to be a trend of increasing heritable genetic variance in favourable environmental conditions (Merilä and Sheldon 2001; Garant et al. 2004; Wilson et al. 2006). However, estimates gained in laboratory studies often suggest the opposite of this trend, with differences between genotypes becoming more apparent under stressful environments (Hoffmann and Merilä 1999; Jia et al. 2000). Laboratory studies have shown GEI between genotypic lines to be commonplace for many life-history traits (Mackay 2001; Schmidt and Conde 2006), male signal traits (Jia et al. 2000; Etges et al. 2007; Mills et al. 2007) and host resistance to parasites (Mitchel et al 2005). GEI for a life-history trait would maintain genetic variance in the trait, as it implies that there may not be a single fittest genotype in all environments (Falconer and Mackay 1996). More studies examining these effects in natural populations are needed before we have a clear picture of any trends, if they exist.

Alternatively, we can also consider GEI within an individual, across different environmental conditions experienced throughout its life to determine if individual plasticity in a trait, in response to changing environmental conditions, has a genetic basis. Individuals may differ in the effects of the environment on their physiological condition depending upon their genotype, and thus there may be genetic variance in how individuals respond to changing climatic conditions (Via et al. 1995; Pigliucci 2005; Nussey et al. 2005a). Alternatively, individuals may differ in their response to the environment due to non-genetic elements such a difference in previous environment or differences in underlying condition or nutritional state (Nussey et al. 2007) as previously outlined. Recent studies using long-term data sets from mammals and birds have revealed phenotypic plasticity of a limited number of traits in response to environmental quality (Nussey et al 2005b), detecting a heritable component for some (Nussey et al. 2005a; Pelletier et al 2007) and suggesting that plasticity may be adaptive (Brommer et al. 2005; Nussey et al 2005a, b; Pelletier et al

2007). Assessing the ability of individuals to adapt to variable environments is key to determining the effects of changing climatic conditions on natural populations.

1.2.4 Environmental covariance between relatives

The environment can also reduce phenotypic differences between related individuals if relatives share a common environment. A good example of this is in many birds species, where many offspring are reared within a single nest. An individual's phenotype develops during the early stages of life and thus the environmental conditions experienced during that development may have large and long-lasting effects on phenotypic expression. To determine these effects, many studies have used experiments that involve cross-fostering offspring to different parents to separate genetic from common environment effects (Merilä and Sheldon 2001). Alternatively, this can be done statistically (Kruuk and Hadfield 2007), by partitioning variance into nest or common environment components (Kruuk and Hadfield 2007). In populations which have parental care, common environment effects often account for a large proportion of the phenotypic differences between individuals, with brood (nest) effects account for 45% of the variance in adult nest helping behaviour in long-tailed tits (MacColl and Hatchwell 2003) and 49% of the variance in body condition in collard flycatchers (Merilä et al. 2001). Environmental causes of similarity between relatives have been identified within natural populations of birds (Charmantier et al 2004), deer (Kruuk et al. 2002), sheep (Wilson et al. 2005), and humans (Pettay et al 2005).

One of the most widely inherited environmental effects is the environment created by females when they produce offspring. When there is a disparity in gamete size, the female will usually determine zygote size, provide a prenatal environment for the zygote to develop, and provide post-natal offspring care (Reinhold 2002). An offspring's phenotype can therefore be greatly influenced by that of its mother, independent of the direct effects of the genes that it inherits (Reinhold 2002). These maternal effects have been shown to be widespread influencing a wide variety of traits (Reinhold 2002). Furthermore, social influences such as maternal effects are unique as they can have both genetic and environmental components. For example,

there may be genotypic differences between mothers in the environment they provide for their offspring and these indirect genetic effects may have implications for evolutionary processes (Wolf et al. 1998). A number of studies have shown maternal effects for many aspects of phenotype in natural populations (e.g. Milner et al. 1999; Kruuk et al. 2000; Coltman et al. 2001) and two studies have separated maternal effects into genetic and environmental components (McAdam et al. 2002; Wilson et al 2005b).

1.2.5 Genetic expression over ontogeny

Many continuous traits develop with age and thus the mean of a given trait will vary over development, creating differences between individuals in relation to their age. Phenotypic variation may also arise through individual differences in the development process during ontogeny (Cheverud et al. 1983). Phenotypic variation may increase as a function of age through genetic and environmental factors. Genetic expression may be continuous over development and thus genetic effects may compound over ontogeny, with a trait expressed later in life inheriting variation from previous events as well as being influenced by new episodes of genetic expression (Atchley and Zhu 1997). As a result additive genetic variance may increase over ontogeny through variance compounding (Houle 1998; Wilson and Realé 2006). Variance compounding of environmental effects may also occur as individuals are subject to different environments throughout life and different sources of environmental variation may occur throughout ontogeny. Alternatively, variance may decrease over ontogeny if the phenotypic value of a given trait is associated with the viability of young individuals, or if there is compensatory growth. Compensatory growth occurs when individuals converge on a reduce range of phenotypes, with the rate of later growth determined by that of early growth (Cheverud et al. 1983). This mechanism may be common in birds and mammals and is characterised by an increase in growth following a period of environmentally induced reduced growth (Riska et al 1984; Cheverud et al. 1993; Badyaev and Martin 2000). It is not only morphological traits which show ontological changes, the genetic variance underlying life-history traits may also vary with age, which has important consequences for the study of senescence as genetic variance may increase

through mutation accumulation or loci may have different antagonistic effects between early and late life (Charlesworth and Hughes 1996; Charmantier et al. 2006; Wilson et al. 2007).

1.2.6 Genetic correlation

Traits often have shared functional, developmental, and genetic properties and therefore differences between individuals during development may influence a suite of phenotypic traits. As a result, components of an individual's phenotype are often associated. For example, different morphological traits are often allometrically related, or the expression of a trait at one age may be conditional on expression at an earlier age. The associations between variance in a set of traits can be summarised in a variance-covariance matrix **P**, where the diagonal elements represent the variances and the off-diagonal elements the covariances. **P** can then be broken down into an additive genetic variance-covariance matrix **G**, and a residual variance-covariance matrix **E**. Traits may not be inherited independently and often individuals resemble each other in many different ways. A given locus may affect more than one trait (pleiotropy), or genes may act independently but there may be non-random inheritance of loci resulting in trait loci becoming linked (linkage disequilibrium; Lynch and Walsh 1998). For a simple example of two traits X and Y, we can estimate the genetic correlation arising from pleiotropy as:

Eq. 1.3 $$r_A = Cov_{AXY} / \sqrt{\sigma^2_{AX}\sigma^2_{AY}}$$

where Cov_{AXY} is the additive genetic covariance (covariance in breeding value) between traits X and Y and σ^2_A is the additive genetic variance of X and Y respectively (Lynch and Walsh 1998). As **P** is a component of both **G** and **E**, the (co)variances described in **P** may reflect those of **G**. However, this may not always be so if environmental relationships between traits resulting from shared environmental effects, or differential allocation of resources between traits oppose relationships at the genetic level (Baker and Wilkinson 2003; Hadfield et al. 2006).

Analysis of the structure of **G** can indicate the extent to which traits are influenced by independent allelic variation and determine the number of independent dimensions of variation described by the traits under consideration (Lande 1982; Cheverud 1982; Blows and Hoffmann 2005). Genetic variance in a trait may be observed if it is genetically correlated with another trait of high variance. This theory forms the basis of the genic capture hypothesis, which predicts genetic variance should be observed if a trait reflects variation in an individual's underlying 'condition' (Houle 1992; Rowe and Houle 1996). If an individual's condition reflects resistance to disease, growth, and ability to convert resources into stored nutrients than it may have a large mutational target as it is influenced by many loci (Rowe and Houle 1992). Similarly, variance in life-history traits such as fecundity or survival is expected to be maintained as it is expected that they are genetically correlated with the traits that influence differences between individuals in survival or fertility (Houle 1992).

Genetic correlations can be measured between traits expressed in either sex, within a population. The sexes share a genome but sex-determining chromosomes may carry not only genes that determine gender, but that also influence other traits. As a result, the contribution of one sex to the genetic value of a trait may be different from the other sex. Sexual dimorphism is a feature of many species, where males and females differ in the form or size of phenotypic traits. For sexually monomorphic traits, expression in both sexes is presumably influenced by the same developmental pathway and genetic correlations are expected to be high (Jensen et al. 2003; Roff 1997). For sexually-dimorphic traits, expectations of between sex correlations are unclear as most studies have shown a lack of sex-biased genetic variance in many traits (Roff 1997; Merilä 1998; Coltman et al 2001; Parker and Garant 2005) and theory predicts that the gene pool that the sexes share prevent morphological divergence (Lande 1980; Cheverud et al. 1985; Reeve and Fairbairn 2001). However, numerous ecological examples exist of sexual size dimorphism (SSD) changing in response to the environment, and the potential exists for differential sex-specific regulatory processes or sex differences in environmental sensitivity may create SSD

despite high genetic correlations and similar levels of genetic variance between the sexes (for review see Badyaev 2002).

We can also estimate genetic correlations within a trait, or between traits, expressed in different environments. Estimating genetic correlations for a given trait expressed across environments is key to determining the mechanisms underlying GEI (Pigliucci 2005; Nussey et al. 2007). There is increasing evidence that genetic correlations between traits can also change as a function of the environment (Sgrò and Hoffmann 2004). Many studies have shown that temperature (Norry and Loeschcke 2002), laboratory versus natural conditions (Simmons and Roff 1996), resource conditions (Messina and Fry 2003), and the presence of predation (Stinchcombe 2002) all influence genetic correlations between traits. Estimating genetic correlations between traits is key to assessing their evolutionary potential and tradeoffs between fitness components which are discussed below (Roff 1992; 2002). However, most studies have been limited to estimating changing (co)variance between traits expressed in only two or three environmental groups and a more constructive approach may be one suggested by Charmantier and Garant (2005), where (co)variance structures are assessed across a continuous environmental gradient which reflects the conditions experienced by populations in the natural world.

Genetic correlations can be negative as well as positive. Negative genetic correlations result from antagonistic effects of loci within the genome and can indicate the occurrence of trade-offs or sexual antagonism (Rice and Chippendale 2001; Roff and Fairbairn 2007). Life-history theory is based on the assumption that evolution is constrained by the presence of trade-offs among traits (Roff 1992; Stearns 1992; Roff and Fairbairn 2007). For example, when resources are in short supply, resources allocated to reproduction are in conflict with resources that must be allocated to somatic growth and maintenance (Williams 1966; Levins 1968). This could create a negative correlation between fecundity and survival which has been empirically shown (Partridge and Sibly, 1991; Stearns 1992; Gustafsson et al 1994; Sinervo and DeNardo 1996; Zuk 1996; Roff 2002; Pettay et al. 2005), although many studies have also shown positive correlations (for a review see Roff 2002). Trade-

offs have also been demonstrated in the relationship between propagule size and number (e.g. egg size and number: Sheldon et al. 2003; Czesak and Fox 2003; Garant et al. 2008) and the relationship between sexual display traits and survival (Höglund and Sheldon 1998; Brooks 2000; Hunt et al. 2004). Sexual antagonism results when a particular genotype may not have the same fitness in males as in females. This may generate a negative genetic correlation in life-history traits between the sexes (Rice 1992; Rice and Chippendale 2001), which has been demonstrated for fitness in Drosophila (Chippendale et al. 2001) and recently in red deer (Foerster et al. 2007). Antagonistic pleiotropy between life-history traits within or between the sexes is thought to result in balanced polymorphisms (Rose et al. 1982) which will maintain additive genetic variance.

1.3 SELECTION

The phenotypic diversity observed in the natural world is shaped by the action of selection. The genetic properties of a population are the result of previous natural selection, combined with the process of mutation and random drift (Falconer and Mackay 1996). The fitness of an individual is the contribution of genes that it makes to the next generation. As a result, natural selection changes gene frequencies in a population, depending upon which genotype provides the greatest contribution to the next generation (Falconer and Mackay 1996). The fitness of an individual is the outcome of its fecundity and its viability, which are in turn influenced by numerous traits. We can therefore consider selection on a trait to be its relationship with lifetime fitness, which is the sum of its relationship with fecundity and viability components (Falconer and Mackay 1996). In this next section, I will consider different forms of selection and their influence on the phenotypic diversity in natural populations.

1.3.1 The process of selection

Selection acts upon a trait if there are differences in fitness between individuals with different phenotypic values of a given trait. Evolutionary biologists have long been

interested in describing and quantifying the action of selection in natural populations as it is the most important determinant of the diversity that we observe in natural populations (Endler 1986; Kingsolver et al. 2001). The type of selection and its intensity can be determined from a plot of fitness against the phenotypic value of a given trait (Falconer and Mackay 1996). We can gain this information from studies of wild populations where known individuals are followed from birth until death, with quantitative trait phenotypes measured throughout their life (Clutton- Brock and Pemberton 2004; Clutton-Brock et al 1982; Grant 1986). We can then estimate the selection differential on trait T (S_T):

Equation 1.4 $S_T = \text{cov}_{P(TW)}$

which is equal to the phenotypic covariance of the character with fitness, where the fitness of an individual is relative to that of others in the population (W). Having determined whether a relationship exists between a trait and fitness we can the estimate the strength and form of selection by estimating the regression coefficient of a given trait on fitness (selection gradient; Lande and Arnold 1983):

Equation 1.5 $b_{TW} = S_T / V_{P(T)}$

where the selection gradient *b* is the correlated selection differential divided by the phenotypic variance of trait T. For comparisons across species, we can we can scale S_T by the phenotypic standard deviation of the trait before selection, to gain a standardised selection differential (Endler 1986).

It should be noted that the strength of selection does not determine whether selection will change a trait value (Falconer and Mackay 1996). For evolution to occur selection must act on underlying genetic variation and there must be genetic covariance between a trait and fitness, as a trait which increases an individual's fitness must be inherited to some degree by their offspring. We can predict a response to selection by multiplying our estimates of the selection gradient by the heritability of the trait (Breeders equation: Falconer and Mackay 1996). However,

selection is mostly quantified using phenotypic values measured in the wild, with the underlying assumption that there is a causal connection (genetic correlation) between fitness and the trait. Recent studies have shown that this may not be the case if there is environmental covariance between the trait and the environment, which influences both the trait and fitness, creating an apparent relationship which has no genetic basis (Rausher 1992; Stichcombe et al. 2002). Therefore, in order to predict a response to selection, we need to determine the genetic correlation between the character and fitness, and the heritability of both the character and fitness (Crow and Nagylaki 1976). Studies should therefore aim to quantify the genetic covariance between a trait and fitness (e.g. Kruuk et al. 2002) in order to estimate a trait's evolutionary potential.

The relationship between a trait and fitness may not be linear and it is unlikely that traits are independently selected. We can quantify the effects of non-linear selection by using a second order polynomial regression (Lande and Arnold 1983), which provide quadratic regression coefficients which can be either negative (stabilising selection where an intermediate optima is preferred) or positive (disruptive selection where opposite ends of the distribution are preferred). We can use partial regression coefficients to estimate the strength of selection for each trait once others are accounted for (Lande and Arnold 1983). We can also include an interaction term between two traits to determine if selection acts upon a combination of trait values (Phillips and Arnold 1989).

1.3.2 Effects of selection on genetic variance

If directional linear selection acts consistently on any heritable trait so that there is always a single optimal genotype, it should induce a permanent change in the distribution of that trait, which is supported by laboratory studies and artificial breeding schemes (Fisher 1958; Endler 1986; Falconer and Mackay 1996). Under these conditions allele fixation is expected, with genetic variance potentially maintained through a balance between mutation which creates variance anew and directional selection which erodes variance (Bulmer 1989; Barton and Turelli 1989; Falconer and Mackay 1996). The likelihood of genetic variance being depleted

depends upon the strength of selection, the traits genetic basis, and the rate of mutation (Barton and Turelli 1989) and it is presently unclear whether the depletion of variance under sustained selection can provide a limit to evolutionary change (Blows and Hoffmann 2005).

Stabilising selection is defined as selection in which an intermediate optimum is favoured. A recent review of empirical studies concluded that there was little evidence of strong non-linear selection (Kingsolver et al. 2001), although it has become clear that we may be looking in the wrong place by not considering all aspects of the fitness surface (Blows and Brooks 2003). Stabilising selection can result from a direct relationship between a trait and fitness or stabilising selection can be apparent as a result of a traits relationship between fecundity and viability which is discussed below. Direct stabilising selection favours genotypes with least variation, increasing the canalisation of development and can move gene frequencies towards fixation, which has been demonstrated empirically (Falconer and Mackay 1996).

If environmental heterogeneity is sufficiently strong to create alternative habitats in a way which makes one phenotypic extreme favoured in one habitat and the opposite phenotypic extreme favoured in another, it may create disruptive selection (Endler 1986). Although it depends upon the specific characteristics of the population (see below), disruptive selection is expected to increase genetic and environmental variance which has been shown experimentally (Prout 1962; Sorensen and Hill 1983) and it is suggested to occur in Darwin's finches (Schluter et al. 1985).

Traits may also be selectively neutral, in that they may have a function, but the exact value of the character is not a determinant of fitness. As a result, provided genetic variation around an optimum has little impact on fitness in any other way, additive genetic variance is likely to be maintained. Traits may also have broad optima across environments if stabilising selection is weak, and thus a large amount of trait variation may not associated with fitness (Falconer and Mackay 1996). Furthermore, although selection in a given year may be strong, if we monitor a population over

many years, selection pressures may vary considerably depending upon population dynamics and environmental conditions (Grant and Grant 2002; Clutton-Brock and Pemberton 2004; Clutton-Brock et al. 1982).

1.3.3 Trade-offs

There may be trade-offs between different traits in their influence on fecundity and viability. For example, in a given breeding event there may be a trade-off between the number of offspring produced and their quality (Stearns 1992; Sheldon et al. 2003; Realè et al. 2003; Wilson et al. 2005b; Garant et al. 2008). There may also be a relationship between increased body size and increased attractiveness but this may be traded-off against increased predation risk as larger individuals are more conspicuous (Covas et al. 2002; Brodin and Johansson 2004). Investment in reproductive traits may also come at a cost to survival (Höglund and Sheldon 1998; Kokko et al. 2002; Hunt et al. 2004). This scenario often results in apparent stabilising selection between a trait and fitness where an intermediate optimum favoured, which is expected to result in a reduction in genetic variance (Falconer and Mackay 1996).

1.3.4 Antagonistic selection

Selection can be antagonistic between different components of fitness, between two traits, over ontogeny, and between the sexes. Antagonistic selection can result from two mechanisms, one where selection pressures are positive but genetic correlations are opposing, and the other where selection pressures are opposing but the genetic correlation between traits is positive. I will briefly outline different mechanisms and their effects on additive genetic variance.

If two traits are genetically correlated, the effects of selection on one trait may induce a response in a genetically correlated trait (Falconer and Mackay 1996). Selection can be antagonistic between fecundity and viability as both are expected to be under positive selection, but may favour different genotypes. Therefore, there is the potential that despite positive selection on both traits, negative genetic correlations will be generated, maintaining variation in fitness components as discussed above

(Roff 1997). Alternatively, if two traits are selected in opposing directions, positive genetic correlations can also acts as constraints. Empirical data is mostly lacking, but studies have tested for antagonistic selection between components of growth (Sinervo and Calsbeek 2003) and associated immune responses (Wilfert et al. 2007). It is unclear whether this type of genetic constraint can limit evolutionary change, particularly considering genetic correlations may not be stable between environments and populations (Roff 1997).

Sexually antagonistic selection pressures can occur between the sexes as each sex may have different selective optima. In many populations females are the limiting resource and this can drive the evolution of sexual size dimorphism as males compete for reproductive opportunities, or it can drive the evolution of display traits as males attempt to attract females by signalling some component of their fitness (Andersson 1994). Despite observable sexual dimorphism in many morphological traits and evidence of different selective optima, most studies have shown highly positive genetic correlations between many traits (Roff 1997; Merilä 1998; Coltman et al 2001; Parker and Garant 2005), which is predicted to constrain morphological divergence (Lande 1980; Cheverud et al. 1985; Reeve and Fairbairn 2001). If there is sexually antagonistic selection and similar genetic control, additive genetic variance is likely to be maintained as the fitness of a given genotype will depend upon the sex in which it is expressed.

Sexual selection is expected to exert strong and continuous directional selection on male secondary sexual traits (Andersson 1994). As a result, the study of sexual selection often concentrates on the selection and genetic basis of male traits in an attempt to understand how genetic variance is maintained under sexual selection (e.g. Kotiaho et al. 2001). However, studies of mate choice have revealed that both males and females may elicit mate choice for the same suite of traits, creating sexual antagonism as different trait combinations may be preferred (Chenoweth and Blows 2005). Furthermore, in many species, females also show reduced expression of the male trait such as the horns or antlers of ungulates, or the display traits of many ‘lekking’ species. This expression was thought to be maintained only through genetic

associations with the male phenotype (Lande 1980) but potentially females may benefit from the expression of these traits if they can be used in intrasexual dominance interactions (West-Eberhard 1983; Amundsen 2000). Therefore, to fully understand the evolution of a trait we need to examine selection pressures in both sexes in which the trait is expressed.

1.3.5 Environmental heterogeneity and selection pressures

There is increasing evidence of variation in selection pressures on a spatial and temporal scale within natural populations (Price et al. 1984; Milner et al. 1999; Garant et al. 2008). If the environment fluctuates so that the optimal phenotype varies between environments then genetic variation may be maintained. Early theoretical models suggested that fluctuating selection could not maintain additive genetic variance (Lande 1977; Turelli 1988; Barton and Turelli 1989). However, recent models have suggested that if the environment is sufficiently variable and generations overlap then genetic variance can be maintained (Sasaki and Ellner 1997). If a certain proportion of the population is insulated from a selection regime within a particular year, then it is possible for a wide range of genotypes to be maintained because of a storage effect (Chesson and Warner 1981). Similarly, if fluctuating selection is age or sex specific, then a proportion of the population are not exposed to the same selection pressures, which may maintain genetic variance (Saski and Ellner 1997; Reinhold 2000; Gorelick and Bertram 2003). Empirical testing of this is lacking and there are studies supporting the hypothesis (Haldane and Jayakar 1963; Mackay 1981), some finding limited evidence (Hendrick 2006; Prout 2000), and some finding population specific effects (Mukai 1988). There is only one example of temporally fluctuating selection in a natural population (although see Chapter four) where the sign and strength of natural selection on many morphological traits fluctuates from year to year in a population of Darwin's finches, resulting in no detectable directional change in any trait (Grant and Grant 2002). The ability of fluctuating selection pressures to maintain genetic variance may depend upon the ecological conditions that specific populations experience and more work is required to assess these effects.

To determine the evolutionary potential of a trait under fluctuating environmental conditions, it may be necessary to simultaneously consider both changes in selection pressures and changes in genetic variance across environments. Recently, a study has found that an environmental coupling between selection and genetic variance may limit the evolutionary potential of birth weight in a natural population of Soay sheep (Wilson et al. 2006). In this population the potential for microevolution was constrained by either a lack of heritable variation in poor environments or a lack of selection in favourable environments (Wilson et al. 2006). To my knowledge there are no studies which determine the genetic correlation between a trait and fitness, a relationship which is required for microevolution to occur (Lynch and Walsh 1998), across fluctuating environmental conditions (although see Chapter four). If there is GEI for fitness, GEI for traits associated with fitness, then there may also be GEI in their relationship and this may be an important factor in the maintenance of variance of many traits.

1.5 STUDY POPULATION

Off the north-west coat of Scotland, UK, lie the islands of St.Kilda which are home to a feral population of Soay sheep (*Ovis aries*). Soay sheep are a breed of the earliest domesticated sheep which spread through Europe during the Bronze Age. In 1930 the last human inhabitants left the main island of Hirta and two years later 107 sheep were established on Hirta to maintain grazing on the island, where they have remained without human interference (Clutton-Brock and Pemberton 2004). The Soay sheep population fluctuates from year to year as they are limited by resources during winter, with most death occurring late winter as a result of starvation (Clutton-Brock and Pemberton 2004). As a result, there are substantial differences between individuals in the environments that they experience at birth and throughout their lives.

Since 1985 over 95% of the sheep born within Village Bay have been individually marked using coloured, numbered ear tags. Tagging is done at birth, when individuals are captured, weighed, their birth weight recorded, and a blood sample

taken for paternity analysis. Individuals are then monitored throughout their life, with morphological measures taken during autumn when over 50% of the population are caught each year. Male behaviour is also observed during the rut late in the year. By monitoring individuals throughout their lives we can understand the ecological factors that contribute to individual differences in survival and fecundity (Clutton-Brock et al. 1992; Clutton-Brock and Pemberton 2004), we can better understand natural selection and its interaction with population dynamics (Milner et al. 1999), and we can better understand the effects of selection on micoevolutionary processes (Endler 1986). Through the development of molecular markers to assess paternity we can now determine patterns of male mating success (Preston et al. 2001; Coltman et al. 1999) and show that components of fitness of both sexes are associated with heritable variation in morphometric (Illius et al. 1995; Coltman et al. 1999; Milner et al. 1999; Wilson et al. 2005a, 2005b, 2006) and polymorphic traits (Moorcroft et al 1996; Clutton-Brock et al. 1997; Gratten et al 2008).

The Soay sheep project has been running for well over twenty years and a wealth of studies have resulted (see Clutton-Brock and Pemberton 2004 for an overview). I shall discuss previous work in detail and describe the measurement of each phenotypic trait in the following chapters, where it is relevant to the work that I present. I should mention here that Soay sheep differ from domestic breeds in many ways but some of the most conspicuous are the polymorphisms in coat colour and horn phenotype observed in this population. In coat colour, Soay sheep are either light or dark and either a solid in colour (self type) or have a white stomach (wild type). Both males and females have horns, with males growing either full (normal) or reduced (scurred) horns, and females growing either full, reduced or no (polled) horns at all. We understand very little about the genetics that underlie these polymorphism or the factors which maintain them (although see Gratten et al. 2008 for the genetics of coat colour). This thesis does not concern the genetic basis of the polymorphism itself but does explore selection pressures on horn phenotype and variation in horn size. The inheritance of horns in Hebridean sheep in general is little understood but work has been conducted on the basic genetic relationships between polled and horned sheep (Dolling 1970). The presence or absence of horns is

expected to be controlled by three alleles at the Horn (Ho) locus: Ho^P controls for polledness, which is incompletely dominant to the other alleles in rams, but dominant in ewes; Ho^+ controls for horns in both sexes; and Ho^{hl} produces sex limited horns in males. In Soay sheep this mechanism of inheritance does not appear to fit the patterns of horn expression that we see (Coltman and Pemberton 2004) and work on this topic is currently underway.

1.6 THESIS AIMS

I aim to assess the mechanisms which maintain variation in both the form and size of horns in a feral population of Soay sheep. To do this I will:

1. Examine the selective pressures on horn phenotype on horn size in each sex, testing for antagonistic selection pressures and trade-offs between components of fitness.

2. Examine the function of horns in females by assessing their use in aggressive interactions between females.

3. Determine the effects of the environment on horn length.

4. Determine the quantitative genetic basis of horn length and examine genetic and environmental variance as a function of age and the environment.

5. Determine the genetic relationships between horn length and other morphological traits and examine the influence of the environment on these relationships.

CHAPTER 2

TRADE-OFFS BETWEEN FITNESS COMPONENTS AND SEXUALLY ANTAGONISTIC SELECTION ON WEAPONRY IN SOAY SHEEP

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2.1 SUMMARY

Males are predicted to compete for reproductive opportunities, with sexual selection driving the evolution of large body size and weaponry through the advantage they confer for access to females. Few studies have explored potential trade-offs of investment in secondary sexual traits between different components of fitness or tested for sexually antagonistic selection pressures. These factors may provide explanations for observed polymorphisms in both form and quality of secondary sexual traits. In this chapter I present an analysis of selection on horn phenotype in a feral population of Soay sheep (*Ovis aries*) on the island of Hirta, St.Kilda, Scotland. Soay sheep display a phenotypic polymorphism for horn type with males growing either normal or reduced (scurred) horns, and females growing either normal, scurred, or no (polled) horns; further variation in size exists within horn morphs. I show that the horn phenotype and the size of the trait displayed is subject to different selection pressures in males and females, generating sexually antagonistic selection. Furthermore, there was evidence of a trade-off between breeding success and longevity in normal-horned males, with both horn type and larger horn size associated with greater annual breeding success but reduced longevity. Therefore, selection through lifetime breeding success was not found to act upon horn phenotype in males. In females, normal-horned females showed reduced annual breeding success but this did not result in a significant difference in lifetime fitness

when compared to scurred individuals as no significant difference in longevity was found. However, increased horn size within this group was negatively associated with breeding success and longevity. Females without horns (polled) suffered reduced longevity and thus reduced lifetime breeding success relative to the other horn morphs. My results therefore suggest that trade-offs between different components of fitness and antagonistic selection between the sexes may maintain genetic variation for secondary sexual traits within a population.

2.2 INTRODUCTION

Natural and sexual selection will act upon a trait if there are differences in fitness between individuals with different phenotypic values of that trait (Falconer and Mackay 1996). When males compete for reproductive opportunities, sexual selection may drive the evolution of exaggerated male weaponry such as horns and antlers, through the advantage they confer in male contests for access to receptive females (Darwin 1871; Clutton-Brock et al. 1992; Andersson 1994). However, if selection acts continuously upon a trait it will presumably favour certain genotypes at the expense of others, resulting in the depletion of genetic variation underlying that trait (Falconer and Mackay 1996; Fisher 1958). The abundant genetic variation underlying many sexually-selected traits which has been observed in natural populations (Pomiankowski and Møller 1995; Rowe and Houle 1996; Tomkins et al. 2004) therefore presents a paradox.

Various explanations have been proposed to account for variation in the form and size of secondary sexual traits, but relatively little testing of these hypotheses has been conducted, particularly in wild populations. First, there may be trade-offs between different components of fitness, with fecundity versus viability selection favouring different genotypes so that no single genotype is optimal. Trade-offs are a major topic of interest in evolutionary biology with several studies focussing on reproduction and survival (Stearns 1989; Gustafsson et al. 1994; Roff 1992, 2000; Roff et al. 2002). A number of studies of sexually-selected traits have demonstrated an association with reproductive success within a given year (e.g. Coltman et al.

1999; Preston et al. 2003) but data on lifetime breeding success are necessary to appreciate potential trade-offs with longevity. To my knowledge, only one study to date has shown that male weaponry increases lifetime breeding success in a wild mammal population (Kruuk et al. 2002; see also Coltman et al. 2005).
Second, there may be contrasting or antagonistic selection pressures acting on the same genotype when expressed in the two sexes (Rice 1992; Chippindale et al. 2001). If two traits are positively correlated in males and females each sex will be constrained from adopting a separate evolutionary pathway (Falconer and Mackay 1996; Merilä et al.1998; Jensen et al. 2003). More notably, recent studies have shown negative correlations between the expression of a particular genotype in males versus in females (Linder and Rice 2005; Rice and Holland 2005; Foerster et al. 2007). To date, there has been little opportunity to explore the effects of the expression of genotypes that are known to be under sexual selection in males but whose function is not understood in females, particularly in wild populations, and hence to test for sexually antagonistic selection pressures.

Finally selection may vary with temporal or spatial environmental variation (Via and Lande 1985; Greenfield and Rodriguez 2004). The strength of selection acting on phenotype may therefore alter in different environments or different phenotypes may be favoured in different environmental conditions (Rose et al. 1998). This latter scenario can lead to a balancing effect, giving equal fitness of different phenotypes in the long term (Shuster and Wade 1991). Alternatively, phenotypic variation may also be maintained by frequency-dependent selection, where one phenotype is most advantageous when rare, and thus is maintained at relatively low numbers within a population (Maynard Smith 1982).

To quantify the magnitude of selection pressures, covariances between measures of different components of fitness and phenotype are required (Endler 1986). In this chapter I examine patterns of selection on the different horn morphologies and horn sizes of males and females in a free-living population of Soay sheep (*Ovis aries*) on the island of Hirta, St.Kilda, Scotland. By examining lifetime breeding success and two component traits, annual breeding success and longevity, I show trade-offs

within, and test for antagonistic selection between, the sexes. Soay sheep have a polymorphism for horn development with males showing either a full horn (normal-horned) or a reduced horn known as a scur (scurred). Females develop smaller horns, and may be either normal-horned, scurred, or show no horn development (polled). Horn type is heritable, and a single-locus three-allele model is the most parsimonious explanation of inheritance in the Hirta population (Coltman and Pemberton 2004). Within normal and scurred horn types, there is also considerable variation in horn size. Prior studies have demonstrated an advantage of large body size in Soay males, with evidence of positive selection through survival, fecundity and lifetime breeding success on hind limb length (Coltman et al. 1999). Horn length in normal-horned males is associated with annual breeding success, independently of body size (Preston et al. 2003), but has not been shown to influence lifetime breeding success. Prior studies have shown evidence of positive selection through survival, fecundity and lifetime breeding success on body size (hind limb length) in Soay males (Coltman et al. 1999).

Results from previous studies of the St.Kilda Soay sheep population suggest the potential for differences in fitness between the different horn types. First, males of different horn type show different mating strategies. Scurred males avoid conflict with other males by mating with females only when they are not guarded by another males (Preston et al. 2003; Stevenson et al. 2004). Although scurred males always gain fewer paternities within a given year, they appear to increase their percentage of mating gained when their frequency in the population is low, and thus it is speculated that the horn polymorphism may be maintained via frequency dependence (Stevenson et al. 2004). Second, scurred males also show a greater over-winter survival rate (Moorcroft et al. 1996). Therefore, as scurred males appear to gain a lower number of paternities per year, this could potentially be balanced by greater longevity generating equal fitness in both groups. Furthermore, scurred females have been shown to have on average higher conception, weaning rates and over-winter survival, relative to a combined class of normal and polled females (Clutton-Brock et al. 1997). This suggests that antagonistic selection may be maintaining the polymorphism, with the advantage of scurred females opposing the selective forces

acting against scurred males (Moorcroft et al. 1996; Clutton-Brock et al. 1997; Milner et al. 2004). However, although we therefore have evidence of several associations between horn phenotype and different components of fitness in either sex, a full comparison of selection on horn phenotype acting through lifetime breeding success in this long-lived species has not previously been conducted.

The long-term data available on the Soay sheep population also provide the opportunity to quantify changes in selection in relation to environmental conditions. Population density has been shown to alter the selection pressures acting on many phenotypic traits within this Soay sheep population (Moorcroft et al. 1996; Clutton-Brock et al. 1997; Coltman et al. 1999; Milner et al. 2004). In particular, cohort specific effects such as density in the year of birth influence breeding success of males (Coltman et al. 1999; Stevenson et al. 2004), and the fitness differences between the horn morphs in females have also been shown to increase with population density (Clutton-Brock et al. 1997). Here, I extend these analyses hare to consider the environmental dependence of selection acting through lifetime breeding success.

In this chapter I explore selective pressures on the form and size of the secondary sexual trait of horns in both males and females of the St.Kilda Soay sheep. I consider the effects of selection on horn type and then horn size in both sexes, acting via lifetime breeding success and its two components, annual breeding success and longevity. This allowed me to test for potential trade-offs between different components of fitness and antagonistic selection to be tested for within and between the sexes.

2.3 METHODS

The present study focuses on an unmanaged population of Soay sheep (*Ovies aries*) which reside in Village Bay on the island of Hirta within the St. Kilda archipelago in the North Atlantic (57°49’ N, 08°34’ W). The population fluctuates between 600 and 2000 individuals as a result of periodic population crashes, with almost all deaths

occurring during late winter, as a consequence of starvation (Clutton-Brock and Pemberton 2004). The population has been the subject of ecological study since the 1960s, and from 1985 about 95% of lambs born within the study site have been ear-tagged, giving intensive sampling of individual level data.

Lambs are ear-tagged shortly after birth in April or May, sampled for genetic analysis and weighed. Lambs are born as either twins or singletons. The population is monitored by census 30 times per year, with individual positions recorded, and by performing systematic searches for corpses in early spring. Soay sheep have a promiscuous mating system in which the onset of the rut is marked by increasing male aggression as rams roam and search for oestrous females. Once located, males fight to gain access to oestrous females, which often involves butting the flanks of rivals and engaging in head-on clashes (Preston et al. 2003).

2.3.1 Pedigree determination

The pedigree structure of the Village Bay population has been inferred by both behavioural observation and genetic analysis. From 1985, daily observations were made from March to May on lambing females, with maternal identities assigned with greater than 99% accuracy when tested by genetic analysis (Pemberton et al. 1999; Overall et al. 2005). Paternities were assigned from genotypic data via the maximum-likelihood method implemented in CERVUS (Marshall et al. 1998) using 18 microsatellite loci (Pemberton et al. 1999). The pedigree structure used in this chapter contains all known maternal links from 1987 to 2004, and all known paternities from 1987 to 2001 where the latter were assigned at a confidence level of 80% or greater with the additional restriction of not more than one locus mismatching between offspring and candidate sire. Since not all lambs were assigned a father, male breeding success was therefore underrepresented, but this bias should not vary between males. The complete pedigree structure contained 5999 individual records with 3536 maternal links, and 1668 paternal links (from 806 distinct dams and 527 distinct sires respectively).

2.3.2 Components of fitness

Annual breeding success (ABS) was defined as the number of lambs sired by a male, or as the number of live lambs born to a female, in a given year. Longevity (LG, in years) was defined as an individual's age at death for animals with a known death date. Lifetime breeding success (LBS) was defined as the sum of annual breeding successes across an individual's lifetime. Therefore selection analyses were divided into short term annual events (ABS) and long term life history traits, such as LG and LBS. All data from all available individuals was included in the analysis. For females, analyses were also conducted using a measure of the number of offspring which survived their first winter, rather than the number of live offspring produced, in order to incorporate the effects of any potential differences in maternal care. This different measure of ABS and subsequently LBS yielded identical conclusions to analyses based on the original measure of ABS, and thus I have not included them here.

2.3.3 Phenotypic traits

The analysis of ABS used morphometric and environmental covariates recorded during the year in which the lambs were conceived. Morphometric measurements were recorded during a two-week period in August, in which 49-67% of the study area population are rounded up each year. Live body weight, hind-leg length and horn length values were therefore taken from measurements made in the August immediately prior to the rut. Horn size was measured as the length of the horn (in mm) from the base along the outer curvature of the spiral to the tip. Hind-leg length was taken as the distance between the tubercalcis of the fibular tarsal bone to the distal end of the metatarsus (in mm). Faecal egg counts of five nematode gut parasites collectively termed strongyles and thirteen small intestinal protozoa termed coccidea, collected in August, were used to estimate parasite burdens. Counts were averaged to provide a yearly estimate of parasite burden, which is unbiased of any temporal trends. Strongyle counts were recorded from 1986 onwards and Coccidea counts from 1991 onwards.

Horn type was recorded at the first point in which an individual was measured and again throughout life. It should be noted that reclassification of horn type in females from polled to scurred may occur over an individual's lifetime, because distinguishing scurred and polled horns during development may be difficult. However, the probability of an individual being reclassified was only 9% and there was no evidence of a change in this value over the study period or with age. Furthermore, I found no significant difference in the fitness of reclassified compared to scurred females, when repeating the analyses below. It is therefore unlikely that error, generated by inclusion of individuals who would never be reclassified because they die young, will affect the associations presented here. Therefore, reclassification is unlikely to affect the conclusions presented here.

The analysis of selection acting through LG or LBS used phenotypic measurements recorded at death. I focused on the traits of horn type and then horn size (measured at death) within each horn type. To compare the relative contributions of different physical attributes to fitness, other phenotypic trait values were incorporated into analyses. Skeletal body size was estimated from a measure of hindleg length at death. Both body size and horn size were standardised to zero mean and equal variance within all age groups (1, 2, 3, 4 and 5+ years). These measures provide a conservative method of comparing the associations of horn and body size with LG and LBS across individuals who survived to different ages. Using this method, two individuals who survived to different ages with large horns for their age can both have the same value, and thus no relationship will be observed between LG and horn size solely as the result of age differences in horn length. Standardizing the variance removes any bias induced by reduced sample sizes at older ages. Birth weights (in kg) were defined operationally as the residuals from a linear regression of capture weight on days since birth (Robertson et al. 1992), since individuals could not be weighed immediately after birth.

2.3.4 Environmental variables

Each year, from 1985 to 2003, the Village Bay population density was estimated on October 1 as the total number of individuals observed from census or caught before

this date and the number of lambs born that year (excluding those known to have died by October 1). Estimates of this population density ranged from 211 to 594 between the years analysed. For analyses of ABS, the value used was the density recorded during the year in which the lambs were conceived, and for LG and LBS measures recorded in the year of birth were used, to test for long-term effects of the environmental conditions experienced during early development. Heft was also included in the analysis of ABS: the study site is divided into three sections or hefts (Coulson et al. 1999). Individuals were assigned to a particular heft based upon the average census position recorded over the year.

2.3.5 Selection through annual breeding success

A series of analyses was used to build a complete picture of the selective pressures on horn type and then horn size in both males and females. First, I considered the relative effect of horn type and then horn size in a given year on the breeding success in that year in for males and females, whilst also taking into account both associated selection through other phenotypic traits and environmental factors. All individuals who were either sighted or caught within the year of the rut were included in the analysis.

Selection analyses were conducted for males and females separately, because the distributions of ABS differed greatly between males and females, and in order to allow comparison with earlier results. Male ABS followed a negative binomial distribution, so a generalized linear mixed model (GLMM), with a negative binomial error structure and logarithmic link, was used to test for associations between horn phenotype and ABS. Female ABS took the values of 0,1 or 2 and so did not follow a standard statistical distribution: I therefore adopted an assumption of normal errors and analysed ABS using a linear mixed model with ABS as the dependent variable. Although this assumption of normality is clearly an approximation, a binomial model of bred/not bred within a given year yielded identical conclusions for female ABS as the model with normal errors. Horn type, hind-leg length, weight, Village Bay population size (density), and the heft within which the individual resided were fitted as fixed effects. Analysis of female ABS also included age and a quadratic term of

age and Village Bay population size (density). Neither measure of parasite burden (strongyle and coccidea count) was shown to have a significant effect on ABS in either sex (males: coccidea Wald statistic = 1.44, $P = 0.230$; strongyle Wald statistic = 0.81, $P = 0.367$; females: coccidea Wald statistic = 0.08, $P = 0.771$; strongyle Wald statistic = 0.75, $P = 0.690$; df = 1 for all parameters). I therefore excluded the parasite measures from the presentation of the results. Scurred and normal-horned males employ different reproductive strategies (Preston et al. 2003, Stevenson et al. 2004); thus, in males, the effects of density and age (divided into three groups) on ABS were nested within the effects of horn type. Nesting allows effects to be quantified independently within each of the horn types. Density dependence in selection pressures was tested for in both models of ABS by including an interaction between horn type and population density in the year of conception.

Mixed models were used to allow for the repeated measures in the data set. Individual identity was fitted as a random effect to account for multiple measures on the same individual, and year as a random effect to account for multiple measures on the same year, accounting for any unmeasured environmental variation attributable to the year of measurement. The significance of the fixed effects was assessed using Wald statistics, on their associated degrees of freedom when fitted last in the model. The models were then repeated, adding horn size nested within horn type to test the effects of horn size within each horn type.

2.3.6 Selection through longevity and lifetime breeding success

Selection through LBS depends upon the sum of ABS values over an individual's lifetime and thus will also depend upon longevity (LG). The same generalized linear model (GLM) framework was used for analyses of LG and LBS as dependent variables in both sexes, using a negative binomial error structure with a logarithmic link. A GLM approach has been used as it enables appropriate significance testing of the associations of factors with LBS and LG, while including other factors and their associations. Hind-leg length (adjusted by age at death), horn type, population density in year of birth, birth weight, and whether the individual was born as a twin or a singleton were fitted as independent variables. A density-by-horn type

interaction was also added to the models as a fixed effect in order to test whether the effect of density on LBS and LG differed between individuals of different horn types, but significant associations were not found in either males or females; thus for clarity the interactions were removed from the models shown. The significance of the fixed effects was assessed using *F*-statistics, on their associated degrees of freedom when fitted last in the model. The models were then repeated, adding horn size nested within horn type to test the effects of the size of horn grown over an individual's lifetime within each horn type.

Having established the statistical significance of any associations between fitness and horn phenotype, using appropriate error structures and significance testing, formal analyses of selection were conducted to gain selection coefficients and gradients. Standardized measures of the selection on horn type through LBS were estimated using selection coefficients, defined as the difference in mean relative fitness between two groups. For males, I therefore estimated the difference between normal-horned and scurred males in relative LBS (estimated as LBS divided by average male LBS); for females, with their three horn types, I estimated the difference between normal-horned and scurred, normal-horned and polled, and then between scurred and polled, in relative LBS. Standardized hind-leg was included in all calculations of selection coefficients to estimate direct selection independent of size, thus accounting for any subtle confounding effects with other factors.

Standardized measures of the total and the direct selection on horn size, through LBS, were then estimated using selection gradients from least-squares regressions (Arnold and Wade 1984). Total selection was estimated by regressing relative LBS on age-standardized horn size at death (age-standardized as above) for each sex and horn type separately. Direct selection on horn size independent of body size was then assessed from standardized selection gradients obtained from a multiple regression including an age-standardized measure of hindleg length at death (Lande and Arnold 1983).

To formally test whether selection through LBS on horn type differed between males and females, selection coefficients and gradients were estimated including sex as a factor and then a sex by horn trait as an interaction. Partial F-tests were then used to compare the unexplained sums of squares from models with and without the interaction term (for details of model building procedure see: Chenowerth and Blows 2005), thus testing whether linear selection on horn type and size differed between the sexes.

All models were carried out using Genstat (VSN International Ltd., Hemel Hempsted, UK), S-Plus 2000 Professional Release 2 (Insightful, Seattle, WA) and R Version 2.1.1 (R Development Team; http://www.r-project.org).

2.4 RESULTS

2.4.1 Variation in morphology

Horn size in normal-horned individuals showed substantial variation with age, and followed a similar pattern of increase as body weight, with both asymptoting at age seven in males and females (Figure 2.1). In contrast, horn size in scurred individuals increased in smaller amounts each year, with substantial variation in females surviving to older ages due to smaller sample sizes (Figure 2.1). A skeletal measure of body size (hind-leg length) reached its maximum by the age of three years in both sexes and thus followed a steeper rate of increase during the first few years of life, than in later years (Figure 2.1). No significant differences were found in body size (age adjusted hindleg length) or birth weight between the horn types in either sex (birth weight: F=0.988, df = 1, P = 0.252; body size: F = 2.622, df = 1, P = 0.106).

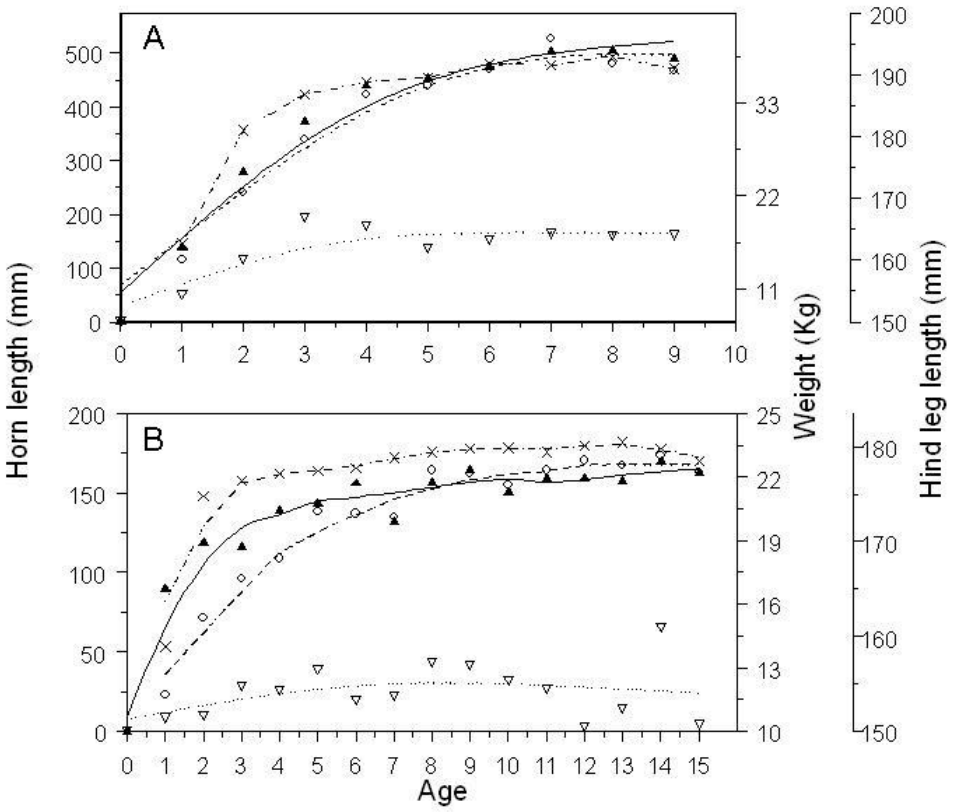

Figure 2.1. Changes with age in mean August live body weight, hind-leg length and horn size in (A) males and (B) females. Crosses: hindleg length with Gompertz growth curve shown by dot-dash line; filled triangles: horn length for individuals with normal-horns with Gompertz curve shown by solid line; open circles: weight with Gompertz growth curve shown by dashed line; open triangles: horn length for individuals with scurred horns with Gompertz curve shown by dotted line.

The number of males known to be present within the population fluctuated between 120 and 381, with the proportion of scurred males varying from 18-42% between years (Figure 2.2). The density of normal-horned males appeared to fluctuate more widely than that of scurred males and thus the population density depended more upon the density of normal-horned males than scurred males (Pearson's correlation with overall density: normal-horned males $t = 114.74$, df = 19, $P<0.001$; scurred males $t = 3.53$, df = 19, $P = 0.06$). In females, there were no differences between the horn types in fluctuations in density (Figure 2.2).

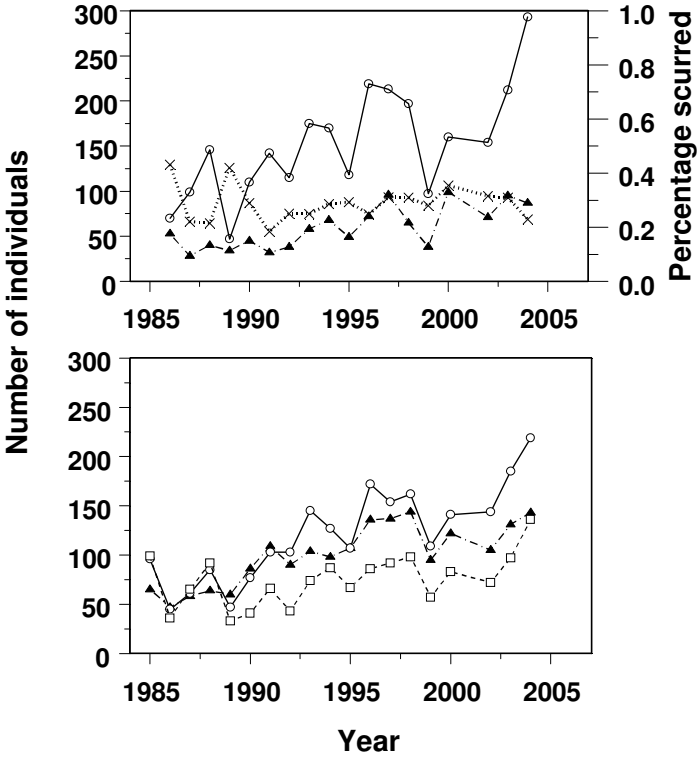

Figure 2.2. Changes in numbers of horn types in males (A) and female (B) with year. Normal-horned individuals are depicted by open circles and solid lines; scurred individuals by solid triangles and dash-dot line; polled individuals by open squares and dashed line. The proportion of scurred amongst all males is also shown by a cross and dashed line in (A).

2.4.2 Annual breeding success

Annual breeding success (ABS) was greatest in normal-horned males between three and six years (Figure 2.3A). Average ABS in scurred males was less variable with age, increasing slightly until four years of age, and then decreasing (Figure 2.3A). As a result of these observations, selection acting upon horn type was tested at three different age groups: a zero to two year group (A1); a three to six year group (A2); and seven years and above (A3).

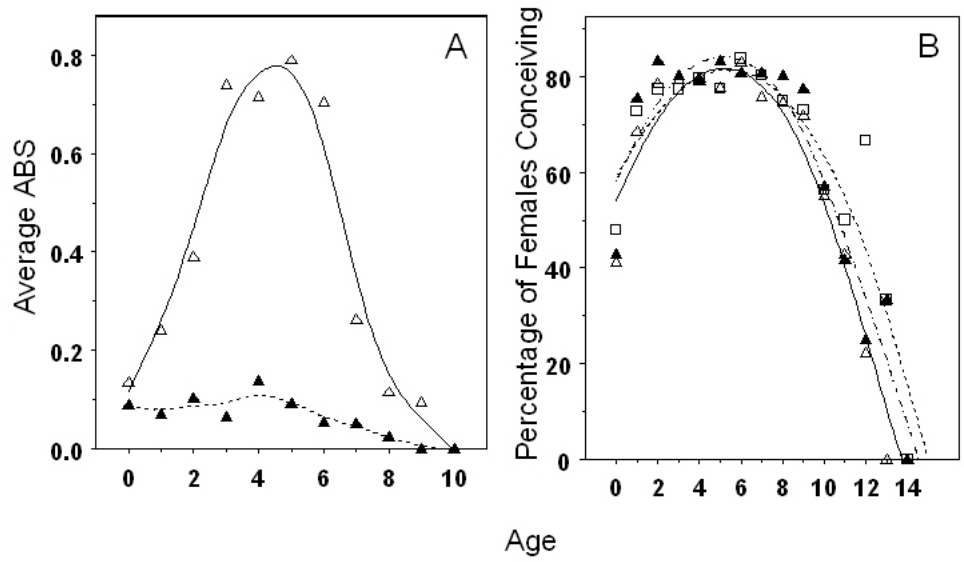

Figure 2.3. (A) Changes in annual breeding success (ABS) in males and (B) the percentage of females conceiving each year with age (in years). Normal-horned individuals are depicted by open circles and solid lines; scurred individuals: solid triangles and dash-dot line; Polled individuals: open squares and dashed line. Lines are fitted using a cubic spline function.

Selection was found to act upon the horn type of males through ABS, with normal-horned males showing significantly higher ABS when compared to scurred males (Table 2.1). This association appears to be driven by normal-horned males showing significantly greater ABS between the ages of three and six, when compared to either scurred males or normal-horned males of other ages (Table 2.1). The associations with age class were maintained when the analysis was repeated considering only males that had lived to at least seven years of age, suggesting that this trend was not driven by the selective appearance or disappearance of successful breeders from the older age classes (results not presented here). Body weight was also positively associated with ABS, but no significant association was found for hind-leg length (Table 2.1). ABS was lower at high population densities for normal-horned males, but there was no significant association for scurred males (Table 2.1). The addition of horn size nested within horn type showed that horn size was significantly positively related to ABS in normal-horned males but had no effect in scurred males (Figure 2.4; Table 2.1).

Table 2.1. Analysis of selection through annual breeding success (ABS) in males. Results are from a generalized linear mixed model with male ABS as dependent variable, negative binomial error structure and logarithmic link function. Individual identity and year were fitted as random effects. Significance of terms was calculated using Wald statistics and indicated by *P* based upon the term fitted last in the model. N denotes the number of observations based upon number of individuals. Nested effects of age groups (A1: 0-2 years; A2: 3-6 years; A3 7+ years) within horn type are compared to those individuals within the first age group. The model was then repeated with horn size nested within horn type. The addition of horn size had a negligible effect on the random effects.

Male ABS	Parameter estimate (SE)	df	Wald statistic	*P*
Heft		2	0.43	0.782
North-west	-0.032 (0.190)			
South-west	-0.036 (0.226)			
Hindleg length	0.002 (0.003)	1	0.67	0.415
Weight	0.031 (0.007)	1	20.67	<0.001
Horn type		1	15.67	<0.001
Normal-horned	0.175 (0.041)			
Horn type: Age (factor)		4	82.99	<0.001
Scurred: A2	0.061 (0.057)			
Scurred: A3	-0.094 (0.074)			
Normal-horned: A2	0.396 (0.044)			
Normal-horned: A3	-0.089 (0.066)			
Horn type: Density		2	37.87	<0.001
Scurred: density	-0.005 (0.003)			
Normal-horned: density	-0.013 (0.002)			

Random effects	Variance component	SE
Individual	0.107	0.011
Year	0.003	0.002
Residual	0.426	0.013
	N = 986 observations (637 individuals)	

Horn type: Horn size		2	31.24	<0.001
Scurred-horn	-0.002 (0.001)			
Normal-horn	0.015 (0.005)			
	N = 946 observations (621 individuals)			

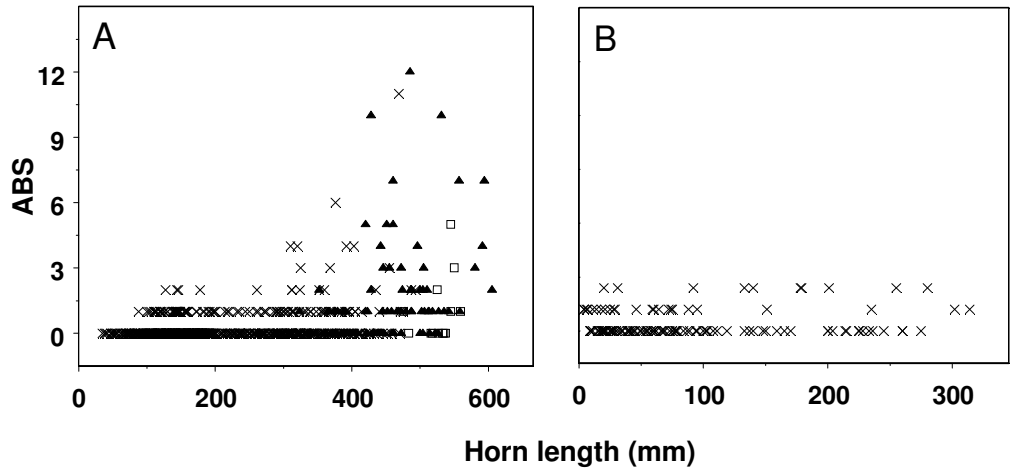

Figure 2.4. Changes in annual breeding success (ABS) with horn length in normal-horned (A) and scurred (B) males. Normal-horned males are divided into three age categories; crosses: age one to three years; triangles: three to seven years; open squares: seven years and above.

The percentage of females conceiving increased with age until the age of eight, when senescence appeared to reduce the percentage reproducing in older age groups (Figure 2.3B). Although no apparent differences were observed across horn types in the percentage of females reproducing with age (Figure 2.3B), once other variables had been corrected for in the full model, normal-horned females showed reduced average ABS across all ages (Table 2.2). Weight and hind-leg length were significantly positively related to ABS in females (Table 2.2), indicating that, as with males, larger individuals were more likely to reproduce in a given year. Population density was also associated with ABS in females, with no significant differences found between the horn types (Table 2.2). Heft was not related to ABS in either sex (Table 2.1; Table 2.2). Both linear and quadratic associations of ABS with age were found in females (Table 2.2), confirming the pattern identified in Figure 2.3.

Horn size nested within horn type was negatively associated with ABS for normal-horned females (Table 2.2), indicating that increased horn growth may be costly, however the coefficients were extremely small (Table 2.2). No significant association was found between horn size and ABS in scurred females (Table 2.2).

Table 2.2. Analysis of selection through annual breeding success (ABS) in females. Results are from a linear mixed model for female ABS. Individual identity and year were fitted as random effects. Significance of terms was calculated using Wald statistics and indicated by *P* based upon the term fitted last in the model. N denotes the number of observations based upon number of individuals. The model was then repeated with horn size nested within horn type (note that polled females are necessarily excluded from the final analysis). The addition of horn size had a negligible effect on the random effects.

Female ABS	Parameter estimate (SE)	df	Wald statistic	*P*
Age	0.151 (0.014)	1	670.10	<0.001
Age^2	-0.016 (0.001)	1	517.76	<0.001
Heft		2	0.20	0.907
North-west	-0.042 (0.028)			
South-west	-0.001 (0.032)			
Hindleg length	0.005 (0.002)	1	24.12	<0.001
Density	-0.001 ($3.0x10^{-4}$)		26.78	<0.001
Weight	0.017 (0.008)	1	8.65	0.001
Horn type		2	6.80	0.010
Polled	0.031 (0.036)			
Scurred	0.000			
Normal-horned	-0.142 (0.056)			
Horn type: Density		3	0.99	0.611
Scurred: density	0.001 (0.001)			
Normal-horned: density	0.002 (0.003)			
Polled: density	-0.002 (0.001)			

Random effects	Variance component	SE
Individual	0.083	0.009
Year	0.017	0.006
Residual	0.232	0.006
	N = 2936 observations (1307 individuals)	

Horn type: Horn size		2	22.49	<0.001
Polled	NA			
Scurred-horn	$-4.5x10^{-4}$ ($4.5x10^{-4}$)			
Normal-horn	-0.001 ($4.5x10^{-4}$)			
	N = 1734 observations (864 individuals)			

2.4.3 Longevity

In males, viability selection acting through longevity favoured scurred individuals, with negative coefficients observed for normal-horned males indicating a survival cost associated with their phenotype (Table 2.3A; Figure 2.5A). Population density at

birth was significantly related to longevity in males and explained the greatest proportion of model deviance, with negative coefficients suggesting that individuals

Table 2.3. Analysis of selection through longevity in (A) males and (B) females. Coefficients (with SE) for terms in an analysis of longevity in males (M_{LG}) and females (F_{LG}) with a negative binomial error structure and logarithmic link. Significance of departures from zero were estimated using F statistics indicated by P. All terms have one degree of freedom with the exception of horn type (2 df) and both residual deviance and degrees of freedom are given. Analysis of horn type: three horn phenotypes are present in females, polled (no horns), scurred (reduced horn) and normal horned; and two in males, scurred and normal-horned. All effects are compared to scurred individuals. The model was then repeated with horn size (recorded at death and age corrected) nested within horn type (2 df). The direction of coefficients remained the same and thus only the effects of the additional terms are shown.

	(A) Male Coefficient (SE)	*F*	*P*	(B) Female Coefficient (SE)	*F*	*P*
Birth weight	0.004 (0.061)	0.003	0.950	0.103 (0.056)	2.52	0.080
Hindleg length	0.011 (0.005)	5.955	0.010	0.017 (0.005)	12.95	<0.001
Horn type		3.629	0.035		8.27	<0.001
Polled	NA			-0.267 (0.086)		
Scurred-horn	0.000			0.000		
Normal-horn	-0.183 (0.094)			0.102 (0.081)		
Density	-0.004 (0.001)	154.888	<0.001	-0.004 (3.3x10-4)	227.49	<0.001
Twin	-0.217 (0.085)	6.691	0.010	-0.299 (0.086)	11.26	<0.001
Residual deviance = 687.68 Residual df = 856				Residual deviance= 829.94 Residual df = 1090		
Horn type: Horn size		3.921	0.020		3.64	0.030
Polled	NA			NA		
Scurred-horn	-0.048 (0.088)		0.586	0.069 (0.192)		0.721
Normal-horn	-0.147 (0.053)		0.006	-0.347 (0.126)		0.006
Residual deviance=660.47 Residual df = 840				Residual deviance = 735.33 Residual df = 876		

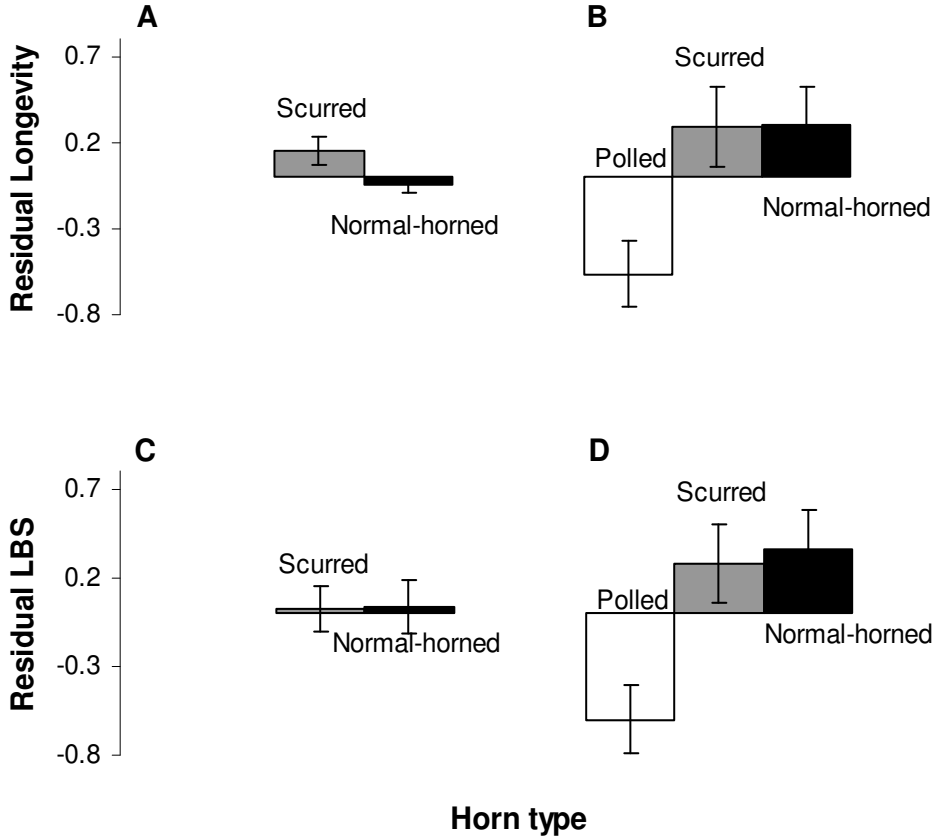

Figure 2.5. Bar charts (with SE) showing average residual longevity and LBS for males (A;C) and females (B; D) within each horn type. Residuals were gained from models of Longevity and LBS containing birth-weight, density in year of birth, twin, and age-adjusted hindleg length.

born at low population densities survived for greater periods of time (Table 2.3A). Age adjusted hindleg length was positively associated with longevity in males (Table 2.3A). Natural selection favours males born as singletons, as shown by the significant negative association between twin status and longevity; but no association with birth weight was found, probably due to the effect being removed by the association with twin status (Table 2.3A).

Longevity was negatively associated with horn size in normal-horned males (Table 2.3A; Figure 2.6A), but there was no significant relationship in scurred males (Table 2.3A; Figure 2.6B).

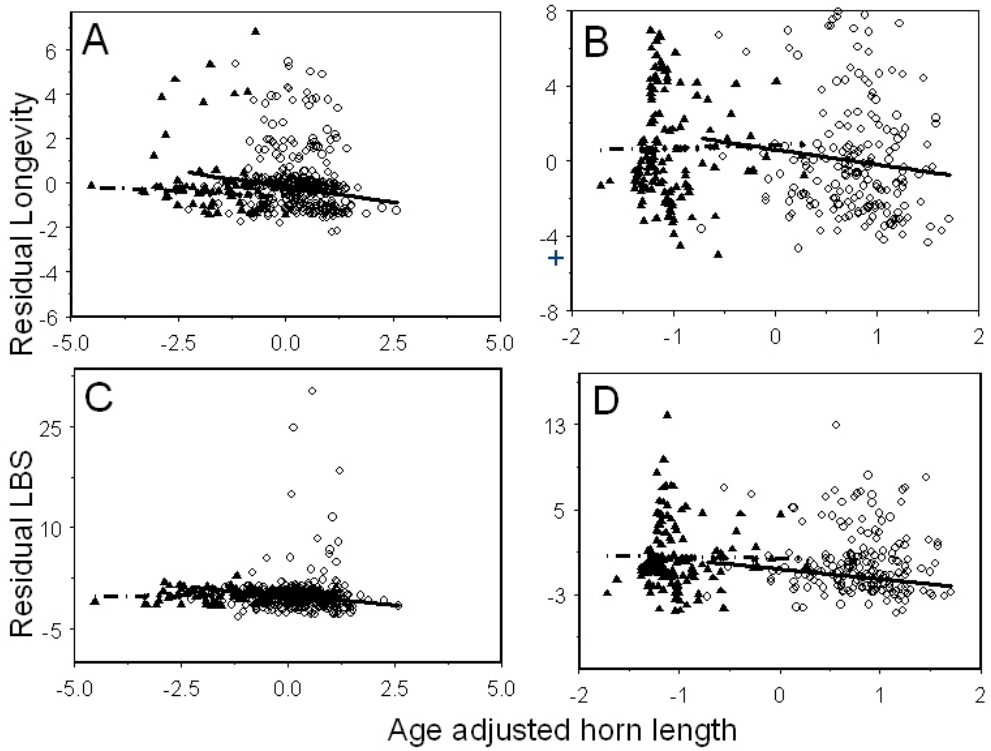

Figure 2.6. Changes in residual longevity (LG) and lifetime breeding success (LBS) with age adjusted horn size in males (A; C) and females (B; D). Residuals gained from a model of hindleg (age adjusted), density at year of birth, twin, and horn type on fitness variables longevity and lifetime breeding success. Horn size is age-standardized for age at death (see methods). Scurred individuals (Sc): solid triangles and dot-dash line; Normal-horned individuals (NH): solid line. Lines show linear regression between two variables.

In females, viability selection favoured scurred and normal horned individuals as opposed to those which were polled, with a negative coefficient observed for polled females (Table 2.3B; Figure 2.5B). Like males, population density at birth was significantly negatively related to longevity in females, and explained the greatest proportion of model deviance, with negative coefficients indicating that females born at low population densities survived for greater periods of time (Table 2.3B). Age adjusted hind-leg length was associated with increased longevity in females, thus confirming positive selection on body size in both sexes (Table 2.3B). Birth weight

was not associated with longevity in females, but females born as singletons had greater longevity than those born as twins, as shown by the significant negative association between twin status and longevity (Table 2.3B).

As for males, horn size in normal-horned females was negatively associated with longevity, with negative coefficients suggesting normal-horned individuals with increased horn length were significantly more likely to die at an earlier age than normal-horned individuals with shorter horns (Table 2.3; Figure 2.6B). No relationship was observed between longevity and horn size in scurred females (Table 2.3; Figure 2.6B).

2.4.4 Lifetime breeding success

Selection pressure acting through lifetime breeding success (LBS) did not differ between horn types in males, suggesting no difference in overall fitness between the two phenotypes (Table 2.4A; Figure 2.5C). Population density at birth had the greatest influence on LBS in males, with negative coefficients suggesting that individuals born at low density produced more offspring. Age adjusted hind-leg length was significantly positively associated with LBS in males suggesting that larger individuals also produced more offspring. Birth weight and being born a twin or a singleton were not associated with LBS in males (Table 2.4A).

There was no association between horn size and LBS in males (Table 2.4A; Figure 2.6C). The positive association of horn size with ABS thus appeared to be masked by the negative association with longevity (Table 2.4A; Figure 2.6A).

Polled females were shown to have reduced LBS, with negative coefficients suggesting a reduction in lifetime fitness (Table 2.4B; Figure 2.5D). Although normal-horned females were shown to have reduced annual breeding success, no difference in LBS was found between scurred and normal-horned females using GLM analysis (Table 2.4B; Figure 2.5D). Population density at birth had the greatest influence on LBS in females, and similar to males, negative coefficients suggested that individuals born at low population density did, on average, produce more

offspring (Table 2.4B). Age adjusted hindleg length was positively associated with LBS in females, with positive coefficients confirming results gained from the analyses of ABS and longevity, that larger individuals had greater lifetime breeding success (Table 2.4B). Birth weight was also positively associated with LBS in females, a result not found in males (Table 2.4B). In females, being born a singleton was also significantly associated with LBS (Table 2.4B). It therefore appears that the circumstances of birth affect lifetime fitness in females to a greater degree than in males.

In normal-horned females there was a significant negative association between horn size and LBS (Table 2.4B; Figure 2.6D). No significant association of horn size and LBS was found in scurred females (Table 2.4B; Figure 2.6D).

Table 2.4. Analysis of selection through lifetime breeding success in (A) males and (B) females. Coefficients (with SE) for terms in an analysis of lifetime breeding success in males (M_{LBS}) and females (F_{LBS}) with a negative binomial error structure and logarithmic link. Significance of departures from zero were estimated using F statistics and are indicated by P. All independent terms have one degree of freedom and both residual deviance and residual degrees of freedom (df) are given. Three horn phenotypes are present in females, polled (no horns), scurred (reduced horn) and normal horned; and two in males, scurred and normal-horned. All effects are compared to individuals who are scurred. The model was then repeated with horn size (recorded at death and age corrected) nested within horn type.

(A) Male				(B) Female		
	Coefficient (SE)	*F*	*P*	Coefficient (SE)	*F*	*P*
Birth weight	0.129 (0.189)	0.489	0.480	0.304 (0.114)	4.367	0.030
Hindleg length	0.049 (0.015)	8.967	0.001	0.043 (0.010)	18.894	<0.001
Horn type		0.019	0.891		5.720	0.001
Polled	NA			-0.478 (0.177)		
Scurred	0.000			0.000		
Normal-horn	0.044 (0.322)			0.136 (0.166)		
Density	-0.011 (0.001)	111.809	<0.001	-0.009 (0.001)	160.842	<0.001
Twin	-0.309 (0.268)	1.331	0.250	-0.636 (0.180)	12.275	0.001
Residual deviance = 678.39 Residual df = 856				Residual deviance= 744.07 Residual df = 986		
Horn type: Horn size		0.726	0.480		3.304	0.037
Polled	NA			NA		
Scurred-horn	-0.083 (0.301)		0.782	0.126 (0.378)		0.739
Normal-horn	0.222 (0.172)		0.198	-0.656 (0.245)		0.008
Residual deviance = 666.73 Residual df = 840				Residual deviance = 502.29 Residual df = 878		

2.4.5 Selection coefficients and gradients

Selection coefficients revealed similar associations between horn type and lifetime breeding success as those gained from the GLM for longevity and LBS. In males, selection coefficients for horn type indicated a non-significant reduction in relative LBS of scurred as compared to normal-horned individuals (Table 5). In females, selection coefficients for horn type indicated no significant difference between scurred and normal-horned individuals and a lower relative lifetime breeding success for polled individuals when compared to either group (Table 5). When directly compared, linear selection on scurred as compared to normal-horned individuals differed significantly between the sexes, with the inclusion of a sex by horn type interaction significantly decreasing model deviance (Table 6).

Calculating selection gradients on horn size for each horn type revealed a contrasting pattern in the relationship between horn size and LBS for normal-horned males and females, with non-significant positive coefficients for males but significant negative coefficients for females (Table 5). This supports the negative associations of fitness and horn size in normal-horned females which were found within all of our analyses. There was no evidence for selection on horn size of scurred individuals of either sex (Table 5). Estimates of direct selection on horn size after taking into account selection on body size through hind-leg length were generally lower than those for total selection (Table 5). There was evidence of significant antagonistic selection on horn size between normal-horned males and females, with the inclusion of the interaction term sex-by-horn size significantly reducing model deviance (Table 6).

Table 2.5. Selection coefficients and standardised selection gradients for lifetime breeding success in (A) males and (B) females. 1) Selection coefficients for horn type in males (normal-horned relative to scurred; n=1815) and females (normal-horned relative to scurred N = 2571; normal-horned relative to polled N = 2730; scurred relative to polled N = 2395), corrected for body size (positive coefficients were found for hindleg in all comparisons and thus is not presented here). 2) Selection gradients for horn size in males and females: (a) total selection; (b) direct selection correcting for body size. The effects of horn size were first tested for all individuals and then for 3) scurred males (N = 174) and females (N = 516); 4) normal-horned males (N = 1643) and females (N = 749) separately.

Variable	(A) Males (SE)	(B) Females (SE)
1) Selection coefficients for horn type:		
a) As compared to normal-horned individuals:		
Scurred	-0.218 (0.148)	-0.069 (0.128)
Polled	NA	-0.824 (0.014)
b) As compared to scurred individuals:		
Polled	NA	-0.789 (0.012)
2) Selection gradients for horn size:		
a) Horn size	0.393 (0.171)	-0.009 (0.187)
b) Horn size	0.151 (0.174)	-0.103 (0.181)
Hindleg	0.991 (0.176)	1.160 (0.191)
3) Selection gradients for horn size in scurred individuals:		
a) Horn size	-0.135 (0.154)	0.412 (0.778)
b) Horn size	-0.175 (0.158)	0.290 (0.768)
Hindleg	0.263 (0.136)	1.136 (0.328)
4) Selection gradients for horn size in normal-horned individuals:		
a) Horn size	0.555 (0.279)	-0.143 (0.087)
b) Horn size	0.071 (0.291)	-0.044 (0.079)
Hindleg	1.156 (0.210)	1.084 (0.249)

Table 2.6. Selection coefficients and standardised selection gradients for lifetime breeding success across the sexes. Note that as all offspring are not assigned a sire, male breeding success is underrepresented, generating the apparent significant differences in average LBS between the sexes; however, this will not be biased across males. Partial *F*-test statistics represent the comparison of the unexplained sums of squares from the regression with and without the interaction term to test whether selection differed between the sexes. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Polled females were excluded from the analysis in section (1).

Variable	Regression Coefficient (SE)	Partial *F*-test statistic
1) Selection coefficients for horn type (N = 2581)		
Sex		
Male compared to female	-1.548 (0.265)	
Hindleg	0.718 (0.088)	
Horn type		
Scurred compared to Normal-horned	0.473 (0.173)	
Sex*Horn type	-0.869 (0.330)	15.72***
2) Selection gradients for horn size in scurred individuals (N = 325)		
Sex		
Male compared to female	-2.225 (0.703)	
Hindleg	0.869 (0.323)	
Horn size	0.177 (0.358)	
Sex*Horn size	-0.693 (0.458)	1.02
3) Selection gradients for horn size in normal-horned individuals (N = 901)		
Sex		
Male compared to female	-2.253 (0.565)	
Hindleg	1.267 (0.156)	
Horn size	-0.783 (0.126)	
Sex*Horn size	0.783 (0.128)	38.96***

2.5 DISCUSSION

I have shown that when a polymorphic trait is displayed in both male and females it may be subject to different selective pressures depending upon the phenotypic type and the sex of the individual. In this population of Soay sheep, differences in selection pressures between the sexes generated sexually antagonistic effects. Furthermore, there was evidence of a trade-off between annual breeding success and longevity in males, with both the normal horn type and (in normal horned males) larger horn size associated with greater annual breeding success but reduced longevity. As a result, selection through lifetime breeding success was not found to act upon horn phenotype in males. When combined, these factors will presumably contribute to the maintenance of the present phenotypic polymorphism for both horn type and size observed in this population.

2.5.1 Trade-offs between components of fitness

The cost of breeding may be the most important control of a mating system, with trade-offs occurring between reproductive success and survival in many systems (Brooks 2000; Liker and Szekely 2005). If investment in sexual traits does not vary in relation to the ability to bear the costs, then a functional trade-off will occur with a negative relationship between male longevity and the expression of sexual traits (Jennions et al. 2001). In this population, the reproductive strategy of male-to-male conflict, typical of normal-horned males, may be more costly than that of the coursing or sneak mating tactics used by scurred males (Stevenson et al. 2004), given the likely costs of fighting as well as of greater investment in weaponry. The fitness of normal-horned males may be maximised by balancing investment in weaponry as they mature with their survival each year (Hansen and Price 1995). However, the results presented here may reflect a survival cost of investment in weaponry (in both sexes), and hence support the suggestion that individuals may not be able to sufficiently regulate their horn growth during periods of harsh conditions.

Several studies have reported associations between a secondary sexual trait and breeding success within a given year, independent of body size (Coltman et al. 2002;

Preston et al. 2003). However in this population, variance in longevity was sufficient to neutralise any breeding advantages of normal-horned males, with selection through longevity favouring scurred males who on average gain a lower number of paternities each year. This is likely to be the reason why no difference in lifetime breeding success was evident between scurred and normal-horned males. In a parallel scenario, male bighorn sheep with large horns achieve higher breeding success in a given year, but hunting pressures reduce their longevity, with the net effect of no association between horn size and lifetime breeding success (Coltman et al. 2002, 2005). Selective forces can act in opposing directions at different stages of life-history (Schluter et al. 1991) and this study provides further illustration of the need to consider lifetime breeding success and its component factors rather than short-term measures of breeding success.

Further evidence for the need to consider long-term measures of breeding success is demonstrated in females, where there were no apparent differences in fitness between scurred and normal-horned females when analysed using a GLM incorporating a range of other variables. Although normal-horned females showed reduced average annual breeding success (Table 2), there was no evidence of a significant reduction in longevity (Table 3), thus neutralising any associations with LBS in the GLM.

2.5.2 Sexually antagonistic selection

Sexually antagonistic associations have received relatively little attention within the literature (although see: Rice and Chippendale 2001; Foerster et al 2007). Previous studies have shown that when selection acts differently on different components of a trait and between the sexes, sexually antagonistic phenotypic selection will occur (Endler 1980; Forsman 1995). I found differences in the patterns of selection acting on horn phenotype in males and females, with significantly contrasting linear selection on scurred as compared to normal-horned individuals between the sexes (Table 6). Furthermore, there was evidence of antagonistic selection in the associations of horn size with LBS, in normal-horned individuals (Table 6). This supports previous studies which have also shown antagonistic selection to be

consistent with the direction of a sexual size dimorphism (e.g. Preziosi and Fairbairn 2000; Schulte-Hostedde et al. 2002). These results suggest that sexually antagonistic selection may therefore contribute to the polymorphism for horn phenotype and variation observed in trait size.

The most significant association in my results was that of polled females with reduced lifetime breeding success. It would therefore be expected that selection should serve to remove alleles generating this phenotype from the population. However, although polled females are the least frequently occurring class, there is no evidence of any decline in the frequency over the study period (linear regression of percentage of polled females over 20 year study period $b = 0.002$ (0.001), $F_{1,19} = 2.28$, $p = 0.157$). Previous work has suggested that the inheritance of horn type can be described with a model containing three alleles with sex-specific effects (Coltman & Pemberton 2004), but when I re-analysed the data using a larger sample size, a less acceptable fit of this model was found. The genetic basis of horn phenotype in the St. Kilda Soay sheep is the subject of ongoing research, and once a reliable model has been established it will be possible to explore the contribution of the phenotypic selection pressures described here to the maintenance of genetic polymorphism.

Selection on body size was clearly evident in both sexes and all horn types, with larger individuals having greater breeding success, supporting previous results gained for this population (Clutton-Brock et al. 1996; Clutton-Brock et al. 1997; Coltman et al. 1999; Milner et al. 1999) and another ungulate species (Coltman et al. 2002; Festa-Bianchet et al. 1998; Festa-Bianchet et al. 1997; Realè et al. 1999).

2.5.3 Selection and the environment

In the wild, variable environments can lead to fluctuating selection pressures, which have been shown to maintain variation in many traits ranging from behaviour (e.g. Dingemonse et al. 2004) to reproduction (e.g. Visser et al. 1998). In this work, population density was shown to be associated with fitness, with density during the rut associated with ABS and the density at which an individual is born associated with both LG and LBS. This confirms previous results of cohort-specific effects in

wild ungulate populations (Coltman et al. 1999), with the deviance of models of LG and LBS being most affected by population density in the year of an individual's birth. Contrary to previous results (Clutton-Brock et al. 1997), there was no evidence that selection pressure on the horn type of either sex differed in differing environments. However in males, population size was associated with ABS in normal-horned males but not scurred males. This is likely to be a result of the mating strategy of normal-horned males, where competition for mates will increase with increasing population density.

2.5.4 Work arising from this chapter

The causal mechanisms underlying the correlations between fitness components and horn type and size in this population may take several forms but are not yet fully understood. Speculation as to the costs and benefits of horns in both males and females has persisted since work by Geist (1971). Currently there is no explanation as to how female mammals use weaponry or if there are any benefits to growing horns which could counteract the negative associations with fitness demonstrated here. A behavioural analysis of horn use in females is presented in Chapter two. An analysis of the ecological determinants of horn size and how males and females respond to the environment may also shed further light on the relative costs and benefits of displaying the phenotypes described and this is presented in Chapter four.

2.5.5 Summary

In summary, I found evidence for selection acting upon horn type and size within both male and female Soay sheep, independent of skeletal body size. This polymorphic trait was subject to different selection pressures depending upon the phenotype displayed and the sex of the individual, thus generating sexually antagonistic selection for both horn type and horn size. Furthermore, estimates of phenotypic selection were dependent upon the specific component of fitness measured, with a balance between the effects of selection through annual breeding success versus through longevity. As a result, there were no differences in lifetime fitness between scurred and normal-horned males which adopt different mating

strategies. These results illustrate the contrasting conclusions that may be drawn when different components of fitness are used in selection analyses, and underline the need to use as comprehensive an estimate of fitness as possible for a full representation of evolutionary processes.

CHAPTER 3

THE USE OF HORNS IN INTRASEXUAL COMPETITION FOR RESOURCES IN FEMALE SOAY SHEEP

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3.1 SUMMARY

In many species, females show reduced expression of a trait that is under sexual selection in males, and this expression is thought to be maintained through genetic associations with the male phenotype. However, there is also the potential for the female trait to convey an advantage in intrasexual conflicts over resources. I tested this hypothesis in a feral population of Soay sheep, in which males and females have a polymorphism for horn development, producing either full (normal-horned), reduced (scurred) or no horns (polled: females only). During the lambing period, females who possessed horns were more likely to initiate and win aggressive interactions, independent of age, weight, and birthing status. The occurrence of aggression was also context dependent, decreasing over the lambing period and associated with local density. These results demonstrate that a trait which confers benefits to males during intrasexual competition for mates may also be utilized by females in intrasexual competition over resources: males use weaponry to gain mates, whereas females use weaponry to gain food.

3.2 INTRODUCTION

In polygynous mammals, intrasexual aggression typically differs between the sexes, with males coming into conflict over breeding opportunities while females come into conflict over resources such as food and space (Thouless and Guinness 1986; Festa-Bianchet 1991). Aggressive competition for reproductive opportunities in males may lead to the evolution of traits such as horns or antlers, which are associated with breeding success (Andersson 1994). In many species, females show reduced expression of a male sexually selected trait. Darwin (1874) explained this through a process of 'inheritance' and Lande (1980) demonstrated that despite costs of displaying the trait, female expression may be maintained through genetic associations between the sexes. An alternative hypothesis exists where females benefit from the expression of secondary traits, with trait size as a signal of condition which females use to assess each other at distance (West-Eberhard 1983; Amundsen 2000). However, previous studies have found that horns and antlers do not directly influence the outcome of female intrasexual interactions, particularly when age is taken into account (e.g. Barrette and Vandal 1986; Holand et al. 2004). If female horns have a direct function in intrasexual conflict the possession of the trait would convey a direct advantage in competition over resources. Therefore, the potential would exist for the trait to be maintained within the population as a result of positive selection.

To assess the function of female horns, I examined the relationship between female intrasexual aggression and horn type, in a free-living population of Soay sheep (*Ovis aries*) on the island of Hirta, St. Kilda, Scotland. Soay sheep display a polymorphism for horn development, with the two sexes producing either a full horn (normal-horned), a reduced horn (scurred) or no horns (polled: females only). They therefore provide an ideal opportunity to examine the relationship between aggressive interactions and secondary trait development in females, independently of body size and age. Furthermore, low grass growth and high female resource demand during early spring, means that requirements for resources will exceed their availability

(Clutton-Brock and Pemberton 2004). Therefore, I also examine female intrasexual aggression in relation to breeding time and density of resource use.

3.2 METHODS

3.2.1 Study population

The present study focuses on an unmanaged, feral population of Soay sheep (*Ovis aries*) residing on the island of Hirta within the St. Kilda archipelago in the North Atlantic (57°49' N, 08°34' W). Since 1985 individuals within the Village Bay study population have been intensively monitored throughout life, with 95% of lambs born being ear-tagged (for a detailed description see: Clutton-Brock and Pemberton 2004). Behavioural observations on aggression have not previously been conducted upon the females of this population.

3.2.2 Behaviour

During a single lambing period (April-May 2006) I conducted fifty, one-hour sampling periods, divided between three areas of similar fixed size. First, I recorded the identities of all females present within each area, determining local density, and recording the reproductive status (whether they had given birth or not) of each female observed. As individuals have been followed from birth, recording their identity allowed us to determine the age and horn type of every individual in the sample. Secondly, all-occurrence sampling (Altmann 1974) was conducted for one hour within each area, recording aggressive interactions between females. Aggressive behaviour was defined as displacement of another individual through movement (move or turn towards), body threats (ears back or legs forward), or head butting. The conflict was considered resolved when an individual withdrew from the area in which they were feeding. A winner and a loser were identified for every interaction, and in all aggressive interactions the winner was the initiator. Myself and two other observers rotated between areas and as there was no evidence of any effect

of area or observer I excluded these factors from our analyses. Censuses recorded the identity of 185 females, a total of 862 times, and over the 50 independent sampling periods we recorded 51 aggressive interactions involving 39 different individuals. Soay sheep forage in single sex herds (Clutton-Brock and Pemberton 2004) and thus I observed only intrasexual interactions.

3.2.3 Morphometric measurements

Morphometric measures are recorded in a two week period in August each year when approximately 65% of the study population are caught and measured. I included measures recorded in August 2005 of the horn length of normal-horned females (measures were available from 40 individuals, which were recorded 165 times), individual body weight (in kg), and hindleg length (distance between tubercalcis of fibular tarsal bone to the distal end of the metatarsus: a measure of body size). Body weight and hindleg measures were available for 75 females upon which 315 observations were made.

3.2.4 Statistical analyses

I divided my statistical analyses into two parts. In the first analysis, I conducted a generalized linear mixed model (GLMM) with binomial error structure to determine factors associated with female aggression. All of the individuals observed in each census were recorded as aggressive (1, i.e. initiating aggression) or non-aggressive (0) in the following sampling period. Fixed effects included age, horn type, reproductive status, the density of females in the area under observation (local density), and the proportion of those with lambs. The model was reduced in a step-wise manner and only the final model is shown. Including morphometric measures reduced the sample sizes; therefore I tested for their effects by adding them to the final model.

Second, I conducted a GLMM with binomial error structure based upon the observed aggressive interactions, to test for differences in age and horn phenotype between the winner and loser of each interaction. A focal individual was selected at random from

each interacting pair and classified as either the winner (1) or loser (0). Relative age (age difference between the focal and the second individual) was included as a linear fixed effect, alongside relative horn type (difference between the focal and the second individual in ranked horn phenotype: polled = 0; scurred = 1; normal-horned = 2). Including both fixed effects within one model separated the effects of age and weaponry.

In both GLMMs, fixed effect significance was assessed using Wald statistics tested against a χ^2 distribution on the appropriate degrees of freedom. Including individual as a random effect accounted for repeated observations on individuals. Analyses were conducted using GENSTAT (VSN International Ltd., Hemel Hempsted, UK).

3.3 RESULTS

3.3.1 Occurrence of aggression

The initiation of female intrasexual aggressive interactions was associated with an individual's horn type, age, and reproductive status (Table 3.1). Normal-horned females were more likely to be aggressive than scurred or polled females (Figure 3.1a). Older individuals within a group were also more likely to be aggressive (Figure 3.1b), with no evidence of an interaction between age and horn type ($Wald_1 = 0.18$, $P = 0.682$). Mothers were less likely to show aggression (Table 3.1). We found no evidence that female body size ($Wald_1 = 0.04$, $P = 0.850$) or body weight ($Wald_1 = 0.13$, $P = 0.718$) was associated with aggression and no evidence that, within normal-horned females, horn size was associated with aggression ($Wald_1 = 0.13$, $P = 0.715$). Horn type remained significant when morphometric measures were included within the model ($Wald_2 = 4.68$, $P = 0.035$).

The occurrence of aggression depended upon the surrounding group structure (Table 3.1). A high proportion of mothers in a group was associated with lower aggression (Figure 3.2a). The higher the local density, the more likely an aggressive encounter

(Figure 3.2b). There was also significant interaction between density and proportion of mothers (Table 3.1). Both results were not dependent upon an outlying high density, high aggression group; removing the observation did not alter their significance when the model was re-run.

Table 3.1. Probability of aggression in all females observed. Generalized mixed model with binomial error structure, showing only significant effects. Non-significant variables included: body weight, body size, and normal-horned female horn size. The random effect of individual accounted for repeated observations on females.

N = 862 observations on 185 individuals.

Variable	Estimate (SE)	df	Wald statistic	*P*
Fixed effects				
Age	0.321 (0.055)	1	33.92	<0.001
Horn type		2	28.59	<0.001
polled	-1.648 (0.774)			
scurred	0.000			
normal-horned	1.152 (0.543)			
Reproductive status		1	37.29	<0.001
w*ithout lamb*	0.000			
w*ith lamb*	-2.628 (0.430)			
Local density	0.017 (0.007)	1	6.14	0.014
Proportion of mothers	-1.402 (0.506)	1	7.66	0.006
Local density* proportion of mothers	-0.156 (0.062)	1	6.36	0.012
Random effects	*Variance (SE)*			
Individual	4.706 (0.934)			
Residual	4.210 (0.225)			

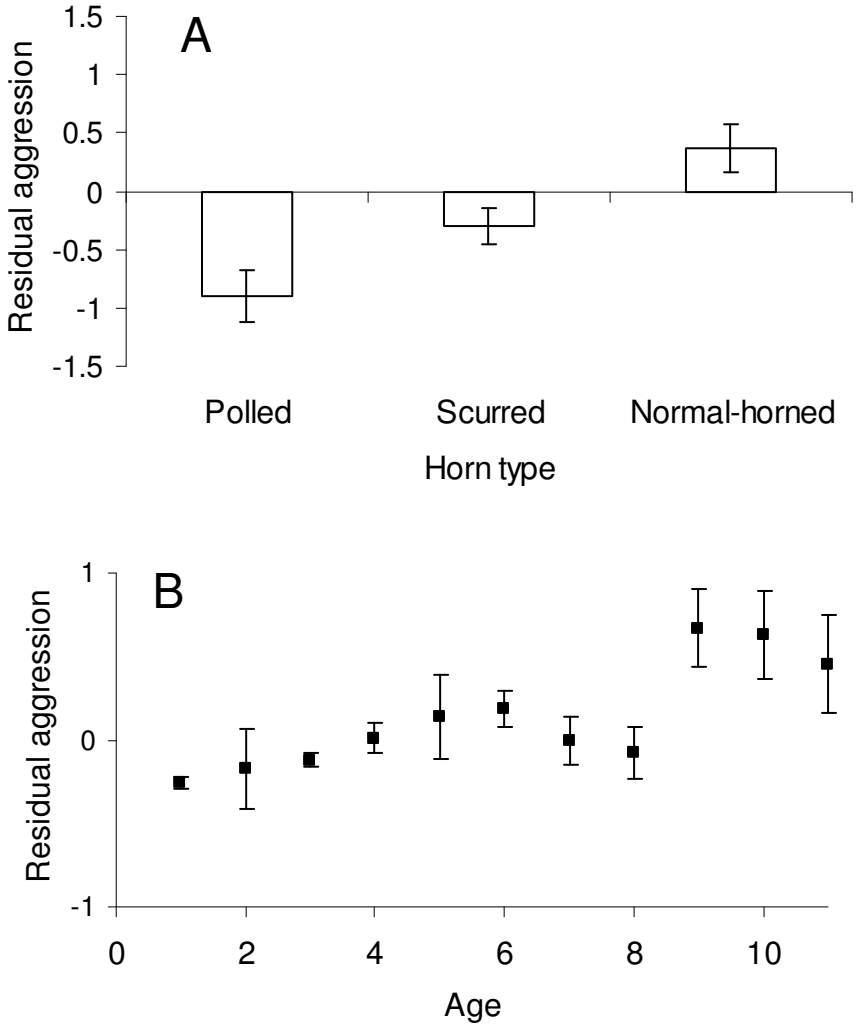


Figure 3.1. Probability of initiating aggression with (A) horn type and (B) age. Error bars represent standard error of the mean. N = 862 observations on 185 individuals.

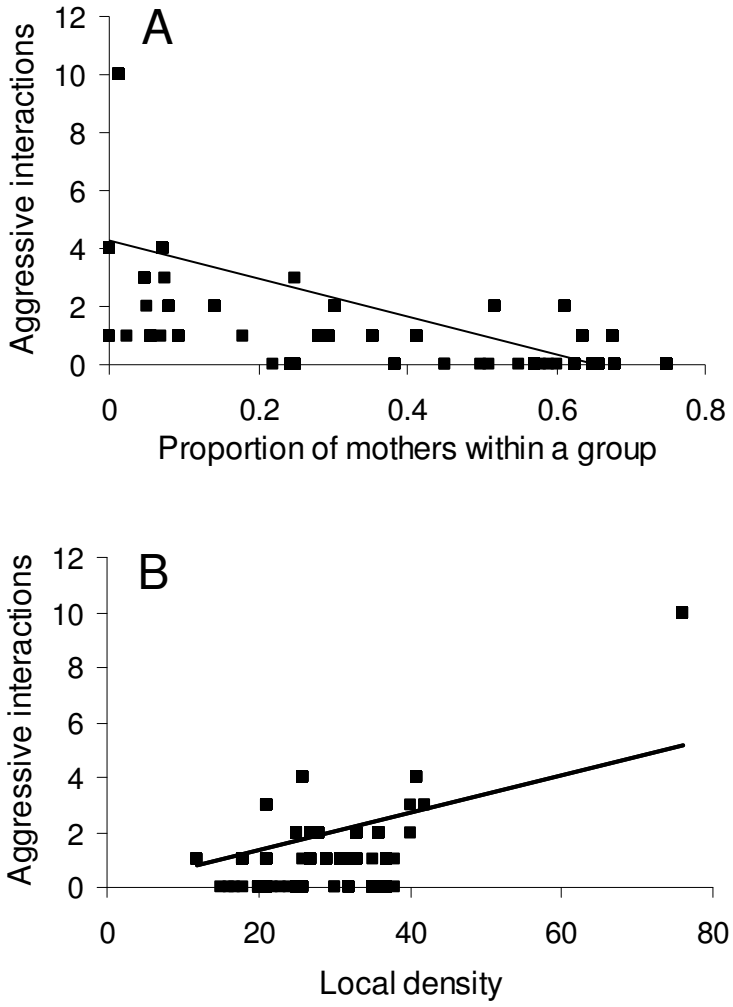


Figure 3.2. Number of aggressive interactions within a group with (A) proportion of mothers and (B) local density. N = 50 observation periods.

3.3.2 Aggressive interactions

Females were more likely to show aggression towards females who were younger, with positive coefficients for relative age (Table 2). Independent of this association, females were more likely to show aggression towards females who displayed a horn phenotype associated with less weaponry then their own (Table 2). We found no evidence that female body size ($Wald_1$= 0.12, P= 0.720) or body weight ($Wald_1$= 1.50, P = 0.220) influenced the outcome of aggression.

Table 3.2. Effects of relative age and relative horn type on the outcome of aggressive interactions in females. Generalized mixed model with binomial error structure. The random effect of individual accounted for repeated interactions of individuals.

N = 51 aggressive interaction involving 39 individuals.

Variable	Estimate (SE)	df	Wald statistic	*P*
Fixed effects				
Relative age	0.732 (0.201)	1	10.73	0.001
Relative horn type	1.142 (0.576)	1	7.98	0.008
Random effects				
Individual	4.663 (3.202)			
Residual	5.786 (1.365)			

3.4 DISCUSSION

I have shown that female horn development has a direct function in intrasexual aggressive competition for resources within a polygynous mammalian species. Females displaced individuals who displayed a horn phenotype associated with less weaponry than their own. This relationship was independent of the association between female aggression and age, with older females more likely to initiate aggression and displace younger ones. Males and females produce horns of a different shape and it has been hypothesised that the broad shape of male horns has evolved to withstand head-on clashes where as female horns have evolved as spikes for displacing individuals (Lincoln 1994). My results support the theory that the form of female horns observed in ungulates is a result of their function. They also indicate that female intrasexual aggression is context dependent. Aggressive interactions increased when local density was high, and are thus related to resource availability (Thouless and Guinness 1986; Festa-Bianchet 1991). Intrasexual aggression also decreased over the lambing period, with females distancing themselves from others to facilitate offspring protection.

However, it is unclear if an advantage in competition over resources conveys long-term benefits. In the short-term, grass growth ceases over winter resulting in high mortality rates in late winter (Clutton-Brock and Pemberton 2004). Lambing follows this period; grass growth begins again in April, but lactation will be costly and neo-natal death is commonplace (Clutton-Brock and Pemberton 2004). Resource demand exceeds availability during this period and we predict that normal-horned females are better able to provision their offspring because of their ability to gain more resources. Previous results from this system demonstrated that normal-horned females do not produce more offspring over their lifetime, when compared to the other horn types (Chapter two). An analysis of offspring fitness in relation to mother's horn type will reveal any benefits to an advantage in resource competition.

Genetic effects, testosterone levels, and early developmental conditions may also influence aggressive behaviour and this may not be the only context in which females display aggression (e.g. Bro-Jørgensen 2002). Lower sample sizes may have resulted in reduced power to detect any effect of body or horn size, and although it is clear that age and horn phenotype directly influence female intrasexual interactions, other factors may be influential. It is clear however, that in studies of sexual selection we must consider that a phenotype under sexual selection in males may also be under selection in females.

CHAPTER 4

ENVIRONMENTAL SENSITIVITY OF HORN GROWTH IN SOAY SHEEP

4.1 SUMMARY

When a secondary sexual trait is displayed in both sexes and sexual selection acts only upon the male phenotype, the male phenotype may show increased sensitivity to environmental variation. Using data from a feral population of Soay sheep (*Ovis aries*) in which both sexes grow horns, I examined the relationship between horn growth and individual-level, population-level, and climatic factors. Soay sheep have a polymorphism for horn growth, with the two sexes producing either a full horn (normal-horned), a reduced horn (scurred) or no horns (polled: females only). Only the sexually-selected trait of horn growth in normal-horned males was sensitive to environmental factors such as summer rainfall, autumn temperatures, and population density, or associated with a measure of parasite resistance. In scurred males and females of both horn types no relationships were found with any climatic or population-level factors. In normal-horned males, environmental sensitivity was determined by both age and population density. At an individual-level, I found no evidence for plasticity in allocation to horn growth relative to body weight, in response to environmental conditions experienced during growth. However, there was evidence that individual-level variance in allocation to horn growth was dependent upon an individual's condition at the start of growth each year. These effects are discussed in relation to selection on individual variation in this secondary sexual trait.

4.2. INTRODUCTION

In a polygynous species, variance in mating success is likely to be greater in males than females (Darwin 1871). It therefore follows that sexual selection acting upon traits associated with mating success should be stronger in males, leading to the development of traits such as weaponry and display plumage (Clutton-Brock et al. 1992; Andersson 1994). However, the evolution of sexually-selected traits may be constrained by the influence of environmental variation (Andersson 1994; Qvarnström 1999; Griffith and Sheldon 2001; Kruuk et al. 2002; Garant et al. 2004), with changing environmental conditions potentially leading to resource-based trade-offs between somatic maintenance and trait growth (Andersson 1994; Kotiaho et al. 2001). As a result of this condition-dependence, sexually-selected traits may be more sensitive to environmental variation than other classes of traits (Parsons 1995; Alatalo et al. 1988; although see Pomiankowski and Møller 1995). In situations in which a secondary sexual trait is under sexual selection in one sex (typically males) but not in the other, a comparison of the environmental associations of the trait between the sexes provides an opportunity to explore the relationship between sexual selection and sensitivity to environmental conditions. Furthermore, it is likely that environmental sensitivity may vary over ontogeny, and that experience of prior environmental stress may have effects on subsequent trait development. In this chapter, I test the effects of fluctuating environmental conditions on the horn growth of Soay sheep.

The ability of individuals within a population to alter the expression of a trait in response to the environment, determines whether the trait is plastic across environments. At a population level, the influence of ecological factors on sexually-selected traits is well-established in many species (Andersson 1994), with large-scale climatic variables (e.g. Post et al. 1999; Møller 2002; Garant et al. 2004), and local ecological conditions such as population density (e.g. Jorgenson et al. 1998; Schmidt et al. 2001; Kruuk et al. 2002; Festa-Bianchet et al. 2004) shown to be important determinants of phenotypic variation in sexually-selected characters, through their influence on resource availability. Individuals may expend different amounts of

energy at different times of the year, may elicit different behaviours, may differ in the costs and timing of reproductive effort, differ in rate of growth, or differ in levels of maintenance required (Stearns 1992). Therefore, the plasticity of a trait in response to the environment may depend upon the age at which the trait is expressed, the sex of the individual in which the trait is expressed, or the dynamics of the population (Stearns 1992; Roff 1992).

At a finer scale, individual-level variation in sexually selected traits may be governed by a link between trait expression and individual condition. Trait expression should therefore be associated with other phenotypic characters which reflect condition such as an individual's body mass (e.g. Festa-Bianchet et al. 2004), or parasite infection (Hamilton and Zuk 1982; Thompson et al. 1997; Møller et al. 1998, 1999). Furthermore, as a result of this condition dependence, individuals may not all respond to fluctuating environmental conditions in the same manner. For example, an ecological variable which influences resource availability, will in turn influence physiological condition (e.g. Albon et al. 1987), but its effects will depend upon individual variation in quality and thus environmental sensitivity (Nussey et al. 2007). Individuals may differ in their environmental sensitivity as a result of their genotype (GxE: e.g. Nussey et al. 2005) or they may differ due to non-genetic components (Nussey et al. 2005; Brommer et al. 2003, 2005, 2008) of individual quality such as differences in previous environmental conditions experienced throughout life. Explicit examination of individual plasticity in wild populations has been largely restricted to timing events such as the timing of offspring production (Nussey et al. 2005a, 2005b; Brommer et al. 2005; Reed et al. 2006) or highly variable traits such as body weight (Pelletier et al. 2007). The examination of morphological characters which are continually grown over ontogeny has received less attention and would further our understanding of the evolutionary dynamics of phenotypic plasticity in the wild.

In this chapter I examine the environmental sensitivity of horn growth in a feral population of Soay sheep, on the island archipelago of St.Kilda, UK, which experience fluctuating environmental conditions (Clutton-Brock and Pemberton

2004). I first test for relationships between horn growth and other phenotypic traits, climatic variables, and population dynamics. Second, I examine the effects of age and population density on environmental sensitivity. Finally, I examine individual-level differences in environmental sensitivity in response to fluctuating environments both during and prior to horn growth.

4.2. METHODS

4.2.1 Study population

This chapter again focuses on an unmanaged, feral population of Soay sheep (*Ovies aries*) which reside in Village Bay on the island of Hirta within the St. Kilda archipelago in the North Atlantic (57°49' N, 08°34' W). The total island population fluctuates between 600 and 2000 individuals as a result of periodic population crashes, with almost all deaths occurring during late winter as a consequence of starvation (Clutton-Brock and Pemberton 2004). The St. Kilda Soay sheep have been the subject of ecological study since the 1960s, and from 1985 about 95% of lambs born within the study site have been ear-tagged, giving intensive sampling of individual level data across the fluctuating environmental conditions which they experience.

4.2.2. Measurements and variables

The methods used to monitor and measure individuals of this population has been described elsewhere (Clutton-Brock and Pemberton 2004; previous chapters) and thus I will restrict my description here to the relevant variables included in the analysis.

Soay sheep have a distinct polymorphism for horn type producing either a full horn (males 86%; females 32%), a reduced horn (or "scur": males 14%; females 28%), or no horn at all ("poll": 40% of females only). The horns of sheep grow cumulatively over life, with horn increments formed when growth stops over winter, forming an

annulus. This provides an annual measure (in mm) of horn growth at each age, which can be measured at any point of an individual's life and after death. The length of every horn increment grown was measured each time an individual was captured, potentially generating repeated measures on the same increment across different years. These repeated measures were averaged across all years, with the exception of the first increment grown. The first horn increment declined significantly in size over an individual's lifetime (by an average of 1.049 ± 0.332 mm per year), presumably due to wear at the tips of the horn. Therefore, only measures of the first increment recorded in the second year of life (either when captured in that year or after death in the first year) were used in the analyses. There was no effect of measurer and no evidence of any change in the length of other increments with increasing age (I do not present results here). I restricted my analyses to horn increment measures grown up until the age of seven as after this point data is reduced, and increments are small and difficult to distinguish apart. Data were available for: 978 normal-horned males (a total of 2487 measures); 196 scurred males (a total of 558 measures); 223 normal-horned females (a total of 1070 measures); and 220 scurred females (a total of 484 measures).

The individual-level and environmental variables used in this study are described below. Variables reflect environmental conditions during the year of increment growth and all available data from between 1985 and 2004 were used.

Individual-level variables:

- Age: the age of the individual in years, determined from its year of birth.
- Dam horn: horn type of the individual's mother (polled, scurred or normal-horned) was included to assess whether there were any effects of the mother's horn phenotype on offspring horn length.
- Twin: record of whether an individual was born as a singleton or as a twin.
- Body weight: the weight of the individual (in kg) as measured in August, standardised within each horn group to zero mean and equal variance.
- Parasite burden: Parasite burdens were determined through faecal egg counts of five nematode gut parasites, collectively termed strongyles (*Teladorsagia spp.,*

Trichostrongylus spp., Chabertia ovina, Bunostomum trigonocephalum, Strongyloides papillosus) and standardised within each horn group to zero mean and equal variance.

Climatic variables:

- NAO: North Atlantic Oscillation; a large scale climatic index describing the difference in atmospheric pressure between south-west Iceland and the Straits of Gibraltar, with values linked to global weather changes (Hurrell 1995). Values were grouped into winter (WNAO: December of year-1 to March; period of high mortality), summer (SNAO: April-July; lambing and start of summer feeding), autumn (ANAO: August-November; end of summer period) of the year.
- Local climatic variables: Seasonal rainfall and average temperatures were used which were collected from the meteorological office at Stornoway (100km from St. Kilda), and are available at http://badc.nerc.ac.uk/home/. Seasons were defined in the same way as the NAO variables above.

Population dynamics:

- Population density: each year, from 1985 to 2006, the study area population size was estimated on October 1 as the total number of individuals observed from census over the course of a year plus the number of lambs born that year (excluding those known to have died by October 1).

4.3. STATISTICAL ANALYSES

4.3.1. Environmental sensitivity of horn growth

I first began by examining which of the variables listed above influence mean horn growth in a given year, and testing if the environmental sensitivity of horn growth varied depending upon the horn type and sex of the individual. I used horn increment measures from scurred females, scurred males, normal-horned females and normal-horned males, which were standardized to a mean of zero and equal variance within

each group and then combined. A single linear mixed model (LMM) was conducted with horn increment length as the response and all the variables listed above, and their interaction with horn type, included as fixed effects. Significant interaction terms would indicate a significant difference between the horn types, in the effects of a given environmental variable. The significance of fixed effect terms was assessed by testing Wald statistics against a Chi-squared distribution on their appropriate degrees of freedom. Different functions of age were tested (linear, quadratic, 1/age) and model deviance compared to determine which function provided the best fit to the data. Identity was included as a random effect to account for repeated measures on an individual over ontogeny. Year of growth was also included as a fixed effect to account for, and test fixed effects against, unexplained year to year variance, alongside year of birth to account for any variance attributable to cohorts. For presentation of the results, I re-ran the model with variables nested within each horn group, as this gives effects sizes within each horn group as opposed to differences in effect size between horn groups. Models were also conducted for each of the four horn groups independently but as the fixed effects showed the same significance as in the combined model, I do not present the results.

4.3.2. Effects of age and population dynamics on environmental sensitivity

Second, I used a LMM with unstandardised horn increment measures of normal-horned males as a response, and tested for interactions of individual and climatic variables with age and population density. A full model was conducted including all variables listed above and all of their interactions. This model was then reduced in a step-wise manner, with non-significant variables removed, until only those significant at the 5% level remained. For clarity I present only the reduced model. Models were also conducted in the same way for the other three horn groups but as there were no significant effects of any environmental or population variables, or their interactions I do not present these results.

4.3.3. Individual-level variation in environmental sensitivity

Finally, I tested whether normal-horned males differ in their allocation to horn growth in response to fluctuating environmental conditions. To do this I first examined the variance in horn growth of each of the first three years of life, in response to the environment experienced both during and prior to growth. Environmental conditions (E_t) during the year of growth were defined as the proportion of individuals alive in that year, which survived the following winter. Environmental conditions of the previous year (E_{t-1}) were defined as the proportion of individuals which survived the winter before growth began. The basic premise behind these measures is that the survival of individuals in a population best reflects the ecological conditions that they have experienced and thus their condition (Wilson et al. 2006; see also later chapters). Both E_t and E_{t-1} were standardized to a zero mean and equal variance, with negative values indicating a poor environment with low survival and positive values indicating a good environment with high survival. The variance of any measure may be a function of the mean of the trait and thus I used coefficients of variation (100*(standard deviation/mean)) to describe changes in population-level variance of horn growth across environments (Houle 1992). Because variance may also be a function of the sample size, individuals were divided into ten equal sized groups based upon the distribution of each environmental variable, and coefficients of variation for horn growth calculated for each group, which were then regressed against E_t and E_{t-1}.

Population-level changes in horn growth allocation across environments should be driven by individual responses to the environment. I tested for individual differences in allocation to horn growth in response to the environment by extending the LMM of horn growth in normal-horned males used above to a random regression mixed model, to model the random effect of the individual term as a polynomial function of E_t and E_{t-1} (Nussey et al. 2005; Nussey et al. 2007). I conducted two models one where weight is included as a fixed effect and one where weight was not included as a fixed effect, to test for plasticity in allocation to horn growth relative to weight, and then in unadjusted horn growth. I used a forward model selection procedure, including random effects and comparing models using log-likelihood ratio tests, with

degrees of freedom as the added number of variance and covariance components estimated. I also compared a series of successively more complex random regression functions, that differed in their order from n=0 (constant individual-level variance across E_t or E_{t-1}) to n=2 (variance change as a quadratic function of E_t or E_{t-1}). There was no evidence of any quadratic changes in variance and thus only linear changes in are presented where they occur. I also tested for individual differences in allocation to horn growth in response to climatic variables and population density in the year of growth, which were again standardised to zero mean and equal variance. Models were also re-run restricting the data to individuals who survived until at least the age of three, and as they revealed the same patterns, I have not presented them here. For the presentation of results I used **I = Z Q Z'**, where **Z** is the vector of orthogonal polynomials evaluated at the values of standardized environmental quality (**Z'** is the transpose of **Z**), to gain a single individual variance-covariance matrix **I** of environment-specific variance estimates for each trait.

4.4. RESULTS

4.4.1. Environmental sensitivity of horn growth

Normal-horned males showed greater horn growth at all ages compared to the other horn groups (Figure 4.1) and patterns of horn growth with age differed over ontogeny between the horn groups (Table 4.1). Individuals of all horn types had smaller horns if they were born to polled mothers, and normal-horned males also showed smaller horns on average if they were born to scurred mothers, indicating that horn size may be associated with the inheritance of an individuals horn type (Table 4.1, 4.2). Being born as a twin only influenced the horn growth of normal-horned males (Table 4.1, 4.2). With the exception of scurred females, horn growth showed allometric scaling with body weight in all horn groups, but effect sizes were larger in normal-horned males (Table 4.1, 4.2). Parasite burden was only associated with the horn growth of normal-horned males (Table 4.1), with increased parasite load reducing horn growth (Table 4.2). Climatic variable of average summer rainfall

and average autumn temperatures were positively associated with the horn growth of normal-horned males (Table 4.1, 4.2). Population density was negatively associated with normal-horned male horn growth (Table 4.1, 4.2). There was no evidence that density or any climatic variable was associated with the horn length of any other horn group (Table 2).

4.4.2. Effects of age and population dynamics on environmental sensitivity

The effects of dam horn type and twin status decreased with age in normal-horned males (Table 4.3). Horn growth became less allometrically scaled with body weight with age, and more allometrically scaled with body weight at high population density (Table 4.3). The effects of parasite burden remained negative and constant across environments (Table 4.3).

There was evidence of age-by-population density interactions for both climatic variables of average summer rainfall and autumn temperatures (Table 4.3). These effects indicate that local weather conditions influence horn growth to a greater extent at later ages, but that their effects decrease when population density is high (Table 4.3).

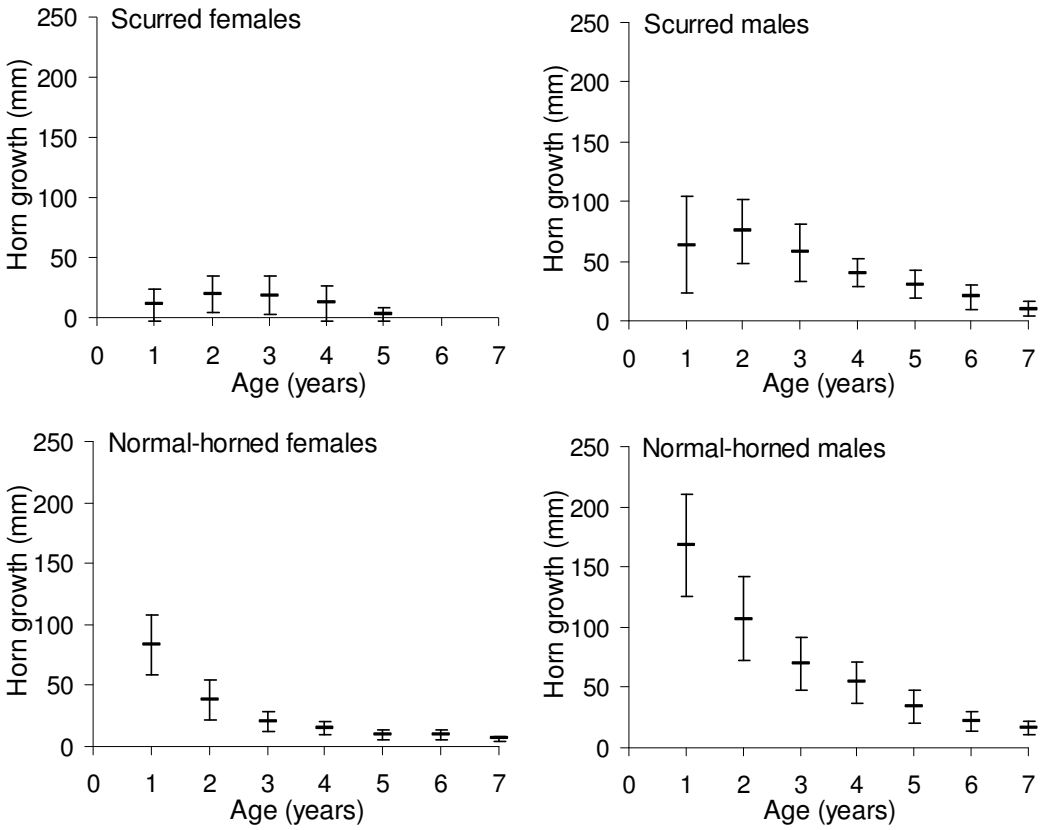


Figure 4.1 Mean horn growth at each age in the four different horn types. Error bars represent one standard deviation from the mean.

Table 4.1. Effects of age, phenotypic characters, climatic variables and population density, and their interactions with horn group, on horn growth. Significance of each variable was assessed when included last in the model using Wald statistics against a Chi-squared distribution. Climatic variables are divided into seasons (W: winter Dec-March; S: summer April-July; A: autumn August-November).

Variable	Wald	df	P
Horn group (H)	3.41	3	0.245
Age	4344.31	1	<0.001
Age2	644.71	1	<0.001
H*Age	147.45	3	<0.001
H*Age2	82.36	3	<0.001
Dam horn type	49.63	3	<0.001
H*Dam horn type	16.39	9	0.063
Twin status	19.48	2	<0.001
H*Twin status	13.66	6	0.034
Body weight	130.81	1	<0.001
H*Body weight	17.49	3	<0.001
Parasite burden	1.75	1	0.186
H*Parasite burden	10.30	3	0.016
WRain	2.07	1	0.150
H*WRain	1.08	3	0.781
SRain	1.27	1	0.260
H*SRain	9.87	3	0.027
ARain	0.20	1	0.655
H*ARain	4.36	3	0.225
WTemp	1.29	1	0.256
H*WTemp	4.36	3	0.225
STemp	0.28	1	0.597
H*STemp	1.53	3	0.674
ATemp	13.99	1	<0.001
H*ATemp	11.36	3	0.010
WNAO	0.44	1	0.505
H*WNAO	1.09	3	0.780
SNAO	0.83	1	0.361
H*SNAO	1.09	3	0.719
ANAO	0.34	1	0.952
H*ANAO	2.61	3	0.455
Population density	6.96	1	0.008
H*Population density	15.17	3	0.002
Random effects	Variance ± SE		
Year of growth	0.018 ± 0.010		
Birthyear	0.017 ± 0.010		
Individual	0.035 ± 0.009		
Residual	0.324 ± 0.011		
Deviance 399.50	df 1426		

Table 4.2. Effects of age, phenotypic characters, climatic variables and population density on the horn length of scurred and normal-horned males and females. Results are gained from a single linear mixed model of standardized horn length, with effects nested within each horn and sex group (see Methods). Significance from zero at greater than the 5% level is indicated in bold based upon t statistics.

	Scurred males	Scurred males	Normal-horned females	Normal-horned males
Constant	-0.227 ± 0.239			
Age	0.510 ± 0.310	0.173 ± 0.173	**-1.376 ± 0.066**	**-1.171 ± 0.031**
Age^2	-0.286 ± 0.128	-0.155 ± 0.035	**0.138 ± 0.010**	**0.102 ± 0.005**
Dam horn				
Polled	-0.301 ± 0.199	-0.352 ± 0.284	**-0.331 ± 0.110**	**-0.204 ± 0.066**
Scurred	-0.232 ± 0.185	-0.181 ± 0.289	-0.041 ± 0.124	-0.103 ± 0.058
Normal	0.007 ± 0.197	0.115 ± 0.356	0.165 ± 0.121	**0.184 ± 0.058**
Twin				
Single	0.113 ± 0.162	0.157 ± 0.289	0.254 ± 0.128	0.015 ± 0.054
Twin	0.194 ± 0.179	0.231 ± 0.302	0.265 ± 0.149	**-0.145 ± 0.047**
Body weight	0.003 ± 0.056	**0.138 ± 0.040**	**0.193 ± 0.051**	**0.247 ± 0.018**
Parasites	0.035 ± 0.078	0.012 ± 0.068	-0.028 ± 0.032	**-0.208 ± 0.019**
WRain	0.001 ± 0.003	0.004 ± 0.003	0.001 ± 0.002	0.002 ± 0.002
SRain	0.005 ± 0.006	0.006 ± 0.006	-0.008 ± 0.005	**0.016 ± 0.004**
ARain	0.001 ± 0.004	-0.007 ± 0.005	0.002 ± 0.002	-0.001 ± 0.002
WTemp	0.031 ± 0.120	0.027 ± 0.101	0.014 ± 0.082	0.021 ± 0.054
STemp	0.025 ± 0.119	-0.127 ± 0.109	0.120 ± 0.087	0.085 ± 0.072
ATemp	-0.024 ± 0.124	-0.009 ± 0.106	0.016 ± 0.089	**0.165 ± 0.049**
WNAO	-0.057 ± 0.057	-0.023 ± 0.046	0.002 ± 0.041	-0.018 ± 0.030
SNAO	0.004 ± 0.023	0.001 ± 0.020	0.001 ± 0.017	0.002 ± 0.014
ANAO	0.040 ± 0.168	0.045 ± 0.152	-0.097 ± 0.124	0.053 ± 0.088
Density	0.033 ± 0.098	0.095 ± 0.081	-0.164 ± 0.083	**-0.264 ± 0.068**

Table 4.3. Effects of age and population density on environmental sensitivity of horn growth in normal-horned males. Results gained from a LMM of horn growth where significance of fixed effects is assessed by Wald statistics against a Chi-squared distribution. N =

Variable	Wald	df	P	Estimate ± SE
Constant				114.100 ± 2.250
Age	5200.61	1	<0.001	-68.991 ± 1.791
Age^2	630.40	1	<0.001	5.935 ± 0.257
Population density	9.15	1	0.002	-3.729 ± 1.088
Dam horn type	31.07	3	<0.001	
Polled				-10.363 ± 3.180
Scurred				-5.555 ± 2.813
Normal-horned				2.109 ± 2.150
Age*Dam horn	12.97	3	0.005	
Polled				2.020 ± 1.984
Scurred				0.938 ± 1.747
Normal-horned				-3.796 ± 1.799
Twin status	11.25	2	0.004	
Singleton				2.037 ± 2.604
Twin				-5.346 ± 2.302
Age*Twin	6.45	2	0.040	
Singleton				0.164 ± 1.572
Twin				4.297 ± 2.204
Body weight	162.93	1	<0.001	11.831 ± 0.926
Age*Weight	67.40	1	<0.001	-5.068 ± 0.617
Pop*Weight	7.28	1	0.007	2.775 ± 1.028
Parasite burden	4.86	1	0.027	-1.769 ± 0.487
SRain	2.48	1	0.115	0.306 ± 0.194
Age*SRain	5.11	1	0.024	0.094 ± 0.042
Pop*SRain	0.03	1	0.869	-0.030 ± 0.177
Age*Pop*SRain	5.19	1	0.023	-0.091 ± 0.039
ATemp	10.35	1	0.001	7.991 ± 3.336
Age*ATemp	15.60	1	<0.001	2.664 ± 0.785
Pop*ATemp	0.88	1	0.348	-2.914 ± 3.277
Age*Pop*ATemp	4.86	1	0.027	-1.390 ± 0.859

Random effect	Variance ± SE
Year of growth	69.10 ± 29.10
Year of birth	71.20 ± 40.40
Individual	91.20 ± 38.40
Residual	875.10 ± 30.90

Deviance 6596.42 df 848

4.4.3. Individual-level variation in environmental sensitivity

There was no evidence that the population-level variance of the first three years of horn growth in normal-horned males was associated with the environmental quality of the year of growth (Figure 4.2). There was also no evidence that variation in the first horn increment was associated with the environmental conditions in the year prior to birth (Figure 4.3). However, there was evidence that variation in horn growth at ages two and three was associated with the environmental conditions experienced before growth begins (Figure 4.3). Individual-level variance in horn growth increased if previous environmental conditions were favourable. I also tested for relationships with climatic variables of average summer rainfall and average autumn temperature and as no relationships were observed, I have not presented these results here.

The LMM of horn growth in normal-horned males was then extended to a random regression model, to test for (A) individual differences in allocation to horn growth relative to body weight and then (B) individual differences in unadjusted horn growth, as a function of environmental quality. There was no evidence that individual differences in allocation to horn growth relative to body weight varied as a function of climatic variables, population density, or environmental quality of the year of growth (Table 4.4). However, the inclusion of a random effect of individual as a first order polynomial function of E_{t-1} significantly improved the model fit, indicating that individuals differ in their allocation to horn growth relative to body weight depending upon the previous environment (Table 4.4). In model B, both environmental quality and population density in the year of growth, and environmental quality prior to growth influenced individual difference in horn growth (Table 4.4).

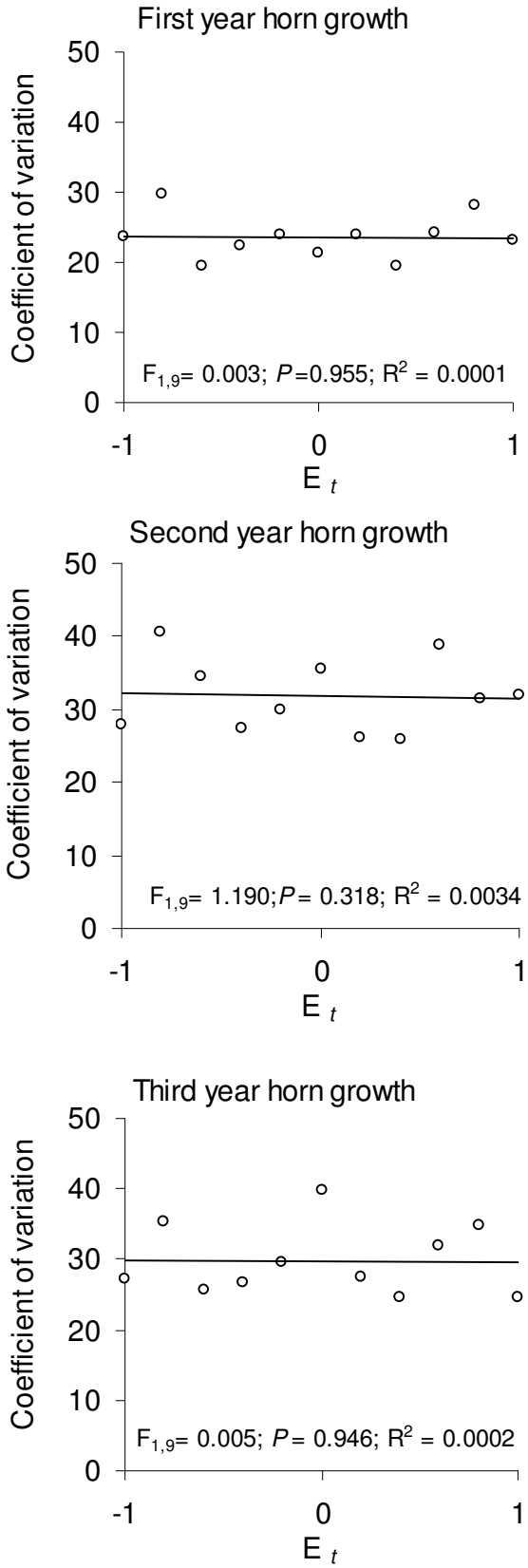


Figure 4.2. Relationship between coefficient of variation for first, second and third year horn growth and the environmental quality of the year of growth.

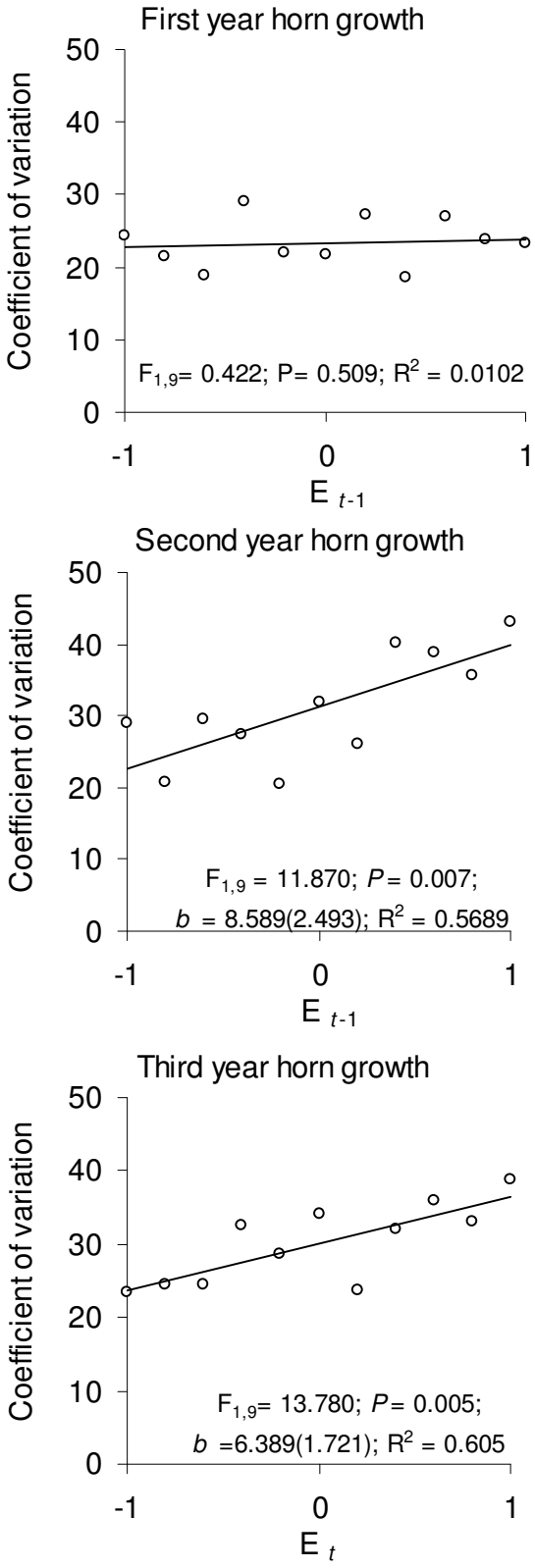


Figure 4.3. Relationship between coefficient of variation for first, second and third year horn growth and the environmental quality of the year prior to growth.

Table 4.4. Hierarchical models describing variation in (A) allocation to horn growth relative to body weight, and (B) horn growth without accounting for body weight, in normal-horned males as a function of environmental and climatic conditions experienced during the year of growth. All other fixed effects are the same as for the LMM listed in Table 4.3. In both A and B, I then tested for the addition of random effects using log-likelihood ratio tests (-2*difference in log-likelihood, tested against a Chi-square distribution). Random effects included were YR: year of growth; BYR; year of birth; ID: average individual effects; *f*(E_t,1).ID: individual effects as a function of E_t; *f*(Pop,1).ID: individual effects as a function of population density in the year of growth; *f*(SRain,1).ID: individual effects as a function of summer rainfall; *f*(ATemp,1).ID: individual effects as a function of autumn temperature; and *f*(E_{t-1},1).ID: individual effects as a function of E_{t-1}. Significance: **P*<0.05; ***P*<0.01.

	YR	BYR	ID	*f*(E_t,1).ID	*f*(Pop,1).ID	*f*(SRain,1).ID	*f*(ATemp,1).ID	*f*(E_{t-1},1).ID	Log-likelihood	Test	df	Statistic
Model A												
1	■								-3329.85			
2	■	■							-3286.09	1 vs.2	1	7.84**
3	■	■	■						-3283.30	2 vs.3	1	5.38*
4	■	■	■	■					-3282.64	4 vs.3	2	1.32
5	■	■	■		■				-3282.20	5 vs.3	2	1.10
6	■	■	■			■			-3283.05	6 vs.3	2	0.50
7	■	■	■				■		-3282.37	7 vs.3	2	1.86
8	■	■	■					■	-3279.25	8 vs.3	2	8.10**
Model B												
1	■								-3887.41			
2	■	■							-3879.86	1 vs.2	1	15.10**
3	■	■	■						-3875.10	2 vs.3	1	9.52**
4	■	■	■	■					-3871.66	4 vs.3	2	6.88*
5	■	■	■		■				-3873.54	5 vs.3	2	1.56
6	■	■	■			■			-3874.00	6 vs.3	2	2.20
7	■	■	■				■		-3873.95	7 vs.3	2	2.30
8	■	■	■					■	-3870.14	8 vs.3	2	9.92**

4.5. DISCUSSION

In this chapter I have shown that allocation to horn growth relative to body weight in normal-horned males is influenced by both climatic variables and population dynamics, supporting previous studies showing environmental sensitivity of sexually selected traits (e.g. Kruuk et al 2002; Festa-Bianchet et al. 2004; Mysterud et al. 2005). In the other horn groups, allocation to horn growth appears to only be allometrically scaled to body weight and there was no evidence of an effect of any climatic variables or population dynamics. These results suggest that where sexual selection drives higher allocation of resources to a trait, allocation becomes sensitive to the environmental conditions that individuals experience (Andersson 1994). Normal-horned males differed in their environmental sensitivity depending upon their age and the level of competition for resources, highlighting the fact that the effects of climatic variables may not be consistent (Stearns 1992; Roff 1992; Merilä and Sheldon 2001). I also found evidence which suggests individual-level plasticity in allocation depends upon previous environmental conditions and thus an individual's condition prior to horn growth in a given year. Environmental variation is likely to be a large component of phenotypic variation in sexually-selected traits (Griffith et al. 1999) and it is clear that this variation may be created by numerous factors and dependent upon the condition of an individual.

In normal-horned males, allocation to horn growth relative to body weight was sensitive to local climate factors during the season of peak grass growth (Clutton-Brock and Pemberton 2004), and thus it is likely that these factors influence horn size through their effects on resource availability. Population density will also influence competition for resources, and at high density resources are likely to be limited which often leads to high levels of mortality in the following winter (Clutton-Brock and Pemberton 1994). The lack of evidence of any environmental effects on allocation to horn growth in the other horn groups may reflect reduced sample sizes, and reduced repeated measures, rather than a lack of effect. However, the LMM of standardised horn length revealed that while standard errors were higher in horn

groups with lower sample sizes, the size and often direction of the effects differed between the horn groups and significant interactions were found. I therefore argue that these effects reflect different patterns of allocation, resulting from differing life-history strategy, rather than a failure to detect an effect.

I found evidence that normal-horned males differed in their susceptibility to climatic variables depending upon their age and the population dynamics they experienced, supporting studies which suggest that environmental effects may not have consistent influence on a trait (Stearns 1992; Roff 1992). It appeared that the first horn increment was more influenced by factors relating to birth than to environmental conditions, with the effects of climatic variables increasing with age, and associations between mother's horn type and twin status decreasing with age. At high density, allocation to horn growth in normal-horned males was allometrically related to body weight to a much greater extent, supporting the theory that in poor environments allocation may be diverted away from secondary sexual traits and towards somatic maintenance (Hoffman and Parsons 1991). The effects of climatic variables were also dependent upon resource availability, with positive effects of summer weather conditions only felt at low density when resources were abundant, resulting in high allocation to horn growth. These results suggest that environmental effects create phenotypic variation in horn growth repeatedly, and with increasing effect across ontogeny, depending upon the fluctuating environments that individuals experience.

There was no evidence for individual plasticity in response to the environmental conditions experienced by normal-horned males during allocation to horn growth in a given year. However, there was evidence that individual-level plasticity in allocation to horn growth was dependent upon individual differences in condition prior to growth. Between individual differences in allocation increased following a year of good environmental quality, when average individual condition is likely to be high. This supports previous studies which suggest increased individual level variance when environmental conditions are favourable (Merilä and Sheldon 2001; Garant et al. 2004; Wilson et al. 2006). It should be noted that an alternative explanation for

these results is possible, in that individuals which survive poor environments may show reduced variance as a result of selection acting upon first year male horn growth in normal-horned males, but it is unlikely that these effects are consistent across ontogeny (see Chapter 5). I also tested for individual plasticity in horn growth not accounting for variation in body weight, showing individual plasticity in horn growth in response to population density both during and prior to horn growth in a given year. This suggests that individuals may be plastic in their response to the environment in their body weight, which creates the appearance of plasticity in horn growth. Previous studies of individual phenotypic plasticity have examined timing traits (Brommer et al. 2003, 2005, 2008; Nussey et al. 2005a, 2005b; Reed at al. 2006), and one study has examined individual plasticity in body weight in another ungulate population (Pelletier et al. 2007). Plasticity in sexually-selected display traits is expected as a result of their condition dependence and has been shown in many laboratory populations (Greenfield and Rodriguez 2004; Price 2006; Mills et al. 2007) and sexual size dimorphism may also be plastic depending upon the environmental conditions experienced (e.g. Bonduriansky 2007).

In summary, phenotypic variation in allocation to a sexually-selected trait is likely to be generated by changing environmental conditions and this may be further enhanced by differences between individuals in their environmental sensitivity. In this population, individual plasticity in allocation to horn growth relative to body weight may be determined by previous environmental conditions. The variation in horn size upon which selection acts may be largely generated by the fluctuating environmental conditions that individuals experience.

CHAPTER 5

ENVIRONMENTAL HETEROGENEITY GENETATES FLUCTUATING SELECTION ON A SECONDARY SEXUAL TRAIT.

This chapter appears as the following publication: Robinson, M.R., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J. M., and Kruuk, L.E.B. 2008. Environmental heterogeneity generates fluctuating selection on a secondary sexual trait. Current Biology 18: 751-757.

5.1 SUMMARY

Allocation to a trait that enhances reproductive success is likely to come at a cost to survival in any population in which resources are limiting. Here, we show that the magnitude of these costs depends upon ecological conditions, generating fluctuating selection on a secondary sexual trait in relation to environmental conditions. In a wild population of Soay sheep (*Ovis aries*), phenotypic and genetic associations between male horn growth and lifetime reproductive success were positive under good environmental conditions (due to increased breeding success) and negative under poor environmental conditions (due to reduced survival). In an unpredictable environment, high allocation to early horn growth is a gamble which will only pay off if ensuing conditions are favourable. Such fluctuating selection may play an important role in the maintenance of genetic variance in secondary sexual traits.

5.2 INTRODUCTION

High allocation of resources to a trait which conveys an advantage in the competition for mates is expected to generate increased breeding success, but such allocation may come at a cost to survival (Stearns 1989; Grafen, 1990; Andersson 1994; Jennions et al. 2001; Roff 2002; Kokko et al. 2003, 2003; Hunt et al. 2004). The relationship between a sexually-selected trait and fitness will therefore represent a balance between its relative impact on fecundity versus viability (Höglund and Sheldon 1998; Roff 2002; Kokko et al. 2002, 2003). Furthermore, because the risk of mortality in a population is likely to be heavily determined by ecological conditions, survival costs will vary as a function of the prevailing environment (Roff 2002). As a result, for populations experiencing heterogeneous ecological conditions, there may not be a single optimal level of allocation (Sasaki and Ellner 1997). These arguments provide an intuitively appealing explanation for the maintenance of genetic diversity in secondary sexual traits (Roff 2002), but they have received surprisingly little empirical support to date, particularly for populations experiencing natural environments (but see Price et al. 1984; Grant and Grant 2002).

The evolution of a trait depends upon the genetic relationship between the trait and fitness and at present, we do not fully understand the effects of the environment on these genetic relationships (Roff 2002). Secondary sexual traits are expected to show condition dependence and allocation may be sensitive to the environments in which they are expressed (Andersson 1994; Greenfield and Rodrigurez 2004). Recently, studies have suggested that genotype-by-environment interactions can potentially maintain genetic variance in secondary characters as one genotype may not produce the same phenotype in all environments (Jia et al. 2000; Greenfield and Rodriguez 2004; Danielson-Francois et al. 2006). However, numerous studies have shown that both mate choice (Cotton et al. 2006) and trade-offs between fecundity and viability (Stearns 1989; Schluter et al. 1991) also vary as a function of the environment and thus selective pressures are also unlikely to remain constant in all environments. As a result, in order to determine the effects of environmental heterogeneity on the evolutionary potential of a trait, both the expression of the trait and the selection

pressures acting across an individual's lifetime, need to be assessed simultaneously across a gradient of environmental conditions.

My aim in this chapter was to assess the genetic architecture of and the selection pressures on a male sexually-selected trait across changing environmental conditions in a population experiencing natural environmental heterogeneity. I did so by examining the covariance (and correlation) between male horn growth and three lifetime fitness measures (average fecundity, longevity and lifetime breeding success) in a feral population of Soay sheep on the Scottish island of Hirta, St. Kilda, UK (Clutton-Brock and Pemberton 2004). The phenotypic covariance between a trait and fitness (equivalent to the selection pressure on the trait Falconer and Mackay 1996) can be broken down into genetic and environmental components. In this way, I estimated the phenotypic, genetic and environmental covariance (and correlations) between horn growth and lifetime fitness in male Soay sheep that experienced different environmental conditions during the year of their birth. The study population is ideal for this purpose as weather conditions, population density and consequently resource availability fluctuate from year-to-year, providing substantial differences between individuals in the environmental quality of their birth year and thus their survival rates (Clutton-Brock and Pemberton 2004). Furthermore, a large volume of multigenerational pedigree, life-history and phenotypic data was available covering a 17 yr period (1985-2001: data was restricted to 2001 to avoid biasing the estimates of later years toward those individuals who had short lives), facilitating the estimation of selection pressures on the horn growth of males across a wide temporal range of environments.

5.3 METHODS

5.3.1 Study population and data structure

Soay sheep (*Ovis aries*) were introduced onto the island archipelago of St.Kilda, NW Scotland in the North Atlantic (57º49'N, 08º34'W) during the Bronze Age (Clutton-

Brock and Pemberton 2004). The unmanaged study population of Village Bay, Hirta was founded in 1932 with the introduction of 107 sheep from the neighbouring island of Soay, and currently fluctuates around an average size of 432 individuals. The population has been the subject of intensive individual-level study since 1985, yielding morphological and life-history data for 6387 pedigreed individuals, including 3626 maternal links and 1699 paternal links (from 807 distinct dams and 495 distinct sires). Maternal identity is known from field observations and paternity is inferred by microsatellite based paternity analysis at a pedigree-wide confidence level of ≥80%, allowing no more than one allelic mismatch between offspring and putative sire, using maximum likelihood methodology implemented in CERVUS (Marshall et al. 1998).

Soay sheep have a distinct polymorphism for horn type producing either a full (normal) horn (86% of males; 32% of females), a reduced horn (14% of males; 28% of females), or no horn at all (40% of females only). I consider only males who grew full horns as this is the only group in which horn size is associated with sexual selection (see Chapter two). The horns of sheep grow cumulatively over the lifetime, with annual increments being apparent when horn growth stops over the winter, forming an annulus. This provides a measure of the horn growth in each year of life, which can be measured at any point of an individual's life and after death. Horn increment data was available for 854 first year measures; 497 second year measures; 327 third year measures; 195 fourth year measures; and 135 fifth year measures (the declining sample sizes represent mortality). I used three relative fitness measures (W): average age-adjusted lifetime fecundity (FEC), longevity (LG), and lifetime breeding success (LBS), with data available for 1691 normal-horned males, after removing animals known to be still alive. I restricted our analyses to individuals born between 1985 and 2001 to avoid biasing the estimates of later years toward those individuals who had short lives.

5.3.2 Random regression model of horn growth over ontogeny

Age-specific quantitative genetic parameters for horn growth were estimated using random regression animal models (Meyer 1998; Meyer and Kirkpatrick 2005;

Wilson et al. 2006) to partition phenotypic variance into genetic and environmental (residual) components. Animal models are a form of linear mixed model implemented in ASReml (VSN International Ltd) using restricted maximum likelihood which are able to accommodate unbalanced datasets and complex pedigrees (Kruuk 2001), and random regression animal models allow different random effects to be modelled as functions of a continuous variable. Fixed effects of age (factor 1-5) and birth year (factor 1985-2001) were included. Birth year was included to remove effects of conditions at birth on mean horn growth and to remove temporal trends in mean values. I then included random effects to model both the additive genetic effects and the year of growth effects as polynomial functions of age. The residual error structure was partitioned to gain age-specific estimates of residual (or environment) variance. At the individual level horn growth phenotype (HG_{AGE}) of individual *i*:

Equation 5.1 $HG_{i,\,AGE} \sim (AGE + BIRTHYEAR)_i + f(\alpha_i, n, AGE_{SD}) + f(YR, n, AGE_{SD}) + e_{i,\,AGE}$

where $f(\alpha_i, n, AGE_{SD})$ is the random regression function of orthogonal polynomials of standardized age (age in years standardized to the interval $-1 \leq AGE_{SD} \geq 1$) with order *n*, of additive genetic merit α_i (or breeding value) of individuals obtained from the pedigree structure; $f(YR, n, AGE_{SD})$ is the random regression function for the year of growth YR; and $e_{i}AGE$ is the age-specific error for individual *i*.

The error term was modelled using a 5x5 unstructured matrix allowing residual errors to be correlated across ages within individuals, removing the need for a permanent environment effect. Adding mother's identity as a random effect did not improve model fit ($\chi^2_1 = 0.96$; $P = 0.327$) and so I do not model maternal effects. Models were fitted using polynomial functions of increasing order, which were compared statistically using log-likelihood ratio tests. Model convergence was not achieved for $n > 3$. The variance-covariance matrix of the random regression parameters obtained for the additive genetic effect (matrix **Q** with dimensions (n +1) x (n +1)) was used to derive age-specific genetic parameters (**G** for HG_{AGE}) and their approximate standard errors (Fisher et al. 2004).

5.3.3 Bivariate random regression model of horn growth and fitness

I then modelled the phenotypic, genetic and residual covariance between horn growth and fitness over fluctuating environmental quality. To do this I used bivariate random regression models, with both fitness (*W*) and first year horn growth (*HG1*) as response variables and ran one model for each of the three fitness measures. Environmental quality (*E*) was defined as the proportion of live born lambs that survived the first winter that following their birth year (standardized to the interval $-1 \leq E \leq 1$; Wilson et al. 2006).

I began by estimating the phenotypic correlations between *W* and *HG1* within different environmental conditions. To do this I fitted a model without any random effects such that all phenotypic variance in both traits was allocated to a residual structure. I standardized both the fitness measure and the horn growth value of each birth year to a zero mean and a unit variance, thus placing both on the same scale. As a result, converting the phenotypic covariance into correlations produced standardized selection differentials for first year horn growth. Thus for each individual (*i*) I fitted a model:

Equation 5.2 $$HG1_i \;\; W_i \;\sim (EG)_i + e_{iEG}$$

where the fixed effect of *EG* is a four level factor (1: very poor, 2: poor, 3: good, 4: very good) produced by grouping birth years based upon the 25% quartiles of the distribution of *E*; and the residual error structure e_{iEG} was partitioned into four EG groups. This gave an estimate of the phenotypic variance of each trait and the phenotypic covariance (converted into a correlation) between the traits within each of the four EG groups. The residual structure was divided to provide roughly equal sample sizes across environments thus maximizing the accuracy of the estimates, and reducing the number of variance components to be estimated. I tested the significance of the phenotypic correlations by re-running the model with the phenotypic covariance between the traits constrained to zero within each EG group, and comparing the models using log-likelihood ratio tests.

I next extended model two to partition the phenotypic covariance between *W* and *HG1* into genetic and environmental components over fluctuating environmental quality. To model the genetic covariance between *W* and *HG1*, a random effect was included which estimates the additive genetic variance of each trait and the genetic covariance between them as a polynomial function of environmental quality. Thus for each individual (*i*) I fitted the random regression model:

Equation 5.3 $HG1_i \;\; W_i \;\sim (EG)_i + f(\alpha_i, n, E) + e_{iEG}$

where $f(\alpha_i, n, E)$ is the random regression function on an orthogonal polynomial of *E*, with order *n*, of the additive genetic merit values α_i of individuals for both fitness and first year horn growth; and e_{iEG} is the environment specific residual error for individual *i*.

For this random regression each individual is only represented once within the data set, as individuals are only ever born into one environment. However, as related individuals are born into different environments, the genetic effects for each trait and the covariance between traits can be estimated as a function of environmental quality. This method represents a more efficient use of the data by avoiding sub-division of records into environment-specific traits (Wilson et al. 2006; Fischer et al. 2004). A first order random regression function provided a better fit for modelling the genetic effects for *HG1* over *E* than a zero order function ($\chi^2_2 = 7.96$; $P = 0.019$), and therefore I used a first order random regression function for the bivariate random regression to model the selection pressures across *E*. The first order random regression term produces a single variance-covariance matrix for the additive genetic effect for both traits (matrix Q with dimensions (2x (n + 1)) x (2x (n + 1))). In this case, I did not find support for models of higher order than n=1, and so consider only the covariance between estimates of intercept and slope of each individual's genetic merit for each trait. I tested for significant genetic covariance between *W* and *HG1* by re-running the models with all four genetic covariances between the two traits constrained to be zero over environmental quality, and comparing the models using log-likelihood ratio tests. This represented a conservative method of testing for a

significant genetic association between the traits over *E* (Meyer 1998; Fischer et al. 2004; Wilson et al. 2006).

Estimates of residual covariance between environments could not be made as multiple records for individuals across environments were not possible. However, estimates of the residual variance of each trait and the covariance between traits within each environment were made. The residual error structure remained partitioned into four levels of *EG*, giving both a residual variance term for each trait, and the covariance between traits, in each of the four environmental groups (i.e. a 2x2 matrix within each *EG* group). I attempted to estimate a residual error structure for each of the 17 birth years but this overparameterized the models, resulting in variance terms which could not be estimated with certainty and thus I do not present this method. I also ran models with a constant residual error structure, giving a 2 x 2 matrix with a single estimate of residual variance for both terms and a single covariance between. These models were not supported by the data, when compared to models with an error structure divided into the four groups using log-likelihood ratio tests (LBS: $\chi^2_9 = 50.82$; $P < 0.001$; FEC: $\chi^2_9 = 18.97$; $P = 0.025$; LG: $\chi^2_9 = 40.97$; $P < 0.001$). As a result, the residual correlations between fitness and first year horn growth represent the associations between the traits which resulted from environmentally determined factors within each *EG* group. I tested the significance of these correlations by re-running model three with the four residual covariances between *W* and *HG1* constrained to zero and compared the models using log-likelihood ratio tests.

For the presentation of the results, I used **G = Z Q Z'**, where **Z** is the vector of orthogonal polynomials evaluated at the values standardized environmental quality (**Z'** is the transpose of **Z**), where **G** is a single additive genetic variance-covariance matrix for both traits. The diagonal of the covariance matrix between the additive genetic variance estimates of both traits provides the estimates of the genetic covariance between both traits across *E*. All covariance estimates were rescaled to give the genetic and residual correlations, providing a dimensionless estimate of association between both traits. Both the covariance and correlations revealed the

same pattern. An analogous method was used to estimate the approximate standard errors (Fischer et al. 2004), which were converted into approximate 95% confidence intervals.

5.4 RESULTS

I first examined the genetic and environmental basis of variation in male horn growth between the ages of 1 and 5 years, when around 92% of horn growth occurs. Soay sheep have a distinct polymorphism for horn development with around 86% of males growing full horns (normal-horned) and 15% growing reduced (scurred) horns. I consider normal-horned males only here, as scurred males do not use their horns to compete for assess to mates, and so there is no sexual selection on horn size in this group (Chapter two).

Using a random regression animal model (Kruuk 2004; Wilson et al. 2006), I combined pedigree and phenotypic data to partition the phenotypic variance of horn growth at each age into a genetic component, an environmental component specific to the year of growth (short-term environmental variance), and a residual (which includes long-term environmental effects) component (see Methods). The genetic component of horn growth was modelled as a polynomial function of age (Wilson et al. 2005), and I selected the polynomial function which best described its distribution over ontogeny. Statistically, the best model fit for the genetic component was a second order function (compared to first order: $\chi^2_3 = 9.34$; $P = 0.025$); estimates of variance components from this model showed that additive genetic variance for horn growth decreased with age (Figure 5.1A). Significant additive genetic variance was only found for horn growth in the first two years of life (Figure 5.1A). The high coefficient of variation for first year growth (Table 5.1) supported previous studies which have shown abundant genetic variance in secondary sexual traits (e.g. Kotiaho et al. 2001). Additive genetic correlations between horn growth at each age were also estimated from the model, and were found to be uniformly positive and close to 1 (Figure 5.1B). The strength of these correlations indicated that variation in growth involves the same loci at all ages. The environmental effect of the year of growth

was also modelled as a function of age, to test for differences between years in changes over ontogeny. Variance in the horn growth of later years was mostly attributable to environmental factors (Table 5.1), a first order function for year of growth gave the best model fit (compared to zero order: $\chi^2_2 = 8.22$; P = 0.016) and coefficients of residual and year of growth variance generally increased with age.

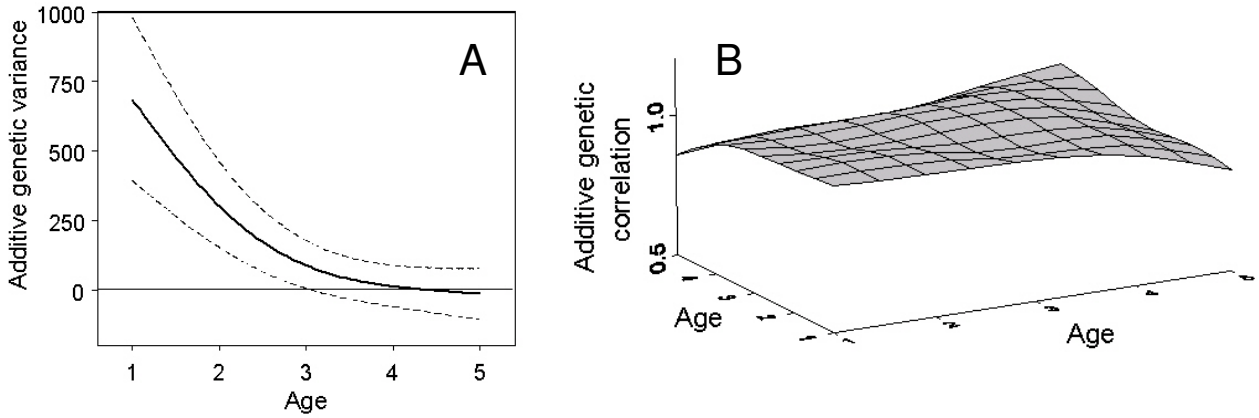


Figure 5.1. Quantitative genetic parameters for horn growth from a random regression animal model. (A): Additive genetic variance with age (in years); (B): genetic correlation between ages. The additive genetic component of horn growth was modelled as a second order quadratic function of age, giving estimates of additive genetic variance at each age which are shown as the solid line in A (dashed lines indicate 95% confidence interval). The analysis produced a genetic covariance matrix for horn growth at each age, which was converted into the matrix of genetic correlations shown in B.

Table 5.1. Age-specific quantitative genetic parameters for horn growth (mm) in normal-horned males, with standard error (SE). Estimates are from a random regression animal model, with second order genetic effects, first order year of growth effects, and an age-specific residual variance structure. Mean age-specific horn growth with standard deviation (SD) in normal-horned male Soay sheep. Quantitative genetic parameters of phenotypic (V_P), additive genetic (V_A), year of growth (V_{YR}), and residual (V_R) variance were converted into coefficients of variance (CV_A, CV_{YR}, CV_R) and estimates of heritability (h^2) are shown. N gives the number of records at each age.

Age (yr)	N	Mean ± SD	V_P	V_A	V_{YR}	V_R	CV_A	CV_{YR}	CV_R	h^2
1	854	178.06 ± 39.27	1643.10 ± 75.18	784.22 ± 167.95	125.50 ± 33.19	781.20 ± 135.7	15.73	6.29	15.70	0.464
2	497	113.69 ± 33.55	1068.43 ± 69.80	180.26 ± 47.76	91.36 ± 33.42	796.44 ± 72.40	11.81	8.41	24.82	0.169
3	327	75.53 ± 21.61	476.90 ± 38.80	73.42 ± 46.61	62.53 ± 33.93	378.58 ± 58.56	11.34	10.47	25.76	0.143
4	195	57.93 ± 16.23	284.50 ± 29.87	42.80 ± 35.15	39.02 ± 36.02	204.72 ± 48.74	11.29	10.78	24.70	0.149
5	135	36.72 ± 12.62	162.91 ± 20.85	3.41 ± 53.62	20.83 ± 42.78	158.65 ± 49.18	5.03	12.43	34.30	0.019

I then investigated whether selection pressures on horn growth were dependent upon the environmental conditions experienced during the first year of life. First, I estimated the phenotypic correlations between horn growth and fitness for males who experienced different environmental conditions. I used an indirect measure of environmental quality (*E*) of an individual's birth year defined as the proportion of lambs which survived their first winter (proportion surviving ranged from 0.05-0.86, with a mean of 0.41), with low survival indicating a poor environment and high survival indicating a good quality environment (for an analogous method see Wilson et al. 2006). I grouped birth years into four groups, corresponding to the quartiles of the distribution of *E,* and used a bivariate linear model to estimate the phenotypic correlation between horn growth and fitness within each group (see Methods). I ran three models, one model for each measure of fitness, calculating an individual's lifetime breeding success (LBS) as the sum of offspring sired over lifespan; fecundity (FEC) as the average age-corrected number of offspring sired per year of life; and longevity (LG) as the total number of years alive.

Secondly, as phenotypic associations may be environmentally driven (Rausher, 1992) as well as having a genetic basis, I extended each model to break down phenotypic associations between horn growth and fitness into genetic and environmental correlations. To do this I conducted a series of bivariate random regression animal models (see Methods). This method simultaneously partitions the phenotypic variance of both traits into genetic and residual (environmental) components, and directly estimates the covariance between the traits in each component. I modelled the genetic component of both traits as a linear function of *E*, estimating the genetic correlation between the traits across a gradient of environmental quality. The residual (environmental) components of each trait and the correlation between them were estimated within each of the four environmental quality groups because estimating a value for each of the 17 years overparameterized the model. The confidence intervals for each parameter are themselves estimates and therefore I generally tested for phenotypic, genetic and residual covariance by re-running the models with the covariance terms of each component in turn constrained

to be zero, and then compared the constrained models to the originals using log-likelihood ratio tests.

Selection on horn growth via lifetime breeding success was positive under good environmental conditions and negative under poor environmental conditions (Figure 5.2; compared to a model with zero phenotypic covariance: $\chi^2_4 = 55.70$; $P < 0.001$). This relationship was driven by the opposing selection pressures on horn growth through fecundity and longevity across environmental conditions. Whilst high rates of first year horn growth were generally associated with increased fecundity (Figure 5.3; compared to a model with zero phenotypic covariance: $\chi^2_4 = 10.87$; $P = 0.028$), they were conversely correlated with significantly decreased longevity in more stressful environments (Figure 5.4; compared to a model with zero phenotypic covariance: $\chi^2_4 = 41.02$; $P < 0.001$).

Figure 5.2. Phenotypic, genetic and environmental (residual) correlations between first year horn growth and lifetime breeding success (LBS). An estimate of environmental quality was gained for each year of birth from 1985-2001 with triangles on the middle panels indicating their distribution. Estimates of the genetic correlation and 95% confidence interval (dashed line) between horn growth and LBS were generated from a random regression animal model allowing additive genetic effects to change as a function of environmental quality. For phenotypic and residual correlations, birth years were grouped based upon the quartiles of the distribution of environmental quality. Estimates of phenotypic and residual correlations were made within each of these four groups and error bars show 95% confidence intervals for the correlations.

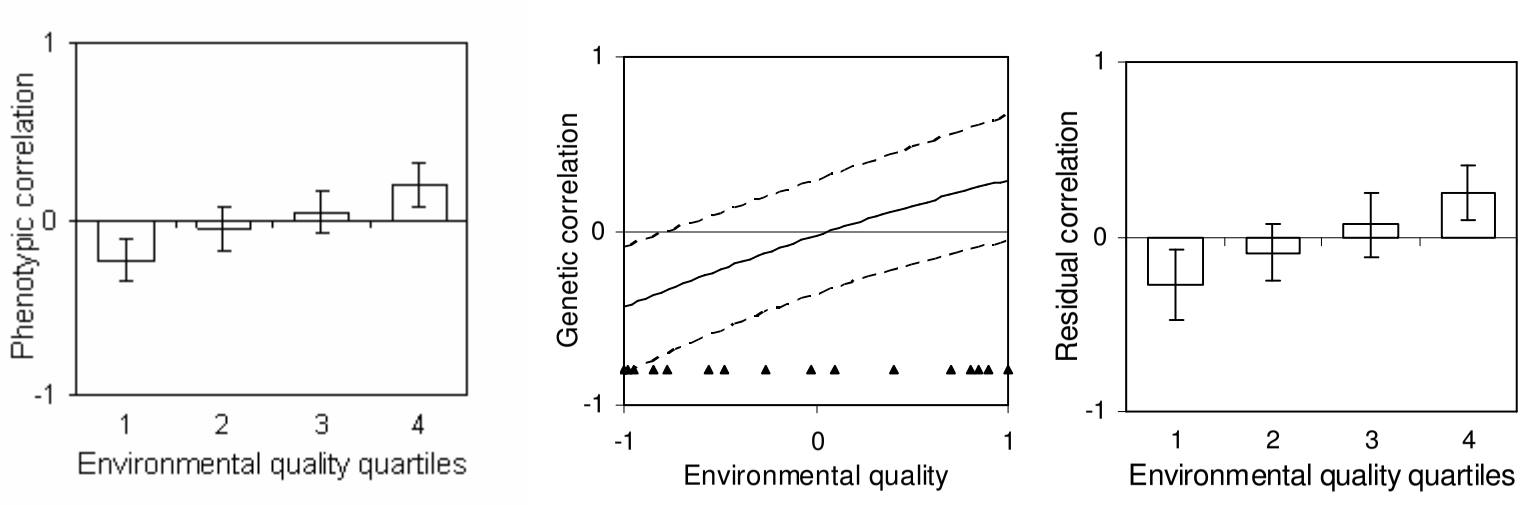


Figure 5.3. Phenotypic, genetic and environmental (residual) correlations between first year horn growth and average lifetime fecundity (FEC). An estimate of environmental quality was gained for each year of birth from 1985-2001 with triangles on the middle panels indicating their distribution. Estimates of the genetic correlation and 95% confidence interval (dashed line) between horn growth and LBS were generated from a random regression animal model allowing additive genetic effects to change as a function of environmental quality. For phenotypic and residual correlations, birth years were grouped based upon the quartiles of the distribution of environmental quality. Estimates of phenotypic and residual correlations were made within each of these four groups and error bars show 95% confidence intervals for the correlations.

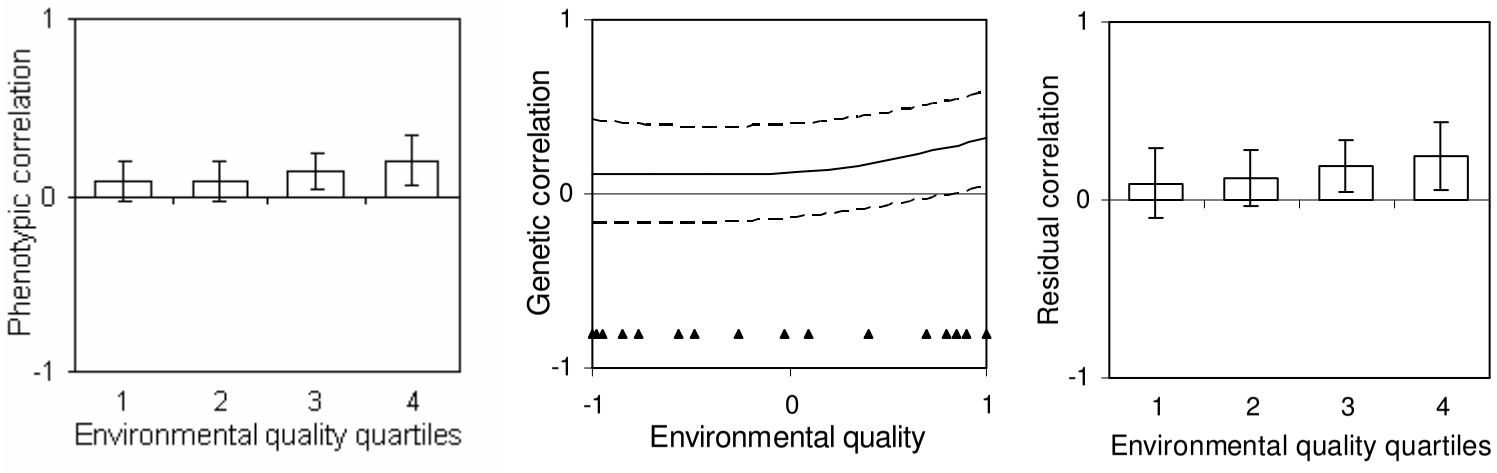
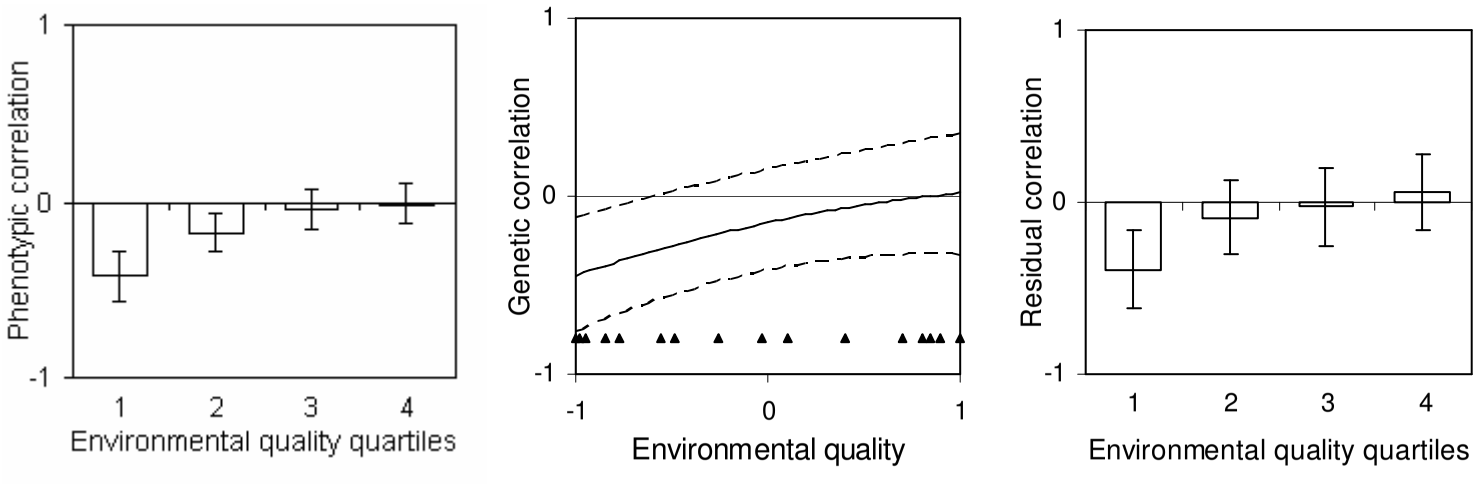


Figure 5.4. Phenotypic, genetic and environmental (residual) correlations between first year horn growth and longevity (LG). An estimate of environmental quality was gained for each year of birth from 1985-2001 with triangles on the middle panels indicating their distribution. Estimates of the genetic correlation and 95% confidence interval (dashed line) between horn growth and LBS were generated from a random regression animal model allowing additive genetic effects to change as a function of environmental quality. For phenotypic and residual correlations, birth years were grouped based upon the quartiles of the distribution of environmental quality. Estimates of phenotypic and residual correlations were made within each of these four groups and error bars show 95% confidence intervals for the correlations.

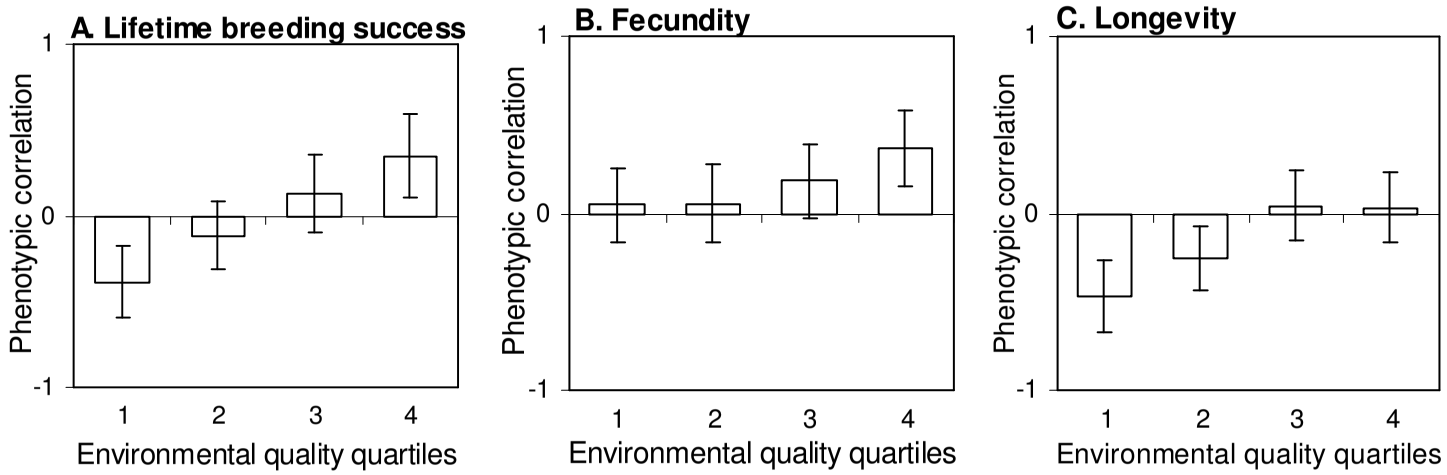


I found significant genotype-by-environment interaction for lifetime breeding success ($\chi^2_2 = 6.79$; $P = 0.034$), and longevity ($\chi^2_2 = 7.96$; $P = 0.019$), but not for fecundity ($\chi^2_2 = 1.74$; $P = 0.419$) or horn growth ($\chi^2_2 = 2.96$; $P = 0.174$). This indicates that within this population, different genes contributed to longevity and thus lifetime breeding success in different environments. For horn growth, this suggests that genetic effects were not influenced by environmental conditions and thus the allocation of a given genotype is the same in all environments. From the same model I also examined the genetic correlations between horn growth and fitness and found the same pattern as the phenotypic correlations (Figure 5.2). Genetic correlations between horn growth and lifetime breeding success showed a reversal from negative to positive across the gradient of environmental quality, suggesting that no single genotype for horn growth is optimal in all environments (Figure 5.2; compared to a model with zero genetic covariance: $\chi^2_4 = 11.16$; P = 0.025). The fecundity benefits were generally greater for individuals of higher genetic merit for horn growth (Figure 5.3; compared to a model with zero genetic covariance: $\chi^2_4 = 9.53$; P = 0.049). The survival costs of investment appeared to be greater for individuals of high genetic merit for horn growth, with negative correlations between longevity and first year horn growth (Figure 5.4; compared to a model with zero genetic covariance: $\chi^2_4 = 10.44$; P = 0.034).

The environmental covariance between horn growth and lifetime breeding success showed the same trends as the genetic covariance, with a reversal from negative to positive (Figure 5.2; compared to a model with zero residual covariance: $\chi^2_4 = 12.86$; P = 0.012). There was some evidence of positive environmental covariance between horn length and fecundity (Figure 5.3; although this was non-significant when compared to a model with zero residual covariance: $\chi^2_4 = 8.16$; P = 0.086). There was also a negative environmental correlation between horn growth and longevity in the worst environmental conditions, implying a trade-off in resource allocation between survival and horn growth (Figure 5.4; compared to a model with zero residual covariance: $\chi^2_4 = 10.84$; P = 0.028). Therefore in an unpredictable environment, high allocation to early horn growth is a gamble, the pay-offs of which depend on the environmental conditions an individual encounters during its first year of life.

Sexually selected traits often show allometric scaling (Bondriansky 2007; Kodric-Brown 2006) and thus I attempted to disentangle selection on first year horn growth from selection on overall body growth by incorporating measures of first year weight as a fixed effect into our models. This enabled me to assess selection pressures on a measure of allocation to horn growth relative to body size. Due to reduced sample size (only 489 males were captured within their first year) the convergence of the genetic models was sub-optimal. However, I found that the phenotypic patterns described above increased in strength when first year weight was included as a fixed effect (Figure 5.5), supporting my results and suggesting that the negative relationship with longevity was driven by allocation to horn growth and not to the potentially confounding factor of body size. Furthermore, males who grow larger horns may potentially show reduced longevity because of increased mating effort within their first year; however, I found no evidence that this is the case as excluding males who successfully bred within their first year did not alter the results that I present here.

Figure 5.5. Phenotypic correlations between first year horn growth and three fitness measures (A) lifetime breeding success; (B) average lifetime fecundity; (C) longevity when first year body weight was included as a covariate. Estimates of phenotypic correlations were made within each of these four groups and error bars show 95% confidence intervals for the correlations.



5.5 DISCUSSION

I have shown that the relationship between a male secondary-sexual trait and fitness is dependent upon ecological conditions and can change sign if environmental conditions are sufficiently variable. A previous study on a natural population revealed that selection on beak size characteristics in a population of Darwin's finches changed direction between droughts and wet years associated with the El Nino climatic cycle (Grant and Grant 2002). This study supports these results and demonstrates that environmental heterogeneity generates fluctuating selection at the genotypic level. As a result, high genetic merit for a secondary-sexual trait may not convey increased fitness in all environments.

There is presently much debate on the role of fluctuating selection in maintaining genetic variation with studies supporting the hypothesis (Dobzhansky 1977; Haldane 1963; Mackay 1981), finding limited evidence (Hendrick 2006; Prout 2000), or finding population specific effects (Mukai 1988). In this population, generations overlap and environmental conditions are unpredictable, two conditions which may be required for fluctuating selection to maintain genetic variance (Sasaki 1997). Furthermore, the coupling of fluctuating selection pressures on a trait with no genotype-by-environment interaction provides unequivocal support to the theory that quantitative genetic variance may be maintained under fluctuating selection pressures. Only by examining fitness through both viability and fecundity, and by accounting for the fluctuating environmental conditions that wild populations experience, can we accurately assess the relationship between a secondary sexual trait and fitness. In the wild, allocation to secondary sexual traits is a trade-off between survival versus fecundity, and our results indicate that in unpredictable environments no single strategy may be optimal.

CHAPTER 6

THE IMPACT OF EARLY ENVIRONMENTAL HETEROGENEITY ON GENETIC ARCHITECTURE IN THE WILD

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6.1 SUMMARY

Studies rarely consider the stability of genetic associations between traits across different environmental conditions. However, the environmental conditions experienced by individuals can shape their development and may be an important factor influencing the evolution of traits in the wild. Here, I examined how the genetic architecture of a suite of sexually dimorphic traits changed as a function of the environmental conditions experienced during development in an unmanaged population of Soay sheep (*Ovis aries*) on St Kilda, NW Scotland. I found significant genotype-by-environment interactions for first year male body weight and parasite load, but not for horn length. I then examined the stability of phenotypic, genetic and environmental (residual) covariance between horn length, body weight and parasite load both within and between the sexes. Genetic associations differed between traits within each sex, with horn length associated with body weight within males but not females. I found evidence that genetic associations between traits both within and between the sexes were dependent upon the environmental conditions experienced during development, with genetic correlations reducing in magnitude in more favourable environmental conditions. My results suggest that in good environments, loci are expressed which have sex-specific effects, which reduce cross-sex genetic correlations, allowing independent evolutionary trajectories between the sexes. This chapter demonstrates that the genetic architecture of traits is not stable under

temporally varying environments and highlights the fact that evolutionary processes may depend largely upon ecological conditions.

6.2 INTRODUCTION

Environmental heterogeneity has long been recognised as an important factor influencing the evolution of fitness-related traits in the wild (Roff 2002). The evolution of a trait depends upon the selection upon it, underlying genetic variation, and to a large degree the genetic relationships with other traits (Lynch and Walsh 1998). There is evidence that selection can vary considerably from year to year (Price et al. 1984, Robinson et al. 2008) and genetic variability in quantitative traits can change in response to environmental conditions (Charmantier and Garant 2005, Hoffmann and Merilä 1999). However, we know surprisingly little about the influence of environmental conditions on the genetic covariances and correlations between traits in wild populations. Laboratory evidence suggests that the environment may influence genetic relationships between traits (Sgrò and Hoffmann 2004), but estimates obtained in a controlled, or in an arbitrary range of conditions show a lack of concordance with those obtained in wild habitats (Conner et al. 2003). As a result, laboratory and environment-specific estimates of genetic covariances or correlations can make predictions for a trait's evolution, but these are valid only for the environment in which they were measured. Therefore, at present, it is difficult to generalise about the evolution of a trait which is expressed in populations which experience variable environmental conditions (Steppan et al. 2002).

Sexually dimorphic traits evolve in response to differing selection pressures between the sexes (Andersson 1994). The most common form of sexual size dimorphism, males being larger than females, is the expected outcome of sexual selection through male-male competition for access to females (Andersson 1994, Darwin 1871). Morphometric traits develop as the result of interactions within the genotype and between genes and the environment (Garant et al. 2004, Kotiaho et al. 2001). Genetic correlations between traits may arise from pleiotropy where a given locus affects

more than one trait, and may be the result of selection for developmental integration (Cheverud 1988, Lynch and Walsh 1998). If two traits are genetically correlated, the potential for those traits to diverge may be limited; alternatively, a low genetic correlation will enable independent evolution of either trait (Lynch and Walsh 1998). For sexually monomorphic traits, we may therefore expect that genetic expression in both sexes is influenced by the same developmental pathway (Jensen et al. 2003, Parker and Garant 2005, Roff 2002). However for sexually dimorphic traits, expectations of between-sex genetic correlations are unclear (Badyaev 2002, Lande 1980). We might expect that the genetic determination of a trait and the patterns of genetic covariance between traits may differ both within and between the sexes, producing the differences in trait growth which are commonly observed (Badyaev 2002, Lande 1980, Roff 2002).

The influence of changing environmental conditions on the **G**-matrix (the matrix of additive genetic variance and covariances corresponding to a set of traits) has been the focus of theoretical quantitative genetic studies (e.g. Jones et al. 2003). There is increasing evidence of genotype-by-environment interaction for many traits expressed in wild populations (Charmantier and Garant 2005) and it is thought that trait heritability may generally be higher in good environmental conditions (Hoffmann and Merilä 1999). There is also evidence that the **G**-matrix may not be stable across different populations of individuals (Roff et al. 2004) and estimates obtained from laboratory populations also suggest that genetic covariances are not stable under different environments (Sgrò and Hoffmann 2004). Therefore, we may expect that associations between traits may depend upon the environmental conditions encountered by an individual during their development. However, to our knowledge, no study has determined whether genetic correlations between traits change across a gradient of the environmental conditions encountered by individuals in the wild (Garant et al. 2008). Therefore, it remains unclear what effects variable environmental conditions have on associations between traits both within and between the sexes.

In this chapter, I aim to assess the stability of phenotypic, genetic and environmental (residual) associations between traits, within and between the sexes, across a range of environmental conditions experienced by a wild population. I focus on the horn length, body weight, and parasite load of a feral population of Soay sheep (*Ovis aries*) from the island of Hirta, St.Kilda, UK. This population is ideal for this purpose as weather conditions, population density, and consequently resource availability fluctuate from year-to-year, providing substantial differences between individuals in the environmental quality of their birth year and thus their survival rates (Clutton-Brock and Pemberton 2004). These varying conditions, combined with a large pedigree and extensive repeated morphological measures, provide an excellent opportunity to assess the potential effects of early environmental heterogeneity on genetic architecture of traits. Previous studies on this population have shown additive genetic variance for many morphological traits (Milner et al. 2000, Wilson et al. 2005, Coltman et al. 2001); genetic correlations between traits in the average environment (Coltman et al., 2001); and genotype-by-environment interactions (Wilson et al. 2006) and we may expect that varying environmental conditions may also influence genetic covariances and correlations between traits. Therefore, I first use a random regression animal model approach to assess the extent to which quantitative genetic parameters of traits measured during the first year of life vary as a function of environmental conditions (Wilson et al. 2006). I then extend this methodology to test whether phenotypic, genetic and environmental covariances between first year traits of horn length, body weight and parasite load depend upon the environmental conditions experienced during the first year of life. Furthermore, there has recently been much interest in assessing genetic correlations between the sexes (Foerster et al. 2007, Poissant et al. 2008, Rice and Chippindale 2001); however, all of these predictions have been made in average environmental conditions. Therefore, I also assess whether conditions experienced during the first year of trait development have long-lasting effects across ontogeny on the genetic covariance between traits in both males and females, and across the sexes.

6.3 METHODS

6.3.1 Study species and data collection

Soay sheep (*Ovis aries*) were introduced onto the island archipelago of St.Kilda, NW Scotland in the North Atlantic (57º49'N, 08º34'W) during the Bronze Age (Clutton-Brock and Pemberton 2004). The unmanaged study population of Village Bay, Hirta was founded in 1932 with the introduction of 107 sheep from neighbouring Soay, and currently fluctuates from 211 to 671 individuals, with an average of 432. These fluctuations occur due to periodic over-winter crashes following years of high population density and poor weather conditions, and as a result there are substantial differences between individuals in the environmental quality of their birth year and in their subsequent survival rates (Clutton-Brock and Pemberton 2004). The population has been the subject of intensive individual-level study since 1985, yielding morphological and life-history data for 6387 pedigreed individuals, including 3626 maternal links and 1699 paternal links (from 807 distinct dams and 495 distinct sires). Maternal identity is known from field observations and paternity is inferred by microsatellite based paternity analysis at a pedigree-wide confidence level of ≥ 80%, allowing no more than one allelic mismatch between offspring and putative sire, using maximum likelihood implemented in CERVUS (Marshall et al. 1998).

I considered the following phenotypic traits, measured in both males and females:

Horn length: Soay sheep have a distinct polymorphism for horn type producing either a full horn (males 86%; females 32%), a reduced horn (or "scur": males 14%; females 28%), or no horn at all ("poll": 40% of females only). I use only horn length measures of full (normal-horned) individuals, as sufficient reliable estimates of size could only be obtained for this group. The horns of sheep grow cumulatively over life, with horn increments formed when growth stops over winter, forming an annulus. This provides an annual measure (in mm) of horn growth at each age, which

can be measured at any point of an individual's life and after death (Chapter five). I did not use horn measures which were recorded in August of the first year of life as they were poorly associated with measurements recorded after the increment had stopped growing later in the year.

Body weight: Measurements of live weight (measured to the nearest 0.1 kg) and parasite load were made during a two-week period in August, in which 49-67% of the study area population are rounded up each year, and during late autumn when free ranging males are sampled during the rut.

Parasite load: As a measure of parasite load, we used measures of faecal egg counts of five nematode gut parasites collectively termed strongyles (Clutton-Brock and Pemberton 2004). These counts represent a measure of nematode parasite infection as: they are correlated with worm burden in Soay sheep (Grenfell et al. 1995); they are associated with other immune measures in domestic sheep (Shaw et al. 1999); and they are associated with over-winter survival in Soay sheep (Illius et al. 1995). Previous work has shown a negative genetic correlation between parasite load and weight within this population (Coltman et al. 2001). Faecal egg counts were transformed by natural logarithm prior to analyses (Coltman et al. 2001).

For males, data of 2032 body weight and 1730 parasite measures from 1685 males, and 2679 horn length measures from 1449 normal-horned males was available. For females, data of 2882 body weight and 3131 parasite measures from 1335 females, and 661 horn length measures from 428 normal-horned females were available. All individuals were born between 1985 and 2005.

In these quantitative genetic analyses, I first assessed the genetic (co)variance of first year male traits to test whether environmental conditions experienced during the

period of their peak growth (Clutton-Brock and Pemberton 2004) affect the genetic architecture. I was not able to conduct a similar analysis for all first year female traits as models convergence was sub-optimal. Second, I then assessed whether conditions experienced during the first year of trait development have long-lasting effects across ontogeny on the genetic covariance between traits in both males and females, and across the sexes.

6.4 QUANTITATIVE GENETIC PARAMETERS

Quantitative genetic parameters were estimated using an animal model, which combines pedigree and phenotypic data to partition the phenotypic variance of each trait into additive genetic, maternal, and environmental components (Kruuk 2004). Animal models are a form of linear mixed model implemented in ASReml (Gilmour et al. 2002) using restricted maximum likelihood, which are able to accommodate unbalanced data sets and complex pedigrees (Kruuk 2004). Here, I used random regression animal models (Chapter five, Wilson et al. 2006) to model the additive genetic effects of each trait as a polynomial function of environmental quality. The environmental quality (E) of an individual's first year was defined as the proportion of live born lambs that survived the first winter that followed their birth year (values ranged from 5-86% and were then standardized to the interval $-1 \leq E \geq 1$).

6.4.1 Effect of environment on components of variance of first year male traits

First, I tested whether variance in first year male traits was influenced by the environmental conditions experienced during the first year of life. Fixed effects included year of birth (BYR: 1985-2005, fitted as a factor) which was fitted for all three traits to remove effects of conditions at birth on mean trait values, and to remove temporal trends in the mean. For body weight and parasite load, day of measurement (DAY: covariate of days since birth) was fitted to account for the fact that measures were taken at different times of year.

Thus for each trait y and individual i I fitted the random regression model:

Equation 6.1 $y_{ij} \sim \mu + (BYR + DAY)_i + f(a_i, n, E) + m_j + e_{iEG}$

where y is one of the three traits measured on individual *i* with mother *j*; μ is the mean of each trait; $f(a_i, n, E)$ is the random regression function of an orthogonal polynomial of the additive genetic merit values a_i as a function of *E*, with order *n*,; m_j is the maternal random effect of mother *j* which has a population-level variance V_M; and e_{iEG} is the environment specific residual error for individual *i* grouped by environment group *EG*, which is a four level factor (1:very poor; 2: poor; 3: good; 4: very good) produced by grouping birth years based upon the 25% quartile of the distribution of *E* (see below).

For this random regression each individual is only represented once within the data set, as individuals are only ever born into one environment. However, as related individuals are born into different environments, the genetic effects for each trait can be estimated as a function of environmental quality. This method represents a more efficient use of the data by avoiding sub-division of records into environment specific traits (Fischer et al. 2004). Estimates of residual covariance between environments could not be made as multiple records for individuals across environments were not possible. I therefore partitioned the residual error structure into four levels of EG, defined by the four quartiles of E, with no covariance between environmental levels. I attempted to estimate a residual error structure for each of the 20 birth years but as this overparameterized the model, resulting in variance terms which could not be estimated with any certainty, I do not present this method.

I began by fitting a model without any additive or maternal effects such that all the phenotypic variance was allocated to the residual structure. This gave the phenotypic variance of each trait, after conditioning on the fixed effects, within each of the four *EG* groups. I tested for significant differences in phenotypic variance over E by re-running the model with a single constant error structure, and comparing models using log-likelihood ratio tests. Subsequently, I added the additive genetic and maternal

effects and, using a forward selection procedure, compared a series of successively more complex random regression models that differed in the order of polynomial function of the additive effects from n=0 (a_i as constant) to n=2 (a_i as a quadratic function of E). Models were compared using log-likelihood ratio tests, with degrees of freedom as the added number of variance and covariance components estimated with increasing function (for example, a comparison of zero to first order models requires two degrees of freedom, to account for the two additional parameters of variance in slope and covariance between slope and intercept). A second order function did not provide a better fit in any model, therefore only zero to first order comparisons are shown. Throughout these comparisons the residual error structure remained partitioned into the four *EG* groups and once the appropriate polynomial function for the additive genetic effects was selected, the model was re-run with a single constant error structure and models compared using log-likelihood ratio tests to test for environmental heterogeneity in residual (environmental) effects.

A random regression function of order n produces a variance-covariance matrix (matrix **Q** with dimensions [(n+1) x (n +1)]): for example, for a first order function, estimating a variance in intercept and slope and the covariance between them. For the presentation of results I used **G = Z Q Z'**, where **Z** is the vector of orthogonal polynomials evaluated at the values of standardized environmental quality (**Z'** is the transpose of **Z**), to gain a single additive genetic variance-covariance matrix **G** of environment-specific (co)variance estimates for each trait. Note that the model structure, with the additive genetic effect fitted as a polynomial function whereas residuals are grouped into four levels, results in estimates of additive genetic variance which vary as a continuous function of E but four discrete estimates of residual variance for each environmental group.

6.4.2 Effect of environment on covariance of first year male traits

Second, I then tested whether the phenotypic, genetic and environmental covariance between first year male traits was dependent upon the environmental quality of an individual's first year. To do this I extended Equation 6.1 to a multivariate random regression, which simultaneously partitions the phenotypic variance of both traits

into genetic, maternal, and residual components, and directly estimates the covariance between traits in each component. I standardized each measure to a zero mean and unit variance, thus placing both measures on the same scale. I modelled the genetic component of the three traits as a linear function of *E*, estimating the genetic covariance (converted to a correlation) between traits across a gradient of environmental quality.

Thus for each individual *i* I extended Equation 6.1 using a multivariate framework:

Equation 6.2 $y_{tij} \sim \mu_t + (\mathrm{BYR} + \mathrm{DAY})_{ti} + f(a_i, n, E)_t + m_{tj} + e_{tiEG}$

where all of the three traits *t* measured on individual *i* with mother *j* were included as response variables; $f(a_i, n, E)$ is the random regression function on an orthogonal polynomial of *E*, with order *n*, of the additive genetic merit values a_i of individuals; m_j is the maternal random effect of mother *j* which has a population-level variance V_M; and e_{iEG} is the environment specific residual error for individual *i* grouped by *EG*.

I fitted a first order random regression function for each trait, producing a single 6x6 variance-covariance matrix (**Q** with dimensions [(3 x (*n*+1)) x (3 x (*n* +1))] where *n*=1). This resulted in estimates of the variance in intercept and slope and their covariance for each trait and the covariance between traits in intercept and slope. I then tested for significant changes in genetic covariance between traits over *E*. Within the 6x6 variance-covariance matrix are three, 2x2 covariance blocks, which give the covariances in intercept and slope between pairs of traits (for example, covariance between intercepts for horn size and body weight, covariance between slopes, covariance between horn size intercept and body weight slope and covariance between horn size slope and body weight intercept). For each pair of traits in turn, I re-ran the model estimating the covariance in intercept and constraining the three slope covariance components to zero. I then compared models using log-likelihood

ratio tests with three degrees of freedom, testing against the null hypothesis that there is no change in covariance over *E*.

I also tested for significant residual covariance between traits. Within each *EG* group estimates of the residual variance of each trait and the covariance between traits could be made (i.e. a 3x3 matrix within each *EG* group). I tested the significance of these correlations by re-running the model three times, once for each pair-wise combination of traits, with the residual covariances within environments constrained to zero for that combination, and compared models using log-likelihood ratio tests.

For the presentation of results I used **G = Z Q Z'**, where **Z** is the vector of orthogonal polynomials evaluated at the values of standardized environmental quality (**Z'** is the transpose of **Z**), to gain an additive genetic variance-covariance matrix of environment specific (co)variance estimates for all traits. The diagonal of the covariance matrices between the additive genetic variance estimates of the traits provides estimates of the genetic covariance between traits across *E*. All covariance estimates were rescaled to give the genetic correlations, providing a dimensionless estimate of the association between both traits. Both the covariance and correlations produced the same patterns. An analogous method was used to estimate the approximate standard errors, which were converted into approximate 95% confidence intervals (Fischer et al. 2004).

6.4.3. (Co)variance between traits over ontogeny

Third, I used repeated measures on each trait collected at different ages over the lifespan of individuals to provide quantitative genetic variance estimates for each trait and the covariance (correlation) between traits in average environmental conditions. Fixed effects included age (factor 1-7+) to control for mean changes over ontogeny and year of growth to control for short-term environmental effects on mean trait values. For weight and parasite load, day of measurement (DAY: covariate of Julian day of the year) was fitted to account for the fact that measures were taken at different times of year.

Thus for each individual *i*, with mother *j* I fitted:

Equation 6.3 $y_{tij} \sim \mu_t + (\text{YEAR} + \text{DAY} + \text{AGE})_{ti} + a_{ti} + m_{tj} + c_{ti} + e_{ti}$

where for each trait *t*, a_i is the breeding value of individual *i* which has a population mean of zero and a variance V_A (additive genetic variance); m_j is the maternal random effect of mother *j* which has a population-level variance V_M; c_i is the individual permanent environment effect which grouped repeated measures on the same individual to quantify any between-individual differences over and above that due to additive genetic and maternal effects, with a population-level variance V_{PE} (Kruuk and Hadfield 2007); and e_i is the trait specific matrix of residual error for individual *i* which has a population-level variance V_R. Random terms were modelled using unstructured 3 x 3 variance-covariance matrices, providing variance estimates for each trait and the covariance between them, which was converted into a correlation.

I used a matrix comparison similar to the maximum-likelihood method of Shaw (1991) to test whether the additive genetic (co)variance matrix differed generally between males and females. I fitted two models to the data: one where the additive genetic (co)variances were free to vary between the sexes but all other (co)variances for the random effects were pooled across the sexes, and one where all the (co)variance estimates of the random effects for all three traits were consistent between the sexes. In both models the residual (co)variances were allowed to vary between the sexes and I used log-likelihood ratio tests to compare the models. For this matrix comparison, phenotypic data were scaled to unit variance to control for scale effects (Hadfield et al. 2007).

6.4.4 Effect of first year environment on (co)variance between traits over ontogeny

Finally, I also wished to test whether traits expressed over life had different relationships depending upon the environmental conditions experienced during the first year of development. First, I did this within males and females separately and

then I estimated correlations for each trait across the sexes. At each age (1-7+), I standardized each measure to a zero mean and unit variance, thus placing both measures on the same scale with the same mean, and removing any effects of increased variance with age.

Thus for each individual *i* I extended Equation 6.3 to a multivariate random regression accounting for repeated measures:

Equation 6.4 $y_{tij} \sim \mu_t + (\text{YEAR} + \text{DAY+AGE})_{ti} + f(a_i, n, E)_t + m_{tj} + c_{ti} + e_{tiEG}$

where combinations of traits *t* of individual *i* and mother *j* were included as response variables; $f(a_i, n, E)$ is the random regression function on an orthogonal polynomial of *E*, with order *n*, of the additive genetic merit values a_i of individuals; m_j is the maternal random effect of mother *j* which has a population-level variance V_M; and e_{iEG} is the environment specific residual error for individual *i* grouped by *EG*. I used the same methodology as described above to assess the significance of the estimates gained, test against a null hypothesis that there is no change in covariance over *E*, and to present the results.

Standard errors for variance and covariance components, as well as the heritabilities and genetic correlations were computed by ASReml. Significance of all estimates was assessed using log-likelihood ratio tests. In all models, estimates of the covariance showed the same trends as the correlations which I present.

6.5 RESULTS

6.5.1 Effect of environment on components of variance of first year male traits

I first examined how the phenotypic, genetic and residual components of variance in each trait in the first year of life varied across a range of environmental conditions in males (Equation 6.1).

There was no evidence that the phenotypic variance of normal-horned male horn length or male body weight varied with environmental quality (Figure 6.1, 6.2). However, phenotypic variance in first year parasite resistance increased with increasing environmental quality (Figure 6.3). There was no evidence that the additive genetic variance of first year horn length increased significantly with environmental quality, indicating that the same phenotype is expressed by a given genotype across different environmental conditions (Figure 6.1). The additive genetic variance of both first year male body weight and male parasite resistance increased with increasing environmental quality, indicating significant genotype-by-environment interactions for these traits (Figure 6.2, 6.3). Despite downward trends in both, there was no evidence that residual (environmental) variance of first year horn length (Figure 6.1) or body weight (Figure 6.2) varied significantly with environmental quality, but there was evidence of an increase in residual variance in parasite resistance (Figure 6.3).

I also examined the additive genetic correlation surfaces for each trait across first year environments. For first year male horn length and parasite resistance, genetic correlations between environments were high and relatively stable (Figure 6.4). However for body weight, there were only very weak genetic correlations across different environmental conditions (Figure 6.4). These results suggest that for body weight, different loci may be involved across environments.

Figure 6.1. Phenotypic, additive genetic and residual variance for first year male horn length as a function of the environmental quality of their first year (*E*; distribution shown by black triangles). Results were gained from model 1 (see methods) where phenotypic and residual variance was estimated by grouping years based upon the quartiles of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good) and error bars show the 95% confidence intervals of the estimates. Additive genetic effects were estimated as a linear function of environmental quality (*E*) and are shown by a solid line, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio comparison of model 1 with a model with constant variance across all environments.

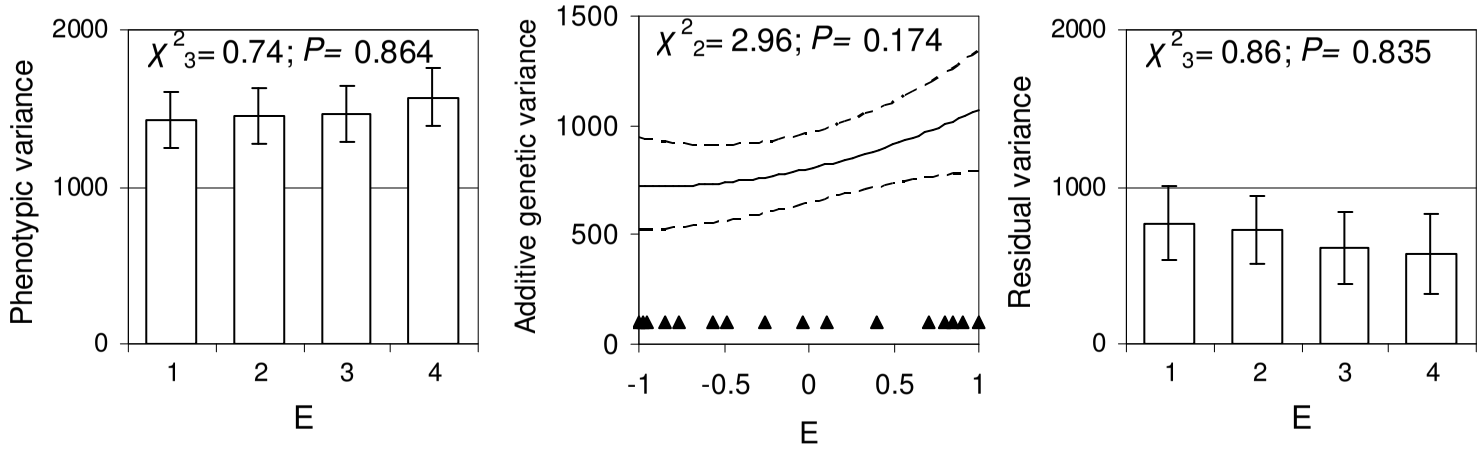


Figure 6.2. Phenotypic, additive genetic and residual variance for first year male body weight as a function of the environmental quality of their first year (*E*; distribution shown by black triangles). Results were gained from model 1 (see methods) where phenotypic and residual variance was estimated by grouping years based upon the quartiles of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good) and error bars show the 95% confidence intervals of the estimates. Additive genetic effects were estimated as a linear function of environmental quality (*E*) and are shown by a solid line, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio comparison of model 1 with a model with constant variance across all environments.

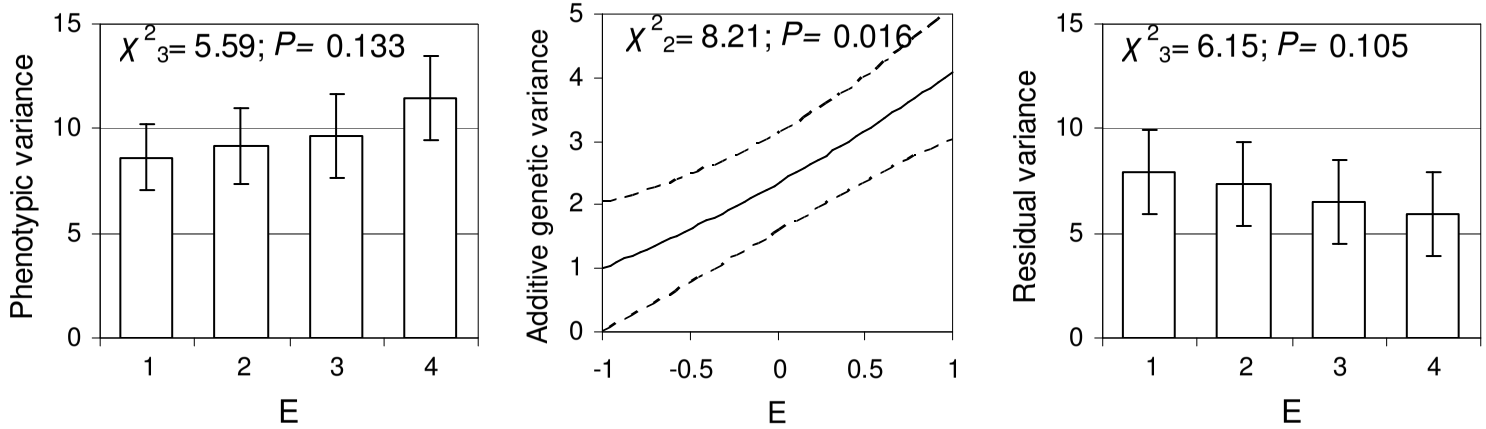


Figure 6.3. Phenotypic, additive genetic and residual variance for first year male parasite resistance as a function of the environmental quality of their first year (*E*; distribution shown by black triangles). Results were gained from model 1 (see methods) where phenotypic and residual variance was estimated by grouping years based upon the quartiles of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good) and error bars show the 95% confidence intervals of the estimates. Additive genetic effects were estimated as a linear function of environmental quality (*E*) and are shown by a solid line, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio comparison of model 1 with a model with constant variance across all environments.

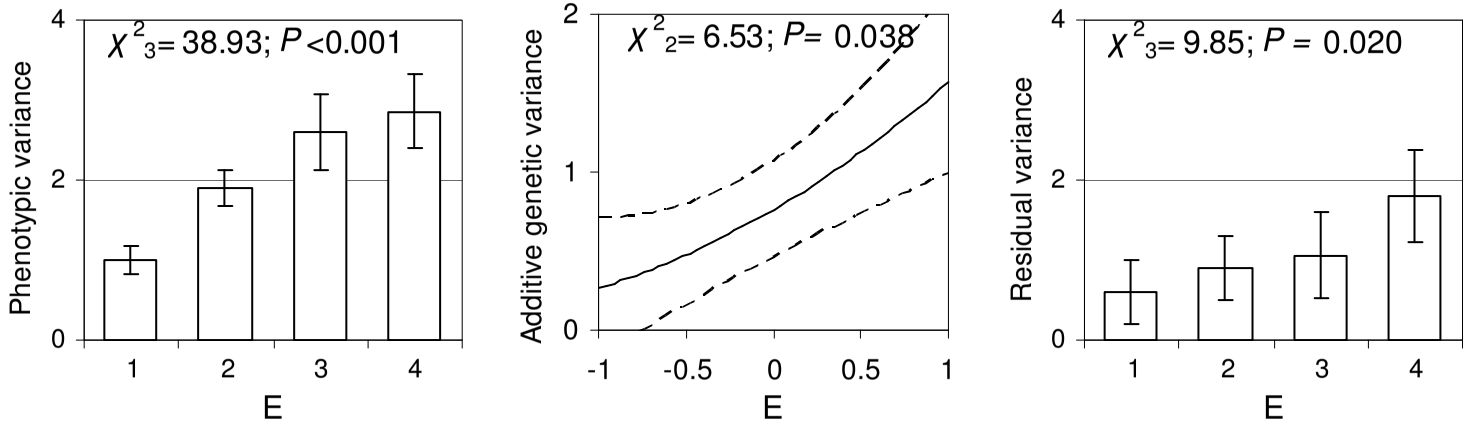
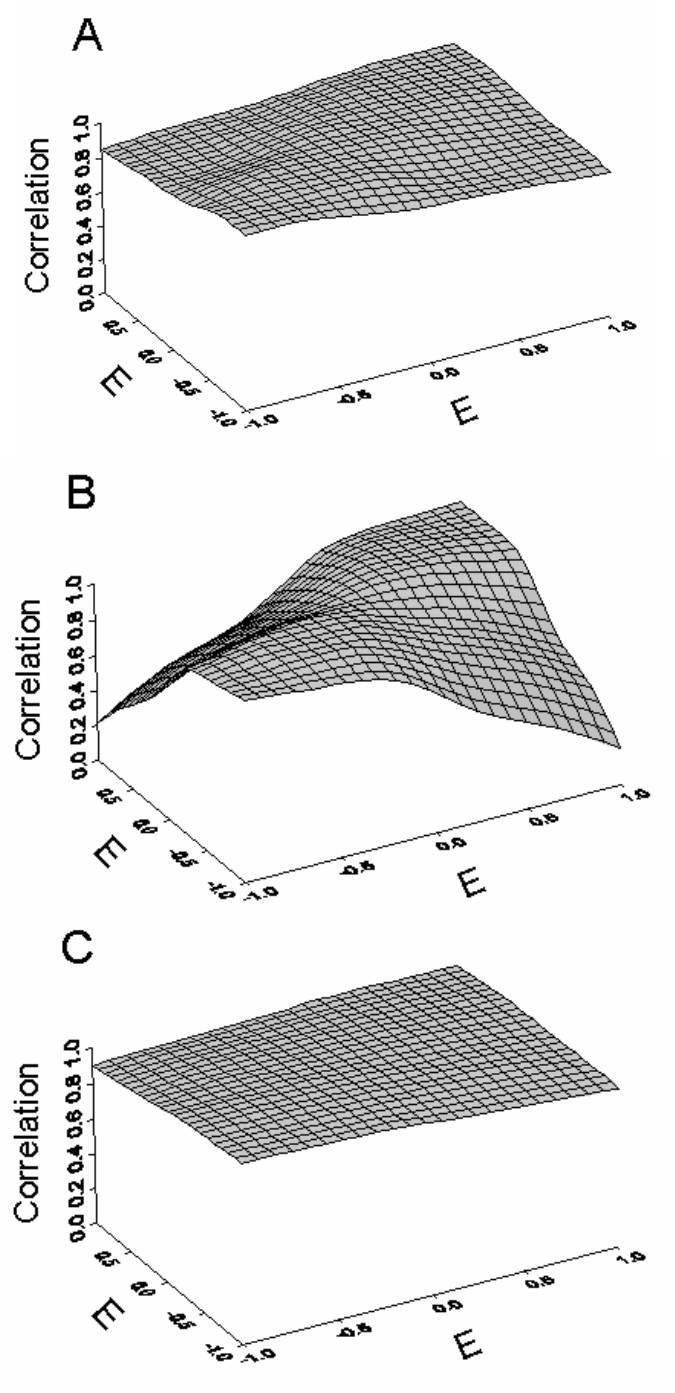


Figure 6.4. Additive genetic correlation surfaces for first year male (A) horn growth; (B) weight; (C) parasite resistance across environmental quality (*E*) gained from model 1 (see methods).



6.5.2 Effect of environment on covariance of first year male traits

Second, I tested whether the phenotypic, genetic and environmental covariances between first year male horn length, body weight, and parasite load varied depending upon the environmental quality experienced (Model 6.2). I found significant phenotypic correlations between all first year male traits, which generally reduced in magnitude as environmental quality increased (Figure 6.5-6.7). Genetic correlations between first year male horn length and both body weight and parasite load were positive and decreased in magnitude with increasing environmental quality (Figure 6.5-6.7). Residual correlations between horn length and body weight were positive implying that resource allocation to body weight was positively associated with allocation to horn growth and increased in magnitude with increasing environmental quality (Figure 6.5-6.7). Residual correlations between horn length and parasite load were negative implying resource allocation to reducing parasite load was associated with allocation to increased horn length (Figure 6.5-6.7). Although I observed a significant phenotypic relationship between body weight and parasite load, which appeared to decrease with increasing environmental quality, I could not significantly demonstrate that this effect was due to changing genetic or residual covariance, although the trend in genetic correlation was only marginally non-significant (Figure 6.5-6.7). This was most likely to be the result of the large standard errors, coupled with smaller covariance estimates for this comparison.

Figure 6.5. Phenotypic, genetic and residual (environmental) correlations between first year male traits of horn length and body weight as a function of environmental quality (*E*) of the first year of life. Results were gained from model 2 (see methods) where phenotypic and residual correlations were calculated within each quartile of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good), with error bars representing 95% confidence interval of the estimates. The genetic correlations were estimated as linear functions of *E* and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio tests, against a null hypothesis of no change in covariance over *E*.

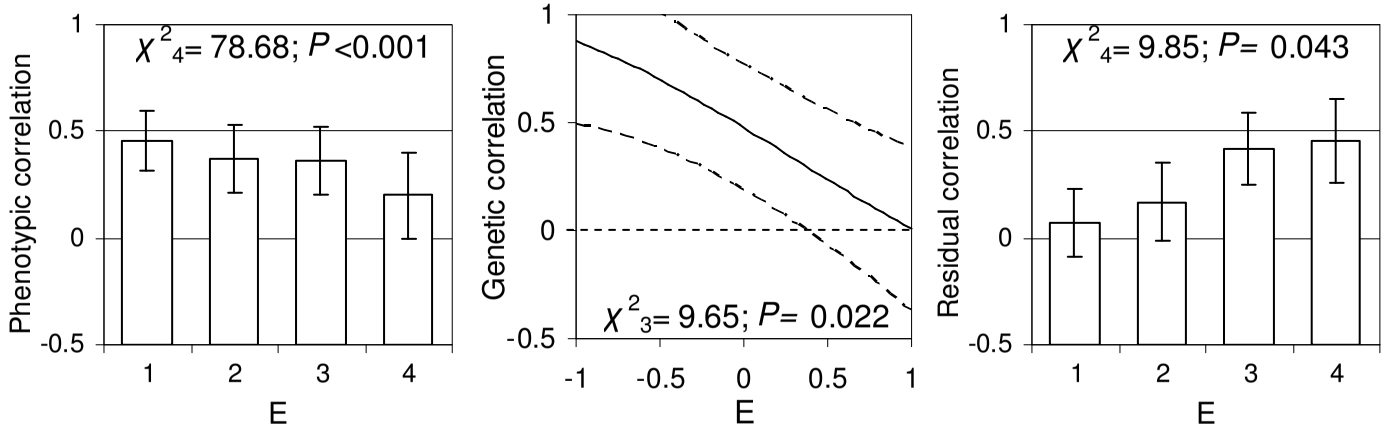


Figure 6.6. Phenotypic, genetic and residual (environmental) correlations between first year male traits of horn length and parasite resistance as a function of environmental quality (*E*) of the first year of life. Results were gained from model 2 (see methods) where phenotypic and residual correlations were calculated within each quartile of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good), with error bars representing 95% confidence interval of the estimates. The genetic correlations were estimated as linear functions of *E* and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio tests, against a null hypothesis of no change in covariance over *E*.

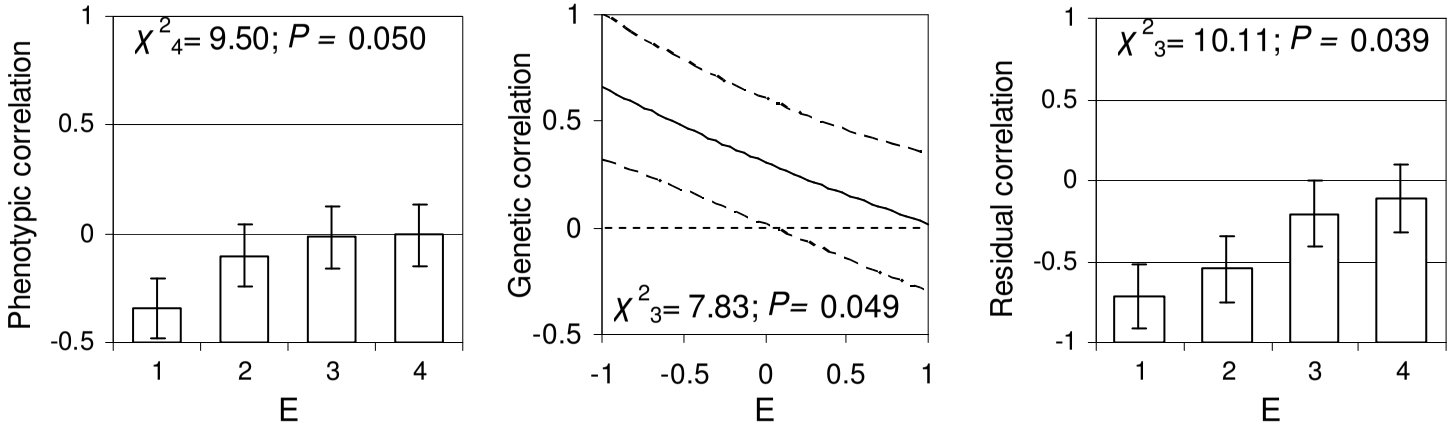
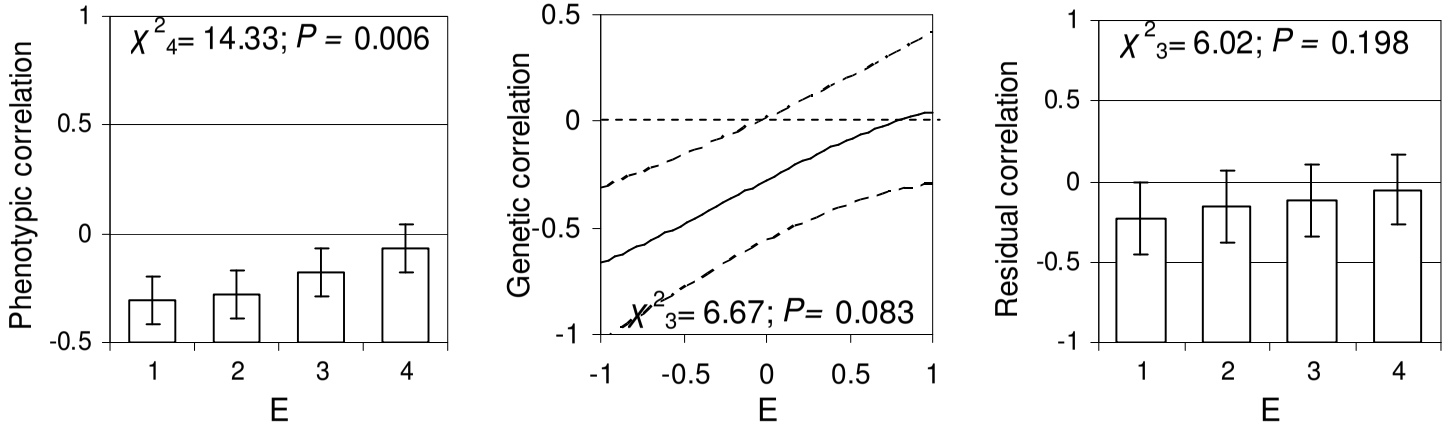


Figure 6.7. Phenotypic, genetic and residual (environmental) correlations between first year male traits of body weight and parasite resistance as a function of environmental quality (*E*) of the first year of life. Results were gained from model 2 (see methods) where phenotypic and residual correlations were calculated within each quartile of the distribution of *E* (1: very poor; 2: poor; 3: good; 4: very good), with error bars representing 95% confidence interval of the estimates. The genetic correlations were estimated as linear functions of *E* and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. Significance testing was conducted using log-likelihood ratio tests, against a null hypothesis of no change in covariance over *E*.



6.5.3 (Co)variance between traits over ontogeny

Third, I then used repeated trait measures recorded over the lifespan of individuals to first examine the relationships between traits in average environmental conditions (Model 6.3). On average, males were larger than females, with larger parasite burdens, and larger horns (Table 6.1). I found significant additive genetic variance for all traits with similar coefficients of additive genetic variance and heritability values between the sexes (Table 6.1).

In the average environment, there was evidence of different genetic relationships between traits within each sex (Model 6.3; general comparison of male and female maticies: $\chi^2_6 = 14.82$, $P = 0.022$). In males, I found significant genetic correlations between horn length and body weight and between body weight and parasite load (Table 6.2). Contrary to the evidence suggesting a genetic correlation between first year male parasite load and male horn length (Figure 6.2), there was no evidence of a significant correlation across all ages (Table 6.2). In females, there was no evidence of a genetic correlation between horn length and any other trait (Table 6.2). Potentially there may be a genetic relationship between female body weight and parasite load ($\chi^2_1 = 3.23$, $P = 0.072$), but I may be unable to significantly demonstrate it due to the large standard error of the estimate.

I found significant genetic correlations between the sexes for body weight and parasite load across ages in the average environment (Model 3; Table 6.2). The cross-sex correlation for horn length was significantly less than one (log-likelihood ratio test against a model where correlation fixed to one: $\chi^2_1 = 7.60$, $P = 0.006$). The cross-sex correlation estimates for body weight ($\chi^2_1 = 1.60$, $P = 0.206$) and parasite resistance ($\chi^2_1 = 0.11$, $P = 0.740$) were not significantly different from one.

Table 6.1. Means, standard deviation (SD), sample sizes (N: number of observations/individuals), and variance components (± standard error) across all ages, in the average environment, for traits measured in males and females. Estimates were obtained from multivariate animal models (see methods, model 3). Significance of the estimates was assessed using log-likelihood ratio tests: * P<0.05; **P<0.01;*** P<0.001.

Trait	Mean	SD	N	V_A	V_{PE}	V_M	V_R	CV_A	h^2
Males									
Horn length (mm)	265.29	108.45	2679/1449	811.70 ± 176.30***	892.20 ± 159.20***	14.71 ± 29.57	479.30 ± 18.71***	10.74	0.37 ± 0.08
Body weight (kg)	21.22	8.08	2032/1685	0.67 ± 0.32*	4.30 ± 0.76***	0.57 ± 0.32	7.38 ± 0.40***	3.86	0.05 ± 0.02
Parasite resistance (log_0 FEC)	5.69	1.75	1730/1685	0.30 ± 0.07***	0.00 ± 0.00	0.00 ± 0.00	2.12 ± 0.09***	9.63	0.12 ± 0.03
Females									
Horn length (mm)	116.90	41.22	661/428	269.40 ± 114.30*	307.30 ± 114.30**	5.56 ± 25.82	95.13 ± 7.95***	14.04	0.40 ± 0.15
Body weight (kg)	18.97	5.20	2882/1335	1.52 ± 0.36**	1.98 ± 0.32***	0.20 ± 0.16	1.92 ± 0.07***	6.50	0.27 ± 0.06
Parasite resistance (log_0 FEC)	4.39	2.64	3131/1335	0.26 ± 0.07***	0.08 ± 0.12	0.03 ± 0.04	4.99 ± 0.14***	11.62	0.05 ± 0.02

V_A: additive genetic variance; V_{PE}: permanent environment effects; V_M: maternal variance; V_R: residual (environmental) variance; CV_A: coefficient of additive genetic variance; h^2: heritability.

Table 6.2. Genetic correlations between males and females (diagonal), between traits in males (above diagonal), and between traits in females (below diagonal) across all ages, in the average environment. Estimates and standard error (± SE) were obtained from multivariate animal models (see methods, model 3). Significance from zero was assessed using log-likelihood ratio tests: * P <0.050; **P <0.010; *** P <0.001.

	Horn length	Body weight	Parasite resistance
Horn length	0.223 ± 0.201	0.541 ± 0.143**	0.151 ± 0.178
Body weight	0.097 ± 0.168	0.793 ± 0.246**	-0.530 ± 0.210**
Parasite resistance	0.093 ± 0.202	-0.278 ± 0.169	0.838 ± 0.273***

6.5.4 Effect of first year environment on (co)variance between traits over ontogeny

Finally, I then extended the analysis of repeated trait measures to examine the effects of first year environmental conditions on genetic covariance between traits expressed over life (Model 6.4). There was no evidence that the average additive genetic variance of parasite load or horn length varied as a function of first year environment (male horn length: $\chi^2_2 = 0.71$, $P = 0.701$; male parasite load: $\chi^2_2 = 1.65$, $P = 0.438$; female horn length: $\chi^2_2 = 0.60$, $P = 0.701$; female parasite load: $\chi^2_2 = 3.68$, $P = 0.159$). However, there was evidence that additive genetic variance of male body weight ($\chi^2_2 = 6.26$, $P = 0.044$) varied as a function of first year environment and results also suggested that female body weight ($\chi^2_2 = 4.72$, $P = 0.096$) also varied as a function of the environment.

There was evidence that genetic correlations both within (Figure 6.8, 6.9) and between (Figure 6.10) the sexes were dependent upon environmental conditions experienced during the first year of life. In males, the genetic correlations between all traits tended towards zero with increased environmental quality of the first year (Figure 6.8). In females, horn length was not significantly associated with any other trait and there was no significant pattern with environment (Figure 6.9). However, results suggested that the genetic correlation between female body weight and female parasite load reduced in magnitude with increasing environmental quality of the first year (Figure 6.9). Genetic correlations between the sexes in both horn length and body weight decreased with increasing environmental quality of the first year of life (Figure 6.10). There was no evidence that the genetic correlation between the sexes in parasite load was dependent upon the environmental conditions experienced during the first year of life (Figure 6.10).

Figure 6.8. Genetic correlation between: (A) horn length and body weight; (B) horn length and parasite resistance; (C) body weight and parasite resistance in males across all ages, as a function of the environmental conditions experienced during the first year of life. Results were gained from model 4 (see methods), where the genetic correlations were estimated as linear functions of E and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. The significance of the trends shown was assessed using log-likelihood-ratio tests, against a null hypothesis of no change in covariance over *E*.

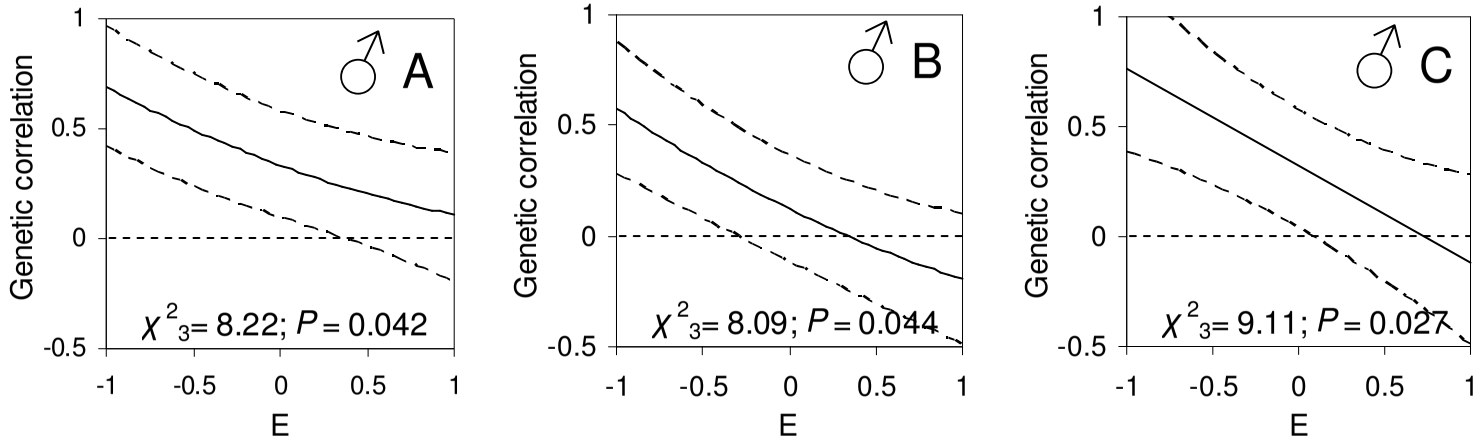


Figure 6.9. Genetic correlation between: (A) horn length and body weight; (B) horn length and parasite resistance; (C) body weight and parasite resistance in females across all ages, as a function of the environmental conditions experienced during the first year of life. Results were gained from model 4 (see methods), where the genetic correlations were estimated as linear functions of E and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. The significance of the trends shown was assessed using log-likelihood-ratio tests, against a null hypothesis of no change in covariance over *E*.

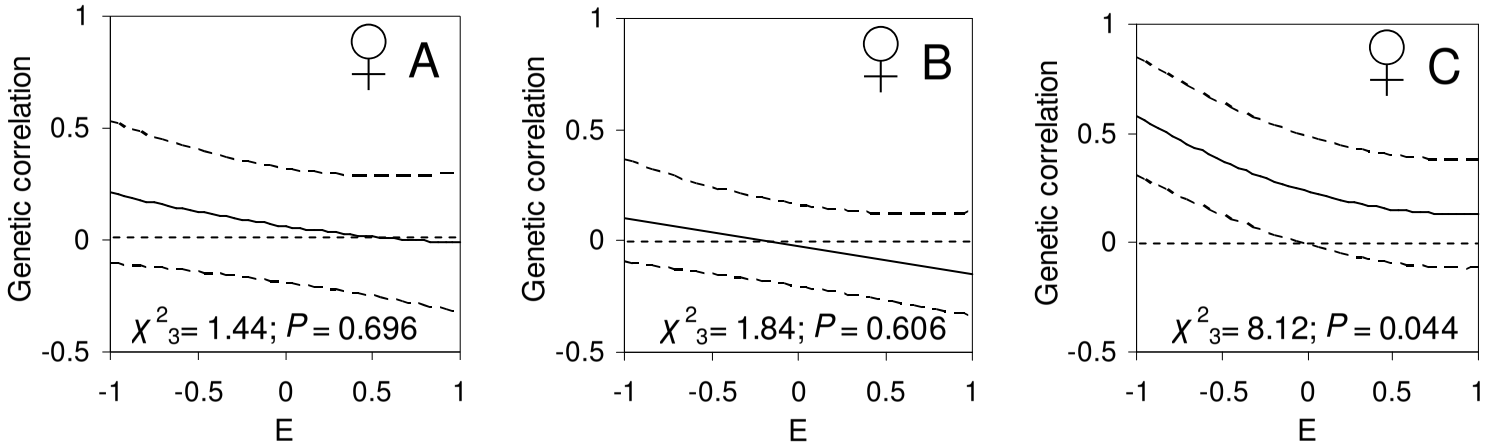
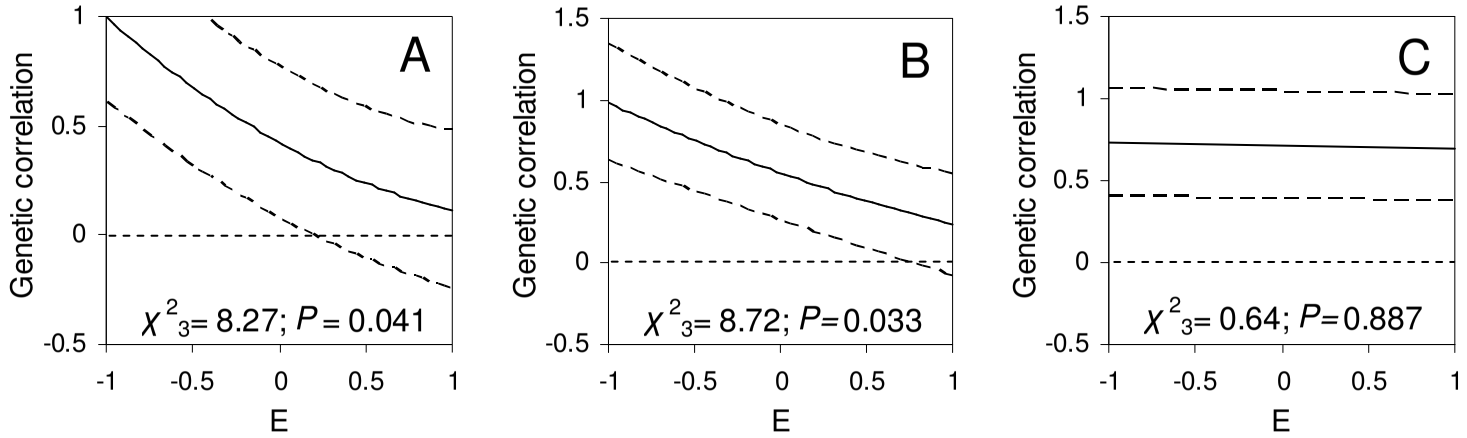


Figure 6.10. Genetic correlations in (A) horn length; (B) body weight; (C) parasite resistance between males and females averaged across ages, as a function of the environmental conditions experienced during first year of life (*E*). Results were gained from model 4 (see methods) where the genetic correlations were estimated as linear functions of *E* and are shown by solid lines, with dashed lines indicating the 95% confidence intervals of the estimates. The significance of the trends shown was assessed using log-likelihood-ratio tests, against a null hypothesis of no change in covariance over *E*.



6.6 DISCUSSION

I have shown that genetic relationships between traits both within and across the sexes can differ depending upon the environmental conditions an individual experiences during early development, supporting previous work which was shown that **G** has the potential to vary across environments (Roff 2002, Sgrò and Hoffmann 2004, Steppan et al. 2002). The environmental conditions experienced during development resulted in genotype-by-environment interactions for both weight and parasite load in first year males. As a result, genetic correlations between traits expressed over life, both within and between the sexes, were also dependent upon the environmental conditions experienced during their development. By assessing the relationships between traits as a function of the environmental conditions in which they were expressed, I was able to show that if first year conditions were relatively poor there was a positive genetic relationship between all traits. However, if first year conditions were relatively good, there was no evidence of significant genetic correlations between horn length and other traits both within males and across the sexes. If relationships between traits had been tested only in the average environment, I would have concluded that highly positive genetic correlations would constrain the evolution of the sexually dimorphic traits of weight and horn length within this population (Coltman et al. 2001) and supported previous studies which have suggested genetic constraint on sexually dimorphic traits (Badyaev 2002, Jensen et al. 2003). My results suggest that in good environments, loci are expressed which have sex specific effects and as a result if environmental conditions on St.Kilda were consistently good, I can predict that both horn length and body weight may be free to move along independent evolutionary trajectories in males. This study highlights the fact that evolutionary processes can only be fully understood with both phenotypic and genetic data (Roff 2002) and that these processes may depend largely upon ecological conditions.

The environment can have a direct influence on quantitative genetic parameters (Hoffmann and Merilä 1999, Hoffmann and Parsons 1991) and numerous studies have shown that the environment can directly influence the genetic determination of

a suite of traits (Charmantier and Garant 2005, Hoffmann and Merilä 1999, Nussey et al. 2007, Via and Lande 1985). In this study, additive genetic variance of both male first year body weight and parasite load increased with increasingly favourable environmental conditions, supporting previous studies of natural populations (Charmantier and Garant 2005, Garant et al. 2004, Merilä 1997). There was no evidence of any change in additive genetic variance of horn length with environment, suggesting that genetic effects are not influenced by the environmental conditions experienced by individuals. Genotype-by-environment interactions can result from genes which have environment specific expression, with will result in low genetic correlations between environments, or from environmentally sensitive allelic effects, which results in genetic correlations which remain close to one across environments (Schlichting and Pigliucci 1993). In this study the genetic correlation between environments for first year male body weight was low, suggesting environment specific expression, where as the genetic correlation for parasite load was high across environments, suggesting environmentally sensitive allelic effects. These patterns did not persist when we considered the repeated measures of traits over the lifespan of individuals. Individuals may experience fluctuating conditions throughout their life and may survive until different ages and therefore patterns of genetic expression for single traits may not be solely associated with birth year environments. Further extension of these models to allow genetic effects to (co)vary as a function of both age and environment may shed more light on this issue.

Genotype-by-environment interactions early in life resulted in changing genetic correlations between male traits across first year environmental conditions, which persisted over the lifespan of individuals. Previous studies have shown that genetic correlations may be influenced by environmental conditions (Sgrò and Hoffmann 2004). Genetic correlations between traits have been shown to change as a function of temperature (Norry and Loeschcke 2002); stress (Stinchcombe 2002); novel environments (Cano et al. 2004, Simmons and Roff 1996); and generally between two populations experiencing different ecological conditions (Begin and Roff 2001, Roff et al. 2004). However, these estimates may have little relevance to natural populations (Hoffmann and Merilä, 1999, Roff, 2002, Sgrò and Hoffmann, 2004)

and correlations have been shown to change between field and laboratory conditions (Conner et al. 2003, Simmons and Roff 1996). As a result, little is known about the stability of the genetic variance-covariance matrix **G** within natural populations under temporally fluctuating environmental conditions (Garant et al. 2008). It remains to be seen whether the patterns of reduced genetic correlations between traits under favourable environmental conditions is a general trend in the wild.

Previous studies on this population have found significant additive genetic variance for parasite load (Smith et al. 1999; Coltman et al. 2001) and for morphometric traits (Milner et al. 2000) in the average environment. Although our variance component estimates were gained from a larger data set, they are similar to those described by Coltman et al. (2001). Furthermore, we found negative genetic correlations between body weight and parasite load in the average environment, in both sexes also supporting previous results and suggesting that individuals with genetically low parasite burdens are likely to experience superior growth, although our estimates and had larger standard errors than those previously obtained (Coltman et al. 2001). This may reflect the larger dataset used here and a different method of analysis which included a permanent environment effect within the model (see Kruuk and Hadfield 2007). I found that first year male horn length was positively associated with parasite load in poor environments implying a genetic trade-off between the two traits and may help to explain the negative association between horn growth and survival previously reported in previous chapters.

I found evidence for sex differences in the genetic architecture of traits which we examined. Currently, very few studies have demonstrated sex-differences in the genetic architecture of sexually dimorphic traits (Jensen et al. 2003, Roff 2002), with many studies concluding high genetic constraint between the sexes (Coltman et al. 2001, Merilä et al. 1998, Parker and Garant 2004). In females, there was no evidence of a genetic correlation between horn length and any other trait, while in males horn length was correlated with both body weight over life and parasite load within the first year of life, in poor environments. Evidence of cross-sex genetic correlations for sexually dimorphic traits is surprisingly rare in the wild (Coltman et al. 2001, Jensen

et al. 2003, Merilä et al. 1998, Parker and Garant 2004, Poissant et al. in press) and no previous study has examined the effects of environmental heterogeneity on these correlations. Genetic correlations between the sexes in horn length and weight decreased as environmental conditions improved, indicating reduced potential for genetic constraint between the sexes under these conditions. In contrast, the cross-sex genetic correlation in parasite resistance remained constant and close to one, suggesting no differences in genetic expression between males and females for parasite resistance. These results suggest that in order to understand the evolution of sexually dimorphic traits we must consider all environments in which they are expressed.

This is the first evidence that in a wild population, genetic covariances between a suite of traits may not be stable under temporally fluctuating environmental conditions. We need to consider the effects of genotype-by-environment interactions at all stages of analysis: from single traits, to multivariate phenotypes, to sexual antagonism studies. This chapter demonstrates the complexities of the relationships between trait both within and between the sexes and provides a way in which these relationships can be modelled for populations experiencing temporally fluctuating environmental conditions.

CHAPTER 7

DISCUSSION

In this thesis I have demonstrated that variation in both the form and size of horns in a feral population of Soay sheep can be created and potentially maintained by numerous factors. In this final chapter, I will first discuss selection on horn phenotype, and then go on to discuss the potential factors which maintain variance in normal-horn male horn size. Selection acts upon the both the form and size of horns in different ways depending upon the sex in which the trait is expressed, generating sexually antagonistic selection which could potentially maintain variance (Chapter two). Horns also have different behavioural functions in males and females, with males using horns to compete for reproductive opportunities and females using horns to compete for resources (Chapter three). Selection pressures on the horn size of normal-horned males are dependent upon environmental conditions, with the genetic covariance between horn length and fitness changing as a function of the environment, meaning that the evolution of horn growth may be constrained as no single genotype has the highest fitness across all environments (Chapter five). A large component of the variation in normal-horned male horn growth may be created by fluctuating environmental conditions and individual differences in their environmental sensitivity (Chapter four). Fluctuating environmental conditions also alter associations between components of phenotype, with the underlying genetic architecture of phenotype varying as a result of GEI for many traits (Chapter six). While the implications of each of these specific findings has been detailed in the previous chapters, I will provide a brief overview of how this work has contributed to our understanding of the maintenance of variance in this trait and more generally, variation in the phenotypes expressed by individuals in the natural environment.

7.1. SELECTION ON HORN PHENOTYPE AND ITS EFFECTS ON TRAIT VARIATION

Overall, I found no difference in either sex in the lifetime fitness of scurred and normal-horned individuals and where selection did act through fecundity or viability it was sexually antagonistic in direction, with both potentially maintaining the observed polymorphism within this population (Chapter two). Normal-horned males have higher fecundity at maturity as compared to scurred males, but this is opposed by lower survival presumably due to the costs associated with their competitive reproductive strategy, supporting the findings of many studies which have the survival costs of sexual selection through male competition (e.g. Höglund and Sheldon 1998; Kokko et al. 2002; Hunt et al. 2004). Normal-horned females on the other hand, show reduced fecundity as compared to scurred females, but on average no reduction in survival. I suggest that reduced fecundity in normal-horned females may be the result of some form of genetic linkage with other traits which reduce fecundity, and similarly in polled females there may be linkage between the horn phenotype they display and genes which influence longevity. Potentially, normal-horned females may be able to balance a reduction in fecundity by an increased ability to provide resources to their offspring (Chapter three), and this may contribute to the finding of equal fitness in this group as compared to scurred females. Increasingly, there are examples of the female expression of a male sexually-selected trait, providing some advantage to females in competition for resources (Amundsen 2000) and this is supported in this population. Further work to assess the genetic basis of this polymorphism will enable us to fully understand the factors which maintain it, as demonstrated by recent work describing the genetic basis of the coat colour polymorphism in this population (Gratten et al. 2008).

Selection also acts on the horn size of both normal-horned males and females. In normal-horned females, horn length is negatively associated with survival (Chapter two) and there is no evidence that it conveys any reproductive benefits (Chapter two and three). In normal-horned males, the relationship between horn length and fitness depends upon the environmental conditions into which an individual is born (Chapter five) and therefore selection through lifetime fitness on this sexually-selected trait,

represents a balance between a negative association with viability, and its positive effects on fecundity through the function of horns in competition for mating opportunities. Many studies have suggested that allocation to a sexually-selected trait represents a balance between viability and fecundity (Stearns 1989; Roff 1997, Höglund and Sheldon 1998), but few studies (Brooks 2000; Hunt et al. 2004) have shown that individuals of high genetic merit for trait production are those which are more likely to suffer a viability cost. While selection pressures in normal-horned males and females are on average antagonistic (Chapter two), I argue that negative selection on female horn length will mirror that in males born in poor environments, which coupled with a high cross-sex genetic correlation in poor environments (Chapter six), would lead to a reduction in horn length if environmental conditions on St. Kilda worsen over time. An improvement in environmental conditions may result in increased sexual antagonism (Chapters two and five) but as cross sex genetic correlations appear to reduce (Chapter six), separate evolutionary trajectories across the sexes may not be constrained (Lande 1980).

If a normal-horned male is born in a good environment, it is likely that that individual will survive until adulthood where selection acts positively on larger horn size (Chapter five). While a very large proportion of male mating success in a given year may be attributed to a few older dominant males with large horn length (Preston et al. 2001, 2003; Clutton-Brock and Pemberton 2004), the fitness of their offspring may be largely determined by the environment they are born into and their sex. Therefore it seems likely that selection on horn length in normal-horned males is zero in the average environment, and potentially stabilising across the environmental conditions experienced by the population so far. In a population of Darwin's finches, the apparent direction of selection has changed over the time in which the study has been conducted and thus the number of different environments sampled (Schluter et al. 1985; Grant and Grant 2002) and this may be the case in this population. Stabilising selection may be the most common form of non-linear selection for many traits (Kingsolver et al. 2001) and is likely to reduce additive genetic variance (Falconer and Mackay 1996). So the question therefore remains as to what maintains the variance in horn length that we observe in this population?

7.2. MAINTENANCE OF TRAIT VARIATION

7.2.1. Environmental heterogeneity

The effects of fluctuating environmental conditions may be a large component of the variance in many secondary sexual traits, through their effects on resource availability (e.g. Griffith et al. 1999; Kruuk et al. 2002; Garant et al. 2004). Environmental conditions in a given year influenced allocation to horn growth in normal-horned males creating variation in horn length (Chapter four). The extent of these effects on variation in horn growth was dependent upon an individual's environmental sensitivity, which in turn was influenced by the condition of an individual prior to growth (Chapter four). Individual plasticity has been demonstrated for many traits expressed in wild populations (e.g. Brommer et al. 2003, 2005, 2008; Nussey et al. 2005a, 2005b; Reed at al. 2006) and an individual's allocation to horn growth in response to the environment in a given year may also be plastic. Selection on horn length later in life may therefore select largely upon chance differences between individuals in the environments that they encounter throughout their lives (Griffith et al. 1999).

The genetic expression of many components of Soay sheep phenotype and the genetic relationships between these components were also dependent upon the environmental conditions that individuals experienced during development. In normal-horned males, there was no evidence that individuals of a given genotype differ the phenotype of their first year horn growth across different environments (Chapters four, five and six), and thus it appears that normal-horned males are unable to adjust their horn length to 'suit' the environment they experience (Chapter five). However, the environmental conditions experienced normal-horned males during development resulted in genotype-by-environment interactions for both first year body weight and parasite resistance and as a result, genetic correlations between traits expressed over life, both within normal-horned males and between normal-horned males and females, were also dependent upon the environmental conditions

experienced during their development. This supports previous work which suggests that **G** has the potential to vary across environments (Roff 2002, Sgrò and Hoffmann 2004, Steppan et al. 2002). However, it remains to be seen whether the trends shown here, of reduced correlations between phenotypic components, commonly occurs in the wild. In good environments, components of phenotype in both males and females may be unconstrained from reaching different selective optima and the low genetic correlation for body weight between environments should maintain additive genetic variance.

7.2.2. Genic capture

Genetic variance in a trait may be observed if it is genetically correlated with another trait of high variance. This theory forms the basis of the genic capture hypothesis, which predicts genetic variance should be observed if a trait reflects variation in an individual's underlying 'condition' (Houle1992; Rowe and Houle 1996). An individual's condition reflects resistance to disease, growth, and ability to convert resources into stored nutrients and thus it will have a large mutational target as it is influenced by many loci and (Rowe and Houle 1992). In normal-horned males, I found positive genetic correlations between body weight and parasite resistance, suggesting a link between horn growth and condition, which coupled with the GEI for fitness and body weight should maintain the additive genetic variance in horn growth that we observe as there is not always a single fittest genotype.

7.3. SUMMARY

The results of this thesis demonstrate that both selective pressures and the variation upon which selection acts is determined by many factors, whose effects vary in relation to an individuals age and sex, the environments that individuals experience and the population dynamics of which they are a part. Only by examining the effects of sex differences and the environment on genetic expression, genetic associations, and selection pressures will we be able to fully understand the facts which maintain the phenotypic diversity observed in the natural world.

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