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The Evolutionary Consequences of Parental Effects

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Abstract

Parents modulate the phenotypes of their offspring, beyond the effects of the genes they pass on. These parental effects can have impacts on the fitnesses of those offspring, as well as the fitness of the parents themselves. Parental investment in offspring is expected to be under antagonistic selection through its beneficial effects to offspring, and its detrimental effects on the parent's own fitness. Evolutionary conflict over parental care is therefore expected to occur, and may cause evolutionary stasis. Furthermore, selection is also expected to act on offspring traits, in order to maximise offspring fitness within a given parental environment, generating predictions of parent-offspring coadaptation.

I tested the predictions of conflict and coadaptation in parent-offspring interactions, using a population of blue tits (*Cyanistes caeruleus*), a species in which adults provide biparental care to their offspring. I found evolutionary conflict over offspring body mass, which may explain stasis in this trait. I also used a cross-fostering design to test for coadaptation between parents and offspring, and siblings. I did not find evidence for parent-offspring coadaptation, nor did I find that siblings were important through either direct interactions, or in mediating parent-offspring interactions, suggesting that there is little family coadaptation in this species. In addition, I investigated whether a maternal effect on hatching time was a passive consequence of environmental changes, or was an anticipatory maternal effect actively placed in eggs to manipulate hatching time. The results from this analysis suggest the latter to be the case, and mothers appear to actively manipulate offspring hatching time to reduce the extent of hatching asynchrony, which may reduce fitness costs to the offspring.

By measuring the effects of interactions between individuals on phenotypes and fitness measures, I was able to show how parental effects on offspring can affect evolutionary dynamics. Such evidence of evolutionary conflicts has not previously been found, due to methodological issues with the ways in which selection has been measured. Thus, I highlight how gaps in knowledge about the evolutionary consequences of parental effects can be addressed using appropriate statistical tools and measures of fitness.

Signed Declaration

I declare that this thesis, *The Evolutionary Consequences of Parental Effects*, and the work presented in it are my own. This work was done wholly while in candidature for a research degree at the University of Oxford. Where I have consulted or paraphrased the published work of others, this is always clearly attributed. I have acknowledged all main sources of help and assistance I have received. I agree to retain an electronic copy of this work until the publication of my final examination result, and to make this available to the examiners if necessary. This thesis is composed of submitted papers (or to be submitted). The contribution of authors to these manuscripts is stated below.

Chapter 2 Submitted to *The Journal of Evolutionary Biology* as: *Thomson, C.E. & Hadfield, J.D. Prenatal maternal effects appear to be insensitive to experimental or natural environmental variation.* I designed the experiment, carried out the field work and statistical analyses and wrote the manuscript. J Hadfield helped design the experiment, designed the statistical analysis, and contributed to writing the manuscript.

Chapter 3 Submitted to *Evolution* as: *Thomson, C.E., Bayer F., Crouch N., Farrell S., Heap E., Mittell E., Zurita M., & Hadfield, J.D. Selection on parental performance opposes selection for larger body size in a wild population of blue tits.* I carried out field work, planned and carried out the statistical analyses and wrote the manuscript. J Hadfield designed the statistical analysis, and contributed to writing the manuscript. All other authors were involved in field work and/or laboratory work.

Chapter 4 Will be submitted after further data has been obtained: *Thomson, C.E. & Hadfield, J.D. The importance of siblings in parent-offspring coadaptation.* I designed the cross-fostering experiment, carried out the field work, carried out the statistical analyses and wrote the manuscript. J Hadfield assisted with experimental design and analysis, and contributed to writing the manuscript.

_____ _____

Signature Date

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Chapter 1

Introduction

Parental effects, the modulation of offspring phenotypes by parents beyond genetic inheritance, are found throughout nature. Whether through changes in the quantities of resources and signals placed in eggs/seeds, or through long-term parental care, these parental effects can have important impacts upon the phenotypes, and even fitnesses, of offspring. Parental effects are therefore likely to be subject to selection through their effects on offspring fitness. However, where these effects are costly to parents, opposing selection may exist. Thus, the evolutionary dynamics of such parental effects are difficult to predict. In this thesis I use data collected from a population of blue tits (*Cyanistes caeruleus*) to address how parental investment in offspring is expected to generate evolutionary stasis, and coadaptation between traits, through the effect of parental effects on the fitness of parents and offspring. In addition, I examine the proximate mechanisms by which a maternal effect on offspring hatching time is mediated in order to evaluate whether it is likely to be an active process in parents in anticipation of future conditions, or a passive by-product of current changing environmental conditions.

1.1 Parental effects

A parental effect is defined as any causal effect of a parent on offspring phenotypes, beyond the genes it passes on (Roach & Wulff, 1987; Mousseau & Fox, 1998; Wolf & Wade, 2009). Whilst some of these parental effects act to increase offspring fitness (termed adaptive maternal effects; Marshall & Uller, 2007), akin to definitions of parental care described below, others may have a neutral or harmful effect on offspring fitness.

Although the term "parental effects" incorporates the phenotypic effects of both male and female parents, maternal effects have historically garnered greater attention. In mammals, fathers generally have less interaction with offspring than females, and so have less scope for expressing traits that generate paternal effects. Addition-

ally, in egg-laying species prenatal effects in eggs are assumed to be maternally derived (e.g. Fox & Mousseau, 1996; Plaistow *et al.*, 2007; Krist, 2011)). However, various paternal effects on offspring phenotypes also exist (Crean & Bonduriansky, 2014). Where males provide postnatal parental care to their offspring, the amount of care is likely to affect offspring phenotypes in a similar way to maternal postnatal care. For example, Buchanan & Catchpole (2000) found that chick mass in sedge warblers (*Acrocephalus schoenobaenus*) was positively correlated with male provisioning rate, demonstrating the effects that males can have on offspring phenotypes. Thus, although less work has been done on paternal effects, I consider both paternal and maternal effects on offspring.

Within this thesis I distinguish between two types of parental effects that affect offspring fitness – direct and indirect. *Direct* parental effects on offspring fitness describe situations in which the parental phenotype directly impacts upon the fitness of the offspring, whereas *indirect* parental effects on offspring fitness are classified as cases where the parents affect some aspect of offspring phenotype, which in turn affects offspring fitness (as with the majority of parental care). I focus on indirect parental effects on offspring fitness. Indeed, although direct and indirect parental effects on offspring fitness have been modelled differently with regards to how they may affect evolutionary change (Willham, 1963, 1972; Cheverud, 1984; Kirkpatrick & Lande, 1992; Hadfield, 2012), many direct effects on offspring fitness may be artefacts from missing traits – they may instead represent indirect effects, but the mediating trait in the offspring that they affect has not been measured. For example, McAdam & Boutin (2003) measured selection on birth date and litter size (maternal traits) through their effects on juvenile survival in red squirrels (*Tamiasciurus hudsonicus*). These effects on offspring fitness is likely to be mediated indirectly, however, through the effect of these traits on offspring growth rate and body mass, and the correlation of those offspring traits with fitness.

Anticipatory maternal effects are a specific class of adaptive maternal effects,

wherein mothers adjust offspring phenotype in anticipation of the conditions that they will encounter (Marshall & Uller, 2007). These are likely represent indirect effects on offspring fitness, as they generally will alter offspring phenotypes and thereby influencing their fitness. There is limited evidence for such effects (Uller *et al.*, 2013), although there are some examples. Fox *et al.* (1997) show that in seed beetles (*Stator limbatus*) mothers adjust egg size in response to the species on which they are laying their eggs, a variable that affects offspring ability to penetrate the seed coats of the different species. In addition, Galloway & Etterson (2007) showed anticipatory maternal effects in response to light environment experienced by the plant *Campanulastrum americanum*, such that there was higher fitness in those offspring raised in an environment that matched the maternal light environment.

In Chapter 2 I investigate the potential for anticipatory maternal effects on offspring hatching time in blue tits. Accelerated development of late laid eggs was found in Hadfield *et al.* (2013b), reducing the extent of hatching asynchrony. This could be mediated through an anticipatory maternal effect, such that females are anticipating the competitive environment into which offspring are likely to hatch, and modulating their phenotype accordingly. Such modulation in anticipation of sibling competition is seen in Plaistow *et al.* (2007), wherein soil mites (*Sansassania berlesei*) lay larger eggs as they age, which may reflect anticipation of increased sibling competition to later laid offspring.

1.2 Parental Care

Parental care and investment are specific types of parental effects, that can be considered to be adaptive parental effects (parental effects that increase offspring fitness; Marshall & Uller, 2007). Trivers (1972) defines parental investment as *any investment by the parent in an individual offspring that increases the offspring's chance of surviving, at the cost of the parent's ability to invest in other offspring*, mediated ei-

ther through reduced survival, reduced fecundity, or reduced care of future offspring. Clutton-Brock (1991) makes a distinction between parental *investment*, as defined above, and parental *care* – care increases the fitness of offspring, but does not necessarily bear a cost to the parent. These two terms are often used synonymously, and indeed the majority of parental care behaviours are likely to come under the definition of investment. Thus, in this thesis I focus on parental care traits that are also considered to be investment.

As described above (Section 1.1), investment can affect offspring survival directly or indirectly. For example, with direct parental investment in offspring fitness, the defence of young against predators increases offspring survival, but is also expected to increase parental mortality risk (Andersson *et al.*, 1980). Similarly, egg incubation or brooding is often necessary for embryonic development, but may reduce parental foraging ability (as in mouth brooding cichlids: Yanagisawa & Sato, 1990; Yanagisawa & Ochi, 1991), and increase predation risk (and therefore decrease survival, e.g. Logerwell & Ohman, 1999; Reguera & Gomendio, 1999; Li & Jackson, 2003).

Herein, I focus primarily on indirect parental investment in offspring fitness, wherein parents affect the phenotype of their offspring, and in doing so influence their fitness. For example, parental provisioning affects offspring growth and body mass, which in turn affects survival probabilities. This can include the effects of prenatal maternal provisioning. In oviparous taxa larger eggs are more likely to hatch (Guisande & Harris, 1995; Krist, 2011) and the offspring are more likely to survive post-hatching (Kraeuter *et al.*, 1981; Springate & Bromage, 1985; Christians, 2002; Krist, 2011), which is probably mediated through the positive effect of egg size on offspring size (shown in Einum & Fleming, 2000; Krist, 2011). Similarly, prenatal provisioning within the womb affects birth mass, and subsequent survival of young (e.g. Jones *et al.*, 2005). Post-natal care is also found in many taxa (see overviews in Balshine, 2012; Trumbo, 2012), and this can be considered either facultative (optional) or obligate (necessary). In the case of facultative care, although

this behaviour increases offspring fitness, young may still survive without care. In burying beetles (*Nicrophorus vespilloides*), for example, parental care increases offspring mass gain, and thereby influences pre-dispersal mass and survival (Eggert *et al.*, 1998). Without this care larvae are still able to obtain food, and although survival is reduced, some offspring still survive. In contrast, postnatal care in the majority of avian and mammalian species is obligate – young will not survive without care. However, even within this category, there is substantial variation between and within species.

1.3 Conflict over Parental Care

The definition of parental investment given by Trivers (1972), sets out the framework in which parental care has subsequently been studied; whilst parental investment provides benefits to individual offspring (either through directly increasing offspring fitness, or by affecting a trait that then affects offspring fitness), it generates a cost to the parent, and thus a trade-off between the fitness of individuals is predicted.

Although multiple definitions of fitness have been used in the context of such studies, here I use the quantitative genetic definition of fitness (i.e. direct fitness) – the total fitness of an individual (i.e. lifetime reproductive success) is the number of zygotes it produces over its lifetime (Arnold & Wade, 1984). However, the effects of parental traits on offspring have also previously been considered using *inclusive* fitness and *weighted* fitness. Inclusive fitness is defined as the sum of the direct fitness and the indirect fitness of an individual, where indirect fitness is the effect a focal individual has on the fitness of others, thus attributing only the fitness caused by the focal individual to that focal individual's indirect fitness, weighted by the relatedness of those that are affected to the focal individual (Hamilton, 1963, 1964; Grafen, 1984). Weighted fitness, on the other hand, is an erroneous measure of fitness that resembles inclusive fitness. This is measured as the total fitness of relatives that

the focal individual has affected, weighted by their relatedness to the focal individual, thus attributing *all* fitness to the focal individual, without consideration of the actual effects that focal individual has on others (Grafen, 1982; Hadfield, 2012). I discuss the problems with such measures of fitness in the discussion (Chapter 5, Section 5.1).

The conflict over parental care can be considered from either inclusive fitness or direct fitness perspectives. In inclusive fitness theory, care increases the fitness of other individuals (i.e. indirect fitness benefits), whilst decreasing the actors own fitness. The benefits of care are weighted by relatedness, and so individuals should favour their own fitness over that of others (as each individual is more related to itself than to siblings/offspring etc.), and thus there is conflict between the benefits and costs of parental care through its effects on inclusive fitness. Where conflict is considered in the context of direct fitness, providing care to offspring reduces and individuals own fitness, and thus is selected against. However, if that care then affects a trait in offspring, which is itself positively correlated with fitness, that offspring trait will be selected for, and so conflict will arise over parental care, mediated by the effect of care on the offspring trait. I focus on the latter perspective in this thesis, but both predict that conflict over parental care can be generated at several levels (Parker *et al.*, 2002), as illustrated in Figure 1.1. Conflict between parent(s) and offspring over optimal levels of parental invesment is expected (Arrows A in Figure 1.1; Section 1.3.1), as is conflict between siblings (Arrows B and C; Section 1.3.3).

Sexual conflict is also predicted between male and female parents (Arrow D in Figure 1.1). A focal parent benefits from the care provided by the other, as this reduces the care the focal individual has to provide, whereas they must provide more care if the other parent provides less, which consequently has costs to their own fitness (Lessells, 1999; Parker *et al.*, 2002; Lessells, 2012). This can manifest at many points through reproductive attempts, driven by differences in parental optima over investment. This conflict is not considered within this thesis, but may

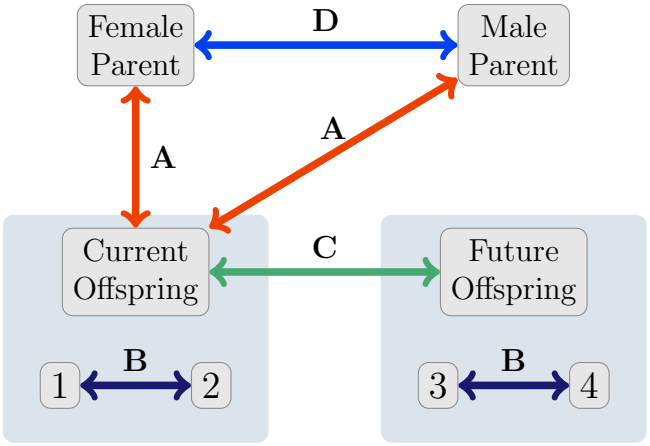


Figure 1.1: Diagram demonstrating the conflicts over parental investment (simplified case of two offspring per breeding attempt). Arrows **A** indicate conflict between parents and their *current* offspring; **B** sibling conflict within the same reproductive attempt; **C** conflict between *current* and *future* offspring; **D** Conflict between parents over investment.

have important consequences on evolutionary dynamics in parental care and conflict.

1.3.1 Parent-offspring conflict

Conflicts between parents and their offspring (Arrows A in Figure 1.1) are expected to arise due to differences in optimal levels of care for each party. Due to the indirect effects of parental care on offspring fitness, offspring are expected to demand more care (and for a longer duration) than parents are willing to provide, as this increases their own fitness (Trivers, 1974; Godfray, 1995) . However, increasing investment in a given reproductive attempt may reduce parental resources for their own somatic maintenance, survival, and future reproduction. Alonso-Alvarez & Velando (2012) show parental care to have numerous physiological costs, including somatic costs, such as oxidative stress and immunosupression. In addition, Merilä & Hemborg (2000) demonstrated an association between brood size manipulation (assumed to manipulate parental care), and a correlate of adult survival (feather wear) in collared flycatchers, *Ficedula albicollis*. Thus, parents appear to pay a cost when parental care is increased.

This conflict between parents and their offspring may result in behavioural disputes over parental investment. Begging behaviour, for example, is used by offspring to solicit care from parents (Kilner & Johnstone, 1997), and is thought to be a signal of offspring need. However, offspring may beg at a higher rate than parents are willing to provision food, and so conflict occurs over the amount of food provided through these behaviours. Gestational diabetes in humans has also been proposed to be a consequence of parent-offspring conflict (Salmon & Malcolm, 2011) – hormones produced by the placenta allow greater glucose uptake by a developing foetus than may be in the mother's interests, by blocking insulin receptivity. Such proximate (including behavioural) conflicts, however, are not sufficient to show the evolutionary conflict predicted by Trivers (1972), as these do not demonstrate that there is an underlying evolutionary conflict in the effects of parental investment on the fitness of both parties (Mock & Forbes, 1992; Kilner & Hinde, 2012).

Kilner & Hinde (2012) highlight two ways in which an evolutionary conflict over parental investment might be detected: (a) optimal parental investment must differ between parents and offspring, i.e. the direct fitness of the two parties must be maximised at different trait values, and (b) a change in the investment level in favour of one party leads to a concomitant decline in the fitness of the other. In the first case, quantifying selection upon parental traits through their effects on parental fitness, and selection upon the offspring traits they affect, allows the quantification of this conflict, with antagonistic selection expected under these predictions.

Such antagonistic selection over parental investment has not previously been demonstrated conclusively, however, and this is likely to be due to problems with the assignments of fitness to individuals. Quantitative genetic models define total fitness as the number of zygotes an individual produces over their lifetime, from the point at which they were a zygote (Arnold & Wade, 1984). This can be split over different life-history components, such as survival to breeding, annual survival as an adult, or annual fecundity. In order to detect antagonistic selection, such measures

of fitness must be used. Many studies, however, measure the fecundity of parents as the number of offspring surviving to a certain point, thereby incorporating the fitness of two generations (*weighted* fitness; Grafen, 1982; Hadfield, 2012). This is incompatible with the quantitative genetic approach, as it conflates selection and inheritance (Wolf *et al.*, 2001), and is likely to mask the selection through pure parental fitness measures. For example, Einum & Fleming (2000) measured selection on egg size in salmon, and found that there was positive directional selection on egg size through the effect on offspring fitness – increased survival from larger eggs. They also measured selection on this trait through the effects on maternal reproductive success, but measured this fitness as the number of eggs multiplied by the subsequent survival of those eggs. The comparison of the two selection measures then becomes restricted, as they both incorporate offspring survival. Similarly, Wilson *et al.* (2005b) attempt to show antagonistic selection acting upon litter size in soay sheep – offspring fitness is negatively affected by being a twin (through reduced body weight), and so twinning is selected against through this effect. They then show a positive selection gradient through the effects on maternal fitness, but again take this fitness measure to include offspring survival.

When present, however, antagonistic selection between parents and offspring may be particularly important in affecting evolutionary dynamics. The paradox of stasis is a common problem in evolutionary biology, wherein directional selection and heritable genetic variation have been detected in natural populations, but no micro-evolutionary change (i.e. change in allele frequencies within populations) has subsequently been observed (Merila *et al.*, 2001). If selection on parentally affected offspring traits that increase in offspring fitness is opposed by the reduction in parental fitness when they provide increased investment in offspring, evolutionary change will be restricted. Conversely, selection upon a trait to increase parental fitness may be opposed by its antagonistic effects on the offspring. Thus, stasis in the trait in spite of directional selection might be expected. This stasis is akin to

the "resolution of conflict" models (Godfray, 1995), wherein an evolutionarily stable strategy is achieved between parents and offspring that is equivalent to the mean trait values at which antagonistic selection leads to stasis.

In Chapter 3 I estimate selection acting upon parents and offspring, measuring fitness as the number of zygotes produced, which is appropriate for quantitative genetic models. Using these, I consider whether stasis in body size may be driven by antagonistic selection. There is generally positive directional selection on this trait (Kingsolver & Diamond, 2011), but Rollinson & Rowe (2015) show that this selection for increased body size is generally found in juveniles, rather than adults. Where parental investment is a large determinant of body size, opposing selection upon parental investment has the potential to restrict evolutionary change, and therefore to explain stasis.

1.3.2 Parent-Offspring Coadaptation

Parent-offspring conflict predicts that there will be opposing selection upon traits related to parental investment, through the effects of these on parental and offspring fitnesses. However, we can also expect that selection should act to maximise offspring fitness within the context of the (heritable) parental environments that they experience, thereby generating parent-offspring coadaptation. This may act to reduce the magnitude of conflict between parents and offspring, with a reduction in the strength of selection acting through offspring fitness. Equivalently, we might expect similar predictions of coadaptation where there are heritable offspring effects on parental fitness, although these are less widely considered.

Thus, although we predict evolutionary conflict between parents and offspring, the joint influence of parental and offspring traits on offspring fitness also results in predictions of genetic coadaptation. Conversely, if heritable offspring effects (on parents) exist, we may also effect coadaptation to be predicted through the joint

influence of offspring and parents on parental fitness. This epistasis for inclusive fitness is expected to drive the evolution of functional integration of these traits (Wolf & Brodie, 1998), such that they are in linkage disequilibrium. Parental traits may drive the evolution of offspring genotypes, such that offspring fitness is maximised for a given parental environment. In turn, parental alleles will become associated with the offspring alleles adapted to it (and vice versa for the effects on parents). Thus, a genetic correlation between traits is expected to occur, although the direction of this correlation depends on the form of selection (Kölliker *et al.*, 2005). This has generally been considered within the context of offspring begging and parental provisioning, and some evidence for positive genetic correlations between these have been found (Kölliker *et al.*, 2000; Lock *et al.*, 2004; Hinde *et al.*, 2009), although no correlation was found in other studies (Estramil *et al.*, 2013, 2014b). Where this coadaptation (and genetic correlation) does exist, this should result in multiple combinations of parental and offspring traits that produce equal fitness (Wolf & Brodie, 1998; Kölliker *et al.*, 2005), such that there should be a ridge of highest offspring fitnesses (see Figure 1.2). Any deviations from these trait combinations will reduce offspring and/or parental fitness, and thus be selected against.

Whilst genetic correlations are necessary to generate coadaptation, they are not sufficient. Fitness effects of these parental and offspring traits are also necessary. These can be tested by using cross-fostering experiments, with the prediction that experimentally moving offspring between nests should cause a phenotypic mismatch between parental and offspring traits, and thus reduce the fitness of one (or both) parties. Positive evidence of coadaptation through effects on offspring fitness was found by Hinde *et al.* (2010), Lock *et al.* (2004) and Hager & Johnstone (2005), but not by Smith & Wettermark (1995), Merilä *et al.* (1999), or Brown *et al.* (2015), and no effect on parental fitness was found in Hinde *et al.* (2010), thus whether coadaptation exists is far from clear. This may be driven by the fact that cross-fostering studies are often varied in methodology, including when offspring are crossed, and

what proportion of the brood are cross-fostered. In addition, they often lack appropriate controls, making it difficult to compare the fitnesses of different classes of individuals. Thus in Chapter 4 I use a cross-fostering design that allows the comparison of the fitnesses of individuals in different family structures, including control (uncrossed) nests.

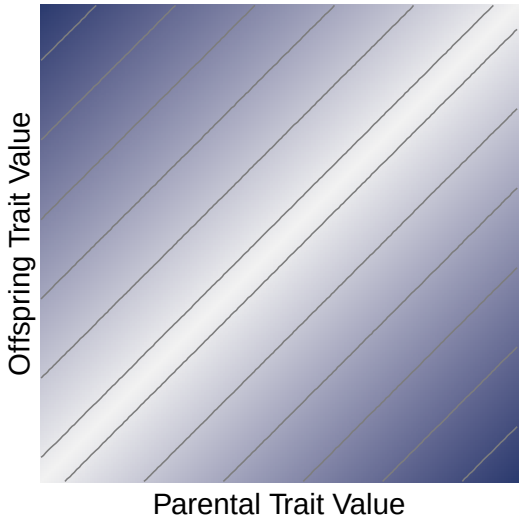


Figure 1.2: A fitness ridge predicted between coadapted offspring and parent traits. Offspring fitness is indicated through the colour in the plot – lighter colours have highest fitness, whilst dark blue indicates a very low fitness. Multiple trait combinations generate the highest fitnesses, but deviation from these result in reduced fitness. Thus, positive genetic correlations between the offspring and parental traits will generate coadaptation

1.3.3 Sibling Conflict and Coadaptation

Where siblings interact with one another, they are expected to be in conflict for access to resources. Each individual gains direct fitness benefits from obtaining more resources for itself, and direct fitness costs when those resources are shared (Mock & Parker, 1997). Thus conflict should occur between siblings, at the intra-brood (Arrow B in Figure 1.1), and inter-brood levels (Arrow C in Figure 1.1). Selfish behaviour is expected to be selected for, where each offspring should demand more than an equal portion of parental resources (Mock & Parker, 1997). This

can occur even in the absence of parents, such as where insect larvae may interact on host plants or animals, and so may be more widespread than parent-offspring conflict. Selfish behaviour of individuals is seen in the extreme in obligately siblicidal species, where dominant offspring routinely kill their siblings, thus claiming parental resources for themselves (as seen in masked boobies, *Sula dactylatra*; Anderson, 1990). Some species may also show sibling cannibalism when food is scarce, such as in the harlequin ladybird (*Harmonia axiridis*; Osawa, 1989). In less extreme cases, this selfishness manifests as competition for parental resources, such as for position within the nest in birds (shown in starlings and great tits: Kacelnik *et al.*, 1995; Kölliker & Richner, 2004), or defence of teats (e.g. in pigs: Fraser & Jones, 1975; Fraser & Thompson, 1991). Offspring winning such competitions are likely to gain more parental resources, which may then translate into higher direct fitness.

As with parent-offspring interactions, however, we may also expect to find sibling coadaptation. Where siblings affect one another's fitness through direct interactions, linkage disequilibrium between such sibling effects and the traits influencing inclusive fitness should be generated. Thus, certain combinations of traits should maximise the fitness of both parties. Such an effect may also be seen if there are pleiotropic effects of alleles determining sibling behaviour, such as competition and negotiation behaviours. This therefore predicts that cross-fostering should be of greater detriment to offspring fitness when only portions of broods are crossed, rather than full broods, as each individual then interacts with a mixture of related and unrelated brood-mates. Such comparisions have not previously been considered in wild studies, although Heim *et al.* (2012) found no difference in survival and body mass between fully and partially cross-fostered young in domestic pigs.

In order to elucidate whether siblings show coadaptation through their effects on fitness, I describe a cross-fostering design in Chapter 4, that allows parent-offspring and sibling coadaptation mechanisms to be distinguished. Although such a design was used by Heim *et al.* (2012) in domestic pigs, this is the first instance in which

it has been used in a wild system to address questions about parent-offspring and sibling coadaptation.

1.4 Introduction to methods

1.4.1 Study Species

In this thesis I carry out manipulative experiments in order to test predictions about parental effects (in Chapters 2, and 4). In addition, I use data from a long term cross-fostering study (particularly in Chapter 3). All of these studies are carried out on a nest-box population of blue tits, *Cyanistes caeruleus*, established in 2009 near Edinburgh, UK (general field methods in Appendix A, field sites shown in map A.1). This species has been used extensively in studies of parental effects and quantitative genetics, and so is ideal for addressing the questions in this thesis. Indeed, there is over a century of history in studying tits in such populations, starting with studies of the great tit (*Parus majors*) in Wageningen in the Netherlands in 1912, and the subsequent establishment of the long-term study in Wytham Woods, Oxford in 1947 (Anderson, 2013). The extensive work already done on this, and closely related, species make it an extremely useful study species.

Blue tits are small passerine birds, which nest in natural or artificial holes and cavities (Cramp & Perrins, 1993). Adult males in the population used here weigh 10.74g on average, whilst females are slightly smaller at 10.60g. Egg laying commences early in the spring, generally mid-April, with variations around this depending on weather and habitat (Lack, 1958), with nest building starting five or more days prior to egg laying (Gibb, 1950). Eggs take around four days to develop within the female, between the rapid yolk development phase and laying (Haywood, 1993), and females lay one egg per day over the course of two weeks. Interruptions in the laying sequence "pausing") are reasonably common (see Chapter 2), such that

females do not lay eggs for one or more days, and then subsequently resume laying. The mean clutch size in the population between 2010 and 2014 was 9.2 eggs. Onset of incubation varies between females, with some starting around three days before clutch completion, and others after all eggs have been laid (Gibb, 1950). Clutches can start hatching from around 13 days after incubation commences (Gibb, 1950), with chicks hatching over a variable period of 1 to 3 days, depending on when incubation was started.

Blue tits provide biparental care to their offspring, although there are differences in the behaviours of male and female parents. Incubation and brooding is carried out by the female alone (Cramp & Perrins, 1993), and she also carries out the majority of nest maintenance and sanitation behaviours (Banbura *et al.*, 2001). Both parents remove faecal sacs and provide food to offspring (a mix of caterpillars, spiders, aphids and other invertebrates), and the male also feeds the female when she is incubating (Perrins, 1979; Cramp & Perrins, 1993). In addition, males have been shown to bring a greater proportion of caterpillars (an preferred food source to offspring) to offspring than females (Banbura *et al.*, 2001). Thus, there has been scope for studies both of parental care and effects. Previous work on parental care has shown that feeding rate increases with brood size (Nur, 1984c), parasite prevalence in the nest (Hurtrez-Boussés *et al.*, 1998), and habitat fragment size (Nour *et al.*, 1998), and that foraging distances varied with habitat quality (Tremblay *et al.*, 2005). Research on offspring behaviour has shown that chicks move toward the male parent when hungry (Dickens *et al.*, 2008). Additional effects of clutch size on phenotypes and behaviour have been found – increased clutch size caused a decline in subsequent female survival (Nur, 1984a), increased energetic costs of incubation (Haftorn & Reinertsen, 1985), decreased current nestling weight (Nur, 1984b), and decreased future breeding success (Nur, 1988). There has also been research on maternal effects in blue tits, including the change in, and effect of, components within eggs: Biard *et al.* (2007) show that maternal carotenoid availability has prenatal maternal

effects on chick immunity after hatching, and Kingma *et al.* (2009) showed that there is a paternal effect on yolk androgen content – mothers altered the pattern of testosterone deposition across the clutch in response to manipulation of male attractiveness.

1.4.2 Cross-Fostering Designs

Cross-fostering offspring between parents allows the relative contributions of genes and the environment to phenotypes to be distinguished from one another. Although most studies cross-foster offspring between nests after hatching, all experiments reported in this thesis cross eggs, rather than chicks. This ensures that broods are of more similar ages than when cross-fostered after hatching, as all individuals within a given nest-of-rearing are receiving the same incubation.

A range of different cross-fostering designs are used, which allows the elucidation of different components of phenotypic variance. In the years 2010-2013, eggs were exchanged in a round robin design between two or more nests, but only every other egg was moved. Thus, the resulting broods are a mix of crossed and uncrossed chicks. This allows several components of variance to be addressed (Illustrated in Figure 1.3): the nest-of-origin effects, which combines the prenatal and genetic effects (these are confounded and cannot be separated), postnatal environmental effects (nest-of-rearing), and sibling effects.

A different cross-fostering design was used in 2014 and 2015 (see Figure 1.4); eggs were crossed reciprocally between pairs of nests, either with every other egg being crossed in a partial design (as before), or with all eggs crossed. Control nests (no eggs exchanged) were also included in this design. This allowed us to detect the effect of cross-fostering (parent-offspring coadaptation), and the effect of siblings (sibling conflict and coadaptation). We can detect fewer effects than in the previous design, however – nest-of-origin and nest-of-rearing effects are confounded when all

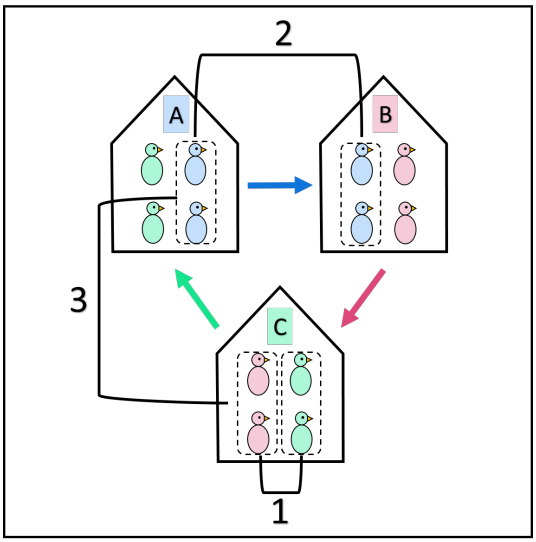


Figure 1.3: Illustration of the cross-fostering design used in 2010-2013. Colours indicate the nest-of-origin, and numbers on brackets indicate the variance components that can be elucidated. **1** – chicks within the same nest resemble each other due to postnatal environmental effects, including parental effects and care. **2** – chicks from the same nest-of-origin resemble one another due to shared genetic and prenatal maternal effects. **3** – chicks in separate nests that share the same siblings resemble one another due to the effects of those sibling's genes.

chicks are crossed or all remain in their natal nests (Figure 1.4), and sibling environmental effects are confounded with nest-of-rearing effects in a reciprocal partial cross-fostering design, although sibling genetic effects can be estimated from the dyad variance.

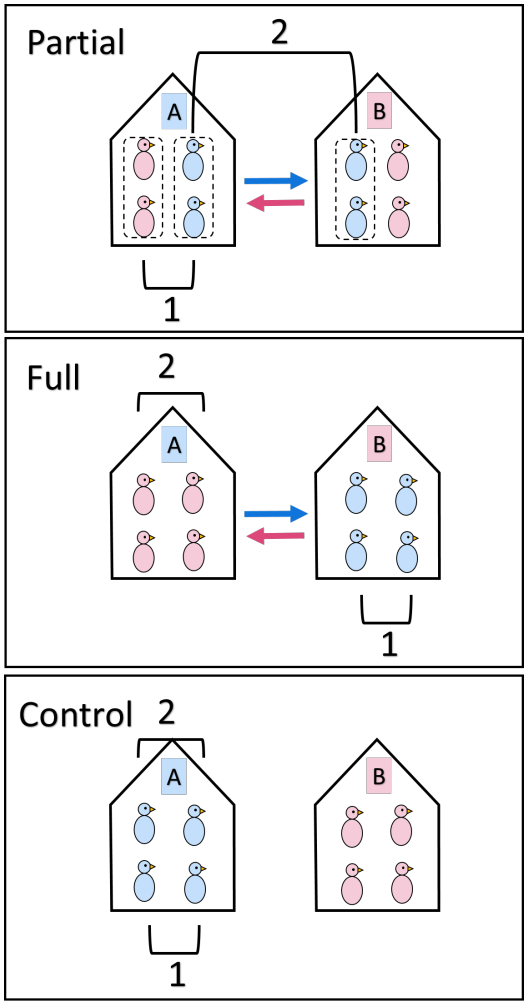


Figure 1.4: Illustration of the cross-fostering design used in 2014-2015. Colours indicate the nest-of-origin, and numbers on brackets indicate the variance components that can be elucidated in the partial design, as in Figure 1.3. **1** – chicks within the same nest resemble each other due to postnatal environmental effects, including parental effects and care. **2** – chicks from the same nest-of-origin resemble one another due to shared genetic and prenatal maternal effects.

1.5 Aims of this Thesis

Overall, this thesis aims to establish how micro-evolution may be affected by parental care and parental effects. Whilst this is investigated primarily through the concept that parents and offspring should be in conflict with one another, I also consider how coadaptation may exist between parents and their offspring. Each of the following data chapters address parts of these aims:

Chapter 2

In this chapter, I investigate the proximate mechanism behind a maternal effect on offspring hatching time, to establish whether it represents a passive effect of environmental change or an anticipatory maternal effect. Specifically, previous work found a difference in the developmental rate between early and late laid eggs, a maternal effect that has downstream effects on offspring fitness. By carrying out a supplementary feeding experiment, I aim to test whether this was driven by environmental changes during the laying period being passed to eggs, or if it occurs independently of environment, and therefore is likely to be an active maternal effect.

Chapter 3

This chapter focuses on how antagonistic selection between parental and offspring traits may explain stasis in body size. I aim to show that selection for increased juvenile body mass is opposed by selection against parental performance for offspring weight, and that this alters predictions of micro-evolutionary change. Thus, this chapter addresses the paradox of stasis, and goes some way towards explaining why no change in body mass is seen, despite the presence of directional selection.

Chapter 4

Whilst the previous chapter focuses on conflict, here I investigate whether there is evidence for coadaptation between parents and their offspring, through a cross-fostering experiment. Within this, I also set out to establish whether siblings are important in mediating parent-offspring coadaptation (or conflict), or if there are direct effects of siblings on growth and fitness, by cross-fostering different proportions of offspring between nests, manipulating family structures. Thus, I show an experimental design that allows the effects of both parents and siblings to be established, with appropriate controls.

Chapter 2

Prenatal maternal effects appear to be insensitive to experimental or natural environmental variation

Abstract

In many birds, hatching asynchrony is a common phenomenon, primarily driven by patterns of incubation behaviour. However, experimental results in blue tits (*Cyanistes caeruleus*) have shown that asynchrony is reduced by intrinsic properties of later eggs that accelerate pre-natal development. These intrinsic differences between early and late eggs could be driven by changes in resource availability to females, which are then passively passed onto the egg. Alternatively, it may be due to an anticipatory maternal effect, wherein some signal or resource is actively placed within the egg, which is beneficial to those eggs laid late within the clutch. In order to distinguish between these hypotheses we designed a supplementary feeding experiment, wherein females were provided with food at certain times during the laying phase. This had no discernible effect on development rate, or other egg characteristics, consistent with anticipatory maternal effects. Using a larger data set we also tested whether natural environmental variation (weather) during egg formation affected maternal investment in eggs. Similarly, egg characteristics were found to be relatively insensitive to the environmental variation, supporting the experimental results.

2.1 Introduction

Parents influence the phenotypes of their offspring, both through the genes they pass on, and by directly modulating the environment offspring experience. These parental effects have been shown to be present in many systems, and can have a major causal contribution to an individual's phenotype (Roach & Wulff, 1987; Mousseau & Dingle, 1991; Mousseau & Fox, 1998; Badyaev & Uller, 2009; Wolf & Wade, 2009). The effects are often context-dependent – varying with the environment that the parents themselves experience (Rossiter, 1996, 1998). This can either

occur through the direct response of a parent to changes in their own environment, or indirectly, through parents using changes in their own environment to anticipate the environment their offspring will experience (Marshall & Uller, 2007). Although the context-dependency of parental effects is unequivocal, whether anticipatory responses are a common feature of context-dependency remains contentious (Uller *et al.*, 2013; Burgess & Marshall, 2014). Anticipatory parental effects (Marshall & Uller, 2007) are expected to occur when the environment experienced by parents is a predictor of the environment that will be experienced by the offspring (Burgess & Marshall, 2014), and selection therefore favours parents producing offspring of an appropriate phenotype for that environment. Whilst there has been some convincing evidence for the presence of such effects, such as adaptation to maternal light environments (Galloway & Etterson, 2007; Galloway, 2005), and transgenerational induction of defences in plants and animals (Agrawal *et al.*, 1999), a recent meta-analysis concluded that anticipatory effects were not widespread (Uller *et al.*, 2013): In those experiments that subjected parents and their offspring to two environments in a fully factorial design, there was only weak evidence that offspring do better when parent/offspring environments are matched rather than mismatched (Uller *et al.*, 2013). Although at face value this finding suggests that there is limited evidence for anticipatory parental effects, such studies are open to the criticism that the environment parents are subject to is not necessarily a good predictor of the environment offspring would have experienced under natural conditions (Burgess & Marshall, 2014).

In species that experience age-related sibling competition, anticipatory parental effects are hypothesised to evolve in order to mediate these age related effects, and may be related to changes in the size and composition of eggs. Unlike those studies reviewed in Uller *et al.* (2013), parents are assumed to know (rather than predict) where offspring of specific eggs will be placed in any age hierarchy, and thus the competitive environment they may experience. As a consequence, anticipatory parental

effects may more easily evolve. For example, Plaistow *et al.* (2007) found that female spider mites increase the size of their eggs as they age, and attributed this change to female anticipation of the level of sibling competition later laid eggs will encounter. In birds, egg size (Slagsvold *et al.*, 1984) and many egg constituents have been shown to vary, both between females and across the laying sequence of individuals (Williams, 2012). These include carotenoids (Royle *et al.*, 2001; Blount *et al.*, 2002; Török *et al.*, 2007; Saino *et al.*, 2002), vitamin E (Royle *et al.*, 2001) and hormones (Gil, 2008). Whilst egg size has downstream consequences on juvenile traits (Krist, 2011), hormonal changes in particular have been invoked as mediators of anticipatory parental effects (e.g. Schwabl, 1993; Schwabl *et al.*, 1997; Lipar *et al.*, 1999; Muller & Groothuis, 2013), and have also been shown to have downstream effects on multiple aspects of offspring competitiveness (reviewed in Groothuis *et al.*, 2005; Williams, 2012), including growth rate (Gorman & Williams, 2005), begging (Schwabl, 1996; Smiseth *et al.*, 2011), and social dominance (Schwabl, 1993).

However, there is usually a strong correlation between the temporal order in which eggs are formed and the relative ages of corresponding offspring. Consequently, the conclusions of such studies are often qualified with compelling alternative explanations, wherein changes in egg characteristics are a passive outcome of the response of females to their own changing environment, condition, or breeding state. In the context of hormones, differences across the laying sequence may reflect the cumulative effect of exposure of eggs to changes in the female's endocrine state (Groothuis & Schwabl, 2008). For example, testosterone peaks a few hours prior to the ovulation of each egg (Johnson, 1986; Schwabl, 1993), which could lead to greater cumulative exposure of later laid eggs. Changes may also be seen in preparation for and upon the onset of incubation, leading to different hormonal exposure of eggs (Goldsmith & Williams, 1980; Sockman & Schwabl, 1999, 2000). Similarly, if dietary carotenoids increase over the season whilst eggs are being laid, then the change in eggs may simply reflect the direct effect of carotenoid availability (Török

et al., 2007).

Previously, we found that prenatal development in blue tits (*Cyanistes caeruleus*) is faster for eggs laid later in the laying sequence, and showed experimentally that this was in part caused by something intrinsic to the eggs at the time of laying (Hadfield *et al.*, 2013b). Although consistent with an anticipatory parental effect that acts to reduce the extent of hatching asynchrony, we offered the alternative explanation that it may simply be a result of females being better able to provision later eggs as the amount of resource available to them increases as spring progresses (Hadfield *et al.*, 2013b). Here, we aim to test this hypothesis by manipulating a female's resource availability during the laying period to see if this accelerates the prenatal development of her offspring. To ensure that any effects detected are the result of changes to the size or composition of the eggs and not due to any effect of the treatment on post-laying behaviour, we cross-fostered half the eggs of each nest on the day they were laid. We are primarily interested in any effects of this treatment on the hatching time of eggs, but also model whether it has any effect on egg weight or hatching success.

The results of our experiment showed little to no effect of the additional resources on prenatal development and other egg characteristics, implying that the effects of the dietary environment are not being passed to offspring. An alternative explanation is that seasonal changes in the environment are causing laying-order effects on pre-natal development, as these are likely to have effects on food abundance and energy demands. For example, the hatching time of winter moth caterpillars, a important food source for breeding blue tits, have been shown to be accelerated by increasing temperatures (Visser & Holleman, 2001). Thus, weather may impact upon resources available during egg formation, and therefore affect prenatal development, but our supplemental feeding treatment alone failed to recapitulate those changes. Using a larger data set we performed a more general (but correlative) test of whether the rate of pre-natal development varied systematically with

three weather variables, which could be drivers of seasonal changes. Some effect of temperature, and other weather variables, was found on the hatching time of eggs, but these effects were relatively small, and suggest that there is little sensitivity of maternally derived egg characteristics to environmental variation.

2.2 Methods

The experiment was carried out during the springs of 2012 and 2013 on a nest box population of blue tits (*Cyanistes caeruleus*) on the Dalmeny estate, Edinburgh, UK. Nest boxes were placed approximately 30m apart, on two study sites – 180 boxes on Cragie Hill (grid reference NT 156 766) and 45 boxes along the Almond River (NT 179 758), shown on the maps A.1 in Appendix A. From early in the spring, next boxes were checked systematically to detect nesting and onset of laying. When nest boxes were empty, or had a very small amount of nesting material within them, they were checked every 4 days. When >15mm of nesting material was present, boxes were checked every 2 days, and once the nest was lined it was checked every day. This method meant that in all but very few cases, nests were checked on the day in which laying began, and thus a single egg was found.

2.2.1 Cross-foster Design

The study was carried out within an ongoing partial cross-foster design used in this population (for full details see Hadfield *et al.*, 2013a). On the day of clutch initiation nests were randomly allocated into groups of three where possible (or two, four, or five if not), between which eggs were switched. First eggs were then moved within triads, such that the egg from nest A was placed in nest B, that from nest B in nest C, and that from C placed in A. One egg is laid per day, so on alternate days, eggs were either moved between nests, or remained within their own nests, and every egg was weighed and marked. Crossing ceased when one or more nests had a laying

pause and resumed when all nests recommenced laying. Daily checks ceased when the female was incubating for the second day in a row, or was found incubating after laying had ceased. The crossing of eggs within a triad stopped when one nest in a given triad finished laying.

2.2.2 Feeding experiment

Within this cross foster design, a feeding experiment was also carried out in order to determine how food availability to parents affects the hatching rate of eggs. We used those nests that were in crosses of 3 or more, and each group was assigned randomly to either a control group or one of two feeding treatments. When a group was assigned a feeding treatment, two nests within that group were fed, and the remaining nest (or nests if the group was larger than 3) remained as a control. Nests in the feeding treatments were provided with twenty wax worms (the larvae of the wax moth, *Gralleria mellonella*) per day, which were pinned to narrow tree branches close to the nest box (<5m) early each morning. This quantity of food was expected to be sufficient to elicit a response, as it constitutes 12kcal (Sauter *et al.*, 2006), a large portion of the daily energy requirements for this species (13.8kcal Gibb, 1957), as well as a higher protein content than an egg (see Discussion; Sauter *et al.*, 2006; Bourgault *et al.*, 2007; Murphy, 1994). On the following day, the number of wax worms consumed was recorded, and any uneaten wax worms were removed. We set up video cameras at some nests to check that the resident birds were taking the food. In some cases other birds were observed taking the wax worms, but this was relatively rare. Blue tits carry out high levels of courtship feeding during the egg laying period (Royama, 1966; Krebs, 1970; Cramp & Perrins, 1993), so we hope that resources from any wax worms taken by the male are passed to feed the female, either directly or indirectly. Nests in the feeding treatments were split into an early group, which were provided with wax worms on days 1 to 4 of the laying sequence, and a late group that received wax worms on days 5 to 8. If development of the

embryo is resource limited we expect chicks from eggs laid in the treated nests to have more rapid prenatal development if the food provided lifts this constraint. We predict that this effect should be more pronounced in chicks from eggs in the early treatment if resource limitation is more acute at the onset of laying than later in the sequence. During 2012 there were 20 nests in each of the early and late treatment, and 18 in each treatment in 2013, so there were equal numbers in each treatment overall. There were 63 control nests in 2012, and 60 in 2013. In addition, those nests that were not in cross-fostering groups of three or more were never included within the feeding treatment, and so are classed as having no treatment, as they were not true controls. There were 29 such nests in 2012, and 17 in 2013.

The clutch sizes of the nests included in each treatment are shown in Figure 2.1, along with the distribution of eggs within the laying sequences from each of these treatments. It is worth noting that the clutch size and maximum egg number (days since the first egg was laid for each egg) of a nest do not necessarily match due to interruptions in the laying sequence. For example, a female may lay a total of 9 eggs, but may pause in laying, such that eggs were not laid on day 3 and 4, and the maximum egg number is 11 rather than 9. Thus although mean clutch size is 8.26, many nests have several eggs with egg numbers substantially greater than this, even if the total number of eggs in that nest is less than this mean. The mean number of wax worms eaten per day in the early treatment was 15.8, and 17.7 per day in the late treatment.

2.2.3 Hatching times

Nests were checked daily for hatching from 11 days after clutches had been completed. As nests were checked daily, we found chicks within twenty-four hours of the first individual hatching. On this occasion (day 0), the identities of any unhatched eggs were recorded, and the same was done on the following two visits (day 1 and

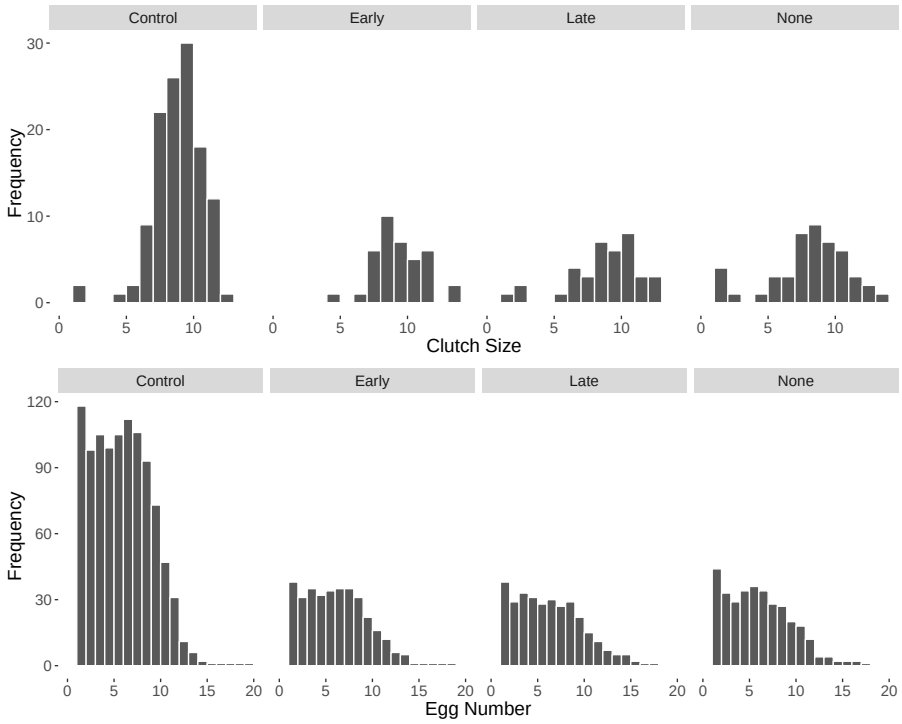


Figure 2.1: The clutch sizes (upper panel) and egg numbers included in the data for each of the treatments. An egg's number is the day on which it was laid (relative to the first egg in the clutch), such that egg 3 is laid 2 days after the first egg.

day 3). No eggs hatched after this point. Thus, the hatching interval of all eggs was known.

2.2.4 Weather

Hourly temperature (°C), rain (ml), and wind speed (knots) measurements were obtained from the Met Office (UK Meteorological Office, 1853-Current). Wind and temperature data was from Turnhouse weather station (NT15988 73905, 2.72km from Craigie Hill, and 2.69km from Almond River), whereas rain data was from Gogar Bank (NT17088 71601, 5.21km from Craigie Hill, and 4.27km from Almond River). Hourly data was used to calculate daily mean temperature, daily mean wind speed, and daily total rainfall.

2.2.5 Analysis

Analyses were carried out in R (R Development Core Team, 2012), using the package MCMCglmm (Hadfield, 2010) to fit Bayesian generalized linear mixed models. For those models which had a binary or ordinal response, residual variances were fixed at 1, as these could not be estimated from the data. For other models a flat improper prior for residual variances was used. For the random effects, parameter expansion was used resulting in scaled $F_{1,1}$ priors on the variances with scale 1000. Fixed effects had independent normal priors with zero mean and large variances (10^8) except in binary/ordinal models. Here, a prior correlation matrix was defined as one in which the fixed effects are identically and independently distributed had the covariates been subject to Gelman's (2008) scaling and centering. The prior correlation matrix was then scaled by six (which in all models was approximately the sum of the variance components) plus $\pi^2/3$ or 1, depending on whether the logit or probit link was used. Feeding models were run for 130000 iterations, with a burn in of 30000 and a thinning interval of 50, whereas weather models were run for 1300000 iterations, with a burn in of 300000 and a thinning interval of 500 as the chains within these models took much longer to mix well.

Fixed effects are considered significant if the 95% credible intervals did not overlap zero, and pMCMC (twice the posterior probability that the estimate is either negative or positive, depending on which is the smaller probability) is less than 0.05. For groups of fixed effects (e.g. year, treatment) their significance was also tested using omnibus Wald tests.

Feeding Experiment: egg-level effects

A series of models were developed to look at the effect of the treatment on individual egg characteristics. The main focus was on hatching time, although we also analysed hatching success, egg weight, and pausing in laying. In all models, clutch size, year,

day of clutch initiation (from 1st April) and whether the egg was laid after a pause in the laying sequence or not were fitted as fixed effects. Where necessary, we distinguish between variables measured in the nest-of-origin and nest-of-rearing using the subscripts o and r respectively. The rank_r of the egg (the time between it and the final egg in the nest-of-rearing being laid), was fitted as a spline to capture any non-linearity in the relationship induced by incubation behaviour (Hadfield *et al.*, 2013b). In all models we excluded eggs that had not been found on the day they were laid, and those from clutches smaller than three eggs (due to early desertion of the clutch). We also excluded three eggs that weighed less than 0.6g, as these were abnormally small (below half the mean egg weight), and do not develop.

For each egg we also included the treatment group of its nest-of-origin (treatment_o) and its nest-of-rearing (treatment_r) as a fixed factor. Nests that were neither fed nor assigned as controls were included in the analysis as a fourth level in treatment group (coded as 'none'). Generally, these were nests that initiated laying early or late in the season when few nests initiated, so that they could not be assigned to groups of three or more.

Eggs within nests of a given feeding treatment vary in when and how many wax worms were consumed by their mother during their development. For example, the fifth egg in an early-treated nest could be affected by up to 80 wax worms provided over the four consecutive days prior to it being laid, whereas the second egg could only be influenced by up to 20 wax worms on the day prior to it being laid. Blue tits are income breeders, and eggs develop over a period of four days (Haywood, 1993), and thus the main eggs expected to be affected by the feeding are those laid around four days after feeding occurs (e.g. egg 5 for extra food given on day 1) if supplementary feeding has a direct effect on eggs. To accommodate this, we included the number of wax worms that were consumed on each day prior to the laying of an egg as multiple predictors. Their effects were modelled using a varying coefficient model (Wood, 2006; Hastie & Tibshirani, 1993): a type of spline that in this context

allows changes in the effect of the predictor (number of wax worms eaten) to be a smooth function of some other covariate (the interval of time between the wax worms being eaten and the egg itself being laid). These models have recently been used in an ecological context (Roberts *et al.*, 2015) and in our context can capture any physiological lags that exist between the consumption of food and any effect of that food on the egg. Separate varying coefficient splines were fitted for the early and late groups.

The degree to which the treatment splines improved model performance was assessed using 20-fold cross-validation (Stone, 1974, 1977), where models were rerun using data with the k^{th} subset removed. The posterior mean predicted values for the omitted data were obtained with the random nest-of-origin and nest-of rearing effects marginalised using posterior predictive simulation. Where responses were discrete, the correct classification of the response variable was used as a measure of predictive ability. For continuous responses the coefficient of determination (R^2) was used. Differences between models in their predictive ability were often small, and so we tested whether an increase in predictive ability relative to that of the null model (i.e. one without treatment splines) was greater than expected from Monte Carlo error (i.e. differences were not solely due to random allocation of observations to subsets). This was achieved using repeat-measure ANOVA with subset as a random effect.

Hatching time was analysed in two ways. Firstly, as an ordinal response, which considers the hatching interval of an egg within a clutch (i.e. the day of hatching relative to the first egg in the clutch to hatch, which could be 0, 1 or 2-3). Secondly, as a censored Gaussian response, which fits the time from the final egg in a nest being layed to the hatching of each egg with the censoring points being the day/time of nest visits between which an egg hatched. Eggs were assumed to be laid at 6am, as the exact laying time was not known, but eggs had always been found after this time (Pullen, 1946; Perrins, 1979). Hatching success was analysed as a binary response

(hatching/not-hatching) and all abandoned nests were excluded. Egg weight was analysed as a Gaussian response, and the probability of an egg being laid as a binary response (where an egg being laid was given the value 1). These two phenotypes are not dependent on post-laying (and therefore post-crossfostering) effects and so nest-of-rearing terms (both fixed and random) were dropped from the model. In addition, clutch size$_o$ was dropped from the model of pausing, as the two are confounded.

Feeding Experiment: nest-level effects

A second set of models investigated the nest-level effects of the feeding experiment, specifically how it affected clutch size and the onset of incubation. The onset of incubation is taken as the number of days before (negative value) or after (positive value) the last egg was laid that the female was found incubating, or the eggs were found to be warm. Both traits were treated as Gaussian and the models had the same form as above, although treatment was included only as a fixed effect, without spline terms, and egg rank was not included in the model. In addition, only nest-of-origin level terms were retained.

Weather

In addition to the main analyses, we ran the same models as those described above, but also included terms to model how weather (a measure of external conditions) affects egg- and nest-level characteristics. In these analyses we used comparable data collected in the previous two years (Hadfield *et al.*, 2013b,a) although egg weight data were unavailable for 2010. Treated nests from 2012-13 were excluded, as were those eggs included in an experiment performed in 2010-2011 (Hadfield *et al.*, 2013b). Consequently any terms associated with treatments were dropped from the model. The effect of weather variables (daily mean temperature, daily mean wind speed, and total daily rainfall) were modelled using varying coefficient splines, as was done for the effect of wax worm consumption. Thus each model had three varying coefficient

splines within it. Daily weather 50 days prior to, and 40 days after, laying of a given egg was used in these splines, so that both long- and short-term effects of weather might be captured. The sample sizes for each model, along with those eggs and nests that have been excluded are shown in Table 2.1.

	Feeding				**Weather**			
	Included		Excluded		Included		Excluded	
	Eggs	Nests	Eggs	Nests	Eggs	Nests	Eggs	Nests
Ordinal Hatching	1649	214	67	3	3076	426	775	39
Censored Gaussian Hatching	1649	214	67	3	3076	426	775	39
Hatching Success	1825	215	49	2	3425	428	829	37
Weight	1979	235	51	12	2754	319	730	91
Pausing	1739	235	0	0	3259	431	794	93
Clutch Size		229		18		409		115
Onset of Incubation		210		24		391		104

Table 2.1: Sample sizes (n), the number of eggs that are excluded, and the number of nests that those eggs are found in, for each model

2.3 Results

2.3.1 Feeding Experiment: egg-level effects

Overall, we found little support for any effects of the feeding treatment on hatching time and other egg characteristics. This is seen particularly in the fitting of treatment splines – in all models the treatment splines did not improve the predictive ability of the model, as evaluated using cross-validation (Table 2.2). Consequently, we report the results of these models with the treatment splines removed in the text and tables. However, in Figures 2.2 and Supp B.2 we present the predictions based on the full model.

The fixed effects for the best ordinal model for hatching rate are shown in Table 2.3. The 95% credible intervals of all fixed treatment effects overlapped zero, and thus there was no significant effect of treatment on hatching day. There were signifi-

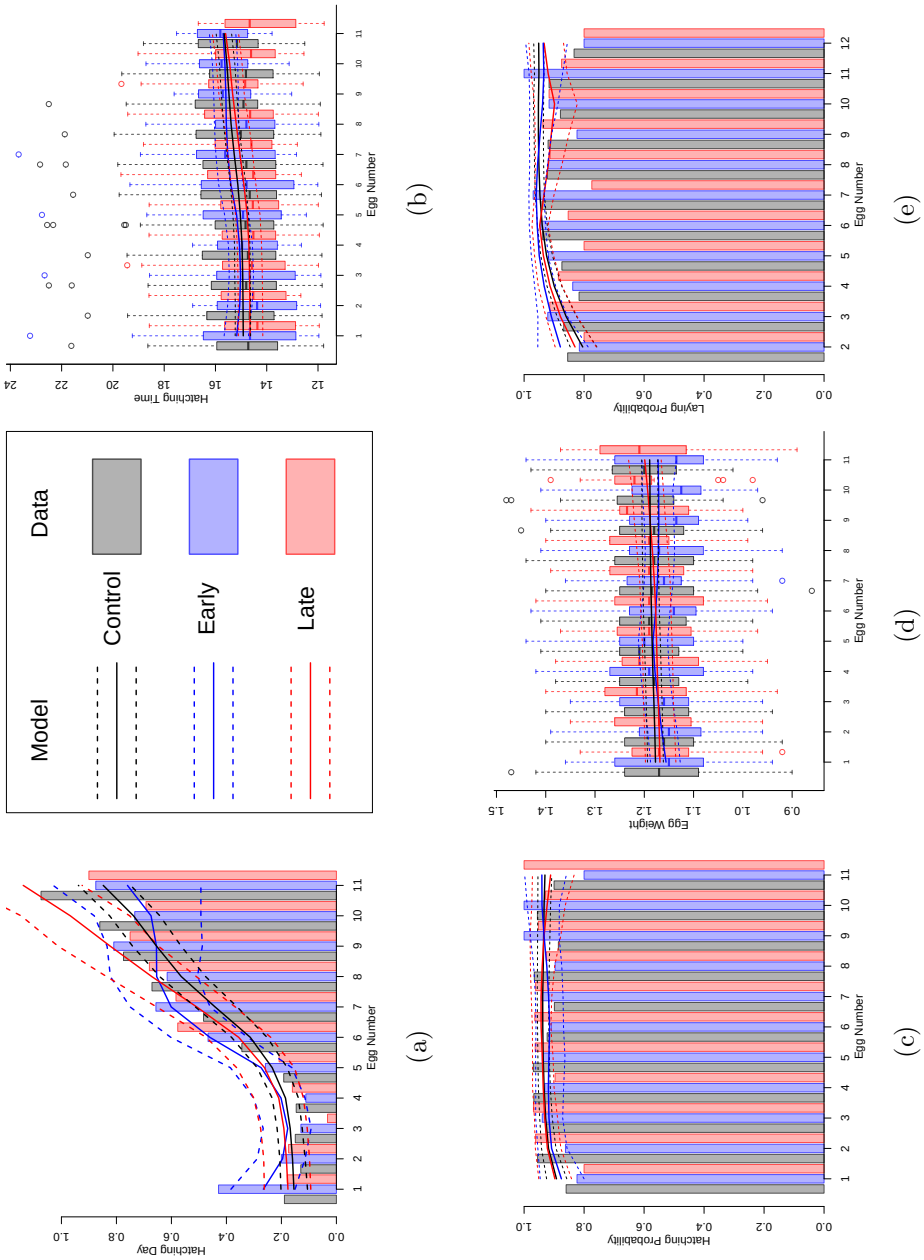


Figure 2.2: The cumulative effect of wax worms eaten on (a) day of hatching within nest, (b) time from laying of the final egg in a nest to hatching, (c) probability of hatching, (d) weight of eggs, and (e) the probability that an egg is laid. Boxplots and barplots show the raw data, split by treatment, together with predictions (solid lines) and 95% credible intervals (dashed lines). Predictions were made holding all predictors at their mean value except rank and the number of wax worms eaten for which we use the mean for each egg-number/treatment combination.

	Full Model	Early Spline Dropped	Late Spline Dropped	Both Dropped	Pr(>W)
Ordinal	0.616	0.615	**0.616**	0.615	0.1240
Censored Gaussian	0.178	0.178	**0.178**	0.177	0.0830
Hatching Success	0.842	0.842	0.842	**0.843**	
Weight	0.094	0.095	0.096	**0.097**	
Laying Success	0.793	0.793	**0.794**	0.793	0.5090

Table 2.2: Mean predictive power from 20-fold cross validation of feeding experiment models, comparing the full model (all fixed and random effects included, along with splines of the effects of the feeding experiment) with those that drop the splines for each of the early and late treatments, and the null model in which both are dropped (but all other fixed and random effects are retained). Ordinal and Censored Gaussian are the two models of hatching time.. For weight, predictive power is measured as an R^2 value, and for other models it is the rate of correct classification. In all cases the predictions are marginal with respect to nest_o and nest_r (where appropriate). The final column is the probability that the estimated predictive power of the best model (in bold) exceeds that of the null model more than would be expected from Monte Carlo error alone.

cant positive effects of clutch size_r, and the day of clutch initiation, showing nests are more asynchronous when clutches are larger and laid later in the season. The spline of egg rank (Supp. Figure B.1a) shows that lower rank eggs hatch later (i.e. late laid eggs hatch late), although this ceases to be the case for eggs of rank > 3 which tend to have equivalent hatching times. Nest-of-origin explained a small amount of variance in the day of hatching (0.068 [0.018 - 0.149]), whereas the nest-of-rearing explained a greater proportion, 0.562 [0.446 - 0.613].

The fixed effects from the censored Gaussian model are summarised in Table 2.4, again this is the best model (without the treatment splines). Overall, the results are broadly similar to the ordinal model with all fixed treatment effects overlapping zero and therefore non-significant. There was a significant negative effect of clutch size_r, with eggs hatching 0.287 days ([0.127 - 0.445], P<0.001) faster for each additional egg in a nest, and eggs in later clutches hatched faster by 0.106 days ([0.074 - 0.137], P<0.001) for each day later in the season. In addition the rank spline (Supp. Figure B.1b) shows that there is a decrease in time from laying to hatching with increasing rank, although this change is most prominent for those eggs of low rank.

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	-6.067	-8.539	-3.654	**<0.001**	
Treatment$_r$.None	0.288	-0.442	1.120	0.464	
Treatment$_r$.Early	-0.200	-1.150	0.675	0.646	0.223
Treatment$_r$.Late	0.731	-0.210	1.588	0.121	
Treatment$_o$.Early	0.585	-0.057	1.285	0.101	0.232
Treatment$_o$.Late	-0.082	-0.789	0.586	0.803	
Clutch size$_r$	0.284	0.105	0.495	**0.002**	
Clutch size$_o$	0.083	-0.048	0.212	0.221	
Year.2013	-0.389	-1.156	0.403	0.308	
Laydate$_r$	0.052	0.014	0.091	**0.007**	
After pause	0.365	-0.131	0.867	0.163	

Table 2.3: Summary of the fixed effects from an ordinal model of the day on which an egg hatched relative to the first day of hatching within the nest in which it was reared. These results are from a model without treatment splines since they did not significantly increase predictive ability (Table 2.2). P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

The variance explained by the nest-of-origin was small, with an intraclass correlation of 0.002 [0.000 - 0.007], but the variance explained by the nest-of-rearing was much larger (0.972 [0.962 - 0.976]) as the censored Gaussian model also includes variation due to differences in incubation behaviour between females. However, the nest-of-origin effect within nest-of-rearing explained more variance (0.079 [0.000 - 0.202]).

For the best model of hatching success, the fixed effects are summarised in Table 2.5 (no treatment splines were in the best model). No significant differences between treatment groups were found, although eggs in none-fed none-control nests were significantly less likely to hatch (-0.863 logits [-1.684 - -0.100] P=0.040). Eggs laid after a pause show a significantly lower probability of hatching than those not laid after a pause. The rank spline (Supp. Figure B.1c) shows that later laid eggs in a clutch (low rank) are more likely to hatch than earlier ones, and Figure 2.2c suggests this is mainly due to first eggs having lower hatching probability. Both the intraclass correlation for nest-of-origin (0.153 [0.000 - 0.264]), and nest-of-rearing (0.167 [0.055 - 0.287]) were reasonably small and of similar magnitude.

The fixed effects from the best model of egg weight are summarised in Table 2.6;

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	21.347	19.327	23.197	**<0.001**	
Treatment$_r$.None	-0.430	-1.106	0.189	0.193	
Treatment$_r$.Early	0.079	-0.675	0.711	0.822	0.663
Treatment$_r$.Late	-0.277	-0.978	0.377	0.438	
Treatment$_o$.Early	0.101	-0.018	0.219	0.107	0.264
Treatment$_o$.Late	0.015	-0.118	0.136	0.818	
Clutch size$_r$	-0.287	-0.445	-0.127	**<0.001**	
Clutch size$_o$	0.018	-0.005	0.040	0.111	
Year.2013	-0.358	-0.972	0.337	0.295	
Laydate$_r$	-0.106	-0.137	-0.074	**<0.001**	
After pause	0.049	-0.038	0.146	0.322	

Table 2.4: Summary of the fixed effects from a censored Gaussian model on the time (in days) from laying to hatching of eggs. These results are from a model without treatment splines since they did not significantly increase predictive ability (Table 2.2). P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

this model did not include any treatment splines. The 95% credible intervals of all treatment effects overlapped zero, thus treatment did not have an effect on egg weight. Eggs were significantly smaller (-0.080 g [-0.111 - -0.049] P<0.001) in 2013 than 2012 and egg weight increased by 0.002 g per day ([0.000 - 0.003], P=0.028) as clutch initiation day increased. There was also a significant effect of being laid after a pause, with eggs laid after an interruption in laying being 0.051 g ([0.043 - 0.059], P<0.001) heavier. The rank spline (Supp. Figure B.1d) shows that there is a general decrease of egg weight with increasing rank, implying that late laid eggs tend to be heavier than earlier ones. A large proportion of the variance in egg weight was explained by nest-of-origin (0.800 [0.768 - 0.832]).

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	0.331	-2.086	3.032	0.809	
Treatment_r.None	-0.863	-1.684	-0.100	**0.040**	
Treatment_r.Early	-0.490	-1.557	0.499	0.349	0.56
Treatment_r.Late	0.258	-0.794	1.319	0.627	
Treatment_o.Early	0.149	-0.830	1.195	0.778	0.918
Treatment_o.Late	-0.155	-1.162	0.899	0.784	
Clutch size_r	0.150	-0.050	0.365	0.160	
Clutch size_o	0.133	-0.024	0.318	0.116	
Year.2013	-0.630	-1.499	0.128	0.125	
Laydate_r	0.034	-0.003	0.076	0.089	
After pause	-0.842	-1.520	-0.126	**0.028**	

Table 2.5: Summary of the fixed effects from a model of hatching success of eggs. These results are from a model without treatment splines since they did not significantly increase predictive ability (Table 2.2). P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	1.160	1.059	1.251	**<0.001**	
Treatment_o.None	-0.005	-0.035	0.027	0.728	
Treatment_o.Early	-0.012	-0.045	0.020	0.467	0.766
Treatment_o.Late	-0.004	-0.037	0.031	0.771	
Clutch size_o	-0.001	-0.009	0.007	0.896	
Year.2013	-0.080	-0.111	-0.049	**<0.001**	
Laydate	0.002	0.000	0.003	**0.028**	
After pause	0.051	0.043	0.059	**<0.001**	

Table 2.6: Summary of the fixed effects from a model of egg weight. These results are from a model without treatment splines since they did not significantly increase predictive ability (Table 2.2). P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

The fixed effects from the best model of the probability of an egg being laid are summarised in Table 2.7 (treatment splines did not improve the model). These results suggest that feeding had no significant effect on the probability that an egg is laid. There is a significant negative effect of the day of clutch initiation, such that pausing occurs more frequently later in the season. The rank spline, Supp. Figure B.1e, shows a negative effect of egg rank on the probability that an egg is laid, such that interruptions in the laying sequence are more likely to happen early

in a clutch. The intraclass correlation for nest-of-origin was moderate: 0.179 [0.088 - 0.312].

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	4.469	3.466	5.530	**<0.001**	
Treatment$_o$.None	-0.530	-1.232	0.082	0.094	
Treatment$_o$.Early	0.394	-0.330	1.116	0.286	0.504
Treatment$_o$.Late	-0.091	-0.782	0.603	0.820	
Year.2013	-0.283	-0.987	0.379	0.399	
Laydate	-0.039	-0.069	-0.004	**0.024**	

Table 2.7: Summary of the fixed effects from a model of the probability that an egg is laid. These results are from a model without treatment splines since they did not significantly increase predictive ability (Table 2.2). P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

2.3.2 Feeding Experiment: nest-level effects

The fixed effects from the model of clutch size are summarised in Table 2.8. Neither treatment had a significant effect on clutch size – early treatment resulted in 0.413 more eggs ([-0.130 - 0.964], P=0.136) than control nests; late treatment resulted in 0.386 more eggs ([-0.197 - 0.947], P=0.170). The difference between early and late treatments overlapped zero (0.026 [-0.640 - 0.676] P=0.917), and the omnibus test showed there was no overall significant effect from the treatments (Wald test χ^2=3.172, P=0.205). There is a significant negative effect of the date on which the clutch started, with females laying 0.073 fewer eggs ([0.049 - 0.095], P<0.001) with each additional day after April 1st that the clutch started.

The fixed effects from the model of incubation onset are summarised in Table 2.9. There was a marginal effect of early treatments on the onset of incubation, with incubation commencing 0.639 days later ([-0.119 - 1.309], P=0.090) than in controls. Although there was no effect of the late treatment, the difference between early and late treatments overlapped zero (0.703 [-0.164 - 1.609] P=0.107). As with clutch size,

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	10.828	10.092	11.564	**<0.001**	
Treatment$_o$.None	-0.112	-0.620	0.467	0.689	
Treatment$_o$.Early	0.413	-0.130	0.964	0.136	0.205
Treatment$_o$.Late	0.386	-0.197	0.947	0.170	
Laydate	-0.073	-0.095	-0.049	**<0.001**	
Year.2013	0.232	-0.312	0.692	0.402	

Table 2.8: Summary of the fixed effects from a gaussian model of clutch size. P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

there was a significant negative effect on the incubation onset of the clutch initiation day – incubation began 0.080 days ([0.048 - 0.114], P<0.001) earlier, relative to the final egg, with each day after 1st of April that the female commenced laying. Additionally, there is a significant negative effect of clutch size, such that incubation onset advanced by 0.261 days ([0.087 - 0.417], P=0.003) for each additional egg in a clutch.

	mean	l-95%	u-95%	pMCMC	Pr(>W)
Intercept	6.185	4.018	8.138	**<0.001**	
Treatment$_o$.None	-0.154	-0.820	0.519	0.687	
Treatment$_o$.Early	0.639	-0.119	1.309	0.090	0.187
Treatment$_o$.Late	-0.064	-0.731	0.715	0.838	
Laydate	-0.080	-0.114	-0.048	**<0.001**	
Year.2013	-0.371	-1.002	0.311	0.298	
Clutch size$_o$	-0.261	-0.417	-0.087	**0.003**	

Table 2.9: Summary of the fixed effects from a gaussian model of the onset of incubation relative to the date on which the last egg in a nest was laid. P-values from wald tests on fixed effects are also presented. The mean is the posterior mean, l-95% and u-95% are the lower and upper 95% credible intervals.

2.3.3 Weather

Generally, we found little support for weather having effects on hatching time, and other egg characteristics. In all models, except the censored gaussian model of hatching time and the model of clutch size, the weather splines did not improve the predictive ability of the model as evaluated using cross-validation (Table 2.10).

The results of the null models were qualitatively similar to the feeding treatment models and are presented in the supplementary information. The main difference was support for inter-annual differences in all models, even for those egg characteristics and nest effects for which no significant differences between 2012 and 2013 were found. In addition, the significant effect of eggs laid after a pause having a lower probability of hatching (-0.842 logits [-1.520 - -0.126] P=0.028), were not replicated in the larger data set (-0.306 logits [-1.016 - 0.370] P=0.377). The splines for each weather variable are shown in Supp. Figures B.3, B.4 and B.5.

	Full Model	Wind Spline Dropped	Temperature Spline Dropped	Rain Spline Dropped	All Dropped	Pr(>W)
Ordinal	0.574	0.592	0.578	0.569	**0.593**	
Censored Gaussian	0.219	**0.219**	0.219	0.218	0.218	**0.0180**
Hatching Success	0.831	**0.831**	0.831	0.831	0.830	0.0730
Weight	**0.066**	0.062	0.063	0.064	0.063	0.4000
Pausing	0.868	**0.869**	0.868	0.868	0.868	0.1990
Clutch Size	0.303	0.305	0.271	**0.308**	0.259	**0.0020**
Incubation	0.243	0.247	0.248	0.249	**0.256**	

Table 2.10: Mean predictive power from 20-fold cross validation of weather models, comparing the full model (all fixed and random effects included, and splines of the effects of temperature, rain and wind) with those with each of the individual weather splines dropped, and the null model in which all three weather splines are dropped (but all other fixed and random effects are retained). Ordinal and Censored Gaussian are the two models of hatching time. For weight, predictive power is measured as an R^2 value, and for other models it is the rate of correct classification. All are run with nest_o and nest_r (where appropriate) marginalised. The final column is the probability that the predictive power of the best model (in bold) exceeds that of the null model more than would be expected from Monte Carlo error alone.

The best supported model for the censored Gaussian model of hatching was that with temperature and rain splines included, however the full model had a very similar classification rate so there was little difference between models with and without wind included (correct classification rate of best model 0.2190; Full model 0.2189; Null model 0.2181). The fixed effects from the best model are shown in Supp. Table B.3, whilst those for the full and null model are shown in Supp. Table B.2.

The weather spline in Supp. Figure B.4b shows that there was a positive effect of temperature on hatching time (days from last egg laid in a nest to hatching), up to three weeks prior to the egg being laid, implying that eggs that are developing in the female when conditions are warmer then hatch later. However, the converse appears to be the case post-laying, where there is a negative effect of temperature on hatching time, such that warm conditions after an egg has been laid leads to an acceleration in hatching time. Both rain (Supp. Figure B.5b) and wind (Supp. Figure B.3b) prior to laying appear to have little effect on hatching time in the full model, although the effect of rain is supported by cross validation. Year has a significant effect on hatching time in the best model (Wald test on year χ^2=13.651, P=0.003), with the biggest estimated difference of an increase of 1.746 days ([0.721 - 2.675], P<0.001) to hatching between 2011 and 2012. However, year effects were larger in the null model with an increase of 2.429 days ([2.045 - 2.847], P<0.001) to hatching between 2011 and 2012 (Wald test on year χ^2=153.13, P<0.001), implying that year and weather effects may be confounded. Similarly, laydate was not significant in the best model, although it was in the null model. In contrast to the feeding model, clutch size$_r$ did not have a significant effect in the full model, but did in the null model, whereas clutch size$_o$ had a significant positive effect on hatching time in both the full and null model.

Inclusion of splines of temperature and wind improved the predictive ability of the model of clutch size (R^2=0.308; Null model R^2=0.259). The weather splines for wind and temperature are seen in Supp. Figures B.3f and B.4f respectively. Wind appears to have a negative effect on clutch size, such that females lay fewer eggs as mean daily wind speed increases. Similarly, temperature negatively affects clutch size, such that smaller clutches are laid as daily mean temperature increases. Year has a non-significant effect (Wald test on year χ^2=0.305, P=0.883), although this then appears significant in the null model. Laydate did not have an effect on clutch size, although this was significant and negative in the null model, suggesting that

seasonal effect as well as year are confounded with weather in this model.

2.4 Discussion

The experiment described above was designed to test whether the increased rate of prenatal development across the laying sequence of blue tits could be directly caused by an increase in the resource availability to the mother at the time of laying. We found little support for supplementary feeding accelerating prenatal development; If anything, there was a positive effect of food consumption on hatching time, i.e. hatching occurred later due to supplementary feeding. Thus, we conclude that the changes in resource availability are not likely to be responsible for the intrinsic acceleration of hatching of late laid eggs observed in Hadfield *et al.* (2013b). We do not see any significant effect of treatment on female behaviour, although the effect size for the fixed effect of treatment on clutch size is reasonable large (increased clutch size by half an egg), suggesting that we may lack the power to estimate this effect.

Our results suggest that differences between eggs across the laying sequence are the result of changes in the female that are independent of either the nutritive or climatic environment. Consequently, these results are consistent with the idea that females are manipulating the hatching rate of their offspring independently of their own state. The mechanism by which they achieve this could be through increasing the provision of resources to eggs laid later. Consistent with this idea is the fact that egg size increases over the laying sequence, and larger eggs hatch faster (Rubolini *et al.*, 2005; Hadfield *et al.*, 2013b). In addition, Ferrari *et al.* (2006) and Alquati *et al.* (2007) found that experimental removal of albumen delayed hatching, thus the relative amount of albumen and yolk within an egg may be important in influencing prenatal development. Maternally deposited hormones, especially androgens, are a more commonly invoked candidate as a mediator of anticipatory

maternal effects. In support of this possibility, androgens generally act to accelerate pre- and post-hatching development (e.g. Eising *et al.*, 2001), reviewed in von Engelhardt & Groothuis (2011), although decreases in development have sometimes been found (Sockman & Schwabl, 2000; Von Engelhardt *et al.*, 2006). In addition Lipar & Ketterson (2000) show that there is a relationship between the laying order, testosterone and the development of the hatching muscle (*musculus complexus*) in red-winged blackbirds (*Agelaius phoeniceus*), and experimentally show that testosterone injected eggs have increased relative complexus mass. Thus it may be that androgens are able to accelerate time to hatching through increased development rate of musculature associated with hatching. However, previous work on blue tits suggests there is relatively little variation across the clutch in androgen concentration, with highest levels of testosterone seen in eggs in the middle of the laying sequence (Kingma *et al.*, 2009); a pattern predicted from interspecific comparisons of initial size differences between first and last hatching chicks and yolk testosterone compensation (Muller & Groothuis, 2013). Thus, it seems unlikely that androgens, particularly testosterone, are driving the differences in prenatal development in this particular case. Nonetheless, if hormones are responsible for the hatching rate variation we see, then our experiment is unable to differentiate between a passive effect of changes in the females endocrine state and an active anticipatory role. It is not known whether females are able to independently control blood plasma and egg hormone concentrations, but most hormones in egg yolks are derived from cells in the developing follicle (Huang *et al.*, 1979; Hackl *et al.*, 2003; Williams *et al.*, 2004; Gil, 2008), and so the possibility of independent control exists (Groothuis & Schwabl, 2008).

It is also possible that laying order effects are a direct maternal response to changing nutritive conditions, but such an effect might not be seen if providing wax worms failed to recapitulate such conditions. However, the wax worms provided to the birds should contain sufficient calorific and protein content to lift seasonal resource con-

straints; twenty wax worms constitute 12kcal and 0.67g protein (Sauter *et al.*, 2006), whereas blue tits require 13.8kcal per day (Gibb, 1957, winter conditions) and each egg contains about 0.12g of protein (Bourgault *et al.*, 2007; Murphy, 1994, given a mean egg weight of 1.18g) . Thus, although there is evidence that responses to feeding experiments depend upon the protein content of the food provided (Nager *et al.*, 1997; Ramsay & Houston, 1997, 1998), we believe our supplementary feeding would lift any constraints imposed by limited protein availability. We might also expect that if a response was to be seen due to changing nutritive conditions, this would be detected in the models using climatic variables as predictors of prenatal development, and such effects are not seen. In addition to macronutrients, it is possible that birds become less constrained by micronutrients as laying progresses. In particular, blue tits do not use skeletal calcium for egg production, so all calcium must be obtained in the diet (Woodburn & Perrins, 1997) mainly through consumption of snail shells, bones and grit (Betts, 1955; Graveland & Berends, 1997; Reynolds & Perrins, 2010). Twenty wax worms contains just 0.230mg calcium, much less than the content of an eggshell (58mg; Graveland & Berends, 1997). However, although the wax worms may be limiting in certain resources, the energy they provide may still allow greater time and resources to be spent in obtaining those micronutrients.

Previous feeding experiments lend support to the idea that supplementary feeding can lift constraints associated with breeding, since supplemented birds often show advanced lay dates and increased clutch size (see Christians, 2002; Robb *et al.*, 2008a). However, these experiments usually commence feeding prior to laying, ranging from several months prior to breeding (e.g. Robb *et al.*, 2008b) to a few days (e.g. Ramsay & Houston, 1997), and the few that have looked at effects on prenatal development often attribute differences to behavioural changes in the female rather than changes in egg characteristics (e.g. Wiebe & Bortolotti, 1994). In the experiment shown here we failed to find any effect of supplementary food on female behaviour, either through clutch size or incubation. Nevertheless, there is limited evidence that

supplementary feeding can alter egg characteristics. Effects on egg size in particular have been investigated, and some increases in response to supplementary food have been found (Wiebe & Bortolotti, 1995; Horsfall, 1984; Ramsay & Houston, 1997), although the results are not always replicated (Nilsson & Svensson, 1993; Nager *et al.*, 1997), and the majority of studies have failed to find any significant effect of feeding (reviewed in Christians, 2002). A recent paper from Ruuskanen *et al.* (2016) carried out a similar experiment to the one shown here, and found increased egg mass in fed nests. However, there appear to be differences between mass of eggs in control and fed treatments prior to feeding, and thus the conclusions from this may be questionable.

In this study we fail to see any major effect of the environment on the prenatal development of chicks, either through the provision of food or through correlations with the weather at the time of laying. Thus it seems that egg characteristics and prenatal development are insensitive to external conditions as experienced by the mother. Consistent with this insensitivity is the high repeatability of egg size (Christians, 2002; Williams, 2012), and other egg constituents (Postma *et al.*, 2014), within females. This suggests that the observed laying-order effects are regulated by the female without regard to her external environment. If the laying-order effects are the result of differential resource provisioning by the female, then we suggest that this is in anticipation of the level of sibling competition the chicks will experience. However, if the laying-order effects are mediated by maternal androgens our results are silent as to whether they are passive by-products of the females endocrinological state or whether they constitute an anticipatory parental effect.

Chapter 3

Selection on parental performance opposes selection for larger body size in a wild population of blue tits

Abstract

There is abundant evidence in many taxa for positive directional selection on body-size, and yet little evidence for microevolutionary change. In many species, variation in body size is partly determined by the actions of parents, so a proposed explanation for stasis is the presence of a negative genetic correlation between direct and parental effects. Consequently, selecting genes for increased body size would result in a correlated decline in parental effects, reducing body size in the following generation. We show that these arguments implicitly assume that parental care is cost free, and that including a cost alters the predicted genetic architectures needed to explain stasis. Using a large cross-fostered population of blue tits, we estimate direct selection on parental effects for body-size, and show that it is negative. Negative selection is consistent with a cost to parental care, and mainly acts through a reduction in current fecundity rather than survival. Under these conditions, evolutionary stasis is possible for moderately negative genetic correlations between direct and parental effects. This is in contrast to the implausibly extreme correlations needed when care is assumed to be cost free. Thus, we highlight the importance of accounting correctly for complete selection acting on traits across generations.

3.1 Introduction

Directional selection acting on a trait causes within-generation change in the mean. Given heritable (additive) genetic variation, part of this change should be passed onto the following generation, causing evolutionary change (Lush, 1943; Lande, 1979; Lande & Arnold, 1983). Kingsolver *et al.* (2001) showed that directional selection on quantitative traits is relatively common, particularly for body size, where positive directional selection predominates (Kingsolver & Pfennig, 2004). However, those studies able to measure microevolutionary change often find that it is absent, despite

estimates of selection and inheritance suggesting it should exist (e.g. Milner *et al.*, 1999, 2000; Charmantier *et al.*, 2004). Many hypotheses for this evolutionary stasis have been proposed (Merila *et al.*, 2001) and investigated (e.g. Kruuk *et al.*, 2001; Siepielski *et al.*, 2009; Morrissey & Hadfield, 2012; Kingsolver & Diamond, 2011), yet none have satisfactorily explained the patterns seen.

One hypothesis to explain the paradox of stasis is that the focal trait under selection may have antagonistic genetic correlations with other traits, which are also under selection. This could restrict microevolution (Lande, 1979; Lande & Arnold, 1983), and evidence for antagonistic genetic correlations between traits have been found in various wild populations (Gratten *et al.*, 2008; Ohno & Miyatake, 2007; Morrissey *et al.*, 2012). Body size in many species is determined by both the genes of an individual, and the genes in its parent(s) that determine the amount of parental care that is provided to them. Under this situation, the type of genetic correlations that act antagonistically become more complex, but a solution is formalised in the Willham model (Willham, 1963, 1972). Rather than explicitly considering all traits underlying parental care, the Willham model considers a composite trait, parental performance. This is a value assigned to an individual which represents the effect that individual has (through all aspects of its phenotype) on the body size of its offspring, measured in units of the offspring trait. If negative genetic correlations exist between body size and parental performance, an individual that possesses alleles for a large body would also possess alleles for lower parental performance, reducing the size of offspring they produce. This restricts net change in body size, as selection for increased body size leads to a concomitant decline in parental performance, and therefore body size in the following generation. Willham (1972) showed that when the selection gradient on body-size is β_b, evolutionary change in body-size has the form:

$$\Delta\bar{z}_b = (g_{b,b} + \frac{3}{2}g_{b,p} + \frac{1}{2}g_{p,p})\beta_b \tag{3.1}$$

where $g_{b,b}$ is the additive genetic variance for body-size, $g_{p,p}$ the additive genetic variance for parental performance (often called the maternal genetic variance) and $g_{b,p}$ is the additive genetic covariance between the two traits. Consequently, if the additive genetic covariance is sufficiently negative, the response to selection may become zero despite selection for increased body size. However, for this to occur, the additive genetic correlation would have to be close to -1. Given that the traits underlying parental performance are likely to be developmentally distinct from body-size, and expressed in different life-stages, such an integrated genetic architecture seems unlikely. The genetic correlation is routinely estimated in the field of animal breeding (e.g. Meyer, 1992; Robinson, 1996) and the mean estimate from domestic ungulates, although negative, is far from -1 (-0.167 $\pm$ 0.026 SE; Wilson & Réale, 2006; Räsänen & Kruuk, 2007). Estimates from non-domestic populations are far fewer, although they have been obtained for captive populations (Blomquist & Williams, 2013), as well as wild animals (McAdam *et al.*, 2002; Wilson *et al.*, 2005a) and plants (Thiede, 1998; Galloway *et al.*, 2009). Wilson *et al.* (2005a) obtained an estimate of -0.41$\pm$0.25 SE for the genetic correlation between birth weight and parental performance in Soay sheep (*Ovis aries*), which is suggestive of a negative relationship, but insufficiently strong to explain evolutionary stasis.

The Willham model (Willham, 1972) was developed in an animal breeding context, where the only target of *artificial* selection is the focal trait, body-size. Cheverud (1984) highlighted that *natural* selection is unlikely to operate in such a manner, and that selection is likely to also operate on parental performance. Under these conditions evolutionary change in body-size has the form:

$$\Delta\bar{z}_b = (g_{b,b} + \frac{3}{2}g_{b,p} + \frac{1}{2}g_{p,p})\beta_b + (g_{b,p} + g_{p,p})\beta_p \tag{3.2}$$

Importantly, selection on parental performance (β_p) is likely to be negative, because parents are expected to pay a cost, in terms of their own fecundity and sur-

vival, when providing care for their offspring (Hadfield, 2012). Through this route, a negative genetic correlation actually facilitates the evolution of increased body-size, and the conclusions drawn from the simple Willham model depend critically on the cost of parental care. Rollinson & Rowe (2015) demonstrate that the majority of positive selection on body size has been measured in juveniles, and trade-off's exist between offspring body size and parental fecundity, highlighting the cost to parents of producing larger offspring, and lending support to this extension of the Willham model.

Support has been put forward that appear to justify the assumption that parental performance is not under negative directional selection, and that $\beta_p = 0$ (or even positive; Einum & Fleming, 2000; McAdam & Boutin, 2004). However, it has been shown that this mistaken notion arises when fitness measures from behavioural ecology, such as the number of recruits an individual produces, are used rather than the fitness measure appropriate for a quantitative genetic model: the number of zygotes (Cheverud & Moore, 1994; Hadfield, 2012). Theoretical predictions show caring should be costly to the parent (Williams, 1966; Trivers, 1972; Stearns, 1992) and this is supported by a large body of empirical work, showing costs to parents in terms of reduced immunity, increased oxidative stress and predation risk, and depletion of micronutrients (reviewed in Alonso-Alvarez & Velando, 2012). These ultimately lead to a reduction in future fecundity (Török *et al.*, 2004), or decreased survival probabilities (Nur, 1984b; Dijkstra *et al.*, 1990; Owens & Bennett, 1994).

Currently, no attempt has been made to estimate the sign and strength of selection acting directly on parental performance. In part, this may reflect the statistical difficulty of relating parental performance (which is measured as an effect on offspring phenotype) to the survival and fecundity of the parents. By developing appropriate statistical methods we measure selection on parental performance using measures of body size, survival and fecundity from a long-term cross-fostering experiment in blue tits (*Cyanistes caeruleus*). We show that when appropriate fitness

measures are used, selection on maternal and paternal performance is negative, and that moderate negative genetic correlations between body-size and parental performance could result in evolutionary stasis.

3.2 Methods

This study was carried out on a nest box population of blue tits, on the Dalmeny estate, Edinburgh, UK (see Figure A.1 in Appendix **??**). This population consists of 225 boxes on Craigie Hill (grid reference NT156766) and beside the Almond River (NT179758), spaced approximately 30m apart. The data used here were collected from 2010 to 2014, with additional data from 2015 used to measure survival. Boxes were visited regularly from early April, such that in the vast majority of cases the first egg of a nest was found on the day it had been laid (see Hadfield *et al.*, 2013a).

3.2.1 Cross-foster Design

On the day the first egg was laid within a nest, the nest was assigned to a cross-fostering group. Where possible these were groups of three nests, and if not possible other group sizes were also used (from 2 to 5). Within each group, eggs were crossed on the day they were laid; the egg in nest A was moved to B, B to C, and C to A. Alternate eggs were crossed within the groups, so the first egg was cross-fostered, the second egg was not, the third was, and so on. In 2014 a different design was used, as part of a separate experiment. All nests were assigned into pairs on the day they initiated laying and each pair was assigned into one of three groups. For the first group the eggs were crossed in the manner explained above, for the second group all eggs were switched between the two nests, and for the third all eggs remained in their nest of origin. When interruptions in the laying sequence happened on a crossing day, the cross was postponed until all nests had laid again. Cross-fostering and nest visits ceased when one of the nests within a group finished laying, or indications of

incubation were found two days in a row (i.e. the female was on the nest or the eggs were warm).

During 2010-2011 a further cross-fostering experiment was implemented. In this experiment, single eggs in 114 out of 276 clutches were cross-fostered into nests that were not part of their original group. These eggs were either the first in the laying sequence or close to the end. In half the cases these cross-fostered eggs replaced eggs that were at the opposite end of the laying sequence. These data were not excluded, as treatment had little effect on chick mass or survival (Hadfield *et al.*, 2013b). In 2012-2013 a feeding experiment was performed, in which 76 out of 247 nests were food supplemented during egg-laying. This had little effect on any pre-natal aspects of development (*Thomson et al. unpublished*), so we do not expect to see any post-natal effects that would affect the analyses here, therefore these data were also included.

3.2.2 Data Collection

Nests were checked daily for hatching from around 11 days after the last egg was laid. In the majority of nests, we found newly hatched chicks on the first day that any hatched (rather than first finding chicks when they were one or more days old). When chicks first hatched (day 0) they were uniquely marked by clipping tufts of down on their head (and if necessary a toe-nail). Any chicks that hatched on subsequent visits (day 1 or 3, no chicks hatched after this point) were also clipped. Nests were visited on days 0, 1, 3, 6, 9, 12 and 15 (what we will call "nest age") when all chicks were weighed and mortality recorded. Blood samples were taken on day 3 from the medial metatarsal vein (see Appendix A), under home office license. On day 9 chicks were ringed. Post-fledging, the nests were revisited and the identities on any unfledged (dead) chicks recorded.

From day 10 onwards, parents were caught in nest boxes, or if necessary by mist

net in front of the box. These adults were identified (if previously caught) or ringed, and blood samples were taken from the ulna vein. Adults were also given colour rings which identified the year in which they were first caught as adults and their sex. The presence of any adults, and the colours of the rings seen (if any) were noted at nest visits during the egg laying and nestling stage.

3.2.3 Genotyping and Pedigree

Genotypes were obtained for all chicks, adults and unhatched (fertile) eggs for which there were available blood or tissue samples. DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), and genotyped at seven polymorphic microsatellite markers (Olano-Marin *et al.*, 2010). See Hadfield *et al.* (2013a) for full molecular methods.

The pedigree of sampled individuals was reconstructed using a Bayesian Markov chain Monte Carlo approach, MasterBayes (Hadfield *et al.*, 2006). Each chick had between one and three potential mothers, due to the cross-fostering design. Maternity was restricted to these females – it was assumed that mixed maternity clutches did not occur. Paternity was not restricted, but the odds that a male caught at a nest was also the sire of the chicks originally from that nest was simultaneously estimated with the pedigree. Likewise, we estimated the rate at which the probability of paternity decays with distance between the nests attended by a male and the nest from which the chicks originally came. Where one or both adults were unsampled from a nest, a non-genotyped dummy adult(s) was included in the model. The size of the unsampled male population was also estimated, representing males that gained paternity but were not associated with a specific nest. Individuals that were assigned to unsampled males with greater than 50% posterior probability were assigned into paternal sibships, using rcolony (Jones & Wang, 2010), see Hadfield *et al.* (2013a).

3.2.4 Assignment of Social Parents

In order to assess selection on parental performance, the social parents (i.e. the caregivers) at each nest need to be known. Any individual caught at a nest was assumed to be the social male/female for that nest. In this manner we assigned social mothers to 493/554 nests and fathers to 413/554 nests. The remaining females were generally not caught as all chicks died too early, and so were assigned dummy identities. The genetic sire with the largest proportion of paternity in a nest was assigned as the social father for the remaining 141 nests where a male was not caught. This could be either a male caught at a different nest that year, or an unsampled male with a dummy identity. In 7 cases two or more males tied, and so a (new) dummy male was assigned to be the social father. Had this method been used to assign a social father in nests where a male had been caught, the correct male would have been assigned in 92% of cases.

The number of adult birds present at a nest, and often their sex-specific colour ring, was recorded during nest visits. This allowed us to get a score of male attendance at each nest, which provided a measure of how much care a male was likely to be giving to his offspring – males that had higher nest attendance were expected to be providing more care. We restricted this to visits made after the first egg had been laid in a nest. This was done to avoid bias, as nests that commence laying later in the season are likely to have been visited a greater number of times than those that lay early, and so would have greater sampling effort if all visits prior to laying were included. We scored male attendance as a 0 if no male had ever been seen, 1 if a male had been seen but was not caught (and therefore providing no, or very little care), and 2 if the male had been caught (therefore providing care, as adults were caught when they were in the nest). There were 31, 126, and 413 nests in each of these categories. In the models for chick traits, the male attendance score for the nest-of-rearing was fitted as a continuous variable. In the models for adult

male fitness components, an individual's average male attendance score over nests in that year for which he was assigned as a social father was used, and fitted as a continuous variable.

The average rate of extra-pair paternity in this population is 18.5%, although this varies between nests depending on the male attendance levels – 17.3% where males have been caught, 21.1% where males have been seen but not caught, and 27.8% where no male has been seen. In addition, 16.4% of males are polygamous (social male at more than one nest), and of these, 22.1% have been caught at both nests at which they are social father.

3.2.5 Statistical Methods

Mass and survival were analysed using a bivariate mixed model. This model included 25900 weights and 28800 survival events (an individual either survives or dies from one age to the next) from 4336 individual chicks in 554 nests, along with 1024 adult survival events from 689 adult birds. We excluded 57 chicks from 8 nests from the analysis used in the dataset. The majority of these were nests found more than one day after chicks had hatched, such that the exact hatching day and age of chicks could not be estimated.

The mass variable in the bivariate model is the juvenile mass across the 7 nest ages at which weights were taken, fitted as a Gaussian response. The model for the body-mass of individual i on day d of year y has the form:

$$b_{iyd} = \mathbf{x}^{(b)}_{iyd}\boldsymbol{\beta}^{(b)} + \mathbf{z}^{(b)}_{iyd}\boldsymbol{u}^{(b)} + (\beta^{(b)}_{f} + d\beta^{(b)}_{f:d}) \cdot f_{ny} + (\beta^{(b)}_{c} + d\beta^{(b)}_{c:d}) \cdot c_{sy} + m_{nyd} + p_{syd} + e^{(b)}_{iyd} \tag{3.3}$$

where $\boldsymbol{\beta}^{(b)}$ are generic fixed effects and $\mathbf{X}^{(b)}$ their associated design matrix. These effects are important to include in the model due to their effects on mass, but are

nuisance parameters with respect to the selection analyses, and include nest age (as a factor), sex, day of hatching within the nest (factor, hatched on day 0, 1 or 3), year, time of day, and overall nest hatching day (days from April 1st). Interactions between all terms and nest age (as a continuous variable) were included to capture changes in the effects of these factors over ontogeny (see Hadfield *et al.* (2013a)). $\beta_f^{(b)}$ is the main effect of female fecundity (f_{ny}; clutch size of nurse/social mother n) on body mass and $\beta_{f:d}^{(b)}$ determines how that effect changes with the age of the chicks. $\beta_c^{(b)}$ and $\beta_{c:d}^{(b)}$ are equivalent terms for male attendance (c_{sy}); subscript s denotes social father. $\mathbf{u}^{(b)}$ are generic random effects and $\mathbf{Z}^{(b)}$ their associated design matrix; these were a nest-of-origin effect, which captures prenatal maternal effects, and the genetic effect, estimated using pedigree information. The age specific random effects were assumed to be drawn from a multivariate normal distribution parameterised by a 7x7 covariance matrix (representing the seven days on which weights were taken). In both cases this covariance matrix was approximated using a lower dimensional first-order antedependence structure similar to the autoregressive structure used in Hadfield *et al.* (2013a), and shown to outperform alternative techniques such as random regression. The six lagged regression coefficients were allowed to vary over age, as were the seven innovation variances.

m_{nyd} and p_{syd} are a third set of random effects associated with the identities of the social parents at a nest, within a year, and together capture the variance in weight at each age due to nest-of-rearing. These therefore estimate the parental performance of the adults caring at each nest. These were fitted as a multimembership model such that the variance of the two effects (male and female) were assumed equal. A small amount of information exists to separate nest-of-rearing effects into nurse and social father effects, as some males are social sires for more than one nest per year. This model would only be weakly identifiable, so we chose to use a multimembership model that explicitly assumes parental performance is equally divided. The 7x7 covariance matrices for these effects were unstructured and we denote them

as $\boldsymbol{V}_m = \boldsymbol{V}_p$. The covariance structure for the residual mass effects ($e^{(b)}_{iyd}$) were also unstructured.

Survival was fitted as a threshold model. Individuals that were alive at time d could either survive (1) or die (0) by time $d + 1$. During the juvenile stage individuals have an event history over 8 ages (0, 1, 3, 6, 9, 12, 15, 25 days, where 25 day represents fledging) characterised by a series of 1's (time periods over which an individual is known to have survived) followed by a 0 (the time period over which an an individual was know to have died) or a 1 (at day 25, if the individual recruited). Adult survival was also modelled as an event history (see equation 3.5 and associated descriptions below), although survival occurs between years rather than days. Observations for time periods prior to hatching, or after death are omitted. The fixed, random, and residual effects on survival were dependent on the life-stage (juvenile or adult) being considered. Juvenile survival from day d to day $d + 1$ has the form:

$$j_{iyd} = \mathrm{I}\left(\mathbf{x}^{(j)}_{iyd}\boldsymbol{\beta}^{(j)} + \mathbf{z}^{(j)}_{iyd}\boldsymbol{u}^{(j)} + (\beta^{(j)}_b + d\beta^{(j)}_{b:d}) \cdot b_{iyd} + e^{(j)}_{iyd} > 0\right) \tag{3.4}$$

where I is the indicator function. The generic fixed effects are the same as those for mass, but also included the nest-of-rearing clutch size and male attendance. This model also includes the effect of an individual's mass on age-specific survival, $\beta^{(j)}_b$, along with its interaction with day, $\beta^{(j)}_{b:d}$. Two generic random effects were included – a nest-of-rearing effect, and a nest-of-rearing by nest-age effect. This nest-by-age effect accounts for any daily variation in nest-specific survival probabilities, for example due to desertion of the brood. Nest-of-origin was not included, as it was found to be of very small magnitude in Hadfield *et al.* (2013a). The residual variance for survival ($e^{(j)}_{iyd}$) was fixed at 1 as in standard probit analysis.

Adult survival from year y to year $y + 1$ has the form:

$$a_{iy} = \mathrm{I}\left(\mathbf{x}_{iy}^{(a)}\boldsymbol{\beta}^{(a)} + \delta_i\left[\beta_f^{(a)} \cdot f_{iy}\right] + (1-\delta_i)\left[(\beta_c^{(a)} \cdot c_{iy}\right] + e_{iy}^{(a)} > 0\right) \tag{3.5}$$

Generic fixed effects ($\boldsymbol{\beta}^{(a)}$) for adult survival were sex and clutch start date (for females). We include two sex-specific fixed effects: the effect of clutch size on female survival ($\beta_f^{(a)}$), and the effect of male attendance on male survival ($\beta_f^{(c)}$). δ_i is 1 when individual i is female, and 0 when male. No random effects were included for adult survival, although importantly the residual adult survival effects ($e_{iy}^{(a)}$) were allowed to covary with the social parent random effect on juvenile mass (m_{nyd}; see SI), giving the 8x8 covariance matrix:

$$\begin{bmatrix} \mathbf{V}_m & \mathbf{c}_{m,a} \\ \mathbf{c}_{m,a}^{'} & 1 \end{bmatrix} \tag{3.6}$$

We assume the male covariance matrix is the same as the female covariance matrix above. $\mathbf{c}_{m,a}$ is a vector of covariances between age-specific nurse effects and residual nurse survival, the variance of which is fixed at one. Below we show how these covariances can be used to estimate viability selection acting on parental performance.

In addition, we ran a univariate model of adult fecundity. Female fecundity was taken to be her clutch size (i.e. the number of eggs she laid). Male fecundity included all those chicks he sired in his own and other nests. This model included separate intercepts for the two sexes ($\beta_{\female}^{(f)}$ and $\beta_{\male}^{(f)}$), as well as male nest attendance (for males only; continuous covariate). Residual variances were estimated separately for males and females. Although fecundity is often fitted as a Poisson response, it tends to be under-dispersed with respect to the Poisson (Kendall & Wittmann, 2010). Our data shows the same distribution, so we treated it as Gaussian. The model has the form:

$$f_{iy} = \delta_i \beta_{\text{♀}}^{(f)} + (1 - \delta_i) \left[\beta_{\text{♂}}^{(f)} + \beta_c^{(f)} \cdot c_{iy} \right] + e_{iy}^{(f)} \tag{3.7}$$

As with adult survival, we excluded unknown adults from this model, as their exact fecundity could not be known.

Both models were fitted in MCMCglmm (Hadfield, 2010) in the statistical program R (R Development Core Team, 2012). For the random effects associated with juvenile survival (nest-of-rearing, and nest by nest age), flat improper priors were used. The nest-of-origin and genetic random effects on juvenile mass had parameter expanded priors for the autoregressive structure, with a high scale (10) for the innovation variances. The autoregressive coefficients had a prior mean of 0, and variances of 10. The random-residual prior covariance (social parent effect on mass and adult survival) followed an inverse-Wishart distribution. The degree of belief parameter was set to the dimensions of the prior matrix minus 3, and the prior scale matrix was set close to zero. Under a simple model, this prior should give posterior modes for the variances and covariances close to the REML estimators. The same prior was also used for the residual covariance matrix for mass. For those parts of the model that could be fitted with ASReml (Gilmour *et al.*, 2009), the posterior modes from this model and the REML estimates were very similar.

3.2.6 Selection gradients

The statistical model allows estimation of how the terms we included in parental performance are related to both survival and fecundity. Figure 3.1 shows how the parental performance traits and offspring body mass are expected to influence both individual fitness components, and ultimately total fitness (lifetime reproductive success – LRS). In order to obtain the selection gradients acting upon these traits, we first transform the results from the model, which allows us to obtain their causal effects on fitness (explained fully in the generation of Equations 3.10, 3.11, and

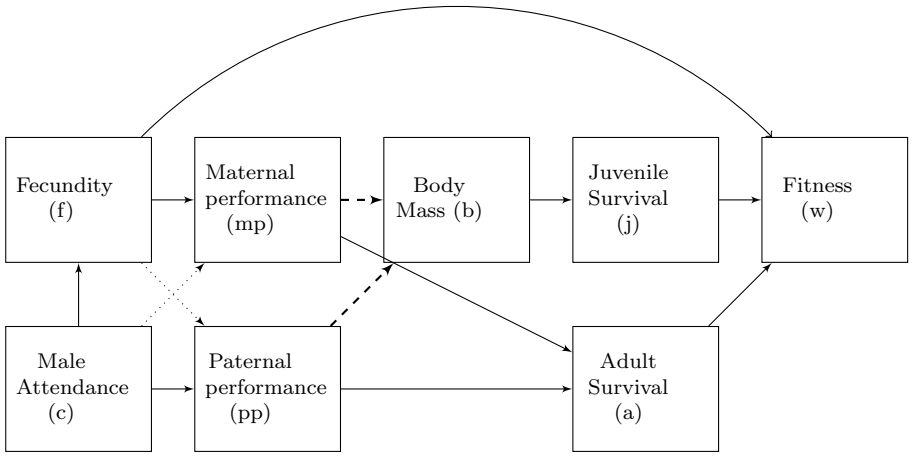


Figure 3.1: Path diagram showing the connections between the traits and fitness components considered within the model. Solid lines represent those connections that are included in the models and involve an individual affecting themselves (e.g. paternal effect an individual provides affects their *own* survival), whereas dashed lines represent parental effects on their offspring (e.g. paternal effect an individual provides affects their *offsprings'* body mass). Dotted lines represent possible connections that are not included in the model, as we are not considering the effects of the two parents on each other. Male attendance is assumed to affect their own fecundity (not that of the female).

3.12 below). These causal effects allow us to relate the traits to individual fitness components. However, we are interested in the selection acting through total lifetime fitness, rather than through single fitness components. We show how these fitness components combine to generate LRS in Equations 3.13 and 3.14. We then take the partial derivatives of LRS with respect to the parameters of interest, whilst averaging over the fixed and random effects that are not of interest with regard to the estimation of selection (Lande & Arnold, 1983) (Equation 3.15). We do this over each MCMC iteration from the statistical model, and so obtain posterior distributions for the selection gradients.

We define total maternal performance *given* (not received) on day d as:

$$mp_{iyd} = \delta_i \left[(\beta_f^{(b)} + d\beta_{f:d}^{(b)}) \cdot f_{iy} + m_{iyd} \right] \tag{3.8}$$

and total paternal performance given as:

$$pp_{iyd} = (1 - \delta_i) \left[(\beta_c^{(b)} + d\beta_{c:d}^{(b)}) \cdot c_{iy} + p_{iyd} \right] \tag{3.9}$$

Where $\beta_f^{(b)}$ is the effect of female fecundity on offspring body mass, $\beta_{f:d}^{(b)}$ its change with offspring age, and mp_{iyd} the female portion of parental performance – the effect on offspring weight attributed to the nurse. $\beta_c^{(b)}$, $\beta_{c:d}^{(b)}$ are the equivalents for the effect of male attendance, and pp_{iyd} the social sire's parental performance.

In order to derive selection gradients for these parental performance traits it is necessary to define them as having a causal effect on fitness, as shown in Figure 3.1, rather than the correlative model defined in Equation 3.6, which shows the parental effect on offspring mass, along with its covariance with residual adult survival. We can consider the regression of adult survival on age specific parental effects as (for maternal effects): $\boldsymbol{\beta}_m^{(a)} = \mathbf{V}_m^{-1}\mathbf{c}_{m,a}$ (where $\mathbf{c}_{m,a}$ is the vector of the covariance of residual nurse survival with age-specific parental performance, from the matrix in equation 3.6), and note that $\boldsymbol{\beta}_p^{(a)} = \boldsymbol{\beta}_m^{(a)}$ by assumption. This gives:

$$\begin{aligned} a_{iy} = \; & I\Big(\mathbf{x}_{iy}^{(a)}\boldsymbol{\beta}^{(a)} + \delta_i \left[\textstyle\sum_d \beta_{m:d}^{(a)} \cdot m_{iyd}\right] + (1 - \delta_i) \left[\textstyle\sum_d \beta_{p:d}^{(a)} \cdot p_{iyd}\right] + \\ & \qquad \delta_i e_{iy}^{(a|m)} + (1 - \delta_i) e_{iy}^{(a|p)} > 0\Big) \\ = \; & F_N\Big(\mathbf{x}_{iy}^{(a)}\boldsymbol{\beta}^{(a)} + \delta_i \left[\textstyle\sum_d \beta_{m:d}^{(a)} \cdot m_{iyd}\right] + (1 - \delta_i) \left[\textstyle\sum_d \beta_{p:d}^{(a)} \cdot p_{iyd}\right], \\ & \qquad 0, \sigma_e^{(a|m)}\Big) \end{aligned} \tag{3.10}$$

$e^{(a|m)}$ is the residual adult survival after conditioning on the maternal random effects, with standard deviation $\sigma_e^{(a|m)} = \sqrt{1 - \mathbf{c}'_{m,a}\boldsymbol{\beta}_m^{(a)}}$. F_N is the cumulative distribution function for the normal distribution. Again, $\sigma_e^{(a|m)} = \sigma_e^{(a|p)}$. It is important to realise that adult survival is determined by the parental performance an individual expresses (rather than receives) in this model.

In the statistical model female fecundity is a predictor of mass, and consequently maternal performance. The fitness function requires the inverse of this, such that maternal performance predicts fecundity, and so we obtain this as $\beta^{(f)}_{b:d} = \left[\beta^{(b)}_{f} + d\beta^{(b)}_{f:d}\right]^{-1}$. Biologically, one can envision an individual deciding how much care it is willing to give, and then modulating its fecundity based upon this decision. The selection model for fecundity therefore has the form:

$$f_{iy} = \delta_i \beta^{(f)}_{\female} + (1-\delta_i)\beta^{(f)}_{\male} + (1-\delta_i)\beta^{(f)}_{c} \cdot c_{iy} + e^{(f)}_{iy} + \delta_i(\sum_{0}^{d} \beta^{(f)}_{b:d}) \cdot b_{iy} \tag{3.11}$$

These transformations represent the model described by the path diagram in Figure 3.1. The model for juvenile survival remains identical to that given in the statistical section, although it can also be written in probit rather than threshold form:

$$j_{iyd} = F_N\left(\mathbf{x}^{(j)}_{iyd}\boldsymbol{\beta}^{(j)} + \mathbf{z}^{(j)}_{iyd}\boldsymbol{u}^{(j)} + (\beta^{(j)}_{b} + d\beta^{(j)}_{b:d}) \cdot b_{iyd}, 0, 1\right) \tag{3.12}$$

Equations 3.10, 3.11, and 3.12 above relate the parameters of interest to each of the three fitness components that comprise total fitness. However, we are interested in obtaining estimates of the total selection that occurs upon body mass and parental performance, and we do this by deriving the expected life-time reproductive success (LRS). This can be considered as the sum of annual fitnesses: the product of age specific fecundity (f_t) and survival to that age ($\prod_{y=1}^{t} s_y$):

$$W = \sum_{t=1}^{\infty} f_t \prod_{y=1}^{t} s_y \tag{3.13}$$

In our model survival to the first breeding attempt is $s_1 = \prod_d j_d$, where d is taken over the measurement days 0, 1, 3, 6, 9, 12, 15 and 25. We assume annual survival

thereafter is constant with age ($s_t = a \; \forall \; t > 1$), as is fecundity. Consequently, Equation 3.13 is a geometric series and can be simplified:

$$W = \frac{f \prod_d j_d}{1 - a} \tag{3.14}$$

We partition the variables that vary over individuals into traits of interest $\boldsymbol{\theta} = [b, mp, pp]^{'}$ and the remaining fixed effect predictors and random effects not of inherent interest $\boldsymbol{\eta}$. The selection gradients on the traits of interest are given by (Lande & Arnold, 1983):

$$\boldsymbol{\beta}_\theta = \frac{E[\partial W(\theta, \eta)/\partial \theta]}{E[W(\theta, \eta)]} = \frac{\int_\theta \int_\eta (\partial W(\theta, \eta)/\partial \theta) p(\theta, \eta) d\theta d\eta}{\int_\theta \int_\eta W(\theta, \eta) p(\theta, \eta) d\theta d\eta} \tag{3.15}$$

The vector of partial derivatives can be calculated analytically (using the Dtensor function in MCMCglmm), but the integration is done numerically. To achieve this we sampled with replacement rows of the design matrices for each model, and simulated random effects according to their inferred distribution (a multivariate normal with estimated parameters). This was done 100,000 times in order to get an accurate estimate of the expectations. This procedure was repeated for every stored MCMC iteration in order to obtain a posterior distribution for $\boldsymbol{\beta}_\theta$.

As $\boldsymbol{\theta} = [b, mp, pp]^{'}$, the selection gradients are estimated for juvenile body mass, maternal performance and paternal performance. These parental performance measures both consist of the performance measure derived from the nest-of-rearing random effect in the model, and each of the sex-specific fixed effects (female fecundity and male nest attendance), as shown in Equations 3.8 and 3.9.

3.3 Results

Juvenile Mass: The fixed effect for mass are summarised in Table 3.1. Broadly, the

	Juvenile Mass					**Juvenile Survival**				
	mean	l-95%	u-95%	pMCMC	p.Wald	mean	l-95%	u-95%	pMCMC	p.Wald
Day 0	0.591	0.455	0.727	**<0.001**		5.057	2.349	8.314	**<0.001**	
Day 1	1.104	0.947	1.248	**<0.001**		4.512	1.733	7.352	**<0.001**	
Day 3	2.563	2.307	2.817	**<0.001**		1.708	-0.841	3.995	0.158	
Day 6	5.590	5.134	6.054	**<0.001**		0.298	-1.851	2.470	0.816	
Day 9	8.541	7.887	9.252	**<0.001**		-2.916	-5.015	-1.035	**0.001**	
Day 12	10.144	9.233	11.043	**<0.001**		-2.986	-4.861	-0.729	**0.004**	
Day 15	10.777	9.717	11.968	**<0.001**		-1.491	-3.959	0.677	0.215	
Day 25						-0.099	-4.651	3.969	0.958	
Sex (M)	0.006	-0.000	0.013	0.067		-0.393	-0.609	-0.186	**<0.001**	
Sex (F) : Day	-0.032	-0.035	-0.029	**<0.001**	**<0.001**	-0.012	-0.027	0.002	0.125	0.120
Hatch Day (0-1)	-0.177	-0.188	-0.166	**<0.001**		-0.128	-0.404	0.121	0.333	
Hatch Day (0-1) : Day	-0.024	-0.029	-0.019	**<0.001**	**<0.001**	-0.006	-0.023	0.010	0.47	0.454
Hatch Day (1-3)	-0.713	-0.770	-0.657	**<0.001**		0.044	-0.546	0.545	0.872	
Hatch Day (1-3) : Day	-0.035	-0.047	-0.024	**<0.001**	**<0.001**	-0.019	-0.056	0.016	0.288	0.293
Year.2011	-0.031	-0.065	0.004	0.072		-0.817	-1.607	-0.005	**0.043**	
Year.2011 : Day	-0.036	-0.055	-0.017	**<0.001**	**<0.001**	-0.106	-0.160	-0.048	**0.001**	**<0.001**
Year.2012	-0.032	-0.064	-0.001	**0.046**		-1.243	-2.012	-0.478	**0.002**	
Year.2012 : Day	-0.029	-0.046	-0.012	**0.002**	**<0.001**	-0.030	-0.079	0.020	0.245	0.244
Year.2013	-0.087	-0.128	-0.047	**<0.001**		-0.619	-1.535	0.434	0.219	
Year.2013 : Day	-0.027	-0.050	-0.006	**0.013**	**0.016**	0.008	-0.068	0.073	0.797	0.829
Year.2014	0.023	-0.012	0.055	0.189		0.501	-0.366	1.442	0.285	
Year.2014 : Day	-0.015	-0.034	0.002	0.1	0.096	-0.066	-0.117	-0.010	**0.017**	**0.016**
Clutch Size	-0.005	-0.010	0.001	0.082		-0.209	-0.358	-0.063	**0.006**	
Clutch Size : Day	-0.007	-0.010	-0.004	**<0.001**	**<0.001**	0.008	-0.002	0.018	0.152	0.151
Nest Hatch Date	0.003	0.001	0.005	**<0.001**		-0.010	-0.053	0.028	0.607	
Nest Hatch Date : Day	-0.002	-0.003	-0.001	**<0.001**	**<0.001**	-0.005	-0.008	-0.002	**0.002**	**0.001**
Male Attendance	0.006	-0.011	0.024	0.537		2.006	1.555	2.453	**<0.001**	
Male Attendance : Day	0.061	0.051	0.073	**<0.001**	**<0.001**	-0.044	-0.079	-0.003	**0.026**	**0.031**
Time	0.561	0.490	0.629	**<0.001**						
Time : Day	0.142	0.118	0.165	**<0.001**	**<0.001**					
Weight						1.639	1.420	1.836	**<0.001**	
Weight : Day						-0.051	-0.064	-0.037	**<0.001**	**<0.001**

Table 3.1: Fixed effect results for the juvenile response variables included in the multivariate model

results for mass showed the same patterns as those found in Hadfield *et al.* (2013a). Clutch size and male attendance were not included in Hadfield *et al.* (2013a) however, and both were found to have significant effects on juvenile mass, particularly after day 0. An increased clutch size led to decreased juvenile mass of -0.005 g/egg ([-0.010 - 0.001], P=0.082) at day 0, and -0.112 g/egg ([-0.161 - -0.063], P<0.001) at day 15, suggesting females provided less care to each individual offspring when they have a larger number to care for. Although male attendance alone had no significant effect on mass at day 0 (0.006 g/attendance-unit [-0.011 - 0.024] P=0.537), there was a strong positive interaction with day, such that there was an increase in juvenile mass of 0.927 g/attendance-unit ([0.769 - 1.099], P<0.001) at day 15 (i.e. a juvenile in a nest with a male feeding would have increased mass compared those where a male has been seen but never caught).

The proportion of variance in juvenile mass explained by the different random effects included in the model is shown in Figure 3.2. This shows that at day 0 the social parents (as a pair) account for 31.4% (26.8 - 35.9) of the variance in mass, which reflects differences between broods that are caused by variation in the time between nests hatching and chicks weighed (nests are visited every 24 hours). After day 6 they are the main driver of juvenile mass, and account for 73% (69.2 - 76.8) of the variance at day 15. The (co)variances in juvenile mass across ages explained by the random social parent effects are shown in Table 3.3, along with the covariance of these measures with adult survival (this is $\mathbf{c}_{m,a}$ from equation 3.6, discussed further in the Adult Survival section below).

Juvenile Survival: The fixed effects for juvenile survival are summarised in Table 3.1. The effect of mass on survival was positive and significant, indicating an increased survival probability with increased weight – survival improved by 1.622 probits/g ([1.420 - 1.836], P<0.001) at day 0. However, there was a negative interaction with day, such that the extent to which increased mass improved survival declined across ontogeny, such that survival was increased by 0.860 probits/g ([0.779

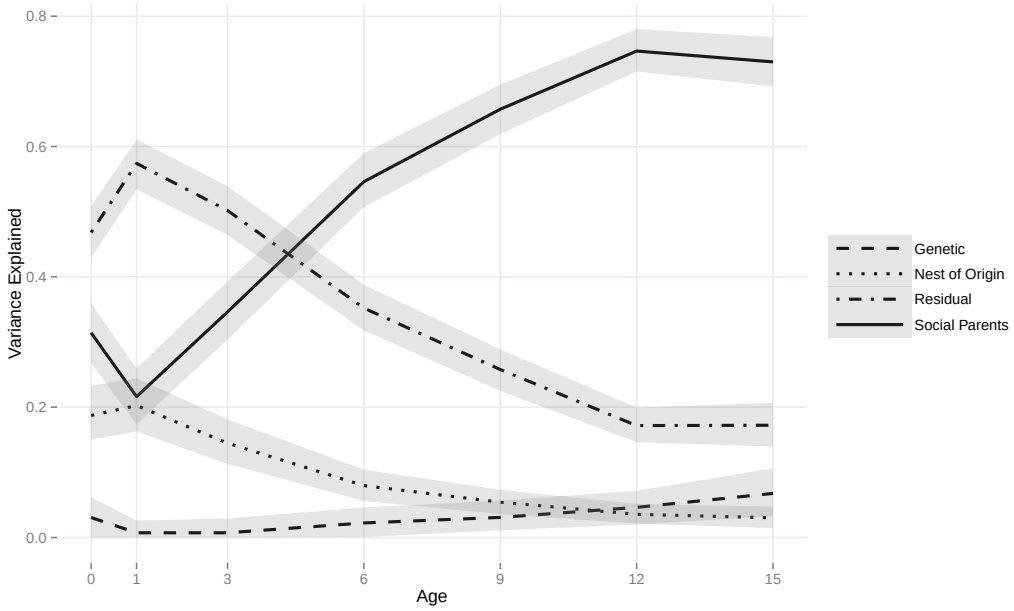


Figure 3.2: The proportion of variance in weight across ontogeny (nest age in days) explained by multiple factors included in the model. Lines are the posterior means, with shaded ribbons representing the 95% confidence intervals. The social parent effects (nest effects) are those that make up part of the parental performance.

	mean	l-95%	u-95%	pMCMC
Sex (F)	-0.000	-1.023	1.083	0.980
Sex (M)	0.120	-0.827	1.161	0.812
Nest Hatch Date (F)	-0.001	-0.015	0.012	0.859
Clutch Size (F)	-0.011	-0.077	0.055	0.741
Male Attendance (M)	-0.118	-0.637	0.391	0.663

Table 3.2: Fixed effect results for the adult survival variables included in the multivariate model

- 0.953], P<0.001) by day 15. The intraclass correlation for nest-of-rearing on juvenile survival was moderate, 0.205 [0.100 - 0.258], whereas the intraclass correlation for nest-of-rearing/day was high, 0.630 [0.556 - 0.711], which represents the fact that chicks in a nest often die in the same time period, due to parental desertion of the brood, as was found in Hadfield *et al.* (2013a).

Adult Survival: The fixed effects for adult survival are summarised in Table 3.2. None of the fixed effect predictors for adult survival were significant, including those that form part of maternal and paternal performance – female survival declined non-significantly with clutch size (change of -0.004 probits/egg [-0.077 - 0.055] P=0.741),

and increased nest attendance caused a non-significant decline in survival in males (-0.130 probits/attendance unit [-0.637 - 0.391] P=0.663). The covariance of adult survival with parental performance can be seen in Table 3.3. Although the estimates of the covariances are negative at later age stages, the confidence intervals overlap zero (e.g. the covariance is -0.0226 (-0.141-0.083) at day 15), and thus there seems to be little or no correlation between measures of parental performance and adult survival.

Adult Fecundity: The fixed effects, and residual variances, of adult fecundity are shown in table 3.4. Both the intercepts and residual variances are different for males and females. Males have higher fecundity than females when they do not attend their nest (male attendance score of zero), but increased male attendance (i.e. care) decreased fecundity by -6.208 eggs/attendance unit ([-7.773 - -4.772], P<0.001).

3.3.1 Selection Gradients

In order to present the selection gradients on an interpretable scale we use a mean standardised measure wherein the selection gradients are the effect on relative lifetime fitness of changing juvenile weight by 10% at the relevant age. Both mass and parental performance are on the same scale and so for juvenile mass this is the direct effect of a 10% change in body mass, whereas for the parental care traits, this is the effect that changing an offspring's mass by 10% has on an individuals own fitness.

Age-specific selection gradients are shown in Figure 3.3, together with age-constant selection gradients (i.e. the change in fitness caused by increasing weight by 10% at *all* ages). As was expected, there was significant positive selection on juvenile mass (age-constant selection 2.308 [1.536 - 3.335] P<0.001). The majority of this selection, however, occurs after nine days, with there being weak selection on mass early in ontogeny, e.g. selection is 0.001 ([0.000 - 0.002], P<0.001) at day

	0	1	3	6	9	12	15	Adult Survival
0	0.0048	0.0041	0.0080	0.0116	0.0078	0.0051	0.0033	0.0036
	(0.004-0.006)	(0.003-0.005)	(0.006-0.010)	(0.006-0.017)	(0.001-0.015)	(-0.004-0.014)	(-0.004-0.011)	(-0.005-0.013)
1	0.7846	0.0057	0.0127	0.0205	0.0167	0.0098	0.0046	-0.0025
	(0.731-0.840)	(0.004-0.007)	(0.010-0.016)	(0.014-0.026)	(0.008-0.026)	(-0.001-0.020)	(-0.004-0.014)	(-0.013-0.008)
3	0.5453	0.7986	0.0443	0.0943	0.1052	0.0832	0.0477	-0.0006
	(0.453-0.628)	(0.750-0.846)	(0.036-0.052)	(0.076-0.111)	(0.083-0.129)	(0.056-0.109)	(0.022-0.071)	(-0.026-0.029)
6	0.3122	0.5076	0.8429	0.2823	0.3761	0.3522	0.2293	-0.0034
	(0.188-0.417)	(0.405-0.606)	(0.807-0.877)	(0.239-0.325)	(0.317-0.440)	(0.283-0.420)	(0.165-0.287)	(-0.067-0.063)
9	0.1410	0.2759	0.6289	0.8913	0.6303	0.6843	0.4873	-0.0188
	(0.020-0.265)	(0.150-0.400)	(0.551-0.690)	(0.870-0.913)	(0.547-0.742)	(0.572-0.793)	(0.389-0.584)	(-0.110-0.084)
12	0.0750	0.1312	0.4040	0.6783	0.8825	0.9534	0.7662	-0.0174
	(-0.059-0.192)	(-0.000-0.262)	(0.296-0.498)	(0.617-0.733)	(0.858-0.906)	(0.820-1.111)	(0.632-0.895)	(-0.136-0.110)
15	0.0532	0.0676	0.2532	0.4829	0.6871	0.8789	0.7965	-0.0226
	(-0.077-0.172)	(-0.057-0.209)	(0.135-0.362)	(0.389-0.565)	(0.620-0.742)	(0.853-0.906)	(0.664-0.939)	(-0.141-0.083)
Adult Survival	0.0516	-0.0334	-0.0031	-0.0064	-0.0237	-0.0178	-0.0252	1.0000
	(-0.080-0.182)	(-0.180-0.109)	(-0.124-0.139)	(-0.123-0.123)	(-0.149-0.095)	(-0.144-0.106)	(-0.154-0.096)	

Table 3.3: Correlations and (co)variances for parental performance on juvenile mass at each age, along with the correlation and covariance of these with adult survival ($\mathbf{c}_{m,a}$ from Equation 3.6). Upper triangle of the matrix contains covariances, with variances on the diagonal. Lower triangle shows the correlations. Values in brackets are the upper and lower 95% confidence intervals.

	mean	l-95%	u-95%	pMCMC
Sex (F)	9.189	9.029	9.333	**<0.001**
Sex (M)	22.340	19.429	25.052	**<0.001**
Male Attendance	-6.419	-7.773	-4.772	**<0.001**
Residual Variances				
Sex (F)	3.030	2.654	3.397	
Sex (M)	13.545	11.719	15.343	

Table 3.4: Fixed effects and residual variances from the model of adult fecundity

0. The magnitude of selection is greatest at day 15 (1.999 [1.020 - 2.754] P<0.001), which results from the mass at this age affecting both the probability of an individual surviving to fledge, and then the probability of those fledged individuals recruiting to breed.

We present the selection gradients acting through different adult fitness measures separately, such that there is a single set of selection gradients acting through adult survival. This assumes that parental care affects survival in the same way for both sexes, along with sex specific selection gradients acting through fecundity. The selection gradients on parental performance acting through survival overlap zero at most ages (Figure 3.3, age-constant selection -0.077 [-0.333 - 0.144] P=0.460), although it is just significant at day 1 (-0.312 [-0.902 - 0.005] P=0.050), giving little evidence for a survival cost to parental care. The selection gradients on parental performance acting through current fecundity are negative. Age-constant selection acting through male attendance was marginal, -0.824 ([-2.014 - 0.125], P=0.053), and age-constant selection acting through female clutch size was significant, -0.282 ([-0.514 - -0.104], P<0.001). The estimates of selection at each age stage for male care are marginally significant and negative, apart from that at day 0, where the 95% confidence intervals overlap zero to a much greater degree. Thus, there is a tendency towards a fitness cost to males for increasing the mass of their offspring through changes in their attendance. There is significant negative selection on female care for all nest ages.

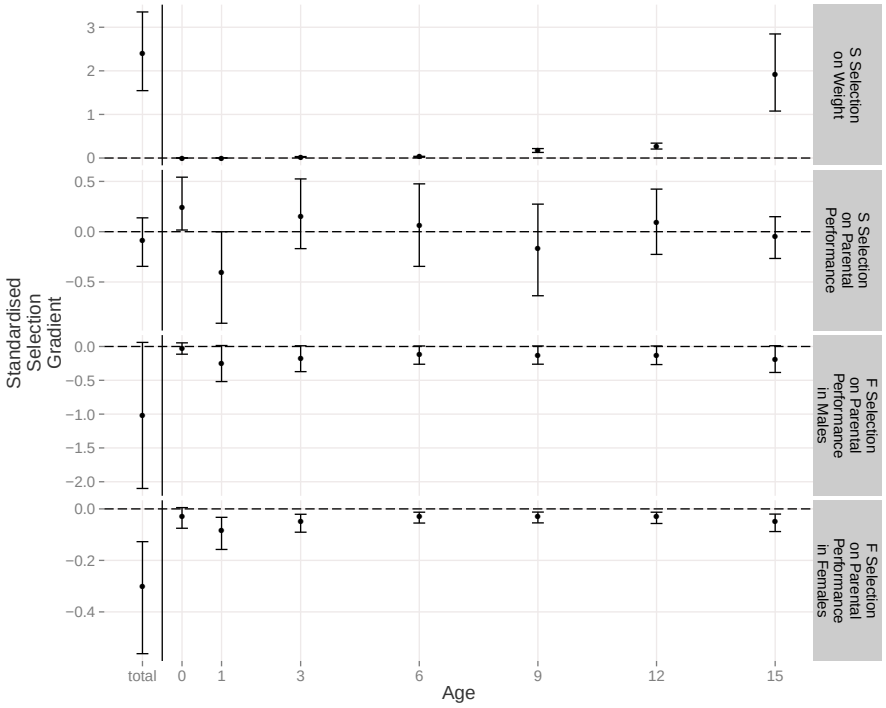


Figure 3.3: The selection gradients occurring upon the weight of juveniles, and then on care parameters of adults. Weight and non-sex-specific care measures affect fitness through their effects on survival (S Selection), whereas the sex specific care measures affect (current) fecundity (F Selection). The summed selection gradients, through their effects on juvenile weight/survival are shown (as total), as well as the split of selection across all ages of ontogeny at which nests are visited. Bars show the 95 percent confidence intervals. Scales of the y-axes are allowed to vary between the different fitness components considered.

3.3.2 Response to Selection

In the absence of parental effects we can use the Lande (1979) Equation to predict evolutionary change in age-specific mass $\mathbf{\Delta \bar{b}} = \mathbf{G}\beta_{\mathbf{b}}$. Using $\mathbf{G}$, the genetic (co)variance matrix of age-specific mass, obtained in the bivariate model, the 95% confidence intervals for the expected response to selection on mass early in ontogeny overlap zero (e.g. for day 0 the expected response was -0.003 [-0.017 - 0.019]). After day six the expected responses to selection become significantly positive (0.094 [0.031 - 0.223] P<0.001), and by day 15 an increase in mass of 0.321 grams per year ([0.134 - 0.570], P<0.001)) would be expected, which is 2.97% of the mean mass at day 15, so would represent a substantial increase in chick mass.

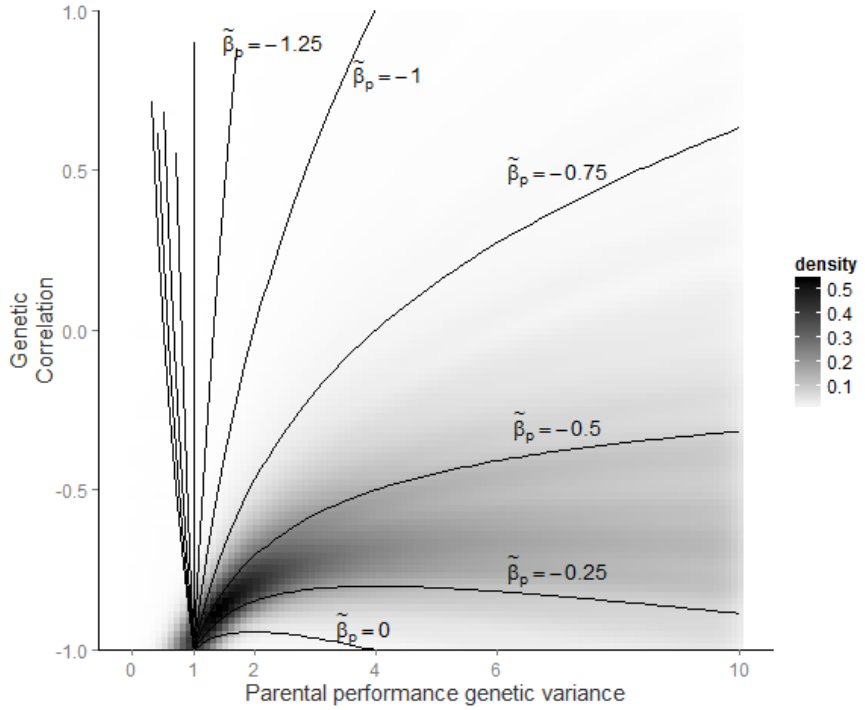


Figure 3.4: Selection on male parental performance, relative to the selection on juvenile weight, demonstrating parameter space in which stasis might be explained, from Equation 3.16. Black lines show the genetic correlation that would be needed to explain stasis for different strengths of selection (relative to selection on juvenile mass). Grey shading represents the density of the posterior estimates of selection from the iterations of the model.

In the presence of parental effects the expected response to selection requires knowledge about the genetic variance in parental performance and the genetic covariance between parental performance and mass. We were not able to estimate these quantities, but we are able to demonstrate the parameter space in which evolutionary stasis would be predicted based on our estimates of selection on parental performance. From Equation 3.2 we can show that stasis is achieved when the genetic correlation between parental performance and mass $r^*_{b,p}$ is:

$$r^*_{b,p} = \frac{-(1 + \tilde{g}_{p,p}(\frac{1}{2} + \tilde{\beta}_p))}{\sqrt{\tilde{g}_{p,p}}\left(\frac{3}{2} + \tilde{\beta}_p\right)} \tag{3.16}$$

where $\tilde{\beta}_p$ is the selection on parental performance relative to selection on mass $\tilde{\beta}_p = \beta_p/\beta_b$, and $\tilde{g}_{p,p}$ is the genetic variance in parental performance relative to the

genetic variance for mass $\tilde{g}_{p,p} = g_{p,p}/g_{b,b}$ (see Section C.1 for full details). Using our estimates of $\tilde{\beta}_p$ we can find the genetic correlation for which an equilibrium is reached ($r^*_{b,p}$) for a given value of $\tilde{g}_{p,p}$. As we are unable to estimate $\tilde{g}_{p,p}$ from our data, we estimate $r^*_{b,p}$ over a range of values of $\tilde{g}_{p,p}$. The posterior distribution of $r^*_{b,p}$ is presented in Figures 3.4 and 3.5. In the case of male parental performance, the mean relative selection gradient is -0.435 [-0.995 - 0.046], and so reduces the need for an extreme negative genetic covariance compared to when there is an absence of selection on the trait. The relative selection on female parental performance is -0.131 [-0.246 - -0.043], so on average does not lift the constraint on stasis as much as for male performance, although the confidence intervals are much narrower.

3.4 Discussion

Here we provide the first estimates of selection upon parental performance, a trait defined through its impact on the phenotype of offspring but caused by multiple unmeasured traits in the individual (such as nest-site selection and food provisioning rate; Willham, 1963, 1972). As expected (Hadfield, 2012), these results show that increasing the mass of an offspring is costly to an individual, when fitness is measured as the total number of zygotes that an individual produces over its lifetime (Arnold, 1985). The cost of care appears to be driven chiefly through reduced fecundity, rather than survival, and the magnitude of this cost seems to be borne differently by the two sexes. Males that show higher levels of attendance (and therefore care) at those nests for which they are the social father pay a current fecundity cost, through reduced rates of polygamy and extra-pair mating, as has been predicted (Werren *et al.*, 1980; Westneat *et al.*, 1990; Houston & McNamara, 2002) and for which there is some previous evidence (Magrath & Elgar, 1997; Schwagmeyer *et al.*, 2012). Increased maternal performance also reduced current fecundity, supporting the prediction of a trade-off between offspring number and investment per offspring (Smith & Fretwell, 1974), as has previously been found (e.g. Badyaev & Ghalam-

bor, 2001; Nakagawa *et al.*, 2007). Indeed, Rollinson & Rowe (2015) demonstrate this trade-off at both phenotypic and genetic levels, with estimates of correlations between size and number being predominantly negative.

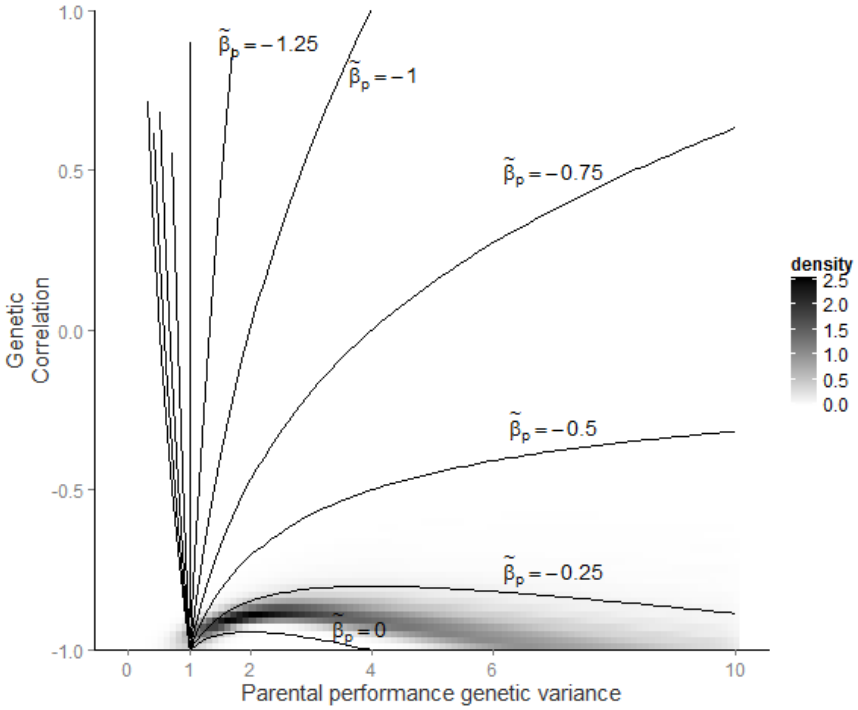


Figure 3.5: Selection on female parental performance, relative to the selection on juvenile weight, demonstrating parameter space in which stasis might be explained, from Equation 3.16. Black lines show the genetic correlation that would be needed to explain stasis for different strengths of selection (relative to selection on juvenile mass). Grey shading represents the density of the posterior estimates of selection from the iterations of the model.

We set out to demonstrate how stasis in body size in the face of positive directional selection might be explained. In the absence of selection acting against parental performance, the Willham model (Willham, 1963, 1972) predicts that genetic correlations between body size and parental performance must be close to -1 for stasis to occur. However, we show that the selection against parental performance that we observe lessens the need for extreme and implausible genetic structures, although a negative genetic correlation would still be needed. However the degree to which this is reduced depends upon the relative magnitudes of the direct and

parental genetic effects on body size. We were not able to estimate these in this analysis, but nonetheless, evidence for heritable variation in parental performance (Wilson & Réale, 2006; Räsänen & Kruuk, 2007) and general parental care traits has been found (MacColl & Hatchwell, 2003; Walling *et al.*, 2008; Dor & Lotem, 2010), although see Wetzel *et al.* (2015). Thus, there is general support for the assumption of genetic variation in parental performance. Estimates of the direct-parental genetic correlation in domestic populations (Wilson & Réale, 2006; Räsänen & Kruuk, 2007) are generally negative, although not strongly so. Thus, it is likely that a negative genetic correlation between parental performance and body mass in blue tits might also be found, but whether these would be sufficient to explain stasis in body size is not currently known. To our knowledge, the only estimates of the genetic parameters from a wild population are those for growth rate in squirrels (McAdam *et al.*, 2002) and birth weight in Soay sheep (Wilson *et al.*, 2005a). In the latter case, $\tilde{g}_{p,p} = 1.42$ and $r_{b,p} = -0.41$. Thus to achieve stasis, $\tilde{\beta}_p \approx -1.3$ would be required, although there is considerable uncertainty about this value given the genetic parameter estimates have very low precision.

Our study employs correlational data to estimate selection, which may underestimate the costs of care compared to experimental studies that manipulate parental care. Differences in individual quality (Reznick, 1985; van Noordwijk & de Jong, 1986; Lessells & McNamara, 2012) mean that some individuals are likely to bear fewer costs through increased performance than others, which correlational studies cannot account for. However, manipulating parental performance effectively is challenging. Those studies that attempt to manipulate parental effort work under the assumption that *total* parental investment of the focal individual may be altered, consequently leading to alteration of parental fitness. However, the costs may instead be passed to offspring (e.g. Einum & Fleming, 2000) such that the investment *per offspring* is reduced, and thus costs to offspring are more frequently found (e.g. Sanz, 1997). Of the various methods to measure the costs of care (Reznick, 1985;

Alonso-Alvarez & Velando, 2012), clutch size manipulations have been the most commonly used (e.g. Nur, 1988; Yoccoz *et al.*, 2002; De Heij *et al.*, 2006; Parejo & Danchin, 2006; Santos & Nakagawa, 2012), although altering the effort made by one parent and monitoring the effect on their partner, as shown in Wright & Cuthill (1990); Sanz *et al.* (2000); Kilner *et al.* (2015), and reviewed in Hinde (2006), are also used to manipulate care. Selection on parental performance could be estimated from such data by considering the change in parental fitness in units of the *total* brood weight change caused by the treatment, accounting for costs borne both by the parents and offspring.

Our results imply that fecundity selection against parental performance differs between the sexes – the mean selection estimate is stronger in males. This might be expected, as males have higher variance in fecundity than females, and thus by increasing care they suffer a higher fitness cost (Trivers, 1972; Smith, 1977). However, the method with which (uncaught) males are assigned to nests may cause an overestimate of the strength of selection – we assign males based on majority paternity, so consequently these uncaught social fathers will naturally have a higher fecundity, and lower male attendance scores. In order to assess the magnitude of this problem we reran the model using only males assigned to nests with over 50% paternity, and believe that our results are relatively robust (see SI). For those males that had been caught at nests, assigning males in this way would result in the correct male being assigned in 94% of cases. Moreover, the trade off between male parental care and fecundity that we measure has previously been predicted (Werren *et al.*, 1980; Westneat *et al.*, 1990) and found (Magrath & Komdeur, 2003). Catching males on territories prior to breeding, or identification using unique colour rings or PIT tags (as in Kidd *et al.*, 2015)), would aid in assigning these uncaught social males to validate this relationship. Similarly, individuals failing in a breeding attempt prior to hatching are lost from the data. It may be that some of these individuals then go on to lay a second clutch, at which they are caught. In these cases, we underestimate

the fecundity of those individuals (e.g. a female who lays a replacement clutch in a second nest after abandoning her first may lay twice as many eggs as are counted in the data). If individuals are not missing at random, estimates of selection may be biased (Hadfield, 2008), and Kidd *et al.* (2015) found that female great tits (*Parus major*) were less likely to be caught (due to early nest failure) if they were immigrants to the population, and when in poor quality habitats.

We do not estimate sex-specific effects of parental performance on survival, as we assume that the effects of changes in performance affect survival in each sex identically. Although parents may provide different *total* amounts of care to their offspring, the survival cost per unit care may not differ. For example, Santos & Nakagawa (2012) found sex differences in survival costs when clutch size is manipulated – males suffered reduced survival when clutch sizes were increased, but females suffered no such cost. This result is likely to be due to males altering their care levels in response to treatment, and thus suffering a survival cost, rather than the treatment altering the cost per unit care between the sexes. On a similar note, we assume that the male and female parents contribute an equal amount to the parental performance for each nest, as a model including separate effects would only be weakly identifiable. In blue tits the female alone builds the nest, incubates eggs and broods the chicks (Cramp & Perrins, 1993), and although both parents feed offspring, evidence of differences exist between feeding rate and duration, and nest sanitation behaviours (Banbura *et al.*, 2001). Thus, it is possible that parental performance is not equally divided between parents. In addition, some aspects of parental performance may also be attributable to the combination of the parents, rather than being divisible between the two – Ihle *et al.* (2015) found individuals pairs resulting from free mate choice had lower offspring mortality than those in forced pairs, implying behavioural compatibility may affect combined parental performance.

Parental performance is the composite effect of multiple traits expressed by parents that then affect offspring body size (Willham, 1963). Consequently, measuring

the inheritance of, and the selection on, the composite effect can only give an approximate prediction for the expected change in body size. An exact prediction requires all traits underlying parental performance to be identified, and the selection upon them quantified, along with their genetic (co)variances (Kirkpatrick & Lande, 1989; Hadfield, 2012; McGlothlin & Galloway, 2014). However, we expect that the results shown here give a reasonable approximation to how selection on parental care traits impact on the evolution of juvenile traits, and how it may help solve the paradox of stasis. The parental performance measured in this study is derived from the variance explained by the nest-of-rearing of chicks, and so also includes traits that may not be parentally derived, such as nest-box location effects, and sibling effects. However, the nest-box effect could be considered to be part of a parent's extended phenotype, through territory selection and defence, although it may not be heritable. Inclusion of specific parental phenotypes, such as feeding rate, could be used to explain some of the nest variance. In addition, sibling effects may predominantly manifest themselves through modulation of parental care, and so inclusion within parental performance may remain valid.

In conclusion, our results here show that, when appropriate measures of fitness are used, there is selection against parental performance. This acts antagonistically to selection upon body size, and goes some way towards explaining stasis in this trait. These are the first estimates of selection upon parental performance, and highlight the need to estimate these parameters when predicting how traits influenced by other individuals may respond to selection. Due to limitations of the data, we did not estimate genetic parameters associated with the traits, and thus any future studies that are able to fully estimate both selection and genetics for these traits will be able to generate more complete predictions as to the way in which stasis is maintained.

Chapter 4

The importance of siblings in parent-offspring coadaptation

Abstract

Parental and offspring behaviours are predicted to act as both the agents and targets of selection, through their interactions with one another. This is expected to generate parent-offspring coadaptation, in which parental and offspring behaviours are genetically correlated in a way that increases inclusive fitness. Cross-fostering is often used to study parent-offspring coadaptation, with the prediction that offspring raised by foster parents should suffer compared to those raised by genetic parents, and that parents should also suffer when raising fostered offspring. Such studies have provided mixed evidence, but differences in experimental design mean some studies keep siblings together, whereas others do not. When parent-offspring coadaptation acts at the level of the brood (such as response to mean begging level), or sibling coadaptation exists, only studies that keep siblings together are expected to show evidence of coadaptation. In order to test whether the mixed evidence is due to sibling effects we performed a cross-fostering experiment in blue tits (*Cyanistes caeruleus*) in which eggs within nests were either all crossed, all remained in the nest they were laid in, or half of them crossed. This resulted in broods of all siblings, with foster or genetic parents, or broods containing a mixture of siblings and non-siblings. We evaluated the effects of these treatments on the weights and survival of chicks throughout ontogeny, and subsequent fitness of parents, and found no evidence of parent-offspring coadaptation effects on chick mass and survival, or on parental fitness. Similarly there was no evidence for sibling coadaptation.

4.1 Introduction

When loci have epistatic effects on fitness, natural selection is expected to favour a build up of linkage disequilibrium between those loci (Fisher, 1930; Lewontin & Kojima, 1960; Lewontin, 1964) and in extreme cases lead to the evolution of co-

adapted gene complexes (e.g. Kronforst *et al.*, 2006; Küpper *et al.*, 2016). When the fitness of an individual is affected by its relatives (either directly or indirectly), selection can favour the formation of linkage disequilibrium between loci that have epistatic effects on *inclusive* fitness (Wade, 1998). In the context of parents and their offspring, this linkage disequilibrium generates parent-offspring coadaptation, wherein the combination of trait values within a family result in the highest fitness (Wolf & Brodie, 1998; Kölliker *et al.*, 2005).

Much of the work on parent-offspring coadaptation has focused on the specific traits expected to mediate these interactions, and the consequences of these for offspring fitness. These fitness benefits to offspring may be generated when heritable traits that cause parental effects are present, and are genetically correlated with the traits in offspring that they affect (Wolf & Brodie, 1998). Conversely, fitness effects to parents are also possible when heritable offspring effects are present, which are genetically correlated with the parental traits they affect (see Hinde *et al.*, 2010, in the context of parent-offspring signalling). Whilist the evidence for heritable parental effects is widespread (Räsänen & Kruuk, 2007), heritable offspring effects are harder to detect, and thus the evidence is more limited (Agrawal *et al.*, 2001). With regard to the traits mediating such effects, parental provisioning and offspring begging have been well studied, with the expectation that if provisioning affects the rate of begging, and/or begging affects the rate of provisioning, genetic correlations may generate coadaptation (Kölliker *et al.*, 2005). Positive parent-offspring correlations between provisioning and begging, attributed to genetic or pre-natal effects, have been found using cross-fostering in great tits (*Parus major*; Kölliker *et al.*, 2000), burying beetles (*Nicrophorus pustulatus*; Lock *et al.*, 2004) and canaries (*Serinus canaria domestica*; Hinde *et al.*, 2009; Estramil *et al.*, 2013), but not blue tits (*Cyanistes caeruleus*; Lucass *et al.*, 2015a). In addition, Hinde *et al.* (2010) demonstrated that, in canaries, the loss of offspring growth when cross-fostered was related to the difference in begging intensities of the biological offspring of the fos-

ter parents, and the foster offspring themselves, suggesting that these traits may underpin any coadaptation. Likewise, Lucass *et al.* (2015b) demonstrated that, in blue tits, the highest masses were achieved by high-begging chicks raised by high-provisioning foster parents, but these chicks showed the lowest masses when with low-provisioning parents, although without a genetic correlation between these behaviours (Lucass *et al.*, 2015a) this does not constitute coadaptation.

Whilst the traits that underpin such coadaptation are of interest, coadaptation is measured ultimately through its consequences on fitness, with the expectation that individuals have higher fitness when interacting with relatives than non-relatives. By comparing the fitness of family members in fully cross-fostered and non-cross-fostered nests of canaries, Hinde *et al.* (2010) found evidence for parent-offspring coadaptation through effects on offspring fitness, but not on parental fitness, although this could not be replicated (Estramil *et al.*, 2013, 2014a). Similarly, partial cross-fostering studies, wherein a subset of offspring are moved between nests (Rutledge *et al.*, 1972), allow comparisons of cross-fostered and non-cross-fostered offspring *within* nests. This experimental design is commonly used to estimate genetic and post-natal maternal effects, but studies that report the difference between cross-fostered and non-cross-fostered offspring are rare. Of those that do report such effects, no differences were found for body mass in starlings (*Sturnus vulgaris*; Smith & Wettermark, 1995), burying beetles (Rauter & Moore, 2002), or domestic pigs (*Sus scrofa domesticus*; Heim *et al.*, 2012), or survival in cliff swallows (*Petrochelidon pyrrhonota*; Brown *et al.*, 2015). In contrast, Giordano *et al.* (2014) showed that non-cross-fostered offspring in great tits, *Parus major*, had faster growth in their original nest, but Winney *et al.* (2015) showed that cross-fostered offspring had higher survival in house sparrows (*Passer domesticus*). The partial cross-fostering design does not allow the consequences of parent-offspring coadaptation on parental fitness to be measured, as all parents rear a mix of related and unrelated offspring.

Thus, the results from these studies are mixed, but these differences may be attributable to the experimental designs used. In particular, the partial and fully cross-fostered designs differ as to whether non-siblings are raised together, and this could result in differences if parental responses to offspring behaviour affect all offspring in the brood equally, rather than affecting each offspring in proportion to its individual effects. For example, parents may respond to the begging behaviour of individuals by increasing the total rate of food provisioning to the brood (Ottosson *et al.*, 1997) and so an individual that begs at a high rate may gain more food for both itself and its siblings. Under this form of parent-offspring coadaptation – which we will call sibling-mediated parent-offspring coadaptation – no differences between the fitness of cross-fostered and non-cross-fostered offspring are expected in the partial cross-fostering design, because parents do not vary in average relatedness to the brood that they raise (see Figure 4.1). Fitness costs would still be expected, however, between fully cross-fostered broods and controls. Additionally, siblings may also have *direct* effects on one-another. These may arise when sibling behaviours, such as negotiation rules (Roulin, 2002; Romano *et al.*, 2012), increase fitness when all nest mates adopt a similar set of behaviours (Mock & Parker, 1997). Should these have a genetic basis, siblings will have more similar behaviours than non-siblings, causing chicks from partially crossed nests to have lower fitness than chicks from fully cross-fostered or control nests, regardless of whether they have been crossed or not (see Figure 4.1).

Here we present the results of a randomised experiment that compares offspring and parental fitnesses across fully cross-fostered, partial cross-fostered and non cross-fostered broods of blue tits. The design allows us to estimate the magnitude of parent-offspring (PO) coadaptation, sibling-mediated parent-offspring (SPO) coadaptation, and sibling (S) coadaptation, as illustrated in Figure 4.1. Under parent-offspring coadaptation (PO) we expect that cross-fostered offspring should have lower fitness than those that are not crossed, as they are raised by non-related par-

ents. Under sibling-mediated coadaptation, whilst we expect that there will be a fitness cost to crossed offspring, this will be of smaller magnitude in partially cross-fostered nests than in fully cross-fostered nests. In addition a cost should also be felt by non-crossed offspring in partially crossed nests. If sibling coadaptation is present, there should be no fitness costs to being cross-fostered in the full treatment, but costs to both crossed and non-crossed offspring in the partial treatment (as they are raised with non-siblings).

4.2 Methods

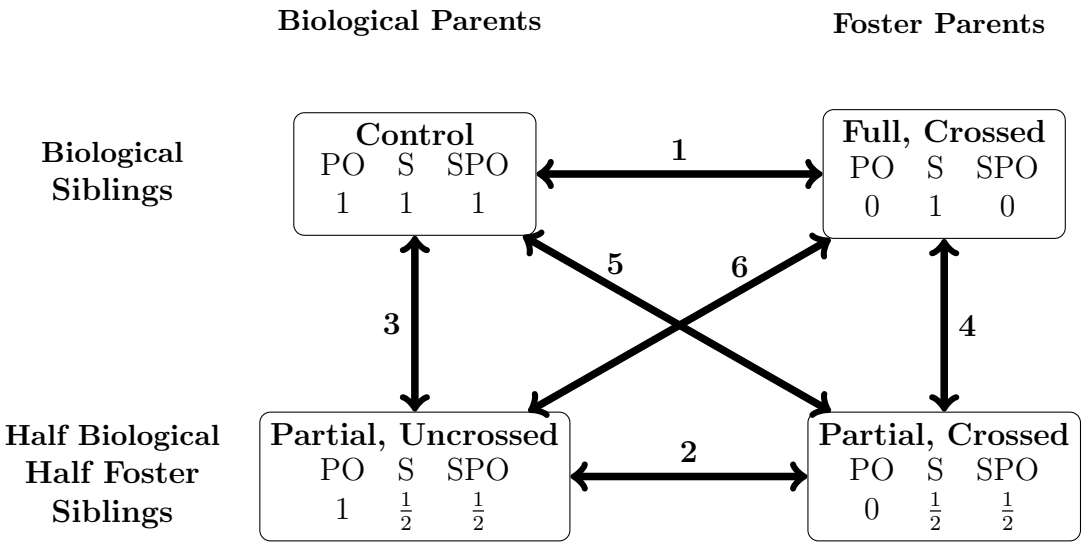


Figure 4.1: The comparisons that can be made between individuals in different types of nest generated by using full cross-fostering, partial cross-fostering, and randomised controls. The codes within boxes stand for parent-offspring coadaptation (PO), sibling-mediated parent-offspring coadaptation (SPO) and sibling coadpatation (S). The numbers underneath each code represent the extent to which individuals gain the fitness benefits of being with relatives through each process, compared with controls. Under certain genetic models these numbers will be proportional to the relatedness of the individual to the adult that raises it (PO) the relatedness of the individuals nest-mates to the adult that raises it (SPO) and the relatedness of the individual to its nest mates (S).

This study was carried out in a nest-box population of blue tits on the Dalmeny estate in Edinburgh, UK (see Figure A.1 in Appendix A). This population has

been studied since 2009, and consists of 180 boxes on Craigie Hill (grid reference NT156766) and 46 beside the Almond River (NT179758), spaced around 30m apart. The experimental data reported here were collected during the breeding seasons of 2014 and 2015, although we also utilise data collected between 2010 and 2013. Nest boxes were visited systematically from early April, such that occupied boxes were known in advance of egg laying, and the first egg was found on the day that it was laid.

4.2.1 Cross-Fostering

2014 and 2015: Nest were paired randomly with another nest on the day that the first egg was laid (in both nests), and assigned to cross-fostering treatments. Pairs of nests were drawn at random and assigned sequentially to treatments. This sequence of treatments was kept constant to ensure a balanced design with a uniform distribution of treatments across the breeding season. There were three treatments: Control, partial cross-fostering, and full cross-fostering. Control nests did not have any eggs exchanged between them, such that all chicks were raised in their nest of origin (30 nests in 2014, 32 in 2015). For partial cross-foster nests, we aimed to reciprocally exchange every other egg between the pair of nests, so the first egg was exchanged, the second was not, the third was, and so on, such that approximately half of the eggs would be crossed (26 nests in 2014, 34 in 2015). For fully cross-fostered nests we aimed to reciprocally exchange every egg between the nests (28 nests in 2014, 34 in 2015). Eggs were exchanged on the day they were laid in both treatments. On some days an odd number of nests commenced laying, such that they could not be assigned to a pair. If the nest would have been assigned a control treatment (if a second nest to pair with was available), this treatment was assigned and the second nest in the pair assigned the following day. However, pairs could not be generated for nests starting on different days in the full or partial treatment, so the unmatched nest was assigned as an "odd" nest, where no eggs were crossed

but it was not in a matched pair (21 nests in 2014, 14 in 2015). The position in the laying sequence was written on each egg using a non-toxic marker.

Blue tits often pause during laying for one or more days, such that there is an interruption in the laying sequence. We did not wish to alter clutch (and subsequently brood) sizes, as these are known to have effects on both chicks and parents (Sanz, 1997; Neuenschwander *et al.*, 2003; De Heij *et al.*, 2006; Parejo & Danchin, 2006), so egg exchanges were not carried out if one of the pair did not lay an egg on a given day. Similarly, crossing of eggs ceased when one of the nests in the pair ceased laying and starting incubating eggs. Thus the partial and full cross-fostering treatments have some variation in the proportion of eggs actually crossed between nests, shown in Figure 4.2.

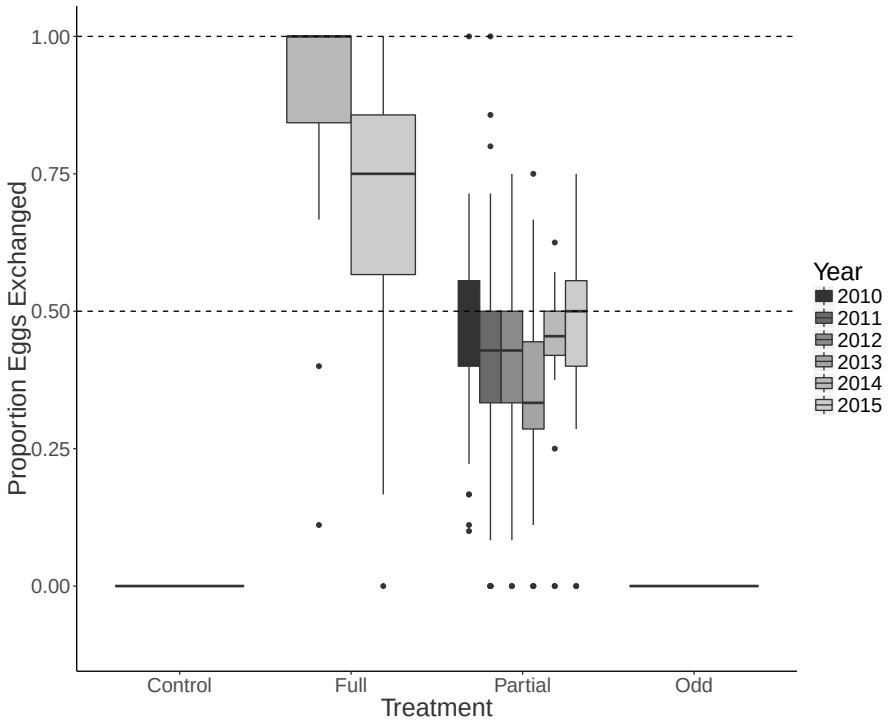


Figure 4.2: The proportion of eggs crossed in each nest within the treatment categories. No eggs were ever exchanged between nests classed as Control or Odd. Boxes are shaded by year. Only 2014 and 2015 included the full treatment as part of the experimental design.

2010 to 2013: During these years all nests were assigned to cross-fostering

groups on the day that the first egg was laid, with a partial cross-foster design being used for all nests. Where possible, nests were assigned into cross-fostering groups of three, and eggs were exchanged in a round-robin design: the egg from nest A was transferred to nest B, that from nest B to nest C, and that from nest C to nest A. Although most nests were in triads, some groups contained two, four, or five nests, if the number of nests commencing laying on a given day was not divisible by three. As with the partial treatment above, eggs were crossed every other day, so that approximately half of all eggs were crossed between nests (Figure 4.2). There were 111 partial nests in 2010, 153 in 2011, 119 in 2012, and 106 in 2013. Nests that could not be assigned to cross-fostering groups are classed as "odd" rather than controls, as they also tend to lie at the extreme ends of the laying period (3 in 2010, 10 in 2011, 15 in 2012 and 7 in 2013). These data were included as they increase the power of the model and improve the estimation of the variance components included. An additional cross-fostering experiment was carried out in 2010 and 2011. In this, first or last laid eggs within nests were exchanged between nests that were not already in the same cross-fostering group, such that the eggs in the clutch came from a total of three different original nests, see Hadfield *et al.* (2013b) for full details.

4.2.2 Data collection

Females were classed as having completed laying and having commenced incubation once eggs were no longer being laid, and she was found incubating, or eggs were warm for the second day in a row. Nests were checked for hatching daily from around 11 days after the final egg had been laid. In the majority of cases this meant that chicks were found on the first day that any hatched. Due to nest failures between the start of laying and chicks hatching, the numbers of nests in each treatment that have chicks are lower than initially assigned (Control - 27 nests in 2014, 26 in 2015; Partial - 26 nests in 2014, 30 in 2015 and 102, 133, 106, and 96 in each of 2010, 2011, 2012 and 2013 respectively; Full - 24 nests in 2014, 30 in 2015; Odd - 18

Treatment	Experimental years	Non-experimental years
Control	396	
Partial Uncrossed	229	2002
Partial Crossed	198	1414
Full Crossed	299	
Full Uncrossed	84	
Odd	202	155

Table 4.1: The number of chicks in each treatment category. Split into experimental years (2014-2015) and non-experimental years (pre-2013)

nests in 2014, 12 in 2015, and 2, 8, 10, and 3 in each of 2010, 2011, 2012 and 2013 respectively). The numbers of chicks in each of the treatments is shown in Table 4.1

The first day that any chicks hatched (day 0) they were uniquely marked by clipping tufts of down on their head, and a toenail if necessary. These chicks were weighed, and the numbers of any unhatched eggs were noted. The same was done on two subsequent visits (day 1 and day 3, no eggs hatched past this point). Chicks were then weighed again on four more days (days 6, 9, 12 and 15), and on each visit all mortality was noted. In addition, blood samples were taken from chicks under home office licence from the medial metatarsal vein on day 3, and chicks were ringed on day 9.

From day 10 onwards adults were caught in the nest box, and if necessary by mist net. If the adult had not been previously caught, it was ringed, with both a metal and a colour ring, which indicated the year that they were first caught as adults, as well as their sex. All adults were weighed, and we took blood samples from the ulna vein.

4.2.3 Genotyping and Pedigree

Genotypes were required in order to determine the nest-of-origin of each chick, as the exact egg from which an individual hatched was generally not known. These genotypes also allowed reconstruction of the pedigree. DNA was extracted from blood

samples taken from chicks, and adults using DNeasy Blood, and from tissue samples of some chicks or unhatched eggs using Tissue kit (Qiagen, Hilden, Germany), and genotyped at seven polymorphic microsatellite markers (Olano-Marin *et al.*, 2010). See Hadfield *et al.* (2013a) for full molecular methods. The sex of each chick was also determined, by amplifying the sex-linked markers P2 and P8 (Griffiths *et al.*, 1998).

We used the program MasterBayes (Hadfield *et al.*, 2006) in R (R Development Core Team, 2012) in order to reconstruct the pedigree of the population. This employs a Bayesian Markov chain Monte Carlo approach. Each chick had one or two potential mothers in 2012-2015, depending the cross-fostering treatment applied to the nest. In 2010 and 2011, there were between one and three potential mothers, due to the extra cross-fostering carried out in some nests, which resulted in nests containing eggs from three mothers. Natural mixed maternity clutches were assumed not to occur, and so maternity for each chick was restricted to the females associated with the nests in its cross-fostering group. There is a reasonably high rate of extra pair offspring in this population, and so paternity was not restricted in the same way. Instead, the odds that chicks originating from a given nest were sired by the male caught at that nest was estimated alongside the pedigree. In addition, we estimated the rate at which the probability of paternity decayed with distance between the nests attended by a male and the nest from which the chicks originally came. Although effort was made to capture both adults at all nests, this was not always possible. Hence, when one or both adults associated with a given nest remained uncaught, a non-genotyped dummy adult(s) was included in the model. We also estimated the size of the population of missing males, which equates to the number of males that would be required in order to assign paternity to all offspring, if these males were drawn from the same 'statistical' population as non-missing males (both sampled and dummy). Offspring whose paternity was assigned to missing or dummy males with a posterior probability greater than 50% were assigned into

Treatment	PO	S	SPO
Control	1	1	1
Full, Crossed	0	1	0
Partial, Uncrossed	1	$\frac{1}{2}$	$\frac{1}{2}$
Partial, Crossed	0	$\frac{1}{2}$	$\frac{1}{2}$

Table 4.2: The expected proportion of fitness benefits from parent-offspring coadaptation (PO), sibling coadaptation (S), and sibling-mediated parent-offspring coadaptation (SPO) experienced by offspring in each cross-fostering category.

paternal sibships, using rcolony (Jones & Wang, 2010), see Hadfield *et al.* (2013a).

4.2.4 Statistical Methods

Chick Mass: The weights of chicks across ontogeny were analysed using mixed-effects models implemented in ASReml-R (Gilmour *et al.*, 2009) in R (R Development Core Team, 2012). Nest age (in days since the first day of hatching) was fitted as a factor. In addition, we included year, sex, and hatching day (day of hatching within the nest relative to the first chick; 0,1 or 3) as factors, and clutch size, day of nest hatching (days since April 1st), and time as continuous fixed effects. All fixed terms, including treatment (see below) were also fitted as interactions with nest age (as a continuous variable), to capture any changes in their effects over ontogeny.

A set of biologically motivated contrasts were set up to capture differences between the four treatment groups (control, full, partial-crossed and partial uncrossed). This resulted in three predictors: parent-offspring (PO) coadaptation, sibling-mediated parent-offspring (SPO) coadaptation and sibling (S) coadpatation, the values of which are shown for each treatment group in Table 4.2 (see Figure 4.1 also). The coefficients associated with these predictors measure the amount of coadaptation that exists by each process when comparing a natural nest to the situation where all individuals (foster parents and offspring) are mutually unrelated. Thus, positive coefficients from the model indicate coadaptation, and negative coefficients indicate family maladaptation.

Figure 4.1 shows the different comparisons that can be made between individuals (chicks) exposed to different treatments. Generally, only comparisons 1 and 2 from this figure have previously been considered (although see Heim *et al.*, 2012), which are unable to distinguish between the three types of effect: in comparison 1 (e.g. Hinde *et al.*, 2010) PO and SPO coadaptation are confounded, and in comparison 2 (e.g. Smith & Wettermark, 1995) only PO coadaptation can be estimated. Our design allows additional comparisons that can distinguish all three types of coadaptation: If PO coadaptation exists, both crossed chicks in full and partial treatments should suffer because they are not related to their foster parents (comparisons 1, 2 and 5). If SPO coadaptation is important, however, crossed individuals in full treatments should suffer relative to controls (comparison 1) and the crossed and uncrossed individuals in the partial treatment should be intermediate (comparisons 3 and 5 versus 4 and 6). This is because in full treatments an individual's foster parents are not related to any nest-mates, but in partial nests an individual's biological/foster parents are related to half of the nest-mates. Finally, if S coadaptation is important then individuals in partial nests should do worse than those in control and full nests (comparisons 3 and 4) because they are only related to half their nest-mates, rather than all.

Because our experimental design was not perfect we also had two additional groups of birds; those from odd nests, and those from uncrossed eggs in the full treatment. Winney *et al.* (2015) showed that cross-fostering studies can produce results contrary to expectations due to non-random assignment of chicks to treatments, which is likely to be the case for these two groups. Odd nests are not assigned to any treatment, as they could not be placed in appropriate cross-fostering groups, and these tend to occur at extreme ends of the laying season compared to controls. Individuals in these nests got the same predictor as control nests, but an additional fixed effect (odd nests = 1, all other nests = 0) was added to account for any bias. Likewise, the full cross-fostering treatment was imperfect due to laying interruptions

and differences in clutch size between pairs of females, and so some eggs were left behind in natal nests, and these tended to be eggs laid later in the laying sequence. These chicks had predictors 1 for PO coadaptation (as they remained with their biological parents) and 0 for S and SPO coadaptation, under the assumption that clutch sizes were large and there were few such chicks. However, again we fitted a unique fixed effect for this group of birds to control for any bias. In order to use partial cross-fostering data from previous years we also fitted contrasts between experimental and non-experimental years for the partial crossed and uncrossed individuals, as well as those in odd nests.

As in the full cross-fostering treatment, earlier eggs within a nest are more likely to be crossed than later ones in partial nests, and this may have consequences on the effect of cross-fostering, as early laid eggs are also likely to hatch earlier, and thus have increased mass (and survival; Hadfield *et al.*, 2013b). Fitting day of hatching as a fixed effect in this model is used to try to eliminate this effect. However, a correlation between egg rank and hatching times (and therefore treatment and hatching times) may remain within a given hatching period, where egg rank is defined as the time in days between a given egg and the last egg being laid, such that the last egg has rank zero, the penultimate egg rank one, and so on. To try and correct for this in the analysis, we fitted the rank of the egg that each chick hatched from. Multiple chicks hatch between visits, however, so we generally cannot assign chicks uniquely to eggs, and consequently this rank measure was the average rank of eggs a chick could have hatched from (based on timing and nest-of-origin). The mean rank of crossed and uncrossed eggs in partial nests was 5.373 and 3.925 respectively, and 4.819 and 2.638 in full crossed nests. As with other fixed effects, this was included in the model, along with an interaction with nest age.

We include three random effects in the model, all fitted as a 7x7 variance covariance matrix across the seven nest ages. Genetic (pedigree) and nest-of-origin random effects are approximated using a first order autoregressive structure (Had-

field *et al.*, 2013a), whereas nest-of-rearing was fitted as an unstructured matrix. Residual mass effects were also unstructured.

Chick Survival: The survival of chicks were analysed in a mixed-effects model in MCMCglmm (Hadfield, 2010) in R (R Development Core Team, 2012). Survival was fitted as a repeat-measure binary trait, as in event-history analysis, using a probit link function. The fixed effects were the same as for the mass models, including the interaction of effects with day. Nest-of-rearing was fitted as a random effect, as was the interaction between nest-of-rearing and nest age, to account for the fact that all chicks within a nest often suffer mortality at the same age due to parental desertion. Flat priors were used for all random effects (zero degree of belief in the inverse-wishart prior), except for the residual variance which was fixed at 1.

Adult Survival and Fecundity: The effects of the treatments on adults were fitted in MCMCglmm; this was modelled only for 2014, as the following year's data is required to estimate subsequent survival and fecundity, and is not yet available for 2016. Treatment was fitted as a fixed effect in these models, as the predictions of the effect of cross-fostering under parent-offspring coadaptation and sibling-mediated parent-offspring coadaptation are identical, and so these could not be fitted as separate predictors. Whilst the model cannot take into account variation in recapture probability, adult recapture probabilities are high in this population, such that the probabilities of correctly scoring a surviving bird are 0.95 (females) and 0.94 (males). In addition, this model assumes that any birds not recaptured the following year have died, rather than emigrated, as there is low movement of adults between areas within the field site.

Fecundity was fitted as a Gaussian trait, and the effect of treatment was fitted separately for each sex within the model. Residual variances were also fitted separately for the sexes, with flat priors as above. Survival was fitted as a binary variable with probit link, again with the effects fitted separately for each sex. The residual

variance was fixed at 1 in the prior.

4.3 Results

Chick Mass: The fixed effects of the model of chick mass are summarised in Table 4.3. There was an overall non-significant effect of PO coadaptation (Wald test p=0.131) in the opposite direction to what might be expected – there was a non-significant reduction in mass at day 0 when with biological parents rather than foster parents (-0.014g±0.010, p=0.079), equivalent to a 1.02% loss of mass; this difference was significant at day 15 (-0.129g±0.071, p=0.034), but this is equivalent to only a 0.66% reduction in mean mass at this age. There was no significant effect of SPO coadaptation (Wald test p=0.753; 0.017g±0.024, p=0.240 at day 0; 0.093g±0.214, p=0.333 at day 15). Likewise, there was no overall effect of S coadaptation (Wald test p=0.288), with a marginal positive effect of siblings at day 0 (0.055g±0.037, p=0.068), and a non-significant positive effect by day 15 (0.289g±0.339, p=0.198). The difference in mean nest weights between treatments at each nest age are shown in Figure 4.3, with chicks from partial nests split into those who have and have not been cross-fostered.

Odd nests were significantly lighter than nests assigned to treatments (Wald test p=0.001), although this was present early in ontogeny but not later (-0.094g±0.026, p<0.001 at day 0; -0.075g±0.243, p=0.379 at day 15). There were no significant differences between odd nests in experimental and non-experimental years (Wald test p=0.621; -0.012g±0.035, p=0.364 at day 0; -0.320g±0.330, p=0.167 at day 15) nor were there differences between partial nests in experimental and non-experimental years for either uncrossed (Wald test p=0.113) or crossed (Wald test p=0.144) chicks. Uncrossed chicks in full treatment nests did not differ from expectation (Wald test p=0.438).

In addition, there are significant effects of time, sex, year, clutch size, hatching

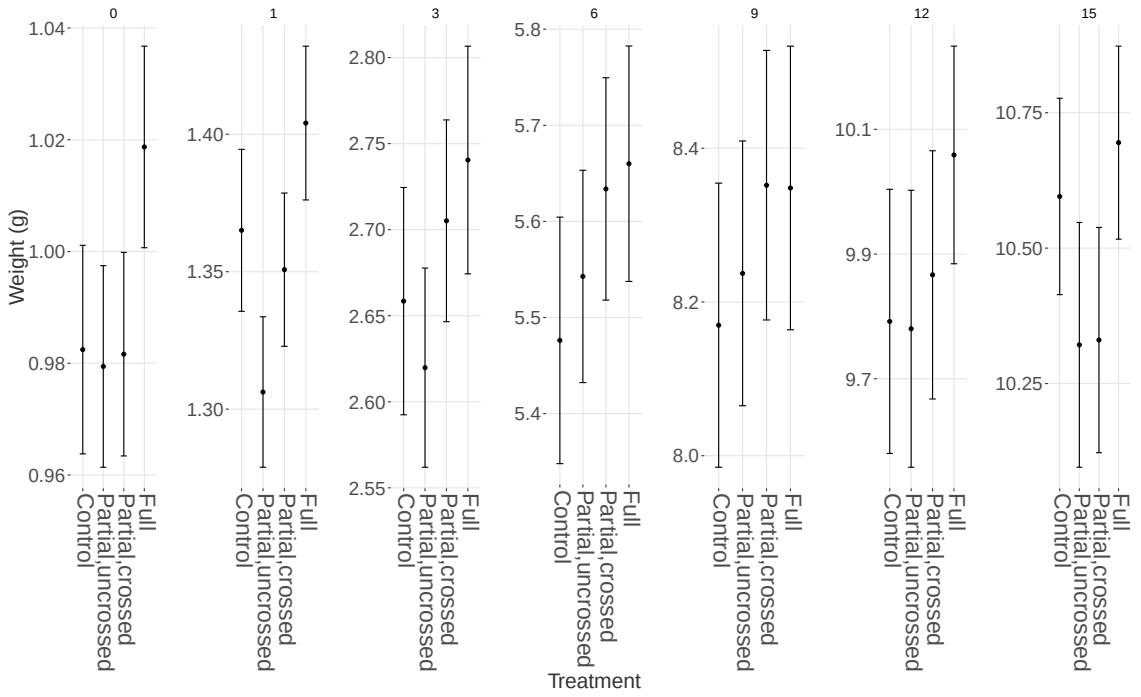


Figure 4.3: Raw means and standard errors of the average chick weights within nests from each of the main treatment categories. Note that the standard errors do not reflect the uncertainty around the treatment *differences*, particularly for crossed and uncrossed chicks in partially treated nests, where the sampling errors are strongly correlated (as crossed and uncrossed chicks are within the same nest, and resemble each other due to nest-of-rearing effects).

date, hatching day within the nest and egg rank on mass (Table 4.3). Egg rank had a positive effect on mass early in ontogeny, such that chicks from early eggs are 0.003g±0.001 heavier per egg rank at day 0 (p=0.009). This is non-significant by the end of ontogeny (-0.001g per rank±0.009, p=0.457), although the test of both fixed effects shows a significant effect overall (Wald test p=0.047).

Chick Survival: The fixed effects included in the model of survival are summarised in Table 4.4. There were no significant effect of any form of coadapation on survival (Wald test: PO Coadaptation p=0.914; SPO coadaptation p=0.534; S coadaptation p=0.270). In the case of the PO coadaptation, chicks remaining with their biological parents had a non-significant increase in survival at day 0 (0.094 probits [-1.119 - 1.075] P=0.857), and day 15 (0.173 probits [-0.346 - 0.726] P=0.546).

	Estimate	SE	Pr(>|Z|)	Pr(>W)
Day 0	0.568	0.063	**<0.001**	
Day 1	1.1	0.075	**<0.001**	
Day 3	2.59	0.134	**<0.001**	
Day 6	5.643	0.243	**<0.001**	
Day 9	8.621	0.355	**<0.001**	
Day 12	10.261	0.468	**<0.001**	
Day 15	10.949	0.579	**<0.001**	
Time	0.554	0.033	**<0.001**	
Time: Day	0.134	0.012	**<0.001**	**<0.001**
Sex (M)	0.006	0.003	**0.019**	
Sex (M): Day	0.033	0.001	**<0.001**	**<0.001**
Year 2011	-0.029	0.015	**0.029**	
Year 2011: Day	-0.034	0.009	**<0.001**	**<0.001**
Year 2012	-0.028	0.015	**0.032**	
Year 2012: Day	-0.018	0.009	**0.027**	**0.041**
Year 2013	-0.091	0.019	**<0.001**	
Year 2013: Day	-0.015	0.012	0.105	**<0.001**
Year 2015	-0.113	0.017	**<0.001**	
Year 2015: Day	-0.014	0.011	0.088	**<0.001**
Clutch Size	-0.005	0.003	**0.024**	
Clutch Size: Day	-0.002	0.002	0.08	0.059
Hatch Date	0.004	0.001	**<0.001**	
Hatch Date: Day	-0.003	0	**<0.001**	**<0.001**
Hatch Day (0-1)	-0.168	0.006	**<0.001**	
Hatch Day (0-1): Day	-0.026	0.003	**<0.001**	**<0.001**
Hatch Day (1-3)	-0.71	0.026	**<0.001**	
Hatch Day (1-3): Day	-0.04	0.006	**<0.001**	**<0.001**
Egg Rank	0.003	0.001	**0.009**	
Egg Rank: Day	0	0.001	0.32	**0.047**
Odd nest	-0.094	0.026	**<0.001**	
Odd nest: Day	0.001	0.016	0.468	**0.001**
Non-Exp Odd	-0.012	0.035	0.364	
Non-Exp Odd: Day	-0.021	0.022	0.171	0.621
Full, Uncrossed	0.052	0.041	0.102	
Full, Uncrossed: Day	0.008	0.023	0.365	0.438
Non-Exp Partial, Uncrossed	-0.016	0.021	0.227	
Non-Exp Partial, Uncrossed: Day	0.023	0.012	**0.033**	0.113
Non-Exp Partial, Crossed	-0.025	0.022	0.127	
Non-Exp Partial, Crossed: Day	0.018	0.013	0.074	0.144
PO	-0.014	0.01	0.079	
PO: Day	-0.008	0.005	**0.046**	0.131
S	0.055	0.037	0.068	
S: Day	0.016	0.022	0.242	0.288
SPO	0.017	0.024	0.24	
SPO: Day	0.005	0.014	0.359	0.753

Table 4.3: Fixed effect results for the model of weight across ages in all years. The mechanisms by which crossed offspring may lose fitness have been fitted as parent-offspring coadaptation (PO), sibling coadaptation (S), and sibling-mediated parent-offspring coadaptation (SPO). In addition, odd nests were fitted as a separate factor, as were uncrossed individuals in the full treatment.

S coadaptation resulted in a non-significant decrease (i.e. maladaptation) in survival at day 0 (-3.382 probits [-6.964 - 0.285] P=0.069) and day 15 (-1.008 probits [-3.761 - 1.687] P=0.463), and similarly there was also a non-significant negative effect of SPO coadaptation at day 0 (-0.912 probits [-2.835 - 1.173] P=0.398) or day 15 (-0.077 probits [-1.924 - 1.578] P=0.938).

Chicks in odd nests did not differ from other chicks in their survival probability (Wald test p=0.789; 0.674 probits [-1.514 - 2.878] P=0.552 at day 0; 0.329 probits [-1.582 - 2.226] P=0.715 at day 15), and there was no difference between experimental and non-experimental odd nests (Wald test p=0.664; -0.091 probits [-2.766 - 2.859] P=0.933 at day 0; 0.681 probits [-1.798 - 3.235] P=0.592 at day 15). Uncrossed chicks in full treatment nests also did not differ significantly from other chicks (Wald test p=0.449; -3.069 probits [-6.991 - 0.737] P=0.119 at day 0; -1.313 probits [-3.949 - 1.719] P=0.363 at day 15). However, there was a significant difference in survival probabilities of offspring in partially cross-fostered nests in non-experimental years compared to experimental years (Wald test: Uncrossed in non-experimental years p=0.002; Crossed in non-experimental years p=0.004). For those that were uncrossed, there was a non-significant decrease in survival at day 0 (-0.938 probits [-2.729 - 1.077] P=0.356), but a positive interaction with day, such that there was a significantly higher survival at day 15 (2.648 probits [1.070 - 4.190] P<0.001). Similarly, for crossed offspring, there was a non-significantly lower survival at day 0 (-0.623 probits [-2.502 - 1.295] P=0.535) and a significantly higher survival by day 15 (2.750 probits [1.290 - 4.425] P<0.001).

Time, sex, and clutch size had no significant effects on survival, but there were significant effects of hatching date and hatching day within the clutch (Table 4.4). Egg rank had a significant overall effect on survival (Wald test p=0.030). Chicks from higher rank eggs (early laid) had non-significantly reduced survival probabilities at day 0 (-0.055 probits [-0.125 - 0.022] P=0.141), but were significantly more likely to survival by day 15 (0.062 probits [0.011 - 0.118] P=0.025).

	mean	l-95%	u-95%	pMCMC	p Wald
Day 0	16.623	10.45	22.74	**<0.001**	
Day 1	16.561	11.059	22.565	**<0.001**	
Day 3	15.348	10.014	20.258	**<0.001**	
Day 6	16.962	12.704	21.747	**<0.001**	
Day 9	16.469	11.996	20.57	**<0.001**	
Day 12	17.086	12.421	21.534	**<0.001**	
Day 15	18.047	12.669	23.014	**<0.001**	
Time	-0.045	-3.39	3.177	0.979	
Time: Day	0.057	-0.219	0.349	0.707	0.698
Sex (M)	0.016	-0.227	0.248	0.896	
Sex (M): Day	0	-0.021	0.022	0.978	0.979
Year 2011	0.22	-1.076	1.763	0.762	
Year 2011: Day	-0.319	-0.424	-0.199	**<0.001**	**<0.001**
Year 2012	-0.329	-1.738	0.969	0.637	
Year 2012: Day	-0.104	-0.211	-0.004	0.052	0.051
Year 2013	0.836	-0.674	2.442	0.31	
Year 2013: Day	-0.076	-0.212	0.037	0.232	0.235
Year 2014	0.892	-0.595	2.465	0.257	
Year 2014: Day	0.07	-0.042	0.2	0.256	0.257
Clutch Size	-0.086	-0.318	0.155	0.472	
Clutch Size: Day	-0.009	-0.028	0.009	0.347	0.344
Hatch Date	-0.078	-0.139	-0.011	**0.017**	
Hatch Date: Day	-0.013	-0.018	-0.007	**<0.001**	**<0.001**
Hatch Day (0-1)	-1.315	-1.673	-0.936	**<0.001**	
Hatch Day (0-1): Day	0.047	0.011	0.078	**<0.001**	**0.007**
Hatch Day (1-3)	-3.299	-4.077	-2.567	**<0.001**	
Hatch Day (1-3): Day	0.129	0.06	0.2	**<0.001**	**<0.001**
Egg Rank	-0.055	-0.125	0.022	0.141	
Egg Rank: Day	0.008	0.001	0.014	**0.023**	**0.03**
Odd nest	0.674	-1.514	2.878	0.552	
Odd nest: Day	-0.023	-0.188	0.141	0.799	0.789
Non-Exp Odd	-0.091	-2.766	2.859	0.933	
Non-Exp Odd: Day	0.051	-0.191	0.283	0.659	0.664
Full, Uncrossed	-3.069	-6.991	0.737	0.119	
Full, Uncrossed: Day	0.117	-0.176	0.422	0.468	0.449
Non-Exp Partial, Uncrossed	-0.938	-2.729	1.077	0.356	
Non-Exp Partial, Uncrossed: Day	0.239	0.084	0.381	**<0.001**	**0.002**
Non-Exp Partial, Crossed	-0.623	-2.502	1.295	0.535	
Non-Exp Partial, Crossed: Day	0.225	0.08	0.385	**<0.001**	**0.004**
PO	0.094	-1.119	1.075	0.857	
PO: Day	0.005	-0.091	0.098	0.923	0.914
S	-3.382	-6.964	0.285	0.069	
S: Day	0.158	-0.121	0.446	0.276	0.27
SPO	-0.912	-2.835	1.173	0.398	
SPO: Day	0.056	-0.116	0.226	0.546	0.534

Table 4.4: Fixed effect results for the effect of treatment on the survival of chicks over ontogeny. The mechanisms by which crossed offspring may lose fitness have been fitted as parent-offspring coadaptation (PO), sibling coadaptation (S), and sibling-mediated parent-offspring coadaptation (SPO). In addition, odd nests were fitted as a separate factor, as were uncrossed individuals in the full treatment.

Treatment	Died	Survived
Control	63	15
Full	62	22
Partial	83	12
Odd	41	7

Table 4.5: The number of adults that died or survived when exposed to each of the treatments in 2014

	mean	l-95%	u-95%	pMCMC
Sex F	-0.051	-0.547	0.479	0.852
Sex M	-0.783	-1.437	-0.132	**0.013**
Male: Odd	0.091	-0.844	1.108	0.821
Male: Full	0.498	-0.376	1.358	0.286
Male: Partial	0.375	-0.57	1.214	0.408
Female: Odd	-0.651	-1.505	0.226	0.137
Female: Full	0.531	-0.209	1.265	0.174
Female: Partial	-0.755	-1.608	-0.039	0.055

Table 4.6: The effects of treatment on survival of adults to the subsequent breeding season.

Adult Survival and Fecundity: The number of adults that died or survived from 2014 to 2015 for each treatment are shown in Table 4.5, and the results from the model of adult survival are in Table 4.6. For females there is a non-significant positive effect of the full treatment on survival (0.531 probits [-0.209 - 1.265] P=0.174), but a marginal negative effect of the partial treatment (-0.755 probits [-1.608 - -0.039] P=0.055), such that there is slightly reduced survival of females raising partially cross-fostered broods. Females raising nests classed as odd have non-significantly lower survival (-0.651 probits [-1.505 - 0.226] P=0.137). For males there is also a non-significant positive effect of the full cross-fostering treatment (0.498 probits [-0.376 - 1.358] P=0.286), partial cross-fostering treatment (0.375 probits [-0.570 - 1.214] P=0.408). There was also a non-significant positive effect of raising odd broods (0.091 probits [-0.844 - 1.108] P=0.821).

The fecundities of adults that survived from 2014 in the different treatments are shown in Figure 4.4, and the results of the model are shown in Table 4.7. For females there was a non-significant positive effect of raising a fully crossed brood (an increase of 0.048 eggs [-1.158 - 1.171] P=0.938) and a non-significant negative effect

of raising a partially crossed brood (-0.148 eggs [-1.789 - 1.393] P=0.872). There was also a non-significant increase in fecundity in those that had odd nests 0.964 eggs [-0.816 - 2.669] P=0.283). For males there was a non-significant decrease in fecundity for the full treatment males (-4.409 eggs [-9.878 - 1.746] P=0.126). The partial treatment had a significant negative effect on fecundity (-6.697 eggs [-12.570 - -1.001] P=0.021), however this was mainly driven by an outlier (a control male with extremely high fecundity in 2015), and if this is excluded the effect of the partial treatment becomes non-significant (-3.138 eggs [-7.962 - 1.356] P=0.179). The effect of raising odd nests was also negative and non-significant (-4.003 eggs [-12.136 - 3.371] P=0.302).

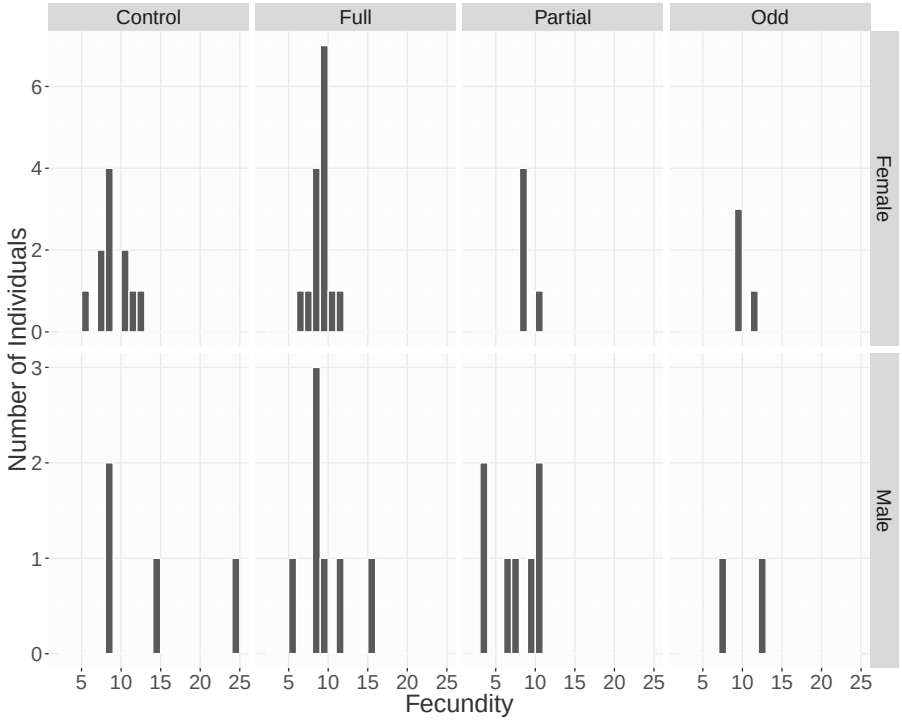


Figure 4.4: The fecundities in 2015 of individuals surviving from 2014 to 2015, split by sex and the treatment they received in 2014.

	mean	l-95%	u-95%	pMCMC
Sex F	8.557	7.647	9.408	**<0.001**
Sex M	13.546	9.067	18.235	**<0.001**
Male: Odd	-4.003	-12.136	3.371	0.302
Male: Full	-4.409	-9.878	1.746	0.126
Male: Partial	-6.697	-12.570	-1.001	**0.021**
Female: Odd	0.964	-0.816	2.669	0.283
Female: Full	0.048	-1.158	1.171	0.938
Female: Partial	-0.148	-1.789	1.393	0.872

Table 4.7: The effects of treatment on fecundity of the surviving adults in the subsequent breeding season.

4.4 Discussion

By carrying out a randomised cross-fostering experiment, this study aimed firstly to test for evidence of parent-offspring (PO) coadaptation in blue tits, and secondly whether sibling (S) coadaptation and/or sibling-mediated parent-offspring (SPO) coadaptation exist. PO coadaptation is primarily detected by comparing the fitnesses of crossed and uncrossed offspring, irrespective of the type of nest (full or partial) that they were raised in (comparisons 1 and 2 in Figure 4.1), with the expectation that crossed offspring will have reduced fitness. We did not find a reduction in offspring mass or survival when offspring were raised by foster rather than biological parents, and instead saw a slight increase in mass when offspring were cross-fostered. This is in contrast to the results found by Hinde *et al.* (2010), who found evidence of PO coadaptation through growth costs to offspring crossed between nests.

The power to detect PO coadaptation effects on offspring was high (changes in mass of $< 1\%$ could have been detected) and so we are fairly confident that if PO coadaptation does exist, the effect is likely to be small. In order for PO coadaptation effects on offspring to exist, there must be linkage disequilibrium between parental effect alleles and the alleles determining the affected offspring traits. Given that Santure *et al.* (2013) have shown that there is low linkage disequilibrium in the

great tit (and assuming similar patterns hold in blue tits), the potential for such coadapted gene complexes to evolve in these species may be limited, and the lack of evidence for PO coadaptation unsurprising (Hadfield, 2012). Indeed, genetic correlations between begging and provisioning were not detected in blue tits (Lucass *et al.*, 2015a), although the power of this study was low. These conditions for PO coadaptation to evolve could be relaxed if there were loci with pleiotropic effects on parental effects and offspring traits. The developmental pathways for these two types of trait are likely to be very different, so the opportunity for pleiotropy may be quite small, although further work is required to verify this. Thus, although the presence of coadaptation between parents and offspring has been widely assumed in behavioural ecology, the results presented here suggest that this assumption may not be valid, and may need to be further tested in other study systems.

Although our arguments have mainly been made in a genetic context, Hinde *et al.* (2010) considered coadaptation from the idea that signals within eggs (prenatal maternal effects) may generate correlations between parents and offspring. Components placed within eggs may act as signals or cues of parental ability, leading to modulation of offspring behaviour in response to the prenatal parental environment (e.g. Hinde *et al.*, 2009; Paquet *et al.*, 2015). Although this involves a fundamentally different mechanism for matching offspring and parental phenotype, the predicted fitness outcomes are the same as those under genetic co-adaptation, and so we believe that our study also rejects a major role for between-brood anticipatory parental effects in blue tits.

If siblings affect each other through non-excludable parental manipulation, then using the test for PO coadaptation alone would miss this mechanism, which we have called sibling-mediated parent-offspring coadaptation. Under this scenario, we would expect to find partially cross-fostered nests to be intermediate between control and fully cross-fostered nests, as some biological offspring of the parents remain at that nest. Although the power to detect this type of effect was lower than for PO

coadaptation, we found little evidence for SPO coadaptation. In addition, if direct interactions between siblings are present, sibling coadaptation may exist, and we would expect chicks in partial cross-fosters to do worse than control and fully cross-fostered chicks. Again, we found little evidence for S coadaptation, thus individuals do not seem to benefit from being with siblings, either through direct or indirect mechanisms.

As well as considering potential effects of coadaptation on offspring, we tested whether there were effects of the cross-fostering treatments on parental fitness. There appears to be no cost to females raising fully cross-fostered broods, and little cost to those raising partially crossed broods, although a marginal negative effect on survival was found. Similarly there were no significant costs to males in either of these treatments. Thus, there is limited evidence for costs to parents through either parent-offspring or sibling-mediated parent-offspring coadaptation. The effect of the partial treatment on female survival is marginal, however, and the lack of significant effects may reflect a power issue, as we currently only include the survival and future fecundity of adults from 2014. Under PO and SPO coadaptation we predicted that adults from fully treated nests should suffer a greater fitness cost than those raising partially treated nests, due to the presence of some biological offspring in partially treated nests. However, if those raising partially crossed broods suffer more than those raising fully crossed broods, this may imply that parents bear the effects of sibling conflict (even if those offspring themselves do not appear to show costs of this).

The absence of coadaptation between traits may be driven by a lack of sufficient genetic variance in either parental or offspring effects, such that genetic covariances will then be absent (or very small). There is direct evidence for heritable parental effects in wild systems (Wilson *et al.*, 2005a), and also indirect evidence whereby traits thought to be responsible for parental effects (e.g. provisioning rates) have been shown to be heritable, in long-tailed tits (MacColl & Hatchwell, 2003), house

sparrows (Dor & Lotem, 2010), and burying beetles (Walling *et al.*, 2008). Direct evidence for heritable offspring effects is more limited (Agrawal *et al.*, 2001), as is the indirect evidence (Dor & Lotem, 2009). Whether this evidence-base reflects real differences between these types of effects, or whether they merely reflect research bias, remains an open question. Nevertheless, it is possible that there may be insufficient genetic variation in one (or both) traits to generate genetic covariances. Instead, individuals may be plastic in their behaviours, such that they adapt to the behaviour of other parties (and the environment). There is evidence that both parents (Ottosson *et al.*, 1997; Rehling *et al.*, 2012) and offspring behaviours (Kedar *et al.*, 2000; Royle *et al.*, 2006; Budden & Wright, 2008; Grodzinski *et al.*, 2008; Riou *et al.*, 2012) are plastic, and so there may not be large genetic components in these, limiting the possibility of coadaptation evolving.

Winney *et al.* (2015) showed an apparent increase in the fitness of house sparrow chicks when raised with and by non-relatives, but attributed this to non-random assignment of chicks and nests to cross-fostering treatment. Although we used a balanced randomised design in order to reduce this type of problem, crossing eggs on the day that they were laid means that crossed eggs tended to be earlier in the laying sequence, when all females were still laying and eggs could be switched. Early eggs tend to hatch earlier (Hadfield *et al.*, 2013a) and are more likely to have been sired by extra pair males (Magrath *et al.*, 2009, an effect confirmed in our population: Table D.1 in SI) both of which are likely to favour higher body-masses and survival. We tried to control for these in our analyses by using hatching time and egg rank as predictors. However, as multiple chicks hatch between visits to the nests we are often not able to assign a chick to the exact egg from which it hatched. In lieu of this information we used mean egg ranks, which may not be able to completely compensate for this bias. Nevertheless, the mean egg ranks of crossed and uncrossed eggs and chicks are similar and the effect of egg rank in the model, although significant, is small once hatching time is accounted for. Consequently,

although we expect that this (unavoidable) inadequacy in our design is likely to bias the estimates of coadaptation, we believe these biases are likely to be small.

The cross-foster design in this study is the first to compare both fully and partially cross-fostered broods, along with appropriate control broods in a wild population. We used these comparisons to test for parent-offspring coadaptation, and sibling effects. We did not find any evidence for parent-offspring coadaptation, either through direct interactions or the effect of siblings, nor did we find any evidence for sibling coadaptation. In order to extend the findings, carrying out similar studies in other species, such as the canaries in Hinde *et al.* (2010), will help to demonstrate whether the results shown here are general, or if there is variation in the degree of coadaptation, and sibling effects, between species.

Chapter 5

General Discussion

The interactions between parents and their offspring have the potential to generate complex evolutionary dynamics, either accelerating evolution or hindering it. In this thesis, I aimed to explore these parental effects, particularly parental care, and how they might affect micro-evolution.

In Chapter 2 I tested a prediction that a maternal effect on prenatal development rate (as found in Hadfield *et al.*, 2013b) might be driven by environmental changes over the laying period of a clutch, and therefore was a passive maternal effect, rather than an active manipulation of offspring phenotype. I did not find evidence for this – eggs laid when females received supplementary food did not accelerate hatching. Thus, the increase in prenatal development of late laid eggs is likely to be an anticipatory maternal effect, in which females are anticipating the sibling competition environment that individuals will experience, which acts independently of the dietary environment of the female. Thus there is an active process occurring where the female causes faster development in late laid eggs in order to reduce the extent of hatching asynchrony.

I found evidence of evolutionary conflicts, in the form of antagonistic selection, between parents and their offspring in Chapter 3, which was mediated through a negative effect of parental performance on parental fecundity, and its positive indirect effect on offspring survival. This finding, when placed in the framework of the Willham model (Willham, 1963, 1972) and its extensions (Cheverud, 1984) shows how antagonistic selection upon offspring traits may explain stasis. Although I was not able to estimate the genetic correlation between these parental and offspring traits, this finding predicts that a negative correlation is still needed in order to explain stasis, although it would not require such an extreme correlation as might be predicted under the assumption of no selection on parental behaviour. Thus, this finding may help to explain stasis in juvenile phenotypic traits (particularly body mass), despite directional selection upon them.

I did not find evidence of coadaptation between parents and their offspring, using the cross-fostering experiment in Chapter 4 – offspring did not suffer reduced mass or survival when exchanged between nests. This is in contrast with previous findings, particularly that of Hinde *et al.* (2010), but is consistent with results from Smith & Wettermark (1995), Brown *et al.* (2015) and Merilä *et al.* (1999). Rather, I found that there was a slight increase in body mass of offspring raised by foster parents. Some bias in which eggs were transferred (especially in the partial cross-fostering treatment) may explain these results, although we attempt to compensate for this in the analysis. There was also no evidence for an effect of sibling coadaptation on individual fitness, nor were there strong effects on parental fitness. Thus, we fail to see any evidence of coadaptation of traits involved in family interactions.

5.1 Estimating Selection upon Parental Traits

The definition of parental investment from Trivers (1974) predicts that there should be evolutionary conflict over parental investment in offspring, due to the antagonistic effects on the direct fitnesses of parents and their offspring (Williams, 1966; Stearns, 1992). There has been some evidence in support of this, particularly from those studies that look at trade-offs over offspring size/quality and number, as predicted by Smith & Fretwell (1974). Charnov & Ernest (2006) show a negative correlation (i.e. trade-off) between offspring size and number in mammals, and reviews in Roff (2002) show evidence of such trade-offs in both observational and manipulative studies. In addition, a cross-species comparison by Badyaev & Ghalambor (2001) has shown that the relative investment of parents in fecundity and parental care vary along altitudinal gradients, with decreased fecundity and increased parental care at higher elevations, reflecting the change in selection pressures with environmental changes.

Evolutionary trade-offs between parent and offspring should, however, manifest

as antagonistic selection between these parties, and thus quantification of selection on parents and offspring should be used to reveal such trade-offs. Whilst there is abundant evidence for selection on offspring traits (especially body mass; Rollinson & Rowe, 2015), evidence of such antagonistic selection is generally lacking. Nevertheless, many of the selection gradients within the meta-analyses on selection in the wild carried out by Kingsolver *et al.* (2001) and in subsequent papers (e.g. Kingsolver & Diamond, 2011) do estimate selection upon parental traits. However, these generally use fitness measures that cross generations – either a composite measure of fitness that appoints offspring survival as part of parental fecundity (weighted fitness, e.g. Sheldon *et al.*, 2003; Charmantier *et al.*, 2006; Chaine & Lyon, 2008), or measuring the direct effect of a parental trait on offspring fitness. Such selection estimates, however, are problematic, as they conflate selection and inheritance, making it difficult to connect the selection measures with the quantitative genetic theory of evolutionary change (Wolf *et al.*, 2001). Therefore, these should be avoided, where possible, and fecundity measured as the number of zygotes an individual produces, and the subsequent survival of a given zygote assigned as the fitness of that zygote itself (Arnold & Wade, 1984; Wolf *et al.*, 2001).

Where the direct effect of a parental trait on offspring fitness is estimated, the resulting selection gradient has been called a parental selection gradient (Kirkpatrick & Lande, 1992; Hadfield, 2012). In this case, the total selection occurring upon traits can be written as:

$$\beta_N = \beta_I + \frac{1}{2}\beta_M \tag{5.1}$$

Where β_I is the direct selection gradient (the partial regression coefficients associated with *offspring* traits from a multiple regression of offspring's *own* fitness on *both* offspring and parental traits), and β_M is the parental selection gradient (the partial regression coefficients associated with *parental* traits from the same multi-

ple regression), scaled by $\frac{1}{2}$ to account for inheritance. Thus, selection is measured through the effect of a trait measured in two generations on the fitness in one generation. The studies in Kingsolver *et al.* (2001), however, instead measure selection on traits measured in *one* generation through its effects on the fitness of individuals in *two* generations (i.e. an individuals own fitness, and offspring fitness). In order to make notation for this clear, I use z to indicate a trait, with the subscript t to indicate when it has been measured in the current generation, and $t+1$ to indicate when it is measured in the next generation. Additionally, I use j and f to denote fitness through juvenile survival and adult fecundity respectively, with equivalent subscripts to indicate the generation in which fitness has been measured. Assuming that the trait value of an individual can affect its own juvenile survival and fecundity, but that the trait value of a parent can only affect the juvenile survival of its offspring, the Kirkpatrick-Lande (K-L) model is written as:

$$\beta_N = \beta^{j_{(t+1)}}_{z_{(t+1)}} + \beta^{f_{(t+1)}}_{z_{(t+1)}} + \frac{1}{2}\beta^{j_{(t+1)}}_{z_{(t)}} \tag{5.2}$$

Thus $\beta_I = \beta^{j_{(t+1)}}_{z_{(t+1)}} + \beta^{f_{(t+1)}}_{z_{(t+1)}}$ (i.e. an individual's *own* trait regressed against its *own* fitness), and $\beta_M = \beta^{j_{(t+1)}}_{z_{(t)}}$ (a individual's *parent's* trait regressed against an individual's *own* fitness). Those studies in Kingsolver *et al.* (2001) that measure selection through the effect of a *parental* trait ($z_{(t)}$) on a fitness measure that includes *offspring* fitness can thus be written as:

$$\tilde{\beta_W} = \tilde{\beta}^{f_{(t)}}_{z_{(t)}} + \tilde{\beta}^{j_{(t+1)}}_{z_{(t)}} \tag{5.3}$$

Where $\tilde{\beta}^{f_{(t)}}_{z_{(t)}}$ is the regression of that individual's own trait on its own fecundity (i.e. number of zygotes). It is important to remember that the regression coefficients using weighted fitness are from univariate regressions, where the K-L regression coefficients are from a multiple regression that include both offspring and parental

traits. When parental and offspring traits are uncorrelated (i.e. not heritable) $\tilde{\beta}^{f_{(t)}}_{z_{(t)}} = \beta^{f_{(t+1)}}_{z_{(t+1)}}$, and $\tilde{\beta}^{j_{(t+1)}}_{z_{(t)}} = \beta_M$. It can be seen therefore, that although these equations relate to one another, they are not equivalent. In the case where the trait is heritable, then it can be shown that

$$\tilde{\beta_W} = \beta^{f_{(t)}}_{z_{(t)}} + \beta^{j_{(t+1)}}_{z_{(t)}} + \frac{1}{2}h^2\beta^{j_{(t+1)}}_{z_{(t+1)}} \tag{5.4}$$

and so direct selection operating through juvenile survival is underestimated by a factor equal to half the heritability, and the parental selection gradient is double what it should be. In cases where parental traits had no direct effect on offspring fitness, after conditioning on offspring traits ($\beta^{j_{(t+1)}}_{z_{(t)}} = 0$) then

$$\tilde{\beta_W} = \beta^{f_{(t)}}_{z_{(t)}} + \frac{1}{2}h^2\beta^{j_{(t+1)}}_{z_{(t+1)}} \tag{5.5}$$

Thus, if direct selection on a trait via fecundity and juvenile survival is antagonistic, such that there is no net selection, using a weighted fitness measure will incorrectly provide evidence for selection for trait values that favour fecundity.

Whilst these considerations are likely to be important when selection is measured on a trait that is expressed concurrently in two generations (such as body size), many studies in Kingsolver *et al.* (2001) instead estimate selection upon traits that are only expressed in adults. For example, selection on phenological traits has been estimated through effects on offspring fitness, including the effects of laying date (Charmantier *et al.*, 2006), parturition date (McAdam & Boutin, 2003), and arrival date (Seamons *et al.*, 2007). Where significant directional selection has been found, this implies that there is a direct effect of the parent's phenological traits on offspring fitness (survival), and as the trait is not simultaneously expressed in the offspring $\beta^{j_{(t+1)}}_{z_{(t+1)}} = 0$ in equations 5.4 and 5.5. However, the parental trait may be correlated with a second trait (y) in the offspring (such as body mass) either through the direct

effect of genes, or through the trait z having a parental effect on y. If the parental trait has no direct effect upon offspring fitness or the offspring trait y, then the selection gradient that has been measured becomes

$$\tilde{\beta_W} = \beta^{f_{(t)}}_{z_{(t)}} + \frac{1}{2} r_G \beta^{j_{(t+1)}}_{y_{(t+1)}} \tag{5.6}$$

where r_G is the genetic correlation between the traits z and y in a given individual, scaled by $\frac{1}{2}$ to account for inheritance. If r_G is positive (i.e. a positive genetic correlation) then the effect on the overall selection will be akin to the effects of heritabilities in equation 5.5. However, a negative genetic correlation will increase the magnitude of the estimated selection gradient if selection is in opposite directions in the two generations, and decrease the magnitude if they are in the same direction.

Furthermore, it is easy to visualise that the parental trait may affect the second trait in the offspring ($y_{(t+1)}$), such as the effect of laying date on offspring body mass, and thereby indirectly affect offspring fitness. Thus:

$$y_{(t+1)} = \quad z_{(t)} m_{y|z} + a_{y_{(t+1)}} + e_{y_{(t+1)}} \tag{5.7}$$

where $m_{y|z}$ is the maternal effect of trait z in the parent on y in the offspring, $a_{y_{(t+1)}}$ is the additive genetic variance for y, and $e_{y_{(t+1)}}$ is error. The fitness of an offspring is therefore $j_{(t+1)} = y_{(t+1)} \beta^{j_{(t+1)}}_{y_{(t+1)}}$, and the selection estimated through offspring fitness then becomes:

$$\begin{aligned} \tilde{\beta}^{j_{(t+1)}}_{z_{(t)}} &= cov(y_{(t+1)} \beta_{y_{(t+1)}}, z_{(t)}) \\ &= cov(\beta^{j_{(t+1)}}_{y_{(t+1)}} [z_{(t)} m_{y|z} + a_{y_{(t+1)}} + e_{(t+1)}], z_{(t)}) \\ &= \beta^{j_{(t+1)}}_{y_{(t+1)}} (m_{y|z} + \tfrac{1}{2} r_G) \end{aligned} \tag{5.8}$$

Assuming throughout that traits are standardised, such that $var(z_{(t)}) = 1$. Ad-

ditionally, it could also be envisioned that the second trait when expressed in parents ($y_{(t)}$) may have an effect on the parents *own* fitness, in addition to the effect of $z_{(t)}$, such that:

$$f_{(t+1)} = \beta_{z_{(t)}}^{f_{(t)}} z_{(t)} + \beta_{y_{(t)}}^{f_{(t)}} y_{(t)} \tag{5.9}$$

and so the selection estimated through parental fecundity becomes:

$$\begin{aligned} \tilde{\beta}_{z_{(t)}}^{f_{(t)}} &= cov(\beta_{z_{(t)}}^{f_{(t)}} z_{(t)} + \beta_{y_{(t)}}^{f_{(t)}} y_{(t)}, z_{(t)}) \\ &= \beta_{z_{(t)}}^{f_{(t)}} + \beta_{y_{(t)}}^{f_{(t)}} r_G \end{aligned} \tag{5.10}$$

The actual selection gradient that has been measured in those cases then become a complex combination of the effect of an individual's focal trait on their own fitness, the effect of the second trait on an individuals own fitness, the effect on the second trait in the offspring ($m_{y|z}$), and the effect of that offspring's trait on offspring fitness:

$$\tilde{\beta_W} = \beta_{z_{(t)}}^{f_{(t)}} + \beta_{y_{(t)}}^{f_{(t)}} r_G + \beta_{y_{(t+1)}}^{j_{(t+1)}} (m_{y|z} + \frac{1}{2} r_G) \tag{5.11}$$

It is clear, therefore, that using weighted fitness to estimate selection is inappropriate, and produces selection gradients that are complex combinations of the effects of parents on offspring fitnesses and traits, and the effect of offspring's own traits on their own fitness. It is unsurprising, therefore, that there has previously been no conclusive evidence of antagonistic selection between parents and offspring.

The method used to show antagonistic selection between parents and offspring in Chapter 3 avoids such issues – I estimate the direct selection upon a parental trait, which also affects an offspring trait that is under direct selection (body mass), but does not directly affect offspring fitness (therefore the parental selection gradient is assumed to be zero). I find, as expected, that selection is generally in oppos-

ing directions, through its effects on parental fecundity and offspring survival, thus confirming antagonistic selection within a size-number trade-off.

5.1.1 Elasticities of Fitness

In Chapter 3 I estimated selection upon juvenile body mass, and parental performance for juvenile body mass. I found that there was strong positive directional selection on juvenile body mass, through its effects on juvenile survival. In addition, I found negative directional selection on parental performance, through its effects on fecundity, and this was of a smaller magnitude than the selection through juvenile survival. This is contrast to the general finding that selection gradients are often found to be of larger magnitude (i.e. stronger selection) through fecundity fitness measures than through survival (Kingsolver *et al.*, 2001; Hoekstra *et al.*, 2001). Whilst this may reflect a specific difference between the traits I measured and the general strengths of selection acting through different fitness components, it may also be due to differences in the way in which selection has been measured. Although I measure selection on traits primarily through their effects on certain fitness measures, I calculate total selection on these traits by showing how fitness episodes relate to LRS, and then take the partial derivatives of LRS with respect to the traits of interest. In contrast, the fitness measures included in Kingsolver & Diamond (2011) and Hoekstra *et al.* (2001) use only a single (or composite) fitness measure, and do not estimate effects on lifetime reproductive success, making it difficult to make comparative statements about the strength of selection in those studies and Chapter 3.

When selection gradients are calculated using episodes of fitness, rather than LRS, weighting selection gradients by the elasticities of vital rates (fitness measures) allows the effect of traits on LRS to be estimated. This therefore allows more complete conclusions to be made about the selection acting through different fitness

measures. These elasticities of fitness are the proportional change in λ (the asymptotic rate of increase of the population) with the proportional change in a given vital rate (i.e. the fitness measure; Caswell, 1989). Therefore, although fecundity selection has been shown to be generally stronger than survival selection (Hoekstra *et al.*, 2001), adult survival has generally been found to have higher elasticity (Crone, 2001), and may therefore contribute a greater amount to lifetime reproductive success. The selection gradients found in Hoekstra *et al.* (2001) should be weighted by the selection gradients of those fitness measures themselves (i.e. the elasticity of the vital rates van Tienderen, 2000). Thus, although (Hoekstra *et al.*, 2001) conclude that selection is generally stronger through fecundity than through survival, weighting such measures by elasticities might generate difference conclusions about the strength of selection (Crone, 2001).

5.2 Maternal effects on eggs

I failed to find any effects of the environment on egg characteristics, and in particular I did not see the expected effect of supplementary feeding on development rate and hatching time. Thus the difference in developmental rate in early and late laid eggs found in Hadfield *et al.* (2013b) is likely to be driven by an active change of some factor in eggs, in anticipation of the competitive environment they are expected to experience.

There is some evidence for prenatal maternal effects on hatching time, although research into this has been limited. Egg size has a small effect on development rate in blue tits (Hadfield *et al.*, 2013b), but this did not completely explain the changes in development rate between early and laid laid eggs. Changes in the relative proportions of yolk and albumen in eggs may also occur over the laying sequence. Ferrari *et al.* (2006) found that albumen mass increased over the laying order in barn swallows (*Hirundo rustica*), whilst yolk mass did not, suggesting that the relative

proportion of the egg that was albumen may change within a clutch. In addition, they found that removal of albumen delayed hatching time (also found in yellow-legged gulls, *Larus michahellis*; Alquati *et al.*, 2007). Thus, changes in the albumen and yolk content of eggs over the laying sequence may be important in mediating these hatching time differences.

There is also evidence for specific substances within eggs impacting upon development rate. Testosterone has been shown to affect hatching time (Sockman & Schwabl, 2000), as has corticosterone (Rubolini *et al.*, 2005). Thus, hatching time effects may be mediated by hormones. Furthermore, Lipar & Ketterson (2000) found that testosterone increased the development of a muscle associated with hatching, and so hatching time effects may also be mediated through this mechanism (without associated increased total development rate). Thus, there is scope for further work to quantify whether differences in such substances within eggs may mediate differences in hatching time over the clutch. Such studies on substances within eggs are limited by methodological problems, however. Quantification of the levels of hormones or carotenoids in eggs requires dissection of those eggs (Schwabl, 1993), and so the traits presumed to be affected by the substances will not be expressed. Alternatively, manipulation of the amount of these substances have been used (e.g. in Lipar & Ketterson, 2000; Sockman & Schwabl, 2000), and the subsequent effects on chicks have been quantified. The true quantities of the amount of manipulated hormone or carotenoids in eggs is unknown, however, and so these represent an imprecise measure of the effects of these substances.

Evolutionary consequences

Such prenatal maternal effects on eggs are an example of how parents can affect the fitness of their offspring, through their effects on a trait (hatching time, and consequent body mass) that then affects fitness. In an evolutionary context, offspring should be under selection to maximise fitness within this maternal effect. If, indeed,

this represents a signal or cue to late-laid offspring that causes increased developmental rate, then offspring may be under selection to increase their response to this cue, and thus reducing hatching asynchrony. However, costs to increased growth rate (Metcalfe & Monaghan, 2001), may balance out this selection. Mauck *et al.* (2005) find that there is a negative correlation between growth rate and the rate of immune system increase in storm petrels, suggesting that there may be a trade-off between immunity and growth. Similar effects were also found in sand martins, but only when food restricted (Brzęk & Konarzewski, 2007).

5.3 Detecting parent-offspring conflict and coadaptation

5.3.1 Manipulative vs. observational studies

This thesis uses a mix of manipulative (Chapters 2 and 4) and observational studies (Chapter 3) in order to test predictions about parental effects. Observational studies are often limited in their ability to detect trade-offs, such as those in Chapter 3, as variation in individual quality (Reznick, 1985; van Noordwijk & de Jong, 1986; Lessells & McNamara, 2012), especially amongst parents, may mask these. Individual quality is defined as *an axis of among-individual heterogeneity that is positively correlated with fitness* (Wilson & Nussey, 2010). Thus, parents of high quality may be able to both provide more care, and produce more offspring in a given attempt, although within a group of individuals of equal quality, a trade-off may remain. Such effects can be seen in experiments where supplementary food is provided to breeding birds. For example, Gill & Hatch (2002) showed that providing extra food to black-legged kittiwakes (*Rissa tridactyla*) increased both clutch size and fledging success, highlighting that increased access to resources can allow increased investment in both fecundity and care. Wild individuals are also hypothesised to vary in the quantity of resources they are able to acquire, generating differences in parental

quality. Thus the effects of quality may overpower any phenotypic trade-offs in observational studies. Indeed, although I find evidence for antagonistic selection in Chapter 3, this may underestimate the true strength of selection relative to manipulative studies.

Manipulative studies, in contrast, may be better able to overcome such issues. Such manipulations to detect the presence and effects of parental care and parental effects can include cross-fostering offspring between different qualities of parents and considering the effects on offspring and/or parents. Alternatively, manipulating the parental care that parents provide may reveal costs of these behaviours, for example using clutch size manipulation (Nur, 1984a; Parejo & Danchin, 2006), or begging playbacks (Kölliker *et al.*, 2000; Hinde, 2006). Such manipulations are expected to increase the amount of care parents provide, and may therefore have downstream effects on parental survival or future fecundity, revealing the direct fitness costs of increased care. As discussed in Chapter 3, however, such studies must also consider any unwarranted effects that the manipulations may have, particularly that costs may be passed costs onto offspring. Indeed, clutch size manipulations are often also used to show how clutch size affects offspring fitness (e.g. Nur, 1984b; Aubret *et al.*, 2003), and so such studies attempting to quantify its effect upon parental traits must also consider impacts upon the offspring.

Sæther & Bakke (2000) found that birds tend to lie along a "slow-fast" continuum in terms of life-history strategy; fast species tend to have short life histories, with high annual fecundity, early maturation and low survival, whereas slow species have higher survival but lower annual fecundity and late maturation. There may be differences in the effects of such parental care manipulations in species along this continuum. Indeed, studies of sea-birds with slow life histories by Mauck & Grubb Jr (1995) and Sæther *et al.* (1993) have shown costs to offspring from parental manipulation, but little cost to parents, such that parents appear to favour their own survival. In contrast, Gustafsson *et al.* (1995) showed that the costs of manipulation

were in part borne by parents in collared flycatchers, a species with a much faster life-history strategy. Thus, the methods to manipulate parental care must also be considered within the context of the life-history of the species in question.

5.3.2 Wild vs. laboratory studies

All research done in this thesis is carried out in the wild. Therefore individuals are expected to be exposed to the natural stresses and challenges of breeding in an unmanipulated, variable environment, although using nest boxes may reduce predation and parasite prevalence. This high level of environmental noise results in the need to use complex statistical models in order to detect relationships in observational data (as in chapter 3), and the effect of experimental treatments (such as the models in Chapters 2, and 4). In contrast, laboratory studies are able to eliminate or manipulate such stresses. Using laboratory studies therefore allows greater control, and reduced environmental noise, and can reduce the need to utilise such complex models. Furthermore, laboratory studies may be able to overcome many of the limitations in this thesis, In particular, I was unable to estimate genetic parameters in Chapter 3, in part due to low rates of recruitment. In a laboratory population, this recruitment may be increased, as mortality and emigration are reduced, and so increase the power available to estimate genetic parameters.

The differences between laboratory and wild based studies may also limit the comparisons that can be made between these. In Chapter 4 in particular I compare the results found with those of Hinde *et al.* (2010), who studied parent-offspring coadaptation in a laboratory maintained population of canaries. The particular conditions in which these birds were allowed to breed were not specified, but are unlikely to include many of the environmental stresses experienced by wild populations. Thus, this difference between studies makes for difficult comparisons. On the one hand, such laboratory studies may result in larger effect sizes of any coad-

aptation or conflict, as other sources of variation have been carefully controlled, but in the wild they are overpowered by natural variation and environmental noise. On the other hand, these individuals have probably also been supplied with ad libitum food, such that the strengths of trade-offs are likely to be weakened compared to a wild population.

Although studies carried out in wild, unmanipulated populations are limited by the effects of environmental noise, they may be more relevant in general when considering how wild populations and species may change in response to selection. Natural selection acts upon phenotypes, and so selection will only act upon the phenotypes that are expressed in the population. Whilst such coadaptation may be seen in laboratory studies, this bears little relevance to how selection will occur in the wild if such phenotypes are not expressed in a wild population. Thus, such laboratory studies may provide little predictive information about evolution. It is important, therefore, to quantify such findings in controlled populations with equivalent comparisons in natural systems. However, it can be considered that model species kept in consistent laboratory conditions for many generations (such as *Drosophila melanogaster*) are likely to have adapted to their conditions, and are thus in their own natural or wild population, thus increasing the ecological validity of such estimates.

5.4 Future work

The genetics of parent-offpsring conflict

Although I was able to quantify the selection gradients acting upon parental and offspring traits, and thus show the presence of antagonistic selection, I was not able to estimate the relevant genetic parameters associated with these traits. In order to predict how evolutionary change will occur and test predictions about stasis, the genetic parameters are required in addition to the selection gradients (Willham, 1963,

1972; Cheverud, 1984). Thus, in order to extend these conclusions, the estimates of genetic variance and covariance between these traits should be estimated. In order to estimate these robustly, increased data from this population will be needed to increase the power of such models. In particular, there is limited recruitment from this population, which limits the estimation of such genetic parameters, and so more data from recruiting individuals is needed.

Maternal effects on hatching time

As discussed above, although I eliminated one possible mechanism by which early and late eggs could differ in hatching time, the true mechanism mediating this difference is still unknown. One possible mechanism is a change in the relative proportions of yolk and albumen within the egg. Over the last two years I have taken photographs of every egg using a light box, so that the yolk and albumen are distinguishable. This should allow quantification of whether these egg components change in a predictable way across the laying sequence, and so address whether this has effects on hatching time. As this is a none-invasive method, we are able to relate these measures with subsequent traits in chicks without compromising hatching probabilities or affecting development rate. In addition, quantifying substances (hormones, carotenoids) within the eggs and comparing those that are laid early and late in the laying sequence may also inform whether these are likely to generate such effects in blue tits. However, these are limited by the problems discussed above – it is methodologically challenging to appropriately quantify these whilst also being able to relate them to offspring phenotypes.

Cross-fostering and Coadaptation

I did not find any evidence for reduced fitness of parents or offspring when cross-fostering was used to manipulate family composition. These findings could be extended and generalised if the cross-fostering design in Chapter 4 were to be used

in other populations and species. In particular, using such designs in the lab-based populations of canaries used in Hinde *et al.* (2010) may highlight whether the coadaptation they find is truly present, and repeatable.

It would also be of interest to carrying out cross fostering in such a way as to eliminate the bias in which eggs are crossed between nests. This could be done by only crossing eggs after all eggs had been laid, and sampling those eggs to be crossed equally across the laying sequence. By removing all eggs to storage incubators and replacing with dummy eggs on the day that they are laid (as is done in Estramil *et al.*, 2013, to synchronise hatching within a clutch) this would reduce any problems of differential incubation by females.

5.5 General Conclusions

I set out to show how parental effects on offspring phenotypes, and ultimately fitness, can generate predictions of conflict and coadaptation between family members. In addition, I wanted to address how a maternal effect on offspring hatching time might be mediated. In Chapter 2, I carried out a supplementary feeding experiment to test whether increased food availability to mothers through the egg laying period was passively generating increased hatching time of eggs laid later in a clutch. I found that supplementary food did not effect hatching time in the way that was predicted, nor were there major effects of natural weather variation, implying that the maternal effect on hatching time acts independently of the environment the mother experiences. Thus, there is scope for further investigation as to how this maternal effect is mediated, and whether offspring are under selection to maximise their response to any signals driving this change. In Chapter 3, I showed that there is conflicting selection between body mass in offspring and parental performance for offspring body mass, which goes some way towards explaining stasis in body mass in juveniles. This highlights the need for appropriate measures of fitness and traits in

order to find such antagonistic selection, which has not previously been shown, and I further explain why previous studies of selection on parental traits have often been inappropriate for finding such effects in Section 5.1. Finally, in Chapter 4, I tested predictions of coadaptation between parents and offspring, and between siblings. I found no evidence for either form of coadaptation through the effects on offspring or parental fitness, and demonstrate a cross-fostering design that allows the elucidation of three mechanisms by which coadaptation could occur. Thus, this thesis addresses several gaps in knowledge as to how conflict and coadapation over parental effects can be generated, and how these can then be estimated using observational data and manipulative experiments.

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Appendix A

Field Methods

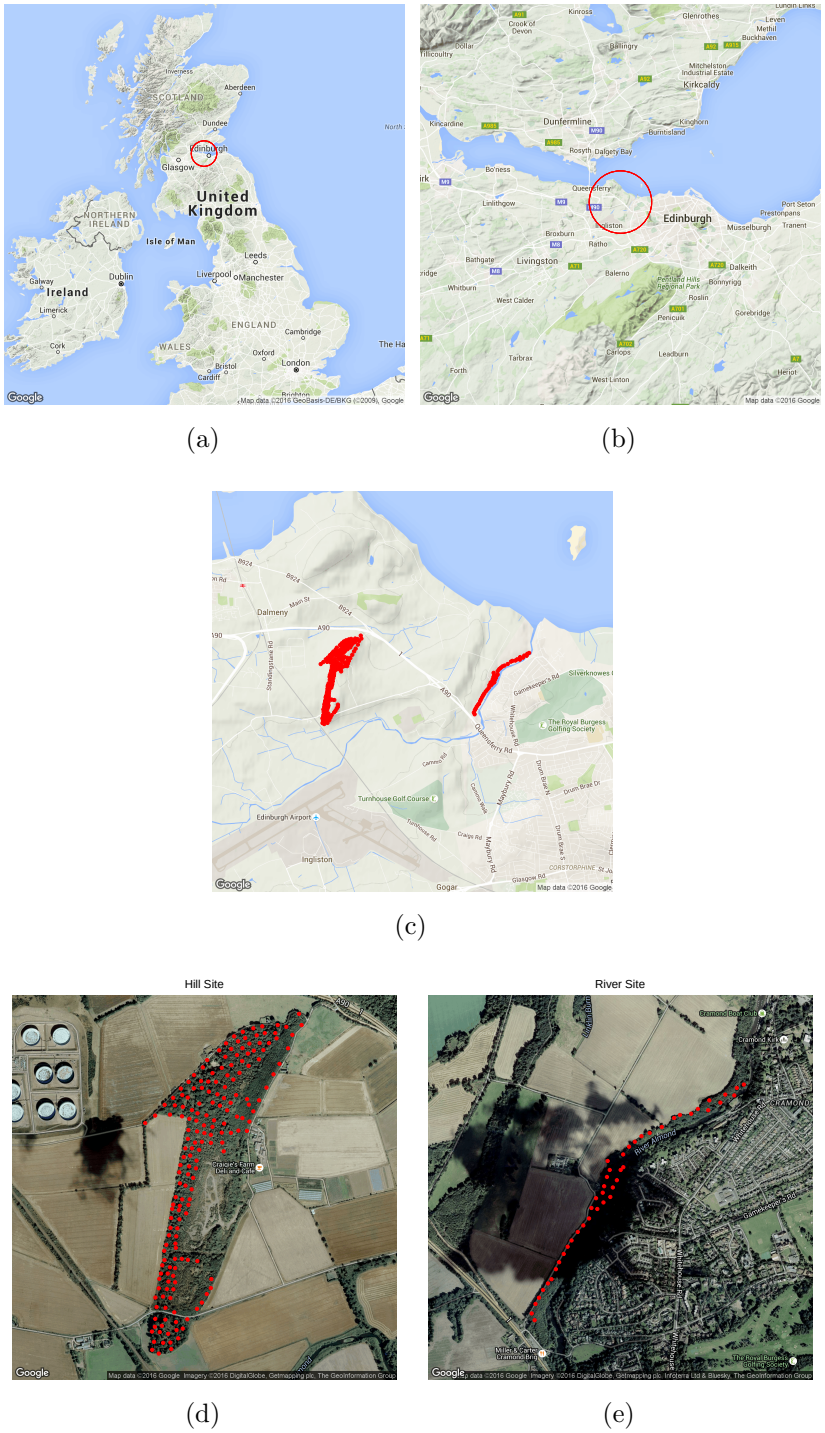
A.1 Field Site

A.1.1 Location

Nest boxes are located on two sites close to Cramond, Edinburgh (Figure A.1). 46 boxes are placed along the west bank of the Almond River (the River site, Grid Reference NT179758). A further 180 boxes are on Craigie Hill, (the Hill site, NT156766), which is located around 2.5km from the river site. All nest boxes are Schwegler woodcrete boxes, with 26mm holes, strapped to tree trunks around 1.5-2m from the ground. Boxes are placed about 30m apart from one another, although this varies across the sites.

A.1.2 Habitat

The prevailing habitat varies thrroughout the field site. On the river site, the majority of boxes are in mixed deciduous woodland, although there are some placed within coniferous woodland. The quantity of woodland around boxes also varies along this site - some boxes are within large sections of woodland, whilst others are



(a) (b)

(c)

(d) (e)

Figure A.1: The location of the two field sites within the UK (a), with closer detail shown in (b) and (c). The lower two maps (d and e) show the distribution of nest boxes within those two sites - each red point is a nest box.

in smaller stands along field margins. As with the river, the hill consists of mainly mixed deciduous woodland, although some portions are coniferous, and others more open birch woodland. In addition, as the hill site spans both sides of a hill, the aspect, and microclimate, vary between boxes on either side of this hill. Thus, the nest boxes are sited along a range of ecological gradients.

A.2 Before egg laying

Nests are visited from early April. As the initiation of nest building and egg laying is highly variable, dependent on weather conditions (particularly temperature), the exact dates from which regular visits commence vary (Figure A.2 shows the nest commencement dates from each year, and Figure A.3 shows the number of eggs laid each day). Surveys of every nest box are carried out every four days, and on these occasions the nesting state of all boxes are recorded - empty, roost (presence of faecal matter), nesting started (a small amount of moss or grass in the box); If there is more than a small amount of nesting material, the height of the nest is measured and recorded (to the nearest 5mm), or if there is lining material (feathers, grass, fur) in the cup of the nest, then the nest is classed as lined. Those nests that are classed as having started nesting (or have a recorded height) are visited every two days, such that they are visited in the time between each full nest survey. In addition, lined nests are visited every day to check for the commencement of egg laying. This surveying method ensures that the rate of change of nests is known, and the first egg laid in a nest is almost always found on the day that it has been laid. Nests are visited every day once egg-laying commenced.

In addition to the nest-status data, the presence of any adult birds is recorded upon visits to the nest. If the adults bear metal rings (from previously being caught as an adult, or a chick from the previous year), these are noted, along with the colour of any plastic rings (see below). We continue this throughout the field season, until

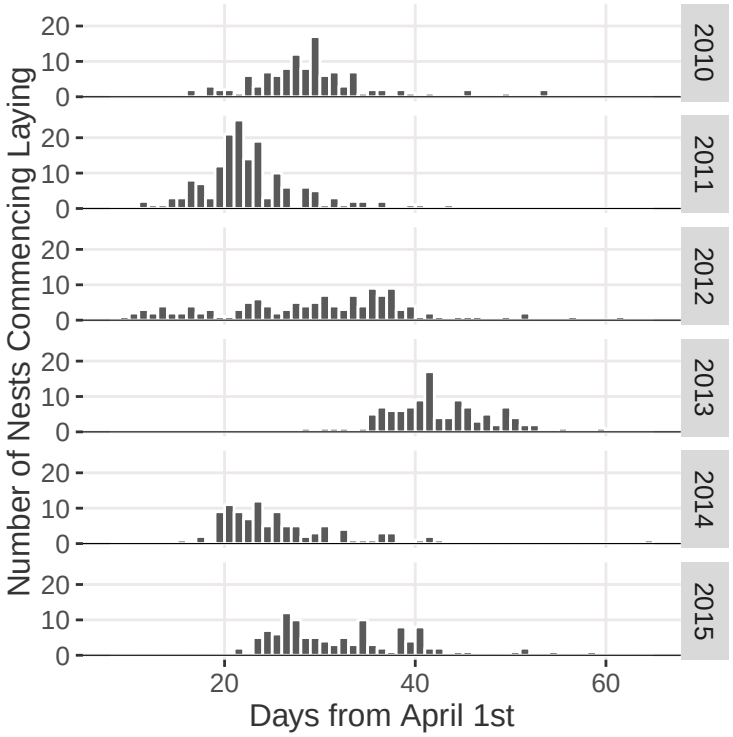


Figure A.2: The numbers of nests commencing laying on each day, within a year

chicks have fledged. This allows a continuous monitoring of how many parents are present at a nest (and therefore likely to be feeding), and is used to generate the measure of male presence scores in Chapter 3.

A.3 Cross-Fostering Eggs

On the first day that an egg is found within a lined nest (almost always the first egg, such that only one egg is in the cup of the nest), that egg is collected from the nest and replaced with a white clay dummy egg. The real egg is placed within a cotton wool lined compartment in a box, and the box marked to indicate the egg's nest-of-origin. Eggs are bought back to a central point by all field workers, whereupon cross-fostering is carried out.

Two different cross-fostering designs have been carried out, although the general methods by which they are carried out remain constant. The designs are explained

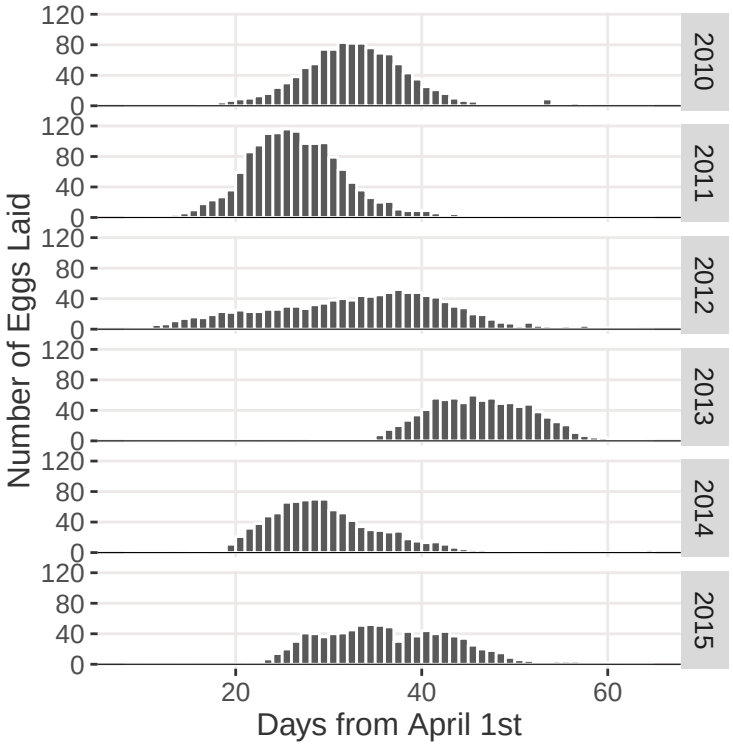


Figure A.3: The number of eggs laid on a given day (since 1st of April) of a given year

below, and shown in Figure A.4.

A.3.1 2010 - 2013 design

On the day that a nest commences laying, it is randomly assigned into a cross fostering group with other nests that commenced laying on that same day. Ideally, cross-fostering groups consisted of three nests. However, the number of nests to be assigned on a given day was not always divisible by three, and so different group numbers (two, four and five) were also used. Within each cross-fostering group, eggs were crossed between nests in a round-robin design - an egg from nest A was crossed into nest B, that from nest B into nest C, and from nest C then into nest A.

All nests were partially cross-fostered, such that every other egg was crossed between nests. The first egg was always exchanged, the second one remained within its nest-of-origin, the third was crossed, the fourth was not etc. All eggs were marked

with their egg number, such that the first egg within a nest was marked with a 1, the second with a 2 etc. This allowed the new (unmarked) egg laid within a clutch each day to be identified. Although the majority of birds lay eggs every day, some had interuptions in their laying sequence, such that no new egg was laid for a day or more. When this happened when cross fostering was due to take place, the eggs were not exchanged between nests, and that cross-fostering group all eggs from that day returned to their nest-of-origin.

2010 - 2011 additional design

An additional cross-fostering design was carried out in 2010 and 2011, explained fully in Hadfield *et al.* (2013a). In this, first or last eggs exchanged between nests not in the same cross fostering group, such that the egg's rank (first vs last) was altered. This generated results which helped to inform and motivate Chapter 2.

A.3.2 2014 - 2015 design

The way in which cross fostering was carried out was broadly similar in 2014 and 2015, although the actual cross-fostering designed was altered (in order to carry out the analysis in Chapter 4. All nests were randomly assigned into cross-fostering pairs on the day they commenced laying, between which eggs were exchanged reciprocally. Three different cross fostering treatments were assigned to these nest pairs. Control pairs had no egg exchanged between them, such that all eggs remained within their nest-of-origin. Partial nests were treated as above, with alternate eggs being exchanged between nests. Fully cross-fostered nests had all eggs reciprocally exchanged (except when one of the pair did not lay on a given day). As nests were in pairs, this design required even numbers of nests to commence laying on a given day. When this was not the case, one of the nests from that day was randomly assigned as an "odd" nest, and did not have any eggs exchanged with other nests.

If the final nest would otherwise have been in a control group (had a second nest to pair it with been present that day), this nest was assigned as a control and the second nest in the control pair assigned the following day.

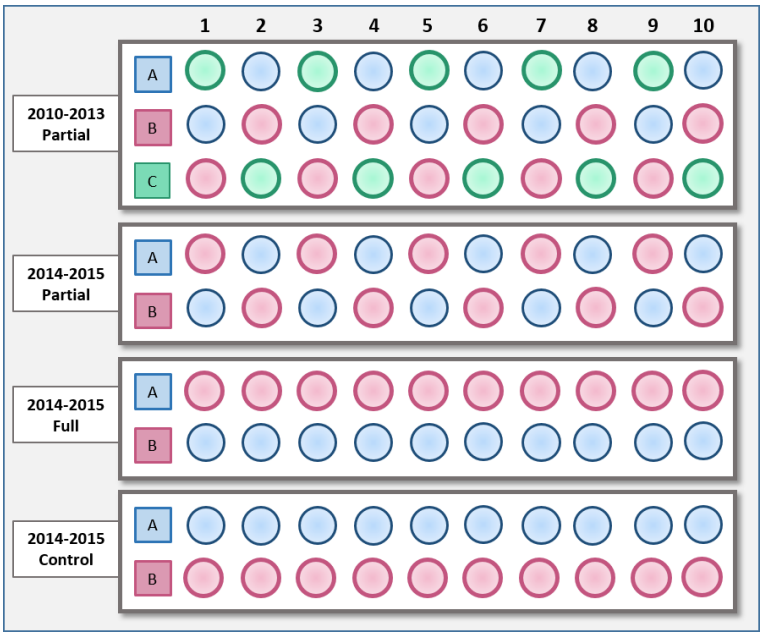


Figure A.4: The different cross-fostering designs used in the data collected for this thesis. Colours indicate the nest-of-origin of the eggs (filled circles). Numbers along the top indicate egg numbers.

A.4 Data collection

A.4.1 Eggs

All eggs are weighed, to the nearest 10mg, on the day that they are laid. In 2010-2013, eggs due to be cross-fostered were weighed at a central point, prior to being returned to their new foster nest, whilst those eggs due to remain in their natal nests were weighed at the nest and returned immediately. In 2014 and 2015 this methodology was altered, such that all eggs were bought to a central location and weighed, prior to being returned to their natal or foster nest. This added in an extra level of control, such that all eggs had been moved around the field sites in the same

manner, eliminating any differences between crossed and uncrossed eggs that this might otherwise cause.

A.4.2 Incubation

If the female was found incubating eggs on a given visit and she had laid eggs the previous day, the female was flushed from the nest and a new egg was checked for. However, if the female had not laid for one or more days previously, she was not flushed from the nest, and it was then classed as an incubating nest. This was also done if the eggs were found to be warm after no new eggs had been laid in a similar way. The expected length of incubation was then calculated based on the time since the final egg had been laid. The nest was not visited again until this expected hatching time.

A.4.3 Chicks

Once the nest was expected to hatch, it was visited daily to check for newly hatched chicks. If the female was on the nest, she was flushed off in order to allow access to the eggs. When chicks were found (day 0), those chicks were weighed (again to the nearest 10mg). Chicks were uniquely marked by clipping combinations of down tufts on their heads, and if necessary a toenail. The numbers marked upon any remaining eggs was noted, such that the hatching date of each egg was known. The nest was revisited upon the following visits (day 1 and day 3) and the same process carried out, such that any new chicks were uniquely marked and all chicks were weighed. No chicks hatched after day 3, so any remaining eggs were removed and dissected in order to obtain embryonic tissue samples for genotyping. Nests were visited a further 4 times to weigh chicks, on days 6, 9, 12 and 15. In addition to weighing chicks, blood samples (5μl) were taken from the medial metatarsal vein under home office licence on day 3, using a 26G needle. Chicks were ringed on day

9, and morphological measurements were taken on day 15.

On each visit, any mortality was recorded, and tissue samples were taken from dead chicks. Nests were not visited after day 15, until after all chicks had fledged from those nests. On this occasion, the identities of any chicks that had failed to fledge were noted.

A.5 Catching Adults

Adults were caught from day 10 onwards. This was done through trapping adults inside the nest boxes when they arrived to feed. In some cases, adults were instead caught by mist net placed in front of the box. Effort was made to catch both the male and female at each nest box, and to avoid catching the same bird twice at a given box. Upon capture, adults were weighed, and their tarsus, head-bill and wing length measured. In addition, blood samples were taken from the ulna vein.

All adults were given metal rings. Those adults that had recruited from the population retained their rings from when they were rung as chicks, and any unrung (immigrant) birds were ringed. From 2011 onwards we also placed plastic colour rings upon adults. These were unique to each year and sex, such that we could identify the sex and year of first capture of the birds visually from these rings (Table A.1 shows the number caught with each colour ring (or caught and given the colour ring) each year.

	2011	2012	2013	2014	2015
Red	63	45	22	15	4
Orange	0	43	20	12	5
Blue	0	0	29	11	4
Black	0	0	0	30	7
Red and Light Blue	0	0	0	0	48
White	96	53	22	10	5
Green	0	49	18	11	7
Yellow	0	0	47	18	11
Pale Blue	0	0	0	44	16
White and Dark Green	0	0	0	0	46

Table A.1: The numbers of adults with each colour ring. The first half of the table are male, the second half (below the line) are females. Rings are assigned in the year they first appear in the table, numbers in subsequent years are those adults that survive to be caught in the next field season.

Appendix B

Chapter 2 Supplementary Materials

B.1 Supplementary Feeding Analysis

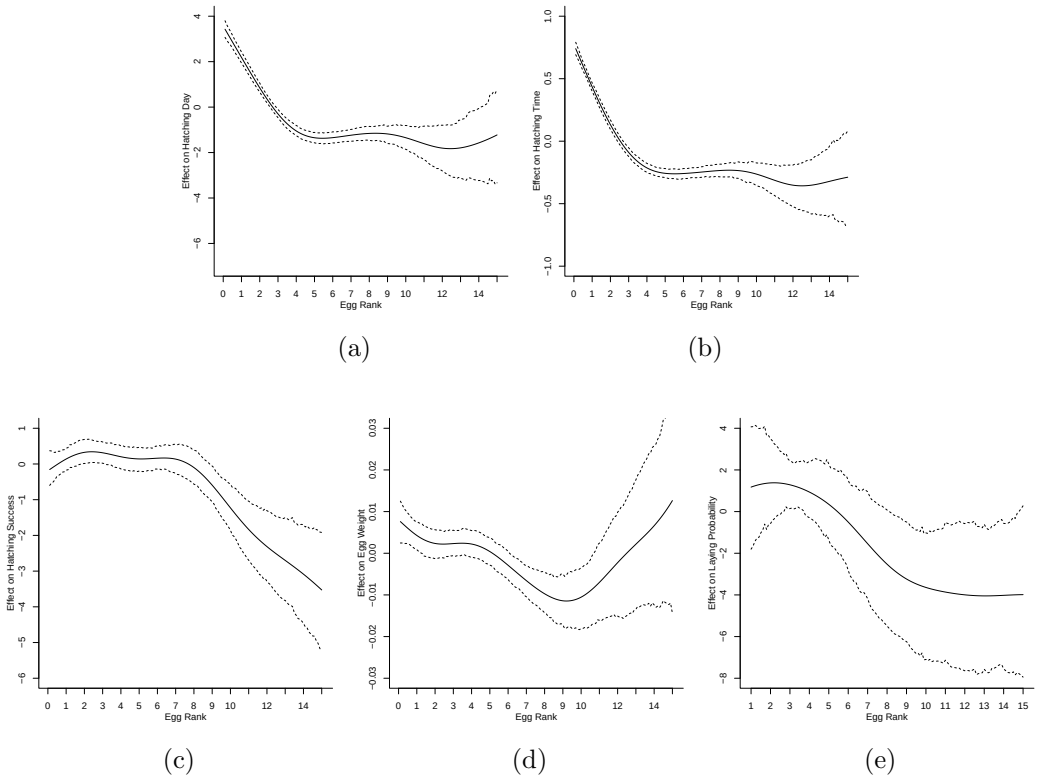


Figure B.1: The effect of egg rank (days between egg being laid and final egg in clutch) on (a) day of hatching within nest (ordinal); (b) time from laying of the final egg in a nest to hatching (censored Gaussian); (c) hatching success (probability of hatching); (d) weight of eggs; and (e) the probability than an egg is laid. Solid lines are the posterior mean estimates, with the dashed lines showing the 95% HPD intervals.

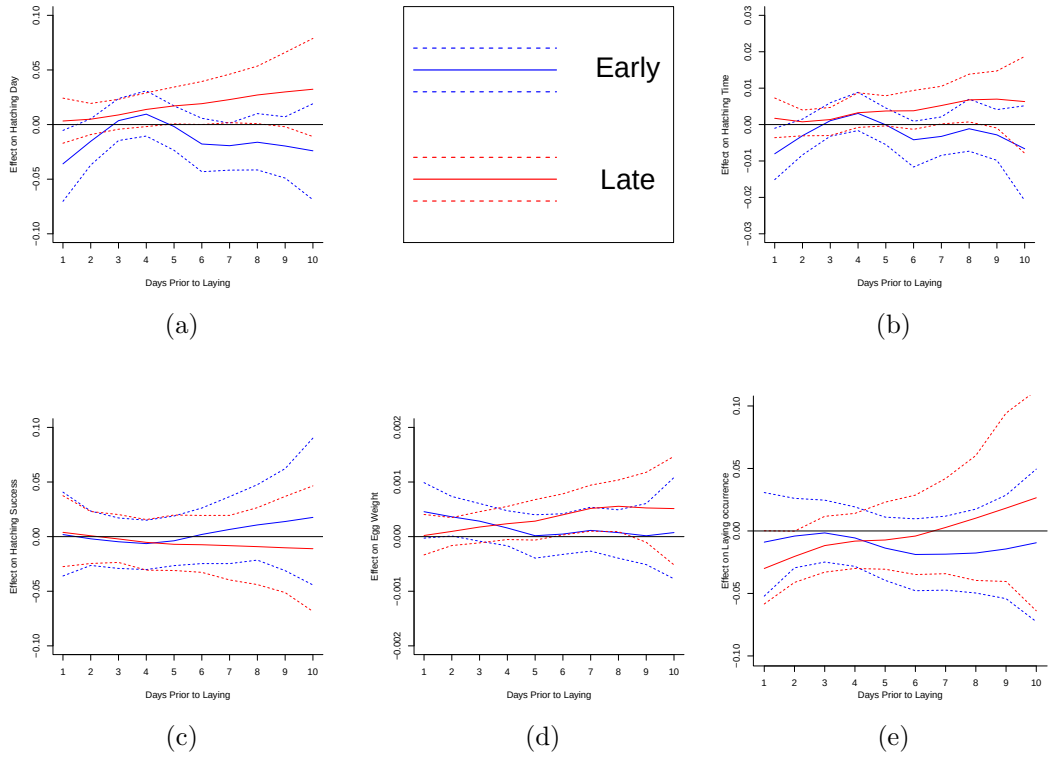


Figure B.2: The effect of the consumption of wax worms (eaten each day prior to the laying of an egg) on: (a) day of hatching within nest (ordinal); (b) time from laying of the final egg in a nest to hatching (censored Gaussian); (c) hatching success (probability of hatching); (d) weight of eggs; and (e) the probability that an egg is laid. The lines represent the effect of a single worm eaten x days prior to the laying of any given egg within the laying sequence, with early and late treatments being plotted separately. Solid lines are the posterior mean estimates, with the dashed lines showing the 95% HPD intervals.

B.2 Weather Analysis

The following tables (B.1-B.9) summarise the fixed effects from the models used to assess the effect of weather on egg characteristics, and female behaviour. In each table the results from the full model (left hand side, all three weather variables included in the model) and the null model (right hand side, no weather variables included in the model) are shown. The varying coefficient splines from each of the three weather variables from the full models are shown in figs. B.3, B.4 and B.5. For censored Gaussian hatching time and clutch size, the results of three models are shown, as the best model from cross validation in each case was shown to be one including two splines (temperature and rain for censored Gaussian hatching; temperature and wind for clutch size), so the results of these, as well as the full and null models are shown.

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	-12.546	-28.469	1.089	0.079	-4.606	-6.489	-2.775	**<0.001**
Clutch size$_r$	0.187	0.029	0.360	**0.028**	0.228	0.101	0.377	**<0.001**
Clutch size$_o$	0.070	-0.029	0.167	0.155	0.070	-0.033	0.163	0.161
Year.2011	0.480	-2.371	3.533	0.718	0.496	-0.056	1.000	0.080
Year.2012	0.188	-1.804	2.133	0.861	-0.319	-0.839	0.287	0.261
Year.2013	-0.543	-3.317	2.363	0.706	-0.706	-1.414	0.013	**0.049**
Laydater	-0.017	-0.124	0.076	0.770	0.040	0.009	0.070	**0.016**
After pause	0.451	0.021	0.878	**0.040**	0.406	0.014	0.876	0.064

Table B.1: Summary of the fixed effects from the full ordinal model of hatching day (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs. B.3a, B.4a and B.5a respectively. Year did not have a significant effect in the full model (Pr(>W)=0.95), but did in the null model (Pr(>W)=0.009).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	18.779	5.886	28.314	**0.009**	18.047	16.557	19.282	**<0.001**
Clutch size$_r$	-0.120	-0.260	0.004	0.053	-0.163	-0.249	-0.059	**0.001**
Clutch size$_o$	0.019	0.001	0.038	**0.048**	0.021	0.003	0.040	**0.028**
Year.2011	-1.137	-3.506	0.590	0.208	-0.786	-1.174	-0.358	**<0.001**
Year.2012	1.379	0.348	2.511	**0.005**	1.643	1.203	2.068	**<0.001**
Year.2013	0.567	-1.162	2.295	0.492	1.299	0.769	1.827	**<0.001**
Laydater	-0.062	-0.169	0.017	0.150	-0.094	-0.117	-0.072	**<0.001**
After pause	0.087	0.009	0.169	**0.044**	0.072	-0.007	0.154	0.078

Table B.2: Summary of the fixed effects from the full censored Gaussian model of hatching time of eggs (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs. B.3b, B.4b and B.5b respectively. Year had a marginal effect in the full model (Pr(>W)=0.059), and this was significant in the null model (Pr(>W)<0.001).

	mean	l-95%	u-95%	pMCMC
Intercept	22.220	17.177	27.626	**<0.001**
Clutch size$_r$	-0.094	-0.202	0.019	0.109
Clutch size$_o$	0.019	0.002	0.037	**0.037**
Year.2011	-0.735	-1.433	-0.076	**0.032**
Year.2012	1.011	0.369	1.706	**0.007**
Year.2013	0.577	-0.472	1.643	0.299
Laydater	-0.035	-0.097	0.017	0.234
After pause	0.084	0.003	0.167	**0.043**

Table B.3: Summary of the fixed effects from the best censored Gaussian model of hatching time of eggs (left), which included temperature and rain splines, but not wind. Visually, the temperature splines in this model are similar to those in B.4b and B.5b respectively. Year had a significant effect (Pr(>W)=0.003).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	14.935	1.501	28.761	**0.032**	2.106	-0.004	4.316	0.051
Clutch size$_r$	0.138	-0.029	0.318	0.135	0.108	-0.046	0.260	0.174
Clutch size$_o$	0.043	-0.117	0.184	0.562	0.046	-0.093	0.190	0.523
Year.2011	0.203	-2.741	3.399	0.888	-1.037	-1.647	-0.394	**0.001**
Year.2012	-0.205	-2.494	2.280	0.858	-0.360	-1.052	0.374	0.322
Year.2013	0.730	-2.317	3.793	0.645	-0.740	-1.585	0.157	0.108
Laydater	0.046	-0.055	0.136	0.367	0.015	-0.023	0.050	0.415
After pause	-0.310	-0.940	0.477	0.390	-0.306	-1.016	0.370	0.377

Table B.4: Summary of the fixed effects from the full model of hatching success (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs. B.3c, B.4c and B.5c respectively. Year did not have a significant effect in the full model (Pr(>W)=0.973), but did in the null model (Pr(>W)=0.004).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	19.839	8.461	34.235	**0.002**	11.323	10.548	12.079	**<0.001**
Clutch size$_o$	0.019	-0.070	0.114	0.680	0.013	-0.045	0.078	0.658
Year.2012	0.071	-2.457	2.638	0.936	0.075	-0.156	0.313	0.537
Year.2013	0.712	-2.140	3.635	0.667	-0.683	-1.014	-0.291	**<0.001**
Laydate	0.026	-0.045	0.091	0.495	0.018	0.003	0.031	**0.012**
After pause	0.562	0.479	0.652	**<0.001**	0.567	0.473	0.656	**<0.001**

Table B.5: Summary of the fixed effects from the full model of egg weight (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs. B.3d, B.4d and B.5d respectively. Year did not have a significant effect in the full model (Pr(>W)=0.857), but did in the null model (Pr(>W)<0.001).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	27.227	12.421	42.887	**<0.001**	4.824	3.745	5.831	**<0.001**
Year.2011	2.435	-0.818	6.037	0.158	-0.186	-0.907	0.417	0.580
Year.2012	-2.922	-5.099	-0.690	**0.011**	-1.178	-1.867	-0.560	**<0.001**
Year.2013	-4.141	-7.272	-0.841	**0.013**	-2.041	-2.878	-1.270	**<0.001**
Laydate	0.164	0.081	0.246	**<0.001**	-0.007	-0.039	0.029	0.666

Table B.6: Summary of the fixed effects from the full model of laying probability (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs B.3e, B.4e and B.5e respectively.Year had a significant effect in both the full model (Pr(>W)<0.001), and the null model (Pr(>W)<0.001).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	12.533	-16.199	38.015	0.335	13.089	12.471	13.778	**<0.001**
Year.2011	-0.597	-5.867	4.862	0.834	-1.515	-1.871	-1.171	**<0.001**
Year.2012	-0.650	-3.348	2.057	0.629	-1.294	-1.661	-0.901	**<0.001**
Year.2013	0.462	-4.035	5.926	0.829	-0.368	-0.959	0.129	0.190
Laydate	-0.135	-0.294	0.023	0.068	-0.107	-0.128	-0.085	**<0.001**

Table B.7: Summary of the fixed effects from the full model of clutchsize (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs B.3f, B.4f and B.5f respectively. Year did had a significant effect in the full model (Pr(>W)=0.883), and this effect was increased in the null model (Pr(>W)<0.001).

	mean	l-95%	u-95%	pMCMC
Intercept	17.027	-9.023	42.093	0.177
Year.2011	-0.228	-5.416	4.827	0.941
Year.2012	-0.642	-2.778	1.800	0.557
Year.2013	-0.245	-3.046	2.536	0.888
Laydate	-0.104	-0.242	0.034	0.130

Table B.8: Summary of the fixed effects from the best model of clutchsize, which contains splines for temperature and wind, but not rain. The splines of these weather variables are visually similar to those in fig. B.3f,and fig. B.4f from the full model. Year had a significant effect (Pr(>W)0.859).

	mean	l-95%	u-95%	pMCMC	mean	l-95%	u-95%	pMCMC
Intercept	16.487	-6.747	43.229	0.152	5.438	3.848	6.934	**<0.001**
Year.2011	0.160	-6.147	7.062	0.995	-1.137	-1.567	-0.665	**<0.001**
Year.2012	0.793	-2.360	4.611	0.628	1.031	0.554	1.516	**<0.001**
Year.2013	1.627	-4.436	8.218	0.596	0.635	0.059	1.221	**0.037**
Laydate	-0.057	-0.164	0.051	0.305	-0.090	-0.115	-0.065	**<0.001**
Clutch size$_o$	-0.224	-0.368	-0.065	**0.003**	-0.258	-0.372	-0.158	**<0.001**

Table B.9: Summary of the fixed effects from the full model of incubation onset (left), and the null model (right). The wind, temperature, and rain splines from the full model can be seen in figs B.3g, B.4g and B.5g respectively. Year did not have a significant effect in the full model (Pr(>W)=0.873), but did in the null model (Pr(>W)<0.001).

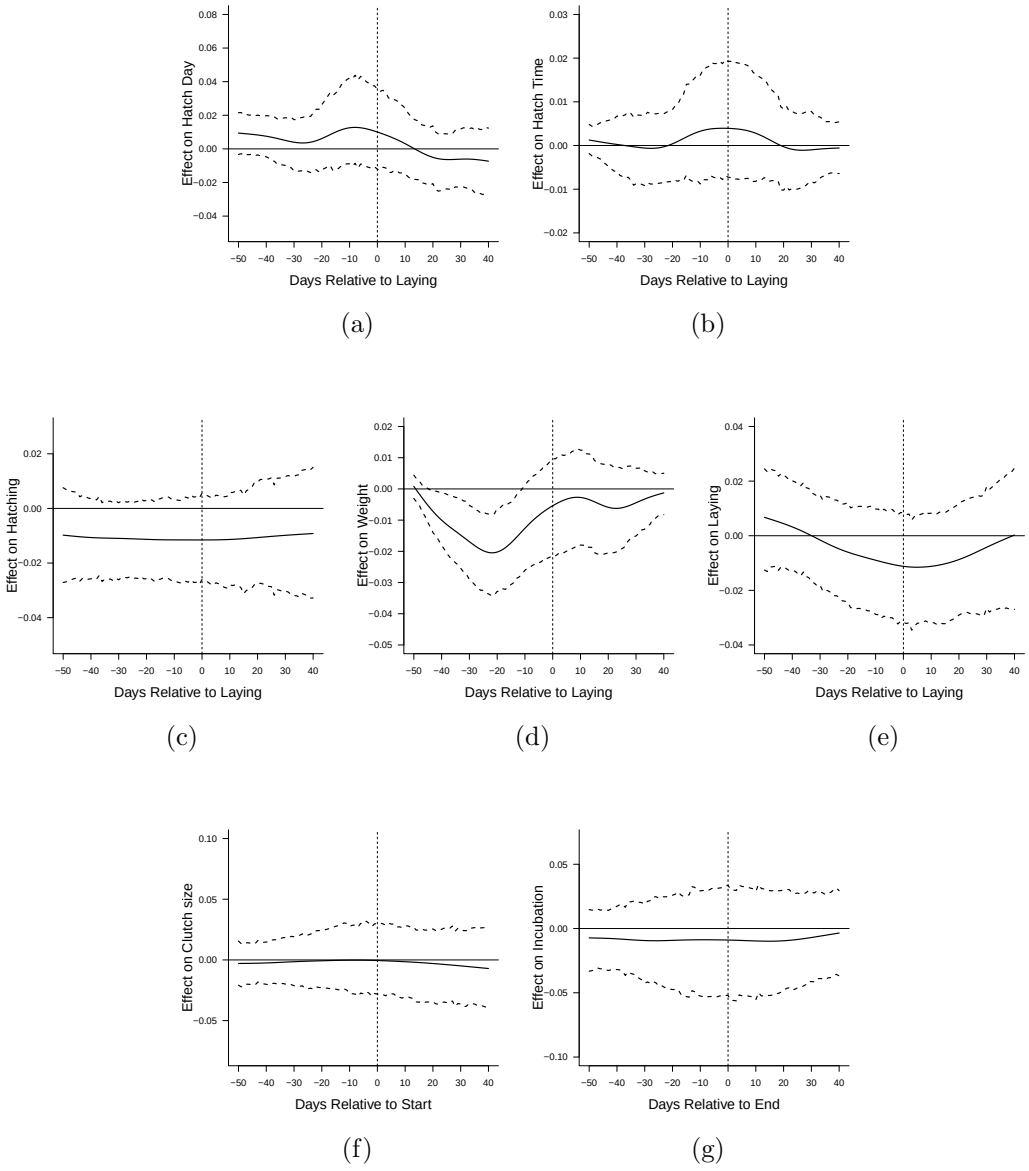


Figure B.3: Varying coefficient splines of wind on each of the response variable measured: (a) ordinal hatching day; (b) censored Gaussian hatching time; (c) hatching success; (d) egg weight; (e) laying probability; (f) clutch size; (g) onset of incubation. The x-axis of each figure shows the time in days relative to an egg being laid (negative numbers before laying, positive after; the dashed line at x=0 indicates the day an egg is laid). Solid lines are the posterior mean estimates, with the dashed lines showing the 95% HPD intervals.

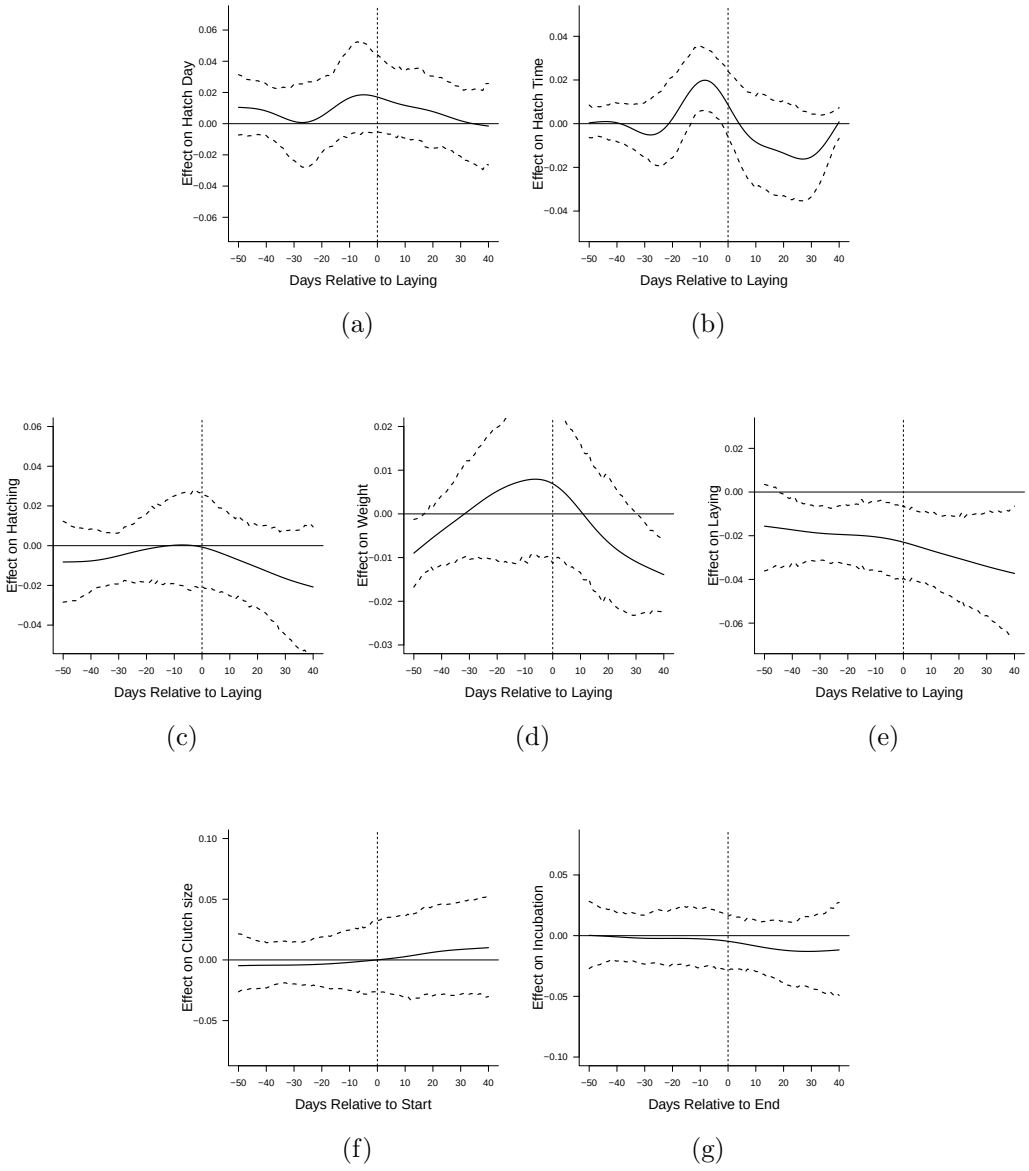


Figure B.4: Varying coefficient splines of temperature on each of the response variable measured: (a) ordinal hatching day; (b) censored Gaussian hatching time; (c) hatching success; (d) egg weight; (e) laying probability; (f) clutch size; (g) onset of incubation. The x-axis of each figure shows the time in days relative to an egg being laid (negative numbers before laying, positive after; the dashed line at x=0 indicates the day an egg is laid). Solid lines are the posterior mean estimates, with the dashed lines showing the 95% HPD intervals.

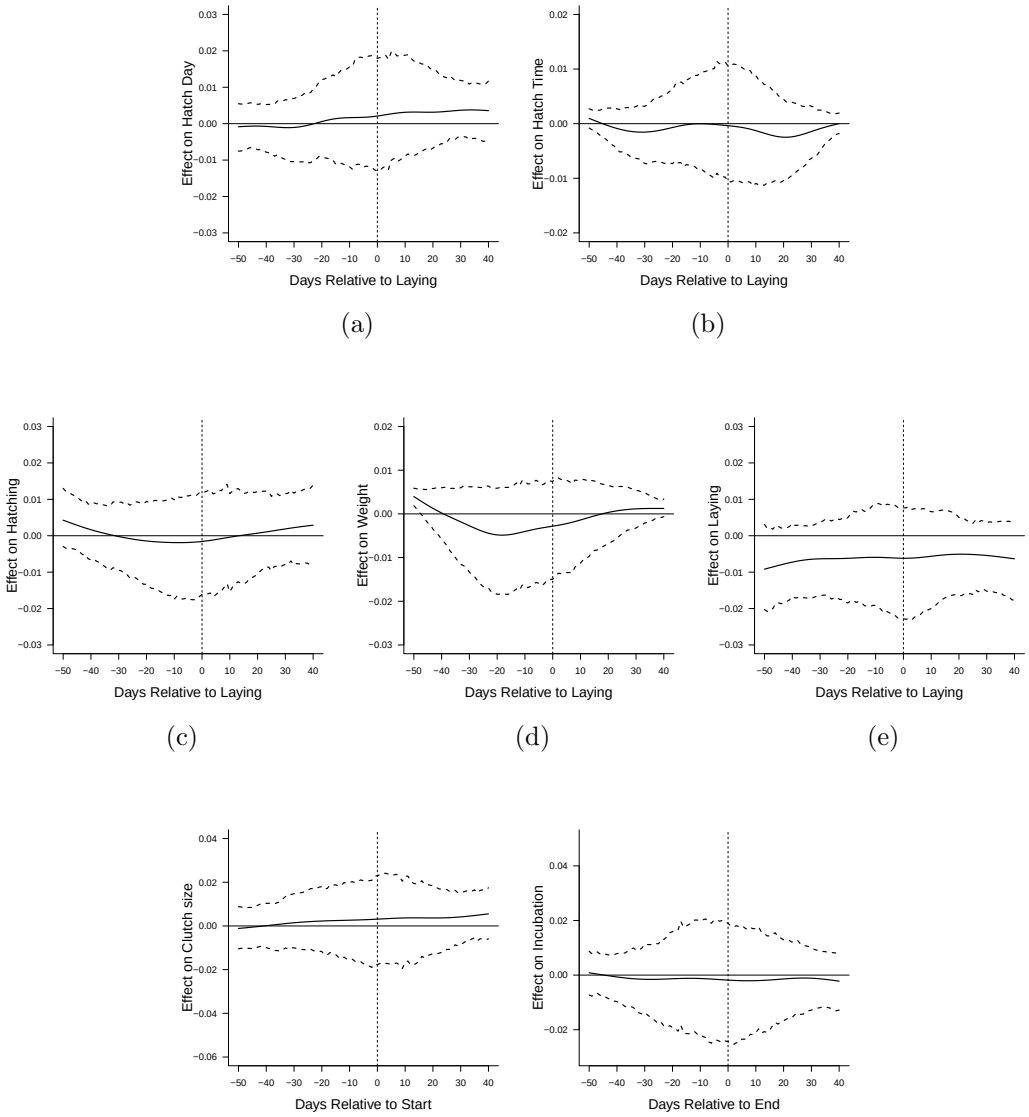


Figure B.5: Varying coefficient splines of rain on each of the response variable measured: (a) ordinal hatching day; (b) censored Gaussian hatching time; (c) hatching success; (d) egg weight; (e) laying probability; (f) clutch size; (g) onset of incubation. The x-axis of each figure shows the time in days relative to an egg being laid (negative numbers before laying, positive after; the dashed line at x=0 indicates the day an egg is laid). Solid lines are the posterior mean estimates, with the dashed lines showing the 95% HPD intervals.

Appendix C

Chapter 3 Supplementary Materials

C.1 Genetic Correlation Estimation

$$
\begin{aligned}
0 = &\ (g_{b,b} + \tfrac{3}{2}g_{b,p} + \tfrac{1}{2}g_{p,p})\beta_b + (g_{b,p} + g_{p,p})\beta_p \\
-(g_{b,p} + g_{p,p})\beta_p = &\ (g_{b,b} + \tfrac{3}{2}g_{b,p} + \tfrac{1}{2}g_{p,p})\beta_b \\
-(g_{b,p} + g_{p,p})\tilde{\beta}_p = &\ (g_{b,b} + \tfrac{3}{2}g_{b,p} + \tfrac{1}{2}g_{p,p}) \\
-(g_{b,p}/g_{b,b} + \tilde{g}_{p,p})\tilde{\beta}_p = &\ (1 + \tfrac{3}{2}g_{b,p}/g_{b,b} + \tfrac{1}{2}\tilde{g}_{p,p}) \\
-(r_{b,p}\sqrt{\tilde{g}_{p,p}} + \tilde{g}_{p,p})\tilde{\beta}_p = &\ (1 + \tfrac{3}{2}r_{b,p}\sqrt{\tilde{g}_{p,p}} + \tfrac{1}{2}\tilde{g}_{p,p}) \\
-(1 + \tfrac{1}{2}\tilde{g}_{p,p} + \tilde{g}_{p,p}\tilde{\beta}_p) = &\ \tfrac{3}{2}r_{b,p}\sqrt{\tilde{g}_{p,p}} + r_{b,p}\sqrt{\tilde{g}_{p,p}}\tilde{\beta}_p \\
-(1 + \tfrac{1}{2}\tilde{g}_{p,p} + \tilde{g}_{p,p}\tilde{\beta}_p) = &\ r_{b,p}\left(\tfrac{3}{2}\sqrt{\tilde{g}_{p,p}} + \sqrt{\tilde{g}_{p,p}}\tilde{\beta}_p\right) \\
r_{b,p} = &\ \tfrac{-(1+\frac{1}{2}\tilde{g}_{p,p}+\tilde{g}_{p,p}\tilde{\beta}_p)}{\left(\frac{3}{2}\sqrt{\tilde{g}_{p,p}}+\sqrt{\tilde{g}_{p,p}}\tilde{\beta}_p\right)} \\
r_{b,p} = &\ \tfrac{-(1+\frac{1}{2}\tilde{g}_{p,p}+\tilde{g}_{p,p}\tilde{\beta}_p)}{\sqrt{\tilde{g}_{p,p}}\left(\frac{3}{2}+\tilde{\beta}_p\right)} \\
r_{b,p} = &\ \tfrac{-(1+\tilde{g}_{p,p}(\frac{1}{2}+\tilde{\beta}_p))}{\sqrt{\tilde{g}_{p,p}}\left(\frac{3}{2}+\tilde{\beta}_p\right)}
\end{aligned}
\tag{C.1}
$$

this is because

$$
\begin{aligned}
r_{b,p} = &\ g_{b,p}/\sqrt{g_{b,b}g_{p,p}} \\
r_{b,p} = &\ g_{b,p}/\sqrt{g_{b,b}\tilde{g}_{p,p}g_{b,b}} \\
r_{b,p} = &\ g_{b,p}/g_{b,b}\sqrt{\tilde{g}_{p,p}} \\
r_{b,p}\sqrt{\tilde{g}_{p,p}} = &\ g_{b,p}/g_{b,b}
\end{aligned}
\tag{C.2}
$$

C.2 Fecundity

We reran the fecundity model, but this time excluded males that had a paternity share in their nest of less than 50%. This excluded 32 males from the model. Results are shown below

	mean	l-95%	u-95%	pMCMC
Sex (F)	9.187	9.034	9.336	**<0.001**
Sex (M)	25.570	22.679	28.371	**<0.001**
Male Attendance	-7.958	-9.440	-6.463	**<0.001**
Residual Variances				
Sex (F)	3.019	2.642	3.395	
Sex (M)	11.337	9.687	12.942	

Table C.1: Fixed effects and residual variances from the model of adult fecundity in which only males with a paternity share of 0.5 or higher were included.

Appendix D

Chapter 4 Supplementary Materials

	mean	l-95%	u-95%	pMCMC
(Intercept)	-3.056	-3.666	-2.472	**<0.001**
Egg Rank	0.154	0.099	0.207	**<0.001**
Year 2011	-1.035	-1.709	-0.305	**0.001**
Year 2012	-0.122	-0.870	0.506	0.768
Year 2013	0.325	-0.399	1.011	0.379
Year 2014	-0.006	-0.714	0.712	0.994
Year 2015	0.535	-0.197	1.299	0.160

Table D.1: The fixed effect results from a model of the rate of extra-pair paternity in nests where the male parent has been caught (an thus extra-pair vs. within-pair paternity can be establised with high certainty). Egg rank is the average rank (days between an egg being laid and the last egg in the nest) of each chick.