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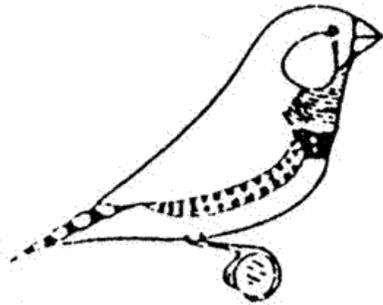
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**Post-natal environmental effects on
behaviour in the zebra finch
(*Taeniopygia guttata*)**



Christine Elizabeth Donaldson

Presented for the degree of Doctor of Philosophy
Division of Ecology and Evolutionary Biology
Faculty of Biomedical and Life Sciences
University of Glasgow

March 2009

Candidate's declaration

I declare that the work recorded in this thesis is entirely my own unless otherwise stated, and that it is of my own composition. No part of this thesis has been submitted for any other degree.

Christine Donaldson

March 2009

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ABSTRACT

Due to mounting evidence that the early environment experienced by a juvenile can affect the morphology and physiology of the adult, there is currently great interest in how environmental variability may shape the behavioural phenotype, and whether such shaping has adaptive benefits. It is clear for example that the developmental environment will have immediate effects on an animal in terms of its survival and performance. Individuals with access to little food or exposed to high levels of predation will have lower survival, and resource-poor surroundings may mean that a young individual is unable to forage successfully, or disperse as normal. However, there is now increasing evidence to suggest that early environmental conditions are also important in determining the success of the adult, meaning that experiences during early development can have significant long-term effects.

In this thesis, I consider the effects of diet quality and exposure to stress in postnatal life on behavioural traits in adulthood, using the zebra finch (*Taeniopygia guttata*) as a model species. There is already evidence to suggest that the early environment can shape behaviour, and consequently, many aspects of behaviour may have important developmental origins that are not a direct result of the genotype.

Two populations of birds were used throughout this collection of studies. The first was raised under different diet regimes, in which both diet quality and consistency were manipulated during the first weeks of life. The second did not undergo any diet manipulation, but were given oral doses of the stress hormone corticosterone or a peanut oil control in the nestling phase. Both populations then underwent a variety of behavioural tests to determine the effects of their early life experiences.

In examining the behaviour of zebra finches, I found that these birds displayed distinct and repeatable behavioural traits, and discuss the idea that they may even exhibit behavioural syndromes, since I was able to show strong correlations between various responses. I studied the effect of both exposure to varying diet quality and consistency in early life on various behavioural traits in adulthood, and found evidence to suggest that certain behavioural responses are linked to the consistency of the nutritional environment experienced by the chick. When tested in adulthood, birds that had experienced a consistent early environment (regardless of food quality) showed a strong trend towards being bolder than birds raised on a variable diet, and this result was replicated in a subset of the original population over a year later, suggesting real longitudinal effects. Such an

outcome may suggest that certain behavioural traits are developed in response to environmental sampling, so as to maximise fitness in the anticipated environment. Similarly, I investigated the behaviour of birds exposed to corticosterone in early life, but found no evidence to suggest that their behavioural traits had been directly shaped by exposure to this hormone. Interestingly however, relative growth rate was linked to individual boldness, a finding which could again possibly be explained through adaptive environmental shaping, in which growth rate is used as a proxy for environmental richness.

Using subsets of the populations of birds that were examined for personality traits, I also considered the effects of the early environment on adult performance in two simple memory tests; specifically studying the ability of birds to search for and remember the location of a food item using environmental cues. In both the diet and hormone manipulated groups, I was able to show that those individuals that had experienced a sub-optimal early environment were compromised in their performance on these simple learning and memory tasks. Again, these were long term effects, since the tests were carried out many months after the birds became adult, suggesting they could not be compensated for, and consequently, since spatial and visual memory are important throughout life, could not be said to be adaptive under any circumstances.

Finally, I also looked for effects of the diet and hormone treatments on the ability of the birds to become dominant. In tests using the birds raised on the different diet regimes, results again suggested that diet consistency was important, as birds raised on a consistent diet were generally more aggressive than those raised on a variable diet, and also showed a trend towards winning more of their individual interactions. Consequently, in this study, nutritional stability made individuals generally more successful in conspecific interactions and thus more likely to hold a higher dominance position, though this is complicated by the fact that dominance was not linked to priority of access to other desirable resources such as potential mates. Using the same experimental design on the birds raised under the hormone treatment, there were trends to suggest that exposure to corticosterone could have negative effects on dominance-related behaviour under certain environmental conditions, but sample sizes were too small to allow firm conclusions.

Overall, this study provides strong evidence for the idea that behaviour can be modified by the environmental conditions an individual experiences in early life. Since behavioural traits will impinge significantly on individual fitness, such effects are of general interest. Since there are also studies that suggest that environmental shaping can be adaptive, I discuss the costs and benefits involved in the different behavioural phenotypes that arise as a result of environmental variation.

Chapter 1

General Introduction

The environment is highly variable, and a developing animal may experience variability in a number of factors, such as food quality and availability, temperature, sibling competition, or predation pressure. The trajectory of normal development may then be altered by such factors. As a consequence, the phenotype of any individual is the combined result of both its genes and the environmental conditions under which it develops. This is because animals can be plastic in their development, so generating a range of phenotypes, allowing individuals to flourish under different conditions, and so maximising the chance of the genotype surviving to the next generation (Gluckman et al. 2005). However, this can require substantial adjustments in morphology or physiology to ensure survival, which can sometimes have significant consequences for the fitness of the animal in later life. For example, growth rate is an adaptively flexible character, and it is possible to see a variety of growth rates within a species (Arendt 1997). By maintaining flexibility, resources can be managed and allocated according to need, so as to maximise the chances of survival when the environment is inconsistent (Gebhardt-Henrich & Richner 1998). As a result, abnormal or adverse conditions during the developmental period (between conception and sexual maturity) can affect growth and development in different ways (Birkhead et al. 1999).

In this study, I examine the extent to which the adult behavioural phenotype is altered by different experiences in the postnatal environment, and consider whether these alterations are potentially costly, whether such costly changes can be mitigated, or whether phenotypes are in fact partially programmed to maximise the chances of survival in the current environment.

Differential effects of the early environment

Certain changes in the developmental environment have been shown to cause damage. Extreme changes to a pattern of normal development can obviously be disastrous, and can lead to an early death. However, even a single nutrient deficiency can lead to severe disruption of development and serious impairment of the animal, compromising both function and survival. For example, human foetuses that experience a deficit of Vitamin A

can go blind (Benton 2008), and the link between folate and normal neural tube development is well known (Benton 2008). Similarly, malnourished rats have only a very poor secretory immune response, making them vulnerable to disease (Sullivan et al. 1993). Early developmental stress is also known to selectively affect certain nuclei in the avian brain that are responsible for the learning and expression of complex song (Buchanan et al. 2004). Because these brain structures develop within the first few months of life (Nowicki et al. 2002), they are at a high risk of being subjected to nutritional and developmental stress, and abnormalities cannot be compensated for in later life, perhaps leading to changes in overall brain size and learning ability (Buchanan et al. 2004).

In these cases, changes in the developmental environment lead to pathology, or a severe decrease in fitness. However, individuals are often able to alter their developmental trajectory to a certain degree without such pathological effects, so mitigating or coping with the change they are experiencing. For example, animals often respond to sub-standard nutrition by altering their growth rate (Gluckman et al. 2005), or by concentrating resources in the most important areas. In birds, tarsus length and body mass are the morphological characters that are most sensitive to early variation in diet (Boag 1987; Ohlsson & Smith 2001), because both increase quickly and reach asymptotes at an early age (Boag 1987). Although feather growth is also rapid, changes caused by neonatal diet are rare. This is because plumage is of high priority to passerine birds, and feather growth follows a strict developmental pattern even when resources are severely limited (Boag 1987). Similar conservation of specific body parts at the expense of others can be seen across a variety of taxa. In humans, for example, the concept of “brain sparing” is well known, whereby cardiac output is redistributed to protect the brain from the effects of malnutrition. This redistribution results in an increased blood flow to the brain, but at a cost to those organs less essential for immediate survival such as skeletal muscles, parts of the intestinal tract, and the kidneys (Nathanielsz & Hanson 2003). If resources cannot be re-allocated in response to under nutrition, individuals may be forced to slow their growth rate in order to survive (Harding & Gluckman 2001). Although slow growth in early life may lead to a small body size in adulthood, which can delay sexual maturation (Roff 1992; Andersson & Andersson 1994), reduce fecundity (Basolo 1998; Dunn et al. 2001), and potentially affect social status and competitive ability (Bumpus 1899; Richner et al. 1989), these costs may be outweighed by the benefits of short-term survival. However, this means that a short-term reduction in food quality or availability that results in the slowing of growth can lead to penalties in later life. Some individuals that experience under-nutrition in early life may attempt to compensate by accelerating their growth at some later point

when conditions have improved, in order to ‘catch-up’ and reach a normal adult size. This kind of trajectory is known as compensatory growth, and it allows individuals to avoid the costs of being a small adult. However, such a rapid acceleration of growth can itself have negative effects. Since compensatory growth requires reallocation of resources from some other function, growth rates may be accelerated by decreasing the accuracy of a developmental program (Arendt 1997). Consequently, animals who have undergone catch-up growth are often less symmetrical than controls or show skeletal abnormalities. Farm animals for example, which are selected for rapid growth to maximise profit, often show disproportionate skeletal development (Ryan 1990). Weak bones and scales in fish can result from periods of rapid growth, and are likely to affect swimming ability and resistance to predation (Arendt et al. 2001), while poor muscle development has been observed in birds and mammals, and will have long-term consequences for dominance and reproductive success (Ohlsson & Smith 2001; Doyle & Leeson 2006). Therefore, although animals may sometimes be able to compensate for an abnormal developmental trajectory in the short-term, such mitigating responses can themselves have associated fitness effects, which can be long-term, and persist into adulthood (Metcalf & Monaghan 2001).

However, far from an abnormal developmental environment leading to a degree of handicap, some scientists propose that it serves to shape the individual to best survive in its current surroundings. A link has been shown in humans between under-nutrition in-utero and the development of diseases such as type-two diabetes and coronary heart disease in adulthood (Hales & Barker 2001). The authors suggest that a poorly nourished foetus can experience adaptations by the endocrine system which help to protect the developing brain from a nutritional shortfall, but which direct resources away from other organs, causing changes that can become costly in later life (Hales & Barker 2001). Observations of patients that were born small due to foetal under-nutrition, but then went on to experience resource rich adult environments, and subsequently developed such diseases, led to an extension of this hypothesis, with suggestions that individuals were in fact programmed to survive best in a poor environment (the ‘thrifty phenotype’ hypothesis; Hales & Barker 2001). It was concluded that such programming took place because developing individuals were, in effect, predicting the conditions they expected to encounter postnatally using the available environmental cues. Consequently, although an animal experiencing poor nutrition may still be impaired relative to one experiencing good nutrition, it was mismatch between the juvenile and adult environments that led to real pathology; when body organs were exposed to resource-rich conditions they had not developed to cope with.

Similarly, the ‘Predictive Adaptive Response’ hypothesis (Gluckman et al. 2005) suggests that an organism can make changes in-utero or in very early life which will adapt it for the predicted post-natal environment. When the environment predicted is indeed the environment experienced, the individual will have increased fitness. However, when there is a mismatch between the predicted and actual environmental conditions, an individual will be compromised, and may become diseased (Gluckman et al. 2005). In this case, the advantages of the adaptations undergone by the developing animal are not seen until adulthood, and these adaptations may be of no benefit to the juvenile, but rather prepare it for the adult conditions which have been forecast. Consequently, there is an interaction between the early developmental environment and the world in which the adult must survive, in determining adult fitness. Such a hypothesis means that individual fitness will also be determined by environmental stability. When the environment is reasonably stable, individuals that are well adapted to that environment will have high fitness. When exposed to minor change, such individuals may also be flexible enough to alter their behaviour without fitness costs (Monaghan 2008). However, if changes become more extreme, there may be fitness costs associated with behavioural change, such that certain life history traits may be traded off against others.

Overall, then, we can conclude that the early environment any animal experiences will play a part in determining its phenotype, that certain environmental conditions will cause pathology, and maybe even death, some will have costs that can be postponed until later life, and some may serve to adapt the individual for maximum survival in its surroundings. However, we know less about how these hypotheses can be applied to behavioural traits.

Early experience shapes behaviour

We know that the early environment an animal experiences can affect a range of aspects of the phenotype, including a variety of behaviours. For instance, growth has the potential to affect behaviour in a number of ways, and effects can be immediate and transient, or delayed and permanent. For example, an animal diverting a large proportion of resources to growth will need a high energy intake and is therefore likely to be hungry, which could potentially influence its risk-taking behaviour or aggression. Alternatively, slow growth in early life may lead to poor muscle development, and prospective behavioural sub-dominance. Predictions by Stamps (2007) suggest that differences in growth rate may encourage differences in personality traits which in turn lead to growth-mortality tradeoffs,

such that growth and personality traits will be correlated across individuals. Although these speculations are very interesting, much of the discussion is focussed on species with indeterminate growth, and more empirical work will be needed to determine how relevant such a hypothesis is for species with a fixed growth period. However, there is much work to suggest that the specifics of early diet can be important. For example, in mice, under-nutrition in early life can change the eating habits of the adult. Mice nursed by mothers fed a low protein diet show a preference for high fat food after weaning, so tend to overeat and put on more weight than controls (Ozanne et al. 2004). Exposure to hormones in the first weeks of life will also be crucial in determining behavioural traits. Young animals exposed to high levels of testosterone tend to be more aggressive and competitive (Groothuis et al. 2005), while individuals exposed to high levels of glucocorticoids, or stress hormones, can show alterations in anti-predator behaviour (Uller & Olsson 2006), emotion (Pryce et al. 2005; Llorente et al. 2007), and even vulnerability to substance abuse (Roman & Nylander 2005). There are numerous other examples of adult behaviour being affected by experiences in early life. Isolation in the pup phase can affect social and feeding motivation in adult rats (Ruedi-Bettschen et al. 2006), sand digging behaviour of adult cuttlefish is affected by exposure to substrates in the first days of life (Poirier et al. 2004), and early nutritional stress can affect singing behaviour in birds (Nowicki et al. 1998; Buchanan et al. 2003). However, much remains to be discovered, and although there is increasing evidence to suggest that the behaviour of an individual reflects its developmental environment, we still know little about whether experiencing an abnormal early environment always results in a compromised individual, or whether it can lead to adaptive shaping.

The zebra finch

The zebra finch (*Taeniopygia guttata*) is a seed-eating finch of the estrildine family, and has become highly domesticated due to its popularity as an avian model since the start of the 19th century. In the wild, birds live in large social groups, feeding, roosting and nesting together (Zann 1996), but the zebra finch also survives well in captivity, breeds freely, and is relatively easy and inexpensive to keep. I chose to use the zebra finch for this study of the effects of the early environment on adult behaviour because it is an altricial bird, and undergoes the majority of its early development in the first weeks after hatching (Ricklefs 1968), making it a convenient model in which to manipulate food and stress in the early part of life. In addition, it has been recently shown by Tschirren et al. (2009, in press) that

when examined for a variety of traits, offspring of domesticated and wild caught zebra finches respond in a very similar way when exposed to favourable and unfavourable conditions during development. Such a finding confirms that it is useful and relevant to study behavioural traits in captive populations, and also that adaptive explanations for differences or changes in behaviour in this species are justified. Zebra finches have also been extensively used to study the long-term effects of the early environment; with studies on growth (Boag 1987; Gil et al. 2006; Arnold et al. 2007), secondary sexual traits (DeKogel & Pijls 1996; Birkhead et al. 1999; Blount et al. 2003), song development (Spencer et al. 2005; Gil et al. 2006), visual learning (Fisher et al. 2006) and reproductive success (Naguib et al. 2006). This collection of studies provided components of this thesis with a strong background of prior work, enabling me to build upon current knowledge, and with the benefit of tried and tested manipulations examine a new avenue of research, namely the behavioural effects associated with environmental variation in early life. Since zebra finches are opportunistic breeders, and will reproduce year-round, I also anticipated good access to chicks and the opportunity to experimentally manipulate their environments. In addition, parents are not significantly disturbed by short-term removal of offspring for measurements or corticosterone administration, and continue to feed and behave as normal.

Personality studies in the zebra finch

Recent work has shown that many animals, including humans, show consistent individual differences in behaviour, in that certain individuals are always more aggressive, bold or active than others, even in homogenous environments (Gosling & John 1999; Carere et al. 2005; Bell 2007; Dingemanse et al. 2007). These characteristic patterns of behaviour are known as ‘personalities’, or ‘behavioural syndromes’, and are thought to allow animals to better cope with the demands they face in the environment (Armitage & Van Vuren 2003; Dingemanse et al. 2004; Carere et al. 2005; Jones & Gosling 2005; Alvarez & Bell 2007). Within individuals, behavioural traits are often correlated (Verbeek et al. 1996; Bell 2005; Freeman et al. 2007), and such correlations mean that animals are frequently assigned to discrete behavioural categories according to their responses to standard tests. With regards to such personality work, the zebra finch has been less studied than some other songbirds and literature on this topic is sparse. In one study, Martins et al. (2007) were able to show that the stress profile of selected lines of zebra finches were linked to their exploratory and risk-taking behaviours, with birds that were highly exploratory and risk-prone generally

displaying a higher response to a standard stressor. However, although this is an interesting result, it remains unclear whether zebra finches display repeatable traits that could be classified as personalities or behavioural syndromes. Therefore, since this is a species that is so widely used for behavioural studies, it is important to conduct some basic work addressing the possible existence of stable behavioural traits in this bird. It is also relevant to examine whether any such traits are the result of (or modified by) experiences in early life.

Cognition studies in the zebra finch

Although there has been much work on avian cognition, the zebra finch has again not been widely studied in this regard. There is evidence that both sub-optimal nutrition and exposure to elevated levels of corticosterone in the nestling phase can have negative effects on song learning in this species (Spencer et al. 2003; Buchanan et al. 2003; Buchanan et al. 2004), with birds experimentally exposed to such conditions singing shorter, simpler songs than their control siblings. Similarly, a more recent study has shown that the early environment can affect learning and memory, with individuals compensating for a period of slow growth significantly less successful in a visual learning task than their well-fed siblings (Fisher et al. 2006). Spatial ability has also been shown to be impaired in zebra finches selectively bred for a high response to stress (Hodgson et al. 2007).

Aggression and dominance in the zebra finch

In zebra finches, no dominance hierarchy is apparent within groups of wild birds, but in captive populations a pecking order may develop, although this can be unstable (Ratcliffe & Boag 1987). Zebra finches may fight over anything, but will come to blows particularly over food, perching and nesting sites (Zann 1996), and if birds are held in single-sex cages, males show more aggression than females (Butterfield 1970). Males will fight over unpaired females (Zann 1996), and plumage and beak colour are important visual cues for perceived dominance and fitness (Burley & Coopersmith 1987; Alonso-Alvarez et al. 2004). A previous study has shown that zebra finches exposed to elevated levels of stress hormone as chicks are less dominant than their control siblings when tested in later life (Spencer & Verhulst 2007). This study provides an interesting starting point for further work in this area, and suggests that experiences in early life can be important for this behavioural trait. In addition, work on fish has revealed that dominance status can be

negatively affected by a period of compensatory growth following a poor start in life (Royle et al. 2005), a phenomenon that has yet to be investigated in birds.

Aims of Thesis

This thesis considers the effects of a range of early diets and associated growth trajectories, and exposure to elevated levels of corticosterone prior to fledging, on behavioural traits in later life. Personality, cognitive ability and dominance behaviour were studied in two sets of birds; one that had undergone different rearing diets designed to alter early growth trajectories, and one that had been given oral corticosterone to mimic heightened stress levels in the nestling phase. Using these two groups, I aimed to address the question of whether the early environment experienced by an individual could have an effect on its later behaviour, and whether such effects were generally adaptive or costly.

Chapters 2 and 3 concern the results of various personality trials, and address the question of whether zebra finches display consistent and repeatable behavioural traits, and discuss the idea that such traits are shaped by early conditions to maximise survival in the surrounding environment. Chapters 4 and 5 present the results of experiments designed to test the learning ability and memory skills of individual birds. These chapters then consider the effect of the early environment on such cognitive abilities and speculate on possible mechanisms for the observed effects. Chapters 6 and 7 examine the effect that early diet and early stress can have on dominance behaviour in males and their offspring, by presenting the results of two experiments using priority of access tests to determine dominance status. In all chapters, I have tried to distinguish between the effects of the experimental manipulations and the growth changes that are often associated with them. Finally, in chapter 8, I bring together these studies of environmental effects on later behaviour, and consider whether the experience of a sub-optimal environment in early life has generally negative effects on individual fitness, or whether such experiences can in fact be adaptive, in shaping the behavioural phenotype for maximum survival.

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Chapter 2

Early nutritional stability shapes personality in the zebra finch

ABSTRACT

Studies of animal personality have revealed that individuals often show correlated suites of behaviours, and can be classified along a proactive-reactive axis. Proactive individuals are bold and aggressive, quickly but superficially explore new environments and readily form behavioural routines; reactive individuals are shy and cautious, pay closer attention to external stimuli, and are more flexible in their behavioural responses. Little is known about the environmental variation that may induce or select for such differences, but early conditions are likely to be important. Here I demonstrate that individual traits may be shaped by the stability of nutritional conditions in early life. I experimentally exposed zebra finch chicks to variable food quality, and examined the effect on personality. Nestlings received either consistent low or high quality food for 30 days after hatching, or a switch between the two at day 15. After fledging, all birds experienced consistent high quality food. I found individual behavioural differences that were linked to diet consistency: birds reared on a consistent diet (either consistently high or low) were bolder than birds on a variable diet. Behavioural differences at the diet consistency level were maintained over time, and males were highly repeatable in their boldness score when re-tested a year later, although females were not. I suggest that environmental shaping may be important in the development of personality, and could tailor traits to the conditions likely to prevail in adulthood. The superficial exploration and routine behaviour of bold individuals might be beneficial in stable surroundings, while being more reactive and flexible could be advantageous in a changing environment.

INTRODUCTION

An individual's behavioural phenotype is enormously important in determining its survival and breeding success (Dall et al. 2004), and in order to maximise fitness, animals should tailor their behavioural responses to suit the environment they find themselves in.

However, although behaviour is always context-dependent to a certain degree, some individuals are consistently more aggressive, bold or active than others, even in homogenous environments (Gosling & John 1999; Carere et al. 2005b; Bell 2007; Dingemanse et al. 2007). Indeed, many animals, including humans, show consistent individual differences in how they respond to novel situations, interact with conspecifics, and deal with various environmental conditions. Within individuals, response behaviours are often correlated (Verbeek et al. 1996; Bell 2005; Freeman et al. 2007), and such correlations mean that animals are frequently allocated to discrete behavioural categories according to their responses to standard tests. Responses to novelty (in the form of a novel object and/or a novel environment) are two such standard ways of quantifying differences in individual behavioural responses (Verbeek et al. 1994). Subjects are presented with novel objects in their home cages, or given the opportunity to explore a novel environment, and their responses in each case are used to assign them scores for various traits such as boldness and speed of exploration. Individual differences are apparent in early life and can persist across time and in a variety of situations, leading to correlated suites of behaviours being labelled as distinct 'personalities' or 'coping styles' (Koolhaas et al. 1999). Such personalities are often assigned along a proactive-reactive continuum, since specific traits such as boldness, aggression and speed of exploration have been found to be correlated within individuals in a number of animal groups (Huntingford 1976; Drent et al. 2003; Armitage & Van Vuren 2003; Carere et al. 2005b; Brown et al. 2007). Proactive individuals are both bold and aggressive, quickly but superficially explore new environments and readily form behavioural routines; while reactive individuals are shy and cautious, pay closer attention to external stimuli, and are more flexible in their behavioural responses (Koolhaas et al. 1999). Although these relationships have not been explicitly demonstrated for our study organism, Koolhaas et al. (1999) have suggested that proactive-reactive personality types may represent fundamental trait characteristics that are likely to be found in many species.

Despite the extensive evidence documenting the existence of animal personalities, the development of different personality types within a population remain poorly

understood. Some individual variation has been shown to have a genetic basis (Sluyter et al. 1995; Sokolowski 2001; Drent et al. 2003), but less is known about the environmental contribution, and whether the environment an individual experiences during early life can shape this part of the behavioural phenotype. We know that conditions during early life can have a significant effect on many aspects of the phenotype (Metcalf & Monaghan 2001; Hales & Barker 2001; Bateson et al. 2004; Ozanne & Constancia 2007), and there is now some evidence that for certain species, environmental variability can shape personality traits; for example, in captive reared fish (Huntingford & Adams 2005), in pigs (De Jong et al. 2000), rats (Ferre et al. 1995; Gregus et al. 2005), and tits (Carere et al. 2005a). However, less is known about whether behaviour is shaped and fixed at some early developmental stage, and whether a sub-optimal developmental environment results in fitness costs or can lead to adaptive shaping. Since there are studies that show that different personality types prosper under different environmental conditions (Verbeek et al. 1994; Dingemans et al. 2004; Huntingford & Adams 2005), if maternal condition or food availability during rearing were accurate predictors of the adult environment, it would make adaptive sense for the offspring's phenotype to be shaped so as to prepare it for the environment it would most likely encounter (Monaghan 2008). Variability in the early developmental environment has been shown to affect behaviours such as dominance and neophobia in zebra finches (Spencer & Verhulst 2007), risk-taking in tits (Arnold et al. 2007), fear responses in rats (Penke et al. 2001; Gregus et al. 2005), and cognitive performance in both mammals and birds (Kitaysky et al. 2003; Akman et al. 2004; Sandstrom & Hart 2005; Fisher et al. 2006). In the main however, such studies highlight the negative long-term effects that unusual or sub-optimal developmental conditions can have on behavioural traits. In this chapter, I hypothesise that environmental variability in early development that is indicative of the conditions likely to be experienced in adulthood can lead to shaping of an individual's personality traits, and discuss the possibility that such shaping is adaptive. Personality type could potentially be influenced either by growth rate as a proxy for environmental richness, or by some other cue to indicate current conditions, such as diet consistency. My objectives in this study were therefore to investigate the effects of early environmental variation, in terms of nutritional quality and stability, on personality traits in the zebra finch, and to determine whether these traits were consistent over time.

METHODS

Subjects

The subjects were offspring of experienced stock adults that bred in the summer of 2006. All parents were housed in standardised cages with access to a wooden nest box, in rooms maintained at 20-22°C, with a relative humidity of 45-55%, under full spectrum artificial light (16:8, L:D cycle). Parents were given a standard diet of *ad lib.* access to a commercial tropical seed mixture (J.E. Haith Ltd, UK), water, shell grit and cuttlefish bone, with rearing and conditioning food (J.E. Haith Ltd UK) and spinach once a week, supplemented with additional egg protein from two weeks prior to pairing until the hatching of their first egg. At this point, broods were randomly allocated to either a high protein (H) or low protein (L) diet treatment. The low protein diet contained approximately 12% protein by dry weight, and the high diet approximately 40%. Families assigned to the high protein treatment were kept on a diet comprising mixed seeds (foreign finch mixture, J.E. Haith Ltd, UK) including some soaked overnight to saturation, plus grit and cuttlefish. They also received 5g of conditioning food (13.6% protein, J.E. Haith Ltd, UK) plus 5g of homogenized hard-boiled hens' egg per family daily. Fresh spinach, Daily Essentials vitamin supplement (The Birdcare Company, UK) and Calcivet calcium supplement (Vetafarm, Australia) were given once a week. Families assigned to the low diet treatment received only mixed seeds (including some soaked overnight to saturation), plus weekly spinach, vitamins and calcium supplement. All families had *ad lib.* access to fresh water.

When half the chicks in any brood reached 15 days of age, the family was again randomly allocated either the H or L diet treatment, such that the first 15 days of growth on one diet was followed by another 15 day period during which the diet was either maintained or switched. This gave four separate diet groups: high/high (HH), high/low (HL), low/high (LH) and low/low (LL). The diet manipulations were designed so as to allow assessment of the effects of growth rate and diet consistency separately. As a result of this protocol, the birds within these four diet groups experienced differences in both diet consistency and quality, with the expectation that they would have different growth trajectories. HH and LL groups experienced a consistent high or low protein diet throughout the nestling period. HL and LH birds experienced a variable diet, with a switch in protein content at 15 days of age in different directions in the two groups. Birds in the HH group grew relatively fast and steadily, birds on the LL diet grew more slowly but also steadily, and birds in the HL and LH groups experienced a variable growth trajectory (fast

growth followed by slow growth and slow growth followed by fast growth respectively) (Fig. 1). Two breeding rounds with the same parents resulted in a total of 189 chicks from a total of 33 pairs. Individual chicks were initially identified by ink markings and later by individual rings, and remained in the parental nests and cages throughout the manipulation, with all food being delivered by the parents. In order to determine the effects of the diet manipulation on growth trajectories, measurements of body mass, wing and tarsus length were taken every five days from hatching until 30 days of age (the end of the diet manipulation period), and finally at 100 days (i.e. when fully adult).

Following the cessation of the dietary manipulation at nutritional independence at 30 days of age, all birds were housed in same-sex groups of six juveniles in standard cages, in visual and auditory contact with other conspecifics. They were all provided with the same standard diet of *ad lib.* mixed seed with protein supplement and greens once a week from then on. Cages were floored with hemp chippings, and contained a seed bowl and water fountain, plus a variety of perches (both wooden rods and natural twigs). A total of 96 birds were then randomly selected from the population for behavioural testing at 40 days of age; 26 HH, 22 HL, 25 LH and 23 LL birds, made up of 47 females and 49 males. This sample contained 28 sibling pairs from the same nest with the same treatment, so allowing us to look for within- and between-family differences in behavioural scores. Since all birds had to be tested individually, subjects were isolated overnight prior to the day of testing in single cages (40 x 43 x 44cm). These cages were of a familiar layout and contained only familiar objects (wooden rod perches, a food dish and a water fountain). The following day each bird received two novel object trials in the morning between 0900 and 1100 hours, which were performed in the familiar cage, and a novel environment trial during the afternoon of the same day between 1200 and 1700 hours, for which each bird was removed from its familiar cage and introduced into the novel environment test arena. In between the two novel object trials, subjects remained alone in the familiar cages in order to minimise handling stress, but could see and hear conspecifics throughout this period.

Novel object test

This test was devised to examine individual behavioural responses to a novel object, a standard way to examine neophobia and exploratory behaviour in birds (Verbeek et al. 1994; Carere et al. 2005c). Tests were conducted on single birds housed in their individual

cages. Each cage contained three perches, labelled 1, 2 and 3 from left to right. At the onset of the novel object trial, perch 1 was removed and replaced with a 'novel perch' bearing object 1 - a blue plastic fish (3cm high x 3cm wide), novel in both colour and shape. Birds always flew onto perch 3 during the introduction of the novel object, and so all trials began with the bird sitting on perch 3. Behavioural responses were then recorded for five minutes from behind a screen placed approximately one metre from the front of the cage as follows: the minimum distance of approach of the bird to the novel object (minimum distance 1, with distances designated as follows; 0 = touching novel object, 1 = on perch 1, within 5cm of novel object, 2 = on perch 1, within 10cm of novel object, 3 = on perch 1, within 18cm, 4 = any point on perch 2, 5 = any point on perch 3), latency to land on the novel perch (land latency 1); number of times the bird landed on the novel perch (times landed 1); and total time spent on the novel perch (time on perch 1). Birds that never landed on the novel perch were given a land latency of 300 seconds, so that I was still able to distinguish birds that did land from those that did not, but was also able to include data for all birds in later analyses. I expected birds with a bold and more 'proactive' personality type to approach the novel object more quickly, more closely and more frequently than birds with a shy, reactive personality type. Following the five minute observation period, the novel perch with object 1 was removed and replaced by the familiar perch 1. The subject was then left for a minimum of 30 minutes before the perches were again swapped, with this time the novel perch holding object 2, a pink plastic frog (4cm high x 3cm wide). The procedure for novel object 2 was exactly the same, and the same behavioural responses were recorded (minimum distance 2, land latency 2 etc). The novel objects were kept concealed from view when not being used so that no bird saw an object until it was presented with it in the cage during testing.

Novel Environment test

A novel environment test arena was set up for the assessment of all birds (Fig. 2.1). A familiar environment structure was provided in the left hand half of a standard cage (60 x 45 x 40cm), containing the usual hemp floor covering and wooden rod perches. The right hand half comprised the "novel environment", designed to look completely different from the familiar cage setup. It had pebbles on the floor, a cardboard screen and a number of plastic plants, but the usual wooden rod perches, placed at the same height as in the familiar side of the cage; all perches were numbered so the movement of the bird could be

tracked throughout the test. A solid opaque divider initially separated the two areas of the cage. At the start of a test, the subject bird was moved from its individual cage into the novel environment side of the test cage. Once the bird was resting on a perch, the solid divider was slowly withdrawn (allowing the bird to move freely between the two halves of the cage) and the period of observation began, with the observer concealed behind a screen one metre from the cage front. The cage was then scan-sampled every 20 seconds for a 20 minute period. The activity observed every 20 seconds was recorded as follows: sitting, hopping, preening, vocalising, feeding and moving, allowing calculation of the proportion of time spent in each of these activities. I also recorded the following: latency to begin exploring the novel environment (latency to explore NE) which was the latency to move from the first perch landed on to another perch in the cage; total time spent in the novel environment (time spent in NE) beginning once an individual moved from the first perch; the total number of perch visits, including repeats (perch visits) and pecks to perches and objects both in the familiar and novel environments (pecks). Birds that never moved from the first perch were assigned a latency to explore of 1200 seconds. I expected birds with a bold, proactive phenotype to begin exploring the novel environment more quickly, and spend more time in it than shy reactive birds. Following this 20 minute session in the novel environment, birds were re-caught, weighed and returned to their group cages.

Both personality tests were repeated on a subset of the original 96 birds over a year later (when birds were between 14 and 16 months old) using the same experimental protocol, but with two different objects (a small straw parrot (3cm high x 2cm wide), and a black stone Buddha statue (4cm high x 3cm wide)) and a new structure for the novel environment, with a selection of new plants and flooring of paper and gravel instead of pebbles. Due to a time constraint, I was unable to retest all birds and so seven HH, HL and LL, and eight LH birds were selected, of which 16 were male and 13 female. The retested individuals were randomly selected from the original four diet groups. This second set of tests was intended to determine whether personalities were stable over long periods of time, and whether early diet quality or consistency affected adult personality.

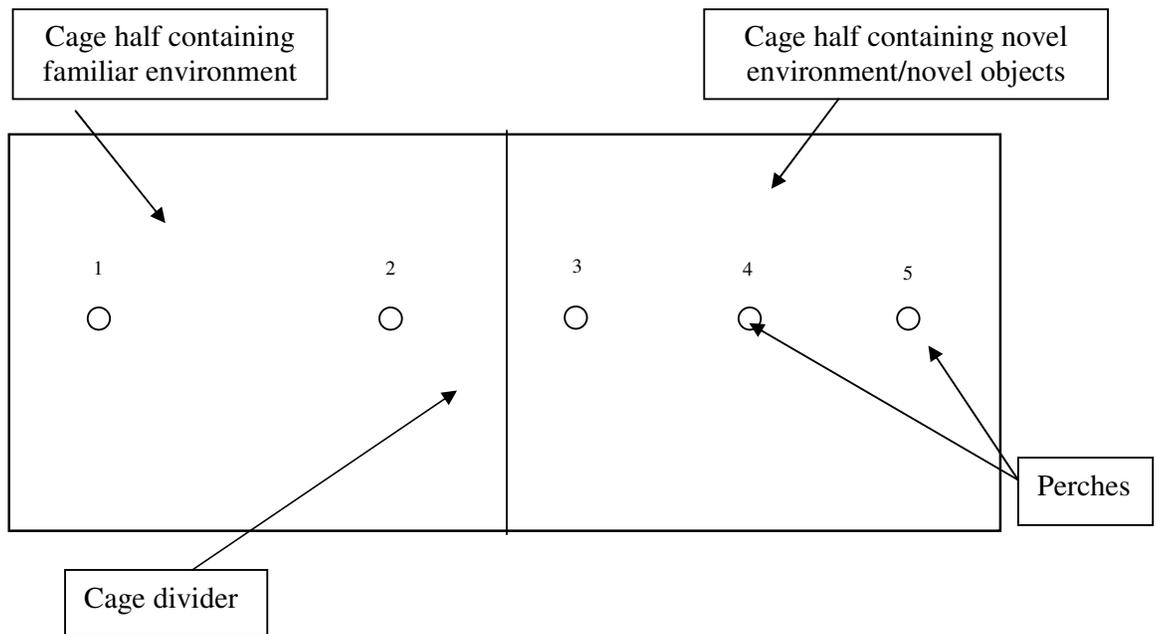


Figure 2.1. Schematic of novel environment test cage

Statistical analysis

Data were mostly analysed using general linear models (GLMs), or general linear mixed models (GLMMs) with family as a random factor where more than one bird came from the same family. Non-significant variables were removed in a stepwise fashion. When assessing bivariate relationships between variables I used Spearman's rank correlations, with sequential Bonferroni correction to account for multiple comparisons. Then, as many of the variables were correlated, I used principal components analysis (PCA) to summarise the behavioural data, after log transformation to account for non-normal skew. To aid in the interpretation of the components, Varimax rotation was performed, which maintains independence between components. The personality scores that were assigned to individuals following this data compression showed a normal distribution, so parametric tests were used to analyse these values.

Many of the behavioural measures taken in the second set of tests were again strongly correlated, and so personality scores from this second data set were calculated using the same factor loadings as in the PCA analysis from the trials at 40 days. To examine changes in these personality traits over time, I used repeated measures ANOVA, with individual scores from the two tests as the repeated measure. Whenever repeatability scores were calculated, I followed the method described by Lessells and Boag (1987) using

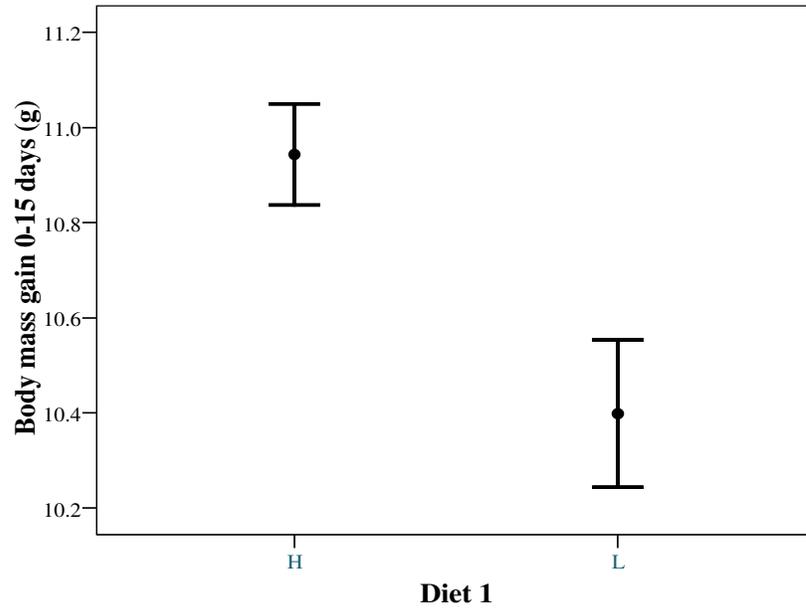
a one-way ANOVA with individual ring number or family as a fixed factor. In all analyses, N=96 unless otherwise stated, and all analyses were conducted using SPSS 15.0.

RESULTS

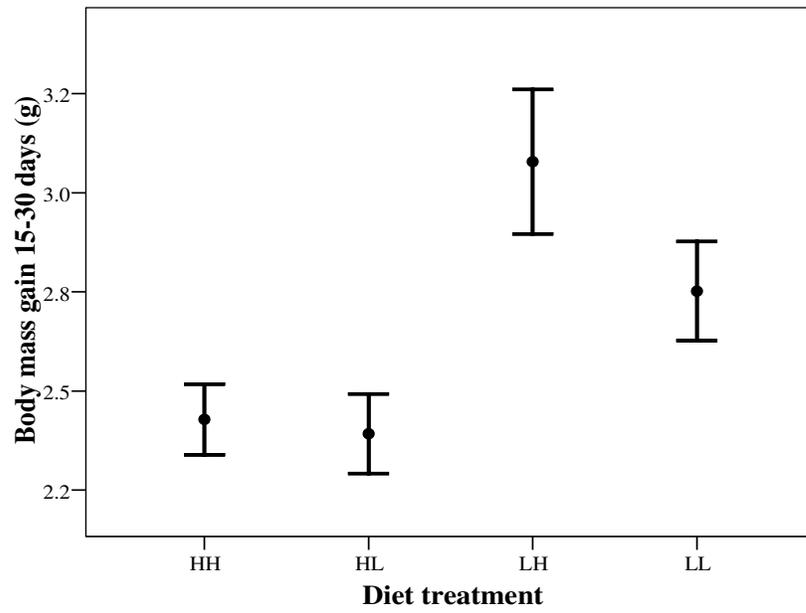
Treatment

As expected, chicks on the different diet treatments showed different growth trajectories. Using a mixed model with sex and diet treatment as fixed factors and family as a random factor, there was no difference in hatching mass between males and females ($p=0.63$) or between chicks allocated to the H or L diet for the first 15 days ($p=0.80$). Between 0 and 15 days of age, sex had no effect on mass gain, and similarly there was no significant interaction between sex and diet treatment (either H or L) (in both cases $p>0.29$). Therefore sex and its interaction with diet treatment were removed from the model. In the resulting simplified model, diet treatment (while controlling for family) had a significant effect on mass gain, with H diet birds gaining significantly more mass than L diet birds (Fig. 2.2 (a), $F_{1, 179.65} = 11.02$, $p=0.001$). After the diet switch at 15 days (at which point there were four different diet groups to consider, rather than just the H group and the L group as there had been for the first half of the manipulation), sex and the interaction between sex and diet group were again non-significant (in both cases $p>0.73$). However, after removing these factors from the model, there was the suggestion of an accelerated rate of growth in the LH treatment group compared to the other groups, as consistent with a transfer to a higher protein diet, although this was not significant (Fig. 2.2 (b), $F_{3, 180.63} = 1.90$, $p=0.13$). Once the treatment ceased at 30 days of age, and all birds were maintained on a standard diet, there was no difference in growth rate between the diet groups (Fig. 2.2 (c), $F_{3, 157.24} = 1.75$, $p=0.16$), though there was a significant effect of sex on growth, with females growing more than males during this period ($F_{1, 177.68} = 10.12$, $p=0.002$). There was no significant interaction effect between sex and diet group between 30 and 100 days ($p=0.39$).

(a)



(b)



(c)

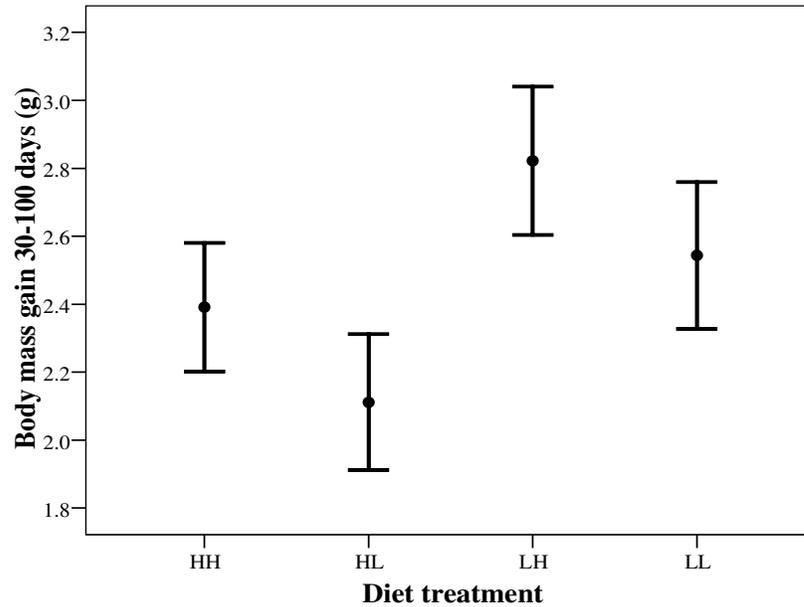


Figure 2.2. The mean \pm s.e. body mass gain (g) of zebra finch chicks (a) reared initially on high (H) or low (L) quality diets between 0 and 15 days, (b) then either maintained on those diets or switched over between 15 and 30 days, making 4 diet treatment groups (HH, HL, LH and LL), and (c) finally maintained on a standard quality diet and monitored until 100 days of age. ($N=189$).

Personality

Behavioural data collected at day 40 revealed that subjects showed consistencies both within and between the two novel object trials, with high correlation coefficients for a number of variables (Table 2.1). For instance, birds that were quick or slow to land on the perch with the first novel object were similarly so for the second novel object, indicative of a ‘bold’ or ‘shy’ phenotype (Fig. 2.3; $r_s = 0.52$, $N=38$, $p = 0.001$; not including 58 birds that did not land in at least one trial, though effect is still strong when including results from all birds; $r_s=0.39$, $N=96$, $p<0.0001$). Subjects that were quick to land on the novel object perch also made more visits to that perch and spent more time on it than birds with a longer land latency (Table 2.1). Individuals also showed consistency within the novel environment trial (Table 2.2). There was a strong negative correlation between exploration latency and the number of perch visits made by any bird, and a positive correlation between the number of pecks and the amount of time spent exploring the novel

environment, with fast explorers visiting the most perches and birds that pecked more in both environments spending more time in the novel environment overall (Table 2.2). Additionally, birds that had a higher explore latency (i.e. took longer to move from their initial perch in the novel environment) spent less of the overall trial moving about.

As well as showing consistencies within each of the types of personality trial, birds also showed consistency across the two types of trial. For example, despite individual variation, the average land latency across both novel object trials showed a negative correlation with the time spent in the novel environment ($r_s = -0.23$, $p = 0.023$, $N = 96$ birds), meaning that birds that were quick to approach the novel objects spent the most time in the novel environment. Similarly, there was a positive correlation between the amount of time any bird spent on the first novel object perch, and the time it then spent in the novel environment ($r_s = 0.23$, $p = 0.023$, $N = 96$ birds). In both cases the correlation was relatively weak, albeit significant.

Table 2.1. Behavioural correlations (Spearman's rank) observed in novel object trials 1 and 2 (only significant correlations shown) See text for variable definitions. N=96 birds.

	Land latency 1	Minimum distance 1	Times landed 1	Time on perch 1	Land latency 2	Minimum distance 2	Times landed 2	Time on perch 2
Land latency 1								
Minimum distance 1	.844**							
Times landed 1	-.899**	-.916**						
Time on perch 1	-.952**	-.851**	.895**					
Land latency 2	.394**	.382**	-.326*	-.366**				
Minimum distance 2					.714**			
Times landed 2					-.689**	-.838**		
Time on perch 2	-.305**	-.307*			-.810**	-.814**	.743**	

*p < 0.05, ** p < 0.01 (both after sequential Bonferroni correction)

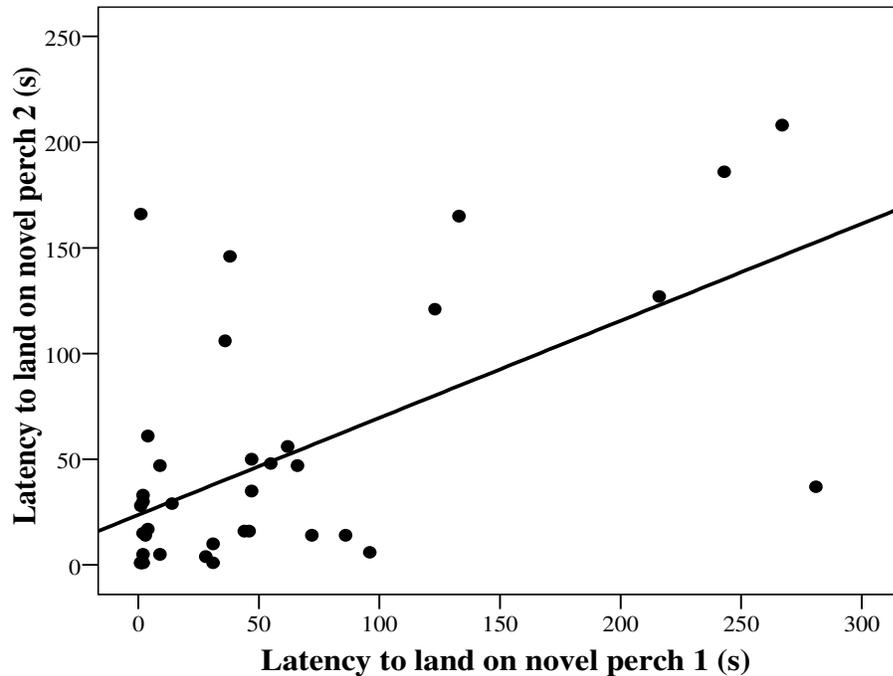


Figure 2.3. The relationship between an individual's latency to land on the perch on which the novel object was mounted in the first (perch 1) and second (perch 2) novel object trials when birds were tested at 40 days. See Table 2.1 for statistical analysis.

The behavioural measures obtained from the separate personality trials were entered into principal component analysis (PCA), in order to summarise the data collected for each individual. This revealed the presence of two components, explaining 25.4 and 18.2% of the variance in behaviour respectively. When the solution was rotated, component 1 explained 22.0% of the variance while component 2 explained 21.6%. The loadings (Table 2.3) suggest that the first component provided a boldness score: it was heavily influenced by a bird's latency to land in the novel object trials, its minimum distance from the novel objects, the number of times landed and the total time spent on the novel object perch, and also the amount of time spent in the novel environment. Thus very 'bold' birds were quick to land on both novel object perches, approached to within a short distance of both objects, landed often, spent a large part of each trial sitting on the novel object perch, and spent long periods in the novel environment. Such birds had strongly negative scores, while 'shy' birds had positive scores. In order to improve the clarity of the graphs to follow, these scores were multiplied by -1, giving bold birds positive scores and shy birds negative scores. Component 2 was primarily influenced by the amount of time a bird was observed sitting, hopping and moving, the number of times a bird landed on the novel object perch, and the total number of perch visits in the novel environment; the

component was thus an index of activity (Table 2.3), with active birds obtaining negative scores. Again, I inverted these scores to make visual interpretation easier. The distribution of scores for the experimental population is shown in figure 2.4. As would be expected given that the two components were independent in the analysis, these scores for boldness and activity were not correlated within individuals ($r_s=0.036$, $p=0.73$).

Table 2.2. Behavioural correlations (Spearman's rank) observed in the novel environment trial (only significant correlations shown). See text for variable definitions. N=96 birds.

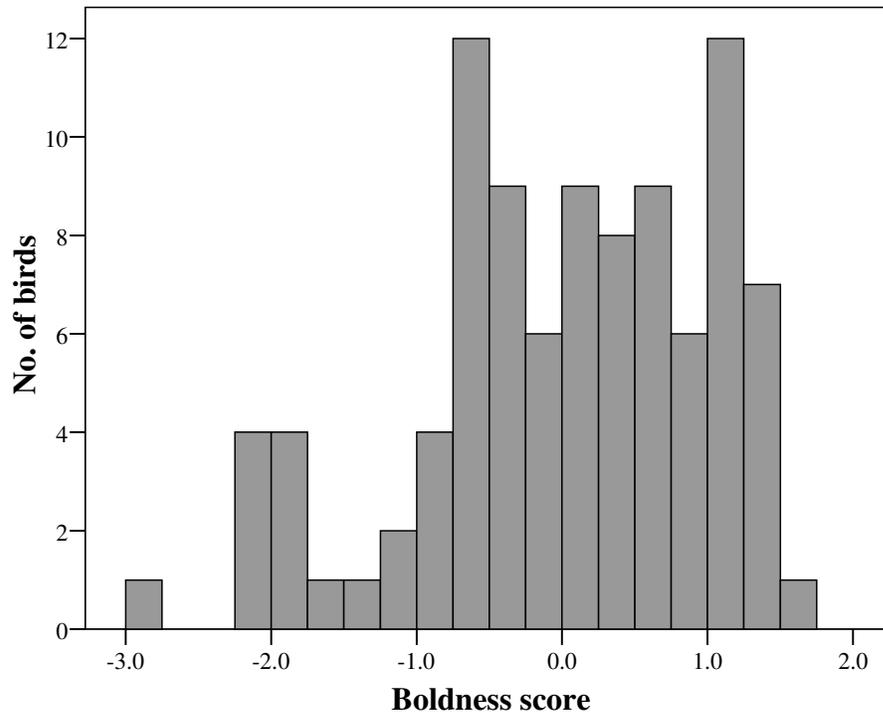
	Latency to explore	Time spent in NE	Perch visits	Pecks	Sitting	Hopping	Feeding	Moving
Latency to explore								
Time spent in NE								
Perch visits	-.427**							
Pecks			.610**					
Sitting	.336*		-.726**					
Hopping			.620**		-.674**			
Feeding			.438**		-.650**	.425**		
Moving	-.372**		.906**		-.702**	.535**	.434**	

* $p < 0.05$, ** $p < 0.01$ (both after sequential Bonferroni correction)

Table 2.3. Principal component analysis of personality measures (Varimax rotation, only loadings over 0.3 displayed). See text for variable definitions. $N=96$ birds.

	Component 1	Component 2
NOVEL OBJECT TEST		
Land latency 1	.663	-.344
Minimum distance 1	.658	-.384
Times landed 1	-.301	.581
Time on perch 1	-.656	
Land latency 2	.762	
Minimum distance 2	.731	
Times landed 2	-.437	
Time on perch 2	-.703	
NOVEL ENVIRONMENT TEST		
Latency to explore NE		-.508
Time spent in NE	-.442	
Perches visited		.764
Sitting		-.855
Hopping		.550
Feeding		.463
Moving		.811

(a)



(b)

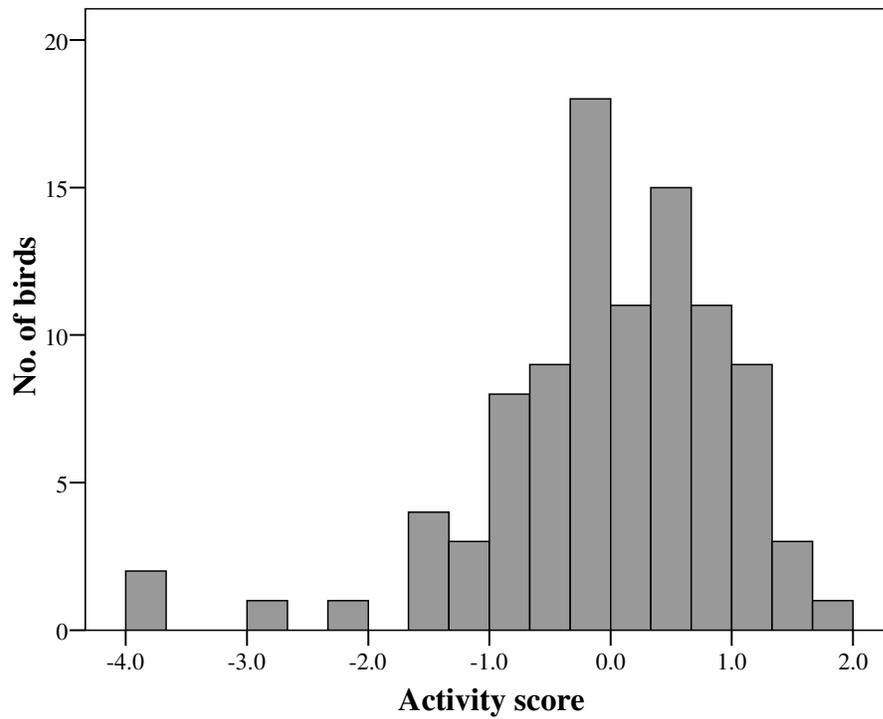


Figure 2.4. The distribution of PCA scores designated as a) boldness and b) activity. $N=96$ birds

Familial effects

To determine whether there was a difference in the amount of variation between and within families in their personality, I calculated a repeatability score (Lessells & Boag 1987) using family as the grouping variable for all families in which there were 2 chicks. Repeatability between full siblings was low (for boldness score (component 1), $r = 0.17$, $F_{27, 28} = 0.72$, $p = 0.81$; for activity score (component 2) $r = 0.26$, $F_{27, 28} = 1.72$, $p = 0.08$). The majority of the siblings were brother/sister pairs, so repeatability was not calculated for the different sexes.

Treatment effects

Boldness

I analysed treatment effects on boldness score using general linear mixed models with family as a random factor, mass gains during the two phases of the dietary manipulation (0-15 days and 15-30 days) as covariates, and sex, diet treatment (and their interaction) and breeding round as fixed factors. There were no significant effects of sex, breeding round or mass gain, nor any interaction effect between diet treatment and sex on boldness score (in all cases $p > 0.46$), and consequently, these variables were dropped from the model. In the simplified model, diet treatment was not an accurate predictor of a bird's boldness ($F_3, 92 = 1.34$, $p = 0.27$). However, examination of the data showed a strong trend for birds on a consistent diet during early life (either HH or LL) to differ in boldness score from birds that had experienced a variable diet (either HL or LH). Therefore I considered a new model using a dichotomous composite variable of diet consistency (either consistent or variable) in place of diet group, with other terms as specified for the above model (sex, breeding round, mass gain and family). For sex, breeding round, mass gain and the sex*diet consistency interaction, p was always > 0.47 , and consequently these factors were dropped from the model. However, following this removal of non-significant factors, diet consistency was found to be important in determining boldness. Birds on a consistent diet during early growth showed a strong trend towards being bolder than birds on a variable diet during this early period (Fig. 2.5; $F_{1, 94} = 3.87$, $p = 0.052$).

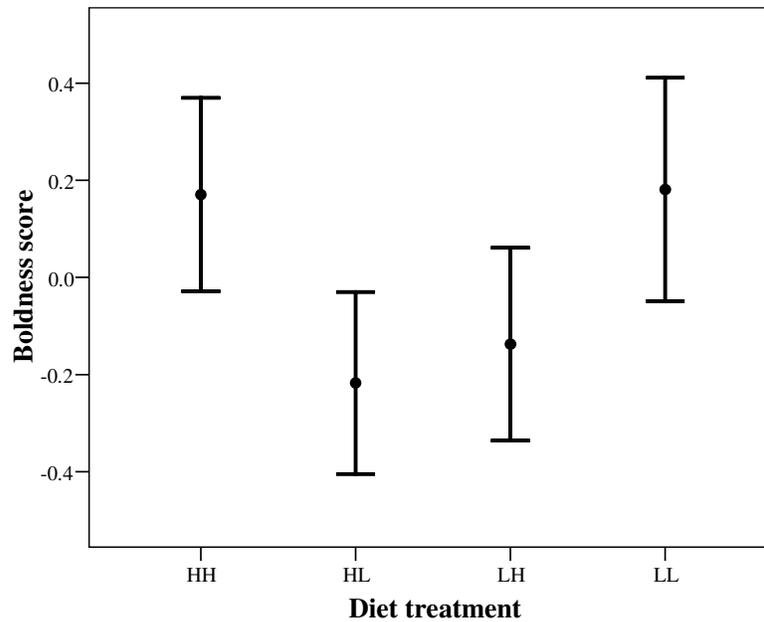


Figure 2.5. Mean \pm s.e boldness scores in relation to diet group for all 96 birds tested at 40 days. Note that birds that received a consistent diet (HH or LL) were bolder than birds that received a variable diet (HL or LH).

Activity

I analysed treatment effects on activity score in the same way as described above, using GLMMs with family as a random factor, mass gain during the manipulation (0-15 days and 15-30 days) as covariates, and sex, diet treatment (and their interaction) and breeding round as fixed factors. There were no significant effects of sex, breeding round or mass gain, nor any interaction effect between diet treatment and sex on activity score (in all cases $p > 0.14$), and consequently, these variables were dropped from the model. In this simplified model, diet treatment was not an accurate predictor of a bird's activity ($F_{3, 86.34} = 0.69$, $p = 0.56$). Again considering a model with diet consistency in place of diet group, and including sex, breeding round, mass gain and family, I again found sex, breeding round, mass gain and the sex*diet consistency interaction to be unimportant in explaining variation in activity score, with p always > 0.47 . These factors were therefore dropped from the model. However, unlike with boldness score, diet consistency did not explain individual differences in activity ($F_{1, 87.44} = 0.15$, $p = 0.70$).

Persistence of personality traits

The personality traits of the birds re-tested at adulthood were analysed using a GLM with sex and diet group as fixed factors, and mass gain during the manipulation as covariates (family was not included in the model, since only one member from each family was included in this subset of the original population). The GLM found no effect of sex, diet group, or mass gain on boldness score (in all cases $p > 0.08$). Using a model with diet consistency in place of diet group; sex and mass gain were similarly unimportant ($p > 0.19$), but once the non-significant terms had been dropped, diet consistency was again found to be significant, with birds on a consistent diet still displaying a bolder phenotype than birds on a variable diet (Fig. 2.6; GLM, $F_{1, 27} = 4.51$, $p = 0.043$). However, there was also a close to significant interaction between sex and diet consistency ($F_{1, 25} = 4.05$, $p = 0.055$). The same models as described above with activity score as the dependent variable in place of boldness found no effect of diet group or diet stability, sex or growth on this particular behavioural trait (in all cases $p > 0.15$).

Since sex was clearly an important factor when considering boldness behaviour over time, I examined differences between males and females more closely. The boldness score for males measured at 40 days and one year later was highly repeatable ($r = 0.65$, $F_{15, 16} = 4.67$, $p = 0.002$), but females showed no repeatability between the two test periods ($r = -0.34$, $F_{12, 13} = 0.49$, $p = 0.89$). Activity score was not repeatable in either sex (Male activity, $r = -0.28$, $F_{15, 16} = 0.56$, $p = 0.87$; Female activity, $r = -0.19$, $F_{12, 13} = 0.67$, $p = 0.76$).

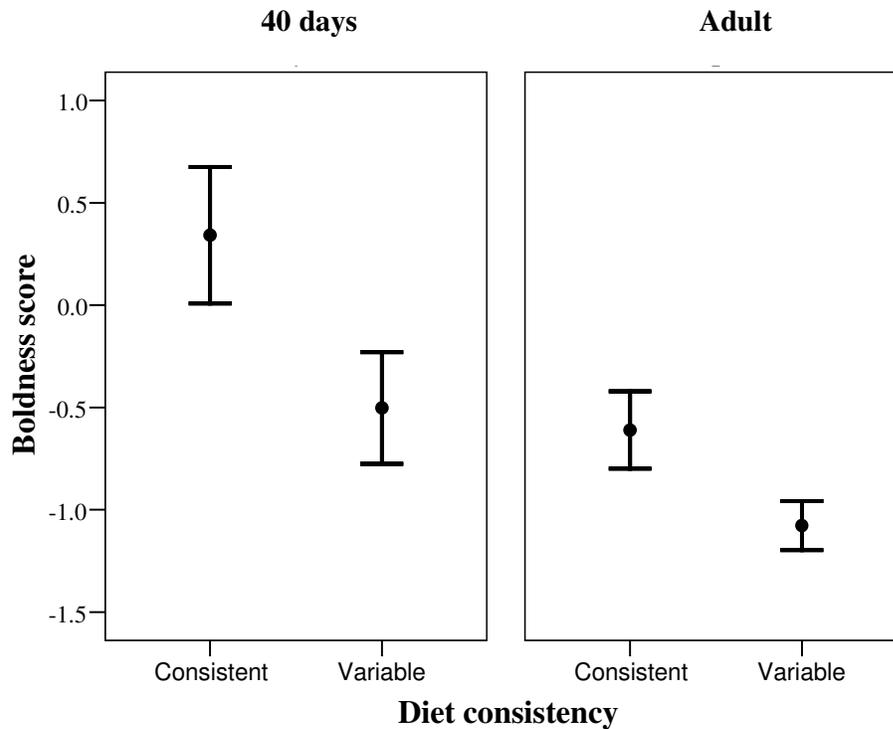


Figure 2.6. Mean \pm s.e boldness scores in relation to diet consistency (consistent diet = HH and LL, variable diet = HL and LH) shown for those birds given personality tests both when 40 days old and as adults (14-16 months of age). $N=29$ birds.

Using a repeated measures ANOVA with boldness scores from the test at 40 days and the test at adulthood as the repeated measure, plus sex and diet consistency as between-subject factors, all birds were less bold as adults than when tested at 40 days (effect of age; $F_{1,25}=16.80$, $p<0.001$). There was no independent effect of sex on overall levels of boldness ($p=0.38$), but there was a significant interaction between age and sex, with females showing a greater change in score between the test at 40 days and the test at adulthood (Fig. 2.7, $F_{1,25}=6.94$, $p=0.014$). As in earlier analyses there was a significant effect of diet consistency on boldness score ($F_{1,25}=6.03$, $p=0.021$), but no significant interaction between age and diet consistency ($p=0.26$).

The equivalent repeated measures ANOVA analysis of activity scores revealed that all birds were also less active as adults than as juveniles ($F_{1,25}=25.07$, $p<0.001$). There was no independent effect of sex on the change in activity score ($p=0.89$), and no significant interaction effect between age and sex ($p=0.95$). There was also no significant effect of diet consistency ($p=0.68$), nor any interaction between age and diet consistency ($p=0.83$).

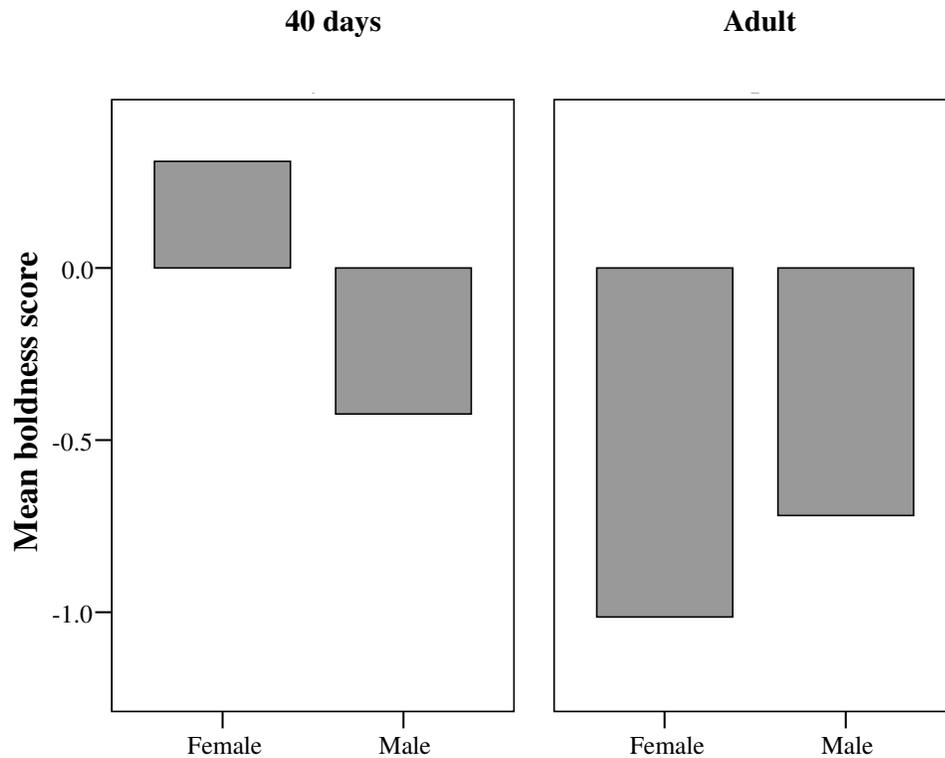


Figure 2.7. Mean boldness scores for males and females given personality tests both when 40 days old and at adulthood (14-16 months of age). $N = 29$ birds.

DISCUSSION

Personality

I found consistent individual differences in the responses of zebra finches to novel objects and a novel environment, and behavioural variables were correlated both within and between the two types of trial. The correlations between traits provide strong evidence for the existence of a bold/shy continuum in this species. The boldness score assigned to each individual is based upon responses in three tests (two novel object tests and one novel environment), and is made up of a number of behavioural observations, including the latency with which birds approached a novel object (a measurement of the speed of exploration), how closely they approached each object, how much time they spent near it and how much time they spent in the novel environment (measures of neophobia). Consequently, birds that were classed as 'bold' explored more quickly and were less neophobic than birds that were rated as 'shy', allowing us to place individuals on a

proactive-reactive continuum by assigning a score for boldness at 40 days of age. When birds were re-tested with the novel object and novel environment over a year after the initial tests, all individuals again showed strong correlations between behavioural measures within the novel object and novel environment tests, suggesting consistent behaviour towards previously unseen stimuli. This work therefore confirms other studies that have demonstrated stable behavioural phenotypes in birds (Dingemanse et al. 2002; Carere et al. 2005b).

Treatment effects

Diet consistency was important in determining boldness, with those that had had a consistent diet during early growth showing both a strong trend towards bolder personalities at 40 days and significantly bolder personalities at adulthood than those that had experienced a change in diet. The diet itself (i.e. whether it was consistently high or low, or the direction in which it changed) was not important in this context. The experimental design allowed separation of the effects of diet consistency and growth trajectory on individual personality, by inducing different growth patterns in birds under both consistent and variable conditions. Since both the fast growing (HH) birds and the slow growing (LL) birds showed bolder personalities, and both kinds of variable growth trajectory (fast/slow (HL) and slow/fast (LH)) resulted in birds that were more shy, I suggest that these findings are the result of differences in the consistency of the early environment experienced, rather than differences in early growth or diet quality. These effects were long-lasting, since the diet treatments ceased at 30 days, and the personality testing took place both at 40 days of age and over a year later. The behavioural differences between individuals observed in these tests are likely to reflect variation in the way any individual responds to its environment and changes within it. The hypothesis for consistent differences in behaviour that are linked with early experience is that this environment provides a ‘weather forecast’ of the conditions individuals are likely to experience later in life (Bateson et al. 2004). Verbeek et al. (1994) discuss the idea that a trade-off exists between personality type and attention to the environment, such that different personalities may prosper in different environmental conditions. They showed that great tits (*Parus major*) display individual differences in exploratory behaviour, and can commonly be described as either ‘fast’ or ‘slow’ explorers. Fast explorers, although they are bold and begin exploring quickly, tend to explore only superficially, and almost always feed in fixed

places without extensive sampling. Such individuals are well adapted to a stable, predictable environment, since they are not disturbed by small changes in their surroundings. However, such birds may find it difficult to adapt rapidly to large changes, e.g. when the location of a food source is altered. Birds that are slow explorers tend to be more thorough in their exploration and so may be at an advantage when environmental conditions change, since they remain alert to such changes and respond to them more effectively. A similar result was also reported in captive great tits trained to find food in different coloured feeders; fast explorers were less able to adapt their routine when the feeder type containing food was changed, while slow explorers were quick to extend their searching (Marchetti & Drent 2000). Similar results suggesting that proactive individuals are less behaviourally flexible than reactive individuals have also been found for mice, *Mus domesticus* (Benus & Rondigs 1997) and chaffinches, *Fringilla coelebs* (Quinn & Cresswell 2005).

If individuals have differential fitness based on the interaction between their personality type and the surrounding environment, it would make sense for personality type to be fashioned in part by an individual's experience early in life if this is predictive of future external conditions. A constant supply of food of the same quality suggests a consistent environment, in which a bold and superficial explorer would be successful. A diet varying in quality over the period of early development suggests greater environmental instability, and hence the prospering of shyer, more thorough and more behaviourally plastic individuals. If it were the case that certain personality traits conferred variable fitness, this could potentially explain the maintenance of dissimilar personalities in natural populations. Dingemanse et al. (2004) discuss how variation in environmental factors can lead to differing selection pressure on different personality types, such that conditions in certain years can favour some behavioural phenotypes over others. Additionally, work on stickleback populations has suggested that habitat stability (in terms of predation pressure) can influence personality traits like boldness and activity (Bell 2005; Brydges et al. 2008). Similar cases describing variable fitness depending on personality and the environment also exist for rats and mice (Vanoortmerssen et al. 1985; Benus et al. 1987). However, although these studies confirm that particular conditions may lead to the increased survival of certain behavioural phenotypes over others, it remains unclear whether environmental shaping can fix personality traits shortly after birth so as to best match those conditions. In one of the few studies to have considered this, Arnold et al. (2007) show that the risk-taking behaviour of juvenile blue tits, *Cyanistes caeruleus*, was influenced by their intake of micronutrients in particular prey types during a developmentally-sensitive phase (and so

could be under parental manipulation). However, this is the first study to suggest that personality traits may be shaped in early life, and show that there may be a link between traits and early diet consistency. If this is the case, then early behavioural programming in response to environmental variation could be important in optimising survival.

Persistence into adulthood

Repeated testing revealed that males showed a strong persistence in their boldness score, but that there was lower persistence for females. In addition, all birds were less bold as adults than as juveniles. The difference between males and females in the persistence of their boldness score is surprising. Females showed a greater change in their boldness score, becoming much shyer as adults than they were as juveniles. This sex distinction could perhaps be linked back to differences in post-dispersal behaviour. In the wild, both male and female zebra finches generally disperse from their natal colony (Zann 1994), but it may be that after dispersal it remains important for males to be bold, since they will defend future mates and nest sites more frequently and vigorously than females. Once a female has successfully dispersed to a new colony, boldness may be less important and may then even be disadvantageous, if it increases the likelihood of aggressive confrontations and the possibility of predator attack.

The reduction in overall boldness in all the birds is interesting, especially since it contrasts with changes found in other small passerines. In studies on tits (Verbeek et al. 1994; Carere et al. 2005b), birds scored as slow explorers at an early age and then re-tested at a later date tended to become faster (i.e. more bold), though in both cases birds were at least partly hand-reared, which may have had an effect on these findings. However, a reduction in boldness in terms of risk-taking behaviour between adolescence and adulthood is a phenomenon that is commonly noted in the mammal literature (including humans; for example, Armitage & Van Vuren 2003; Stansfield & Kirstein 2006). Young birds are perhaps likely to be bolder than older birds, since after fledging, juveniles will need to disperse to find their own territories and feeding grounds, and will need to explore the surrounding area in order to do this. This early life requirement could mean that birds are bolder shortly after fledging than at any other time in their life, a period coinciding exactly with the age of my birds when they were first tested. In this study, birds were maintained in single sex groups on a standard diet for a year before being re-tested, experiencing highly stable conditions. Birds could therefore become settled in their respective

environments, and less inclined to actively explore anything novel, or alternatively, have become used to a variety of initially novel objects and so find them less interesting. The shift in individual scores is unlikely to be due to habituation to the test, since both novel objects and the environment were changed so that they were still novel, both testing sessions were of short duration, and tests were conducted over a year apart. Alternatively, changes in boldness could be the result of different individual experiences or of some degree of adaptation to the environment experienced after fledging. An important thing to note however, is that despite these changes at the individual level, the same overall trends were maintained, so that birds raised on a consistent diet were still bolder than those raised on a variable diet, suggesting that the early environment can have a significant and prolonged effect on behavioural phenotype.

In conclusion, behavioural differences at the diet consistency level were stable over time, despite some changes in individual boldness scores. I suggest that bold birds are more likely to arise from a consistent diet in the nest because with fast exploring, routine-like behaviour they will flourish in a stable environment. Shy, slow explorers that are better able to adapt to environmental change, will have higher fitness in a more unpredictable environment, where close attention to detail is important for survival, and so will be more likely to arise from nests in which the diet quality has been variable. The obvious future direction based on these findings is to determine whether such environmental shaping really is adaptive in terms of survival and breeding success, and what costs are incurred when the environment experienced is different from the one anticipated.

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Chapter 3

The effect of early corticosterone exposure on adult personality in the zebra finch

ABSTRACT

It has now been shown that personalities or behavioural syndromes exist in a variety of animal species, in that individuals show consistent and repeatable differences in certain behavioural traits. The underlying cause of this variation however, remains unknown. Behavioural phenotype will be partly shaped by individual genes, but an animal's surroundings, or more importantly, its developmental environment, may also play a large role in determining such traits. Here I considered whether personality traits such as boldness and activity could be shaped by exposure to different levels of the stress hormone corticosterone in the nestling phase, using the zebra finch (*Taeniopygia guttata*) as a model species. Sibling pairs of chicks were given oral doses of corticosterone or a peanut oil control between 12 and 28 days after hatching. Birds were shown to display consistent differences in their behaviour, and behavioural traits were correlated across situations. However, there was no effect of corticosterone exposure on the measured personality traits. Such a finding suggests that personality is not directly shaped by corticosterone exposure in the nest. Examination of relative growth rates within pairs of siblings suggested that growth may be important in the shaping of personality, and this is discussed in the context of predictive adaptive responses to environmental variation.

INTRODUCTION

Variability in environmental conditions during early development has recently been demonstrated to be important for a number of life history traits. Differences in early conditions are thought to be significant in the shaping of the adult phenotype, not only in terms of morphology (Birkhead et al. 1999; Metcalfe & Monaghan 2001; Blount et al. 2003), but also physiology (Criscuolo et al. 2008) and behaviour (Nowicki et al. 2002; Kitaysky et al. 2003; Spencer et al. 2003; Spencer & Verhulst 2007). Adverse changes in the environment during early life can lead to an elevation of stress hormones in the developing animal, and differential exposure to such hormones during the developmental phase can alter the stress response in adulthood (Anisman et al. 1998). Stress experienced during postnatal development has been shown to result in exaggeration of the adult stress response in some studies (Kuhn et al. 1990; Hayward & Wingfield 2004; Spencer et al. 2009), and attenuation of the response in others (Casolini et al. 1997; Vallee et al. 1997), depending on the type and duration of the stressor. Such findings suggest that the neuroendocrine stress axis is fairly malleable in early life, and current thinking is that exposure to stress hormones during development may be important in shaping the adult phenotype for optimal survival in the environment that animal is likely to experience (Anisman et al. 1998; Spencer & Verhulst 2007).

There is also evidence that individual differences in stress reactivity could be linked to differences in behaviour (Satterlee et al. 1993; Ferre et al. 1995; Carere et al. 2003; Gregus et al. 2005; Martins et al. 2007). Recent work has shown that a variety of animals differ in their 'personalities', or 'coping styles', and show characteristic patterns of behaviour that apparently allow them to better cope with the demands they face in the environment (Armitage & Van Vuren 2003; Dingemanse et al. 2004; Jones & Gosling 2005; Carere et al. 2005a; Alvarez & Bell 2007). Such studies suggest that animals can often be classified along a proactive-reactive axis. Individuals at the proactive end of the scale are bold and aggressive, quickly but superficially explore new environments and readily form behavioural routines; while reactive individuals are shy and cautious, pay closer attention to external stimuli, and are more flexible in their behavioural responses. Novel object and novel environment tests are two standard ways of quantifying personality types. In these tests, animals are presented with novel objects in their home cages, or given the opportunity to explore a novel environment, and their behavioural responses are used to assign them scores for various traits such as boldness and speed of exploration, so allowing

them to be placed on the proactive-reactive continuum. Recently, some studies have demonstrated links between such traits and stress reactivity. For example, Carere et al (2003), show that great tits (*Parus major*) classified as shy and reactive showed a much higher corticosterone response to social stress than birds classified as aggressive and proactive. Similarly, Martins et al (2007) have demonstrated that zebra finches (*Taeniopygia guttata*) selected for different responses to stressors also differ in their exploratory and risk-taking behaviours, and Overli et al. (2004) have linked aggression and dominance status to stress profiles in rainbow trout (*Oncorhynchus mykiss*). However, exactly why individuals develop different personality types, and why these should be linked to the stress response remains unknown. Overall, the developmental mechanisms that lead to individual differences in personality are poorly understood. Some individual variation has been shown to have a genetic basis (Sluyter et al. 1995; Sokolowski 2001; Drent et al. 2003; van Oers 2005), but less is known about the environmental contribution, and whether the environment an individual experiences during development can be important in shaping the behavioural phenotype.

Since there is evidence that the neuroendocrine stress axis can be shaped by early conditions, and also evidence that animals tested for their stress reactivity in adulthood show links between their stress response and certain personality traits, the question remains as to whether the stress experienced during development could directly shape the personality of the adult animal. In one of the few studies to have addressed this, Spencer and Verhulst (2007) found that young zebra finch males exposed to an elevation of the stress hormone corticosterone (CORT) in the nest were less neophobic than controls, showing reduced latencies to approach a novel object. In contrast, Chung et al (2005) found that rats born to stressed mothers showed increased anxiety behaviour as adults, as did rats that endured 24 of maternal deprivation at day 14 after birth (Penke et al. 2001). In general, mammal work suggests that while mild stress (such as short periods of handling) during early postnatal life reduces adult fearfulness and anxiety in response to a stressor (Meaney et al. 1991; Weaver et al. 2000), more severe stress (like prolonged maternal deprivation) can have the opposite effect, and increase fearful behaviour in adulthood (Cirulli et al. 1994; Anisman et al. 1998). However, fewer studies have been conducted in avian species, and there is still much uncertainty as to how stress experienced in early life may be important in the shaping of adult personality.

In this study, I manipulated corticosterone levels during the nestling phase of the zebra finch, with the aim of establishing whether personality traits in this altricial bird could be partly determined by environmental conditions during postnatal development. I

used sibling pairs, elevating corticosterone levels in one sibling and using the other as a control. Subjects then underwent personality tests after becoming nutritionally independent. Since the corticosterone treatment also had an effect on growth trajectory, I also monitored individual growth rates to determine whether individual alterations in growth could be linked to behavioural profiles.

METHODS

Corticosterone manipulation

To investigate the effect of early corticosterone (CORT) elevation on personality traits in juvenile and adult zebra finches, same-sex sibling pairs of chicks had their corticosterone levels experimentally manipulated during postnatal development. To obtain these chicks, adult birds were randomly paired in 60 x 50 x 50 cm cages and allowed to breed. Pairs were given *ad lib.* access to a commercial tropical seed mixture (Haiths Ltd, UK), water, shell grit and cuttlefish bone, and were given rearing and conditioning food (Haiths Ltd UK) and spinach once a week. On the hatching of their offspring, all families received daily conditioning food. Chicks were sexed at 5 days post hatching using a standard DNA test, and same sex sibling pairs were randomly assigned to either the CORT or control group (with a maximum of two chicks per brood per treatment) (Spencer et al. 2008). Hatch order was counterbalanced across the groups to control for individual differences in basal CORT levels. Exposure to corticosterone was manipulated while captive-bred young zebra finches were still in the nest. Those in the CORT treatment group received small and biologically relevant (Spencer & Verhulst 2007; Spencer et al. 2008) amounts of CORT each day between 12 and 28 days of age. CORT doses were scaled to mimic the peak stress response of young birds to a capture-handling-restraint protocol, and doses were calculated for two age classes (12-15 and 16-28 days), as age related changes in peak CORT responses were noted during a pilot study (Spencer et al. 2008). The hormone was dissolved in a peanut oil carrier and chicks were removed from the nest daily and fed with CORT-oil directly using a dropper. The hormone was administered at a concentration that would elevate plasma concentrations to approximately 1 s.d. above the mean peak concentration for each age class (Spencer et al. 2003). Therefore, a concentration of 0.124mg of CORT per ml of peanut oil was used from 12-15 days, and from 16-28 days of

age this was increased to 0.163mg/ml; 0.025ml of the appropriate solution was fed to CORT birds twice daily, while control birds were dosed at the same time with the peanut oil carrier. Body mass and tarsus measurements were taken at 12, 28 and 60 days of age.

Following the cessation of the hormonal manipulation (at 28 days), by which time the chicks were nutritionally independent of their parents; the experimental birds were sorted into same-sex groups of 6 juveniles according to treatment. Therefore, CORT birds were housed together and control birds likewise in standard cages, in visual and auditory contact with other conspecifics. From this time onwards, all juveniles were provided with the same standard diet of *ad lib.* mixed seed, grit and cuttlebone, with protein supplement and greens once a week. Cages were floored with hemp chippings, and contained a seed bowl and water fountain, plus a variety of perches (both wooden rods and natural twigs). At 60 days of age, stress responses were assessed using a standardised capture handling restraint protocol. There was no effect of the treatment on baseline CORT levels in either CORT or control birds, but birds that had received the CORT treatment demonstrated significantly elevated peak CORT concentrations following exposure to a standard stressor relative to their control siblings (Spencer et al 2008).

The behavioural testing was carried out when the birds reached approximately 80 days of age. Testing was carried out on a total of 42 sibling pairs, comprising 26 pairs of brothers and 16 pairs of sisters. Since subjects had to be housed alone for behavioural observations, on the evening before the tests, birds were caught and placed singly in cages to acclimatise overnight (40 x 43 x 44cm). These cages were of a familiar layout and contained only familiar objects (wooden rod perches, a food dish and a water fountain). The following day each bird received two novel object trials in the morning between 0900 and 1100 hours, which were performed in this cage with the familiar layout and familiar objects, and a novel environment trial during the afternoon of the same day between 1200 and 1700 hours, for which each bird was removed from its single cage and introduced into the novel environment test arena. In between the two novel object trials, subjects remained alone in their single cages with the familiar layout and familiar objects in order to minimise handling stress, but could see and hear conspecifics throughout this period.

Behavioural testing

Birds were assessed for personality using the novel object and the novel environment tests exactly as described in chapter 2. Both the novel object and the novel environment tests

were repeated on a subset of the original 84 birds six months later (when birds were around 9 months old), again following the exact protocol for the repeated tests described in chapter 2. 15 pairs of male siblings were selected to be re-tested as adults, since initial analysis had shown sex to have no effect on personality scores (see results). This test was intended to determine whether personality scores were consistent over long periods of time, and whether the corticosterone manipulation was having prolonged or delayed effects.

RESULTS

Effects of CORT treatment on growth

Birds exposed to elevated corticosterone showed a significantly reduced growth rate compared to controls during the treatment (Fig. 3.1(a) GLM, $F_{1, 82}=4.23$, $p=0.043$), such that by 28 days, there was a significant difference in the mean body mass of the two treatment groups (Paired $t_{41}=2.12$, $p=0.041$). Birds in the CORT group then showed a trend towards an increased rate of growth following the cessation of the treatment compared to controls (Fig. 3.1(b) GLM, $F_{1, 82}=2.87$, $p=0.094$), and by 60 days of age, there was no difference in body mass between the two groups (Paired $t_{41}=1.024$, $p=0.31$). The effect of the induced difference in growth rate on the personality variables was therefore also examined.

Behavioural phenotypes

The behavioural data revealed that subjects were consistent in their behavioural responses both within and between the two novel object trials, with high correlations between a number of variables (Table 3.1). For example, birds that were quick to land on novel object perch 1 were also quick to land on novel object perch 2, indicative of a 'bold' phenotype. Subjects that were quick to land on a novel perch also landed more frequently and spent more time on that perch than birds with a long land latency (Table 3.1). Individuals also showed consistency within the novel environment trial (Table 3.2). There was a strong

negative correlation between explore latency and the number of perch visits made by any bird, and a negative correlation between explore latency and the total time spent in the novel environment, with fast explorers spending more time in the novel environment overall (Table 3.2). Additionally, birds that took longer to begin exploring (i.e. longer to leave their initial perch in the novel environment) spent less of the overall trial in movement.

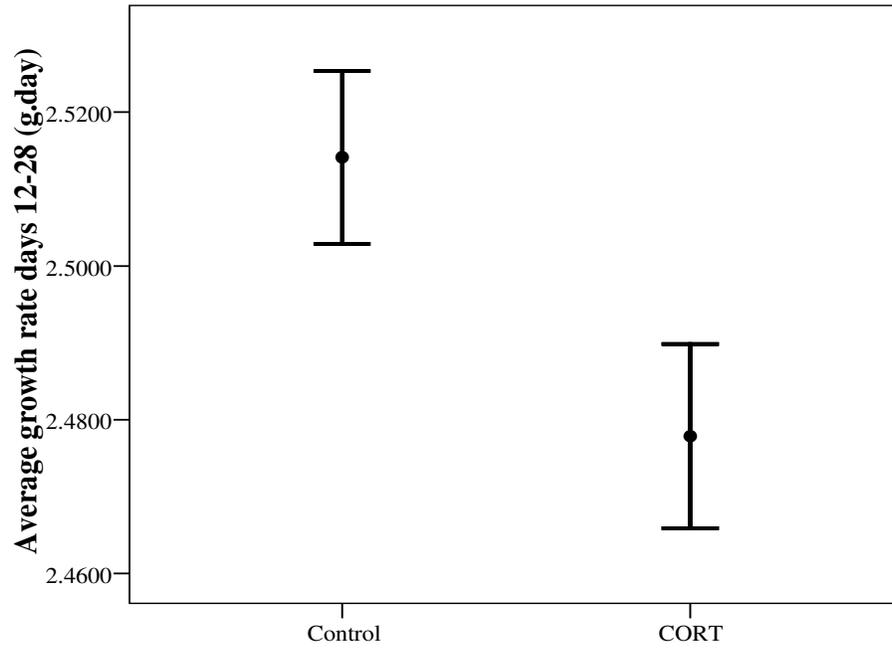
As well as showing consistencies within each of the types of personality trial, birds also showed significant individual stability between the two types of trial. Thus the land latency of a bird in novel object trial 1 showed a significant negative correlation with the number of perches it visited in the novel environment ($r_s = -0.25$, $N=84$, $p=0.022$), and similarly, there was a positive correlation between the time any individual spent on novel object perch 2, and the time it spent in the novel environment ($r_s = -0.312$, $N=84$, $p=0.003$). In both cases however, the correlation was relatively weak.

Since each bird was given two novel object trials at 80 days of age, I was able to determine individual consistency over the short-term for the same test type by calculating repeatability scores for various behavioural measures observed on the same day. Repeatability is the proportion of phenotypic variation explained by the individual, and was calculated for all individuals ($N=84$) using a one-way ANOVA with individual ring number as a fixed factor (after Lessells & Boag 1987). Latency to land was found to be repeatable between the two novel object trials ($r=0.37$, $F_{83, 84}=2.19$, $p<0.001$), as was minimum distance ($r=0.42$, $F_{83, 84}=2.48$, $p<0.001$), the number of times landed on the perch ($r=0.58$, $F_{83, 84}=3.7$, $p<0.001$,) and the total time spent sitting on the novel object perch ($r=0.21$, $F_{83, 84}=1.55$, $p=0.024$).

The behavioural measures obtained from the two novel object trials and the novel environment trial for all 84 birds were entered into a principal component analysis (PCA). This revealed the presence of two components, explaining 26.35 and 20.50% of the variance in behaviour respectively. To aid in the interpretation of these components, Varimax rotation was performed, which maintains independence among the mathematical factors. The rotated solution (Table 3.3) explained a total of 46.84% of the variance, with component 1 contributing 23.90% and component 2 contributing 22.94%. The interpretation of these two components was that the first provided an activity score (with a positive score indicating a high level of activity), since it was heavily influenced by the amount of time a bird was observed sitting, hopping, moving, feeding and preening (proportions arcsin transformed), and the total number of perch visits in the novel environment. Component 2 was primarily influenced by a bird's latency to land in the

novel object trials, its minimum distance from the novel objects, the number of times landed and the total time spent on the novel object perch, and so it was interpreted as a boldness score. Thus 'bold' birds were quick to land on both novel object perches, approached to within a short distance of both objects, landed often, and spent a large part of each trial sitting on the novel object perch. Such birds had strongly positive scores. 'Shy' birds were opposite in their responses, and had negative scores.

(a)



(b)

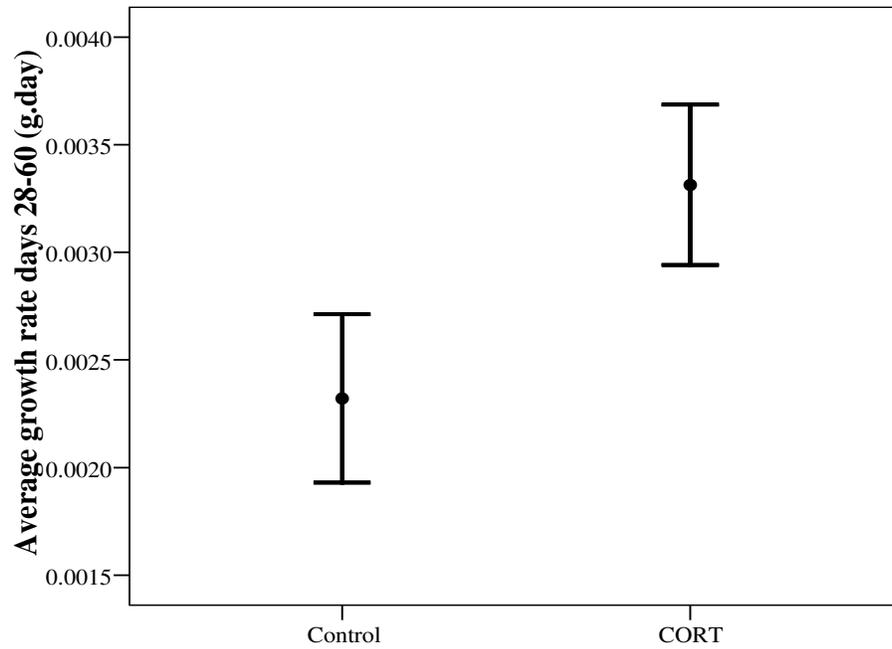


Figure 3.1. Mean \pm s.e. growth rate for the control and CORT treatment groups, (a) during the CORT manipulation (days 12-28), and (b) once the CORT manipulation had ceased (days 28-60). $N=84$ birds

Table 3.1. Behavioural correlations observed in novel object trials 1 and 2 (only significant correlations shown). See text for variable definitions; Land latency 1 refers to data from trial 1 etc. N=84 birds.

	Land latency 1	Minimum distance 1	Times landed 1	Time on perch 1	Land latency 2	Minimum distance 2	Times landed 2	Time on perch 2
Land latency 1								
Minimum distance 1	.839**							
Times landed 1	-.824**	-.914**						
Time on perch 1	-.876**	-.881**	.876**					
Land latency 2	.375**	.405**	-.378**	-.396**				
Minimum distance 2		.451**	-.466**	-.394**	.736**			
Times landed 2	-.348*	-.497**	.485**	.451**	-.780**	-.849**		
Time on perch 2		-.377**	.336*	.350*	-.885**	-.737**	.790**	

*p < 0.05, **p < 0.01 (both after sequential Bonferroni correction).

Table 3.2. Behavioural correlations observed in the novel environment trial (only significant correlations shown). See text for variable definitions. $N=84$ birds.

	Latency to explore	Time spent in NE	Perch visits	Pecks	Time spent sitting	Time spent hopping	Time spent moving
Latency to explore							
Time spent in NE							
Perch visits	-.328*	-.451**					
Pecks	.704**	.515*					
Time spent sitting	-.537**	-.699**	-.637*				
Time spent hopping	.523**	.552**	.605**	-.830**			
Time spent moving		.397**	.940**	.503**	-.721**	.590**	

* $p < 0.05$, ** $p < 0.01$ (both after sequential Bonferroni correction).

Treatment effects

Using the personality scores obtained from the principal components analysis, I used a paired samples t-test to investigate the effect of CORT treatment within sibling pairs. There was no effect of elevated CORT exposure during early growth on activity ($t_{41}=0.17$, $p=0.87$) or boldness score ($t_{41}=0.16$, $p=0.87$). Using a GLM with family as a random factor to control for the non-independence of siblings, there was also no effect of sex on either activity ($F_{1,4}=0.91$, $p=0.40$), or boldness score ($F_{1,4}=1.26$, $p=0.33$), so the sexes were pooled for subsequent analysis. A one way ANOVA with family as a fixed factor showed that siblings were more similar in their boldness score than non-siblings, regardless of treatment ($F_{34,49}=1.73$, $p=0.039$). However, the same similarity between siblings was not found for activity score ($F_{34,49}=0.97$, $p=0.54$).

Table 3.3. Principal component analysis of behavioural measures (Varimax rotation, only loadings over 0.3 displayed).

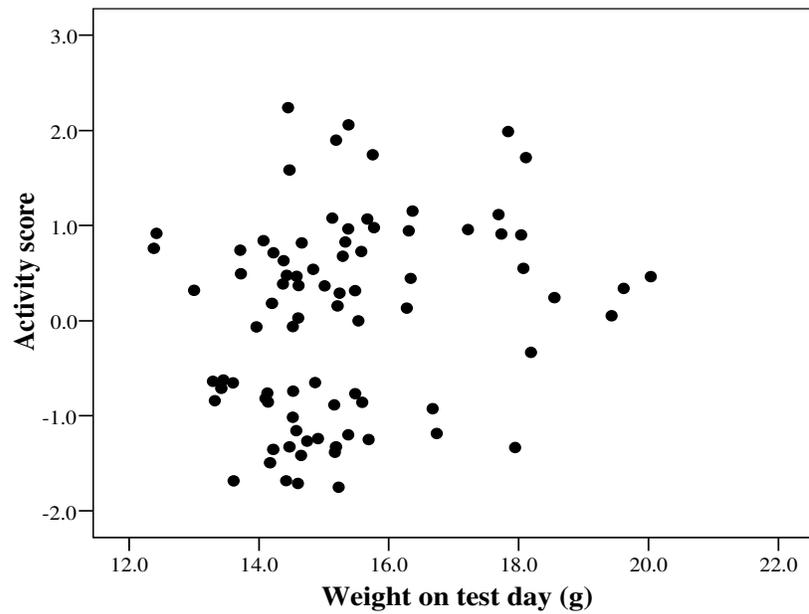
	Component 1	Component 2
Land latency 1		-.679
Minimum distance 1		-.811
Times landed 1		.703
Time on perch 1		.661
Land latency 2		-.706
Minimum distance 2		-.716
Times landed 2		-.730
Time on perch 2		.585
Perches visited	.636	
Novel perch visits	.711	
Pecks	.537	
Time spent sitting	-.958	
Time spent preening	.473	
Time spent hopping	.778	
Time spent feeding	.600	
Time spent vocalising	.455	
Time spent moving	.805	

Growth effects on personality traits

Body mass on the day of testing showed a significant relationship with both measures of personality (Fig. 3.2(a) activity score $F_{1,84}=4.06$, $p=0.047$; Fig. 3.2(b) boldness score $F_{1,84}=7.88$, $p=0.006$), and including sex in the model as a fixed factor showed there to be no effect of sex on these relationships between weight and personality ($p>0.21$ for both activity and boldness). Heavier birds of both sexes were more active, but less bold than lighter birds.

To investigate possible links between early growth rate and adult personality, I considered growth rate of each CORT bird relative to its control sibling. Relative growth rate was calculated as the difference in growth rate (defined as mass gain per day (g)) between sibling pairs (i.e. by subtracting the growth rate of the control bird from that of its CORT sibling). I found that the relative boldness of birds was influenced by their relative growth rate during the period of the stress manipulation (days 12-28). CORT birds that gained more mass than their control sibling during this period tended to be less bold as adults than their sibling, while CORT birds that grew less than their control sibling tended to be bolder than their siblings (Fig. 3.3, Spearman rank correlation $r=-0.37$, $p=0.016$, $N=42$ sib pairs). There was no correlation between relative growth rate following the manipulation (days 28-60) and this measure of personality ($r_s=0.11$, $p=0.483$, $N=42$ sib pairs), and no significant correlations between relative growth rate and activity score (relative growth rate days 12-28, $r_s=0.038$, $p=0.81$; relative growth rate days 28-60, $r_s=-0.103$, $p=0.517$, $N=42$ sib pairs).

(a)



(b)

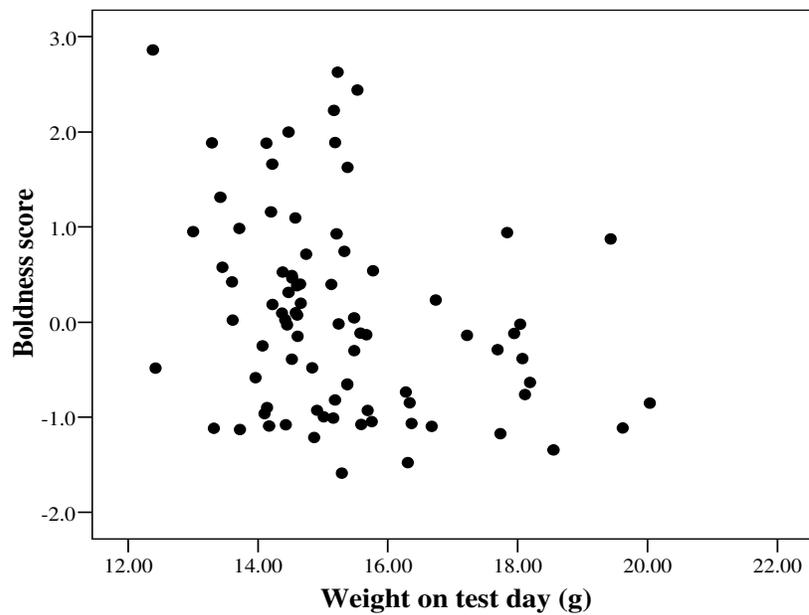


Figure 3.2. The relationship between body weight on the day of testing and (a) the activity score calculated from the trials given at 80 days and (b) the boldness score calculated from the trials given at 80 days. $N=84$ birds See text for statistical analysis.

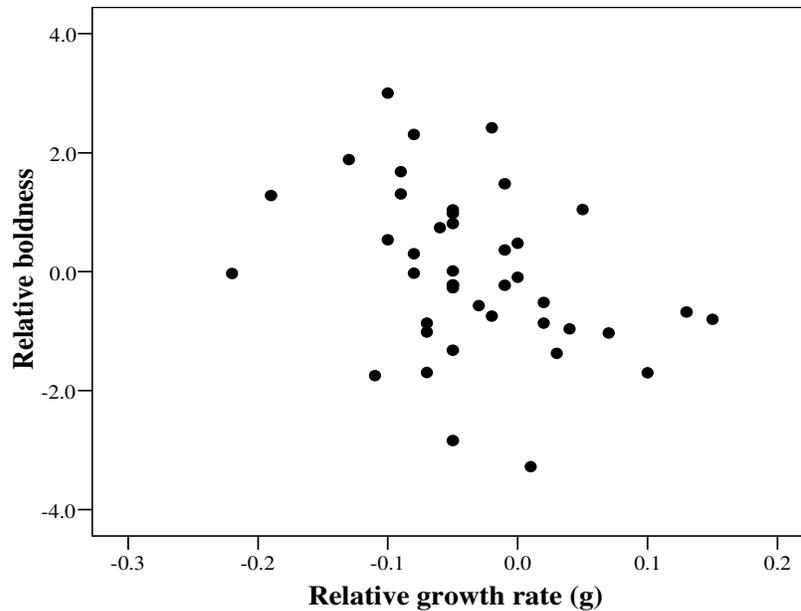
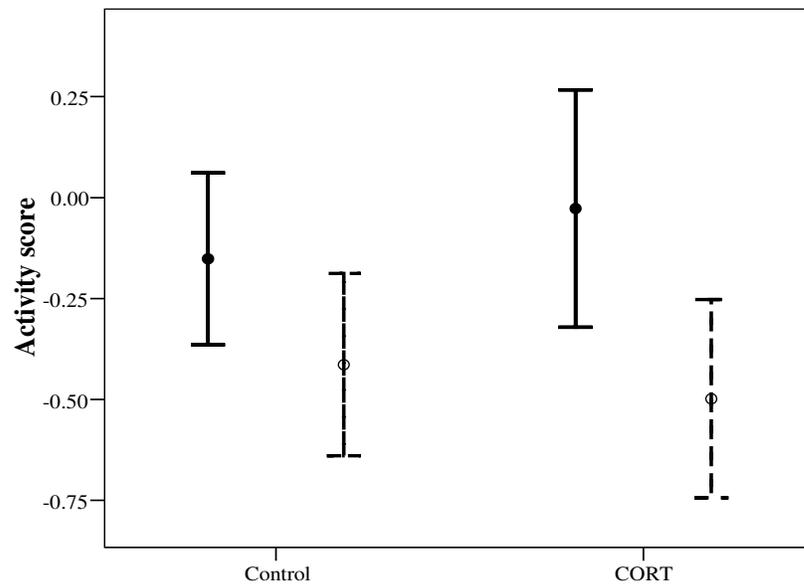


Figure 3.3. The relationship between relative growth rate (mass gain in grams per day) during the period of corticosterone treatment (days 12-28), and relative boldness at 80 days of age. Each data point represents a sibling pair; a positive value for relative growth rate indicates that the CORT sibling gained more weight than the control sibling. Similarly, a positive value for relative boldness indicates that the CORT sibling was scored as more bold on the PCA analysis than the control sibling. $N=42$ sibling pairs.

Persistence of personality traits

As described above, both personality tests were repeated on a subset of 30 male birds six months after the initial experiment. Many of the behavioural measures taken in this second set of tests were again strongly correlated, and so personality scores from this second data set were calculated using the same factor loadings as in the PCA analysis from the trials at 80 days. All birds again showed high repeatability scores within the two novel object tests given at 9 months for land latency ($r=0.53$, $F_{29,30}=3.27$, $p=0.001$) and minimum distance ($r=0.59$, $F_{29,30}=3.83$, $p<0.001$), though not for the number of times landed on the novel object perches ($F_{29,30}=1.37$, $p=0.2$), or the total time spent on the novel object perches ($F_{29,30}=1.58$, $p=0.12$). I also found that these siblings were no more similar in their boldness scores than non-siblings ($F_{14,15}=1.73$, $p=0.15$), though interestingly, activity scores generated from this second test were similar between siblings ($F_{14,15}=7.24$, $p<0.001$).

(a)



(b)

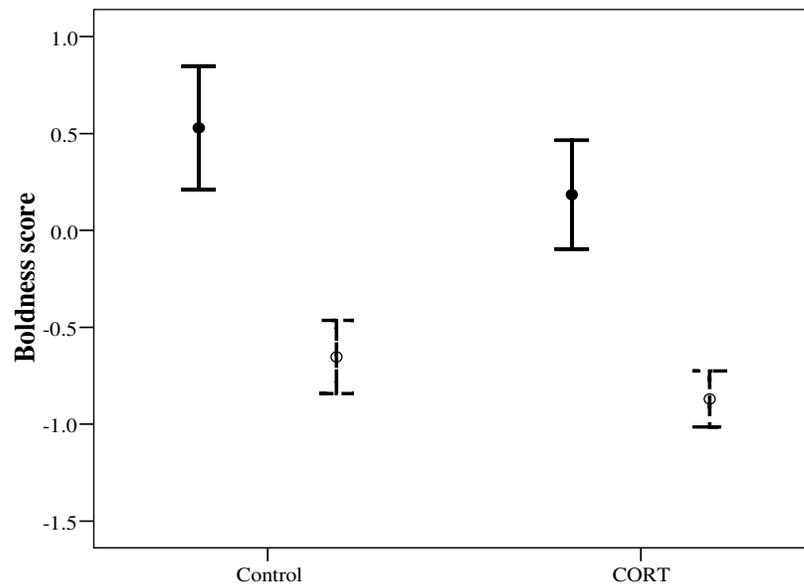


Figure 3.4. Mean \pm s.e personality scores for the 15 control and 15 CORT males given both the initial personality test at 80 days (solid circles and solid lines) and the repeated test at 9 months (hollow circles and dashed line). (a) Activity scores, with positive scores indicating an active individual and negative scores an inactive individual, and (b) boldness scores, with positive scores indicating a bold individual and negative scores a shy individual.

Having personality scores for tests conducted both at 80 days and 9 months of age for these 30 males, I was then able to look at individual consistency across time by calculating repeatability for activity and boldness. There was low repeatability both for activity ($F_{29, 30}=0.75$, $p=0.79$) and boldness ($F_{29, 30}=0.61$, $p=0.91$). Closer inspection of the data revealed that all birds showed a trend towards being less active as adults, although this was not significant (Fig. 3.4(a); Repeated measures $F_{1, 28}=1.88$, $p=0.182$), and all birds were scored as less bold as adults than as juveniles (Fig. 3.4(b); Repeated measures $F_{1, 28} = 23.74$, $p<0.001$).

DISCUSSION

Behavioural phenotypes

When I looked for personality traits in birds that had experienced different developmental environments, I found no effect of CORT exposure on either boldness or activity scores within sibling pairs. Such a finding suggests that personality is not directly shaped by corticosterone exposure in the nest. This result is the opposite of what has been found in many mammal studies, which suggest that stress hormone exposure in early life is in some way involved in the development of the behavioural phenotype (Meaney et al. 1991; Cirulli et al. 1994; Weaver et al. 2000; Penke et al. 2001; Chung et al. 2005), though the exact effect depends very much on the type and duration of the stressor. The paired design, in which siblings reared in the same nest experienced different levels of CORT exposure during growth, was a robust way of looking for differences in personality mediated by the effects of CORT. The failure to find any difference between siblings is interesting, especially since behavioural differences as a result of variable stress hormone exposure have previously been demonstrated in this species by Spencer et al (2007). It may be that the inherited component of personality was so strong as to mask any effect of the CORT treatment, or that personality traits are simply shaped by some other component of the early environment. One important thing to note is that in the study by Spencer and Verhulst (2007), sibling pairs of zebra finches were treated with CORT in the same way as described above, but administration began at day 7 and concluded at day 18; so beginning and finishing earlier than in the current study, in which the treatment was administered between days 12 and 28. Since the authors found an effect of this treatment on male

neophobia, it is possible that personality traits are set soon after hatching, so that it is exposure to CORT in the very first days of life that is critical in determining behaviour, and that stress experienced after this time is much less important. In addition, results from chapter 2 suggest that the nutritional stability of the early environment may be important in determining personality type, since such information could provide signals about the quality and stability of the environment outside the nest, and serve to shape the phenotype of the bird to best cope with the conditions it is most likely to find upon fledging. If this is indeed the case, then since all birds in this study received a high quality diet that remained stable throughout the chick phase, we might not expect to see any differences in personality between siblings on different treatments.

I also found consistent individual differences in the responses of zebra finches to novel objects and a novel environment, and behavioural variables were correlated both within and between the two types of trial, allowing us to score individuals for both activity level and boldness at 80 days of age. When a subset of brothers were retested with a new novel object and novel environment at 9 months of age, all individuals again showed correlations between behavioural measures collected in this second round of tests, suggesting consistent behaviour towards unseen stimuli. This work therefore confirms other studies that have demonstrated stable behavioural phenotypes in avian species (Dingemanse et al. 2002; Carere et al. 2005a).

Family effects

We would expect siblings to be reasonably similar to one another in their personality scores, since such phenotypic traits have been shown to have a heritable component (Dingemanse et al. 2002; van Oers et al. 2004; van Oers et al. 2005). I found that siblings were more similar than non-siblings in their boldness score despite having received different treatments, but that related individuals were no more similar than non-related individuals in their activity score. Why this might be is difficult to explain, especially since treatment also had no effect on individual activity. It may be that with small numbers from each family in combination with different treatments, it is hard to detect similarities between related individuals in all aspects of the behavioural phenotype. There is also the possibility that because of our matched design within broods, parents may have responded differently to CORT and control chicks and in some way influenced their behaviour. There are studies to show that elevation of CORT can, for example increase the frequency of

begging behaviour (Kitaysky 2003), and any changes in CORT chick begging may have had knock on-effects for parental provisioning, and so for the control birds in the same nest.

Growth effects

CORT birds grew less than controls during the manipulation but more than controls once the manipulation was over. That is, they then showed compensatory growth. Birds in the two groups did not show a significant difference in body mass at 60 days, so although birds showed different growth trajectories, they were not different in size at adulthood. Consequently, the stress manipulation affected growth trajectory, but not overall adult size. These differences in growth trajectory did not translate directly into differences in personality score. However, when I considered differences in growth rate between same-sex pairs of siblings, it transpired that boldness score was affected by the rate of growth during the period of the corticosterone manipulation, and not specifically the growth suppression. Previous studies have found that the adult phenotype can be affected by the amount of compensatory growth that an animal undergoes following a period in which growth has been restricted (Nowicki et al. 1998; Ozanne & Hales 2004; Fisher et al. 2006). CORT birds had their growth rate depressed during the manipulation but were able to compensate and reach full adult size by growing more rapidly in the period after the manipulation. However, in this study, relative growth rate during this period of compensation does not appear to be important in determining behavioural phenotype. It may be that the personality of an individual is determined by conditions in the nest, rather than conditions after fledging. If this was the case, then a period of compensatory growth following nutritional independence may not alter the personality type of an individual because it has already been set. This would suggest the existence of a sensitive period at some point during development as has been shown for example in song learning (Bottjer 1997), in which case the timing of events during early life may be important. However, exactly why growth difference between siblings should be linked to personality in the way they are is unclear. It is possible that the nest conditions could tailor the behavioural responses of each individual to suit the environment they are most likely to encounter, in order to maximise fitness. CORT birds that grew less than their siblings during the manipulation may anticipate an environment in which resources are limited and so develop bolder personalities in order to effectively compete for those resources, and explore new

potential food supplies, so maximising the chance of survival and reducing their likelihood of death through starvation. On the other hand, those CORT birds that grew more than their control siblings during the manipulation may anticipate an environment that is richer in resources and so develop personalities that minimise unnecessary mortality risk, such as foraging in the presence of predators, on novel food items, or exploring new areas. In this way, personality type could potentially be influenced by growth rate as a proxy for environmental richness, with personality traits conferring different amounts of fitness depending on variables such as food availability and population density. Cases describing variable fitness depending on the match between personality and the environment exist for rats and mice (Vanoortmerssen et al. 1985; Benus et al. 1987), and also for birds (Marchetti & Drent 2000; Dingemanse et al. 2004).

An alternative explanation is that a bold personality is more costly, making it less likely that those individuals that devoted the most energy to growing will also be able to become bold. In a study on salmon (Vollestad & Quinn 2003), it was found that an increased growth rate is negatively correlated with aggressiveness, and the authors suggest this is due to the increased energy expenditure and risks associated with aggression (which are similar for boldness), including increased risk of predation and violent conspecific interactions, making an aggressive personality too costly to sustain alongside or following a period of fast growth. However, lifetime growth patterns in fish are very different from those of birds, making comparisons difficult, and since few studies have considered such correlations in avian species, this is purely speculative.

Persistence of personality traits

Birds showed repeatability in their raw behavioural responses to tests delivered on the same day, but the 30 males that were retested 6 months after the initial test showed low repeatability for the personality scores calculated from from each set of trials using a PCA (boldness and activity). Birds were in fact shyer and showed a trend towards being less active at 9 months than at 80 days (Fig. 3.4). The reduction in overall boldness in all the birds is interesting, especially since it contrasts with changes found in other small passerines. In studies on tits (Verbeek et al. 1994; Carere et al. 2005a), birds scored as shy, slow explorers at an early age and then re-tested at a later date tended to become faster (i.e. more bold), though in both cases birds were at least partly hand-reared, which may have had an effect on these findings. However, a reduction in boldness in terms of risk-taking

behaviour between adolescence and adulthood is a phenomenon that is commonly noted in the mammal literature (including humans) (for example see Stansfield & Kirstein 2006). Young birds are perhaps likely to be bolder than older birds, since after fledging, juveniles will need to disperse to find their own territories and feeding grounds, and will need to explore the surrounding area in order to do this. This early life requirement could mean that birds are bolder shortly after fledging than at any other time in their life, a period coinciding closely with the age of my birds when they were first tested. In this study, birds were maintained in single sex groups on a standard diet for a number of months before being re-tested, experiencing highly stable conditions. Birds could therefore become settled in their respective environments, and less inclined to actively explore anything novel, or alternatively, have become used to a variety of initially novel objects and so find them less interesting. The shift in individual scores is unlikely to be due to habituation to the test, since both novel objects and the environment were changed so that they were still novel, both testing sessions were of short duration, and tests were conducted more than 5 months apart, although its possible that birds felt more at ease, due to familiarity with the testing procedure. Alternatively, changes in boldness could be the result of different individual experiences or of some degree of adaptation to the environment experienced after fledging. Indeed, some recent studies suggest that coping styles could be more flexible than previously thought and could be changed as a result of experience: Ruiz-Gomez et al. (2008) showed in work on rainbow trout that fish from two strains selected for proactive and reactive personality types switched behavioural profile following a period of starvation during transport.

In summary, exposure to elevated levels of CORT in the first weeks of postnatal life appears to have no effect on individual personality traits in this study, though this may have been due to the CORT manipulation falling outside some critical period in the shaping of the behavioural phenotype. When viewed in combination with other work on this species (Spencer & Verhulst 2007), this study could suggest that CORT may be important in determining personality traits, but only when exposure occurs in the very early stages of hatchling growth. Examination of relative growth trajectories suggested that growth rate may also be important in influencing the behavioural differences I observed between individuals. Exactly how growth can impact upon personality traits is unclear, but there is the possibility that growth is used to indicate current environmental conditions, and so personality is shaped so as to aid survival in the environment the individual is likely to encounter. Overall, evidence is accumulating that the early environment is critical in the phenotypic development of an animal, though more integrated studies are needed to

address the mechanisms behind the individual differences in behaviour that are so commonly observed.

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Chapter 4

Early nutritional quality affects adult performance in a simple learning task

ABSTRACT

Numerous studies have shown that cognitive ability can be impaired by sub-optimal conditions during early development. However, whether the basis of this impairment lies in nutritional deprivation or nutritional quality reduction, or happens as a result of growth compensation that can follow a period of poor nutrition, remains unclear. An experiment was conducted to address this issue, by raising young zebra finches on four different diets, allowing separation of the effects of a poor quality diet from the effects of compensatory growth. Chicks raised on these treatments were tested on their ability to learn and perform in both a spatial and a visual food location task in adulthood. All birds that had experienced a low protein diet in the first 15 days of life were poorer at learning a basic foraging task when two years old, regardless of the degree of compensatory growth that they had undergone following the diet treatment. Individual growth trajectories showed no relationship with ability to learn the initial task, or with individual cognitive performance. Consequently, this study demonstrates that a reduction in nutritional quality in early life may be more important in determining adult cognitive ability than the growth trajectory that follows, suggesting that even transient, short-term protein reduction during early development can have a profound impact on cognitive ability for the entire life of an animal.

INTRODUCTION

The environment any animal experiences during development is likely to be variable, and the growth rate of an individual can be significantly altered by environmental factors such as local food availability, weather, or parental attentiveness. If normal growth is disrupted in some way, it may be possible for an animal to compensate at a later date, either by growing more rapidly once the nutritional limitation is removed, or by delaying maturation and independence and growing more slowly over a longer period (Mangel & Munch 2005). However, although such compensation is adaptive in the short-term, and can lessen the drawbacks of being a small adult, it can also carry costs. Compensatory growth has been linked to delayed maturation, earlier onset of adult disease, and reduced longevity (Metcalf & Monaghan 2001).

Various studies have shown that impairment in cognitive ability can result from some degree of nutritional shortfall during early development (Galler et al. 1983; Castro & Rudy 1989; Bedi 1992; Pravosudov et al. 2005), with particularly strong effects on tasks involving spatial and visual cues. A study by Akman and colleagues (2004) showed that food deprivation in the first few weeks of life in young rats was associated with reduced brain neurogenesis and some impairment of visual-spatial memory; Bedi (1992) found that undernourished rats performed less well in spatial tasks than controls, and Castro et al. (1989) found that malnourished rats were compromised in their ability to successfully complete a variety of visual tasks. However, since nutritional stress in early life is almost always accompanied by some degree of growth perturbation, it is unclear whether the cognitive effects associated with such nutritional deficits are a direct result of food shortfall, or are due to the subsequent growth adjustment. In humans, cognitive limitations have been linked both to nutritional deficits (Morley & Lucas 1997) and to the degree of compensatory growth that an individual must undergo in order to reach a normal adult size (for example see Morley et al. 2004). In other mammals, there is evidence that reductions in performance on cognitive tasks may also be linked to compensation (Akman et al. 2004), though as far as I am aware, this has not been tested directly. Despite evidence for the general fitness costs of catch-up growth, little is known about the effects of such compensation on cognition in non-human animals.

A number of studies have shown that sub-optimal nutrition in early life can have negative effects on song learning in birds (Nowicki et al. 2002; Spencer et al. 2003; Buchanan et al. 2003), but less is known about whether other types of learning and

memory are also sensitive to such fluctuations in this group. Nutritional deficits during early development have been shown to negatively affect spatial but not visual learning in the Western scrub jay, *Aphelocoma californica*, with nutritionally-deprived birds developing relatively smaller hippocampi with fewer neurons than controls (Pravosudov et al. 2005). However, it seems likely that nutritionally deprived birds in this study underwent a period of compensatory growth, since they were given *ad lib.* access to food once they began to feed themselves, and did not differ in mass from controls when measured at adulthood. Consequently, although this study confirms that early nutrition is important in determining cognitive ability, it does not tease apart the effects of the actual deprivation from those of the rate of growth that follows. However, a recent study on the zebra finch has shown that inferior performance on a visual learning task following a period of poor nutrition in early life is not influenced by the early diet itself, but by the degree of catch-up growth that an individual undergoes relative to a control sibling (Fisher et al. 2006).

However, Fisher et al's study is one of the few to have considered whether cognitive impairment is a direct effect of nutritional deprivation (in terms of either the total amount of food or a specific micronutrient), or an indirect effect caused by changes in growth trajectory. Consequently, more studies are needed in this area. Altricial birds are a good model group for answering this kind of question: young passerines grow extremely quickly in the first few weeks of life, and can be more than 80% of their adult size only 10 to 20 days after hatching (Ricklefs 1968). This fast growth rate allows young birds to spend as little time as possible in the vulnerable juvenile phase, but is a potentially risky strategy in that any environmental fluctuations leading to nutritional stress during this period of rapid development can have significant consequences for the morphological and behavioural phenotype of the adult bird.

In this study, I asked whether cognitive ability in the adult zebra finch is affected by either diet quality or associated growth differences during very early development. To do this both diet quality and growth trajectory were manipulated in young lab-reared zebra finches. Laboratory studies allow manipulation of the early environment in a controlled way without the 'noise' associated with food supply in the natural environment. The effects of early nutrition on both spatial and visual learning performance were examined.

METHODS

Subjects

The subjects were selected from the population of birds that had undergone the dietary manipulations fully detailed in chapter 2. When two years old, a total of 40 males from this experimental population were selected to complete a simple learning task; 10 from each of the four diet treatment groups (HH, HL, LH, and LL). Two pairs of males shared the same parents, but were hatched during different breeding rounds and so were not raised in the same nest. These males were placed individually in single cages (40 x 43 x 44 cm), where they remained throughout training and testing. Each cage was standardised with three wooden rod perches, a water dispenser and a food bowl. Cage floors were covered in paper only, to allow easy removal of excess seed prior to deprivation periods. Although kept singly, birds were always in visual and acoustic contact with conspecifics, and were not observed to be behaving abnormally. However, during training and testing a large screen was placed in front of the banks of cages involved, so that birds elsewhere in the room were not able to see others' reactions to, or use of, the test tray. Birds were kept on a 14:10 hour L: D schedule throughout the experimental period.

Training

Using a method similar to that established for testing spatial learning in small passerines (Hodgson & Healy 2005), I used food rewards in an ice cube tray in order to test spatial and visual learning and memory. The yellow plastic tray had 12 identical wells, which were rectangular holes of approximately 3cm by 4cm and 2cm deep. In the first phase of training, a tray containing a few seeds in each well was placed in each bird's cage for 30 minutes twice a day between 0900 and 1700 hours. The birds underwent a short period of food deprivation before each training session in order to maximise motivation to feed (2 hours in the morning and 1.5 hours in the afternoon, with at least 3 hours of normal feeding in between; the 2 hour deprivation period represented only 15% of available daylight hours, a period of time well within the natural range for this small granivorous bird (Zann 1996)). The tray was always placed in the centre of the cage, with the long sides parallel to the wire cage front. Once birds had learnt to feed on uncovered seeds in the tray, the task was made steadily harder by covering the seed with successively larger pieces of white

cotton wool. The training thus proceeded through 5 separate phases, until by phase 5, all wells were completely covered with cotton balls that the birds needed to physically remove in order to access the seed underneath. In addition, in the final phase, only six of the 12 wells were rewarded, so that birds often had to remove more than one cotton ball in order to gain a reward, so learning that wells were not always rewarded (see appendix 1 for more details of these training phases). For a bird to be successful in a phase, it needed to find the food in the tray and feed within a 30 minute training session. Birds were given the spatial and visual tests only after reaching and successfully completing phase 5 on two consecutive days.

Spatial test

As in training, birds received the ice cube tray at a randomised time between 0900 and 1700 hours, following 2 hours without food. This 2 hour deprivation period represented only 15% of available daylight hours, a period of time well within the natural range for this small granivorous bird (Zann 1996). In this test, every well in the food tray was fully covered with a white cotton ball (as in stage 5 of training) but only three of the 12 wells contained hidden seed, making the ratio of rewarded to un-rewarded wells 1:3. These rewarded wells were unmarked but were in the same spatial location in every trial and for every bird. This protocol was intended to test whether birds were able to learn and use spatial cues to locate hidden food and so minimise searching time.

Each bird was presented with the tray and allowed to search freely for up to 30 minutes until finding and feeding from one rewarded well. During this first presentation, termed presentation A, behavioural observations were made from behind an opaque material screen placed approximately 1 metre from the cage front. Latency to approach the tray after it was placed in the cage (approach time) was recorded, along with time to find and eat from a rewarded well after this first approach (search time), the total number of cotton balls removed, the order in which they were removed and the number removed from empty wells (mistakes). On finding a rewarded well, birds were permitted to feed for 1 minute. The tray was then removed for 5 min, during which time the 3 rewarded wells were replenished and all the cotton balls replaced.

After this 5 minute break, the tray was returned to the cage and birds were given time to search again for up to 20 minutes, a time deemed sufficient to allow birds to search and find food without depriving those that were unsuccessful of food for an extended

period of time. On finding a reward, subjects were left to feed for 2 minutes. During this second presentation, termed presentation B, I recorded the same behaviours. Once a rewarded well had been found in presentation B and the bird had fed for 2 minutes, the tray was removed from the cage and the subject's normal food dish returned. Birds that failed to locate a seed-filled well in presentation A did not receive a B presentation. In these cases the tray was removed, testing for that day terminated and the food dish returned as normal. The same testing procedure, with the same spatial layout was used on 5 consecutive days, so allowing us to examine subjects' memory from day to day over the week.

Visual test

In this test, birds again received the test tray once a day following two hours of deprivation, and the ratio of rewarded to un-rewarded wells was again 1:3. However, this time the cotton balls used to cover the wells were pink and blue instead of the familiar white. One colour was always used to cover wells containing seeds, and the other only to cover empty wells, although the spatial location of the different colours in the grid varied between trials. Therefore the only cue to identify a rewarded well in this test was visual. The colour chosen to indicate a rewarded well was randomised for each bird and between sexes to control for any colour preference, but was kept constant for a given bird.

The protocol for presentation A was exactly the same as in the spatial test, and the same variables were recorded. Following presentation A, the tray was removed for 5 minutes, and the spatial distribution of rewarded wells and their associated colour cotton balls altered for presentation B. Birds were once more presented with the tray and allowed to search while the same behavioural observations were made. Due to a time constraint with the experimental subjects, this protocol was followed once a day for 4 consecutive days instead of 5 as in the spatial trial.

Statistical methods

Most of the data were analysed using general linear models (GLMs) and repeated measures ANOVA, with either diet 1 and 2 entered as fixed factors, or mass changes as covariates. To determine the relationship between diet treatments and the likelihood of success in the

training schedule, I used chi squared with Yates's correction. When further considering differences in size and growth rate between birds that had been successful in the training and birds that had not, I used independent samples t-tests, with learnt/not learnt as the grouping variable.

Test performance in both the spatial and visual tasks was analysed using a repeated measures ANOVA with day and presentation as test variables, and approach time, search time and the number of mistakes made as the various repeated measures. If birds were learning the spatial and visual 'rules' behind each task, we would expect them to find a food reward more quickly, and do so with fewer mistakes in successive trials. Moreover, if the diet manipulations had any effect on the extent of learning, these measures would differ between the diet treatment groups. For the first two measures of performance, I pooled the data from the A and B presentations, since I was interested in overall improvement from day to day. When analysing approach and search time, I log-transformed the data to account for non-normal distributions, which tended to be left-skewed. The distribution of mistakes did not deviate significantly from normality. In these repeated measure models, I included diets 1 and 2 as between-subject factors, and removed non-significant variables in a stepwise fashion. I was particularly interested in the effects of diet 1, since the majority of chick growth occurs during the first 15 days of life. I also used a GLM, with diet 1 and 2 as fixed factors, to investigate the effect of these diet parameters on the number of mistakes made by any individual over consecutive trials. I analysed the mean number of mistakes made in A and B presentations separately, and used a mean value to account for the fact that birds did not approach the test tray in some trials, and so had no result for the number of mistakes made.

From a total of 40 birds, only 18 were able to consistently complete phase 5 of the training successfully. These were therefore the only 18 that could be considered to have learnt the basic task, and consequently, were the only birds able to undergo spatial or visual testing. Of these 18 birds, 6 were from the HH treatment, 7 from the HL treatment, 3 from the LH treatment, and 2 from the LL treatment. One of these birds subsequently fell sick and had to be excluded from testing, and another completed the visual test but never approached the test tray in the spatial test, and so had no analysable results for this trial. Therefore, analyses of test performance were conducted on a total of 17 males for visual tests and 16 males for spatial tests. All analyses were conducted using SPSS 15.0.

RESULTS

Treatment effects on growth

For a full discussion of the treatment effects on growth, see the results section of chapter 2 (pages 30-32).

Successful training

Birds that had received a high protein diet between 0 and 15 days were more likely to successfully complete the training than birds that had received a low protein diet (Fig. 4.1; $\chi^2=4.95$, $df=1$, $p=0.026$), and this success was unaffected by the diet received between days 15 and 30 (For H protein birds, $\chi^2=0.22$, $df=1$, $p=0.64$; for L protein birds, $\chi^2=0.27$, $df=1$, $p=0.61$). Successful and unsuccessful birds did not differ significantly in their early growth rate (mean mass gain 0-15 days, successful birds = 11.23 ± 0.36 g, unsuccessful birds = 10.81 ± 0.23 g, $t_{38}=0.99$, $p=0.33$; mean mass gain 15-30 days, successful birds = 2.64 ± 0.27 g, unsuccessful birds = 2.39 ± 0.18 g, $t_{38}=0.82$, $p=0.42$).

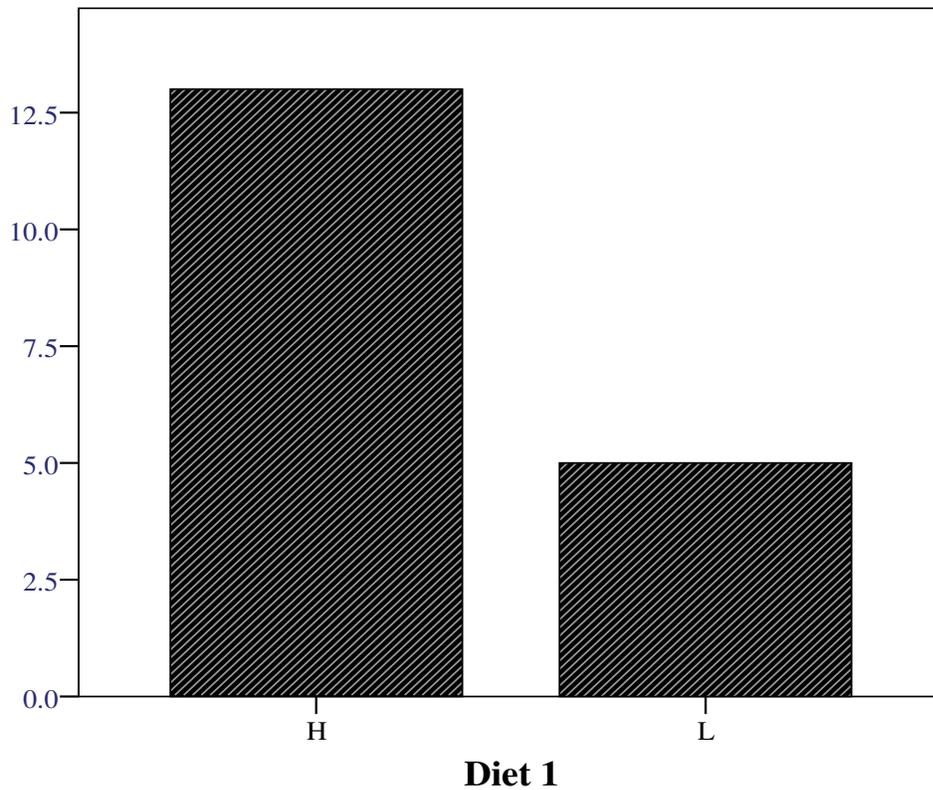


Figure 4.1. The number of birds that successfully completed cognitive training and went on to be tested shown according to the diet they received for the first 15 days of life (H=high protein, L=low protein, N=18 birds).

Spatial test

The time taken to approach the feeding tray in each trial showed a negative trend over time, with day of testing having a significant effect on the mean approach time for each A and B presentation, indicating that birds approached the tray more quickly in later trials ($F_{4,60} = 3.14$, $p=0.021$). Diet quality in the first 15 days of life (diet 1) had no effect on mean approach time (mean for A presentations, $F_{1,12}=0.75$, $p=0.40$; mean for B presentations, $F_{1,12}=0.75$, $p=0.40$), nor did diet quality between 15 and 30 days (diet 2) (mean for A presentations, $F_{1,12}=0.17$, $p=0.68$; mean for B presentations, $F_{1,12}=0.62$, $p=0.45$) or the interaction between these two diet periods (diet 1*diet 2 =diet treatment) (mean for A presentations, $F_{1,12}=0.19$, $p=0.67$; mean for B presentations, $F_{1,12}=0.31$, $p=0.59$).

The time taken to locate the first food item after initial approach (i.e. search time) also changed significantly with day of testing, meaning that birds became quicker at

finding food in later trials ($F_{4,60}=4.12$, $p=0.005$). However, there was no effect of diet 1 (mean for A presentations, $F_{1,12}=0.81$, $p=0.39$; mean for B presentations, $F_{1,12}=0.95$, $p=0.35$), diet 2 (mean for A presentations, $F_{1,12}=0.38$, $p=0.55$; mean for B presentations, $F_{1,12}=0.18$, $p=0.68$) or diet 1*diet 2 (mean for A presentations, $F_{1,12}=0.59$, $p=0.46$; mean for B presentations, $F_{1,12}=0.41$, $p=0.54$). Similarly, there was no effect of diet 1 on the mean number of mistakes made in each of the presentations (A presentations, $F_{1,12}=0.95$, $p=0.35$; B presentations, $F_{1,12}=0.006$, $p=0.94$), of diet 2 (A presentations $F_{1,12}=0.41$, $p=0.93$; B presentations, $F_{1,12}=0.38$, $p=0.55$), nor diet 1* diet 2 (A presentations, $F_{1,12}=0.021$, $p=0.89$; B presentations, $F_{1,12}=0.51$, $p=0.49$). As with approach and search time, there was a significant effect of day of testing on the number of mistakes made ($F_{4,40}=2.93$, $p=0.033$), with birds making fewer mistakes on later test days.

Growth and spatial test performance

Early growth had no effect on an individual's mean approach time either in presentation A (mass gain days 0-15, $F_{1,14}=0.42$, $p=0.53$; mass gain days 15-30, $F_{1,14}=0.96$, $p=0.34$) or presentation B (mass gain day 0-15, $F_{1,14}=0.005$, $p=0.95$; mass gain days 15-30, $F_{1,14}=0.13$, $p=0.72$). Nor did it have an effect on mean search time in presentation A (mass gain days 0-15, $F_{1,14}=0.16$, $p=0.70$; mass gain days 15-30, $F_{1,14}=0.007$, $p=0.94$) or B (mass gain day 0-15, $F_{1,14}=0.201$, $p=0.66$; mass gain days 15-30, $F_{1,14}=0.011$, $p=0.92$). Additionally, there was no effect of early growth on the mean number of mistakes made by each individual across all presentations (mass gain days 0-15, $F_{1,14}=0.90$, $p=0.36$; mass gain days 15-30, $F_{1,14}=0.57$, $p=0.47$).

Visual tests

The time taken to approach the feeding tray in each trial once again showed a significant negative trend over time, indicating that birds approached the tray more quickly in later trials ($F_{4,64}=5.05$, $p=0.001$). However, there was no effect of diet quality between days 0 and 15 (diet 1) on mean approach time (mean A presentations $F_{1,13}=0.96$, $p=0.34$; mean B presentations, $F_{1,13}=0.001$, $p=0.98$). There was also no effect of diet quality between days 15 and 30 (diet 2) on mean approach time (mean A presentations, $F_{1,13}=1.86$, $p=0.20$; mean B presentations, $F_{1,13}=0.46$, $p=0.51$), and no significant interactions between the two

diet periods (diet 1* diet 2) (mean A presentations, $F_{1,13} = 1.20$, $p=0.29$; mean B presentations, $F_{1,13} = 0.13$, $p=0.73$).

There was also a significant effect of day of testing on mean search time (the mean time taken to locate the first food item after initial approach), as birds became quicker at finding food in later trials ($F_{4,64} = 6.28$, $p < 0.0001$). However, there was no effect of diet 1 (mean for A presentations, $F_{1,13} = 0.008$, $p=0.93$; mean for B presentations, $F_{1,13} = 0.043$, $p=0.84$), diet 2 (mean for A presentations, $F_{1,13} = 1.87$, $p=0.20$; mean for B presentations, $F_{1,13} = 0.54$, $p=0.82$) or diet 1*diet 2 (mean for A presentations, $F_{1,13} = 0.16$, $p=0.70$; mean for B presentations, $F_{1,13} = 0.083$, $p=0.78$). Similarly, there was no effect of diet 1 on the mean number of mistakes made in each of the presentations (A presentations, $F_{1,13} = 1.017$, $p=0.33$; B presentations, $F_{1,13} = 2.21$, $p=0.16$), of diet 2 (A presentations $F_{1,13} = 0.00$, $p=1.00$; B presentations, $F_{1,13} = 0.37$, $p=0.55$), nor diet 1* diet 2 (A presentations, $F_{1,13} = 0.75$, $p=0.403$; B presentations, $F_{1,13} = 0.37$, $p=0.55$).

Growth and visual test performance

Early growth rate had no effect on an individual's mean approach time either in presentation A (mass gain days 0-15, $F_{1,15} = 0.22$, $p=0.64$; mass gain days 15-30, $F_{1,15} = 0.006$, $p=0.94$) or presentation B (mass gain day 0-15, $F_{1,15} = 0.58$, $p=0.46$; mass gain days 15-30, $F_{1,15} = 0.96$, $p=0.18$). Early growth also had no effect on an individual's mean search time in presentation A (mass gain days 0-15, $F_{1,15} = 0.93$, $p=0.35$; mass gain days 15-30, $F_{1,15} = 0.72$, $p=0.41$) or B (mass gain day 0-15, $F_{1,15} = 0.703$, $p=0.42$; mass gain days 15-30, $F_{1,15} = 0.78$, $p=0.39$). Additionally, there was no effect of early growth on the mean number of mistakes made by each individual across all presentations (mass gain days 0-15, $F_{1,15} = 0.79$, $p=0.39$, mass gain days 15-30, $F_{1,15} = 0.23$, $p=0.64$).

DISCUSSION

The dietary treatments were successful in affecting the growth rates of the subjects, with birds on the H protein diet for the first 15 days growing significantly more than birds on the low protein diet for this period. Birds on the LH diet treatment experienced a period of low quality food followed by a period of high quality food, and the group means suggest

that a period of compensatory growth occurred between days 15 and 30. The graphs also indicate that LH birds also prolonged this increased growth rate beyond the duration of the diet treatment (Fig. 2.2 a-c, pg. 31/32), and continued to grow at a slightly elevated rate until at least 100 days, well after the time period when these birds would normally cease growing. Birds on the LL diet grew more slowly and steadily, and did not undergo the same growth acceleration. Interestingly, birds that received a high quality diet during the first half of the treatment (i.e. diet 1) were significantly more likely to complete the cognitive training as adults than birds that received a low quality diet during this time, and early growth rate had no effect on this success. Therefore, despite undergoing different growth trajectories, it appears that LH and LL birds were equally compromised in their ability to successfully proceed through the training. Since this is the case, I suggest that it is the diet quality that birds experience in the first 15 days of life which is important in determining their adult learning performance, rather than the extent of growth compensation which may follow.

The difference in aptitude for the basic task between birds that received H and L protein during the first 15 days could have arisen because the early diet treatments affected a subject's ability to register the relevant environmental cues, or some stage of memory retention or formation. This could be due to interrupted or incomplete development in parts of the brain, caused by shortage of building material, or be a result of reduced neurogenesis, as has been found in some bird and mammal species (Akman 2004; Jordan 1982; Pravosudov 2005). Alternatively, it could be that those birds that were unsuccessful in the training were in some way impaired in their motor or visual skills, and so found the removal of cotton balls more difficult. However, although physical impairments have been found in cases following reasonably severe levels of food deprivation (e.g. Jordan 1979), activity levels in birds following a less severe food restriction have been unaffected (Pravosudov 2005), so changes to motor skills are unlikely to result from these mild changes in diet quality. We must also consider the possibility that birds differed in their motivation to complete the learning tasks, if for example some were hungrier than others following the short period of food removal. However, in all trials, I observed birds approaching the tray and hopping around the cage floor actively searching for food. I therefore considered all birds to be highly motivated, in that they were all attempting to find the food reward. Despite this general desire to locate the hidden seeds, some subjects were still unable to grasp the basic concept of the task, suggesting differences in learning rather than motivation. Since this point is only anecdotal rather than empirical however, further studies may consider whether weight loss following a period of deprivation is

correlated with exploration behaviour in such a task, so allowing more convincing separation of the effects of motivation and test performance. The exact mechanism behind this result therefore remains unclear, and further experiments are needed.

When considering the performance of birds that were successfully able to complete the training, approach time and search time decreased over the week of testing in both spatial and visual trials. However, diet treatment had no effect on this decrease, and on average, birds reduced their approach and search time regardless of their nutritional history. This improvement in performance is likely to be a result of the subjects learning something about the rule behind each of the tasks, so allowing them to locate food based on the available cues rather than through extensive searching, an explanation supported by the associated reduction in the number of mistakes made by all birds in the spatial test (see below). Alternatively, the reduction in approach and search time could be due to some sort of habituation to the test protocol.

In the spatial trial, all birds also showed a decrease in the number of mistakes they made as the week progressed, though again, this was unaffected by nutritional history. This improvement in performance is a further indication that learning is occurring, and birds are able to find food in this test using the spatial cues available. However, the results suggest that the acquisition of this knowledge or the implementation of information provided by the cues does not differ in birds that received different developmental diets. The fact that there was not a similar reduction in the number of mistakes made in the visual trials is interesting. Birds from all four diet treatments showed no improvement in the number of mistakes made from day to day. Such a result may suggest that while these birds are able to use spatial cues to locate a food item more quickly, they are less able to use visual cues for the same purpose. The reduction in approach and search time in the visual test may therefore be down to habituation and faster searching rather than use of the available signals.

To further support the suggestion that it is diet quality rather than compensatory growth that is important in determining performance in this task, there was no evidence that individual growth rates had any effect on performance during the training, or the spatial and visual tests. Of course it may be that the 40 males used in the test had significant overlap in their growth trajectories, making it difficult to spot real differences between the treatments that might have been linked to test performance. Alternatively, my relatively small sample size may not have been able to show differences in test performance, especially since so few birds on the LH and LL treatments underwent testing. However, since I have demonstrated no correlations between growth trajectory and initial

learning ability, or later test performance, such a result supports the suggestion it is that the quality reduction experienced in the first few days of life that is having an effect on the learning capacity of these birds, rather than differences in growth.

Overall then, it appears that it is only the very early diet period (diet 1, 0-15 days) that is important in determining test performance in these individuals. Indeed, studies on mammals would suggest that the earlier animals are exposed to sub-optimal nutrition, the worse the effect on learning (Smart 1986). Since the first few days of life are likely to be of critical importance in the development of the brain and associated neural structures, this is perhaps unsurprising. Additionally, although the manipulation of diet quality was followed by a substantial period (2 years) of high quality nutrition before the memory tests were carried out, it also seems that the damage done during very early life has not been rectified even after a prolonged period on an improved diet. This is in contrast to some mammal studies (e.g. Bedi 1992) which show that a short period of nutritional rehabilitation can result in an improvement in the spatial abilities of previously undernourished rat pups.

If it is indeed the case in this study that diet quality is more important than growth trajectory in determining performance in this learning task, then these results contrast sharply with those found by Fisher et al. (2006). This study on zebra finches suggested that it is the amount of compensation undergone rather than the diet experienced that is central to later performance on a similar test. However, Fisher et al.'s test did not involve groups that had received a low quality diet but had not undergone extensive growth compensation, so making it difficult to draw out the different effects of diet quality and growth. Fisher et al. also carried out a slightly different test, involving associative learning of visual cues, which may be more or less difficult than the learning tasks carried out here, and could possibly account for some of the differences in findings.

One potentially confounding variable and a problem that has arisen from previous work in this area is that it remains unclear whether variation in nutritional quality is really the cause of the differences in behaviour that have been documented. Some previous studies have demonstrated impairments in learning and memory as a result of food deprivation in early life, but such studies often have an increase in stress associated with their food restriction. For example, Akman et al. (2004) highlight this difficulty in their study on cognition in young rats. They limited the nutritional intake of their subjects by removing pups from their mothers. Such an intervention is likely to cause considerable stress, and so it is not possible to separate the effects of food deprivation from the effects of increased stress hormone secretion on subsequent spatial learning and memory performance. Indeed, in a study which controlled for this confounding factor in which rats

were food deprived but remained with their mothers, there were no effects of the deprivation on the measured cognitive ability (Slob 1973). The diet manipulation in the present study should not have involved any significant increase in stress levels, and the early environment of the subjects was minimally disrupted. Chicks were fed by their parents as normal and birds on all diet treatments underwent handling infrequently and only for morphological measurements. In addition, food was not in short supply, and only quality was reduced, so that chicks should not have suffered from deprivation stress. If disturbance or deprivation stress is indeed the mechanism behind the impairment of learning and memory which has been found in some animals, then this experimental design may be the reason I found no difference in test performance between birds from the different diet treatments.

In this study, I addressed the question of whether learning and memory can be impaired as a result of poor nutrition in the nest, or as a result of compensatory growth. My results suggest that diet quality in very early life has a significant effect on the ability of adult birds to learn a simple foraging task, but I found no evidence that growth compensation was important in this skill, or in determining performance in a spatial or visual task. Since the learning abilities of an animal will be critical to its survival and success once it has become independent, not only for finding food, but also for predator avoidance, territory and mate acquisition, and successful parenting, these are important questions, with implications that very much extend to our own species; namely that adverse early conditions can result in cognitive deficits that will have significant consequences for individual lifetime fitness.

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Chapter 5

Early stress impairs learning and memory in adult zebra finches

ABSTRACT

Animals are potentially exposed to a variety of environmental conditions during their early development, some of which may be perceived as physiologically stressful, and there is a growing interest in the long-term effects of exposure to early stress on the adult phenotype. Cognitive abilities such as learning and memory are essential components of many behaviours and allow an individual the behavioural flexibility to cope with environmental change. In this regard, neural development is thought to be influenced by stress in early life, and negative effects on neural development that cannot be compensated for later could have significant fitness consequences. I investigated the learning and memory skills of adult zebra finches that had been experimentally exposed to varying levels of the stress hormone corticosterone as nestlings. Birds were tested on their ability to find food using either a visual or spatial cue. Individuals that had experienced elevated levels of corticosterone as chicks were less able at adulthood to use both types of cue to locate a reward. These birds continued to make a large number of mistakes in repeated trials, while controls were able to reduce the number of mistakes made over successive tests. These results suggest that corticosterone elevation in early life is important in shaping adult learning, either by affecting memory or a subject's ability to register or attend to environmental cues.

INTRODUCTION

There is a growing body of literature to suggest that the early environment can have profound effects on phenotypic development, not only in terms of morphology (Birkhead et al. 1999; Metcalfe & Monaghan 2001; Blount et al. 2003), but also physiology (Crisuolo et al. 2008) and behaviour (Vallee et al. 1999; Penke et al. 2001; Nowicki et al. 2002; Kitaysky et al. 2003; Chung et al. 2005; Spencer & Verhulst 2007). The environmental conditions experienced during early growth can vary considerably, and some circumstances might invoke a stress response (Sutanto & Dekloet 1994; Wingfield 2003). Some degree of stress during this early life stage - such as may be caused by low food availability, bad weather or sibling competition - is likely to occur in all young animals. In vertebrates, such stressors lead to the release of corticosteroids which promote behavioural and physiological alterations that aid an individual's ability to deal with a threat, or any ensuing stressful situation (de Kloet et al. 1999; Cockrem 2007; Wingfield 2008). These corticosteroids shield the brain from the negative effects of stress (de Kloet et al. 1999) and are essential in normal cognitive performance, as they are involved in the coordination of the networks that underlie complex learning in the hippocampus (de Kloet et al. 1999). However, if a stressful situation persists, the elevation can become chronic, and such long-term exposure can cause permanent changes in the neuroendocrine system and cognitive abilities of some animals.

Effects of early exposure to elevated glucocorticoid hormones have been relatively well studied in mammals. For example, rat pups that undergo prolonged (6 hour) daily periods of social isolation in the third week of life show an associated elevation of the stress hormone corticosterone in the peripheral circulation, and, as adults, these stressed animals perform less well in a spatial maze task than controls (Sandstrom & Hart 2005). Similar effects are observed in rat pups that received corticosterone in their food (Dachir et al. 1993). In contrast, a completely different outcome is observed if pups are exposed to corticosterone through their mothers. Catalani et al. (1993) and Casolini et al. (1997) demonstrated that rat pups nursed by corticosterone-dosed mothers are able to perform better in a spatial task after weaning than controls and have lower plasma concentrations of corticosterone. Such conflicting results have led to the conclusion that the effects of corticosteroids on mammalian cognition appear to follow an inverted U-shaped relationship, with low doses of corticosterone improving cognitive performance, and higher doses associated with a reduction in learning ability (Kovacs et al. 1976).

Relatively little is known, however, about the long-term effects of early stressors on subsequent cognitive abilities in other vertebrate taxa. Corticosterone is the main glucocorticoid stress hormone in birds as well as in rodents (Cockrem 2007) and although concentrations of corticosterone in adulthood have been linked to learning and memory task performance in a number of avian studies (reviewed in Pravosudov 2005), only a few have shown any relationship between exposure to the hormone in early life and ability in adulthood. Kitaysky and colleagues (2003) showed that young kittiwake chicks (*Rissa tridactyla*) implanted with corticosterone for four weeks increased their food intake through amplified begging but were poorer than controls in both a visual learning task as young birds and a spatial learning task as adults. These results would suggest that there was a short-term survival benefit to increased corticosterone exposure but a long-term cost in terms of reduced cognitive ability. Corticosterone administration in early life has also been shown to reduce the size of the higher vocal centre in male zebra finches, leading to the development of shorter, simpler songs (Buchanan et al. 2004), which has negative implications for reproductive fitness. Such studies suggest that resource allocation to brain development could be modified in adverse early conditions, leading to alterations in functional performance, although the underlying mechanisms are still unclear. However, the long-term effects of elevated stress during development on adult learning ability remain uncertain, and to my knowledge, there are no studies on avian species which have investigated such longitudinal effects on both visual and spatial learning ability in adulthood. Here I investigate the effects of exposure to increased levels of corticosterone in very early life on the ability of adult zebra finches (*Taeniopygia guttata*) to use both spatial and visual cues in a simple foraging task. The results demonstrate that short-term post-natal exposure to elevated corticosterone (within the natural range) has negative effects on adult performance in two kinds of simple learning task.

METHODS

Subjects

Birds were selected from the population that had undergone differential exposure to corticosterone (CORT) while in the nest, as described in chapter 3. These birds were fully adult (around 300 days of age), and 16 unrelated birds per treatment (8 males and 8

females) were randomly selected for cognitive testing. Subjects were trained and tested for both spatial and visual learning and memory exactly as described in chapter 4.

Sample sizes

From a total of 32 birds, 15 were able to consistently complete phase 5 of the training. Therefore, only these 15 could be considered to have learnt that food could be found in the trays and thus were the only birds to undergo testing. Of these 15, 8 were control birds (5 males and 3 females) and 7 CORT birds (3 males and 4 females). In all analysis of the spatial trials, $N=15$ unless otherwise stated. In the visual test some birds occasionally failed to lift any cotton balls or to find food in the B presentation, and so data were missing for some variables, leading to variation in sample sizes for the visual test analysis. Thus, in the examination of approach and search time, 6 control (4 males and 2 females) and 6 CORT birds (2 males and 4 females) had sufficient results for analysis. Fewer birds had a full complement of data points for the number of mistakes made in each trial, so analysis was conducted upon 5 control birds (3 male and 2 female) and 5 CORT birds (1 male and 4 female).

Statistical analysis

Data were mostly analysed using general linear models (GLMs) and repeated measures ANOVA, and non-significant variables were removed in a stepwise fashion. For the analysis of success in training, I used a binomial logistic regression with learnt/not learnt as the dependent variable.

Approach and search time in both the visual and spatial trials were analysed using repeated measures ANOVA, with individual birds as subjects and time each day (pooled for A and B presentations, as I was interested in how approach and search time changed overall from day to day) as the repeated measure. Prior to all analysis, time data were log-transformed to account for non-normal distributions, which tended to be left skewed. In these repeated measure models, treatment group was included as a between subjects factor. To investigate progress from presentation A to B, data were analysed using a GLM with individual search time in presentation B averaged across all test days as the dependent

variable, treatment as a fixed factor and individual search time in presentation A averaged across all test days as a covariate.

The overall rate of learning in both the spatial and visual tasks was quantified for each individual by plotting the number of mistakes made per trial against trial number, excluding the data for the very first trial since the number of mistakes made on that occasion was randomly distributed (a total of nine trials, as there were five test days with two presentations per day). Trial number was expressed on a logarithmic scale, since improvement was nonlinear with respect to time. The gradients of these relationships indicate the rate of learning. In the spatial trial, one control bird made no mistakes, thus had no calculated gradient and was removed from this part of the analysis (reducing my sample size to 14). To compare gradients and the mean number of mistakes made between the treatment groups, I used independent samples t-tests, with treatment as the grouping variable. All analyses were conducted using SPSS 15.0.

RESULTS

There was no significant difference between treatments in the likelihood of success in the training phase (Wald's statistic=0.13, $p=0.72$) and also no significant difference in the number of males and females that were successful in training (Wald's statistic=0.13, $p=0.72$).

Spatial task

There was no difference between males and females in performance of the spatial task (mean approach time $F_{1, 13} = 2.17$, $p=0.17$; mean search time $F_{1, 13} = 0.24$, $p=0.63$; mean number of mistakes for both A and B presentations, $F_{1, 13} = 0.018$, $p=0.90$; error regression gradient $F_{1, 13} = 0.4$, $p=0.54$). Males and females were therefore pooled for subsequent analyses.

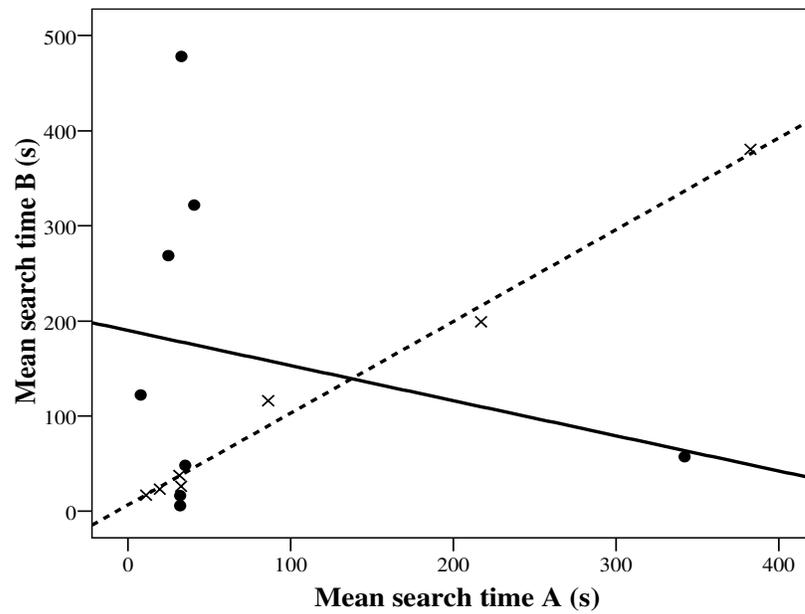
The day of testing had a significant effect on mean approach time, with birds approaching the tray more quickly in later trials ($F_{4, 48} = 4.69$, $p=0.003$). However, there was no difference in time to approach the feeding tray between the CORT and control groups ($F_{1, 11} = 1.658$, $p=0.227$), and no interaction between day and treatment ($F_{4, 40} =$

0.581, $p=0.678$). The time taken to locate the first food item after initial approach to the tray (i.e. search time) also showed a significant negative relationship with time ($F_{4, 48} = 2.77$, $p=0.039$). Again, there was no difference in search time between the CORT and control groups ($F_{1, 11}=0.27$, $p=0.62$), and no interaction between day and treatment ($F_{4, 44} = 4.76$, $p=0.75$).

Mean individual search time in presentation B was found to be significantly affected by an interaction between mean individual search time in presentation A and treatment (Fig. 5.1(a); $F_{1, 11} = 4.91$, $p=0.049$). Therefore, while control birds that were slow to find food in presentation A were quick to find it in presentation B, CORT birds that were slow in presentation A were also slow in presentation B. There was no independent effect of treatment ($F_{1, 11} = 4.37$, $p=0.061$), nor search time in presentation A ($F_{1, 11}=0.98$, $p=0.34$) on mean search time in presentation B. However, the plot of the data (Fig. 5.1(a)) shows an obvious outlier in the control birds. One control bird had a long mean search time in presentation A but a short mean search time in presentation B. A repeat analysis with the outlier removed caused the above interaction to become non-significant ($F_{1, 10}=0.102$, $p=0.76$).

Comparison of the rate of learning between groups (based on a gradient calculated from the errors made in each trial) indicated that control birds showed a negative gradient (-0.56 ± 0.39 (S.E.), $n = 7$) since they made successively fewer mistakes over time, whereas CORT birds had a positive gradient (0.49 ± 0.31 , $n = 7$) as they became worse at performing the task as the trials progressed (Fig. 5.2). This difference between the groups in the rate of learning was close to statistical significance ($t_{12} = 2.12$, $p=0.055$).

(a)



(b)

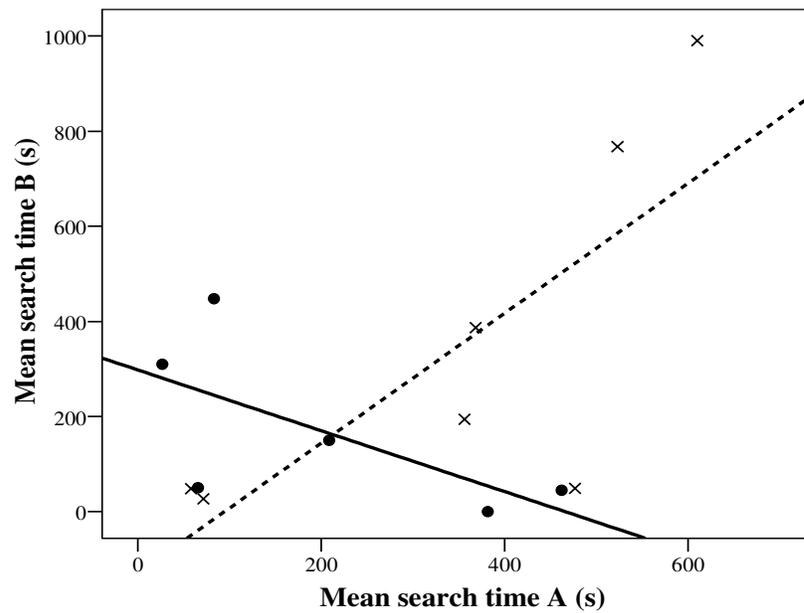
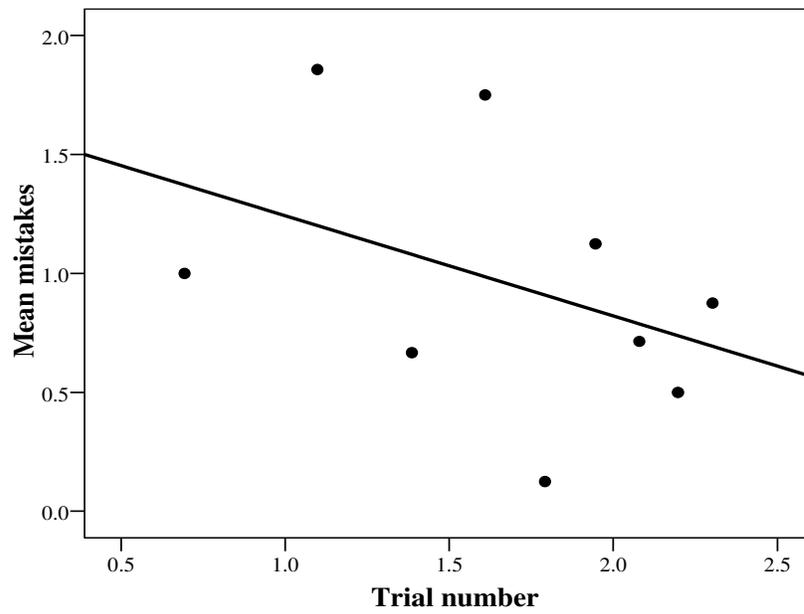


Figure 5.1. The relationship between mean individual search time prior to finding food in all A and all B presentations for control (solid circles and solid line) and CORT-treated (crosses and dashed line) birds in (a) the spatial learning test, and (b) the visual learning test.

(a)



(b)

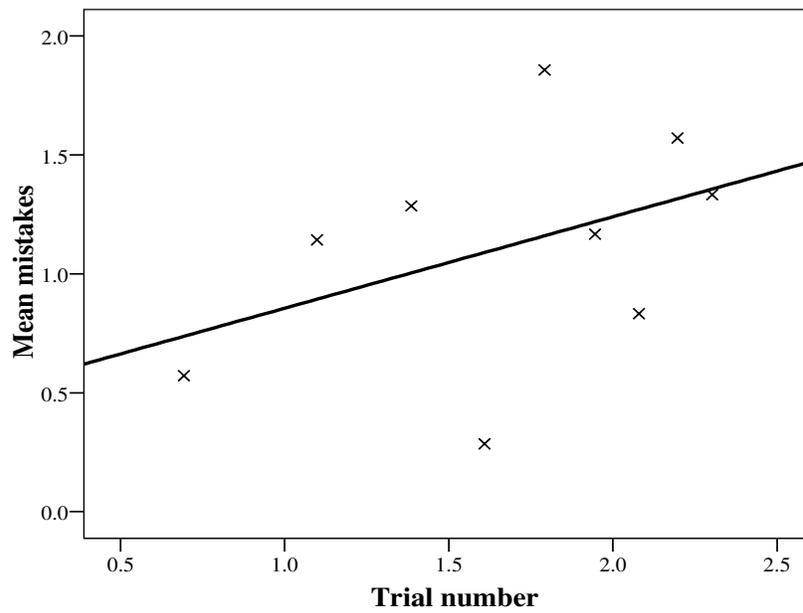


Figure 5.2. The mean number of mistakes (i.e. empty wells visited prior to finding a rewarded well) made by (a) control and (b) CORT birds in relation to $\ln(\text{trial number})$ in the spatial learning test. Lines indicate linear regressions; see text for analysis.

Visual task

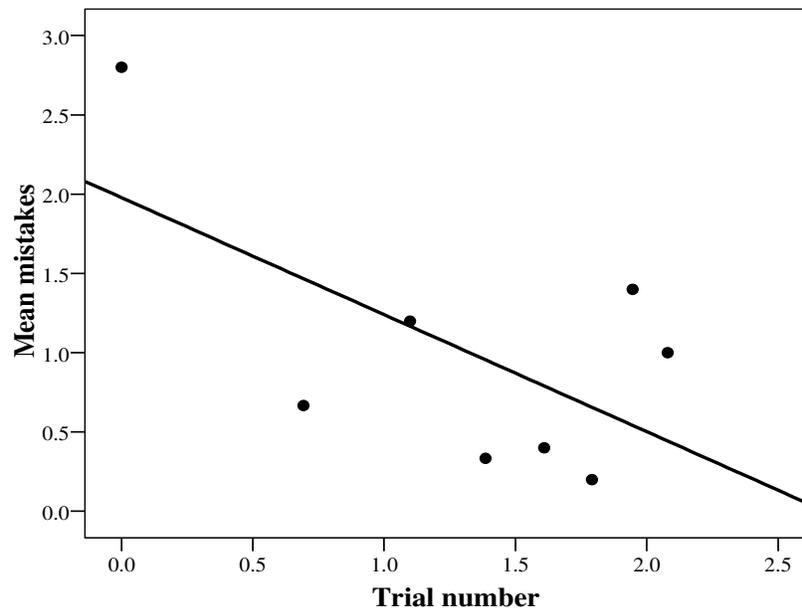
I found no effect of sex on performance in the visual task (mean approach time $F_{1,13} = 0.90$, $p=0.36$; mean search time $F_{1,13} = 0.58$, $p=0.46$; mean number of mistakes for both A and B presentations, $F_{1,11} = 0.17$, $p=0.69$; error regression gradient $F_{1,8} = 1.65$, $p=0.24$). Data from males and females were, therefore, pooled for subsequent analyses.

As with the spatial test, there was a significant effect of day on approach time ($F_{1.5,15.4} = 4.81$, $p=0.031$, $N=12$), with birds approaching the tray more quickly in later trials. There was no effect of treatment on approach time ($F_{1,7} = 0.10$, $p=0.76$), nor any interaction between day and treatment ($F_{1,7} = 0.60$, $p=0.62$). There was also a significant effect of day on search time ($F_{3,21} = 3.23$, $p=0.043$, $N=12$), with all birds reducing their search time from day to day, but no significant effect of treatment on search time ($F_{1,4} = 0.01$, $p=0.92$) nor any interaction between day and treatment ($F_{3,12} = 0.24$, $p=0.87$).

As with the spatial trial, there was a significant interaction between individual search time in presentation A and treatment on individual search time in presentation B (Fig. 5.1(b); $F_{1,9} = 7.94$, $p=0.02$). The relationship was similar to that observed in the spatial trials, with control birds showing a reduction in search time from presentation A to presentation B, whereas CORT birds showed no improvement. There were no outliers to consider in this case.

Similarly, when comparing rates of learning between the treatments, control birds showed a strongly negative gradient (-0.82 ± 0.17), with a reduction in the number of mistakes made on successive days, while in CORT birds there was no significant change in the number of mistakes made over the week of testing (-0.10 ± 0.19 ; Fig. 5.3). There was thus a significant difference in the rate of learning between the two treatment groups ($t_8 = 2.83$, $p=0.022$).

(a)



(b)

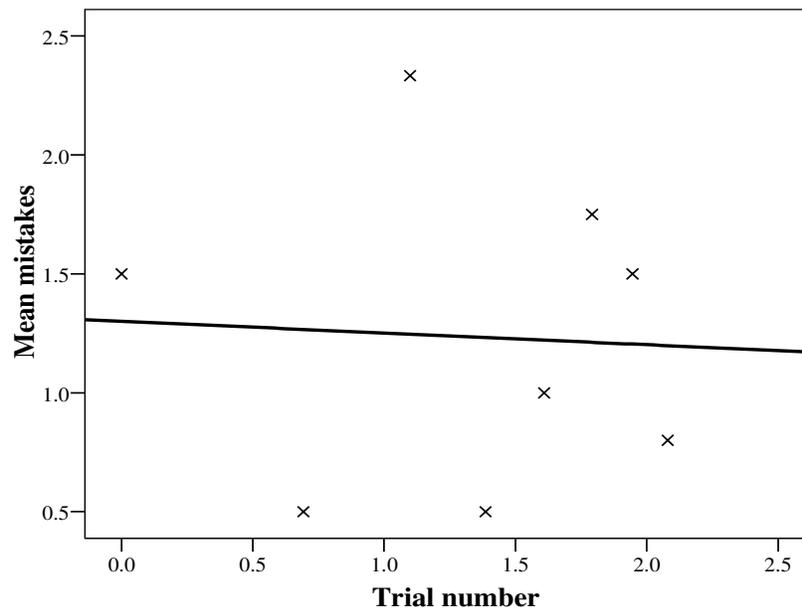


Figure 5.3. The mean number of mistakes (i.e. empty wells visited prior to finding a rewarded well) made by (a) control and (b) CORT birds in relation to $\ln(\text{trial number})$ in the visual learning test. Lines indicate linear regressions; see text for analysis.

DISCUSSION

Relatively few studies have examined the effects of early stress or exposure to CORT on learning and memory in avian species, but this study clearly demonstrates clear trends to suggest negative effects of elevated corticosterone concentrations in early life on adult performance in spatial and visual learning tasks. As control birds performed better than CORT birds in both spatial and visual tasks in adulthood, the results indicate that early exposure to an episode of high CORT levels during early development could have a profound impact on learning and memory evident much later in life.

These results are consistent with those found in some mammal studies (Dachir et al. 1993; Sandstrom & Hart 2005), which show negative effects of early stress on adult cognitive ability, but are in opposition to a number of others. Some studies have reported no change in adult learning performance following experience of early stress, and indeed, have even suggested that some exposure to early stress can improve the efficiency of the negative feedback system associated with glucocorticoid release, so delaying the cognitive deficits that appear naturally as a result of aging (Meaney et al. 1988; Meaney et al. 1991). Since control birds generally showed a faster rate of learning than CORT birds in this study in both visual and spatial tasks, it seems that the type and severity of the stress experienced is critical when considering effects on test performance. It may be that a smaller CORT elevation or a shorter period of administration would have buffered the CORT birds against the effects of later stress, and improved their ability to perform in learning tasks in later life.

However, not all aspects of learning and memory were affected equally in the current study. For example, in both tests mean overall search time (i.e. the time taken to find a rewarded well) decreased as the week progressed, with birds finding the food more quickly in later trials. However, this aspect of learning and memory was not significantly affected by CORT exposure. Whilst in control birds the reduction in search time might be attributed to the associated drop in the number of mistakes made (see below), the same trend was also seen in the CORT birds in which the number of visits to such empty wells remained constant. Therefore, the explanation may at least in part be due to a faster rate of searching as birds became experienced in the test protocol, so that search time was independent of treatment. Such an explanation is supported by the reduction in approach time from day to day in birds from both treatment groups.

Results also suggested that search times changed in opposite directions between presentations A and B for the CORT and control birds. This relationship, which was found

in both types of learning trial, may have arisen because control birds used the available cues to reduce their search time, whereas CORT birds searched more randomly. Control birds that took longer to find food in presentation A were likely to have removed a number of balls before finding a rewarded well, so would have learned that not all cotton balls covered seed (i.e. the longer they searched, the more they could have learned). In contrast, those birds that by chance found food very quickly in presentation A would probably have removed fewer balls overall, and so would not have had the chance to learn that only a minority of wells were rewarded. Consequently these birds may have spent longer searching in presentation B, because they had not determined the underlying rule behind the task. CORT birds, on the other hand, showed no change in search time between the two presentations. This could suggest that this measure may have been an indicator of their behavioural phenotype – if they were fast searchers in presentation A, they would be fast searchers in presentation B, a trait that is independent of learning. Kitaysky et al. (2003) showed a similar long-term effect of early corticosterone exposure on kittiwake chicks, with CORT-treated birds unable to reduce the time taken to solve a spatial task as quickly as controls. Such a result also suggests that CORT birds were less able to improve their performance from presentation to presentation, perhaps because they weren't learning anything about the underlying rules. However, the findings for control birds in the spatial trial were heavily influenced by one outlier, which when removed, rendered the interesting interaction between search time and treatment non-significant (Fig. 5(a)), meaning we should interpret these findings with caution. Even with the outlier removed however, there are clear differences between the two treatment groups, and if we also consider the relationship found in the visual trials, it seems likely that CORT and control birds are showing genuine differences in their searching behaviour. It may be that a larger sample size would have made interpretation of these differences easier.

A separate measure of performance used in the present study was the number of empty wells each bird visited before finding one containing food (i.e. the number of mistakes). In this study, controls showed a steeper reduction in the number of mistakes they made in both the spatial and visual tests than birds exposed to CORT. This would suggest that the memory capability of control birds was significantly better than that of their CORT-fed conspecifics. This result is unlikely to be a consequence of differences in motivation, since during the trials, all birds were seen to approach the tray and engage in some level of searching, whether that was removing cotton balls or hopping around the tray. Searching behaviour suggests that the subjects were motivated to find food, although it is not possible to say whether the levels of motivation caused by hunger varied between

individuals. However, since all birds were attempting to do the task, and some just made more mistakes than others, it seems likely that differences in performance were the result of ability rather than incentive. Consequently, despite treatment having no effect on an individual's ability to learn the basic protocol of this task (i.e. to progress beyond phase 5 of training), CORT birds seemed unable to use spatial and visual cues in the same way as controls.

Since there was no difference in the number of control and CORT birds that completed the training phase and proceeded to testing, it seems unlikely that elevated CORT levels during development had a negative effect on motor skills, and indeed, since CORT birds continued to make a high number of mistakes, they clearly had no difficulty in physically removing cotton balls to retrieve food. The difference in the ability to learn could therefore arise because the early administration of corticosterone either affects a subject's ability to register the relevant environmental cues or some stage of memory retention or formation (Kitaysky et al. 2003). This could be as a result of reduced neurogenesis or interrupted development in parts of the brain associated with visual processing or spatial learning, as has been found in various mammal species (Gould & Tanapat 1999).

There is also some evidence that adrenal steroid receptors in the brain may be affected by exposure to elevated corticosterone concentrations, which can lead to alterations in spatial memory. For example, Hodgson et al. (2007) found that zebra finches selected for a high corticosterone response to acute stress had significantly reduced densities of mineralocorticoid receptors in the hippocampus and as a consequence were impaired in their ability to perform a spatial task compared to controls. Such a result suggests that corticosterone may in fact have an organisational effect on the brain, something that is likely to occur in early life but which will have long-lasting, if not permanent effects. However, although changes in the hippocampus may explain the spatial impairment in my CORT birds, it is not clear what could be causing them to also perform badly in the visual task. In Hodgson et al.'s selected zebra finches, high CORT birds actually showed a tendency to perform better than controls on a visual task, which was suggested may be due to some degree of visual compensation as a direct result of the spatial impairment (Hodgson et al. 2007). As the CORT birds in the present study performed badly on both spatial and visual tests, it may be that some region analogous to the hippocampus in its importance for visual tasks has been affected in this study, or alternatively, some part of the brain that is more fundamental for general learning and memory. Also, since the subjects used in this study were chronically exposed to elevated

corticosterone levels, we might expect to see more severe changes in their learning performance than in birds simply selected for a high peak stress response.

Consequently, in order to understand the full effects of stress hormones on learning and memory, and exactly how they may be shaping specific areas of the brain important in certain types of learning, more integrative studies are needed, combining techniques to deduce the mechanisms behind observed behaviours. Future work on avian species could also consider whether corticosterone elevation in early life could have biphasic effects on cognitive ability as has been demonstrated in mammals (Casolini et al. 1997; Sandstrom & Hart 2005), and in addition, the effects of embryonic exposure to corticosterone. Since various studies show that behavioural phenotype can be altered by maternal effects (Nordgreen et al. 2006; Wada et al. 2008) it would be interesting to look for associated changes in learning performance. Another interesting consideration is at what age the effects of exposure to early stress are manifest. One study conducted on rats showed that early exposure to increased stress not only had negative effects on the ability of adult animals to successfully complete spatial tasks, but also that these effects were absent when the rats were given the same tests at a younger age (Brunson et al. 2005), suggesting a delayed, progressive impairment of cognitive ability as a result of early stress.

Food distribution is always patchy, so it is an advantage for any animal to be able to remember the location of a food source or relocate it, using environmental cues. This study suggests that a short-term increase of CORT in the nestling phase could have a significant impact on an individual's ability to forage successfully, such that survival and reproductive success could be compromised. Additionally, attention to spatial and visual cues available in the environment is likely to be important in predator avoidance, having a further effect on survival probability. Other studies (Kitaysky et al. 2001, 2003) suggest that there are also benefits associated with increasing CORT secretion during the period of parental care, since high levels of CORT amplify the frequency of begging behaviour and so may boost food allocation. However, although such a strategy may increase the chance of survival in the short-term, the long-term consequences may have serious negative implications for adult fitness. This study has revealed a significant cost associated with exposure to early stress, similar to that shown by Buchanan et al. (2004), who demonstrated a negative effect of developmental stress on song learning and performance in adulthood, which is likely to be harmful for breeding success. However, this is the first study that I know of to demonstrate that early corticosterone exposure can have negative effects on both spatial and visual learning and memory in adulthood, a cost that has potential implications not only for breeding success, but also for survival with regards to

foraging and predator avoidance. I have demonstrated that an elevation of stress hormones during development can have effects on learning and memory long after that stress has ceased, and if increased exposure to corticosterone during this important developmental phase can cause cognitive deficits that can never be rectified, then such an elevation could have significant implications for lifetime fitness.

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Chapter 6

The effect of early growth and nutrition on adult dominance behaviour in the zebra finch

ABSTRACT

Despite extensive study of the costs and benefits of dominance status, relatively little is known about exactly why some individuals become consistently more successful than others in gaining access to resources. With more and more evidence accumulating to suggest that the early environment experienced by an animal is important in determining the adult phenotype, it is important to know how environmental variability might influence behaviours that contribute to competitive performance. Here I investigate whether diet quality and growth trajectory in very early life can affect adult dominance behaviour. I experimentally exposed male zebra finch chicks to variable food quality, and examined the effect on dominance in adulthood through a priority of access test. Nestlings received either consistent low or high quality food for 30 days after hatching, or a switch between the two at day 15, leading to differences in growth trajectory between the groups. I found individual behavioural differences which were linked to diet consistency: birds reared on a consistent diet (either consistently high or low) were generally more successful in male-male interactions than birds on a variable diet, though this success was not linked to priority of access. Dominance hierarchies with regards to conspecific interactions were also consistent over time, suggesting stable phenotypic differences. These differences were not linked to individual growth trajectories. However, I did find that birds which grew the least in the first 15 days of life had priority of access to a group of potential mates in my test. I discuss these results in the context of the early environment shaping dominance related behavioural traits in order to maximise survival probability in current surroundings.

INTRODUCTION

Dominance is a complex behavioural trait, often simplified and defined as “success in contests over critical resources” (Qvarnstrom & Forsgren 1998). In many populations, dominance status can be an important determinant of overall fitness, since these critical resources include food, shelter, and mates, which are essential for survival and reproductive success (Robinson-Wolrath & Owens 2003). In certain animal groups a dominance hierarchy is formed, where some group members are subordinate to others. These hierarchies are determined either by aggressive or affiliative interactions, or by the use of cues such as aggressiveness, body size, or fighting weaponry (Qvarnstrom & Forsgren 1998), and each individual is aware of his or her approximate ranking. Additionally, these ranks are affected by sex, age and body size (Richner 1989). Males usually dominate females, adults dominate juveniles, and larger individuals tend to dominate smaller (Richner 1989). Preferential access to resources means that in the majority of vertebrates, dominants enjoy higher survival and reproductive success than subordinates (Richner 1989; Creel 2001; Verhulst & Salomons 2004), and in some cases, reproduction in subordinates can be prevented or suppressed entirely (Guhl 1956). However, the benefits of a high social status are somewhat balanced by the associated costs. Dominant individuals regularly suffer a higher risk of predation (Qvarnstrom & Forsgren 1998); dominance and body size are often highly correlated (Richner 1989), so dominance can be associated with increased energy stress (Qvarnstrom & Forsgren 1998) and dominants are often required to fight with or threaten others to obtain and maintain a high social position, which can be costly in terms of energy and time, and carries the dangerous possibility of wounding or death (Huntingford & Turner 1987). Regular contests and aggressive interactions can also lead to an elevation of stress hormones, which can have negative effects over a prolonged period (Creel 2001). A high level of testosterone, which is important in aggressive behaviour, is often traded off against effort invested in parental care, such that offspring survival can be compromised (Sargent 1985; Qvarnstrom 1997; Verhulst & Salomons 2004). Consequently, despite dominance conferring many advantages, such costs to high social status must also be considered.

Although the costs and benefits of social status have received much attention, there is less known about the mechanisms influencing the development of behaviours related to dominance. Several recent studies have demonstrated that the early environmental conditions experienced by an animal can shape its phenotype not only in terms of

morphology, but also in terms of behaviour (Nowicki et al. 2002; Kitaysky et al. 2003; Fisher 2006; Spencer & Verhulst 2007), but it remains unclear whether early developmental conditions can potentially influence dominance status in later life. Currently, there are suggestions that the nutritional environment and subsequent growth trajectory experienced shortly after birth could have an effect on traits linked to dominance in adulthood. Most studies in this area have been conducted in fish. Johnsson et al. (1996) increased dominance status in trout by administering growth hormone, suggesting that the behavioural change came about as a result of increased growth escalating energy demand and therefore competitive ability. A similar result was found in salmon, where faster growing fish demonstrated higher aggression, most likely as a consequence of an increased energy requirement (Nicieza & Metcalfe 1999). In contrast, in a study of male swordtails, individuals that experienced slow growth followed by a period of rapid growth as juveniles displayed subordinate behaviours, while conspecifics that had grown normally were much more likely to exhibit aggressive, dominant behaviours (Royle et al. 2005). Similarly, rainbow trout that had undergone a period of fast growth early in life then went on to become subordinate in paired interactions (Overli et al. 2004). In these cases, the behavioural outcome seems to be linked to a period of accelerated growth, though with opposite effects. Since rapid growth requires a massive input of energy, one could argue that such growth has the potential to either increase or decrease competitive ability and aggressive behaviour. It is possible that with a higher energy demand, rapidly growing individuals will be more highly motivated to compete aggressively for resources, as was suggested by Johnsson et al. (1996) and Nicieza & Metcalfe (1999). Alternatively, it may be that since extensive resource allocation to rapid growth carries a variety of costs (Metcalfe & Monaghan 2001), individuals that are undergoing, or have undergone, growth acceleration may not be able to meet the added costs of aggression and vigilance required to obtain and maintain a high dominance position (Royle et al. 2005). However, far less is known of this phenomenon in other vertebrate taxa. Unlike fish, birds generally have fixed and relatively short growth periods. Young passerines grow extremely quickly in the first few weeks of life, and can be more than 80% of their adult size only 10 to 20 days after hatching (Ricklefs 1968). Therefore, if specific growth trajectory were linked to behaviour, changes in normal development could have significant consequences for the adult phenotype, and subsequent survival and fitness.

In this study, I aimed to determine whether early variation in nutrition, leading to differences in growth trajectory, could influence the dominance status of an individual in adulthood. To do this, I manipulated diet quality and subsequently growth trajectory in

male zebra finches and examined the effect on dominance related behavioural traits in adulthood. In captive populations of this animal, there is relatively little competition over food and perches, since these resources are always in plentiful supply (Ratcliffe & Boag 1987). However, access to mates is limited and there is fierce competition between individuals for access to potential partners. Since this competition is most intense between males, I chose to focus on male-male interactions in the presence of females, and examine behavioural differences associated with variability in the early environment.

METHODS

Subjects

The subjects were selected from the group of birds that had undergone the various diet manipulations described in chapter 2, with a total of 48 males randomly selected and tested for dominance. Testing took place once the subjects were fully adult (>120 days). A fortnight prior to testing, subjects were caught and re-housed in new standard holding cages with same sex non-related individuals. Birds to be tested together were housed in separate holding cages. These holding cages contained both normal perches and small perches only large enough to allow one bird to perch at any one time (single bird perches). Subjects remained in these randomised groups for a minimum of 7 days before being tested in the observation cage.

Dominance testing

The outcome of competition for access to mates was assessed using a display perch and viewing window. Groups of test males were placed together in one half of a cage and groups of focal females in the other half, with the two halves separated by wire mesh. The males were able to hear the females in the other cage half and so were motivated to display and investigate. However, the mesh divide was mostly covered with an opaque screen, so that the males could only see the females through a small window near the top (Fig. 6.1). A perch was made to run through both sides of the cage, spanning the whole length of the female half and just passing through to the male half (Fig. 6.1). The small perch on the male side was the only place the males could perch to see the females. The proximity of

potential mates increased the motivation of the subjects to compete for access to the viewing window.

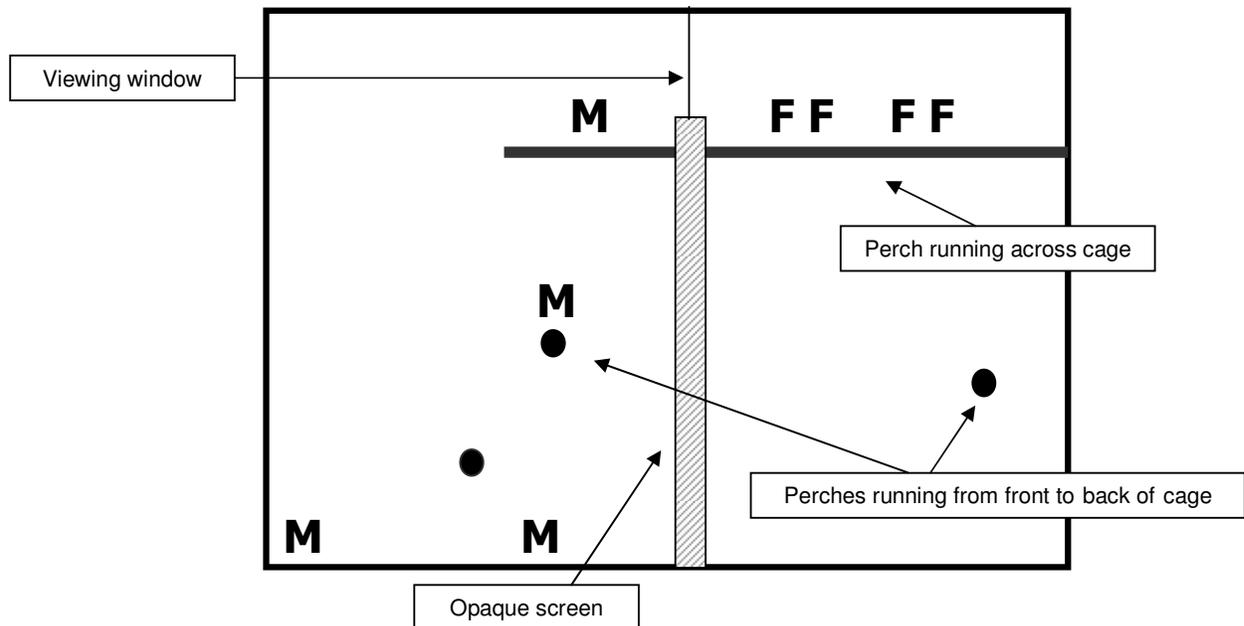


Figure 6.1. Priority of access test cage showing a male (M) viewing the females (F) on the other side through the viewing window.

Test groups comprised four males representing each of the four diet treatments (HH, HL, LH, LL), resulting in a total of 12 groups. Within test groups, birds were not related, had never been housed together, and were grouped depending on hatching order, with first hatched birds in quartets and last hatched likewise. Each test group was allocated four focal females. These females were unrelated to the test males, were young, and of mixed family origin. The males forming the test group and their focal females were introduced simultaneously meaning that all birds entered the new cage at the same time, so reducing the likelihood of aggression based on a perceived prior residence/intrusion asymmetry.

Within test groups, birds were individually identified using small white dots on the head. White is not thought to influence the perceived status of an individual zebra finch (Cuthill 1997), and so the markings should not have altered individual behaviour, plus they could not be seen by the females. Following the release into the cage, observations began immediately from behind a cloth screen placed approximately one metre from the cage

front and completely obscuring the observers from view. Each test lasted for 30 minutes, during which time only the males were observed. All aggressive encounters were recorded, specifying the type of interaction and the identity of the birds involved. The interactions were categorised as chasing, pecking, fighting and displacing. Birds were recorded as chasing if one individual flew after another; as pecking if one bird attacked another with its beak; as fighting if birds attacked one another with their wings and/or feet; and as having displaced a cage mate if by landing on a perch they caused another bird to leave that perch. In recording these interactions, one bird was identified as the initiator of the aggression, and the other or others as the recipient/s. These observations were translated directly into wins and losses for each bird, with subjects doing the chasing, pecking, fighting or displacing considered winners, and those receiving the pecks/being chased etc the losers. The observers also used a scan sampling method to record the identity of the bird at the viewing window every 20 seconds. The number of interactions each bird was involved in, the number won and lost, and the number of times seen on the perch were then totalled for each bird. I defined dominant individuals as those that won the majority of the aggressive encounters they were involved in, and expected that this competitive edge would allow dominants to spend a greater proportion of time at the viewing window, so having the best access to potential mates. At the end of the test period, all birds were recaptured and returned to their holding cages. 8 weeks after this initial dominance test, four of the original test groups (16 birds) were re-tested using exactly the same procedure.

Statistical methods and data handling

Data were mostly analysed using general linear models (GLMs) and non-significant variables were removed in a stepwise fashion. From the behavioural observations, I was able to determine the total number of aggressive interactions within each test group, the number of interactions each bird was involved in, and the total number of interactions won by each individual. All interactions between birds resulted in one bird being defined as the 'winner' and one as the 'loser'. The number of wins and losses attributed to each individual were totalled and expressed as a proportion of the total number of interactions any individual was involved in. Since the number of interactions in each test group varied considerably (ranging from 38 to 395 with an average of 136), I also considered the total number of interactions each individual was involved in expressed as a proportion of the total interactions that took place in the cage. The number of times each individual was seen

at the viewing window was also converted to a proportion, based on the total number of times any bird was recorded at the window during the trial. Since these proportion scores were normally distributed, I used Pearson's correlations.

Repeatability scores for various behavioural measures were calculated for the 16 males retested 8 weeks after the initial test, using a one-way ANOVA with bird identity as a fixed factor, after Lessells and Boag (1987). All analyses were conducted using SPSS 15, and $N=48$ males unless otherwise stated.

RESULTS

Treatment

As expected, chicks on the different diet treatments showed different growth trajectories. Chicks allocated to the H or L diet at hatching showed no difference in their hatching mass ($F_{1, 185}=0.088$, $p=0.77$). However, between 0 and 15 days of age, H diet birds gained significantly more mass than L diet birds ($10.94 \pm 1.03\text{g}$ and $10.40 \pm 1.48\text{g}$ respectively; $F_{1, 184} = 8.49$, $p=0.004$). After the diet switch at 15 days, there was an accelerated rate of growth in the LH treatment group compared to the other groups, as consistent with a transfer to a higher protein diet (HH mass gain, $2.43 \pm 0.62\text{g}$; HL mass gain, $2.39 \pm 0.69\text{g}$; LH mass gain $3.08 \pm 1.30\text{g}$; LL mass gain, 2.75 ± 0.81 ; $F_{3, 184} = 6.17$, $p=0.001$). Birds that had grown more slowly to begin with were able to fully compensate, and there was no significant difference between the treatment groups in body mass at 100 days ($F_{3, 185}=1.69$, $p=0.17$). There was no effect of sex on growth during the diet manipulation (mass gain 0-15 days, $F_{1, 184} = 0.66$, $p=0.42$; mass gain between 15 and 30 days, $F_{1, 184}=0.019$, $p=0.89$).

Dominance traits

There was a strong positive correlation between the proportion of individual encounters a male won and the proportion of total cage encounters he was involved in ($r=0.64$, $N=48$, $p<0.001$), although surprisingly, there was no correlation between the proportion of individual interactions won by a male and the proportion of time he spent at the viewing window ($r=0.049$, $N=48$, $p=0.74$).

Diet treatment had no effect on the proportion of individual encounters won ($F_{3,44}=1.56$, $p=0.21$) or the proportion of total cage encounters any bird was involved in ($F_{3,44}=0.26$, $p=0.86$). On average, males on a consistent diet (HH or LL) showed a trend towards winning more of the encounters they were involved in than males on a variable diet (HL or LH) (Fig. 6.2; $F_{1,46}=3.79$, $p=0.058$), but there was no effect of diet consistency on the proportion of total cage encounters any bird was involved in ($F_{1,46}=0.073$, $p=0.79$). Diet treatment had no effect on the proportion of time any male spent at the viewing window ($F_{3,44}=1.09$, $p=0.36$) and nor did diet consistency ($F_{1,46}=0.51$, $p=0.48$).

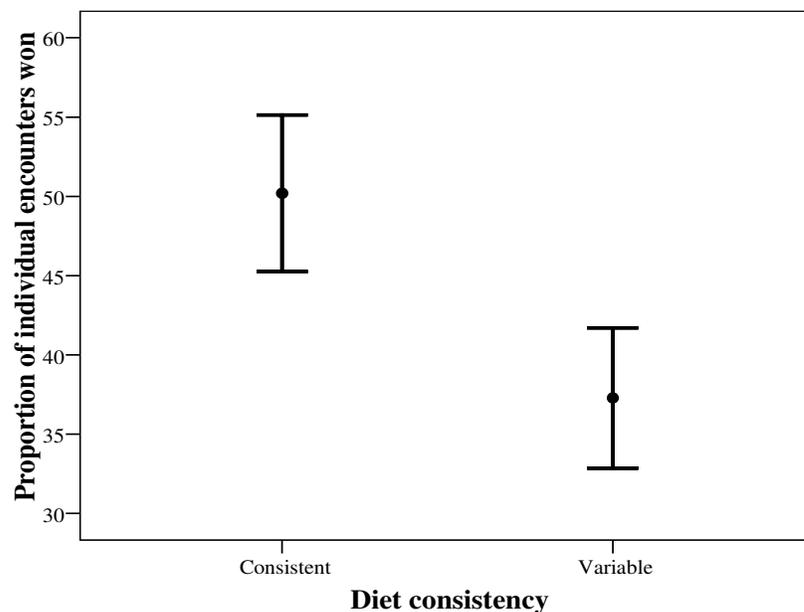


Figure 6.2. The proportion \pm s.e. of individual interactions won by males on a consistent diet (HH/LL) and males on a variable diet (HL/LH) when tested for dominance at adulthood.

Early growth was not important in determining either the proportion of individual encounters won (mass gain days 0-15, $F_{1,46}=0.04$, $p=0.84$; mass gain days 15-30, $F_{1,46}=0.25$, $p=0.62$) or the proportion of total cage encounters a bird was involved in (mass gain days 0-15, $F_{1,46}=0.51$, $p=0.48$; mass gain days 15-30, $F_{1,46}=0.04$, $p=0.85$). However, early growth rate was significant in access to the viewing window. Males that grew the most between days 0 and 15 spent the least time at the viewing window, and males that grew the least between days 0 and 15 spent the most time at the viewing window (Fig. 6.3; $F_{1,46}=4.83$, $p=0.033$). There was no effect of growth rate between 15 and 30 days on access

to the window ($F_{1,46}=2.70$, $p=0.11$). Adult body mass was also not significant in determining these dominance measures (proportion of individual interactions won, $F_{1,46}=0.63$, $p=0.43$; proportion of total interactions involved in, $F_{1,46}=0.00$, $p=0.99$; proportion of time at the viewing window, $F_{1,46}=1.85$, $p=0.18$).

Using behavioural measures from the four test groups that underwent both the original test at 120 days, and the repeated test 8 weeks later, birds were highly repeatable in the proportion of individual encounters won ($r=0.49$, $F_{15,16}=2.92$, $p=0.02$, $N=16$). Subjects also showed a trend towards being repeatable in the proportion of time they spent at the viewing window ($r=0.34$, $F_{15,16}=2.01$, $p=0.089$, $N=16$).

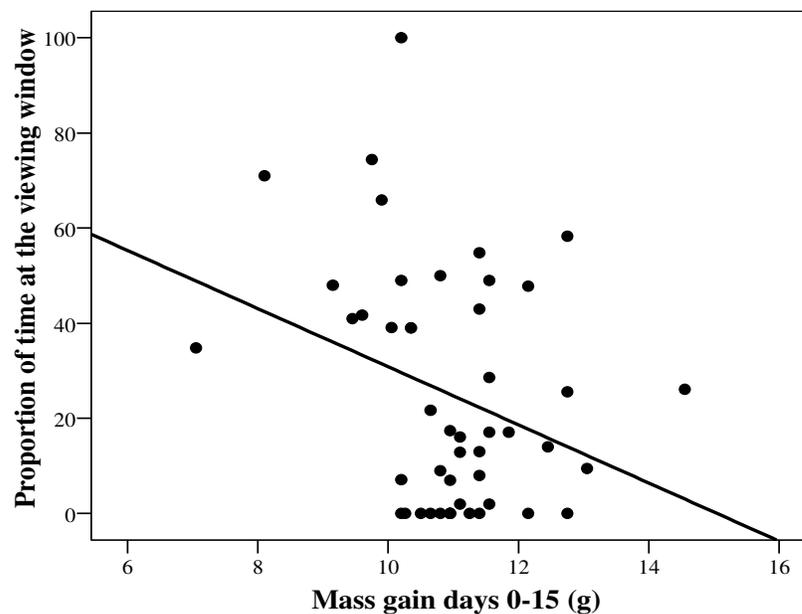


Figure 6.3. The relationship between mass gain during the first 15 days of life and the proportion of time spent at the viewing window by individual males when tested for dominance at adulthood.

DISCUSSION

Birds raised on a consistent diet showed a trend towards winning more of their individual interactions than birds raised on a variable diet, potentially suggesting that nutritional stability made individuals generally more aggressive and successful in conspecific interactions, and thus more likely to hold a higher dominance status. Since both kinds of bird on the stable growth trajectory (fast growing HH birds and slow growing LL birds)

showed a tendency to be more aggressive and dominant in terms of male-male interactions than both kinds of bird on the variable growth trajectory (fast/slow HL birds and slow/fast LH birds), I propose that these findings are the result of differences in the consistency of the early environment experienced, rather than differences in nutritional quality and subsequent rates of growth. In chapter 2, I showed that diet consistency was also important in determining certain personality traits, with birds raised on a consistent diet being reliably bolder than birds raised on a variable diet. I suggested that this may be due to some kind of environmental shaping, in which personality types are influenced by early developmental conditions, to give individuals the best chance of surviving the environment they are born into (Bateson et al. 2004; Monaghan 2008). In terms of behavioural syndromes, boldness and aggression are often correlated (Huntingford 1976; Drent et al. 2003; Armitage & Van Vuren 2003; Brown et al. 2007), so the fact that the proportion of encounters won here is also linked to diet consistency may suggest that such behavioural syndromes exist in this species, i.e. if birds raised on a consistent diet are bold, they are also aggressive. As discussed in chapter 2, a consistent diet experienced in early life may forecast a stable environment in which population density is likely to be high, so favouring bold, aggressive birds that can outcompete conspecifics for access to resources. A variable diet, on the other hand, could suggest an unstable environment, in which population density might be lower, and could arguably lead to shy, less aggressive males, since a high level of aggression may be an unnecessary cost in such surroundings. The early programming of social dominance has also been observed in the rat (Rosen 1961; Becker & Ezinga 1969), though there is no suggestion from these studies that such programming is adaptive.

Looking more closely at individual growth trajectories, I found no evidence to suggest that growth was linked to aggression. Growth during the first 15 days of life appeared to be important in determining access to the viewing window however, with individuals in all diet groups that grew the most in this early life stage spending the least time in view of the females. Exactly why early growth should be linked to window access in this way is difficult to explain, but could perhaps again be the result of environmental shaping. If males that grow little in the first 15 days anticipate a poor environment, in which survival probability is low, they may be eager to breed at the earliest opportunity, and may therefore spend more time trying to attract potential mates. It is interesting that early growth had no effect on aggression in this study, and also that growth acceleration in the second phase of the diet treatment (days 15-30) which would have taken place in the LH diet group, seemed unimportant. A link between early growth and overall dominance

status has previously been found by Richner et al. (1989) in carrion crow juveniles, with birds raised on limited food showing depressed growth rates and suffering subsequently lower social status than their control siblings. However, this manipulation resulted in experimental birds reaching a significantly lower final weight than control birds fed *ad lib.*, and so growth effects cannot be separated from the effects of body size, which is often strongly correlated with social status (Richner 1989; Malyon & Healy 1994; Clutton-Brock et al. 2006). In the present study, adult body mass was not linked to any of my dominance measures, presumably since slow growing birds were able to compensate for a poor start and individuals were not significantly different in their adult body mass (see chapter 2). Many studies have considered the effect of dominance status on growth rate (Nakano 1995; Martin-Smith & Armstrong 2002; Sloman et al. 2008), but few have looked for the reverse effect as we have here, so further work is required to confirm exactly how early growth is important in later dominance behaviour.

There were clear differences in the proportion of interactions won by the subjects, such that in each test cage, some birds were plainly dominant over others. High repeatabilities for the proportion of interactions won by each individual indicate that the results obtained from this experimental method are reflecting real behavioural differences and dominance relationships between individuals, and are not just the results of random chance. However, although birds winning most of their own interactions were also involved in a high number of the total cage encounters, it is surprising that there was no correlation between the proportion of individual interactions won by any male and the time then spent at the viewing window, as I expected birds winning the most encounters to monopolise the perch. Thus in this case, dominance as assessed from male-male interactions did not correspond to potential mate access. However, this could perhaps be explained by considering the elevated costs of being dominant in a larger group, when the desirable resource (in this case, potential mates) is essentially clumped. It could be that in a group of four, dominant males have to spend so much time asserting their status that they do not actually reap the benefits of their position, while males who are lower in the hierarchy are involved in fewer interactions and are able to sneak onto the viewing perch while the dominant male is otherwise engaged. This may mean that subordinates actually have greater access to potential mates in a group of four than they would have in a pair, but by using a sneaking strategy, rather than competing directly with more aggressive opponents. A study showing such an effect of resource distribution on the benefits of dominance is described in Monaghan and Metcalfe (1985), where when foraging in groups, dominant hares (*Lepus europaeus*) do less well when food is clumped because they must

spend more time defending a clumped food patch as group size increases, so that they have less opportunity to feed themselves. There, as here, competitive dominance does not necessarily result in increased access to the desirable resource, and hence may not increase individual fitness in such a scenario. Other work showing that competitive dominance is not always directly linked to priority of access has been carried out on carrion crows (Richner 1989).

Although groups of contestants showed clear and consistent differences in competitive ability, there was no indication that the quality of the early diet treatment made certain individuals competitively superior. However, experiments with invertebrates have shown that the type of dietary restriction may also be important in determining competitive ability. In a study of Argentine ants (*Linepithema humile*) (Grover et al. 2007), behavioural traits associated with dominance, such as aggression, were shown to be affected by carbohydrate, but not protein scarcity. Ants that had limited access to a carbohydrate food source were less aggressive and less active than controls, but no such behavioural changes were associated with protein restriction. This study shows that competitive performance can be influenced by nutrient availability, but that protein may be less significant for dominance behaviours than other macronutrients, perhaps since protein is used for growth and body repair rather than as the principal metabolic fuel that might be needed in aggressive conspecific interactions. If this were the case for birds, then we may not expect to see pronounced differences between individuals from the different diet treatments, since only protein availability was manipulated.

The study of environmentally induced differences in phenotype and their consequences for individual fitness is of great importance, since there are many environmental factors that are subject to a large degree of variability and consequently, depending on conditions, certain phenotypic traits will give some animals advantages over others. In addition, there is currently much interest in the degree to which environmental shaping could bring adaptive benefits. However, much of the work in this area has been conducted on fish. Fish often show indeterminate growth, instead of the fixed growth period we see in mammals and birds, meaning that further studies on a wider range of vertebrates are required. Although here, there are suggestions that such adaptive shaping could be occurring for dominance related traits, my sample sizes are perhaps too small to draw strong conclusions. Overall though, this study provides yet more evidence that early developmental conditions can potentially shape the behaviour of the adult animal, and that as such, even small environmental changes will have significant effects on individual fitness.

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Chapter 7

Does corticosterone exposure in early life affect adult dominance status in the zebra finch?

ABSTRACT

With increasing evidence that the early environment experienced by a young animal can affect the morphology and physiology of the adult, there is currently great interest in how environmental variability may shape the behavioural phenotype, and whether such shaping has adaptive benefits. The ability of an individual to compete for access to some desirable resource will have a significant effect on the survival and success of that individual throughout its life, and so any environmental effects on behavioural traits associated with dominance will be important. Here I consider whether developmental stress shapes the dominance status of an individual in adult life, whether shaping causes variable fitness under different environmental conditions, and whether phenotypic changes can be passed to offspring. I compared adult birds that had undergone a period of elevated stress during early postnatal life with controls, and measured dominance status through pair-wise interactions in a priority of access test. Much of the work in this area has been conducted on fish, which often show indeterminate growth, instead of the fixed growth period we see in mammals and birds, meaning that further studies on a wider range of vertebrates are required. The tests were carried out under both predictable and unpredictable food conditions. Although no significant effects of developmental stress on dominance status were found, there were trends in the data to suggest that early stress may have a negative effect on the ability of an individual to be dominant as an adult, plus a suggestion that there may be carry-over effects of this stress into their offspring. Subjects exposed to increased levels of corticosterone while in the nest showed a tendency to win fewer interactions than controls in pair-wise tests when experiencing unpredictable environmental conditions. The offspring of postnatally stressed and control birds showed the same trend, but control juveniles also tended to win a greater number of interactions under predictable environmental conditions. I therefore found no evidence to suggest that a phenotype developed under stressful conditions would prosper in a more stressful adult environment. These trends suggest further work is required in this area.

INTRODUCTION

Dominance is an important behavioural trait that has significant consequences for the lifetime fitness and reproductive success of any animal, since dominant individuals gain greater access to limited resources (Qvarnstrom & Forsgren 1998). Despite the extensive literature on the costs and benefits of being dominant or subordinate however, and the link between individual social status and stress profile having been a topic of much interest for a number of years, little is known about the effect of the early hormonal environment on the outcome of later dominance interactions. Varying levels of glucocorticoid stress hormones such as corticosterone (CORT) and cortisol have been linked to competitive ability and subsequent dominance rank, but it remains unclear whether these stress hormones are the cause or the consequence of this status. Subordinates have frequently been shown to exhibit higher baseline levels of glucocorticoids, or higher peak levels in response to stressful circumstances (Louch & Higginbo 1967; Schwabl et al. 1988; Sloman et al. 2001; Overli et al. 2004), but more recently, numerous studies have shown that in some social groups, it is the dominant individuals that are suffering the costs of elevated stress (Creel 2001; Pravosudov et al. 2003; Sands & Creel 2004; Muller & Wrangham 2004). To add to this confusion, other studies have found no relationship at all between dominance status and stress hormone profiles (Poisbleau et al. 2005; Taillon & Cote 2008), suggesting no particular subordination stress or dominance cost, while a review by Sloman and Armstrong (2002) considers the possibility that testing animals in unnatural situations in the laboratory may give an exaggerated view of the stress experienced by individuals regardless of their status.

In general, the majority of studies have focussed on the stress profiles of individuals once their dominance status has been determined, rather than the hormonal mechanisms that may underlie status acquisition. However, there is some evidence that hormone concentrations might actually be responsible for individual differences in social status. For example, rainbow trout (*Oncorhynchus mykiss*) with naturally high levels of cortisol preceding competitive interactions are more likely to become subordinate (Sloman et al. 2001), and similarly, trout treated with cortisol prior to the formation of a dominance hierarchy have been shown to be at a competitive disadvantage (Gregory & Wood 1999). Such studies suggest that rather than the social status of an animal determining its stress levels, dominance rank could in fact be constrained by the hormonal profile. But exactly how the hormonal profile of an individual is determined remains unclear. Adverse changes

in the environment during early life can lead to an elevation of stress hormones in the developing animal, and differential exposure to such hormones during the developmental phase can alter the stress response in adulthood (Anisman et al. 1998). Stress experienced during postnatal development can cause either exaggeration (Kuhn et al. 1990; Hayward & Wingfield 2004; Spencer et al. 2009), or attenuation of the adult stress response (Casolini et al. 1997; Vallee et al. 1997), depending on the type and duration of the stressor. This suggests that the neuroendocrine stress axis is fairly malleable in early life, and it is possible that exposure to stress hormones during development may be important in shaping the adult phenotype, and may even play a part in programming an individual for optimal survival in the environment it is likely to experience (Anisman et al. 1998; Spencer & Verhulst 2007; Love & Williams 2008).

In terms of dominance related behaviours, it is clear that the physiological status of an individual will be important in determining competitive ability. Body condition and energy reserves for example, are likely to affect an animal's willingness and capacity for aggressive interactions, but less is known about hormonal effects. If the stress axis could indeed be programmed to a degree by early experience, and the stress profile of an individual were to predispose it to either a dominant or subordinate status, then the conditions an animal encounters in early development could have significant consequences for its fitness in later life. However, the potential for early hormone exposure to influence the dominance status of an animal remains an intriguing possibility about which little is known. In one of the few studies to have considered this directly, Spencer et al. (2007) exposed nestling zebra finches to elevated levels of the stress hormone corticosterone (CORT) and demonstrated that this early exposure had a negative effect on the subjects' ability to be dominant when between 50 and 60 days of age, with CORT treated birds showing a reduced ability to compete for a perch against a size- and age-matched conspecific control. The authors suggest that this difference comes about as a result of a direct effect of CORT exposure on the stress response, causing elevated peak levels or a faster stress reaction in those birds given the hormone in early life (Spencer & Verhulst 2007). Since this effect of the early environment has already been shown to exist, I was interested in whether such hormone exposure in early life could have consequences for dominance status that persisted into adulthood. However, adult fitness will also be mediated by the current environment, and individuals that do well under one set of environmental conditions may not necessarily do well if conditions change. High and low responses to stress in individuals from the same population may represent alternative coping strategies, which may have different adaptive value depending on the current

environmental conditions (Blas et al. 2007). Therefore, I also asked whether early stress exposure could lead to changes in dominance behaviour that might serve to shape an individual for the environment it expects to experience later in life (Monaghan 2008). If animals that are programmed for a high response to stress have in fact been shaped to be better adapted to cope with a changing environment, we might predict that they may have higher fitness in unpredictable surroundings. To this end, I tested the dominance behaviour of adult zebra finches that had undergone corticosterone manipulation as nestlings, under both predictable and unpredictable diet conditions, with the aim of establishing whether dominance status in the adult bird could be partly determined by environmental conditions during postnatal development, and whether status could be altered by environmental predictability. Since hormonal effects can be long lasting, and can even be carried over into the next generation (Naguib et al. 2006; Naguib et al 2005), I was also interested in looking for possible transgenerational effects of exposure to early stress. I therefore also conducted the same tests on the offspring of these birds.

METHODS

Subjects

Birds that had undergone the corticosterone manipulation detailed in chapter 3 were sorted into single-sex groups at sexual maturity. At around 15 months of age, these birds were then paired with unrelated individuals from the same treatment groups and allowed to breed. Offspring were raised on a standard diet as described in chapter 3, which was the same diet their parents had received as chicks, but did not undergo any CORT manipulation themselves. Pairs were then split and returned to single-sex groups after breeding. Once the chicks were fully fledged and independent, 16 unrelated adult males (~1.5 years old, from the original manipulation group), and 16 unrelated juvenile males (~80 days, their offspring), were randomly selected for dominance testing. Of the 16 adults, 8 had received the CORT treatment as nestlings and 8 were controls. Of the 16 juveniles, 8 were the offspring of parents that had both received the CORT treatment, and 8 were the offspring of parents that had received only the peanut oil control.

Dominance testing

Since zebra finches in captivity have an approximate and fluctuating status system (Ratcliffe & Boag 1987), it was important to offer subjects something desirable and biologically relevant to encourage them to compete. These birds are known to compete for access to a mate (Zann 1996), and so this experiment measured dominance in the context of competition for access to a perch from which males could see and display to females, exactly as described in chapter 6. Because I had only two treatments in this case (CORT and control), birds were tested in pairs instead of in groups of four as described previously. The test cage was set up in exactly the same way as for the diet birds (see Fig. 6.1, chapter 6), and behavioural observations were carried out precisely as described in chapter 6, with interactions between pairs categorised as chasing, pecking, fighting and displacing, and birds again individually identified using white spots on the head. Pairs of males were therefore placed together in one half of a cage, and four focal females in the other half, with the two halves separated by wire mesh.

Test pairs of males comprised one CORT treated adult and one control adult, or for the juveniles, one bird whose parents were both raised on the CORT treatment and one bird whose parents were both raised on the control treatment. All males were matched for age and body mass (within 1.5g). Four focal females were randomly drawn from a pool of 16 for each test. As with the previous dominance experiment described in chapter 6, test males and focal females were removed from their different holding cages and placed into darkened carry boxes for five minutes, then introduced simultaneously so that all birds entered the new cage at the same time, so reducing the likelihood of dominance asymmetries based on prior residency. At the end of the 30 minute test period, all birds were recaptured and returned to their holding cages. Males were re-tested with the same partner under a different diet regime (see below) two weeks after this initial test.

Diet treatment

For 7 days prior to dominance testing, birds were exposed to one of two feeding regimes, a predictable diet (P), or an unpredictable diet (U). On the P diet, birds received the standard diet as described above; on the U diet, birds received the same diet components in the same quantities, but food was removed for a total of 3 hours per day at randomised times between the hours of 08.00 and 19.00. Half of the adult and juvenile pairs received their

first test after seven days on the P diet, and their second test after seven days on the U diet. The remaining pairs underwent testing with the sequence of diet regimes reversed, to control for order effects.

Statistical methods and data handling

All interactions resulted in one bird being defined as the ‘winner’ and one as the ‘loser’, so that from the behavioural observations I was able to determine the total number of interactions won and lost by each individual. The number of wins attributed to each subject and the number of times each bird was seen at the viewing window were used in analyses after the appropriate tests for normality. I used repeated measure ANOVA to look for effects of the CORT treatment and the two diet regimes in both adults and juveniles, comparing each individual against its test partner. I calculated the difference between the number of wins by a control bird and its CORT partner and the number of window visits likewise, and entered these values into a repeated measures analysis, with the diet regime as a between-subjects factor. All analyses were conducted using SPSS 15, and N=8 control and 8 CORT adult males, and 8 control and 8 CORT juvenile males unless otherwise stated.

RESULTS

Adults

Figure 7.1 shows the relationships between hormone treatment, diet regime, and the average number of interactions won. There was no significant effect of the hormone treatment ($F_{1,7}=1.07$, $p=0.34$), meaning that control birds were no more likely to be dominant than their CORT partners, and no effect of diet on the number of interactions won by any bird ($F_{1,7}=2.80$, $p=0.14$). Similarly, there was no significant effect of hormone treatment on the number of times each individual was seen at the female viewing window ($F_{1,7}=0.09$, $p=0.78$), or any effect of the diet regime ($F_{1,7}=0.00$, $p=1.0$). Finally, there was no correlation between the number of interactions won and the number of times any individual was seen at the viewing window on either the P ($r=-0.31$, $N=16$, $p=0.25$) or U diet ($r=0.24$, $N=16$, $p=0.38$).

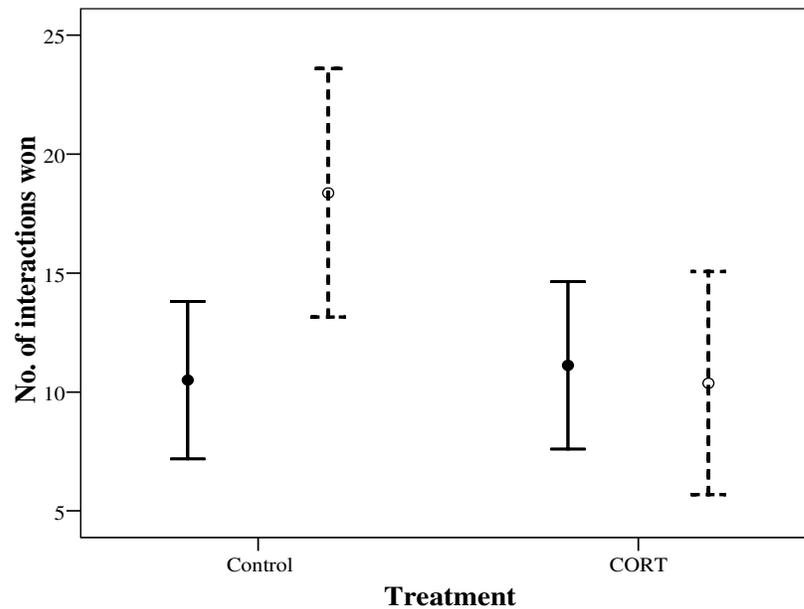


Figure 7.1. The mean \pm s.e. number of interactions won by control and CORT treated adults, when on a predictable diet (solid circles and solid lines), and when exposed to an unpredictable diet (hollow circles and dashed lines). $N=8$ birds per treatment. See text for statistical analyses.

Juveniles

Figure 7.2 shows the relationships between the hormone treatment of the parents, diet regime, and the average number of interactions won. There was no significant effect of parental hormone treatment ($F_{1,7}=2.46$, $p=0.16$) and no effect of the different diet regimes on the number of interactions won by any bird ($F_{1,7}=0.41$, $p=0.54$). Similarly, there was no significant effect of parental hormone treatment on the number of times each individual was seen at the female viewing window ($F_{1,7}=0.20$, $p=0.67$) or any effect of the diet regime ($F_{1,7}=0.23$, $p=0.64$). There was no correlation between the number of interactions won and the number of times any individual was seen at the viewing window on the P diet ($r=-0.21$, $N=16$, $p=0.94$), but on the U diet, there was a positive correlation between the number of interactions won and the number of times any individual was seen at the viewing window ($r=0.59$, $N=16$, $p=0.015$).

Additionally, when tested under the P diet, pairs of juveniles engaged in a greater number of interactions than pairs of adults (mean total adult interactions = 21.63 ± 3.58 ; mean juvenile total interactions = 60.25 ± 8.70 ; independent samples $t_{30}=4.12$, $p<0.001$)

and showed a non-significant trend in the same direction when tested under the U diet (mean total adult interactions = 28.75 ± 6.08 ; mean juvenile total interactions = 48.88 ± 9.55 ; independent samples $t_{30}=1.60$, $p=0.12$).

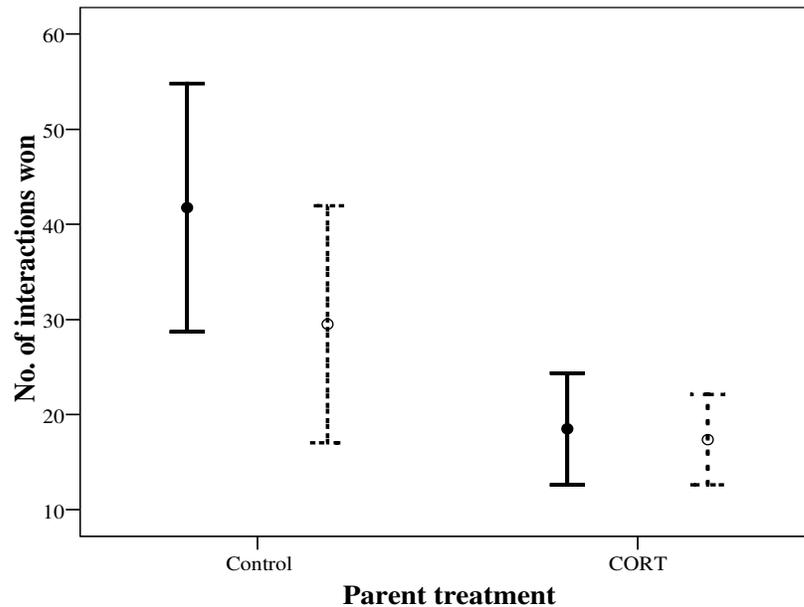


Figure 7.2. The mean \pm s.e. number of interactions won by juveniles whose parents received either the control or CORT treatment, when on a predictable (solid circles and solid lines), and an unpredictable diet (hollow circles and dashed lines). $N=8$ birds per treatment. See text for statistical analyses.

DISCUSSION

This study tested the dominance behaviour of adult zebra finches that had undergone corticosterone manipulation as nestlings, under both predictable and unpredictable diet conditions, with the aim of establishing whether dominance status in the adult bird could be partly determined by environmental conditions during postnatal development, and whether any such effects could be altered by environmental predictability. From the raw data shown in the figures above, both control adults and the offspring of control adults showed a trend towards winning a greater number of interactions than their CORT rivals when under the unpredictable diet conditions. Although this was only a trend, it is suggestive, especially since it was present both in the originally manipulated birds and in their offspring, and since a similar effect of early corticosterone exposure on dominance

behaviour has been found in this species when tested for dominance between 50 and 60 days of age (Spencer & Verhulst 2007). It may be that although birds from the different treatments do not differ in their competitive ability under normal, predictable food conditions, CORT birds and their offspring are less able to mobilise the energy stores that are required when competing under conditions of unpredictable food. Similarly, control birds could simply become more aggressive under a variable environment, when individual survival and reproductive success is less certain, which may be a cost that CORT birds cannot afford. Aggression levels have been shown to increase in adult sticklebacks (*Gasterosteus aculeatus*) when the environmental conditions became more variable, placing extra energy demands on individuals (Sneddon et al. 2006). In Sneddon et al.'s study, dominant individuals either became more aggressive and dominant under variable environmental conditions, or were unable to maintain their status positions and were deposed. Behavioural differences between individuals related to aspects of the current environment have also been shown in other fish species (Dingemanse et al. 2007; Salonen & Peuhkuri 2007). Since the benefit of winning a fight in the current study (i.e. impressing a number of potential mates), may increase under an unpredictable environment, we might expect birds to increase their level of competition. However, Game theory predicts that when the perceived cost of fighting increases, the relative benefit of contesting decreases and, as a consequence, animals may be less willing to compete (Bradbury 1998). Therefore, if the cost of fighting is higher for CORT birds in general, they may be less willing to engage in lengthy interactions under unpredictable conditions, and as a result, win fewer overall. Control adults showed no trend for winning more interactions under a predictable diet than their CORT-fed rivals, although the juveniles of control parents did. This is interesting, and suggests that the area deserves further exploration. This outcome may be an artefact of the generally higher rate of aggression demonstrated by the juvenile birds. When tested after experiencing the predictable diet regime, juvenile pairs engaged in a greater number of interactions than adult pairs, and also showed a trend in the same direction when tested under the unpredictable diet regime. This may suggest that juveniles are generally more aggressive, or that access to potential mates is more important for them than for the older birds. Young birds are perhaps likely to be more aggressive than older birds, since in the wild they would have to disperse from their natal ground to find their own breeding territory (Zann 1996). Alternatively, it may be that adults are able to more quickly assert their status, or have more experience in assessing an opponent's competitive ability using visual signals, and so engage in fewer aggressive encounters to minimise unnecessary energy expenditure.

It is interesting that there were no effects of the treatment on access to the viewing window in either the adults or their offspring, and surprising that there was frequently no relationship between the number of interactions won by any individual and its access to the viewing window. The only trial in which success in encounters and window access were positively correlated was with the juveniles under conditions of unpredictable food; in all the other tests there was no such correlation. This suggests that although some birds were consistently winning more interactions than their competitors, this competitive success was not necessarily linked to better mate access in terms of the amount of time spent at the window. It could be that males notice the females and then engage in conspecific competition in order to impress them, rather than simply displaying at the window. Though there is much evidence that female zebra finches choose males on the basis of their secondary sexual traits such as beak colour and song complexity (Burley & Coopersmith 1987; Ratcliffe & Boag 1987), less is known about the importance of conspecific aggression in determining female mate choice, though one study suggests it may be of some relevance (Forstmeier & Birkhead 2004). Since it was clear to the observer that in the majority of cases males were very interested in the proximity of potential mates, it seems likely that their aggressive interactions were mediated by the females, meaning that male-male competition could well be important in mate selection in this species.

From the means presented at the end of the results section, it appears that adults tended to show an increase in the total number of aggressive encounters they engaged in when under unpredictable food, while juveniles tended to show a decrease. This could indicate something about the costs of fighting when the environment is changeable. Older birds may be more focused on finding a partner to breed with under these conditions, and so be prepared to expend more energy in competition. On the other hand, young birds could be more concerned with expending resources on body maintenance and survival.

I found no evidence to suggest that early hormone exposure had programmed CORT birds and their offspring to prosper in an unpredictable environment, since CORT birds and their offspring showed no signs of being able to outcompete controls when under conditions of unpredictable food. Instead, it seems likely, from this study and related work (Spencer & Verhulst 2007), that birds exposed to stress in early life may be compromised in their ability to be dominant, both as young birds, and as adults. Indeed, studies on other avian species have shown that individuals with a high peak stress response, as shown for the CORT treated birds, have lower fitness than low responders in terms of survival and reproductive success (Romero & Wikelski 2001; Brown et al. 2005; Blas et al. 2007).

In general, the raw data are suggestive of trends in the same direction as the findings of Spencer & Verhulst (2007), namely that birds exposed to elevated CORT in early life were compromised in their ability to be dominant as adults, with effects that may even have extended to their offspring. One important difference between this work and that of Spencer and Verhulst (2007), is that in the latter, sibling pairs of zebra finches were treated with CORT in the same way as described above, but administration began at day 7 and concluded at day 18, so beginning and finishing earlier than in the current study, in which the treatment was administered between days 12 and 28. Since these authors found a significant effect of this treatment on male competitive ability, whereas the present study found only trends, we could speculate that dominance traits are set soon after hatching, so that it is exposure to CORT in the very first days of life that is critical in determining behaviour, and that stress experienced after this time is less important. This might suggest the existence of some sort of critical period for the development of behavioural phenotype, as has been shown for song learning (Bottjer 1997). Though equally, it may just be that with a larger sample size (20 pairs of birds), treatment differences between individuals were more clearly defined.

The fact that I have only indentified trends in this study is most likely a result of the small sample size, which was limited by other studies requiring use of the same birds. As a consequence, the discussion of these results is naturally highly speculative. However, although I was not able to detect significant differences here, the trends found provide a potentially interesting basis for future work, in that environmental effects on phenotypic development are likely to be at least partly a result of hormonal differences between individuals. The effect of the early environment on the adult phenotype, and whether behaviour is adaptively shaped to suit current conditions, remains a subject of great interest, and further studies with more extensive integration across disciplines are required in order to further explore this exciting new set of questions.

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Chapter 8

General Discussion

Summary

As outlined in the Introduction to this thesis, it has been suggested that the early environment experienced by animals can have a range of effects on the adult phenotype (Metcalf & Monaghan 2001; Bateson et al. 2004; Gluckman et al. 2005; Monaghan 2008). In each experiment outlined in this work, I aimed to determine whether behavioural phenotype could be affected by conditions in early life, and to consider, where possible, whether sub-optimal conditions would have generally negative effects on adult fitness, or could positively shape the individual to survive best in its surroundings.

In chapters 2 and 3, zebra finches were shown to display distinct and repeatable behavioural traits, and the existence of correlations between traits led me to suggest that this species, along with many others, exhibit behavioural syndromes. In studying the effect of the early diet on behaviour, I found strong evidence to suggest that personality traits could be shaped by the consistency of the nutritional environment experienced by the chick, with birds that had experienced a consistent early environment (whether food quality was good or bad) repeatedly bolder than birds raised on a variable diet (chapter 2). When considered in terms of environmental stability throughout life, one can argue that this provides an example of adaptive shaping (Gluckman et al. 2005), with behavioural traits being developed in response to environmental sampling. In investigating the effect of early stress, contrary to some mammal studies (Meaney et al. 1991; Weaver et al. 2000; Penke et al. 2001; Chung et al. 2005), I found no evidence that behaviour was directly shaped by exposure to the stress hormone corticosterone in the early weeks of life (Chapter 3). However, as discussed in chapter 3, there were some interesting effects of growth rate on behaviour. Chicks exposed to elevated corticosterone that gained more mass than their control siblings during the treatment period tended to be less bold than their siblings when tested as adults, while corticosterone exposed birds that grew less than their control sibling tended to be bolder than their siblings. I suggested that this could again be explained using the idea of adaptive environmental shaping, in which growth rate is used as a proxy for environmental richness. The absence of any direct relationship between stress treatment and the measured behavioural traits may also have been the result of the timing of the

manipulation; if, for example, it fell outside some critical period for behavioural development (Bottjer 1997).

In both experiments concerned with the effect of the early environment on adult cognition (chapters 4 and 5), I showed that the sub-optimal early environments experienced by the birds had significant negative consequences for performance on simple learning and memory tasks in later life. These effects were apparent many months after the birds became adult, suggesting they could not be compensated for, and consequently, could not be said to be adaptive under any circumstances. Instead, it seems that rather than preparing the individual for a stressful adult environment (Hales & Barker 2001; Monaghan 2008), these early conditions had a negative effect on the cognitive abilities involved in searching for and remembering the location of a food item using environmental cues. This was evidence that stress in early life, whether nutritional or otherwise, can lead to a compromised individual, since spatial and visual memory will surely be important in finding food throughout life. Since these effects were present in adulthood, many months after the exposure to stress, it also appears that these effects cannot be reversed by non-stressful periods in later life.

In chapters 6 and 7, I set out to test the effect of the early environment on adult dominance behaviour. When considering the dominance status of birds raised on the different diet regimes, the results again suggested that diet consistency was important (Chapter 6). Birds raised on a consistent diet were generally more aggressive, and showed a trend towards winning more of their individual interactions than birds raised on a variable diet. Consequently, in this study, nutritional stability made individuals generally more successful in conspecific interactions and thus more likely to hold a higher dominance position. However, winning a high proportion of interactions was not linked to access to potential mates as might have been expected. If diet stability is linked to aggressiveness however, this may again be evidence of environmental shaping. A number of studies have shown that boldness and aggression can be linked (Huntingford 1976; Drent et al. 2003; Armitage & Van Vuren 2003), and if this were the case, such behavioural coupling may promote survival in a stable environment where population density is high.

In chapter 7, there were trends to suggest that exposure to corticosterone may have negative effects on dominance-related behaviour under certain environmental conditions, but the sample sizes were not large enough to enable me to draw firm conclusions on this point. I certainly found no evidence to suggest that the early environment had programmed birds and their offspring to prosper in particular conditions. Indeed, it is possible that birds

exposed to stress in early life are compromised in their ability to be dominant, both as young birds, and as adults.

Overall then, this work adds weight to previous studies (Nowicki et al. 2002; Kitaysky et al. 2003; Buchanan et al. 2003; Spencer & Verhulst 2007) that have shown that behaviour, as well as other aspects of the phenotype, can be modified by the environmental conditions an individual experiences in early life. This collection of experiments has also shown that although sub-optimal developmental conditions can be costly, it is not always the case that an animal born into a poor environment will be handicapped. Instead, I have shown some evidence to suggest that under certain conditions at least, adaptive shaping may occur.

Individual differences in behaviour

Individual differences in behaviour are undoubtedly partly the result of a particular genotype, and although more attention is now being paid to the importance of environmentally induced differences, most studies continue to be lab-based. The use of domesticated birds is widespread and studies on captive zebra finches have shown strong effects of the early environment on traits such as growth (Alonso-Alvarez et al. 2007), mortality (de Kogel 1997), song learning (Spencer et al. 2005; Gil et al. 2006), and sexual attractiveness (Birkhead et al. 1999; Naguib et al. 2008). Although such studies are both important and revealing, we must always make a point of considering the experiences of animals in the wild, and how these match up to captive experiments. Natural environments are constantly changing, not just from year to year, but also between seasons, and even, in terms of weather, from day to day. So, with regard to the collection of studies presented in this thesis, to what extent are zebra finches exposed to nutritional instability and stress in the wild? Zebra finches live in arid and semi-desert areas of Australia, and pairs of birds can lay up to 7 eggs (Zann 1996), so potentially have large broods to rear. Young birds may suffer from lack of food in general if resources are scarce, or lack of a particular nutrient if breeding has occurred outside the optimum time period. If birds are born into a particularly large brood, parents can struggle to feed all of their offspring adequately, and in surroundings where competition for food is high, or predation events very common, there may be a high chance of losing one or both parents, meaning either that the food supplied by one adult will have to split amongst the brood, or that the chicks may starve. These are scenarios that are possible in the life of any young bird. Therefore, exposure to

differing diet quality and diet consistencies is likely to be very common. Additionally, brood size has also been shown to have effects on various phenotypic traits such as sexual attractiveness (de Kogel & Prijs 1996), hormone levels and immunocompetence (Naguib et al. 2004; Verhulst et al. 2005; Naguib et al. 2008). Similarly, young birds will be exposed to a variety of potential stressors. An insufficient amount or an inconsistent provision of food is likely to cause the release of stress hormones. Extremes of weather or temperature may also elevate stress levels (de Kloet et al. 1999; Cockrem 2007), and in the desert where the heat can be fierce during the day, but temperatures drop substantially at night, both cold and heat stress are likely to be important for young chicks (Zann 1996). Families are also at risk of attack from a variety of predators, including lizards, snakes and rats, increasing the chances of losing either one or both parents (Zann 1996), and putting chicks in danger of starvation.

Taking the ecology of this bird into consideration then, are zebra finches likely to have evolved behavioural development based on environmental sampling? In an environment that underwent large and frequent fluctuations in conditions, it would be disadvantageous for individuals to develop a phenotype based on sampling over a relatively short time period, while in a relatively stable environment, sampling could have considerable fitness benefits. For at least a reasonable part of the year, the arid Australian habitat these birds populate could provide a predictable, stable environment, meaning it would be advantageous for chicks to make judgements about their surroundings and develop accordingly. Alternatively, since wild zebra finches breed opportunistically following rainfall, the environment into which chicks are born could be generally stable. If this is the case, then we might expect to find birds sampling their environment before fixing their behavioural phenotype in order to maximise their chances of survival and success. If this did happen, birds breeding in different areas could show different personality traits or dominance status, or birds born at different times of years may have dissimilar suites of behaviours. Differences in behavioural syndromes between populations have already been shown to exist in sticklebacks (Dingemanse et al. 2007), with predator density affecting behaviours such as aggression and activity. In wild populations, there is also likely to be selection for particular behavioural phenotypes after the juvenile phase, because individuals with different sets of traits are likely to have different fitness. Bold, aggressive animals will most likely gain better access to resources, but may suffer increased predation or limited behavioural flexibility if conditions change. Shy individuals may lose out in competitions for food or mates but survive better in environments with a variety of predators (Verbeek et al. 1994; Benus & Rondigs 1997; Marchetti & Drent

2000; Quinn & Cresswell 2005). Consequently, there may be different distributions of behaviours following particular environmental events or at certain times of year.

Currently, there is much interest in the field of animal personalities because correlated suites of traits are presumed to have important ecological and evolutionary consequences for populations (Lindström 1999; Bell 2007). In showing evidence that zebra finches display particular sets of personality attributes, this study adds to the large body of knowledge that exists about this bird, but also opens up possibilities for future studies on the fitness consequences of particular phenotypes in certain environments, and the potential evolutionary constraints associated with consistent behavioural responses over plasticity.

With regard to the experiments on learning ability and dominance behaviour, it is again relevant to turn to the ecology of the wild bird. In much of the zebra finch's range, the environment is arid and resources are patchily distributed (Zann 1996). Birds forage in flocks on food patches, and tend to deplete one patch before moving to the next, but are often seen returning to previous locations (Zann 1996). Therefore, it is likely that birds will be required to use both spatial and visual cues when finding and returning to such foraging sites (Sanford & Clayton 2008). Individuals that are compromised in their ability to do this and so waste time in searching will have a reduced time in which to feed themselves and their chicks, so having lower Darwinian fitness (Lemon 1991). Since there is a high chance that a number of birds in any population will undergo developmental stress of some kind, the cognitive cost associated with that stress is considerable, and could have important consequences for both wild individuals and populations.

In the wild, birds also live in large social groups, feeding, roosting and nesting together (Zann 1996). Consequently, the dominance status of an individual will influence access to resources, including food, shelter and potential mates, and so contribute significantly to overall fitness. Wild zebra finches compete over food, perching and nesting sites, with males tending to show more aggression than females, although both sexes often exhibit aggressive behaviour (Butterfield 1970). Therefore, the study of environmental effects on this type of behaviour will also help us understand why certain birds are more successful than others, and determine the potential costs and benefits of individual differences.

Future work

Long-term programmes such as this where individuals are tested for behaviour early in life and then later on in adulthood provide the potential to identify patterns over the life of the animal, and to determine if and when behavioural traits might be shaped. However, to show conclusively the adaptive nature of some behavioural phenotypes over others, it is necessary to study the reproductive success of the birds and also the recruitment probability of their offspring. Consequently there is currently still a long way to go in this field, and studies must begin to span generations in order to answer the questions that remain. Also, in considering whether the effects of the early environment on adult behaviour are costly or adaptive, it would be best to assess the fitness of adults in a variety of environments. Such studies would allow us to look for fitness costs associated with a mismatch between the developmental and adult environments such as has been shown in work on our own species (Gluckman et al. 2005). Although this thesis directly measures behavioural differences between individuals in an attempt to link phenotypes back to a certain developmental environment, and we can draw some conclusions about overall fitness with regard to dominance behaviour and cognitive ability, we can only speculate about adaptive shaping of personality traits, and the potential consequences of mismatch between sampling and environment. With reference to the different personality types demonstrated in this work, there is no obviously superior phenotype, because traits will be differentially adaptive depending on the environment experienced. Future work might therefore consider whether individuals shown to possess certain personality traits show greater survival or fecundity under particular environmental conditions, or suffer increased costs if the surroundings are different from those anticipated during development.

Also, in addressing this interesting set of questions, we must ask whether zebra finches are the most appropriate study organism. When comparing across the entire animal kingdom, these birds are relatively long-lived species that in all probability will experience a variety of environmental conditions in their lifetime. More light may be shed on the idea of adaptive shaping by considering species that live only for one year, or even for one season. The lifetime fitness of such animals would of course be hugely affected by any developmental programming that took place as the result of environmental sampling and any mismatches that then occurred. Additionally, it would be interesting to examine behavioural changes associated with more and less extreme changes in developmental conditions. Perhaps it is the extent to which the developmental trajectory deviates from the optimum that is important in determining whether a behavioural change will be costly or

adaptive. We might expect that extreme disruption of the normal developmental trajectory would always have negative effects on Darwinian fitness (Gluckman et al. 2005), but it remains unclear at what point a disruption can be compensated for, or when it is possible for predictive adaptive changes to take place.

The mechanisms that underlie the behavioural differences observed here also remain unknown. Therefore, the detailed study of behaviour also needs to be more frequently linked with studies of physiology. Such integration across disciplines will ensure that not only are we able to discover more about the effects of the early environment on later behaviour, but that we start to understand the proximate causes behind individual differences.

Finally, we always intend our laboratory experiments to mimic real life situations. However, in this study, birds that were experimentally stressed in early life were not naturally stressed, but were given oral doses of a stress hormone. Although we know that this indeed raises the level of the stress hormone in the body, and so imitates the natural response to a stressor, we cannot know the extent to which this varies from a real life situation, in which the cause of the stress (e.g. restricted food) may also be important in the developing phenotype. Therefore, as with all behavioural studies, it is important that similar tests be carried out on wild animals in their natural environment.

Closing remarks

In this thesis, I have shown that, as with other aspects of the phenotype, the early environment experienced by any animal can be extremely important in determining its behaviour. I have also demonstrated that these early effects can have long-term implications for adult fitness, with the suggestion that certain changes may even be adaptive under particular conditions. I have shown that environmentally-induced behavioural change can lead to a compromised individual, but also has the potential to increase fitness through shaping. In our constantly changing world, such environmental effects may lead to the success of some species and the decline of others. If even small environmental changes can have significant implications for individual fitness, then the study of such changes is not only interesting, but hugely important.

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Appendix 1

Description of the five training phases used in the cognitive tests described in chapters 4 and 5. The table shows the measurements taken in each phase plus the requirements which had to be met in order for a bird to progress to the next phase. ‘Wells’ refers to the 12 wells in the ice-cube tray; ‘rewarded’ indicates whether the wells contained food.

Phase	Description	Measurements	Requirements
1	12 wells rewarded Birds learn to feed from tray	Latency to approach tray Latency to feed No. of food wells exploited	Minimum of 4 repeats. Subjects feed on 2 consecutive trials to progress
2	12 wells rewarded 5 half covered with cotton Birds feed from tray in presence of cotton pieces	Cotton pieces removed No. of food wells exploited	Minimum of 4 repeats Subjects feed on 2 consecutive trials to progress
3	12 wells rewarded 12 half covered with cotton Birds remove cotton to feed	Cotton pieces removed No. of food wells exploited	Minimum of 4 repeats Subjects feed on 2 consecutive trials to progress
4	12 wells rewarded 12 fully covered with cotton so no food visible. Birds feed from wells by manually removing balls	Cotton balls removed No. of food wells exploited	Minimum of 6 repeats. Subjects feed on 2 consecutive trials to progress
5	6 wells rewarded 12 fully covered with cotton Birds may have to lift more than one ball for reinforcement	Cotton balls removed No. of food wells exploited	Minimum of 6 repeats Subjects feed on 2 consecutive trials to progress to test