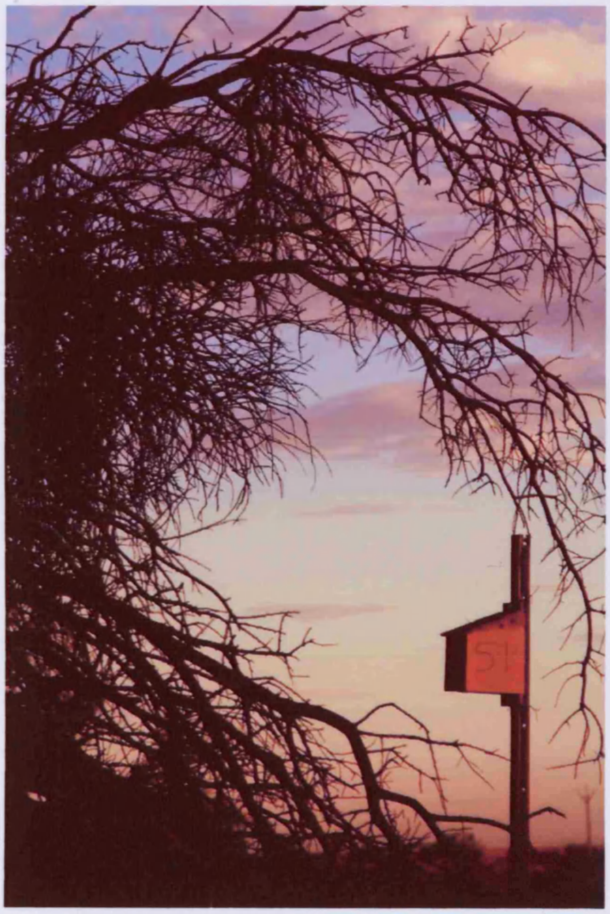
Song, stress and female preferences in the zebra finch, *Taeniopygia guttata*



Joseph L. Woodgate

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School of Biosciences
Cardiff University

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You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird. So let's look at the bird and see what it's doing - that's what counts. I learned very early the difference between knowing the name of something and knowing something.

Richard Feynman (1918-1988)

The next best thing to being clever is being able to quote someone who is.

Mary Pettibone Poole, A Glass Eye at a Keyhole, 1938

STATEMENT 3

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Summary

The developmental stress hypothesis proposes that complex songs evolved as honest signals of developmental history. Developmental stress has been shown to affect sexual traits including song in male songbirds, as well as the song control nuclei in the brain, thereby reducing a male's attractiveness to females. Although female birds use these nuclei for assessing song, the effects of stress on female preferences remain largely unaddressed. I tested the mate choice behaviour of female zebra finches, *Taeniopygia guttata*, that had been reared under control conditions or nutritional stress. In a visual mate choice experiment, neither control nor stressed females showed agreement in preference, but stressed females were less active than controls and moved less often between stimulus males. In an acoustic choice experiment, females preferred complex songs to simplified ones, but stressed and control females did not differ in the direction or strength of song preferences. These findings suggest that quality of the rearing environment does not affect females' ability to discriminate between male signals, but could have long term, context-dependent effects on choosiness or other aspects of female choice behaviour. In contrast to previous studies, I found no difference between stressed and control males in brain or song characteristics. I calculated the relative influence of environmental and genetic factors on neural development and song structure, finding evidence that song might signal information regarding both developmental history and genetic quality. I recorded male songs from wild zebra finches in a breeding colony in New South Wales. Song structure in this population predicted a male's reproductive success but not his morphology, or that of his mate. Finally, I summarise the evidence for the developmental stress hypothesis and suggest areas for further research. Together, my results illustrate the importance of understanding the consequences of early environmental conditions for female choice and male attractiveness.

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Chapter 1: Introduction

1.1 Sexual selection

Sexual selection, a form of selection arising from differences between individuals in reproductive success, has been the subject of much research since it was first proposed by Darwin (1871), and is now accepted to be the main selective force driving the evolution of many elaborate secondary sexual traits (Andersson, 1994). Secondary sexual traits are those forms of sexual dimorphism which play no direct, mechanical, role in reproduction (as opposed to the primary sexual traits, the gonads and copulatory organs), but whose function is to increase reproductive success by conferring an advantage in mate competition (Andersson, 1994). Sexual selection favours traits that enhance an individual's reproductive success, whether through competition between males for access to females (e.g. size, strength, weaponry such as horns or spurs), or female choice for preferred males (e.g. conspicuous appearance, behaviour or acoustic signals, such as bird song; Andersson, 1994). It is worth noting that many male traits serve dual functions in courtship and in male-male competition (Berglund et al., 1996), including bird song in some species (Searcy and Andersson, 1986; Catchpole and Slater, 2008). It has been hypothesised that females utilize pre-existing traits, involved in competition or dominance among males, as indicators of male quality (Berglund et al., 1996).

Two main sets of hypotheses have been proposed to explain the mechanism by which female choice for male cues could evolve. Fisherian Run-Away models (Fisher, 1930; O'Donald, 1977; Lande, 1981; Pomiankowski and Iwasa, 1998), suggest that heritable female preferences for a beneficial male trait will become coupled to genes which produce that trait in males. This is because females showing a preference for a particular trait will mate with males that possess the trait and will have both sons with a fitness advantage over other males, and daughters with a preference for the trait. This can lead to a 'run-away' process of positive feedback resulting in both the trait, and female preference for the trait, becoming elaborated over many generations. Such traits can evolve to a point where they carry a significant cost to the bearer, the disadvantages being offset by the advantage it confers in terms of reproductive success (Lande, 1981). However, Run-Away processes are inadequate to explain the evolution of sexual traits when females incur a cost by expressing choice (Pomiankowski et al., 1991; Eshel et al., 2002).

Secondly, a number of indicator models (Zahavi, 1975; Hamilton and Zuk, 1982; Folstad and Karter, 1992) propose that male traits provide females with information regarding males' genetic or phenotypic quality, allowing females to choose the fittest males and thereby maximise their reproductive success. Females may obtain direct benefits from choosing attractive males, such as food, high quality territories, protection from predators, or better parental care for their offspring (Andersson, 1994). In other species, males may only offer indirect, genetic benefits ('good genes', Andersson, 1994). It is likely that indicator traits could only evolve if there is a mechanism by which their honesty is maintained, as males will be selected to produce signals that maximise mating success, regardless of their honesty, while females are under selection pressure to ignore unreliable signals and only to discriminate between males on the basis of traits that accurately reflect male quality.

The Handicap Principle (Zahavi, 1975) is the best known indicator model, and suggests that honesty of male signals is enforced by the fact that such signals are costly to produce or maintain. A trait can act as a signal of some aspect of quality if it incurs a cost and the cost is not equal for males of different quality (Grafen, 1990b). Each male signals enough that a worse male would be unable to afford the cost of producing the same signal, so cheating does not pay and the signal remains honest. The Handicap Principle has received support from a number of theoretical models (Grafen, 1990b, a; Iwasa and Pomiankowski, 1991; Eshel et al., 2002).

In addition to these two sets of hypotheses, there is the possibility that male traits may evolve to exploit pre-existing female sensory bias (Endler and Basolo, 1998; Collins, 1999; Arnqvist, 2006; Clark and Feo, 2010). That is to say that females' senses will be attuned to the reception of particular stimuli, either because these are adaptive in other contexts (for foraging, predator avoidance, etc.), or simply as by-products of sensory evolution. Females may be predisposed to respond favourably to male traits that emulate these preferred stimuli (Arnqvist, 2006). Note that sensory bias hypotheses seek to explain the origin of male signals and female preferences and do not preclude the possibility that they are maintained and elaborated by Run-Away or Handicap processes (Arnqvist, 2006).

One of the most important predictions of the Handicap Principle is that male traits or ornaments show heightened condition dependence by comparison to non-sexual traits (Pomiankowski, 1987; Grafen, 1990a). Condition dependence here is used in a broad sense to refer to the long-term or short-term effects of any of a large number of potential environmental stressors (e.g. food quantity or quality, parasite loads, infections, temperature or photoperiod manipulations, brood size manipulations) on trait expression. Numerous studies have found positive relationships between male traits and various measures of quality or condition (reviewed in Andersson, 1994; Johnstone, 1995), but much of this evidence is simply correlational. Many experimental studies failed to use appropriate controls or account for variation in body size (Cotton et al., 2004). Nonetheless, Cotton et al. (2004) identified a number of well-designed studies providing evidence for a causative link between male condition and trait expression in crickets, stalk-eyed flies, wolf spiders, guppies and several species of bird (e.g. red jungle fowl, *Gallus gallus*, Zuk et al., 1990; house finch, *Carpocadus mexicanus*, Hill, 2000; brown-headed cowbird, *Molothrus ater*, McGraw et al., 2002).

1.2 Bird song

Song is a sexually selected acoustic signal (Andersson, 1994; Catchpole and Slater, 2008) produced by oscine birds and is both learned and innate (see section 1.2.2, below). Song performs dual functions in territory defence (Krebs et al., 1978; McDonald, 1989; Nowicki et al., 1998b; de Kort et al., 2009), and attracting a mate (Searcy and Andersson, 1986; Catchpole, 1987; Kroodsma and Byers, 1991; Brenowitz et al., 1997). It has been suggested that simple songs are sufficient for territory defence and that more complex songs play a more important role in mate attraction (Catchpole, 2000; Podos et al., 2004).

Both the structure and complexity of song varies enormously across species (DeVoogd et al., 1993; Catchpole and Slater, 2008). Because of this variability, song complexity can be difficult to define. In some species, such as the zebra finch, *Taeniopygia guttata*, individuals perform a single, stereotyped song phrase that may vary between individuals in the number of notes or syllables it contains (Zann, 1996; Riebel, 2009). Some 72% of songbird species produce a repertoire of different song types that can be sung interchangeably (MacDougall-Shackleton, 1997), each characterised by its unique sequence of syllables. These song repertoires can range in size from only a few songs, for example in the great tit, *Parus major* (Krebs et al., 1978), up to several hundred, as in the nightingale, *Luscinia megarhynchos* (Kipper et al., 2006). Still other species, the sedge warbler, *Acrocephalus schoenobaenus*, for example, do not sing stereotyped song phrases at all, but a fluid song in which a variable number of different elements can be recombined in different orders (Catchpole, 1976). As a result, researchers have tended to define repertoire size as either the number of syllables or song types produced by an individual, depending on their study species. Gil and Gahr (2002) have suggested that repertoire length (the sum in seconds of all the different song elements in a male's repertoire, regardless of whether it is a repertoire of songs or of syllables; Irwin, 2000), would be a relatively unbiased measure for between species comparisons of song complexity. However, this measure has not been widely adopted and I will defer to the definitions of repertoire size used by the individual authors throughout this thesis.

1.2.1 Female preferences

Female birds generally exercise choice over potential mates and show preferences for a number of sexually selected traits displayed by males including song (Andersson, 1994; Catchpole and Slater, 2008). Bird song is a complex signal comprising multiple traits, each of which may bear different costs and so signal different information about male quality (Gil and Gahr, 2002). These include performance related traits, such as singing rate, amplitude of song or the length of song bouts, as well as structural traits relating to the acoustic properties of song elements, their variety and temporal ordering within song.

Female preferences for males with a high rate of singing have been documented in both field and laboratory studies over a range of species. Male barn swallows, *Hirundo rustica*, that sang at a higher rate were found to have a higher proportion of paternity in their nest (Møller et al., 1998), although song rate was also correlated with tail length, known to be used as a cue in mate choice. Song rate was correlated with how early in a season male willow warblers, *Phylloscopus trochilus*, attracted a mate (Radesater et al., 1987), while Alatalo et al. (1990) manipulated singing rate in pied fly catchers, *Ficedula hypoleuca*, by providing food supplements to some males, and found that they attracted mates earlier than those not receiving additional food. In laboratory mate choice studies, female birds preferred males that sang at a higher rate (zebra finch, Houtman, 1992; Collins et al., 1994; house finch, Nolan and Hill, 2004), and these preferred males were more successful at obtaining extra-pair copulations (Houtman, 1992).

Sexual signals are presumed to advertise direct or indirect benefits to females. There is evidence that song rate predicts territory quality (willow warbler, Radesater and Jakobsson, 1989; blackcap, *Sylvia atricapilla*, Hoi-Leitner et al., 1995), male parental care (stonechat, *Saxicola torquata*, Greig-Smith, 1982), male participation in nest defence (stonechat, Greig-Smith, 1982; blackcap, Hoi-Leitner et al., 1993) and overall reproductive success (blackcap, Hoi-Leitner et al., 1993). The song rate of male zebra finches (measured as the duration of song produced over 15 minutes in the presence of a female) predicts that of their sons (Houtman, 1992), which might indicate either the indirect benefit of heritable song rate or the direct benefit of improved song tutoring of the offspring. The honesty of these signals is probably enforced by the trade-off between time spent singing and time spent on foraging and other behaviours, and perhaps also by the energetic costs of song production (Gil and Gahr, 2002; Catchpole and Slater, 2008). Studies investigating the metabolic costs of singing have had mixed results, with some finding that intensive singing incurs substantial costs (Eberhardt, 1994; Hasselquist and Bensch, 2008), while others suggest that the cost of singing may be rather lower (Oberweger and Goller, 2001; Ward et al., 2003; Ward et al., 2004), in which case it might not be sufficient to mediate the honesty of song output as a signal of male quality.

Playback experiments in which song recordings were played at high or low amplitude have found female preferences for high amplitude song in red-winged blackbirds, *Agelaius phoeniceus*, and zebra finches (Searcy, 1996; Ritschard et al., 2010). Also, male dusky warblers, *Phylloscopus fuscatus*, that were able to maintain a higher amplitude when singing, obtained more extra-pair paternity (Forstmeier et al., 2002). Song amplitude may be an honest signal of male quality due to the energetic costs of producing loud song (Gil and Gahr, 2002; Ritschard et al., 2010). Alternatively, amplitude may be constrained by the physical size of the vocal tract and syringeal muscles. However, while vocal amplitude appears to signal body size in anurans (Gerhardt, 1975; Arak, 1988), there is little evidence for this in birds (Brumm, 2009).

Some structural song traits, known to be involved in female song preferences, might be attractive because they are difficult to sing. These traits could act as honest signals of male quality by displaying how close a male can approach the physical and physiological limits on the sounds he can produce (Nowicki and Searcy, 2004). For example, female canaries, *Serinus canaria*, respond with high levels of sexual display to a particular type of complex song element, known as 'sexy syllables' (Vallet and Kreutzer, 1995; Vallet et al., 1998; Nagle et al., 2002). Vallet et al. (1998) have hypothesised that these rapid two-note syllables might convey information about motor constraints on song production, while Leitner and Catchpole (2004) found that the proportion of sexy syllables in a male's song was correlated with the size of a song control nucleus (HVC) in the forebrain, indicating there might be neural constraints on production. Similarly, males might vary in performance quality as defined by the apparent trade-off between trill rate and frequency bandwidth in the song structure of many species, which is likely to reflect physical constraints on performance (Podos, 1996, 1997). Although the frequency of acoustic signals indicates body mass in several species (Hardouin et al., 2007; Mager et al., 2007; Forstmeier et al., 2009), this relationship has been most commonly observed in non-learned calls rather than song. Indeed, Forstmeier et al. (2009) showed that male and female calls in the zebra finch had a stronger genetic correlation with body size than did male song. Frequency is strongly affected by vocal learning (Williams et al., 1989), so frequency traits in bird song are unlikely to provide an accurate signal of male morphology.

The song trait for which female preferences have been most commonly demonstrated is repertoire size (Catchpole and Slater, 2008). In laboratory studies, female zebra finches and Bengalese finches, *Lonchura striata*, performed more copulation solicitation displays to, or preferred to listen to, recordings of more complex songs consisting of a greater number of syllable types (Clayton and Prove, 1989; Neubauer, 1999; Vyas et al., 2009; and see chapter 3). Sedge warblers do not produce stereotyped song phrases, but females prefer songs formed from a larger repertoire of syllable types (Catchpole et al., 1984), and males with larger repertoires have been found to be more likely to acquire a mate (Airey et al., 2000a), and to acquire mates earlier.

Females from a number of other species have been shown to respond more to songs comprising a greater repertoire of song types, in laboratory studies (song sparrow, *Melospiza melodia*, Searcy and Marler, 1981; Searcy, 1984; great tit, Baker et al., 1986; yellowhammer, *Emberiza citronella*, Baker et al., 1987; brown-headed cowbird, Hosoi et al., 2005). Field studies have found that male birds with larger repertoires are more likely than those with small repertoires to mate (song sparrow, Reid et al., 2004), acquire mates earlier (European starling, *Sturnus vulgaris*, Mountjoy and Lemon, 1996), obtain a greater number of extra-pair fertilizations (great reed warbler, *Acrocephalus arundinaceus*, Hasselquist et al., 1996) or attract more females in polygynous species (red winged blackbird, Yasukawa et al., 1980; great reed warbler, Catchpole et al., 1985;

European starling, Eens et al., 1991). Males with larger repertoires also produce a greater number of offspring (great reed warbler, Catchpole, 1986; great tit, Lambrechts and Dhondt, 1986; song sparrow, Hiebert et al., 1989; European starling, Eens et al., 1991; willow warbler, Gil and Slater, 2000; marsh warbler, *Acrocephalus palustris*, Bell et al., 2004; song sparrow, Reid et al., 2005), or father offspring that are more likely to survive long enough to reproduce themselves (great tit, McGregor et al., 1981; great reed warbler, Hasselquist et al., 1996; Hasselquist, 1998).

However, in a recent review of 47 papers, examining the evidence for female preferences for larger repertoires, Byers and Kroodsma (2009) noted that many studies had failed to demonstrate a female preference for repertoire size (snow bunting, *Plectrophenax nivalis*, Hofstad et al., 2002; great reed warbler, Forstmeier and Leisler, 2004; house finch, Nolan and Hill, 2004; willow warbler, Gil et al., 2007). Moreover, the majority of the positive results reported come from laboratory studies, which could reflect the different proxy measures used for female choice (field studies tend to rely on pairing date or measures of reproductive success while laboratory studies often measure female copulation solicitation displays or time spent near different stimuli), or might indicate that preferences expressed in a laboratory setting do not translate into choices made in nature (Byers and Kroodsma, 2009). Given that, in species that have been the subject of multiple investigations, there is often little agreement between studies regarding female preference for repertoires, one should be cautious in concluding that song repertoires are the result of strong selection for repertoire size, by female choice (Byers and Kroodsma, 2009).

In terms of the potential benefits females might obtain from choosing males with a larger repertoire, there is evidence that repertoire size can signal a male's reproductive experience (red-winged blackbird, Yasukawa et al., 1980), or the quantity or quality of parental care he will provide (stonechat, Greig-Smith, 1982; red-winged blackbird, Yasukawa et al., 1990; sedge warbler, Buchanan and Catchpole, 2000; whitethroat, *Sylvia communis*, Halupka and Borowiec, 2006). It has also been suggested that repertoire size signals the quality of a male's territory (great tit, Krebs et al., 1978; red-winged blackbird, Yasukawa, 1981; great reed warbler, Catchpole, 1986), although song repertoire did not predict territory size or how soon a male was able to acquire a territory, in song sparrows (Beecher et al., 2000; Reid et al., 2004). However, the fact that male great reed warblers with large repertoires were more likely to obtain extra-pair paternity (Hasselquist et al., 1996), and that barn swallows with large repertoires fathered a greater percentage of the offspring in their own broods than did those with smaller repertoires (Møller et al., 1998), indicates that females also obtain indirect benefits by preferring large repertoires. Further evidence of indirect benefits for preferences for complex song comes from the fact that such preferences exist in species such as zebra finches, where males do not control territories or other resources (Zann, 1996).

One potential indirect benefit may be that repertoire size apparently signals male age in several species (red-winged blackbird, Yasukawa et al., 1980; great tit, McGregor et al., 1981; song sparrow, Hiebert et al., 1989; sedge warbler, Birkhead et al., 1997; European starling, Eens, 1997; willow warbler, Gil et al., 2001; great reed warbler, Forstmeier et al., 2006; sedge warbler, Nicholson et al., 2007). Although some of these species, such as red-winged blackbirds or starlings, can continue to learn songs in adulthood (Yasukawa et al., 1980; Eens, 1997), most are 'closed-ended learners' in which little or no modification of the song occurs after an initial period of song learning (see section 1.2.2, below). This means that any link between longevity and song repertoire is likely to be due to differential levels of mortality between males with relatively simple or more complex songs (McGregor et al., 1981; Lambrechts and

Dhondt, 1986; Hiebert et al., 1989; Forstmeier et al., 2006). There may be good genes benefits to choosing older males, because longevity is likely to indicate viability, at least in their present environment (Kokko, 1998).

Several field studies have suggested song repertoire size is correlated with other measures of male quality, finding relationships between song repertoire size and male condition (pied flycatcher, Lampe and Espmark, 1994; European starling, Mountjoy and Lemon, 1996; song sparrow, Pfaff et al., 2007) or male size (nightingale, Kipper et al., 2006). No such relationship between repertoire size and morphology was observed in several other species, however, despite song repertoire being predictive of a male's reproductive success (great tit, McGregor et al., 1981; great reed warbler, Catchpole, 1986; great tit, Lambrechts and Dhondt, 1986; European starling, Eens et al., 1991; and zebra finches, see chapter 5). Working on domesticated zebra finches, Holveck and Riebel (2007) reported a relationship between certain measures of song performance and male mass and tarsus length, but these results were not replicated by Forstmeier et al. (2009).

Song repertoire has been found to be correlated with the size of the brain nucleus HVC in several species (canary, Nottebohm et al., 1981; sedge warbler, Airey et al., 2000a; zebra finch, Airey and DeVoogd, 2000; song sparrow, Pfaff et al., 2007), and Garamszegi and Eens (2004) found strong evidence that song traits were related to the volume of song nuclei in a meta-analysis of 41 previous studies. It appears that this relationship arises because neural development limits the capacity of the bird to learn or produce complex song, rather than some aspect of singing behaviour influencing brain development (Burek et al., 1991; Brenowitz et al., 1995). In the zebra finch, at least, the volume of the song control nuclei of the forebrain has been found to be heritable (Airey et al., 2000b). Consequently, by choosing males with more complex song, females could obtain genetic benefits for their offspring. HVC volume is correlated with the overall volume of the forebrain in zebra finches (Airey et al., 2000b), so males with more complex song may also have a greater capacity for other neural tasks such as learning and remembering (DeVoogd, 2004). Boogert et al. (2008) demonstrated that male zebra finches with more complex song were more proficient at solving a novel foraging task, indicating that song structure might signal male learning ability.

Despite the apparently widespread preference for complex songs, and evidence that song repertoires can honestly signal both direct and indirect benefits, summarised above, it is not immediately clear how selection for large repertoires could come about as there do not appear to be obvious costs associated with producing large repertoires as opposed to repetitions of a smaller repertoire. One possibility is the sensory exploitation hypothesis (Ryan et al., 1990), which postulates that sexual selection favours traits that exploit females' preexisting sensory bias toward complex song. Evidence in favour of this hypothesis comes from three studies that showed a female preference for artificially created song repertoires in zebra finches (Collins, 1999; Vyas et al., 2009), and in common grackles, *Quiscalus quiscula* (Searcy, 1992), two species in which males do not have a repertoire of songs. If females do have an innate preference for repertoires, this preference could be because females habituate less quickly to more complex songs (great tit, Krebs, 1976). However, no such preferences were found in two other species that also lack repertoires, field sparrows, *Spizella pusilla*, and white-throated sparrows, *Zonotrichia albicollis* (Searcy and Marler, 1984).

The immunocompetence handicap hypothesis (Folstad and Karter, 1992; Sheldon and Verhulst, 1996) proposes that the development or expression of ornamental traits may be limited by trade-offs between immune defense and trait expression. However, a meta-analysis by Roberts et al. (2004) found no evidence that testosterone affected direct measures of immunity across a range of taxa, although increased testosterone was correlated with increased ectoparasite load in several studies. In songbirds, while there is a strong interspecific correlation between repertoire size and the relative size of the spleen (Møller et al., 2000), there is little direct evidence for song traits being limited by immunocompetence within any species (but see Pfaff et al., 2007 on song sparrows). Another possibility is the developmental stress hypothesis, discussed in detail in section 1.3, below.

1.2.2 Song learning and the neural control of song

Song is at least partially learned in all oscine species (Kroodsma and Baylis, 1982; Catchpole and Slater, 2008). Juvenile males from the majority of species must learn their song by imitating the song of a mature male (reviewed in Catchpole and Slater, 2008), although males from at least two species appear to be able to develop normal songs without any previous exposure to song models (grey catbird, *Dumatella carolinensis*, Kroodsma et al., 1997; sedge warbler, Leitner et al., 2002). The process of memorising a song model begins very early in life, in many species, long before they themselves begin to sing (chaffinch, *Fringilla coelebs*, Thorpe, 1958; marsh wren, *Cistothorus palustris*, Kroodsma and Pickert, 1980; song sparrow, Marler and Peters, 1987).

Learning in most species, known as 'closed-ended learners', appears to be restricted to a sensitive phase during development, and birds are unable to learn or modify their song after this period (chaffinch, Thorpe, 1958; white-crowned sparrow, *Zonotrichia leucophrys*, Marler, 1970; song sparrow, Marler and Peters, 1987). Several species, however, have been shown to be capable of modifying their songs from year to year (Catchpole and Slater, 2008). These 'open-ended learners' include the European starling (Eens et al., 1992; Mountjoy and Lemon, 1995), and canary (Nottebohm et al., 1986). Learning follows diverse paths and occurs over different timescales across songbird species making it difficult to identify a typical learning program (Beecher and Brenowitz, 2005; Brenowitz and Beecher, 2005), but there appears to be a general need for young males to compare auditory feedback from their own developing song to an internal model. The developing song is highly variable to begin with but gradually converges upon the model (white-crowned sparrow, Marler, 1970; zebra finch, Tchernichovski et al., 2001). Young males from several species that were deafened after having memorised song, but before they began to produce it, developed highly atypical song, showing that auditory feedback is necessary for successful song learning (Konishi, 1964, 1965; Marler and Sherman, 1983). Female song preferences are likely also to be learned during the early stages of development (zebra finch, Riebel, 2000, 2003).

Both learning and production of song are controlled by complex neural pathways in the brain, involving a number of discrete brain nuclei and the connections between them (reviewed in Brenowitz et al., 1997; Brainard and Doupe, 2002). Songbirds are one of only six animal groups to exhibit vocal learning (Nottebohm, 1972; Jarvis et al., 2000), and one of only four that have been found to have dedicated areas in the forebrain associated with learned communication (along with parrots, hummingbirds and humans; Jarvis and Mello, 2000; Jarvis et al., 2000; Jarvis, 2004). There are two main pathways in the song control system (Fig. 1.1). The posterior forebrain pathway, also known as the motor pathway, controls the production of song in adults (Brenowitz et al., 1997; Brainard and Doupe, 2002). If the major brain nuclei that comprise the motor pathway (HVC [used as a proper name, Reiner et al., 2004], and the robust nucleus of the archistriatum, RA) are inactivated, male canaries may adopt the correct posture and produce beak movements associated with singing behaviour but are unable to produce song (Nottebohm et al., 1976). Neurophysiological activity in these nuclei is temporally correlated with singing in several songbird species (McCasland, 1987; Chi and Margoliash, 2001). It is hard to assess whether the motor pathway plays a role in the acquisition of song as the song produced by a bird is the only assay of what it has learned, and damage to the motor pathway disrupts a bird's ability to produce song, but it is generally assumed to also play a role in song learning (Brenowitz et al., 1997).

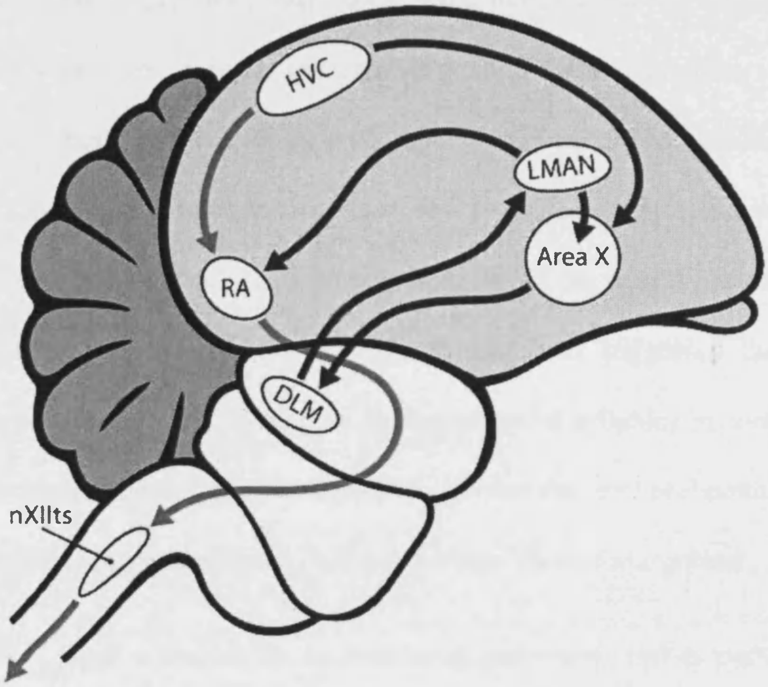

Figure 1.1: Schematic sagittal view of the songbird brain showing the two major pathways of the song system. The posterior forebrain pathway or motor pathway (grey arrows) connects the HVC and the thalamic nucleus uvaeformis (Uva, not shown) to the nucleus robustus archistriatalis (RA) and ultimately leads to the neurons of the nucleus hypoglossus pars tracheosyringealis (nXIIts) that project to the vocal muscles. The anterior forebrain pathway (black arrows) indirectly connects the HVC to the RA via Area X, the nucleus dorsolateralis thalamis, pars medialis (DLM), and the lateral part of nucleus magnocellularis anterioris (LMAN). Figure taken from Nottebohm (2005).

The second major song pathway, the anterior forebrain pathway, plays a role in the learning of song and song recognition (Doupe and Solis, 1997; Margoliash, 1997). Inactivation of brain nuclei that comprise this pathway, or the connections between them, does not disrupt the production of crystallised song by adult birds (Nottebohm et al., 1976; Bottjer et al., 1984), but prevents the formation of normal adult song if it occurs in juveniles (Bottjer et al., 1984; Scharff and Nottebohm, 1991). If Area X is disrupted, juvenile zebra finches reach the plastic song stage but song never crystallises, and as adults birds they produce a long and rambling song with unusually variable notes (Scharff and Nottebohm, 1991). Birds with damage to area lMAN on the other hand, produce song with an aberrant, monotonous and repetitive, but stable structure. It is suggested that area lMAN plays a crucial role in ensuring the neural plasticity necessary for learning (Scharff and Nottebohm, 1991). It has been suggested that the RA is responsible for learning and producing the fine structure of syllables in song and that the HVC and other areas of the song control pathway control the temporal patterning of these elements to produce song (zebra finch, Vu et al., 1994; Yu and Margoliash, 1996).

The HVC plays a major role in both song pathways, and is perhaps the most important nucleus for song learning and production. There appears, for example, to be a hierarchy of song nuclei within the motor pathway, with the HVC encoding higher-level song structure than RA (zebra finch, Yu and Margoliash, 1996). Furthermore, Halle et al. (2003b, a) found that lesions that reduced the volume of the HVC in male canaries caused a reduction in repertoire size in adulthood, suggesting a direct link between HVC structure and singing ability. More evidence for the unique role of HVC in the song system is provided by the discovery that up to 300 genes are expressed differently in the HVC compared to surrounding areas (Lovell et al., 2008), and singing behaviour apparently induces changes in gene expression within the HVC (Jarvis and Nottebohm, 1997).

As noted above, variation in HVC volume is correlated with variation in song complexity within a number of songbird species. A similar pattern is found across species, with differences in song complexity apparently correlated with differences in the song control system. For example warblers of the genus *Acrocephalus* have complex songs with a larger repertoire of syllables than members of the closely related *Locustella* genus, as well as having larger HVC volumes (Szekely et al., 1996). Comparisons across species are difficult to interpret because shared ancestry rather than independent evolution may account for apparent correlations between traits. DeVoogd et al. (1993) controlled for these phylogenetic effects in an analysis of species differences in the size of song control nuclei, the number of songs in a species' repertoire and the number of different syllables per song across 45 oscine species. They found that the relative volume of the HVC (controlling for differences in overall brain volume) was correlated with the number of songs in a species' repertoire but not with the number of different syllables in a song (DeVoogd et al., 1993). This relationship was specific to the HVC rather than the overall size of the song control system, and implies that the complexity of a species song and the amount of brain space devoted to the song control system have coevolved (DeVoogd et al., 1993).

1.2.3 Sex differences in the song control system, and the function of HVC in females

The song control nuclei are found in both sexes, but only the male sings in most species of songbird, and the song nuclei of males are several times larger than those of females (Nottebohm and Arnold, 1976; MacDougall-Shackleton and Ball, 1999). In the zebra finch, which has the most sexually dimorphic brain structure of any species yet studied (Arnold, 1980), the HVC and RA of the male are five times larger than those of the female, and Area X is often not visible in females (Williams, 1985; MacDougall-Shackleton and Ball, 1999). The connections between HVC and RA are reduced or absent in female zebra finches (Williams, 1985). Females do produce song in some species, and the degree of sexual dimorphism in neural structure is correlated with the degree to which the sexes differ in song complexity (Brenowitz et al., 1997), but it can be difficult to draw conclusions from cross-species studies as species may share common ancestry. MacDougall-Shackleton and Ball (1999) used a method of contrast scores to control for phylogenetic effects and found that sexual dimorphism in HVC volume was correlated with dimorphism in song repertoire size, while dimorphism in RA volume correlated with sex differences in song output. This evidence suggests that sex differences in the song control system probably coevolved with sex differences in singing behaviour.

The female song control system, in particular the HVC, is likely to play an important role even in species in which females do not sing. Female canaries with HVC lesions were shown to respond to a heterospecific song with copulation solicitation display whereas intact females respond only to conspecific song (Brenowitz, 1991; Del Negro et al., 1998; Halle et al., 2002). The nature and size of the females' response to conspecific song was unchanged, providing strong evidence that damage to the HVC eliminated the females' discrimination between conspecific and heterospecific song, rather than causing a general change in their response to acoustic stimuli. It is unknown, however, whether females with HVC damage were unable to discriminate between songs, or whether they could perceive the species differences but did not respond to them. Interestingly, Halle et al. (2002), found that females regained their discriminatory abilities over a period of several months, even though the lesions caused permanent loss of HVC tissue, and concluded that the lateral portion of the HVC, which was unharmed in their study, took over the function of the damaged medial portion. Leitner & Catchpole (2002) found that female canaries that discriminated more strongly between songs containing different proportions of 'sexy syllables' had larger HVC volumes, and Del Negro et al. (2000) found neurons in the female HVC that responded selectively to these syllables. These studies indicate that the HVC is important for females' ability to discriminate between or show preferences based on the quality of male song.

The song control system of songbirds is the only known sexual dimorphism of avian brains (Arnold, 1997). Estrogens have a masculinising effect on the song system during development in both male and female zebra finches (Gurney and Konishi, 1980; Simpson and Vicario, 1991; Adkins-Regan et al., 1994; Jacobs et al., 1995). Testosterone also masculinises the developing song system (Gurney, 1981) but is apparently less important than estrogen in this regard (Grisham and Arnold, 1995). Treatment of adult female canaries with testosterone causes the HVC to double in volume, and causes the female to start producing male-like song (Nottebohm, 1980). Songbird brains have high concentrations of enzymes that metabolise sex steroids (e.g. aromatase which converts testosterone to estradiol), allowing the relative concentrations of sex steroids in the brain to be regulated independently of the rest of the body (Schlinger, 1997). Sex steroid receptors in the avian brain are found largely in and around the song control nuclei, and estrogen receptors increase in number at around day 5 in the zebra finch, coinciding with the point at which sex differences in the brain start to appear, and decline in number again towards the end of the sensitive period for song learning (Gahr and Konishi, 1988; Schlinger, 1997). This evidence suggests that sexual dimorphism in the avian brain is the result of differential concentrations of gonadal steroid hormones during critical periods in neural development, but inhibition of estrogen synthesis fails to feminise the developing brain (Schlinger and Arnold, 1991; Wade et al., 1994; Springer and Wade, 1997). Females that develop with large amounts of testicular tissue but little ovarian tissue, nonetheless develop a typical female song control circuit (Wade and Arnold, 1994; Springer and Wade, 1997). Furthermore it has been suggested that neural sex differences can develop independently of gonadal sex steroids (Arnold, 1992; Balthazart and Ball, 1995), indicating that sex steroids are not wholly responsible for the sexually dimorphic development of the song control system. Arnold (1997) proposed that a non-hormonal gene product, expressed in sexually dimorphic concentrations during development, could be responsible for regulating the neural development of the song system.

1.3 The developmental stress hypothesis

As discussed in section 1.2.1, although there is considerable evidence that complex male song repertoires have evolved through female choice, it is not immediately obvious how producing a repertoire of song types or syllables incurs a cost to the singer, and thus how the honesty of such a signal could be maintained. Nowicki (1998a) proposed the nutritional stress hypothesis, which suggested that the neural development of nestling songbirds could be limited by their nutritional state, and that brain capacity would in turn limit the structure of the song they were able to produce in adulthood. This hypothesis was later extended to encompass all potential stressors acting during the early stages of nestling development, under the name of the developmental stress hypothesis, by Buchanan et al. (2003).

The brain nuclei that comprise the song control system develop later than other brain areas (Alvarez-Buylla et al., 1994), and this development often coincides with important sensitive periods for song learning (Brenowitz et al., 1997; Nottebohm, 2005). Songbirds are altricial and nestlings are totally dependent on their parents for provisioning between hatching and fledging and often for some time thereafter (Ricklefs, 1968). Young birds undergo rapid growth during this period, but their growth rate is dependent on the amount of food they receive, thereby leaving them vulnerable to undernutrition (Ricklefs, 1968; Nowicki et al., 1998a). The song control system develops during just this early stage of development, when young birds are at the greatest risk of undernutrition and other stressors (Kirn et al., 1999; Kittelberger and Mooney, 1999; Brainard and Doupe, 2002). Therefore, nutritional restriction during this period, or reallocation of essential resources in response to other stressors, is likely to impact negatively on the development of the song control system. Given that the capacity of the song control system probably limits an individual's ability to develop complex song (Burek et al., 1991; Brenowitz et al., 1995), it is hypothesised that the quality of song produced in adulthood will reflect either the level of stress experienced or how well a male was able to cope with environmental stressors during development (Nowicki et al., 1998a; Nowicki et al., 2002b; Buchanan et al., 2003).

Developmental stress has long term effects on various proxies for fitness including morphology, fecundity, quality of offspring, antioxidant defenses and potentially longevity (Birkhead et al., 1999; Metcalfe and Monaghan, 2001; Blount et al., 2003; Metcalfe and Monaghan, 2003; Arnold et al., 2007). Thus, by attending to repertoire size or other aspects of song complexity, females might obtain information about the phenotypic quality of potential mates. Song may also signal a male's capacity for other neural tasks such as learning and remembering (DeVoogd, 2004; Boogert et al., 2008). In addition to simply reflecting phenotypic quality, it is likely that individuals of higher genotypic quality will be more robust in their development when facing a given level of stress (Andersson, 1994; Nowicki et al., 1998a). If there is any stress by genotype interaction involved in determining adult song structure, then phenotype could accurately signal information about male genetic quality (Nowicki et al., 1998a; Nowicki et al., 2002a). Airey et al. (2000b) showed that the volume of the song control nuclei, as well as overall brain mass, is heritable in the zebra finch, as are the number of HVC neurons (Ward et al., 2001) and the rate of neurogenesis in the HVC of adults (Hurley et al., 2008). Forstmeier et al. (2009) found rather lower estimates for the heritability of various song traits in zebra finches. None of these studies, however, have attempted to quantify the effects of genotype-by-environment interactions on the development of song traits or brain morphology, which would be necessary in order for developmental stress to mediate an honest signal of genetic quality.

Since its proposal in 1998, the developmental stress hypothesis has received considerable attention from researchers, and its key predictions, that stressors experienced during development should have a deleterious effect on the size of the adult song control system and the structure of male song, and that females should prefer the song of males that experienced a lesser degree of stress, have been the subject of both laboratory and field studies (summarised in chapter 6, table 6.1). The majority of these studies have investigated the effects of nutritional stress on development, either by manipulating the amount of parental effort required to provide food to their nestlings or through brood size manipulations, which increase or decrease the number of nestlings that each set of parents must feed and are presumed thereby to affect the quality of parental care that each one receives. Other studies have used unpredictability of the food supply (Buchanan et al., 2003; Spencer et al., 2004), parasite load (Buchanan et al., 1999; Spencer et al., 2005a), or artificially raised corticosterone levels (Spencer et al., 2003; Buchanan et al., 2004) as stressors. Corticosterone is naturally released in response to stressful conditions and chronically elevated levels have a number deleterious effects including reduced neural development (McEwen and Sapolsky, 1995; Wingfield et al., 1998; de Kloet, 2000), so might be the mechanism by which stress could mediate the development of the song control system.

1.3.1 Effects of developmental stress on the song control system

Nowicki et al. (2002a) provided the first experimental evidence for an effect of developmental stress on brain development, by hand rearing nestling male swamp sparrows, *Melospiza georgiana*, on either an *ad libitum* diet or on 70% of the food volume consumed by the control diet. Those reared on the restricted diet had smaller HVC and RA nuclei in adulthood, as well as a smaller overall telencephalon volume, compared to birds in the control group. Swamp sparrows have small song repertoires with little variation between individuals, and no effect of the nutritional stress treatment on repertoire size was detected, but males in the experimental group produced less accurate copies of their father's song as adults (Nowicki et al., 2002a). Buchanan et al. (2004) reared zebra finches under two types of stress. One group had their food supply limited by mixing their seed with inedible husk in a 1:3 ratio, while another group of nestlings had corticosterone administered daily in an oral dose. In adulthood the male offspring were sacrificed and males from both the stress treatment groups were found to have smaller HVCs than those reared under control conditions, although neither RA volume nor overall brain mass were affected by the treatments (Buchanan et al., 2004). These results provided further evidence that HVC development is vulnerable to the effects of developmental stress and suggested that the HVC might selectively vulnerable to the effects of adverse early conditions compared to the rest of the brain.

The corticosterone and nutritional restriction treatments applied by Buchanan et al. (2004) had very similar effects on HVC development which suggests, perhaps, a common mechanism by which anything that induces a physiological stress response might impair HVC development. This suggestion received further support from a study in which juvenile canaries were experimentally injected with a common avian blood parasite, *Plasmodium relictum* (Spencer et al., 2005a). The infection depressed haematocrit levels soon after injection, confirming the physiological effects of the treatment, and males in the treatment group had significantly smaller HVCs than did controls (Spencer et al., 2005a).

Although the studies cited above provide evidence of a link between developmental stress and the development of the song control system, they do not demonstrate a direct causal link. An alternative hypothesis is that stress could reduce song learning or complexity by other means and that this reduced singing ability could itself limit brain development. MacDonald et al. (2006) showed that nutritionally restricted song sparrow nestlings already had reduced HVC volumes, compared to controls on an *ad libitum* diet, at 23-26 days post-hatching. This coincides with the onset of song acquisition (Marler and Peters, 1987), so differences in HVC volume apparently precede any differences in song structure. Taken together, the studies I describe above provide evidence that adult HVC volume is selectively impaired by developmental stress, although a study by Gil et al. (2006), found no effect of brood size manipulation on the volume of any song control nuclei, including HVC, in male zebra finches.

1.3.2 Effects of developmental stress on song

The evidence regarding the effects of developmental stress on song structure is less clear cut. For example, three studies have induced nutritional stress in nestling zebra finches by mixing their seed supply with husk in a 1:3 ratio. Spencer et al. (2003), using the same birds whose HVC volume was found to be reduced by stress (Buchanan et al., 2004), found that males from both the nutritional stress and corticosterone groups sang shorter song phrases consisting of fewer syllables compared to controls. There was also a non-significant trend for birds from the treatment groups to have fewer unique syllable types in their song phrase than controls (Spencer et al., 2003). Finally, the maximum frequency reached by birds in the experimental treatments was not as high as that of controls (Spencer et al., 2003). Once again, males treated by administration of corticosterone exhibited similar effects to those exposed to nutritional stress, implying a common mechanism of action. Zann and Cash (2008) found comparable, but not identical, effects of developmental stress on song structure, although males from the food restriction treatment actually produced longer phrases than controls. By contrast, Brumm et al. (2009) found no effect of the food stress treatment on phrase length or the number of syllables in a song phrase. Brumm et al. (2009) also quantified the similarity of male's songs to that of their fathers and found that those from the stress group had produced less accurate copies of their father's song as well as having a non-significant trend toward copying fewer syllable types from their fathers. This apparent effect of stress on song learning supported the results of Nowicki et al. (swamp sparrow, 2002a).

Two further studies used brood size manipulations to manipulate levels of stress in nestling zebra finches. Gil et al. (2006) found no effect of treatment on song phrase length, the number of different element types in the song phrase or the proportion of syllables that were accurately copied from their tutor's song. Although the birds in the this study did not differ in song or brain structure, both male and female nestlings from enlarged broods were lighter, had higher levels of circulating testosterone and showed reduced immune response, compared to those from smaller broods (Naguib et al., 2004), and the brood size manipulation apparently led to transgenerational effects on the morphology of the next generation (Naguib and Gil, 2005). Holveck et al. (2008) looked specifically at the effects on song learning of brood size manipulation in zebra finches.

Males reared in small broods did not learn a greater proportion of syllables from their tutor's song (in line with Gil et al., 2006), but were more likely to copy syntax (e.g. groups of elements that followed one another), than males from large broods (Holveck et al., 2008). Males originating from small broods also sang songs that were more consistent in their sound duration (the total duration of sound within the song phrase, excluding silences between syllables) between phrases (Holveck et al., 2008).

Two studies on other songbird species have provided less ambiguous evidence for an effect of stress on song structure. Parasitised canaries had smaller repertoires of syllable types than uninfected males (Spencer et al., 2005a). European starlings that were caught as fledglings and subjected to an unpredictable food supply by removing their food for periods of time at random, had smaller repertoires of song phrase types in adulthood than those that had been kept on *ad libitum* food (Spencer et al., 2004). The song control system of starlings develops during this post-fledging period so detrimental effects of stress on neural development might be responsible for this effect, but it must be noted that starlings are open-ended learners in which the song control nuclei continue to develop with age (Bernard et al., 1996), so it is far from clear whether the effects of stress on song structure reported by Spencer et al. (2004) were permanent.

There is also decidedly mixed evidence for the proposition that developmental stress might affect performance related aspects of song. Blue tits, *Parus caerulus*, raised in experimentally enlarged broods sang shorter bouts of song than those from small broods (Dreiss et al., 2006). Starlings that had experienced an unpredictable food supply also sang shorter bouts than controls, and spent less time singing overall (Buchanan et al., 2003). Male zebra finches reared on a normal seed diet or high protein diet did not differ in song rate (Birkhead et al., 1999). Those reared in experimentally enlarged broods sang less than controls in one study (Naguib et al., 2008), but the same birds did not differ in singing activity on a prior occasion (Gil et al., 2006), and Tschirren et al. (2009) found that males from large broods actually sang more.

1.3.3 Effects of developmental stress on male attractiveness

A key prediction of the developmental stress hypothesis is that, if song complexity has evolved as an honest signal of a male's developmental history, females should prefer males that have experienced a lesser degree of stress during development. Four studies to date have tested this prediction. Spencer et al. (2005b) allowed female zebra finches to choose between male songs using an operant test on purely acoustic cues, in which the birds were able to trigger playback of recorded songs by landing on computer controlled perches. This experimental design is similar to a testing paradigm in which female preferences have been shown to predict preferences for live males (Holveck and Riebel, 2007). By presenting a choice between songs recorded from males reared under stress or control conditions (Spencer et al., 2003), Spencer et al. (2005b) were able to show that females preferred the songs of unstressed males.

Prior to the proposal of the developmental stress hypothesis, de Kogel and Prijs (1996) tested the effects of brood size manipulation on male attractiveness, using a combination of acoustic and visual cues by presenting female zebra finches with a choice between males reared in experimentally enlarged or reduced broods, in two-way mate choice tests. Males from small broods were larger, had redder beaks and sang at a higher rate during choice tests, and were found to be more attractive to female than those reared in enlarged broods. However, in a similar mate choice experiment, Naguib et al. (2008) found that females did not spend more time with males originating in smaller broods, nor did they express a preference for males with a larger repertoire of syllable types. Blount et al. (2003) manipulated the quality of diet provided for nestling zebra finches but found that diet quality did not affect male attractiveness in two-way choice tests.

In a further relevant study, Naguib and Nemitz (2007) housed juvenile zebra finches in groups that were given diets of different quality between days 35 and 60 post-hatching. At six months of age, males from the lower quality diet treatment were less attractive to females in two-way mate choice tests. These males had smaller orange cheek patches than those in the high quality diet treatment and were still at least partially in the plumage that was developed during the period of nutritional stress (Naguib and Nemitz, 2007). It is unclear, however, whether the effects of nutritional restriction on male plumage or attractiveness were permanent and the authors did not investigate whether the treatment affected male song in any way.

1.3.4 Further evidence for the developmental stress hypothesis

Several field and aviary studies have provided correlative evidence that suggests that developmental stress might affect song structure and male quality in wild birds. A long term study of great reed warblers showed that the length of the innermost primary feather of male nestlings (a measure of developmental rate) was positively correlated with first year song repertoire size (Nowicki et al., 2000). Repertoire size also had a non-significant correlation with nestling body mass, despite methodological problems associated with trying to use a single-point measurement of body mass as a measure of growth (Nowicki et al., 2000). Doutrelant et al. (2000) found that repertoire size in blue tits was correlated with tarsus length, which has been shown to be a good proxy for developmental conditions (Merila and Fry, 1998). Song sparrows that showed a greater corticosterone response to transient stress had smaller syllable repertoires and were less likely to return to breed the following year (MacDougall-Shackleton et al., 2009), suggesting that males with more complex song are better able to cope with stress and that this ability affects survival.

Male Bengalese finches from large broods were smaller in body size than those from smaller broods and sang less complex songs, in a free flight aviary (Soma et al., 2006). In an extensive correlative study, Bolund et al. (2010b) monitored, but did not manipulate, the early growth rate of more than 800 zebra finch nestlings in a laboratory population. Although mass at day 8 was found to predict adult body size and mass in both sexes, it did not appear to influence male plumage coloration or any aspect of male song, including the number of syllables in the song phrase and phrase length (Bolund et al., 2010b). Early growth also did not predict male attractiveness as measured both in choice chambers and by female responses to male courtship in free-flying aviary conditions. The authors conclude that there is no evidence that male sexual traits or attractiveness show heightened condition dependence (Bolund et al., 2010b). Males that were heavier at day 8 did enjoy higher reproductive success, fertilizing more eggs in the free-flight aviary, and the authors suggest that was mediated by long lasting differences in body size (Bolund et al., 2010b).

Finally, Ritchie et al. (2008) constructed the first formal model of the developmental stress hypothesis. This was a population genetic model and modeled developmental stress as affecting both sexes, resulting in either a high or low quality bird, so that quality was not inherited. High quality males were able to learn either of two song types but low quality ones were only able to learn the 'simpler' type of song. Furthermore, low quality birds (both males and females) were unable to differentiate between the two song types. Both the song type males preferred to learn, and the song type females preferred in a mate, were determined by a genetic component, modeled as a single gene with two loci. This genetic bias toward learning from, or mating with, a male with a particular song type could only be expressed if the bird heard that song type as an infant. Both male and female quality affected fitness, defined as the number of viable offspring a mated pair produced, but only direct benefits were provided by males to females. This model demonstrated that a genetic bias (in both males and females) toward learning particular song types, which reveal a male's level of developmental stress, can be selected for, over a range of conditions and in both monogamous and polygynous mating systems. Cultural transmission of these harder to learn song types can be stable, despite the inability of stressed birds to acquire them.

Ritchie et al. (2008) were also able to make predictions about the particular conditions under which preferences for hard to learn songs are more or less likely to evolve. This system is less likely to evolve if the level of developmental stress experienced by juvenile birds is typically high, so that there are fewer high quality birds in the population. In addition, it will be more likely to arise in species in which females select males from a large pool of potential mates and, in monogamous species, where high quality females are able to acquire a mate faster than low quality females. Finally, as the fitness cost to a high quality female of mating with a low quality male decreases, so does the likelihood of a mating system evolving in which songs act as honest signals of males' developmental history.

1.3.5 Summary

Taken together, the studies described in sections 1.3.1-1.4.4, above, demonstrate that there is empirical support for the idea that detrimental environmental conditions after hatching can inhibit the development of the song control system, and weaker evidence that developmental stress impairs song learning or adult song structure. Despite this broad support, there is considerable disagreement about the exact effects of developmental stress and little is known about the mechanisms that underlie any relationship between developmental stress and song. Two main experimental stressors have been used in investigations of the developmental stress hypothesis: nutritional restriction and brood size manipulation. Although both are presumed to have similar effects, reducing the resources available to individual chicks, the results of these two sets of experiments are often very different. Broadly speaking, many nutritional restriction studies have found large and significant differences in neural developmental and song structure between the experimental and control groups (e.g. Nowicki et al., 2002a; Spencer et al., 2003; Buchanan et al., 2004; MacDonald et al., 2006), whereas the majority of brood size manipulation studies have found no effects, or only subtle differences such as in the quality of song syntax learning (Gil et al., 2006; Holveck et al., 2008; see table 6.1). This might reflect differences in the degree of stress induced by the two types of experimental manipulation, or even in the effects each has on developing chicks. For example, raising brood sizes not only adjusts the provisioning of each chick by the foster-parents, but also competition between nestlings (great tit, Neuenschwander et al., 2003). Song learning can be inhibited by male siblings in the nest (zebra finch, Tchernichovski and Nottebohm, 1998), although Gil et al. (2006) attempted to control for this by transferring their birds to tutor groups of equal size during the sensitive period for song learning. At the very least, differences in the results obtained by these two, superficially similar, approaches to investigating the effects of developmental stress indicates that the nature of the stressor can influence the nature of an individual's response to stress.

1.3.6 Effects of developmental stress on female mate choice

Individual variation in female preferences and choice behavior, and the causes of this variation, have received little attention (Jennions and Petrie, 1997; Widemo and

Saether, 1999), but there is a growing body of evidence, across a range of taxa, to suggest that female mate preferences are condition dependent (reviewed in Cotton et al., 2006). Most of these studies have found that low quality females have weaker preferences than high quality females (e.g. Hingle et al., 2001b; Hunt et al., 2005; Hebets et al., 2008), but the direction of preferences might also be affected by condition. For example, female sticklebacks, *Gasterosteus aculeatus*, in good condition showed different preferences for male colour than did those in poor condition (Bakker et al., 1999).

Several studies have investigated long term effects of the developmental environment on female choice behaviour. Manipulation of the quality of rearing diet of female black crickets, *Teleogryllus commodus*, demonstrated that females reared on a high quality diet showed stronger preferences for male calls than those reared on a low quality diet (Hunt et al., 2005). A similar experiment on *Schizocosa* wolf spiders found that females reared on a high quality diet showed strong preferences for males reared under similar conditions, while females reared on a low quality diet showed random mating preferences (Hebets et al., 2008).

Developmental stress is not a phenomenon unique to males. Female songbirds will also experience stressors during development and it has been previously shown that female preferences are affected by learning during early life (Riebel, 2000, 2003). Indeed, the brain nuclei thought to be involved in female perception of, and preference for, male song are the very same as those controlling song production in males and that have been shown to be adversely affected by developmental stress (see sections 1.2.3 and 1.3.1, above). If stress has similar deleterious effects on the neural development of females, it could impair the ability of females to discriminate between males on the basis of their song or other signals. MacDonald et al. (2006) found that female song sparrow reared under nutritional stress had reduced HVC volume at 23-26 days and that the magnitude of this effect was similar to that seen in males. It is unknown whether this reduction in HVC volume would have persisted to adulthood, but this result does indicate that stress could affect female mate choice.

Even in the absence of any effect of stress on female trait perception, poor quality females are predicted to suffer higher costs from choosiness than high quality females and so be less discriminating (Cotton et al., 2006). Females might even express a preference for a mate whose developmental history matches their own, either to minimise the costs of competition for mates (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005), or because matched pairs might have higher reproductive success than mismatched ones.

Four studies have investigated the effects of developmental stress on female choice in birds, to date, two of which used brood size manipulations on zebra finches (Riebel et al., 2009; Holveck and Riebel, 2010), and two which used nutritional stress and comprise chapters 2 and 3 of this thesis (Woodgate et al., 2010; Woodgate et al., in press). Riebel et al. (2009) tested female preferences for familiar and unfamiliar songs using an operant design in which subjects could trigger playback of recorded songs by pecking keys. All preferred the song of their tutor (the unrelated male with which they were housed after nutritional independence) to unfamiliar songs. However, when given the choice of two unfamiliar songs, females from small broods showed stronger preferences than those from enlarged broods (i.e. they showed a greater bias toward playing their preferred song). The majority of females from both treatment groups expressed a preference for one or other stimulus song but, despite the fact that the song stimuli had been chosen to differ in phrase length, neither group showed a preference for longer songs over shorter ones (Riebel et al., 2009). Riebel et al. (2009) provide no data regarding whether the song stimuli differed in complexity or other structural parameters.

subjects were reared under benign laboratory conditions with no attempt to induce stress, and no measure of early growth rate or condition was taken.

The second issue involves the fact that the developmental stress hypothesis specifically predicts that song complexity or repertoire size acts as a signal of developmental conditions (Nowicki et al., 1998a; Buchanan et al., 2003). This raises the important question of whether developmental stress could affect females' ability to discriminate between songs on the basis of complexity, or otherwise affect their preferences for males whose songs differ in complexity. If females that experienced developmental stress were less able to discriminate between male songs this would have significant implications for the developmental stress hypothesis, as lower quality females would have reduced access to information about male quality. There may be selection on all females, even those that experienced developmental stress themselves, to maintain the ability to acquire information from male song regarding their developmental history but no existing studies have addressed this directly. The stimulus songs used by Holveck and Riebel (2010) differed subtly in their consistency and the accuracy with which they had been learned (Holveck et al., 2008), and females were evidently able to distinguish between the songs of males from small or large broods on the basis of these or other differences. However, there is no evidence that the brood size manipulation affected syllable number or other measures of complexity as has been found in prior experiments (Spencer et al., 2003; Zann and Cash, 2008). Likewise, although Riebel et al. (2009) used stimulus songs that differed in phrase length, all originated from males reared under benign conditions and it is not known whether they differed in complexity. Furthermore, it is not clear whether the apparent assortative mating preferences found by Holveck and Riebel (2010) are due to the effects of developmental stress or whether they represent female preferences for song characteristics that are intrinsically linked to differences in brood size.

1.4 The stress response

In the context of this thesis, stress can be defined as the physiological response of an organism to adverse or unpredictable conditions. If the effects of stress on neural development and song, if any, are mediated by the release of corticosterone, the structure of bird song in adults could reflect the impact of any environmental factor that causes a stress response during development. Acute stressors cause the release of catecholamine neurotransmitters (e.g. dopamine, adrenalin, noradrenalin), which cause a rapid increase in alertness and arousal and promote behavioural responses such as the cessation of feeding, increased vigilance and escape flight (Broom and Johnson, 1993). This initial response is followed within minutes by a surge in glucocorticoid hormones, of which corticosterone is the most important in birds. Glucocorticoids act in a number of different ways to promote the typical physiological responses to a stressor, including shutting down digestive processes, increasing peripheral blood supply and mobilising stored energy (Broom and Johnson, 1993; Wingfield and Kitaysky, 2002). This response to acute stressors has been described as the emergency life history stage (Wingfield et al., 1998; Wingfield and Kitaysky, 2002) and serves to reallocate resources from nonessential or less immediate functions to life-preserving processes, helping an animal to survive short periods of extreme stress.

A chronic stress response over days or weeks can have deleterious long-term effects. Raised glucocorticoid levels suppress the production of insulin so that stored energy continues to be mobilised, leaving an animal susceptible to fatigue and disease. Immunosuppressive effects of raised glucocorticoid levels increase susceptibility to disease and can lead to a collapse of immune function (Brown, 1994; Svensson et al., 1998). There is evidence from rodents, primates and humans that chronic exposure to glucocorticoids causes neural damage (Uno et al., 1990; Woolley et al., 1990; McEwen and Sapolsky, 1995; Magarinos et al., 1997; Bremner, 1999), and inhibits neural regeneration (Gould et al., 1998). Long term overproduction of glucocorticoids can have detrimental effects on sleep, learning, memory, exploratory behaviour and other cognitive functions (Bremner, 1999; de Kloet et al., 1999; Roozendaal, 2002). Comparable deleterious effects on muscle mass, immune function and cognition have been demonstrated in birds (Wingfield et al., 1995; Wingfield et al., 1997; Raberg et al., 1998; Wingfield et al., 1998; Saino et al., 2003).

The same stressor can have very different effects on different individuals, so to quantify the amount of stress experienced by an individual it is necessary to measure a suitable parameter, correlated with the animal's physiological stress response. The most common way of measuring stress is via levels of circulating glucorcorticoids in the blood (Broom and Johnson, 1993), which has the advantage that the time between the onset of stress and the release of glucocorticoids is at least two minutes, making it possible to measure an animal's baseline level without the results being confounded by the stress of sampling. Pravosudov and Kitaysky (2006) hand reared western scrub-jays, *Aphelocoma californica*, on control or food restricted diets and found that those on the restricted diet had elevated corticosterone levels after ten days, by comparison with the controls. This difference was still apparent 22 days after the onset of treatment but the difference between the stressed and control nestlings was much reduced, and by one year of age there was no difference between the two groups, indicating that the differences in circulating glucocorticoid levels caused by developmental stress are transitory, although the effects of stress may be permanent.

Another problem with measuring stress hormone levels is that it remains unclear whether an individual showing an increased stress response is actually more stressed, or simply applying an adaptive strategy to cope with a given level of stress (Buchanan, 2000; Wingfield and Kitaysky, 2002). An alternative way to quantify the effects of stress might be to look directly at a trait of interest. For example it has been frequently reported that nutritional stress causes a reduction in the growth rate of nestling birds (Boag, 1987; Nowicki et al., 2002a; Spencer et al., 2003; Arnold et al., 2007; Zann and Cash, 2008; Brumm et al., 2009), although they are frequently able to compensate and reach the same adult mass as controls.

1.5 The zebra finch

As described above, by far the most common model species for studies investigating the developmental stress hypothesis has been the zebra finch. In this thesis I present the results of three further laboratory experiments on domesticated zebra finches and one study on wild zebra finches. The zebra finch is a small, seed-eating, socially monogamous estrildid finch, native to Australia (Zann, 1996). It is thought to be the most primitive (i.e. least changed from the ancestral form) of the *Estrildinae* (Christidis, 1986). The zebra finch is perhaps the most important model passerine system (Griffith and Buchanan, 2010), and in particular has served as the major model species for understanding both the neural basis of song learning and production (reviewed in Zann, 1996; Brainard and Doupe, 2002), and the effects of song on mate choice (reviewed in Riebel, 2009). The zebra finch genome was published in 2010 (Warren et al.), only the second bird species (and the first songbird) to have had its genome sequenced, reflecting its importance as a model species.

1.5.1 Why study zebra finches?

There are several aspects of the life history and behaviour of the zebra finch which make it an ideal model for studying the effects of developmental stress on song, neural development and mate choice. Zebra finches are hardy and easy to keep in captivity (Morris, 1954; Zann, 1996), and have long been one of the most popular and numerous caged birds in the world. Their ready availability through the pet trade, and the ease with which they are kept in the laboratory make them an obvious choice of songbird model. Furthermore, zebra finches breed readily in captivity (Morris, 1954; Zann, 1996) and are opportunistic breeders that can be induced to breed at any time of year (Zann et al., 1995; Williamson et al., 2008). They have an extremely short generation time with captive female zebra finches laying their first eggs as early as 90 days of age (Sossinka, 1980) and wild females producing eggs as early as 62 days post-hatching (Zann, 1994). These traits render them extremely useful for studies of sexual selection since breeding populations can be established in only a short period of time and it is a simple matter to investigate the effects of manipulations applied during early development on adult sexual displays and preferences, and even to investigate trangenerational effects.

Zebra finches are closed-ended learners, producing a relatively simple song (Fig 1.2), consisting of a single, stereotyped song phrase which is unique to the individual (Sossinka and Bohner, 1980; Zann, 1990; Nordeen and Nordeen, 1992; Zann, 1993). Not only does this mean that song complexity is constant throughout an individual's life, and is easier to quantify than in species with a repertoire of song types, but it makes it possible to analyse and compare males' songs to the songs of their father or other tutor, even using automated programmes (Tchernichovski and Mitra, 2002), in order to measure the accuracy of song learning. Zebra finch song has a final advantage for researchers investigating sexual selection by female choice, in that males do not possess or compete for territories so song serves no territorial function. Males show no aggression toward singing birds and singing males do not behave aggressively toward others (Zann, 1994, 1996). Since zebra finch song lacks a territorial function it is likely that song structure in this species primarily reflects the result of selection by female choice.

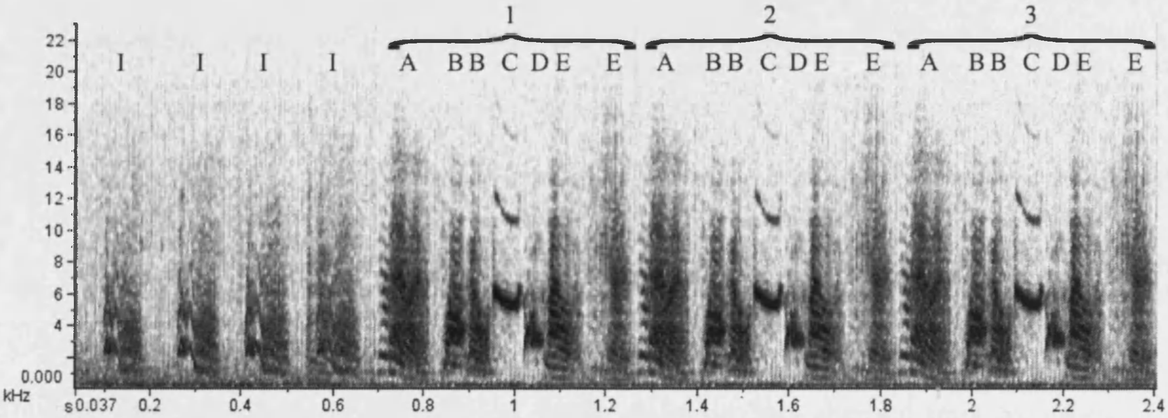

Figure 1.2: Example spectrogram of a typical zebra finch song. A song bout typically consists of a series of repeated introductory syllables (labeled I) followed by several repetitions (range 1-23) of the song phrase (Sossinka and Bohner, 1980). The song phrase consists of 3-14 syllables (constituent elements of the song, separated by short silences or by sudden changes in frequency), sung in a fixed order (Zann, 1993). The song phrase shown here is repeated 3 times and consists of a total of 7 syllables. There are 5 unique syllable types in the phrase, 2 of which (B and E) are repeated.

1.5.2 Development of zebra finch song

Zebra finches are closed-ended learners (section 1.2.2), with a single sensitive phase for learning, extending from day 30-65 (Eales, 1985, 1987; Roper and Zann, 2006), although it may extend up to 80 days if a suitable tutor is not found within 65 days

(Jones et al., 1996; Zann, 1996). After this period zebra finches are unable to learn new song elements, and once song has crystallised (at around 90 days) it does not change throughout the individual's life, although syllable insertions or deletions can occur when a bird is singing (Helekar et al., 2000). In fact, one study (Sturdy et al., 1999) found that up to 44% of song phrases sung by domesticated zebra finches deviated from their predominant syllable order. Song development begins with subsong, toneless irregular sounds, around days 25-36 post-hatching (Zann, 1996), but by day 50 there is a resemblance to adult song in both the syllable structure and organization (Arnold, 1975). By day 90, stereotyped adult song is produced, although the song phrase may shorten due to a decrease in the intervals between syllables, and the number of phrases sung per song continues to increase (Arnold, 1975).

Male zebra finches learn their song from a tutor. If they are not exposed to adult male song during the sensitive period, young males produce aberrant song consisting of improvised syllables, though retaining the species typical organisation into phrases and bouts (Morrison and Nottebohm, 1993; Zann, 1996). In the wild, zebra finches learn their songs from their father (Zann, 1990, 1996), as do domesticated zebra finches kept in aviaries, even when multiple potential tutors are available (Mann and Slater, 1995). Mann and Slater (1994) found evidence that this tendency to learn their father's song was the result of young males preferring to learn from males with which they were housed prior to the onset of the sensitive phase (usually the father), from a male that is paired to the female that raised them (usually the mother), and secondarily from males that have a mate.

A number of studies have set out to investigate how tutors are chosen by providing a choice of potential tutors to males that were reared by females only, and observing which they copy their song from. Males tend to learn predominantly from one tutor, even when several are available (Slater et al., 1991; Mann and Slater, 1995), although some syllables from other males will often be incorporated in their song (Mann and Slater, 1995), and Williams (1990) found that all juvenile males in an aviary population copied syllable types from at least two tutors. If males are given a series of tutors, they will often learn syllables from several of them but copy more of their song from later tutors (Slater et al., 1991). Young male zebra finches and Bengalese finches preferred to learn their songs from a male of their own species, even if that male had learned his own song from the other species (Clayton, 1988b), implying that visual cues are important in song tutor choice. Further evidence for the importance of visual cues comes from the fact that young males also prefer to learn from a tutor belonging to the same colour morph (Mann et al., 1991), and that they will copy the song of a tutor when they are separated by a wire lattice, but not if they are separated by an opaque barrier (Eales, 1989). When given a choice between two unrelated tutors, the only variable that appears to predict which one young males will learn from is aggressiveness, with young males preferring to learn from males that act aggressively toward them (Clayton, 1987a; Jones and Slater, 1996), although Williams (1990), in an aviary study, found that two males that were disproportionately chosen as tutors interacted more with fledglings and provided more parental care.

1.5.3 Mate choice in the zebra finch

Zebra finches form stable pair bonds which are broken only by death or the removal of one partner from the colony although, due to the high mortality and migration rate experienced by zebra finch colonies, an individual may have several partners during its lifetime (Zann, 1996). Both male and female zebra finches discriminate between potential partners and display preferences (Clayton, 1990a; Wynn and Price, 1993) but, in practice, females are more successful in mating with their preferred males than vice versa and female choice can be said to be the most important factor in pairing (Clayton, 1990a). Both males and females with high reproductive success within their pair bond also get more extra-pair copulations (Zann, 1996), but extra-pair paternity is rare in wild zebra finches and has been found to affect as few as 1.7-2.4% of nestlings in 5-8% of broods (Birkhead et al., 1990; Griffith et al., 2010).

Zebra finches consist of two subspecies, those from the Australian mainland (*T. guttata castanotis*) and those from the Lesser Sunda islands (*T. g. guttata*) and many early investigations into mate choice cues in the zebra finch looked at the preferences of females from these subspecies. Females from both subspecies showed preferences for males from the same subspecies in preference tests (Clayton, 1990b). However, females from each subspecies that had been cross-fostered and reared by the opposite subspecies preferred males whose subspecies matched that of their foster-parents rather than their own (Clayton, 1990c), indicating that female preferences are learned rather than innate. Cross-fostered females also showed preferences for the songs of their foster-subspecies rather than their own (Clayton, 1990b). *Taeniopygia. guttata castanotis* males have a thicker black breast band than *guttata* males and Clayton (1990b) artificially increased the size of the breast band in *guttata* males to resemble those of *castanotis*. In preference tests *castanotis* females preferred these manipulated males to unmanipulated *guttata* males, whereas *guttata* females preferred the unmanipulated males, demonstrating that the breast band is used as a mate choice cue (Clayton, 1990b).

Female preferences have also been demonstrated for bill colour, with females preferring males with redder bills (Burley and Coopersmith, 1987; Houtman, 1992). Swaddle and Cuthill (1994b), manipulated the symmetry of the barred feathers on the chest of male zebra finches and demonstrated that females preferred more symmetrical males. This preference for symmetry generalised to an artificial ornament in the form of leg bands (Swaddle and Cuthill, 1994a), and symmetrical males went on to have greater reproductive success than asymmetrical males (Swaddle, 1996). Captive birds are often banded with coloured leg bands to facilitate identification, but Burley et al. (1982) discovered that the colour of these bands affected female preferences in mate choice tests. Females preferred males with red bands but avoided blue or green banded males (Burley et al., 1982; Hunt et al., 1997), perhaps due to a generalization from female preferences for red coloration of other male traits. Interestingly, colour bands have since been shown to affect male mortality (Burley, 1985), dominance (Cuthill et al., 1997), condition and singing rate (Pariser et al., 2010).

Female song preferences in laboratory experiments predict their preferences for live males in mate choice chambers (Holveck and Riebel, 2007), which in turn predict their choice of mates in aviary conditions (Clayton, 1990a), suggesting that male song is the most important cue for mate choice in zebra finches. Female zebra finches preferred to approach a male of their own species than a Bengalese finch, but only if auditory cues were available (Brazas and Shimizu, 2002). This result was apparent even when these auditory cues provided no information about the male's location, indicating that auditory cues are required to facilitate visual mate preferences. Further evidence for the importance of song as a mate choice cue in zebra finches comes from Tomaszycki and Adkins-Regan (2005), who showed that males that were temporarily muted, or had their song performance altered by surgery, were less attractive to females in two-way choice tests and took longer to from pair bonds in an aviary.

Considerable attention has been paid to what aspects of male song are attractive to females. Female preferences have been demonstrated for complex songs with a larger repertoire of syllables (Neubauer, 1999; Holveck and Riebel, 2007; Vyas et al., 2009; and see chapter 3). Several studies have also indicated a preference for longer song phrases

(Clayton and Prove, 1989; Neubauer, 1999), but this may be due to the confounding variable of syllable number (Riebel, 2009). Leadbeater et al. (2005), did not find any preference for longer songs in operant song-choice tests and female preferences were better explained by syllable repertoire than phrase duration in operant and phonotaxis preference tests (Holveck and Riebel, 2007). Females prefer males that sing at a higher rate (ten Cate and Mug, 1984; Houtman, 1992; Collins et al., 1994), and those that sing more frequently, although it remains unclear whether song rate is the cause or effect of female preference (reviewed in Riebel, 2009). Higher amplitude songs have also been shown to be more attractive in operant choice tests (Ritschard et al., 2010). Females show a preference for the song of their father or foster father (Miller, 1979a; Zann, 1996), the song of familiar males (Riebel, 2000; Riebel and Smallegange, 2003), songs which resemble that of their father (Clayton, 1987b), or songs they were exposed to during the first 25 days after hatching (Riebel, 2000). Collins et al. (1999) found that females showed a preference for an artificially created repertoire of song types over the species-typical stereotyped song, although they did not differentiate between this repertoire and the songs of four different males so it is not clear whether they were expressing a preference for a repertoire of songs over a simple song, or for several different males over a single male. Despite the evidence for female preferences for a range of acoustic features, the relative importance of these various cues remains unclear and it has not been possible to derive a 'catalogue' of traits that determine the attractiveness of a zebra finch song (Riebel, 2009).

1.5.4 Wild and domesticated zebra finches

By far the majority of work on zebra finches, including investigations into developmental stress, neural control of song and female choice, has been carried out on laboratory populations of zebra finches. Zebra finches have been well established in captivity, both in Australia and Europe, since the 1870s and introduction of wild stock to the captive gene pool has been extremely low since the early 20th Century (Zann, 1996). This raises the question of whether findings in domesticated zebra finches actually reflect the history of natural and sexual selection on wild birds or whether domesticated and wild stocks have diverged, either through genetic drift or differing selection pressures. Forstmeier et al. (2007), found that all domesticated populations of zebra finches that they tested had reduced genetic variability compared to wild populations, but that there was no evidence for any severe bottlenecks. Several recent studies have found differences between wild and domesticated populations in several traits. Wild zebra finch females have been shown to prefer wild males to domesticated ones, and vice versa (Rutstein et al., 2007), and wild and domesticated birds differ in nestling growth rate, adult mass and size, and immune response (Tschirren et al., 2009). Despite these differences there was no difference in song rate and brood size manipulation appeared to affect wild and domesticated zebra finches in similar ways (Tschirren et al., 2009), suggesting that the life history trade-offs underlying development are fundamentally unaltered by domestication. This is reassuring, but it should be born in mind that laboratory studies on domesticated populations may not fully reflect the selection pressures at work in the field, and future studies should focus on better understanding the relationship between male song, female choice and reproductive success in wild populations.

1.6 Aims

In this thesis I have used zebra finches as a model species to investigate the practical implications of the effects of developmental stress on song and neural development in two areas. The first two chapters report studies in which I examined the effects of developmental stress on female mate choice, using mate choice techniques that are commonly used to investigate consensus by females regarding male attractiveness. This allowed me to investigate whether developmental stress can cause differences in female preferences that could be expected to lead to biologically relevant differences in mating behaviour in wild populations. In the second half of the thesis I turned my attention to the effects of developmental stress on males, asking what information about male quality might be signaled by song structure (chapter 4). I have also investigated, for the first time in a wild zebra finch population, whether male song structure is predictive of male quality or reproductive success (chapter 5).

In chapter 2 (published in Animal Behaviour as Woodgate et al., 2010), I tested the preferences of female zebra finches that had been reared under control conditions or conditions of nutritional stress, for live males in a four-way choice chamber. This experiment aimed to quantify whether developmental stress affected female choice in adulthood. In this experiment, I found that females from the stress treatment group were less active than controls, so I also present the results of a second experiment which attempted to elucidate whether this difference in activity reflected a general difference in female behaviour or energy levels, or whether it was context dependent.

Chapter 3 (Woodgate et al., in press at Behavioral Ecology), investigates specifically whether nutritional stress in early development affects female preferences for songs that differ in complexity. I investigated whether control and stressed females differed in either the strength or direction of their preferences for recordings of male song that had been edited to differ only in complexity, using an operant choice experiment.

In chapter 4, I calculated the relative influences of heritability, shared rearing conditions and environmental conditions on the neural development, body mass and song structure of male zebra finches reared under control conditions or nutritional stress in a partial cross-fostering experiment. By calculating the heritability of these traits and whether there was a significant genotype-by-environment interaction, I sought to determine whether the effects of stress on male phenotype could convey information regarding a male's genetic quality or developmental history.

Finally, chapter 5 describes a study into whether male song reflects fitness and reproductive success in a wild zebra finch population. I recorded the songs of breeding males and investigated whether males' song structure predicted their morphology or reproductive success. Some of the offspring in this colony were cross-fostered to other nests, allowing me to investigate whether any relationship between song and reproductive success reflected an effect of genes or parenting, by comparing the success of offspring within the nest to those reared in foster-nests.

In concert, the research described in these chapters enhances our understanding of the effects of developmental stress, both on male traits and on female mate choice behaviour. As such they highlight the important role early environmental conditions might play in the evolution of condition dependent male sexual traits, and question whether female preferences or other aspects of mate choice behaviour might themselves be condition dependent.

Chapter 2: Developmental stress and female mate choice behaviour in the zebra finch

2.1 Introduction

Female mate choice is generally accepted to be a fundamental selective force in driving the evolution of elaborate male sexual traits (Andersson, 1994). Yet while individual variation in male signal production has been widely studied, factors contributing to individual variation in female choice behaviour remain poorly understood (Jennions and Petrie, 1997; Widemo and Saether, 1999). Mounting evidence suggests that, like many male ornaments, female preferences can often be condition dependent (reviewed in Cotton et al., 2006), and several recent studies have shown long-term effects of the developmental environment on female choice in a small number of invertebrate species. Female black field crickets, *Teleogryllus commodus*, reared on a high-protein diet respond more rapidly to male call playbacks and show stronger preferences than those reared on low-protein diets (Hunt et al., 2005). Hebets et al. (2008) manipulated rearing diet in *Schizocosa* wolf spiders and found that females reared on high-quality diets showed strong preferences for males reared on a high-quality diet while females reared on a low-quality diet showed random preferences. In vertebrates, Riebel et al. (2009) found that female zebra finches, *Taeniopygia guttata*, reared in large or medium-size broods exhibited weaker song preferences than those from small broods, while Holveck & Riebel (2010) found that female zebra finches reared in small or large broods preferred the song of males reared under similar conditions and suggested that this might be evidence of assortative mating by phenotypic quality. These studies suggest that developmental conditions can be an important source of variation in female choice.

However, the potential effects of environmental factors during the rearing period on adult female mate choice remain largely unaddressed.

There are several ways in which environmental factors experienced during early development could affect female choice. These explanations can be understood at both the mechanistic and functional levels (Tinbergen, 1963). First, when considering the mechanism for such an effect, developmental stress may impair the ability of females to rank potential mates according to the mate choice criteria normally used by females exposed to superior developmental regimes. This may be caused by the deleterious effects of stress on their higher order neural-processing mechanisms (as has been shown previously to occur in males, Buchanan et al., 2004), or even on their visual or auditory systems. According to this hypothesis, stressed females would not rank males in the same way as unstressed females and may show random choice. However, careful experimental tests of discriminatory ability would be needed to determine that this random choice was due to changes in trait perception. Stress has well-documented detrimental effects on neural development (McEwen and Sapolsky, 1995; de Kloet, 2000), and the inhibitory effect of stress on the development of the song control system is hypothesised to mediate the honesty of song complexity as a signal of past developmental stress (Nowicki et al., 1998a; Nowicki et al., 2002a). Although female songbirds of many species do not produce song, most retain the nuclei of the song control system, albeit often at greatly reduced volumes (Nottebohm and Arnold, 1976; Ball and MacDougall-Shackleton, 2001), and these are involved in female perception of, and preferences for, male song (Brenowitz, 1991; Del Negro et al., 1998; MacDougall-Shackleton et al., 1998; Leitner and Catchpole, 2002). It is plausible, therefore, that the effects of developmental stress on the developing brain may affect female preference. The effects of stress on neural development are not limited to the song control system (McEwen and Sapolsky, 1995; de

Kloet, 2000), so the ability of females to evaluate and choose between potential mates on the basis of other male signals could also be disrupted by developmental stress.

Alternatively, several functional hypotheses suggest that developmental stress could affect mate choice even without a direct effect of developmental conditions on the mechanisms by which females perceive male traits. Theory suggests that poor-quality females will bear higher costs of choosiness and be less discriminating in their mate choice (Cotton et al., 2006). Poor neonatal nutrition has been shown to have detrimental effects on growth rates as well as long-term effects on a number of life history traits including morphology, fecundity, quality of offspring and possibly longevity (Birkhead et al., 1999; Metcalfe and Monaghan, 2001, 2003; Arnold et al., 2007). Less choosy females may benefit, therefore, by investing less time and resources in sampling potential mates (Cotton et al., 2006) and reduced choosiness could affect female preferences in two ways: females may exhibit random choice which would, in practice, be difficult to distinguish from the effects of reduced perceptual ability in mate preference tests; Alternatively, theory suggests that less choosy females might use different traits as mate choice cues, or prioritise cues differently to choosier females (Fawcett and Johnstone, 2003b). According to this hypothesis, stressed females would show consistent mate choice preferences as a group, but these preferences would be uncorrelated with those of unstressed females.

Another, functional hypothesis suggests that females may show an active choice for a mate that matches their perception of their own rank, as determined by their developmental history, leading to assortative mating according to developmental stress exposure. If there is costly competition for mates, poorer quality individuals may seek to minimise the costs they incur by preferentially targeting low-quality partners (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005), leading to assortative mating by individual quality. This would further benefit females if pairs matched according to early experience have higher reproductive success than mismatched pairs, if, for example, developmental history is related to environmental variation. There is little empirical evidence so far that female quality could affect the direction of mating preferences, but Holveck & Riebel (2010) found that females raised in experimentally enlarged broods preferred the song of males raised under similar conditions.

The nutritional stress hypothesis (Nowicki et al., 1998a), later extended to encompass a number of potential environmental stressors under the name of the developmental stress hypothesis, relates specifically to the development of male song as a mate choice signal (Buchanan et al., 2003). It suggests that song complexity in songbirds, a sexually selected signal used by females in mate choice decisions, may act as an honest signal of how well a male was able to cope with nutritional or other stress during early development. A number of recent studies have provided empirical support to the hypothesis (Nowicki et al., 2000; Nowicki et al., 2002a; Buchanan et al., 2003; Spencer et al., 2003; Buchanan et al., 2004; Spencer et al., 2004; Spencer et al., 2005b; MacDonald et al., 2006; Zann and Cash, 2008); although see Gil et al. (2006) and Naguib et al. (2008). However, although the effects of developmental conditions on the expression of male traits are well established, there have been no empirical tests to date of whether developmental stress can affect female choice for live males.

In this study I tested the hypothesis that female mate choice behaviour based on visually assessed, or other non-acoustic, traits may be sensitive to the effects of environmental conditions during early development. I investigated the effects of nutritional restriction on female choice in a model species for sexual selection studies, the zebra finch. Female zebra finches were reared under control conditions or conditions of nutritional stress, using an established experimental paradigm for developmental stress that has been found by several previous studies to reduce nestling growth rates and reduce song complexity of adult males (Spencer et al., 2003; Zann and Cash, 2008), and which mimics a form of stress to which young birds are particularly vulnerable (Nowicki et al., 1998a). In adulthood, I tested female mate choice in an extensive series of 128 four-way male choice tests using a randomised block design.

My aim in this study was to quantify whether developmental stress in females affects their choice of males in adulthood. My experimental design allowed me to elucidate in part the mechanisms that underlie any such effect. I predicted that if stressed females could not perceive male sexual traits as effectively as controls, there would be no correlation between the mate choice preferences of stressed and control females. In this case stressed females would show random choice but would invest as much time and energy in choice as controls. If perceptual ability was unaffected but stressed females were less choosy, they may also exhibit random choice but would invest less in sampling potential mates. If stressed females exhibit consistent preferences as a group, but these preferences are uncorrelated with those of controls, it would imply an effect of stress on the way females assess male cues, particularly if stressed females also showed reduced sampling behaviour. Since, in this experiment, I did not manipulate males' developmental history, it would not be possible to detect assortative mating on the basis of developmental stress.

2.2 EXPERIMENT 1: FEMALE CHOICE WITH NONACOUSTIC CUES

2.2.1 Methods

2.2.1.1 Experimental subjects

Subjects were 32 adult female zebra finches raised at the Max Planck Institute for Ornithology in Seewiesen, Germany, in compliance with the German animal protection law, as part of an experiment designed to test the heritability of neural morphology in male zebra finches. These females were the offspring of 20 pairs of laboratory-raised birds which were partnered randomly and allowed to raise two broods in succession. Each pair raised both broods either under control conditions or under conditions of nutritional stress, with this treatment allocated randomly. Unavoidably, some of the experimental females were genetic siblings.

Experimental manipulations, designed to induce different levels of nutritional stress, took place between days 5 and 30 after the chicks hatched. A modified version of the feeding protocol described by Spencer et al. (2003) was used, in which control nests were provided with *ad libitum* seed mix while those in the stress treatment received a limited mass of seed daily, the amount of seed provided being adjusted for brood size and the age of the chicks. In addition, the seed provided for nests under the stress treatment was mixed with husks in a 1:2 ratio by volume. After 30 days of age all cages received *ad libitum* seed.

The aims of the larger study necessitated a partial cross-fostering design in which half of the chicks in each brood were cross-fostered to a different brood of similar age (hatching dates within 1 day of each other), within 3 days of hatching, maintaining the original brood size. Seven pairs of genetic sisters originating from the same brood were used in the experiment, of which six pairs had been separated by the cross-fostering and were raised in different nests. One pair of sisters was raised in the same foster nest under control conditions.

Until 60 days of age offspring were housed with their parents or foster-parents in cages measuring 40 x 40 x 40 cm. At 60 days they were removed from their parent's cage but remained housed with their siblings. From day 80 onwards, birds from all treatment groups received identical treatment and were housed together in two sex-specific aviaries with large outdoor (416 x 242 cm and 302 cm high) and indoor (403 x 301 cm and 200 cm high) compartments. Throughout the breeding and rearing period the temperature was maintained at 21°C on a 12:12 h light:dark photoperiod. Of 47 broods, 24 were raised under control and 23 under stressed conditions (mean brood size [number of chicks hatched in each brood] ± 1 SD: control broods: 2.8 ± 0.8; stressed broods: 3.2 ± 1.1).

As adults (mean age in days ± SE = 189.7 ± 8.2), the female offspring were transferred to the University of Bristol for mate choice trials. All birds were housed in groups of six to eight per cage (118 x 50 cm and 212 cm high, Terenziani, Montichiari, Italy) and maintained at 21°C on a 12:12 h light:dark photoperiod, which coincided with the natural day length in Seewiesen at the time the birds were transferred to Bristol. There were eight possible combinations of the different treatment factors (control or stress treatment, cross-fostered or not, brood 1 or 2), and four females from each combination were used in the mate preference tests. Of the females originally transferred to Bristol, three birds died before the experiment started and a further two were excluded as they were lame and unable to perch. From the remaining pool of birds, four females were selected at random from each of the eight possible combinations of treatments to give a total sample size of 32 females.

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2.2.1.2 Morphological measurements

The offspring were weighed daily until 35 days post-hatching and at days 40, 50, 60, 70 and 80, allowing me to look for effects of the stress treatment on nestling growth rates. At day 30, when the stress treatment ceased, I calculated the effect size (*d*) of the effect of the stress treatment on nestling mass as the difference between the mean mass of nestlings from the control and stress treatment groups divided by the pooled standard deviation in mass. After the completion of experimental trials (mean age in days ± SE = 649.8 ± 13.4) I measured mass, mean wing length (mean length of both wings) and mean tarsus length (mean of three measurements from each leg) of all but one of the subjects from experiment 1 (one female had died before the morphological measurements were taken), and all participants in experiment 2.

2.2.1.3 Mate choice trials

Female preferences for live males were tested in a mate choice apparatus previously described by Bennett et al. (1996; 1997) in earlier mate choice experiments on zebra finches and Pearn et al. (2001) on budgerigars, *Melopsittacus undulates* (Fig. 2.1). This experiment was conducted under approval from the Bristol University Ethics Committee, UIN number: UB/06/028. At the start of each trial, barriers that initially confine a subject female to a central arena are removed using a pulley system operated by the experimenter from outside the room. This leaves the subject female free to enter any of four viewing arms arranged in the shape of a cross, each of which terminates in a stimulus chamber containing a male. The stimulus chambers are separated from the rest of the apparatus by a clear Plexiglas filter such that the female can have visual (and potentially olfactory), but not physical, contact with the males. In an attempt to mask any auditory cues from the males, I played a sound recording of zebra finch vocalizations from their home cage throughout the trials (Pearn et al., 2001; Evans et al., 2006). This was also intended to reduce the stressful effects of isolation.

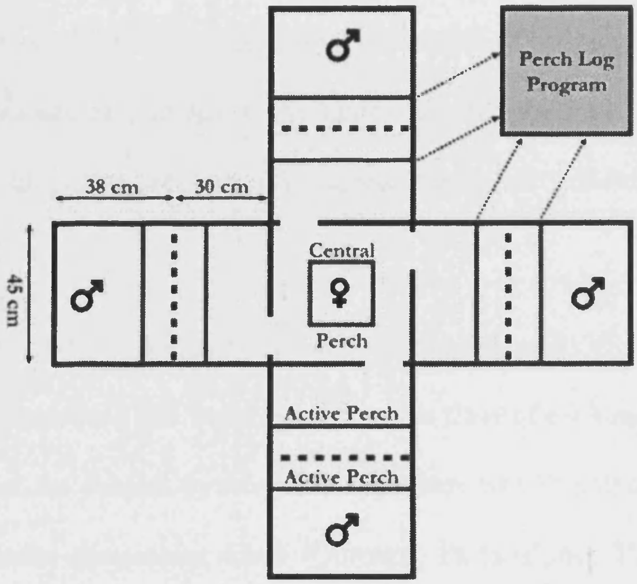

Figure 2.1: Plan view of the mate choice apparatus. Dotted lines indicate the positions of the Plexiglas filters.

Electronically monitored perches in each viewing arm logged the number of hops made in front of each male, which has been found to be indicative of female preferences in a number of previous studies (Bennett et al., 1996; Bennett et al., 1997; Evans et al., 2006). Opaque barriers beneath the perches ensured that females could only view the males from the perches and not when sitting on the floor. From the perch log data I also deduced how often the female moved from one viewing arm to another and thus how many opportunities she had to sample the potential mates. Although the perch log data indicated when a female switched from one viewing arm to another, it is not possible to determine whether females had returned to the central arena between consecutive hops in front of the same male or whether they remained in the viewing arm but did not move. It is likely, therefore, that while I accurately measured the number of switches from one male to another, this will underestimate the total number of sampling visits since several consecutive visits to the same male will be treated as one. Although it is impossible to determine from the perch logs the frequency or duration of visits to the central arena, observations of a subset of females in the apparatus indicated no difference between females from the control and stress groups. Indeed, the majority of females made few or no visits to the feeders.

During each trial the temperature was maintained at 21°C and all birds were provided with *ad libitum* seed and water, placed on the floor of each male chamber and in the central arena for the subject female. The apparatus was illuminated by 12 equally spaced 100 W Truelite fluorescent tubes (Durotest, Philadelphia, PA, U.S.A.), which provide spectral emissions similar to natural skylight, powered by high-frequency (> 30 kHz) ballasts (Tridonic, Basingstoke, U.K.), to ensure that female preferences would not be affected by artificial lighting conditions (Bennett et al., 1996; Evans et al., 2006), and that lighting conditions were the same as earlier mate choice experiments using zebra finches (Bennett et al., 1996; Bennett et al., 1997).

Four quartets of stimulus males were used (*N*=16) and every quartet was presented to each of the 32 females over the course of the experiment resulting in a repeated measures design. The four males of each quartet were always presented together allowing me to compare the preferences expressed by different females. Each trial lasted 3.5 h and two trials were conducted per day. Each stimulus male was used in 32 trials over the course of the experiment and appeared in no more than one trial every 2 days. Females experienced four trials each with no less than 1 week between each trial. The order in which females were tested was determined randomly without replacement, such that no female experienced a second trial until all 32 had completed their first trial. The four quartets of males were presented to the four females from each combination according to a randomised block design. In addition, the stimulus chamber in which each male of a quartet was placed was also randomised so that the four females of each treatment combination did not view the same males in the same locations. The time of day was balanced across females so that each female experienced two morning and two afternoon trials.

2.2.1.4 Acclimatization trials

Over the course of 2 weeks, immediately prior to the start of experimental trials each female experienced four acclimatization trials lasting 3.5 h each, intended to allow them to become familiar with the structure of the apparatus and to overcome any neophobia they might experience. During these acclimatization trials females were released in the apparatus in groups of six to reduce isolation stress and promote exploration of the apparatus. The four stimulus chambers each contained a conspecific female, rather than a male, to ensure that no association was formed between particular arms of the apparatus and preferred males, which could later bias behaviour in the experimental trials.

2.2.1.5 Stimulus males

The males used in the stimulus chambers of the mate choice apparatus during experimental mate choice trials were obtained from local breeders in the Bristol area. All males were adults (>100 days) at the time they were acquired but no further data were available regarding their ages. Sixteen wild-type males were randomly assigned to the four quartets.

Between trials, males were housed one quartet to a cage in the same room as, but not within visual contact of, the females. Measurements of wing length (mean length of both wings), tarsus length (mean of three measurements from each leg) and mass were taken at the completion of experimental trials.

2.2.1.6 Statistical analysis

Statistical analyses were performed using Minitab 15 (Minitab Inc., State College, PA, U.S.A.) and SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.). To determine the effect of the stress treatment on nestling growth rates, I used two-sample t tests to compare the mean mass of nestlings from each treatment at the start of the experimental manipulation (5 days post-hatching), and at day 30 when the stress treatment ceased. Two-sample t tests were also used to investigate the effects of the stress treatment on the mean adult wing length, tarsus length and mass of all the birds used in experiments 1 and 2.

To investigate the effects of the treatments on female choice I ran a repeated measures general linear model (GLM) using the total number of perches made in front of each male in a quartet over the course of each trial as the dependent variable. The perching data were log transformed to normalise the residuals. Female identity was the random term in the model. The original model included stress treatment, cross-fostering treatment and brood number as between-subjects factors and had two within-subjects terms: quartet and male identity, nested within the quartet with which each male appeared. The original, full-factorial model was simplified by sequential deletion of nonsignificant terms. There was a significant interaction between stress treatment and male quartet ($P < 0.05$). However, this interaction was judged not to be of major interest since it merely reflects variation in the effect of the stress treatment on female activity among randomly chosen male sets. The stress treatment*male quartet interaction term was, therefore, removed from the final model to allow me to calculate accurately the main effect of the stress treatment across all four quartets of males. The final model included stress treatment as the only between-subjects factor, and male quartet and nested male identity as within-subjects factors. Constraining the model to retain the other two between-subjects variables, cross-fostering treatment and brood number, or to retain the stress treatment*male quartet interaction, had no qualitative effect on the results.

There were six pairs and three trios of genetic sisters used in the experiment. To control for the potential nonindependence of sisters I ran a linear mixed model on the number of perches in front of each male, including both female identity and genetic family of origin as random terms. Stress treatment was the only fixed factor in the model.

To test whether trial order had an effect on female activity I ran a GLM using as the dependent variable the total number of perches made in front of all males over the course of a trial. The initial model included female identity as a random term, stress treatment, cross-fostering and brood as between-subjects factors and trial number as a within-subjects factor. After a process of model simplification by sequential deletion of nonsignificant terms the final model retained just subject identity, stress treatment and trial order. The total number of perches made during just the first hour of each trial was also analysed using the same model.

To investigate the level of agreement between different females that had experienced the same treatment I calculated the repeatability of females' ranking of males relative to the other males of their quartets. I performed two nested ANOVAs, one for the rankings made by stressed females and one for control females, in which 16 males were nested within their four quartets. I then calculated repeatability using the method outlined by Lessells and Boag (1987), but comparing only the variation within quartets to the within-individual variation, and ignoring that component of the variation attributable to differences between the quartets (Forstmeier and Birkhead, 2004). Fisher's *z* transformation was calculated to compare the repeatability of the rankings made by control and stressed females.

The number of switches made from one viewing arm to another did not follow a normal distribution and could not be normalised by transformation. A Kruskal-Wallis test was used to compare the total number of switches made across all four trials by females in the control and stress treatment groups. I also compared the mean number of hops made per visit to a viewing arm (total number of hops across all four trials/total number of switches across all four trials) by stressed and control females using a Kruskal-Wallis test.

2.2.2 Results

In concordance with previous studies (Spencer et al., 2003; Zann and Cash, 2008) the stress treatment had a significant effect on nestling growth rates (Fig. 2.2). At the start of the treatment (day 5) there was no difference between the groups in body mass ($t_{31} = 0.8$, $P = 0.429$), but by the end of the treatment period (day 30) there was a small but significant effect of stress treatment on nestling mass ($t_{25} = 2.49$, $P = 0.02$; effect size, $d = 0.34$). Control nestlings were significantly heavier than those in the stress group. In adulthood there was no difference between the stressed and control females in wing length, tarsus length or mass (all $P > 0.05$).

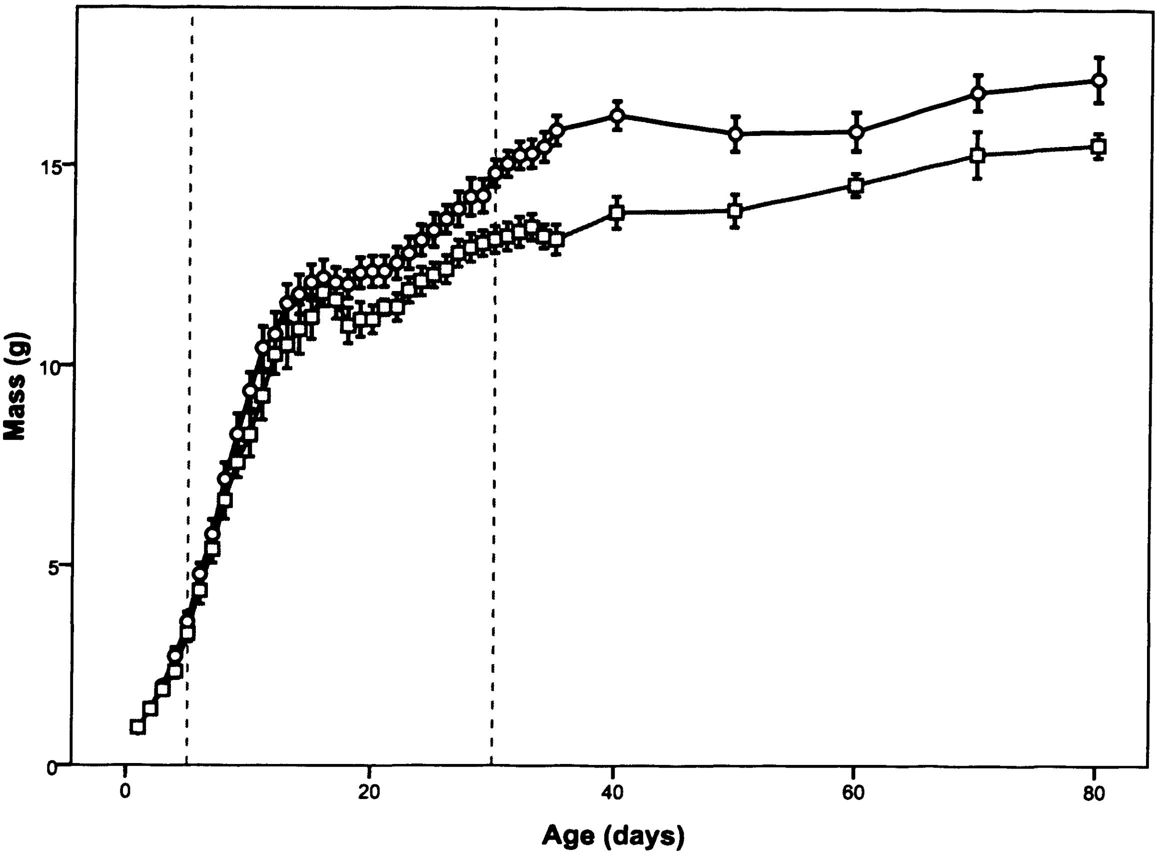

Figure 2.2: Growth rates of nestling zebra finches (mean mass of nestlings in each brood ± 1 S.E.) under control (open circles) and stress (open squares) treatments. Dotted lines indicate the start (day 5) and end (day 30) of experimental manipulations.

There was a significant treatment effect on female activity in the mate choice trials (Table 2.1). Control females were significantly more active than those in the stress group (Fig. 2.3) and made almost three times as many hops (mean hops per trial ± 1 SE: controls: 654.1 ± 219.9; stressed: 230.9 ± 115.6; effect size, d = 0.62; Table 2.1). In addition, there was a significant effect of stress treatment on movement within the mate choice apparatus. The number of switches between viewing arms differed significantly, with control females moving between arms more often (H_1 = 5.49, P = 0.019). However, there was no difference between the treatments in the mean number of hops made per visit to a viewing arm ($H_1 < 0.01$, $P > 0.99$).

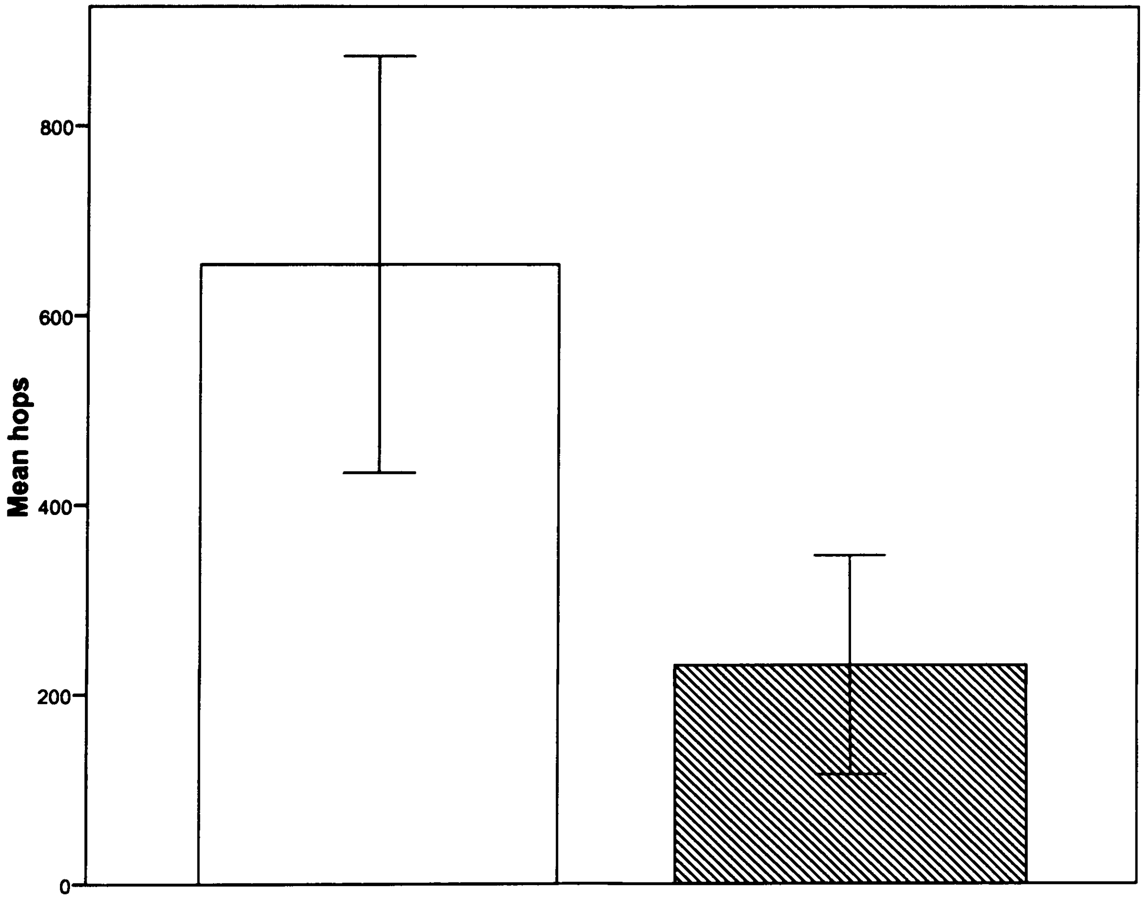

Figure 2.3: Mean number of hops (± 1S.E.) made per 3.5 hour trial by control (white bars) and stressed (shaded bars) females.

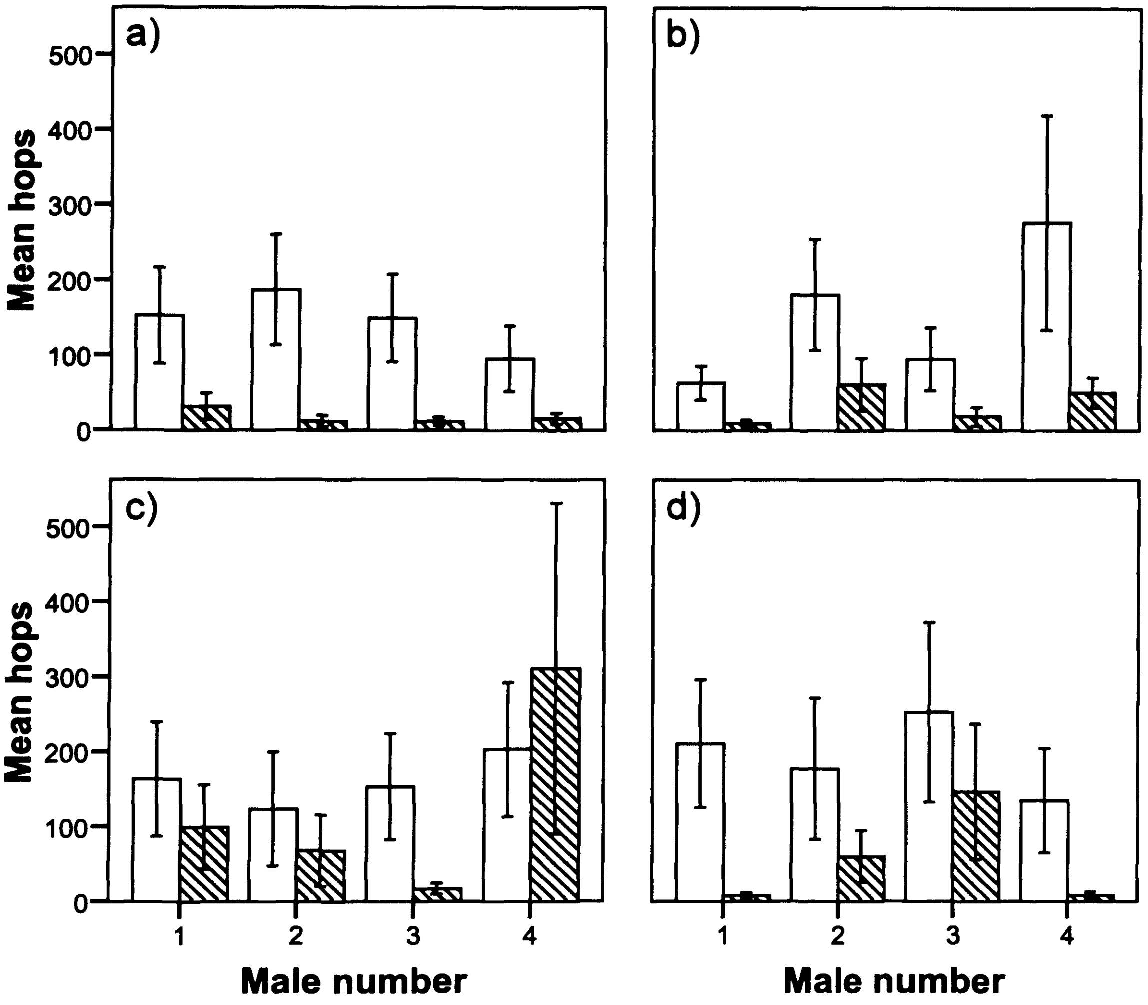

Figure 2.4: Mean number of hops (± 1S.E.) made in front of each stimulus male by control (white bars) and stressed (shaded bars) females. a) – d) represent the four quartets of males.

Figure 2.4 illustrates the activity of control and stressed females when viewing each different stimulus male and shows that female hopping rates (for both control and stressed females) differed significantly between different males (Table 2.1), implying that some males were preferred to others. There was no difference in female activity rates between quartets of males (Table 2.1), indicating that, although attractiveness varied between males, no quartet of males received significantly more attention than others.

After I controlled for the difference in activity rates noted above, there was no effect of the stress treatment on the difference in female activity between stimulus males ($P > 0.05$), so the stress treatment*male identity interaction term was therefore excluded from the final model. There was no significant difference in activity rates or preference between females raised in their natal nest or a foster nest, nor between females from the first and second broods, so cross-fostering and brood number were also dropped from the final model.

The effect of stress treatment on female activity remained significant when genetic family was included as a random term in the model ($F_{1,29.8} = 7.813$, $P = 0.009$), as did the effect of female identity (Wald $Z = 2.184$, $P = 0.029$). Genetic family did not have a significant effect (Wald $Z = 0.072$, $P = 0.943$).

There was no effect of trial order on female activity, nor was there a significant interaction between stress treatment and trial order (Table 2.1). The significant effect of stress treatment on activity levels noted above emerged even during the first hour of the trials (Table 2.1). During the first hour there was no interaction between trial order and treatment, nor a main effect of trial order (Table 2.1).

The relative rankings given to each male were significantly repeatable when the males were ranked by females from either control or stress treatments. However, this repeatability of male attractiveness was low (control females: $r = 0.06$, $F_{12,240} = 2.05$, $P = 0.021$; stressed females: $r = 0.06$, $F_{12,240} = 1.98$, $P = 0.027$) and there was no treatment difference in repeatability ($z = 0.01$, $P = 0.992$).

Table 2.1: Results of GLMs testing the effects of the stress treatment on the number of hops by females in front of different males; and whether the total number of hops in each trial or in the first hour of each trial were affected by stress treatment and trial order. The random factor in each model, subject identity, is indicated in bold type. Significant *P* values are indicated in bold.

Dependent variable	Model term	*F*	d.f.	*P*
Hops in each viewing arm	Stress treatment	8.00	1, 30	**0.008**
	Male quartet	0.27	3, 465	0.849
	Male identity (nested within quartet)	3.07	12, 465	**<0.001**
	Subject identity	8.86	30, 465	**<0.001**
Total number of hops per trial	Stress treatment	8.85	1, 30	**0.006**
	Trial number	1.52	3, 90	0.215
	Treatment * Trial order	0.34	3, 90	0.794
	Subject identity	5.30	30, 90	**<0.001**
Total number of hops during first hour of trial	Stress treatment	5.41	1, 30	**0.027**
	Trial number	0.46	3, 90	0.708
	Treatment * Trial order	1.24	3, 90	0.299
	Subject identity	6.32	30, 90	**<0.001**

2.3 EXPERIMENT 2: EFFECT OF SOCIAL CONTEXT

2.3.1 Methods

2.3.1.1 Experimental subjects

The results of experiment 1 indicate an effect of stress treatment on activity rates in the mate choice trials. In experiment 2 I tested whether this effect was specific to a mate choice situation by looking at activity rates in different social contexts. I monitored the activity of control and experimentally stressed females in three contexts: alone, in visual, but not physical, contact with an unfamiliar female, and in visual contact with an unfamiliar male. Of the 32 subjects used in experiment 2, 25 had previously been used in the mate choice trials and the remaining seven chosen at random from a pool of five to seven available females from each combination of treatments. This was because experiment 2 took place 8 months after experiment 1 during which time four of the females used in those trials had died.

2.3.1.2 Activity rate trials

Females were transferred to a cage (118 x 50 cm and 50 cm high), identical to their home cages, except that it had a central divider of wire mesh, preventing physical contact but otherwise allowing birds on either side to have visual, acoustic and olfactory contact with one another. Each half of the cage contained two perches, those on the side containing the focal female being monitored so that the number of hops on each was electronically logged by a program (PerchLog, P.G. Lovell, School of Psychology, University of St Andrews, U.K.). The focal female was placed in one half of the cage and the number of hops she made was monitored over a 1 h trial, while the other side of the apparatus contained (1) no bird, (2) an unfamiliar female or (3) an unfamiliar male.

Four stimulus males and four stimulus females were used in this experiment, all of which were acquired from local breeders. Each stimulus male and female was used in eight trials and experienced a maximum of two trials per day. Each subject female experienced three trials, one in each social situation. The order in which females were used was determined randomly without replacement, such that no female experienced a second trial until all 32 had completed their first trial. The order in which females were exposed to the three social situations was allocated according to a randomised block design, and the half of the cage (right or left) in which the female was placed was randomised. Each of the four stimulus males and the four stimulus females was viewed by eight subjects, one from each treatment combination so than no two subject females from the same treatment viewed the same stimulus birds.

2.3.1.3 Statistical analysis

To investigate the effects of stress on female activity under different social conditions I ran a GLM in which the total number of hops made by each subject female during each 1 h trial was the dependent variable and subject identity the random factor. Again, the hopping data were log transformed. The original model included stress treatment, cross-fostering treatment and brood number as between-subjects factors, and a single within-subjects term, trial type, which specifies the three social conditions (subject female alone, visual contact with a conspecific female, visual contact with a conspecific male). The original, full-factorial, model was simplified via sequential deletion of nonsignificant terms until a final model was specified, with one between-subjects term (stress treatment), a single within-subjects factor (trial type) and the interaction term between these factors. Constraining the model to include the other two between-subjects variables, cross-fostering treatment and brood number, had no qualitative effect on the results.

I investigated whether female activity levels in experiment 2 were consistent with activity levels in experiment 1. There were 25 females that participated in both experiments and for these birds I used the Spearman rank correlation to compare the rank order of their total number of hops across all four trials in experiment 1 with their total activity across all three trials in experiment 2.

2.3.2 Results

Females were more active during trials in which they had visual contact with an unfamiliar conspecific than when they were alone ($F_{2,60} = 4.93$, $P = 0.01$; Tukey's test, pairwise comparison of female activity when alone versus conspecific female present: $t = -2.87$, $P = 0.016$; conspecific male present: $t = -2.55$, $P = 0.035$; Fig. 2.5). However, there was no difference in activity between trials in which the subject female could view a female or male conspecific (Tukey's test: $t = 0.32$, $P = 0.946$; Fig. 2.5).

Significant and large differences between the activity rates of control and stressed females were seen in the preference trials in experiment 1 but I found no effect of stress treatment on activity rates in experiment 2 ($F_{1,30} = 1.42$, $P = 0.243$; Fig. 2.5). There was a nonsignificant tendency for stressed females to be less active than controls under two of the three social contexts (alone; visual contact with unfamiliar male), but the activity differences were not significant in any of the three trial types (Tukey's test, pairwise comparison of mean activity of control with stressed females in different trial types: female alone: $t = 1.62$, $P = 0.591$; conspecific female present: $t = 0.36$, $P = 0.999$; conspecific male present: $t = 0.87$, $P = 0.952$), and there was no interaction between stress treatment and trial type ($F_{2,60} = 0.40$, $P = 0.671$).

Females' ranked activity levels in this experiment showed a significant correlation with their activity in experiment 1 (Spearman rank correlation: $r_S = 0.48$, $N = 25$, $P = 0.016$).

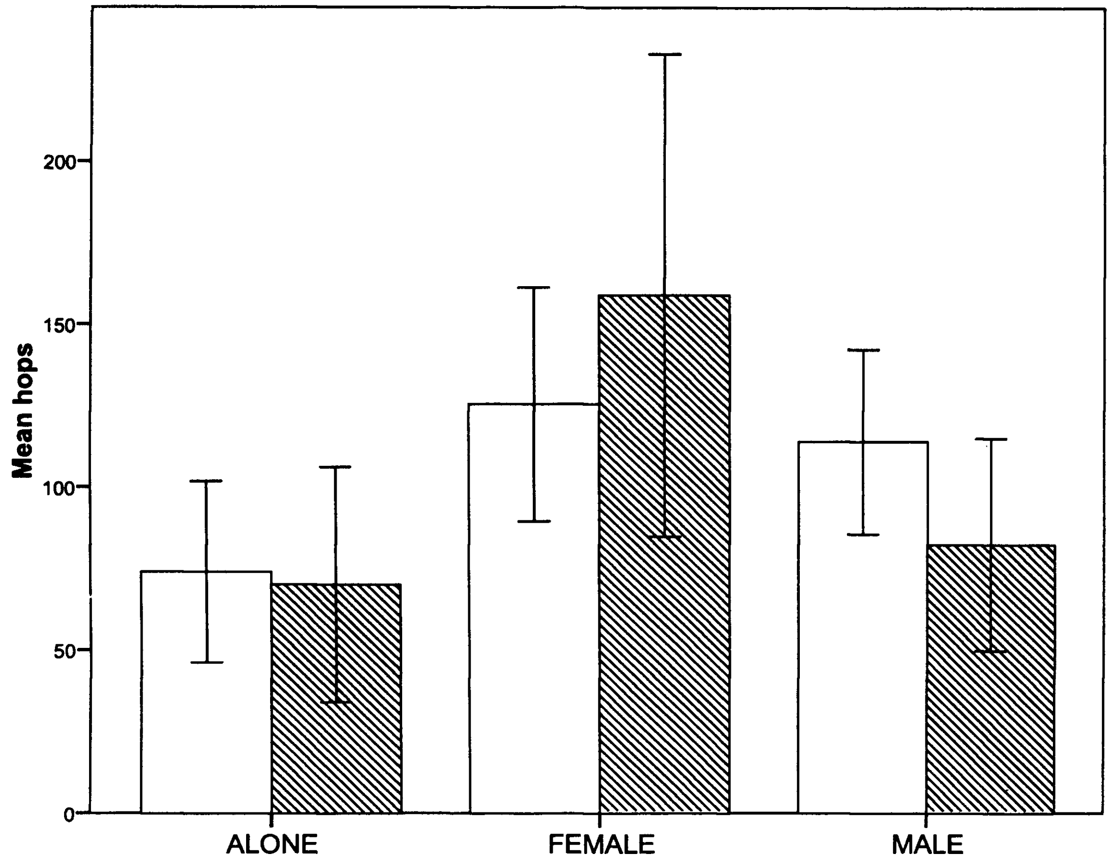

Figure 2.5: Mean number of hops (± 1 S.E.) made by control (white bars) and stressed females (shaded bars) in three different social contexts: ALONE = focal female alone in apparatus; FEMALE: focal female had visual contact with an unfamiliar female; MALE = focal female had visual contact with an unfamiliar male.

2.4 Discussion

In recent years there has been considerable interest in how developmental conditions can affect male signalling and attractiveness (Andersson, 1994). The condition dependence of many male signals is now evident (Buchanan et al., 1999; Griffith et al., 1999; Nowicki et al., 2002a; Buchanan et al., 2003; Spencer et al., 2003, 2004; Spencer et al., 2005a; Spencer et al., 2005b; Gil et al., 2006; Naguib and Nemitz, 2007; Naguib et al., 2008; Zann and Cash, 2008), even when the signal is produced a long time after the cessation of the stressor (Buchanan et al., 2003; Spencer et al., 2003; Spencer et al., 2005a; Naguib and Nemitz, 2007; Zann and Cash, 2008). This study constitutes the first experimental test of the effects of developmental stress on female preferences for live males.

Stressed females were almost three times less active than controls during the mate choice trials. The subjects in this study included several sets of genetic sisters. Since female activity levels in mate choice tests are likely to be heritable (Forstmeier, 2005), the activity of sisters cannot be considered to be fully independent. However, the effect of stress treatment remains robust when genetic family is accounted for as a random effect. This demonstrates the potential for mate choice effects, owing to a reduction in the participation or possibly the motivation of stressed females for mate assessment. If such a reduction translated into a change in male assessment in the wild I predict that one would see altered mate choice behaviour in individuals that experienced stress during early development. Similar reductions in activity levels in a choice chamber have been observed in inbred female zebra finches (Bolund et al., 2010a), perhaps implying a general difference in mate choice behaviour between high- and low-quality females.

I found a clear effect of male identity on female activity, demonstrating that males varied in their attractiveness to the test females. However, despite this, and despite the significant effect of the stress treatment on female activity, I found no difference between the treatment groups in their preferences for males. However, differences in the way females sample potential mates could still affect the outcome of mate choice even where females do not differ in preference since females that sample few potential mates will show less bias in their mate choice than those that choose between many (Wagner, 1998).

Two- and four-way mate choice trials have previously detected strong, population level female preferences when a trait of interest in the stimulus males has been manipulated (e.g. Burley et al., 1982; Swaddle and Cuthill, 1994a; Bennett et al., 1996), but since I was investigating the possibility of an effect of stress on females' ability to discriminate between males, rather than on preference for a particular trait, I chose to allow subjects to choose between unmanipulated males on the basis of naturally occurring variation between them. Several studies have successfully used these experimental protocols to investigate female preferences for unmanipulated stimulus males (e.g. Bennett et al., 1997; Evans et al., 2006), and Holveck & Riebel (2007) found that female preference for live males in a two-way choice test was repeatable across several test paradigms and predicted female choice for song alone. However, Forstmeier & Birkhead (2004) found low levels of between-female agreement for unmanipulated males in a similar choice environment. Although the relative rank preferences accorded to the stimulus males in experiment 1 were found to be repeatable, the repeatability was extremely low, in line with (although even lower than) those reported by Forstmeier & Birkhead (2004). These low levels of agreement between females imply that their preferences were relatively weak or that they varied between females, making it difficult to identify any treatment effect on preference. There was no effect of the stress treatment on the level of agreement between females.

Another criticism levelled at this methodology is that it is not possible to separate preference from sampling behaviour (Wagner, 1998; Rutstein et al., 2007). If an individual samples potential mates more often before making a choice it can be harder to detect a preference (Wagner, 1998): the female is likely to be more active in front of the less preferred males as part of her sampling behaviour, making it appear that her preference is weaker than that of a female that makes a choice after less sampling. It seems likely that, when the differences between stimulus males are less obvious, females will have to sample each male more often before making a choice, so my results may underestimate the strength of female preferences in this study. Additionally, the apparatus constitutes a novel environment and may promote stress in females. This could lead to reduced willingness to move around the apparatus with the result that females were unable to sample males sufficiently to make a choice. A final way in which my methodology may have underestimated preference strength is due to the stimulus males and experimental females being drawn from different populations. Genetic differences have been demonstrated to exist between populations (Forstmeier et al., 2007), and female preferences for various traits are known to be learned (e.g. Burley, 2006), so it is possible that females might exhibit stronger preferences for males from their own population and be less able to assess attractiveness in males from other populations.

Females' relative activity levels in experiment 1 were significantly correlated with those in experiment 2, indicating that female activity is consistent over time and across different behavioural contexts. However, stress treatment had a significant effect on the overall activity rates of females during four-way mate choice trials in experiment 1 but not in no-choice trials in experiment 2. This difference in activity was not observed under any of the three trial types in experiment 2, allowing me to conclude that stress does not have a fundamental effect on activity rates per se, but instead has a context-dependent effect on behaviour. The two experiments differed in a number of ways (including novelty of the apparatus, length of the trials, time of year, age of the females, masking of potential acoustic cues, etc.), so it is impossible to draw clear conclusions from these data about exactly what behaviour is affected by stress. There are, however, several intriguing possibilities that should be addressed by future studies.

Experiment 1 used a traditional four-way mate choice design of a type that does not separate female preference from sampling behaviour (Wagner, 1998; Rutstein et al., 2007), whereas the methods used in experiment 2 can be thought of as a no-choice trial in which sampling behaviour is not measured. An effect of stress on mate-sampling strategies would lead to a difference between control and stressed females in their behaviour in experiment 1 but remain undetected in experiment 2. If stressed females expend less time and resources on sampling potential mates this would support the prediction that females of lower quality should be less choosy (Cotton et al., 2006) and, moreover, could affect the outcome of mate choice in a natural context even if stress has no effect on preference function (Wagner, 1998). In experiment 1, the number of switches made between viewing arms by control females (an estimate of how many sampling visits were made to the potential mates) was significantly greater than that of stressed females, but there was no effect of stress treatment on the mean number of hops they made per visit. This supports the hypothesis that the difference in activity rates was the result of reduced sampling by stressed females. If there had been a difference in hopping activity per se, I would expect to see no difference in the number of switches, with stressed birds making fewer hops per visit than controls. Although the switching data may underestimate the number of visits females made to the viewing arms, since consecutive visits to the same arm could not be detected, observation of a subset of females in the apparatus suggests this bias is consistent across the treatment groups. I suggest, therefore, that my conclusion that control females made more sampling visits, switching more often between males, is robust, implying their mate choice sampling behaviour allows them greater opportunities to compare the available males.

There were several factors that differed between the two experiments and might have potentially led to a difference in behaviour unassociated with mate choice. One such factor was the novelty of the apparatus. In experiment 2 this was an adapted home cage while the mate choice chamber used in experiment 1 was largely unfamiliar. Females from the control and stress treatments differed significantly in their behaviour in this novel environment but not in the more familiar setting. A large body of evidence suggests that individuals differ consistently in suites of behavioural and physiological traits known as personalities or behavioural syndromes which are often measured by individual differences in behaviour in a novel environment (Sih et al., 2004; Groothuis and Carere, 2005). It is an intriguing possibility that my results might represent an effect of early environmental conditions on females' propensity to explore a novel environment, and one that it would be interesting to see addressed in future studies. However, if the difference in activity levels between stressed and control females in experiment 1 was due to differences in exploratory behaviour or coping styles in a novel environment, one might expect an interaction between stress treatment and trial order with the difference between the treatment groups being reduced in later trials as the females became more acclimatised to the mate choice apparatus, which was not the case.

The duration of the trials in the two experiments was also markedly different: 3.5 h in experiment 1 and only 1 h in experiment 2. If the effects of developmental stress underlying the difference in activity levels observed in experiment 1 displayed a temporal pattern, emerging only over long periods of activity, the trials of experiment 2 might have been too short for them to appear. However, analysis of just the first hour's activity in experiment 1 reveals the same pattern as can be seen in the main analysis, with controls hopping significantly more often than stressed females.

Experiment 2 took place 8 months after experiment 1, so seasonal variation in mate choice behaviour could underlie the lack of consistency between the two. Wild zebra finch populations often breed continuously for 7 - 10 months of the year and breeding appears to be triggered by rainfall and the ripening of new supplies of seed (Zann et al., 1995; Zann, 1996). Unlike periodically breeding species, zebra finch females' ovaries are maintained in a medium-developed resting state allowing rapid ovulation (Sossinka, 1980). Because of these traits the zebra finch has traditionally been considered a nonseasonal, opportunistic breeder and it has been assumed that if diet, photoperiod, humidity and temperature are kept constant, as in my two experiments, there will be no effect of season on breeding behaviour. However, Williamson et al. (2008) found seasonal differences in maternal reproductive investment even under constant conditions. It is unknown whether there may be similar seasonal effects on behaviour in mate choice contexts.

Because of the difference in timing of the two experiments, the subject females were 8 months older during experiment 2 than they were at the end of experiment 1. The possibility cannot be discounted that the difference in hops observed in experiment 1 is due to age-dependent rather than context-dependent effects of developmental stress. Age-dependent changes in reproductive success are found across the majority of bird species, reproductive success usually increasing with age (Martin, 1995). If such age-dependent effects interacted with the effects of the stress treatment it is possible that any differences in activity levels or choosiness between control and stressed females reduce with age.

The results of this study clearly demonstrate that early developmental stress caused a reduction in female activity in a mate choice situation, which might potentially have profound consequences for both mate assessment and reproductive behaviour in natural populations. Both stressed and control females showed preferences for some males over others, which indicates that developmental stress did not reduce females' ability to differentiate between potential mates. There was no significant difference between the preferences of stressed and control females which one would expect if females preferred males with similar developmental histories to themselves, or if control and stressed females were assessing males according to different criteria. Stressed females were less active in a mate choice context than controls, and made fewer visits to the viewing arms, consistent with the hypothesis that stressed females invest less in sampling potential mates. Reduced sampling effort is an expected outcome of reduced choosiness in females.

Although my results do not allow me to differentiate clearly between all possible hypotheses, they are most consistent with the possibility that developmental stress causes reduced choosiness in females. This hypothesis is in concordance with several previous studies that have looked at the effects of the rearing environment on female choice (Hingle et al., 2001a; Hunt et al., 2005; Hebets et al., 2008). The long-term repercussions of the early environment on adult behaviour, and their implications for mate choice, have been hitherto largely neglected, but this study demonstrates the importance of understanding the contribution of environmental factors to variation between individuals in mate choice behaviour.

Chapter 3: Developmental stressors that impair male song learning do not appear to affect female song preferences in the zebra finch

3.1 Introduction

The importance of female choice as a powerful and widespread selective force is well documented (Andersson, 1994). However, relatively little attention has been paid to the role individual variation in female preference plays in mate choice (Jennions and Petrie, 1997; Widemo and Saether, 1999; Qvarnstrom et al., 2000; Cotton et al., 2006). In particular, although there is good empirical support for the condition dependence of female preferences (Cotton et al., 2006), the contribution to female preference of variation in environmental factors that might influence growth has rarely been addressed. Hunt et al. (2005) found that the strength of the mating preferences of female black field crickets (*Teleogryllus commodus*), was affected by the quality of the diet on which they were reared, with those raised on a high protein diet showing stronger preferences and more sexual responsiveness in phonotaxis tests. Female *Schizocosa* wolf spiders raised on a high nutrition diet preferred males reared on a similar diet whereas those reared on a diet of lower quality showed no preference (Hebets et al., 2008). In vertebrates, Holveck and Riebel (2010), found that the brood size in which female zebra finches (*Taeniopygia guttata*) were reared affected the direction of their preference for male songs in adulthood, while Riebel et al. (2009) found that female zebra finches reared in large or medium size broods exhibited weaker song preferences than those from small broods when offered a choice of two unfamiliar songs. Using the same model species, I found that zebra finch females reared under conditions of nutritional stress were less active than controls during mate choice trials, suggesting a difference in mate choice strategies, in particular the possibility that stressed females might invest less in sampling potential mates (chapter 2, published as Woodgate et al., 2010).

There are several mechanisms by which unfavourable conditions during development could affect female choice in adulthood. Detrimental effects on development might impair the ability of females to accurately assess male secondary sexual traits, for example via the well documented deleterious effects of stress on neural development (McEwen and Sapolsky, 1995; de Kloet, 2000). Even in the absence of a direct effect on trait perception, adverse developmental conditions can lead to reduced phenotypic quality (e.g. Birkhead et al., 1999; Metcalfe and Monaghan, 2001, 2003; Arnold et al., 2007). Low quality females may prioritise mate choice cues differently to higher quality females (Fawcett and Johnstone, 2003b), and are predicted to invest less time and resources sampling potential mates and to be less discriminating in their mate choice (Cotton et al., 2006). Finally, low quality females may seek to limit the cost of competition for mates, and minimise the risk of failing to mate, by expressing a preference for low quality males (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005).

The potential effects of developmental stress on song preferences in songbirds are of particular interest since there is mounting evidence that the complexity of male songs may function as an honest signal of how an individual was able to cope with developmental stress. This was proposed as the nutritional stress hypothesis by Nowicki et al. (1998a), and later extended to cover all potential environmental stressors as the developmental stress hypothesis (Buchanan et al., 2003). A number of studies over the past several years have provided evidence that developmental stress may have a detrimental effect on neural development in the HVC region of the songbird brain, a nucleus associated with the learning and production of song (Nowicki et al., 2002a; Spencer et al., 2003; Buchanan et al., 2004; Spencer et al., 2005a; MacDonald et al., 2006). Stressors experienced during early development have been found to have a detrimental effect on song learning in the swamp sparrow, *Melospiza georgiana* (Nowicki et al., 2002a), and zebra finch (Holveck et al., 2008; Brumm et al., 2009); and on measures of song complexity in the European starling, *Sturnus vulgaris* (Spencer et al., 2004), canary, *Serinus canaria* (Spencer et al., 2005a), and zebra finch (Spencer et al., 2003; Zann and Cash, 2008). Female zebra finches prefer complex songs (Clayton and Prove, 1989) and the less complex songs of stressed male zebra finches are less attractive to females than those of control males (Spencer et al., 2005b). In wild song sparrows, *Melospiza melodia*, adult males displaying a larger stress response both sang less complex songs and were less likely to return to breed the following year (MacDougall-Shackleton et al., 2009). However, some studies have failed to demonstrate any effect of developmental stress on neural developments, song structure or attractiveness, in male zebra finches (Gil et al., 2006; Naguib et al., 2008), and Bolund et al. (2010b) found no evidence for a heightened effect of developmental conditions on sexually selected traits such as song, compared to non-sexually selected traits, in zebra finches.

Female songbirds of many species also possess the song-control nuclei of the brain, albeit often much reduced in volume (Nottebohm and Arnold, 1976; Ball and MacDougall-Shackleton, 2001), and a number of studies have provided evidence that these neural structures play a role in the perception of, and preference for, male song (Brenowitz, 1991; Del Negro et al., 1998; Halle et al., 2002; Leitner and Catchpole, 2002). MacDonald et al. (2006) found that nutritional stress during development led to reduced HVC volume in female as well as male song sparrows. If nutritional stress has a detrimental effect on females' ability to discriminate between male songs this would have important implications for the developmental stress hypothesis, since low quality females would be unable to acquire information pertaining to male quality, but no studies have directly addressed this question to date. Two studies have, however, investigated the influence of developmental conditions on female song preferences: Riebel et al. (2009) allowed female zebra finches from small or large broods to choose between recordings of unmanipulated male songs in operant song preference tests and found that those from small broods exhibited stronger preferences than those from medium or large broods when choosing between unfamiliar songs. When forced to choose between an unfamiliar song and that of their tutor, females from all treatments preferred the tutor song, implying that any effect of developmental stress in song discrimination ability was not large enough to prevent recognition of the tutor song. In a similar study, Holveck and Riebel (2010) found that female zebra finches raised in small broods had a significant preference for the song of males raised in small broods while those from larger broods preferred the song of males raised in large broods, implying assortative preferences by phenotypic quality. Again, these results suggest that there was no effect of treatment on females' ability to discriminate between the stimulus songs.

The developmental stress hypothesis proposes that male song complexity has been selected for as a sexual signal because it reflects developmental conditions (Nowicki et al., 1998a; Nowicki et al., 2002a). I sought to test the hypothesis that developmental stress experienced by females could alter the strength or direction of their preference when offered a choice between songs of differing complexity. Female zebra finches raised under stress or control conditions were able to trigger playback of song recordings that had been digitally edited to vary in complexity. Female preferences were evaluated using an operant song preference design similar to previous studies, making it possible to measure female preference in isolation from other male traits and without the influence of male preferences (Riebel, 2000). Similar choice tests predict preferences for live males (Holveck and Riebel, 2007), and have been used in previous studies investigating the effects of developmental conditions on female preference (Riebel et al., 2009; Holveck and Riebel, 2010).

3.2 Methods

3.2.1 Experimental subjects

Subjects were 32 adult female zebra finches raised at the Max Planck Institute for Ornithology in Seewiesen, Germany under control conditions or nutritional stress. These females were the offspring of 21 pairs of laboratory raised finches and included 7 pairs and 3 trios of genetic sisters. The experimental manipulation of nestling condition was achieved by limiting the food intake rate of nestlings and so impairing growth rates, as in previous studies (Lemon, 1993; Spencer et al., 2003). Briefly, each pair of nesting zebra finches raised two broods in succession under identical conditions. Pairs were allocated at random either to the control treatment, in which they were provided with *ad libitum* seed mix, or to the nutritional stress treatment in which a limited mass of seed was provided, mixed with husks in a 1:2 ratio by volume (Spencer et al., 2003; Buchanan et al., 2004; and see chapter 2). The mass of seed provided to nests under the stress treatment was adjusted for brood size and the age of the chicks. Half of the chicks in each brood were cross-fostered, at random, to another brood of similar age within three days of hatching. These treatments are described in greater detail in chapter 2.

The female offspring were transferred to the University of Bristol as adults (mean age in days ± S.E. = 189.7 ± 8.2) in September 2006, where they were maintained at 21°C on a 12:12 light:dark photoperiod and provided with *ad libitum* seed mix and water.

There were eight possible combinations of the three treatment factors: control or stress treatment, cross-fostered or not, brood 1 or 2. Four females from each combination of treatments were chosen as subjects in the song preference trials, giving a total sample size of 32. If more than four females were available from a particular treatment combination, four subjects were selected at random. Twenty eight of the subjects had previously been used in visual mate choice trials, described in chapter 2.

3.2.2 Song discrimination apparatus

I tested female song preferences using a song discrimination apparatus adapted from that used by Spencer et al. (2005a). This experiment was conducted under approval from the Bristol University Ethics Committee, UIN number: UB/06/028. The apparatus consists of an L-shaped wooden box with two 'active perches', one in each arm of the apparatus (Fig. 3.1). These perches were connected via microswitches to a computer (RM, UK) which logged the number of hops made by a female on each perch (PerchLog, P.G. Lovell, UK). Upon activation PerchLog also played a pre-recorded sound from a speaker (Sony, UK) mounted above the perch, with maximum amplitude of 80.4 dB at the level of the perch (23cm beneath the speaker). Whilst a playback was in progress, further perches were logged but did not trigger another playback. Food and water were provided *ad libitum* in dishes mounted above the active perches. A third, central, perch was situated between the arms of the apparatus and was not electronically monitored. To encourage females to spend their time on the perches rather than the floor, the bottom of the apparatus was filled with water to a depth of 1cm. This had the desired effect of encouraging females to sit on the perches, only rarely landing on the bottom of the apparatus. Birds spent a maximum of one hour per day in the apparatus and spent a total of seven hours in the apparatus over the course of the experiment.

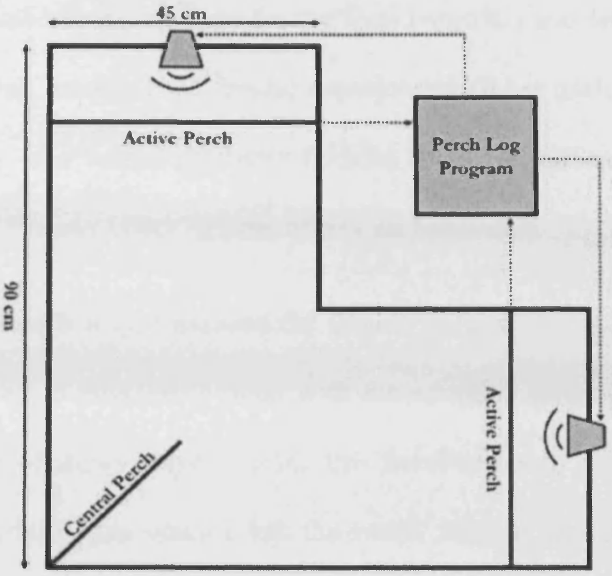

Figure 3.1: Plan view of the song discrimination apparatus.

3.2.3 Song preference tests

During the experiment, each female underwent three acclimatization trials on consecutive days to familiarise them with the novel apparatus and with the experimental protocol. On the two days following the last acclimatization trial they experienced two experimental trials, each with a different pair of stimulus songs. Approximately two to five weeks later each female underwent a further two trials on consecutive days with the same two pairs of stimulus songs they had experienced in the initial trials. Thus each subject experienced three acclimatization trials and four experimental trials: two trials with each of two pairs of stimulus songs. All trials, acclimatization and experimental, lasted one hour each.

Females were allocated at random to one of six groups and the order in which each group underwent their trials was determined at random. Thus, all females in a group underwent five consecutive days of trials before the females of another group began their trials. Females remained in their groups for the final two trials and were used in the same order as before. Within groups, each female experienced all her trials at the same time of day but time of day was balanced across females from the different combinations of treatments to control for any effect of time of day on behaviour.

At the start of each trial, I induced the female to land several times on each of the active perches in order to familiarise them with the stimulus songs played back by each perch (mean number of songs played during this familiarization period ± 1S.D.: 25.8 ± 3.2). The one hour trial began when I left the room. During acclimatization trials, one active perch triggered playback of a male zebra finch song, while the other triggered playback of a sample of guitar music of identical length and mean amplitude. These sounds were assigned to the left and right perches at random.

In the experimental trials, landing on one perch triggered playback of a zebra finch song and the other played a simplified version of the same song. The allocation of these songs to the left and right perches was balanced within individual females, such that if the complex song was triggered by the left perch in the first experimental trial, it was triggered by the right perch in the second trial with that pair of stimulus songs. In each trial, I measured three response variables: 1) the total number of hops made on both active perches; 2) preference strength, defined as the proportion of total hops that was made on the perch that received the greater number of hops, regardless of whether that perch triggered playback of the control or simplified song; and 3) preference for the control (more complex) song, defined as the proportion of total hops that were made on the active perch that triggered playback of the control song.

3.2.4 Stimulus songs

In each experimental trial the female could choose between a pair of stimulus songs that differed only in complexity, consisting of one control and one simplified song. Six pairs of stimulus songs were created, from recordings of six male zebra finches. Each female experienced trials with two of the six pairs of stimulus songs. Song pairs were assigned in a balanced manner across treatment groups such that each song pair was used with equal numbers of control and stressed females. All the stimulus songs were unfamiliar to the subjects in this experiment.

Stimulus songs were constructed by editing the source recordings using Raven 1.2 sound analysis software (Cornell, USA). Control song files consisted of a single phrase excerpted from the source recording and repeated five times to form a song bout. The first repetition of the phrase was preceded by four introductory notes taken from the same source recording. Four song files were constructed for each song, each using a different recording of the song phrase, selected at random from the same source recording. During the experiment, each hop on the relevant perch triggered the playback of one of the four song files at random. This allowed me to control for subtle variation in song production.

Simplified songs were constructed by selectively deleting syllables from the control song files (Fig. 3.2). In this way, I produced songs that mimicked the simplified song structure of males that have experienced developmental stress (Spencer et al., 2003) but were identical to the control songs in all other respects. I removed two syllables from each song phrase, one that was repeated elsewhere in the phrase and one that was unique. The first syllable of a phrase was never removed and two consecutive syllables were never removed. Of the remaining syllables, that fulfilled these requirements, the two to be deleted were chosen at random.

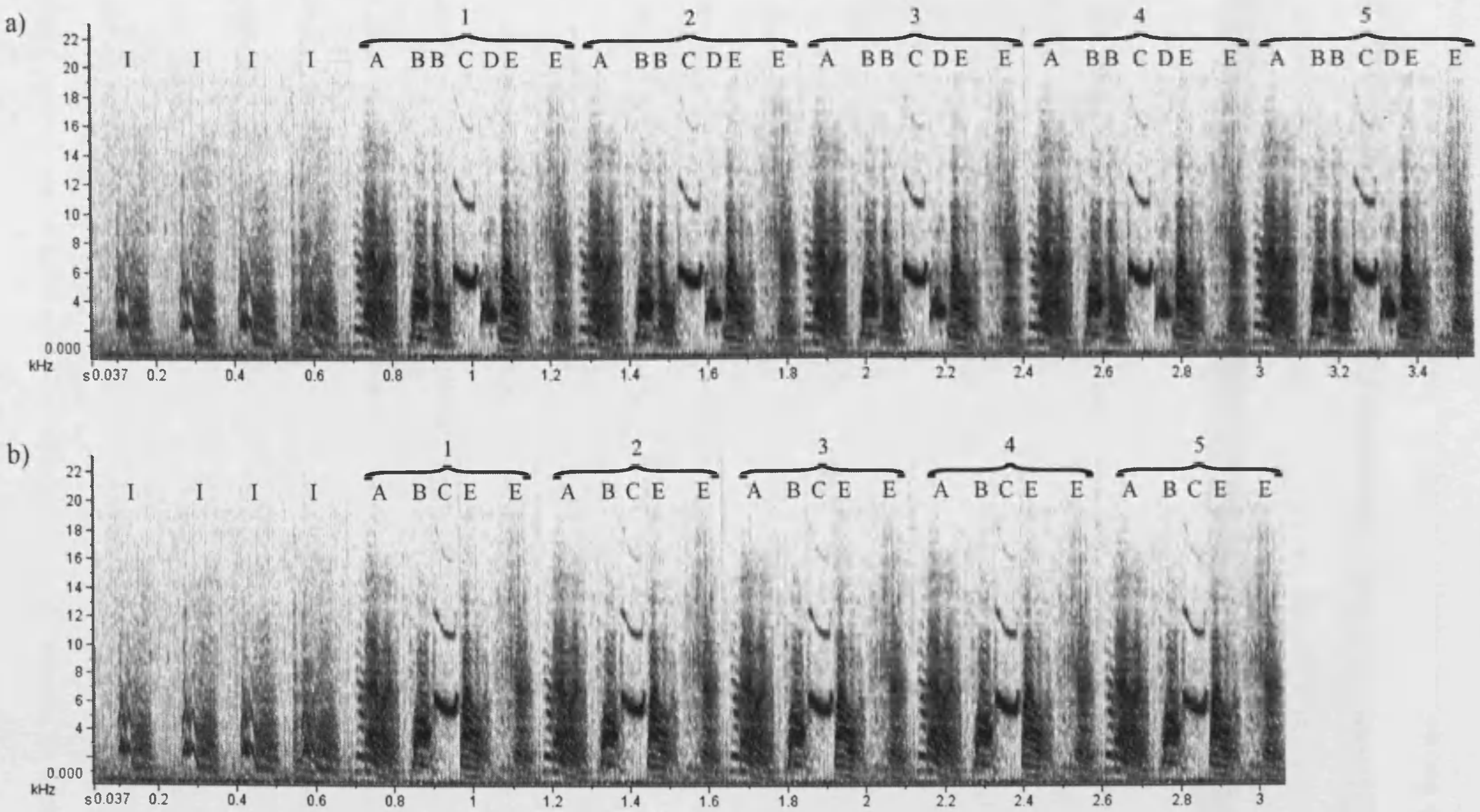

Figure 3.2: Example spectrograms of stimulus songs used in the song preference tests. Song a) is a control song bout consisting of four introductory syllables (I) followed by five repetitions of the song phrase. Song b) is the corresponding simplified song, created by deleting two syllables: D and the second repeat of syllable B.

Control songs were significantly longer than simplified songs (mean phrase length [s] ± 1 S.E. control = 0.54 ± 0.03; simplified = 0.40 ± 0.03; $t_9 = 3.34$, $P = 0.009$), and contained more syllables (mean number of syllables per phrase ± 1 S.E. control = 7.33 ± 0.49; simplified = 5.33 ± 0.49; $t_{10} = 2.86$, $P = 0.017$). The reduction in the number of different syllable types in the song phrase of the simplified stimulus songs fell short of statistical significance (mean number of different syllables per phrase ± 1. S.E. control = 5.83 ± 0.54; simplified = 4.83 ± 0.54; $t_{10} = 1.30$, $P = 0.222$). The characteristics exhibited by the manipulated songs are consistent with the songs of stressed males (Spencer et al., 2003).

3.2.5 Statistical analysis

All analyses were carried out using Minitab 15 statistical software (Minitab Inc., Pennsylvania, USA). The data used in the analysis of the song preference tests were the mean activity level and preference ratios from the two trials each female experienced with each pair of stimulus songs. I used the mean activity level data to test whether there was a treatment effect on females' motivation to sample the song of potential mates, and the preference ratios to test whether stress affected the direction or strength of female preference. One female from the stress treatment group did not hop at all during any of her four trials so was excluded from the analysis, reducing the total sample size to 31. Hopping data were normalised by log transformation and the preference ratios by arcsine square root transformation. These data were analysed with repeated measures general linear models (GLMs). I started with a full-factorial model with stress treatment, cross-fostering treatment, brood number and stimulus song pair as fixed factors, and female identity (nested within each combination of treatments) entered as a random effect. Non-significant interaction terms, and then factors, were sequentially deleted from the model, resulting in a final model that consisted of stress treatment and song pair as fixed factors, and female identity as the random factor.

To test whether females showed an overall preference for complex songs I calculated the proportion of each female's total hops, across all four trials, that were made on the perch triggering the more complex song of a pair. A Wilcoxon signed rank test was used to test whether this differed from 0.5.

The subjects in this experiment included several sets of genetic sisters. Since activity levels in a different type of mate choice apparatus are known to have a heritable component (Forstmeier, 2005), I used another set of GLMs to test for a genetic family effect on female behaviour. The data used in this analysis were the mean hops and preference ratios across all four trials experienced by each female. Again, the hopping data were log transformed and the preference ratios arcsine square root transformed to normalise them. The model consisted of stress treatment as a fixed factor and genetic family as a random effect.

3.3 Results

There was no treatment effect on the total number of hops made during preference trials, suggesting that females raised under control conditions or nutritional stress were equally motivated to hear male song (Fig. 3.3; mean hops per trial ± 1 S.E. controls = 27.5 ± 16.7; stressed = 16.3 ± 6.09; $F_{1,29} = 0.03$, $P = 0.875$). Females from the control and stress treatments did not differ in overall strength of preference (Fig. 3.4; mean proportion of hops made on preferred perch ± 1 S.E. controls = 0.90 ± 0.04; stressed = 0.90 ± 0.03; $F_{1,29} = 0.54$, $P = 0.467$). The six pairs of stimulus songs did not differ in terms of the overall activity they elicited ($F_{1,26} = 0.52$, $P = 0.758$), or the strength of preference females displayed for their preferred song ($F_{1, 25}$ = 1.44, P = 0.245).

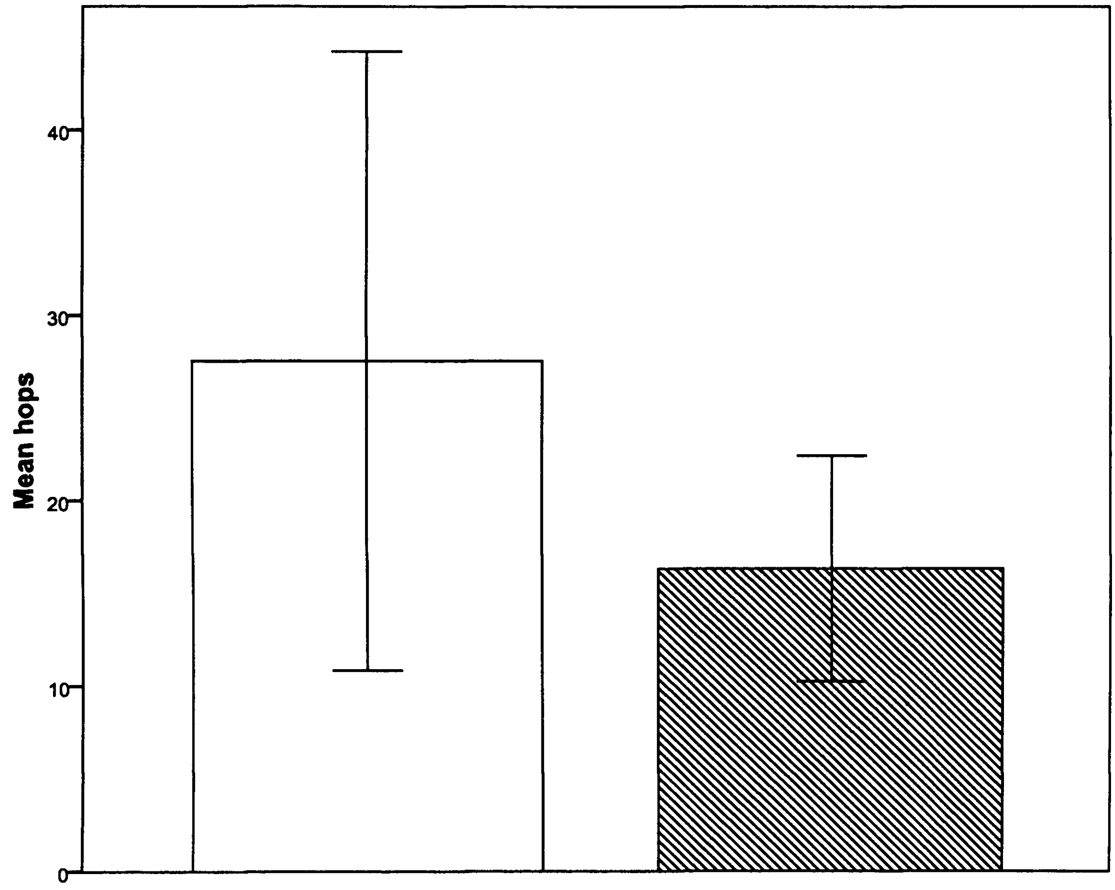

Figure 3.3: Mean number of hops (± 1 S.E.) made on both active perches per 1 hour trial by control (white bars) and stressed (shaded bars) females.

Control and stressed females did not differ in their degree of preference for the more complex song in a pair of stimulus songs (Fig. 3.5; mean proportion of hops triggering control song ± 1 S.E. controls = 0.64 ± 0.08; stressed = 0.63 ± 0.06; $F_{1,29}$ = 0.12, P = 0.732), nor was their preference affected by which stimulus song pair was presented ($F_{1,25}$ = 0.37, P = 0.863). Across all song pairs, females preferred the more complex song in the pair (W = 331.5, N = 31, P = 0.01).

There was no effect of the genetic family of origin on females' activity levels ($F_{20,9}$ = 0.83, P = 0.652), nor on the strength of preference they showed for either their preferred song in each pair ($F_{20,9}$ = 1.61, P = 0.233) or for the control songs ($F_{20,9}$ = 0.91, P = 0.597).

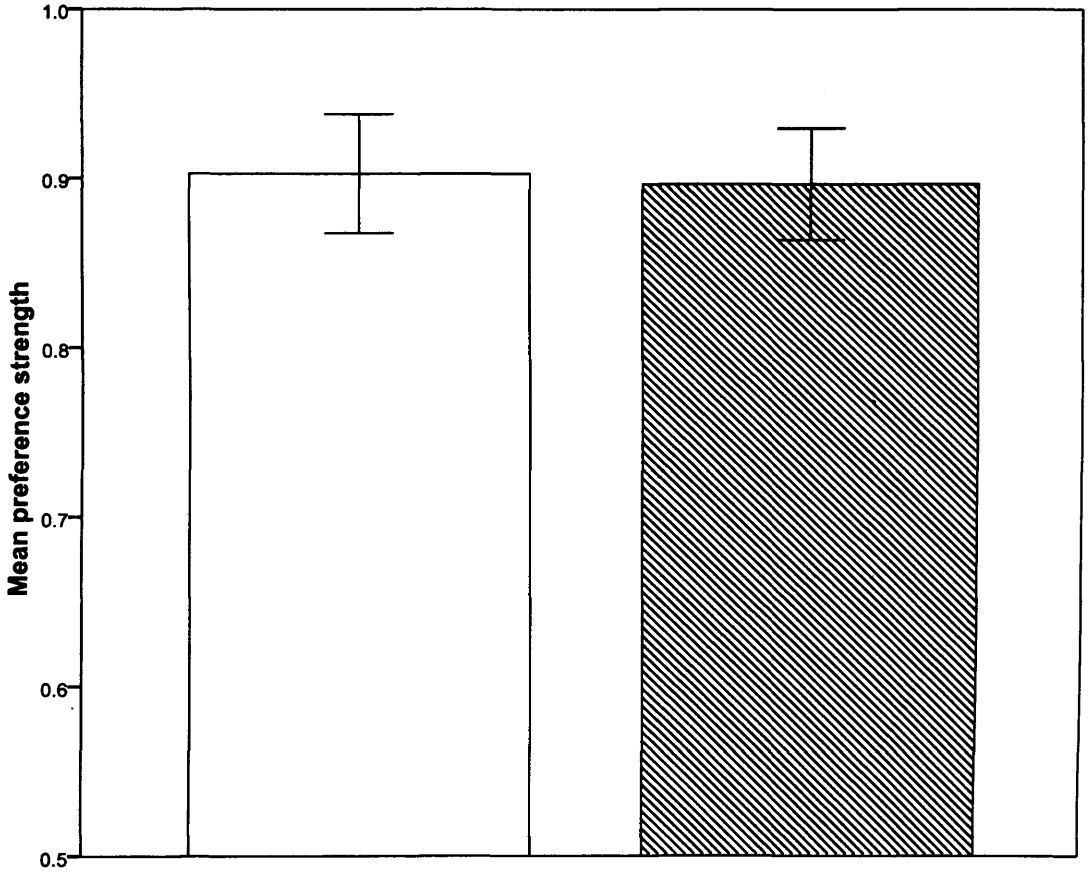

Figure 3.4: Mean preference strength (± 1 S.E.) of control (white bars) and stressed (shaded bars) females, for preferred song. Preference strength is calculated as: number of hops on the perch that received the greatest number of hops / total number of hops across both active perches. The scale starts at 0.5 since it is not possible for the preferred song to receive less than 50% of the total hops.

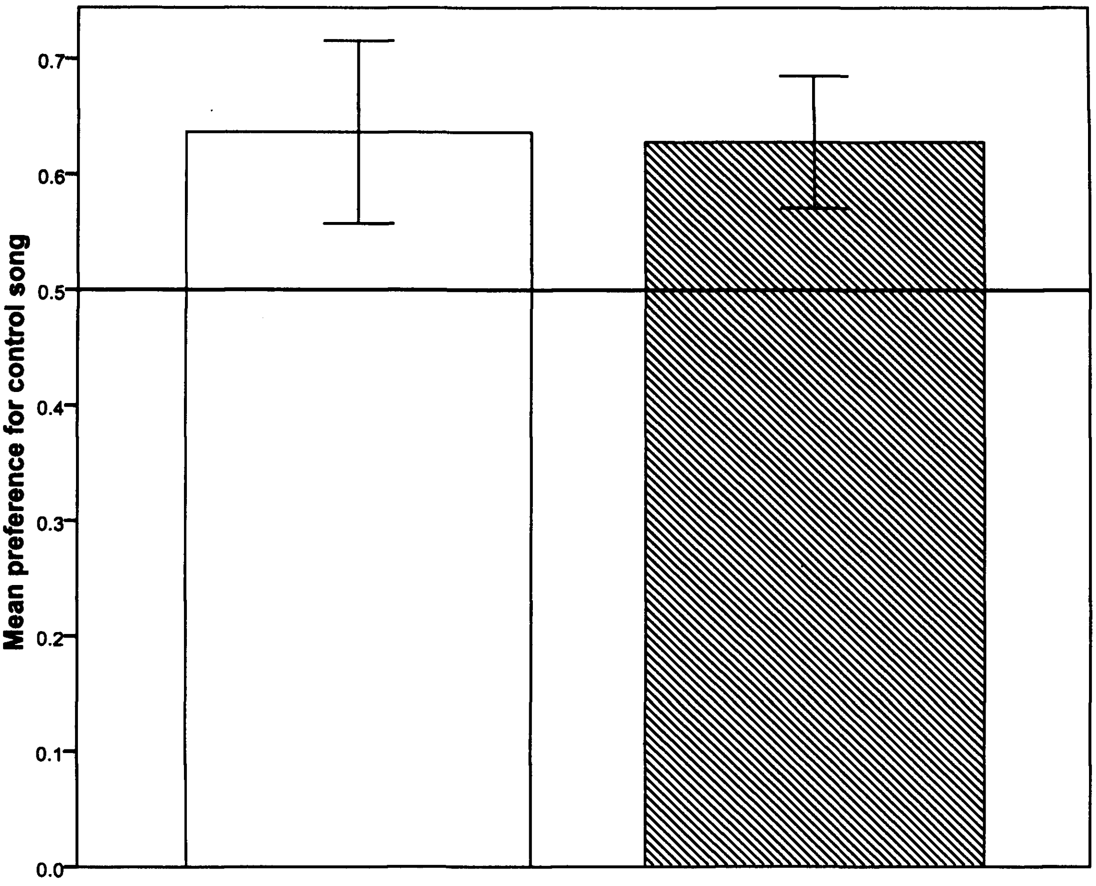

Figure 3.5: Mean preference (± 1 S.E.) for the control song in a pair of stimulus songs, by control (white bars) and stressed (shaded bars) females. Preference is calculated as: number of hops on the perch that triggered playback of the control song / total number of hops across both active perches. A ratio greater than 0.5 indicates a preference for the control song.

3.4 Discussion

Within the oscine group, complex songs are thought to have evolved as a result of female preferences (Catchpole and Slater, 2008) and song complexity is hypothesised to honestly reflect how well the singer fared with stressors in the rearing environment (Nowicki et al., 1998a; Nowicki et al., 2002a). There is evidence that female zebra finches have an inherent bias toward more complex songs (Clayton and Prove, 1989; Collins, 1999; Vyas et al., 2009). However, female preferences in many species have found to be influenced by treatments that manipulated early growth during development (Hunt et al., 2005; Hebets et al., 2008; Riebel et al., 2009; Holveck and Riebel, 2010; Woodgate et al., 2010, chapter 2) and female birds are subject to the same variability in developmental conditions as males. I tested whether female preferences for song complexity are affected by developmental stress, which would have implications for the potential of song complexity to serve as an honest signal. When control females or females that had experienced nutritional stress during development were allowed to choose between songs of differing complexity, I found that females from both treatments preferred the more complex song in a pair but there was no difference in the strength of this preference between the treatment groups.

There are several possible explanations for the observed lack of a treatment effect on female preference. Firstly, is it possible that the experimental paradigm I used to investigate song preferences is not capable of detecting real differences in female preferences? Previous studies suggest this is unlikely: Operant song choice set ups similar to that used in this study, in which females can choose to trigger song playbacks by landing on a perch or pecking a button, have frequently been used to investigate song preferences (Riebel and Slater, 1998; Collins, 1999; Gentner and Hulse, 2000; Spencer et al., 2005b; Holveck and Riebel, 2007), and preferences expressed in such experiments are consistent with choices made between live males (Holveck and Riebel, 2007). Although fewer studies have used this paradigm to investigate differences in preferences between groups of females, it has been used successfully to detect differences in the strength or direction of preferences between groups that differed in previous exposure to male song (Riebel, 2000), or the brood size in which they were reared (Riebel et al., 2009; Holveck and Riebel, 2010). Secondly, perhaps the stimulus songs, which were created by editing the same source files, were not sufficiently different from one another to elicit different responses from the subjects. This is unlikely for two reasons: in terms of duration and complexity, the simplified stimulus songs resemble those of stressed males recorded by Spencer et al. (2005b) which were shown to be less attractive to females than those of control males using the same song discrimination apparatus as in the present study. In addition, across both groups of females in this study, I found a significant preference for control songs, strongly suggesting that females were able to differentiate between the control and simplified songs and that they elicited different levels of preference. A third possibility is that the nutritional stress treatment used in this experiment might not have been sufficiently severe to induce differences in female development, but that more extreme levels of stress might have greater effects on female choice behaviour. The stressor applied in this study was designed to mimic levels of nutritional stress that are known to deleteriously affect survivorship and reproduction in zebra finches (Lemon, 1993). I have previously shown that females reared under the stress treatment in this study had reduced nestling growth rates and were less active than controls in a mate choice context (see chapter 2), confirming that the treatment did induce stress in the subjects. I cannot, however, rule out the possibility that more drastic levels of developmental stress might have greater effects. Nonetheless, perhaps the most parsimonious explanation for my results is that adverse rearing conditions did not affect the strength or direction of female preferences for songs of differing complexity.

To date there is weak support for an effect of developmental stress on female preferences. Two other studies have examined the effects of developmental stress on female song preferences in zebra finches: Riebel et al. (2009) found that females stressed by a brood manipulation differed in preference strength (those from small broods having a stronger preference) when choosing between unfamiliar songs, although when one song was that of their tutor there was no difference in preference. Holveck and Riebel (2010) reported assortative preference for songs with females preferring the songs of males raised in a similar brood size. By contrast, Schielzeth et al. (2010) found no evidence for early rearing effects on female preference in an unmanipulated population of zebra finches. In terms of the underlying mechanism, MacDonald et al. (2006) found that nutritional stress had a deleterious effect on HVC growth in juvenile female song sparrows, but no studies have looked at the effects of developmental stress on female brain development in zebra finches or whether the differences in HVC volume found by MacDonald et al. (2006) extended into adulthood. The results of the present study, however, suggest that any effect of stress on brain development is insufficient to prevent females discriminating between biologically relevant levels of variation in song complexity.

Theory suggests that low quality females might preferentially mate with low quality males under certain conditions, for example if they cannot bear the costs of a prolonged search for a high quality mate or are likely to lose out in competition for mates with higher quality females (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005). This would lead to assortative mating by phenotypic quality. Holveck and Riebel (2010) found that female zebra finches preferred the songs of males that were raised in similarly sized broods to themselves and argue that this is evidence that low quality females prefer low quality males. Preference strength is expected to reflect the costs and benefits of expressing a preference (regardless of the direction of preference), so that preferences will be weaker when costly and stronger if the benefits of being discriminatory are large (Cotton et al., 2006). The difference between the ability of high and low quality females to bear the costs of choice is predicted to lead to reduced preference strength in low quality females. In the present study, stressed and control females did not differ in the strength or direction of their preferences suggesting that the potential benefits of using song complexity in mate choice decisions are large enough to counteract the costs of strong preference. One such benefit of attending to differences in song complexity is that complexity may accurately signal information about the male's developmental history (Nowicki et al., 1998a; Buchanan et al., 2003; Spencer et al., 2003). Riebel et al. (2009) found that females originating from smaller broods expressed stronger preferences than those from large broods only when choosing between two unfamiliar songs. Females showed no preference for longer songs suggesting that there was little variation in the attractiveness of the stimulus songs. When the benefits of discriminating between songs are relatively small, as when Riebel et al. (2009) presented a choice between unfamiliar males, females that experienced adverse developmental conditions may be less able to bear the costs of choosiness and display weaker preferences than females raised under more benign conditions. By contrast, it is likely that when the benefits of choice are greater, such as when songs differ in a way that might signal differences in male fitness or when females have learned preferences for a song they were exposed to early in life (Miller, 1979b; Clayton, 1988a; Riebel et al., 2002), even females of low quality can maximise their fitness by expressing strong preferences.

The stimulus songs used by Holveck and Riebel (2010) were recorded from males raised in large or small broods. Interestingly these songs did not differ in length or complexity, although both the phrase duration and the duration of sound production within the phrase (excluding silences between syllables) were found to be more variable in males from large broods (Holveck et al., 2008). Males from large broods also showed less accuracy in syntax learning from their tutor's song but since Holveck and Riebel (2010) only found evidence of assortative preferences when females were tested with unfamiliar songs it seems most likely that they used differences in singing consistency to distinguish between males from small and large broods. Rather than present song recordings from males raised under different conditions, in the present study I digitally edited recordings of zebra finch songs to create stimulus sets that differed only in complexity and in phrase duration (shorter songs being an inevitable consequence of removing sections of the original phrase). Thus I was able to control for variation in all other respects (e.g. variation in singing performance, peak frequency, amplitude, rate of syllable production, types of syllables present in the song, syllable order etc.). This raises the question of whether the effects of brood size on song preferences so far documented (Riebel et al., 2009; Holveck and Riebel, 2010) are due to the effects of developmental stress or represent preferences for characteristics that are intrinsically linked to brood size.

Female zebra finches respond to a number of auditory, morphological and behavioural traits when choosing a mate (Burley and Coopersmith, 1987; Swaddle and Cuthill, 1994a; Zann, 1996; Williams, 2001; Riebel, 2009). The strength and direction of preferences for some or many of these cues are likely to be condition dependent (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005; Cotton et al., 2006) and females of differing quality might prioritise cues differently (Fawcett and Johnstone, 2003b), so the outcome of mate choice may differ between stressed and control females despite their preferences for song complexity being unaffected. A final way in which developmental stress could affect female choice is by reducing the time and resources females invest in sampling potential mates. In this study, I found no significant effect of the stress treatment on the total number of hops made on active perches, and previous studies found no effect of brood size manipulations on the number of key pecks in similar song choice experiments (Riebel et al., 2009; Holveck and Riebel, 2010). This suggests that, in this situation, developmental conditions have little or no effect on motivation to sample male songs, perhaps because the costs of sampling (i.e. flying to the relevant perch and hopping on it or pecking a key) are too low to have a measurable effect on behaviour.

The results of the present study suggest that there was no effect of developmental stress on the ability of female zebra finches to discriminate between male songs that differ in complexity. Females are under selection pressure to respond only to male signals that contain reliable and valuable information, when choosing a mate (Dawkins and Krebs, 1978). If male song accurately signals information pertaining to the male's own developmental history then all females, even those that experienced developmental stress themselves, may be under selection to maintain the ability to access this information and to utilise it in mate selection. My results are consistent with the developmental stress hypothesis (Nowicki et al., 1998a; Nowicki et al., 2002b; Buchanan et al., 2003), since the value of an honest signal rests on the ability of the intended recipient to access the information it encodes.

In this study, nutritional stress did not affect the direction or strength of female preferences for song complexity. Although I did not directly address females' ability to discriminate between songs of differing complexity, the results strongly imply that it was not affected by the stress treatment. It is increasingly clear that environmental factors experienced during early life can affect female mate choice behaviour (Hunt et al., 2005; Hebets et al., 2008; Riebel et al., 2009; Holveck and Riebel, 2010; Woodgate et al., 2010), but preferences for complex song may be robust to developmental stress, perhaps owing to an inherent sensory bias toward complex songs (Collins, 1999), potentially providing a strong selective force in the evolution of complex song.

Chapter 4: Genetic and environmental components of phenotypic variation in brain and song structure of male zebra finches

4.1 Introduction

Bird song is an elaborate trait whose evolution is driven by natural and sexual selection (Searcy and Andersson, 1986; Catchpole and Slater, 2008). The honesty of such signals is commonly thought to be enforced by the cost of producing them (Zahavi, 1975; Grafen, 1990b). It is not immediately clear, however, what costs might be associated with several traits that are associated with a song's attractiveness to females, such as complexity or repertoire size (Nowicki et al., 1998a; Nowicki et al., 2002a). The developmental stress hypothesis (Nowicki et al., 1998a; Buchanan et al., 2003), suggests that song will accurately reflect the effect of detrimental environmental conditions on early development, because the nuclei in the oscine forebrain that control song learning and production develop during early life when nestlings are at the greatest risk of undernutrition and other stressors (Kirn et al., 1999; Kittelberger and Mooney, 1999; Brainard and Doupe, 2002). A growing number of empirical studies have addressed the nutritional stress hypothesis, providing some support for its predictions (see chapter 1, section 1.3).

The volume of the HVC (proper name), a key telencephalic nucleus involved in song learning and production, has been found to be deleteriously affected by both nutritional restriction and injections of corticosterone during development in the zebra finch, *Taeniopygia guttata* (Buchanan et al., 2004), by food restriction in the swamp sparrow, *Melospiza georgiana* (Nowicki et al., 2002a), and song sparrow, *Melospiza* *melodia* (MacDonald et al., 2006), and by high parasite burden in the canary, *Serinus canaria* (Spencer et al., 2005a). Another song nucleus, the robustus archistriatalis (RA), was also affected by nutritional restriction in the swamp sparrow (Nowicki et al., 2002a). These studies support the hypothesis that the development of forebrain areas associated with song reflects early environmental conditions, although Gil et al. (2006) found no effect of artificially increased brood sizes (a manipulation thought to induce developmental stress by reducing levels of parental care), on the development of any song control nuclei in zebra finches.

A number of studies have also found effects of developmental stressors on adult song structure, although with less agreement about which aspects of song might honestly signal developmental conditions. Spencer et al. (2003) found males reared under conditions of nutritional stress or with artificially elevated corticosterone levels produced shorter songs containing fewer syllables and with a lower maximum frequency. Similar results were found by Zann and Cash (2008), although phrase length was actually longer in males from the stress group. However, several studies have failed to find an effect of food restriction (Brumm et al., 2009) or increased brood size (Gil et al., 2006; Holveck et al., 2008) on a number of measures of song structure including syllable number and phrase duration. In other oscine species, developmental stress led to reduced song repertoires in canaries (Spencer et al., 2005a) and European starlings, *Sturnus vulgaris* (Spencer et al., 2004), and shorter singing bouts in blue tits, *Parus caeruleus* (Dreiss et al., 2006). In addition to these experimental studies, observational studies have indicated a relationship between various proxy measurements thought to reflect developmental condition and song complexity or repertoire size in the blue tit (Doutrelant et al., 2000), song sparrow (MacDougall-Shackleton et al., 2009), Bengalese finch, *Lonchuria striata* (Soma et al., 2006), and great reed warbler, *Acrocephalus arundinaceus* (Nowicki et al., 2000). Several studies have reported that males raised under conditions of developmental stress sang less than controls (European starling, Buchanan et al., 2003; zebra finch, Naguib et al., 2008), although Tschirren et al. (2009) found that male zebra finches raised in experimentally enlarged broods sang at a higher rate over a timed five minute period than those from small broods. Finally, the accuracy of song syntax learning in zebra finches has been shown to be impaired by both nutritional restriction (Brumm et al., 2009) and increased brood size (Holveck et al., 2008), although the proportion of syllables copied from the song tutor was unaffected by developmental stress in either study.

Despite the evidence that developmental stress affects neural development, song learning and complexity, it remains unclear what information regarding male quality is actually signaled by song structure. If aspects of male song honestly reflect the amount of developmental stress an individual experienced, then it could act as a signal of phenotypic quality (Nowicki et al., 1998a; Nowicki et al., 2002a), since stress during development has long term effects on various components of fitness including morphology, fecundity, quality of offspring, antioxidant defenses and, potentially, longevity (Birkhead et al., 1999; Metcalfe and Monaghan, 2001; Blount et al., 2003; Metcalfe and Monaghan, 2003; Arnold et al., 2007). Additionally, it seems likely that individuals of higher genotypic quality will be more robust in their development when facing a given level of stress (Andersson, 1994; Nowicki et al., 1998a), so if there is an effect on male phenotype of stress by genotype interaction, phenotype might accurately signal information about male genetic quality (Nowicki et al., 1998a; Nowicki et al., 2002a). To quantify the relative influence of genotypic or phenotypic quality on male song, it is necessary to determine the relative contributions of genetics, environmental conditions and genotype-by-environment interactions to the development of the song control system. No previous study of developmental stress has been able to do this, because most involved the offspring either being raised by their genetic parents (Nowicki et al., 2002a; Brumm et al., 2009) or cross-fostered as entire broods (Spencer et al., 2003; Buchanan et al., 2004; Zann and Cash, 2008). These experimental designs make it impossible to disentangle the effects of genotype from those of a common rearing environment. A partial cross-fostering design (one in which half the offspring are raised by foster parents while their siblings are raised by their genetic parents), is necessary to separate these effects, but the only studies to use such a design have done so in order to manipulate brood size (Dreiss et al., 2006; Gil et al., 2006; Holveck et al., 2008), leaving the effects of cross-fostering confounded with those of the brood size manipulation.

The best estimates of the heritability of song control nuclei in the oscine brain come from Airey et al. (2000b), and suggest that HVC volume, along with the volume of other song control nuclei and overall brain and body mass, are moderately heritable in the zebra finch (HVC volume heritability, $h^2 = 0.38$; brain mass, $h^2 = 0.49$; body mass, $h^2 = 0.32$; Airey et al., 2000b). Those nuclei that make up the posterior forebrain pathway (see chapter 1, Fig. 1.1), thought to control song production (Brenowitz et al., 1997), were found to be substantially more heritable than those of the anterior forebrain pathway, which is thought to play a role in song learning (Doupe and Solis, 1997; Margoliash, 1997), implying a greater role for environmental conditions in determining the quality of song learning. However, all the zebra finches used in this study were raised by their genetic parents, making it impossible to separate the effects of genotype and common rearing environment. Because of this, all the heritability calculations are likely to be over-estimates. Furthermore, Airey et al. (2000b) give no estimate of the effect of genotype-by-environment interaction on brain development. There is evidence that both the number of HVC neurons (Ward et al., 2001) and the rate of neurogenesis in the HVC of adult zebra finches (Hurley et al., 2008) is predicted by their nest of origin, but all offspring in both studies were raised by their genetic parents so it is again unclear whether these effects can be accounted for by variation in genotype or in the rearing environment (e.g. parental care, song exposure). In humans, twin studies have shown than brain volume is highly heritable (reviewed in Peper et al., 2007), as are many specific brain regions (Peper et al., 2007; Kremen et al., 2010). Both common environment and unique environmental conditions explain much of the individual variation in volume of several brain regions in humans (Peper et al., 2007; Kremen et al., 2010).

Several studies have attempted to quantify genetic and environmental influences on song and other sexual traits in songbirds. Forstmeier et al. (2009) used pedigree-based animal models to calculate the heritability of a number of call and song characteristics in a captive population of 808 zebra finches reared in cages or aviaries. Female call traits, which are unlearned, had higher heritability values than male calls or song (e.g. fundamental frequency of female calls, $h^2 = 0.67$; male calls, $h^2 = 0.16$; male song, $h^2 = 0.10$; Forstmeier et al., 2009). Male songs had a greater component of environmental variance than did female calls, and structural song traits such as syllable number or song phrase duration had extremely low estimates of heritability (syllable number, $h^2 = 0.11$; phrase duration, $h^2 = 0.18$; Forstmeier et al., 2009). Bolund et al. (2010b) used natural variation in growth rates in zebra finch nestlings that were reared on *ad libitum* food supply, to examine correlations between early growth (assumed to reflect variation in environmental conditions) and variation in a number of adult traits, including morphology as well as song traits and measures of beak and feather coloration. Although early environmental effects on growth correlated with measures of adult morphology and beak colour, they had no effect on song structure or singing rate, or on male plumage coloration or measures of female choice, implying that zebra finch nestlings are highly resilient to the effects of developmental stress and that secondary sexual traits in the zebra finch do not accurately reflect developmental stress.

As sexual signals usually have a large component of environmental variance, it is important to understand whether there are genotype-by-environment interactions in determining phenotype since they determine whether an ornament can signal heritable quality (Cotton et al., 2004). It is well established that different genotypes differ in their response to environmental changes in the determination of a number of life-history traits (Stearns, 1992), but the potential for such genotype-by-environment interactions to determine the expression of secondary sexual traits is less well documented. Qvarnstrom (2000) showed that the size of the white forehead badge of male collared flycatchers, *Ficedula albicollis*, was predicted by that of their father during years with favourable weather conditions, or if raised in artificially reduced broods, but that no such relationship could be identified in males raised in enlarged broods or during poor years. There is an interaction between larval density and genotype in determining wing length, a sexually selected character, in *Drosophila melanogaster* (Wilkinson, 1987). Estimates of heritability of several male song characters in *Drosophila montana* and *D. littoralis* were lower when fathers were wild caught than when both generations were raised in the laboratory, indicating the influence of genotype-by-environment interactions (Aspi and Hoikkala, 1993). Strong genotype-by-environment interactions have been shown in the determination of sexually selected traits in male lesser waxmoths, *Achroia grisella*, and it is proposed that these interactions play a role in maintaining additive genetic variation in sexual traits (Jia et al., 2000; Danielson-Francois et al., 2006).

Theory suggests that strong genotype-by-environment effects are likely to disrupt the reliability of signals of male quality by 'blurring' the relationship between genotype and phenotype (Greenfield and Rodriguez, 2004; Higginson and Reader, 2009), which would imply that female choice for such traits is less likely to evolve. Under certain conditions, however, Kokko and Heubel (2008) suggest that weaker genotype-by-environment interactions can help maintain additive genetic variation in male secondary sexual traits, particularly if there is little mixing of individuals from different developmental environments.

In this study, a partial cross-fostering design was used to investigate the effects of nutritional stress on brain development and adult song characters in zebra finches. This design allowed me to separate the effects of heritability of trait expression and common rearing environment, and to determine for the first time whether there was any stress treatment by genotype interaction. My aim was thus to determine which aspects of song structure have the potential to signal information about a male's developmental history, genetic quality or aspects of both, and whether these effects are mediated through environmental or genetic control of neural morphology. Specifically, I predicted that there would be a large environmental component to variation in male song and brain structure, allowing song to act as a signal of developmental condition. I also predicted that there may be significant genotype-by-environment interactions in the determination of these traits and that developmental stress could affect the strength of the relationship between sons' song structure and brain morphology, and that of their fathers.

4.2 Methods

4.2.1 Experimental subjects

In 2006, zebra finches from the breeding colonies at the Max Planck Institute for Ornithology, Seewiesen, Germany, were paired up at random to form 32 breeding pairs. Each pair was kept in a breeding cage (40 x 40 x 40 cm) with *ad libitum* food supply and a 12:12 L/D photoperiod. Birds were provided with coconut fibres and cotton strings and allowed to breed in a nest box. Experimental manipulations, designed to induce nutritional stress, took place between days 5 and 30 after the chicks hatched. A modified version of the feeding regime described by Spencer et al. (2003) was used, in which pairs raised under control condition received *ad libitum* seed mix (Exotenfutter, Supravit GmbH, Heufeld, Germany) while those in the stress treatment received a limited mass of seeds daily, adjusted for brood size and the age of the chicks. The seed provided for nests under the stress treatment was mixed with husks in a 1:2 ratio by volume. Each breeding pair laid four clutches. Half of the breeding pairs raised their first two clutches under control conditions and the last two clutches under the stress treatment (n = 16 pairs), while the other half raised their first two clutches under stress treatment and the last two clutches under control conditions (n = 16). Half of the chicks in each brood were cross-fostered to a brood of similar age in the other treatment group, one to three days after hatching. Brood sizes were not altered. There were four treatment groups: 1) offspring reared by their genetic parents under the control treatment, 2) offspring reared by foster parents under the control treatment, 3) offspring reared by their genetic parents under the stress treatment, and 4) offspring reared by foster parents under the stress treatment.

Nestlings were weighed daily until 35 days post-hatch and then every ten days from post-hatch days 40-80. Fathers and foster-fathers were weighed once their final brood reached adulthood. At day 30-35 post-hatch on consecutive days a blood sample was taken from each bird within a clutch in order to measure plasma corticosterone levels as part of a subsequent study. From 60 days onwards, birds were separated from their parents and housed in compartments (40 x 40 x 40 cm) adjacent to their home cage. Finally, at adulthood after 80 days of age, they were transferred to a large sex-specific aviary with large outdoor (416 x 242 cm and 302 cm high) and indoor (403 x 301 cm and 200 cm high) compartments.

A total of 107 broods were produced, 56 reared under control and 51 under stressed conditions. In total 169 males and 152 females were produced in this experiment (mean brood size [number of chicks hatched in each brood] ± 1 SD: control broods: 3.0 ± 1.1; stressed broods: 3.0 ± 1.0). In order to calculate heritabilities, I compared the brain and song traits of male offspring with their genetic and foster siblings. Not all nests produced adult males originating from all four treatment groups so the sample size available for this study was 48 males. These 48 subjects were drawn from 12 nests, 6 of which reared control then stressed broods and 6 that reared stressed then control broods. Four males from each nest were selected: one genetic- and one foster-son from control broods and one genetic- and one foster-son reared under the stress treatment. When more than one male was available from each combination of treatments, the subjects were selected at random.

4.2.2 Song recording and analysis

For song recording, offspring males were transferred to individual recording cages at 169 (± 40) days old, together with an unfamiliar female (selected at random from the breeding colony). Fathers' and foster fathers' songs were recorded at the end of the breeding period in the recording cages, together with their mate. All recordings were made using a Sennheiser ME67 directional microphone (Sennheiser, Wedemark, Germany) connected to a Sony TCD-5M tape recorder. At least 20 songs were recorded from each male. The recordings were digitised using Canary 1.2.1 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) on a Power Macintosh 7200/90. The sampling rate was 22 kHz with a 16 bit sample size, and the frequency/time resolution was 342 Hz with a FFT size of 256 points. Raven 1.2 sound analysis software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) was used to analyse all song recordings (Hann weighting, 1024 point DFT size, 140 Hz filter bandwidth). I examined the spectrogram of each male's recording by eye to determine the composition of the stereotyped song phrase and made accurate measures of a number of song parameters (summarised in Table 4.1) from five phrases selected at random, using the measuring tools in Raven. The mean of these five phrases was taken to give accurate measures for each male.

Table 4.1: Definitions of the six song structure parameters.

Parameter	Definition
Syllable number	The total number of syllables that make up the song phrase, excluding introductory syllables
Unique syllables	The number of different syllable types in the song phrase
Phrase length (s)	Duration of the song phrase
Peak frequency (kHz)	The frequency of maximum power in the phrase
Maximum frequency (kHz)	The highest frequency reached during the phrase
Proportion unique	Proportion of unique syllables per song (= Unique syllables / Syllable number)

4.2.3 Brain measurements

After the song recordings (offspring age in days, 169 ± 40), the male offspring, and their fathers and foster-fathers, were killed by decapitation and their brains were removed immediately by dissecting them out of the skull. The brains were weighed using a Sartorius BA110S balance (Sartorius AG, Goettingen, Germany). Afterwards, brains were frozen over liquid nitrogen and stored at −80°C. Brains were cut on a cryostat (Leica Microsystems, Wetzlar, Germany) into 30 μm sagittal sections. Sections were mounted onto Superfrost Plus slides (Menzel Gläser, Germany), Nissl-stained with 0.1% thionin and cover slipped. Slides were analysed under bright-field illumination using a Leitz Aristoplan microscope (Leitz, Wetzlar, Germany). Brain sections were video-digitised on a PC equipped with an image analysis system (Meta Morph, Visitron, Germany) and HVC area was measured using the built-in measurement tools. HVC volumes were calculated as the sum of the area sizes multiplied by section interval and section thickness.

4.2.4 Statistical analysis

Statistical analyses were performed using Minitab 15 (Minitab Inc., State College, PA, U.S.A.) and SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.). I investigated the effect of the stress treatment on nestling mass at two points during development, with two sets of general linear models (GLMs), using as dependent variables nestling mass at the start of treatments (day 5 post-hatching) and at day 30 (the end of the experimental manipulation and point of nutritional independence). Male and female nestling mass was analysed separately using a model in which stress treatment was a fixed factor and rearing nest was included as a random effect to control for different rates of provisioning from the parents. Genetic father was initially included as a random term but had no significant effect on nestling mass so was removed from the final model. Effect sizes (*d*) were calculated for significant effects as the difference between the means divided by the pooled standard deviation.

I tested for an effect of the stress treatment on adult male body mass and on brain and song variables, using GLMs. Although both syllable number and the number of unique syllables in each song phrase were measured, they were found to be highly correlated (Pearson correlation, $r_{46} = 0.85$, $P = <0.001$) so unique syllables were dropped from the analysis. I calculated the mean value for each response variable of the two males raised under each treatment in each nest. The basic model structure used to analyse each response variable was the same and included stress treatment and the order in which the treatments were applied to each nest (i.e. whether the parents raised broods under control then stress treatment vice versa) as fixed factors, along with the interaction term between stress treatment and treatment order. The identity of the rearing nest, nested within treatment order, was included as a random factor. The analysis of HVC volume initially included males' brain and body mass as covariates, while the analysis of brain mass included body mass as a covariate, and analyses of all song variables included HVC volume and brain mass as covariates. For each response variable the model was simplified separately by sequential deletion of non-significant interaction terms and covariates, with the final model constrained to retain the main effects of stress treatment, treatment order and rearing nest.

To test the relative contributions of genetic and environmental factors in determining the phenotypes of males in this experiment, I ran a series of father-son regressions. Each brain, body and song variable was analysed as the dependent variable in a GLM, which included the corresponding value from both their genetic father and the male that reared each son as covariates. (The rearing male was the genetic father of all non-fostered offspring and the foster-father of those in the cross-fostering treatment.) The initial model included stress treatment, cross-fostering treatment and brood number as fixed factors, along with the interaction terms between the stress treatment and cross-fostering, brood number and rearing- and genetic father's phenotypes. This initial model was simplified by the sequential elimination of non-significant interactions and terms, with the final model constrained to retain the main effects of stress treatment and the two covariates. There were no significant effects of cross-fostering or brood number, or significant interactions between these terms and the stress treatment, for any dependent variables so both terms were dropped from the final models. The heritability (h^2) was estimated as twice the slope of the father-son regression. It should be noted that this method unavoidably includes some pseudoreplication, as the same parental males acted as rearing- or genetic father to several offspring.

Consequently, I employed a second method of estimating heritabilities, this time using sib-sib comparisons. I estimated the genetic, environmental and genotype-by-environment components of the variation in brain, body and song variables, following Merila (1996) and Christe et al. (2000). GLMs were used to calculate these variance components using restricted maximum likelihood estimates. The model included stress treatment as a fixed factor and nest of origin and nest of rearing as random effects, as well as the interaction term between stress treatment and nest of origin. The total phenotypic variance for each trait (V_P) was calculated as the sum of the variance components from the GLM ($V_P = V_A + V_{GE} + V_{EC} + V_E$). The variance due to nest of origin estimates half the additive genetic variance (1/2 V_A) but also includes one quarter of the dominance variance (V_D) and any variation attributable to maternal effects (V_M), if present. I estimated V_A as double the nest of origin variance. The term 'nest of rearing' estimates variance due to effects of the common environment (V_{EC}), including variation in parental care, song tutoring etc. The stress treatment by nest of origin interaction indicates whether the response of chicks to developmental stress is dependent on genotypic quality and represents part of the variance due to genotype-by-environment interactions (V_{GE}). Finally, the error component of variance includes any environmental effects (V_E) not attributable to comment rearing environment (including any effect of the stress treatment) and the remaining portion of V_{GE} not due to stress by genotype interaction, as well as ½ V_A and ¾ V_D. Heritability was calculated as $h^2 = V_A/V_P$. I also calculated the proportion of phenotypic variance attributable to a common rearing environment (V_{EC}/V_P), and to stress treatment by genotype interaction (V_{GE}/V_P). The coefficient of variation for phenotypic variation (CV_P), additive genetic variation (CV_A), variation due to common rearing environment (CV_{EC}) and variation due to stress treatment by genotype interaction (CV_{GE}), was calculated as $CV = \sqrt{V}/X$ where X was the mean value for each trait (Charlesworth, 1984; Houle, 1992). I used a jack-knifing procedure to generate a resampling distribution of the variance components, and used this distribution to calculate heritability estimates and their standard errors. Heritability estimates were considered statistically significant if they were more than two standard errors greater than zero (Merila, 1996).

4.3 Results

At the start of the experimental manipulation (day 5), neither male nor female nestlings from the two treatment groups differed in body mass (Fig. 4.1; males: $F_{1,59}$ = 3.47, P = 0.067; females: $F_{1,59}$ = 0.09, P = 0.769). When the stress treatment ceased at day 30, there was still no treatment effect on males (Fig. 4.1a; $F_{1,43}$ = 0.07, P = 0.79) but females from the control treatment were significantly heavier than those from the stress group (Fig. 4.1b; $F_{1,53}$ = 10.09, P = 0.002, d = 0.73). Between days 30 and 60 male nestlings showed a non-significant trend toward reduced mass in males from the stress treatment (Fig. 4.1a), but even at day 35, when the difference in mass between the groups was greatest, this was not statistically significant ($F_{1,35}$ = 0.26, P = 0.613). Control females were still heavier than those from the stress group at day 35 ($F_{1,33}$ = 4.39, P = 0.044, d = 0.93).

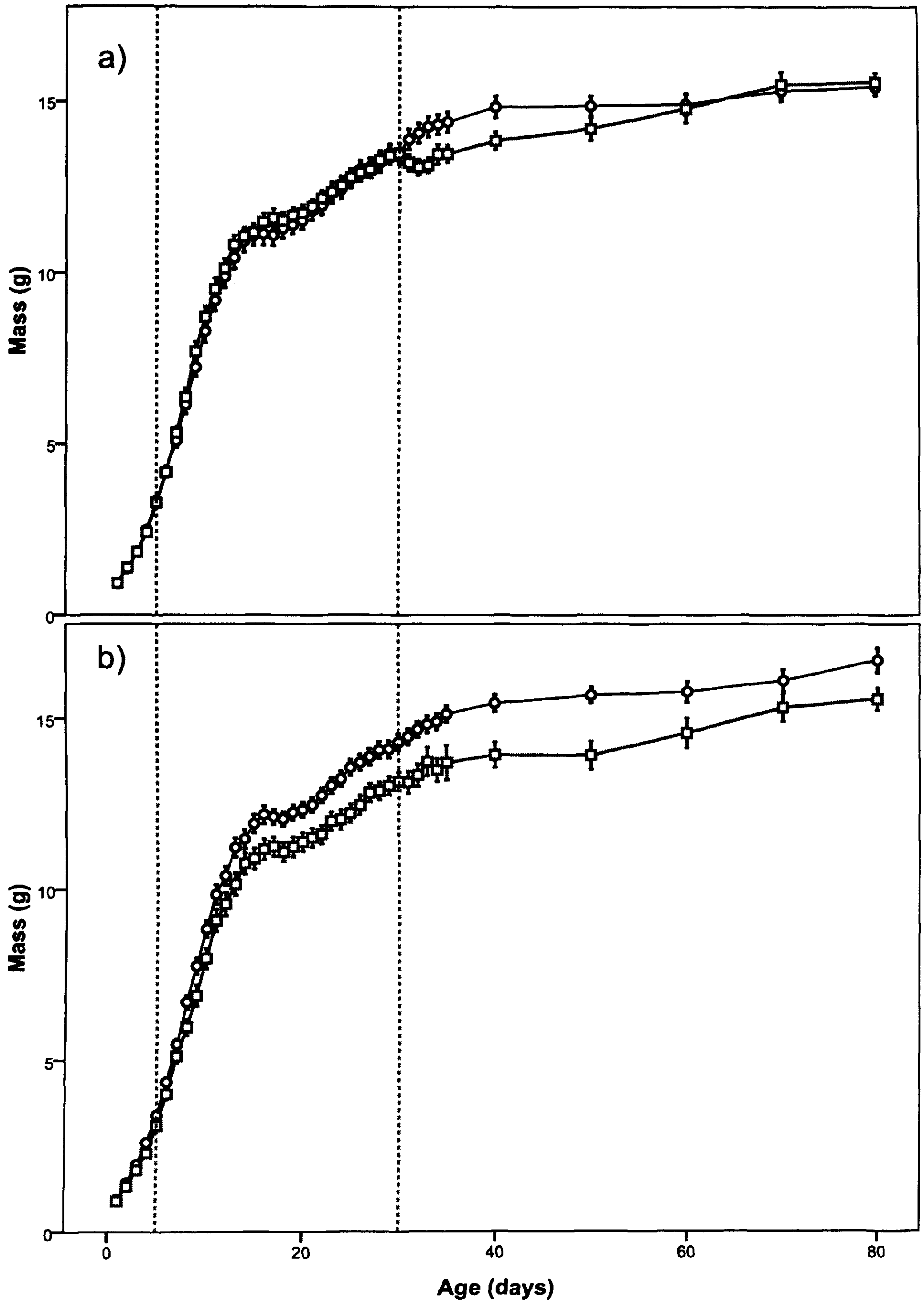

Figure 4.1: Growth rates of a) male and b) female offspring reared under control (open circles) and stress (open squares) treatments. Dotted lines indicate the start (day 5) and end (day 30) of experimental manipulations.

The stress treatment did not affect adult body mass, brain mass or HVC volume (Fig. 4.2; Table 4.2). Of the song variables, only syllable number and peak frequency were affected by stress (Fig. 4.2; Table 4.2). Males raised under the control treatment had fewer syllables in their song phrase than those raised under the stress treatment (Fig. 4.2d; mean syllable number ± SE: controls: 6.52 ± 0.44; stressed: 7.83 ± 0.59; d = 0.52). Control males had lower peak frequencies than those from the stress treatment (Fig. 4.2f; mean peak frequency [Hz] ± SE: controls: 2850 ± 215; stressed: 3417 ± 187; $d = 0.59$), while there was a non-significant tendency toward the maximum frequency being lower in males from the control group (mean maximum frequency [Hz] ± SE: controls: 9972 ± 189; stressed: 11680 ± 238; $d = 0.28$). Neither phrase length nor the proportion of unique syllables in the song phrase were affected by the stress treatment. Peak frequency varied significantly with brain mass (Table 4.2), but no other song traits were significantly predicted by brain mass or HVC volume (all $P > 0.05$). There was no effect of treatment order on any of the traits measured.

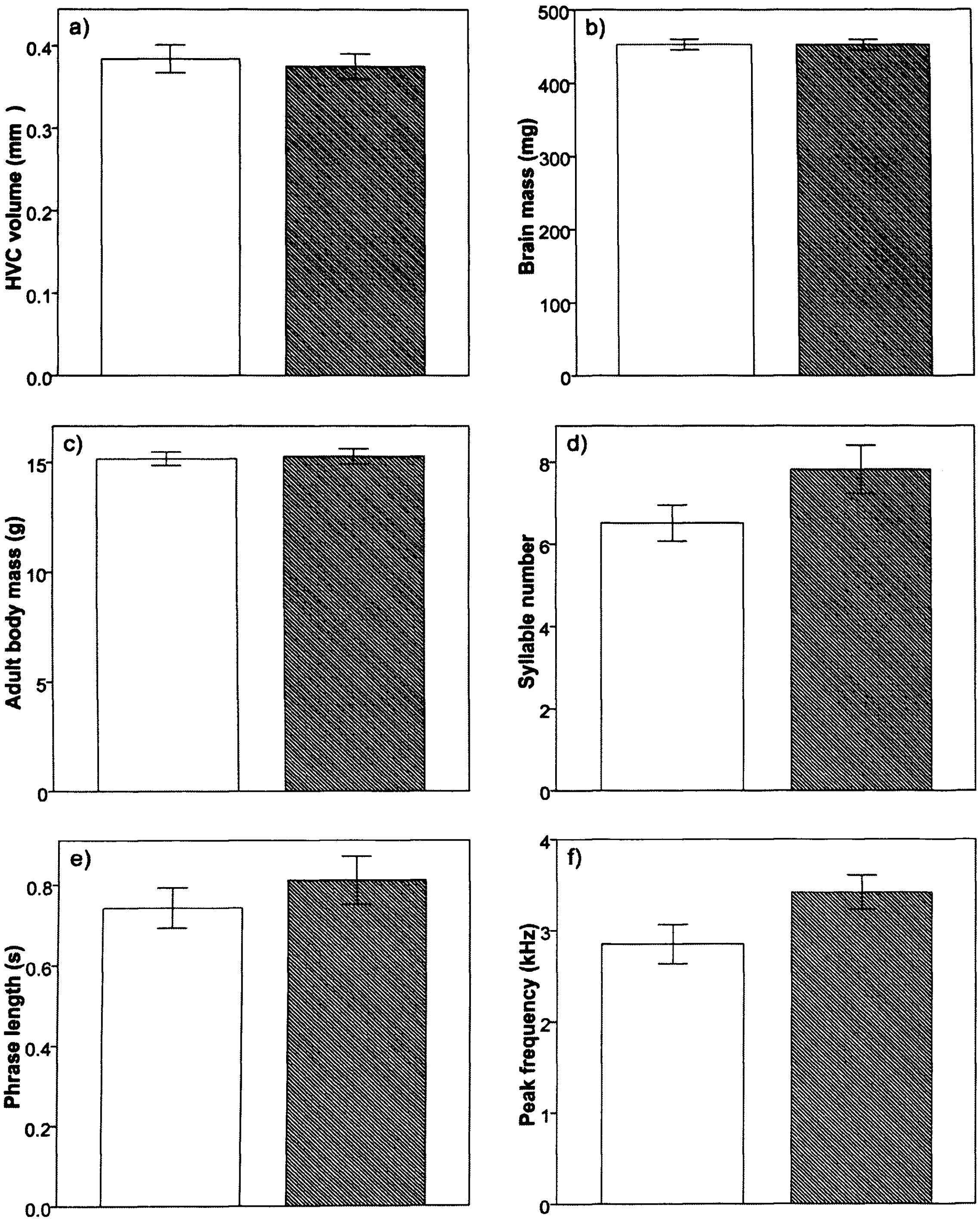

Figure 4.2: Effects of the stress treatment on a) HVC volume, b) brain mass, c) adult body mass, d) syllable number, e) phrase length and f) peak frequency. White bars indicate males reared under control conditions and shaded bars those reared under the stress treatment.

Table 4.2: Results of GLMs testing the effects of the stress treatment on eight brain, body and song variables. The random factor in each model, nest of rearing, is indicated in bold type, and covariates, when retained in the model, are in italics. Significant *P* values are indicated in bold.

Dependent variable	Model term	*F*	d.f.	*P*
HVC volume	Stress treatment	0.15	1,11	0.705
	Treatment order	1.09	1,10	0.322
	Rearing nest	0.90	10,11	0.559
Brain mass	Stress treatment	<0.01	1,11	0.967
	Treatment order	1.33	1,10	0.276
	Rearing nest	1.50	10,11	0.258
Body mass	Stress treatment	0.05	1,11	0.823
	Treatment order	0.44	1,10	0.523
	Rearing nest	1.13	10,11	0.419
Syllable number	Stress treatment	5.25	1,11	**0.043**
	Treatment order	3.32	1,10	0.099
	Rearing nest	2.49	10,11	0.075
Phrase length	Stress treatment	0.96	1,11	0.348
	Treatment order	3.25	1,10	0.102
	Rearing nest	1.71	10,11	0.197
Peak frequency	Stress treatment	9.25	1,10	**0.012**
	Treatment order	2.63	1,10	0.134
	Rearing nest	4.72	10,10	**0.011**
	Brain mass	7.31	1.10	**0.022**
Maximum frequency	Stress treatment	4.47	1,11	0.058
	Treatment order	0.67	1,10	0.432
	Rearing nest	9.40	10,11	**<0.001**
Proportion unique	Stress treatment	0.01	1,11	0.941
	Treatment order	<0.01	1,10	0.962
	Rearing nest	3.26	10,11	**0.033**

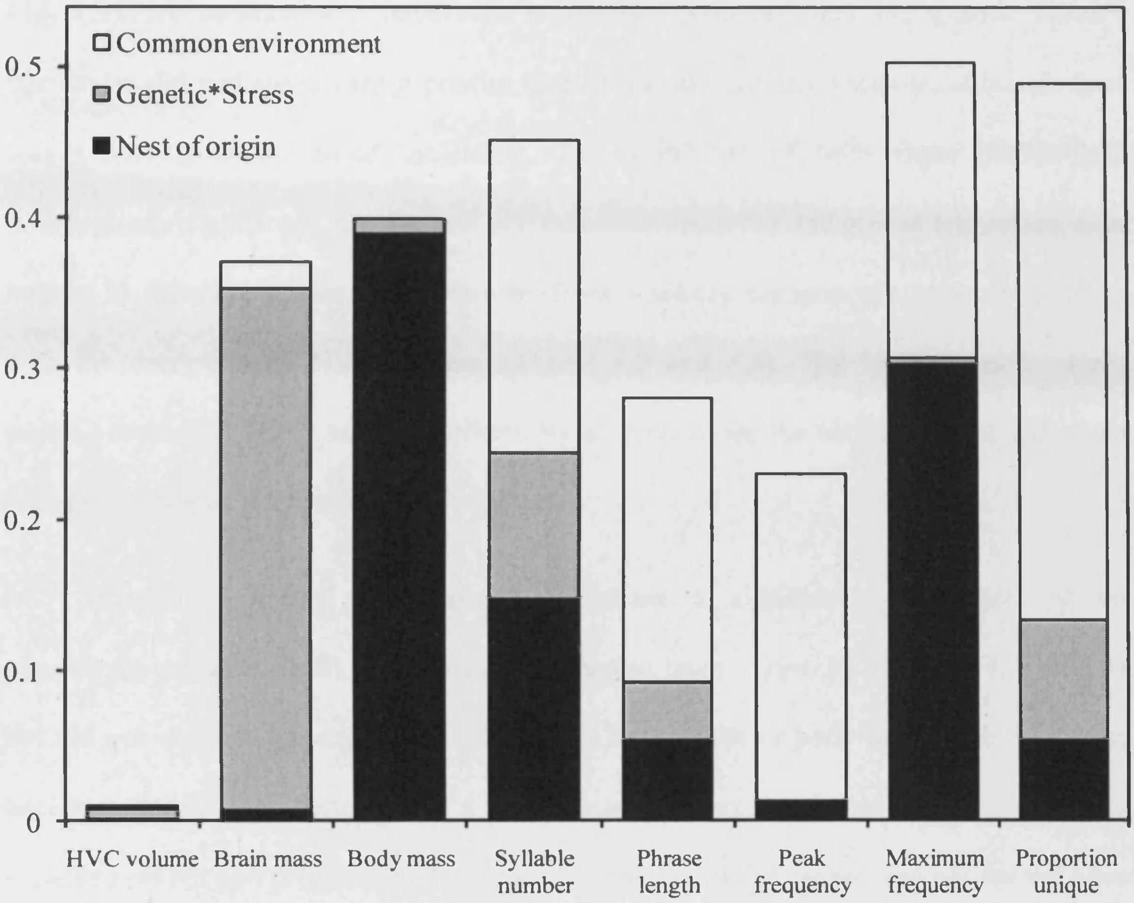

Figure 4.3: Causal components of variance in brain, body and song traits. Variance components were estimated using restricted maximum likelihoods and are expressed as a proportion of the total phenotypic variance (V_P). The error variance (not shown) accounts for the remainder of the variance for each trait. Note that the effect of nest of origin estimates ½ V_A.

The relative contributions of nest of origin, common rearing environment and interaction between genotype and the stress treatment are shown in Figure 4.3. Analysis of the variance components indicated that all the song variables, with the exception of peak frequency, were heritable (Table 4.4). Heritability was low for phrase length and the proportion of unique syllables in a phrase, and moderate for syllable number and maximum frequency (Table 4.4, Fig. 4.3). Body mass was highly heritable (Table 4.4,

Fig. 4.3). By contrast, the father-son regressions indicated that the genetic father's phenotype did not significantly predict that of the son for any variable, although there was a non-significant trend indicating high heritability of body mass (Table 4.3). Although nonsignificant, the heritability estimates from the father-son regression were largely in agreement with those derived from variance components between siblings, with the exception of HVC volume (Tables 4.3 and 4.4). The variance components method estimated HVC volume heritability at zero while the estimate from father-son regression indicated a heritability of 0.21.

Common rearing environment explained a significant proportion of the phenotypic variance in all song variables, ranging from 0.19-0.36 (Table 4.4, Fig. 4.3), but did not account for any of the variance in brain traits or body mass. The regression analysis showed that foster-father's song phenotype predicted that of the son for the syllable number and proportion of unique syllables in a song phrase, but not for the other three song variables (Table 4.3). There was a significant effect on sons' brain mass of the stress treatment by genetic father's brain mass interaction (Table 4.3), and analysis of the variance components indicates that the stress treatment by genotype interaction accounted for 0.34 of the phenotypic variance in brain mass (Fig. 4.3). However, repeating the father-son regressions for males from the control and stress groups separately reveals no significant effect of genetic father's brain mass for either group (controls: $F_{1,21} = 0.79$, $P = 0.384$, $h^2 = 0.51$; stressed: $F_{1,21} = 3.23$, $P = 0.087$, $h^2 = -0.76$), although this might reflect the reduced sample size that resulted from splitting the dataset. The stress treatment by genotype interaction also explained a small proportion (0.04-0.10) of the variance in syllable number, phrase length and the proportion of syllables in a phrase that are unique (Fig. 4.3). There was a significant interaction between stress treatment and foster-father's phenotype on the proportion of unique syllables (Table 4.3). When analysed separately, the relationship between foster-father's and sons' proportion of unique syllables remained significant for sons from the control group but not for stressed sons (controls: $F_{1,21} = 15.81$, $P = 0.001$; stressed: $F_{1,21} = 0.20$, $P = 0.656$).

Table 4.3: Results of father-son regressions testing the effects of the stress treatment, genetic factors and common rearing environment on eight brain, body and song variables. Covariate terms are indicated in italics. Significant *P* values are indicated in bold.

Dependent variable	Model term	*F*	d.f.	*P*	Slope	h^2
HVC volume	Stress	0.17	1,44	0.685		
	Genetic father	0.19	1,44	0.668	0.11	0.21
	Rearing male	0.11	1,44	0.746	0.08	
Brain mass	Stress	5.42	1,43	**0.025**		
	Genetic father	0.1	1,43	0.754	-0.05	-0.11
	Rearing male	0.04	1,43	0.849	-0.03	
	*Stress*Genetic father*	5.49	1,43	**0.024**	0.34	
Body mass	Stress	0.01	1,44	0.919		
	Genetic father	3.65	1,44	0.063	0.31	0.62
	Rearing male	1.41	1,44	0.241	0.18	
Syllable number	Stress	5.10	1,42	**0.029**		
	Genetic father	0.68	1,42	0.415	0.12	0.23
	Rearing male	4.22	1,42	**0.046**	0.46	
Phrase length	Stress	1.27	1,42	0.266		
	Genetic father	0.01	1,42	0.919	0.02	0.03
	Rearing male	<0.01	1,42	0.970	<0.01	
Peak frequency	Stress	4.77	1,42	**0.035**		
	Genetic father	0.42	1,42	0.519	-0.09	-0.18
	Rearing male	1.28	1,42	0.264	0.14	
Maximum frequency	Stress	3.62	1,42	0.064		
	Genetic father	2.41	1,42	0.128	0.28	0.56
	Rearing male	0.23	1,42	0.634	0.08	
Proportion unique	Stress	5.63	1,41	**0.022**		
	Genetic father	2.85	1,41	0.099	0.23	0.47
	Rearing male	7.66	1,41	**0.008**	0.39	
	*Stress*Rearing male*	6.32	1,41	**0.016**	0.33	

Table 4.4: Phenotypic (V_P), additive genetic (V_A), common rearing environment (V_{EC}), genotype-by-stress (V_{GE}) and environmental (V_E) components of variance for eight brain, body and song variables. Also shown are estimates of heritability (h^2); the proportion of variation attributable to common rearing environment (V_{EC}/V_P), and to stress treatment by genotype interactions (V_{GE}/V_P); and the coefficients of variation for each of these factors. Each column shows the mean ± SE, derived from the jack-knifing procedure, for each component. Significant values of h^2, V_{EC}/V_P, and V_{GE}/V_P are indicated in bold type.

Dependent variable	V_P	V_A	V_{EC}	V_{GE}	V_E	h^2	V_{EC}/V_P	V_{GE}/V_P	CV_P	CV_A	CV_{EC}	CV_{GE}
HVC volume	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± 0.01	0.21	0.01	0.01	0.02
Brain mass	1222.34 ± 6.38	16.22 ± 8.48	22.76 ± 6.20	423.02 ± <0.01	768.45 ± 11.74	0.01 ± 0.01	0.02 ± 0.01	**0.34** ± 0.01	0.08	0.01	0.01	0.05
Body mass	2.53 ± 0.01	1.97 ± 0.03	<0.01 ± <0.01	0.03 ± 0.01	1.52 ± 0.02	**0.78** ± 0.01	<0.01 ± <0.01	0.01 ± 0.01	0.10	0.09	<0.01	0.01
Syllable number	6.66 ± 0.03	1.96 ± 0.10	1.38 ± 0.04	0.65 ± 0.06	3.65 ± 0.05	**0.29** ± 0.02	**0.21** ± 0.01	**0.10** ± 0.01	0.36	0.20	0.16	0.11
Phrase length	0.07 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	0.05 ± <0.01	**0.11** ± 0.01	**0.19** ± <0.01	**0.04** ± 0.01	0.35	0.11	0.15	0.07
Peak frequency	1.05 ± <0.01	0.03 ± 0.01	0.23 ± 0.01	<0.01 ± <0.01	0.81 ± 0.01	0.02 ± 0.01	**0.22** ± <0.01	<0.01 ± <0.01	0.33	0.05	0.15	<0.01
Maximum frequency	1.19 ± 0.01	0.72 ± 0.04	0.23 ± 0.01	0.01 ± 0.01	0.59 ± 0.01	**0.60** ± 0.03	**0.20** ± 0.01	0.01 ± 0.01	0.11	0.08	0.05	0.01
Proportion unique	0.02 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	**0.11** ± 0.01	**0.36** ± 0.01	**0.08** ± 0.01	0.17	0.06	0.10	0.05

4.4 Discussion

In this study, I aimed to quantify the contributions of the rearing environment and genetics to male brain, body and song traits, and to discover whether developmental stress affected the strength of these relationships. Unexpectedly, the stress treatment had no effect on males' HVC volume, in contrast to the results of several previous studies (Nowicki et al., 2002a; Buchanan et al., 2004; Spencer et al., 2005a; MacDonald et al., 2006). This lack of an effect is perhaps unsurprising given that the stress treatment also had no apparent effect on male nestlings' growth rates, indicating that it had little adverse affect on development. Gil et al. (2006) found that brood size manipulation had no effect on the size of any song control nucleus, although in that case the brood size manipulation had a detrimental effect on growth rate (Naguib et al., 2004). There is less consensus from previous studies regarding the expected effects of developmental stress on song but my finding that the songs of males in the stress treatment group had a greater number of syllables and higher peak frequency than those from the control group appears inconsistent with the predictions of the developmental stress hypothesis (Nowicki et al., 1998a; Buchanan et al., 2003). Nonetheless, in the absence of any difference in HVC volume or brain mass between the treatment groups it is unsurprising to find no reduction in song complexity in the stress treatment group, since any effect of developmental stress on song is predicted to be mediated by detrimental effects on brain development (Nowicki et al., 1998a; Nowicki et al., 2002a).

Several previous studies have found little or no effect of sub-optimal environmental conditions on song structure (Gil et al., 2006; Holveck et al., 2008; Brumm et al., 2009), while those that have form little consensus on what song traits are affected or to what degree (Buchanan et al., 2003; Spencer et al., 2003, 2004; Dreiss et al., 2006; Naguib et al., 2008; Zann and Cash, 2008). Several recent studies have suggested that developmental stress might have rather more subtle effects, influencing aspects of song learning such as the accuracy with which syntax is copied from the tutor (Holveck et al., 2008; Brumm et al., 2009). I did not quantify the accuracy of song learning in this study so it remains unclear whether it might have been affected by the nutritional stress treatment. However, the fact that the proportion of unique syllables in a foster-father's song phrase predicted that of their foster-sons under the control but not the stress treatment hints at the possibility that stress may have a detrimental effect on the quality of song learning.

The nutritional stress treatment had a significant adverse effect on the growth of female offspring but not that of males. The detrimental effect of developmental stress on growth rates, in both males and females, is a frequently reported phenomenon (Boag, 1987; de Kogel, 1997; Nowicki et al., 2002a; Spencer et al., 2003; Naguib et al., 2004; Arnold et al., 2007; Zann and Cash, 2008; Brumm et al., 2009). Many of the studies in which it has been observed appear not to have looked for sex differences in susceptibility to stress (Nowicki et al., 2002a; Spencer et al., 2003; Zann and Cash, 2008; Brumm et al., 2009). Those that have included sex as a factor in their analysis have found no effect of sex or any sex by treatment interaction on growth rates (Boag, 1987; de Kogel, 1997; Naguib et al., 2004; Arnold et al., 2007). However, Kilner (1998) showed that zebra finch broods had female biased nestling mortality when food availability was low; Arnold et al. (2007) found that females, but not males, in the food restriction treatment had shorter wings as adults; and De Kogel (1997) found that females from larger broods had a higher adult mortality rate, but that brood size did not affect male mortality. These studies imply either that females are more vulnerable to the effects of developmental stress than males or that there are sex differences in the way young birds compensate for poor developmental conditions, perhaps investing limited resources in structures that will maximise reproductive potential. If females are more susceptible to suboptimal developmental conditions, this could be a result of biased parental care toward males, female offspring being outcompeted by males for access to parental care, or because resource availability has a greater impact on the development of female than male chicks. Martins (2004) hand reared zebra finch chicks on diets of different quality and found, in the absence of any parent-offspring or sib-sib interactions, that female growth rate, but not that of males, was lower on a restricted food supply, which suggests that females experience greater costs of a poor rearing environment than males. If it is the case that females are more susceptible to nutritional stress than males, then the finding that females, but not males, were affected by the treatment in this study might indicate that the difference between the quality of rearing environment experienced by the control and stress treatment groups was too small to have a measurable effect on male development, despite affecting the development of female nestlings. This raises the potentially interesting possibility that differences in the effects of developmental stress on neural development and song traits reported by previous studies might reflect differences in the degree of stress experienced by the subjects, and this might be a fruitful direction for future research.

Body mass was the only trait I examined for which nest of origin explained a large portion of the variance. The coefficient of additive genetic variation (CV_{A}) standardises the additive genetic variance of a trait with respect to the mean value of that trait, and represents a measure of evolutionary potential of the trait to selection (Charlesworth, 1984; Houle, 1992). Despite the high estimate of heritability, body mass actually had a relatively low CV_A (Table 4.4), suggesting that there are other constraints that limit the degree of variation in body mass, and that it is not under strong selection as an indicator of genetic quality. This is in agreement with previous findings that CV_As are significantly greater for sexual than non-sexual characters across a range of taxa (Pomiankowski and Møller, 1995). By contrast, although syllable number had a relatively low heritability, it had a CV_A of 0.2, roughly twice that of body mass (Table 4.4) and comparable to the mean value of 0.17 that Pomiankowski and Møller (1995) found for secondary sexual traits in their meta-analysis, across a range of species. Pomiankowski and Møller (1995) hypothesised that long term directional selection will favour genetic modifiers that increase the phenotypic variation in sexual traits even if they have no effect on the mean trait value. Syllable number in zebra finch song could thus be under selection as a signal of male genotypic quality. The other song parameters I investigated have lower values of CV_A and are unlikely to act as signal of genotypic quality.

A common rearing environment explained between 19% and 36% of the variation in all five song variables I examined, but did not account for any of the variance in brain structure or body mass. This reflects the fact that bird song is learned from a tutor (Marler, 1990; Catchpole and Slater, 2008). Three of the song parameters, syllable number, phrase length and peak frequency, had a coefficient of variation attributable to common rearing environment (CV_{EC}) of 0.15-0.16, roughly comparable to the CV_As of sexual signals discussed above. These measures of song structure have the potential to signal the quality of an individual's song tutor, which might indirectly signal genetic quality in spite of the low heritability of song traits, since song is typically learned from the father (Miller, 1979b; Clayton, 1987b; Zann, 1996). There was an interaction between the stress treatment and tutor's song in determining the proportion of unique syllables in the song phrase. Analysing males from the control and stress treatment group separately indicated that control males' proportion of unique syllables was predicted by that of their foster father but that no such relationship existed for sons raised under conditions of nutritional stress. This might suggest that, while the quality of the tutor's song places an upper limit on that achievable by the son, developmental stress can disrupt song learning, in which case the proportion of unique syllables in a song phrase might signal aspects of both the learning environment and phenotypic quality.

An interaction between the stress treatment and genotype is responsible for more than one third of the observed variance in brain mass, although CV_{GE} for this trait was very low, implying that this interaction plays little role in selection. Although fathers' brain mass did not significantly predict that of sons' when the control and stress treatments were analysed separately, there was a non-significant positive relationship between the brain mass of sons from the control group and that of their genetic father while this trend was reversed in sons originating in the stress treatment group. This result might suggest that any link between brain mass and genotype is disrupted by variation in the quality of the rearing environment, so any trait that signaled brain mass would be of little use in advertising genetic quality. Indeed, I found that brain mass did not predict HVC volume or any song trait, with the exception of peak frequency.

There were small stress treatment by genotype effects on the expression of three song traits: syllable number, phrase length and the proportion of syllables in the song phrase that are unique. These accounted for only 4-10% of the variance in these traits, and CV_{GE} was low at 0.05-0.11 but, given that the stress treatment in this experiment had little or no overall effect on phenotype, it is unsurprising that the interaction between treatment and genotype did not have a major influence on phenotypic expression. If more extreme levels of stress have more drastic effects on neural development and song structure, as has been found by previous studies (e.g. Spencer et al., 2003; Buchanan et al., 2004; MacDonald et al., 2006), the significant stress treatment by genotype interactions found here suggest that males of differing genotypic quality may respond to developmental stress in different ways or be able to compensate for it to different degrees. It is not clear how this could affect sexual selection for these traits. Under certain conditions, genotype-by-environment interactions are predicted to promote female choice (Kokko and Heubel, 2008). This is particularly likely to be the case if there is not too much variation in the environmental conditions experienced by males. For example, if the majority of developmental stress is due to population level stochasticity in environmental conditions (e.g. periodic droughts), rather than differences in parental care or sibling interactions, then females might be able to derive information about a male's genetic quality from the way his song structure developed in the face of that stress. If there is much greater diversity in males' environmental backgrounds, male traits cannot function as reliable indicators of offspring fitness and female choice for these traits is likely to be eroded (Greenfield and Rodriguez, 2004; Kokko and Heubel, 2008; Higginson and Reader, 2009). This is likely to be the case if levels of environmental stress due more to individual level effects, such as differences in parental care, than to population level events, and is likely to be exacerbated by relatively high levels of dispersal in zebra finches (Zann, 1996).

Virtually all of the variance in HVC volume, and the majority of variance in all the other traits examined in this study, was attributable to unique environmental effects (i.e. not due to the shared rearing environment) and to any genotype-by-environment interactions, apart from the stress treatment by environment effects discussed above. The nutritional stress treatment must account for a portion of this variation in syllable number and peak frequency, but the stress treatment had no significant effect on any of the other variables.

Estimates of the heritability of brain variables and body mass did not agree closely with those obtained by Airey et al. (2000b). Airey et al. (2000b) estimated that both HVC volume and total brain mass had moderate heritability, of 0.38 and 0.49 respectively. I estimated HVC heritability at zero using variance components of sib-sib differences, and even the more generous estimate obtained from father-son regression puts the heritability of HVC volume at 0.21, roughly half that indicated by Airey et al. (2000b). I also found little or no heritability of brain mass. The heritabilities calculated by Airey et al. (2000b) are almost certainly overestimated since they also include the effect of common rearing environment. However, I found only a negligible influence of common rearing environment on HVC volume and brain mass so differences in the experimental design are unlikely to account for all of the differences between my estimates of heritability and those of Airey et al. (2000b). Perhaps a more important difference between the two studies is that Airey et al. (2000b) did not investigate the possibility of any genotype-by-environment interaction influencing brain development. I found a significant effect of the stress treatment by genotype interaction on total brain mass which accounted for 37% of the total variance in brain mass. This interaction is likely to blur any link between genotype and phenotypic expression of this trait (as appeared to be the case with father-son regressions looking at males from the two treatment groups separately), so might account for the discrepancy between my estimates of heritability and those found by Airey et al. (2000b). I calculated fairly high estimates for the heritability of adult body mass which were more than twice as large as those given by Airey et al. (2000b). Forstmeier et al. (2009) found generally low heritability for a number of male song traits in the zebra finch, a result which is in general agreement with my findings in this study, although my estimate of the heritability of syllable number in the song phrase is roughly twice that reported by Forstmeier et al. (2009).

Although I was able to separate the effects of genotype and common rearing environment on the determination of brain and song traits, and to test for interaction between the stress treatment and genotype, there are several methodological issues which may reduce the reliability of my heritability estimates. The most important of these is the relatively low sample size I had available. Only 12 nests produced adult males from all four combinations of cross-fostering and stress treatment, leaving a total sample of 48 sons (from 18 genetic fathers), smaller than the 38 fathers and 68 sons used by Airey et al. (2000b) and falling considerably short of the population of 429 males employed by Forstmeier et al. (2009), who examined vocal and morphological characters, but not neural traits. The method I employed to test the relationship between the phenotypes of foster- and genetic fathers and that of their sons, also had shortcomings. Since each foster-father raised four males (and most genetic fathers had more than one son in the experiment), the father-son regressions may include pseudoreplication. It is not possible to use mid-son values for each rearing or genetic father since the aim of the analysis is to tease apart differences between males raised by different fathers and under different stress treatments. I also calculated variance components contributing to phenotypic differences between and among siblings. These two approaches yield relatively similar estimates of heritability for most of the traits measured, which is encouraging and implies that my estimates are not wildly inaccurate.

My aims in this study were, firstly, to quantify the influence of environmental and genetic factors in determining male brain morphology and song structure; and secondly to discover whether developmental stress could affect the strength of any relationship between genotype and trait expression, thereby having the potential to mediate signals of genetic quality. The experimental stressor used in this study had little or no effect on male growth, neural development or song structure making the second aim difficult to accurately address. Regarding the first aim, the majority of variation in brain and song traits was attributable to unique environmental effects. If the lack of stress effects on male phenotype reported here was the result of males experiencing only low levels of stress, more extreme stress has the potential to have a larger influence on brain and song development. In addition, I found that several male song traits, particularly syllable number, have the potential to signal information about a male's genetic quality or the quality of his song tutor. This information could result from a direct link between genotype and phenotype, in the case of syllable number, or be mediated by an interaction between genotype and developmental stress. Of the song structure parameters considered in this study, syllable number appears to have the greatest potential to act as a signal of genotypic quality.

Chapter 5: Does song structure predict male quality in wild zebra finches?

5.1 Introduction

Oscine song is a secondary sexual trait whose evolution is driven by sexual as well as natural selection (Searcy and Andersson, 1986; Catchpole and Slater, 2008). It is supposed that such traits advertise direct or indirect benefits to females from choosing mates with the most attractive songs and that the honesty of these signals is mediated by their cost to the singer (Zahavi, 1975; Grafen, 1990b). Bird song is one of the most well studied secondary sexual traits and a great number of studies have demonstrated that a number of male song traits are involved in attracting females (Catchpole and Slater, 2008). Constraints on the development or production of some, but not all, of these traits have been identified, which enforce their honesty as signals (Gil and Gahr, 2002; Catchpole and Slater, 2008).

Female preferences have been demonstrated for several performance related traits such as a high rate of singing (e.g. in the zebra finch, *Taeniopygia guttata*, Houtman, 1992), high amplitude song (zebra finch, Ritschard et al., 2010), or longer songs or bouts of song (see chapter 1, section 1.2). The honesty of these signals is probably enforced by the trade-off between time spent singing and time spent on foraging and other behaviours, and possibly also by the energetic costs of song production (Gil and Gahr, 2002; Catchpole and Slater, 2008). Female birds also show preferences for various structural song traits. Some of these might be attractive because they are difficult to sing, such as so-called 'sexy syllables' in canary song, *Serinus canaria* (Vallet et al., 1998). Similarly, maximum performance quality in many songbird species appears to be defined by a trade-off between trill rate and frequency bandwidth (Podos, 1996, 1997). The song trait for which female preferences have been most commonly demonstrated is repertoire size, measured as either the number of syllables in a song or the number of different song types performed by a male, depending on the biology of individual species (reviewed in Catchpole and Slater, 2008; and see chapter 1, section 1.2.1). Despite this widespread preference for complex songs, it is not immediately clear how selection for large repertoires could come about, as there do not appear to be obvious costs associated with producing large repertoires as opposed to repetitions of a smaller repertoire. A number of potential resolutions have been suggested to this problem, including exploitation by males of female sensory bias (Ryan et al., 1990), or the possibility that song complexity is limited by immunocompetence (Folstad and Karter, 1992; Sheldon and Verhulst, 1996) or developmental conditions (Nowicki et al., 1998a; Buchanan et al., 2003). The evidence for these is considered in detail in chapter 1, sections 1.2 and 1.3.

The essence of sexual selection theory is that certain traits will be selected for if they enhance the reproductive success of individuals that exhibit them (Andersson, 1994), and many field studies have demonstrated links between males' song performance or structure and various indicators of reproductive success, across a range of species. There is evidence that males with larger repertoires may attract a mate earlier (*Acrocephalus* warblers, Catchpole, 1980; European starling, *Sturnus vulgaris*, Eens et al., 1991; Mountjoy and Lemon, 1996; sedge warbler, *Acrocephalus schoenobaenus*, Buchanan and Catchpole, 1997), obtain a greater number of extra-pair fertilizations (great reed warbler, *Acrocephalus arundinaceus*, Hasselquist et al., 1996) or attract more females in polygynous species (red-winged blackbird, *Agelaius phoeniceus*, Yasukawa et al., 1980; great reed warbler, Catchpole et al., 1985; European starling, Eens et al., 1991), produce a greater number of offspring (great reed warbler, Catchpole, 1986; great tit, *Parus major*, Lambrechts and Dhondt, 1986; song sparrow, *Melospiza melodia*, Hiebert et al., 1989; European starling, Eens et al., 1991; willow warbler, *Phylloscopus trochilus*, Gil and Slater, 2000; song sparrow, Reid et al., 2005), or father offspring that are more likely to survive long enough to reproduce themselves (great tit, McGregor et al., 1981; great reed warbler, Hasselquist et al., 1996).

The zebra finch is a small, seed-eating, socially monogamous estrildid finch (Zann, 1996), thought to be the most primitive (i.e. least changed from the ancestral form) of the *Estrildinae* (Christidis, 1986). Zebra finch males do not produce a repertoire of different song types, but produce a single, highly stereotyped song phase, unique to the individual (Zann, 1990; Nordeen and Nordeen, 1992). A song phrase typically consists of 3 to 14 syllables produced in a fixed order and 73% of songs contain one or more repeated syllables (Zann, 1996; see Fig. 1.2 for a spectrogram of a typical zebra finch song). Zebra finches are closed-ended song learners, with a single sensitive phase for learning, extending from day 30-65 (Eales, 1985, 1987; Roper and Zann, 2006). Zebra finches are not territorial and males show no aggression toward singing birds, while singing males do not behave aggressively toward others (Zann, 1996). This lack of a territorial function of song implies that selection by female choice is the primary selection pressure on the development of zebra finch song, making them an ideal model species for the study of song and selection by female choice.

Little is known about female zebra finches' mate choice criteria in the wild, but song preferences in laboratory experiments predict their preferences for live males in mate choice chambers (Holveck and Riebel, 2007), which in turn predict their choice of mates in aviary conditions (Clayton, 1990a). These experiments indicate that male song plays an important role in mate choice in this species. Female preferences have been demonstrated for complex songs with a larger repertoire of syllables (Neubauer, 1999;

Holveck and Riebel, 2007; Vyas et al., 2009; and see chapter 3). Although several studies have indicated a preference for longer song phrases, this is likely to reflect the confounding variable of syllable number (Riebel, 2009), and female preferences were better explained by syllable repertoire than phrase duration in operant and phonotaxis preference tests (Holveck and Riebel, 2007). Female zebra finches also appear to prefer males that sing at a higher rate (ten Cate and Mug, 1984; Houtman, 1992; Collins et al., 1994), although it remains unclear whether song rate is the cause or effect of female preference (reviewed in Riebel, 2009). The proportion of sound versus silence within a song phrase, and syllable syntax, may also play a role in female song preferences (reviewed in Riebel, 2009), although few studies have addressed these directly.

The zebra finch is perhaps the most important model passerine system (Griffith and Buchanan, 2010), and in particular has served as the major model species for understanding both the neural basis of song learning and production (reviewed in Zann, 1996; Brainard and Doupe, 2002), and the effects of song on mate choice (reviewed in Riebel, 2009). However, the vast majority of these studies have been carried out on populations that have been bred in captivity for many generations. The structure, geographical variation and development of zebra finch song in wild populations have been extensively described (see Zann, 1996), but almost nothing is known about the song preferences of wild females or whether male song is predictive of reproductive success in the field. Wild zebra finch females have been shown to prefer wild males to domesticated ones, and vice versa (Rutstein et al., 2007), and wild and domesticated birds differ in nestling growth rate, adult mass and size, and immune response (Tschirren et al., 2009). Despite these differences, Tschirren et al. (2009) found no difference in song rate and brood size manipulation appeared to affect wild and domesticated zebra finches in similar ways. Forstmeier et al. (2007), found that all domesticated populations of zebra finches they tested had reduced genetic variability compared to wild populations, but that there was no evidence for any severe bottlenecks. Laboratory studies on captive populations may not fully reflect the selection pressures at work in the field so it is important to better understand the relationship between male song, female choice and reproductive success in wild populations. In this study, I recorded the songs of male zebra finches in a wild population, in New South Wales, and investigated whether song structure predicted reproductive success. I also tested whether song structure can predict a male's morphology or the morphology of his mate, as might be expected if males with more complex songs attracted higher quality females. I predicted that males with more complex songs would be larger and produce a greater number of surviving offspring than those with simpler songs.

5.2 Methods

5.2.1 Study site

This study was conducted between September and December 2008 at the Fowlers Gap Arid Zone Research Station of the University of New South Wales, New South Wales, Australia (31°05' S, 142°42' E). The station is located in the semi-arid zone of far western New South Wales, experiencing seasonally highly variable temperatures with cold winters and hot summers, and receiving a mean annual rainfall of 240 mm (Griffith et al., 2008). Breeding attempts were monitored during this period in nest boxes at the Gap Hills site on the station. A total of 199 plywood nest boxes (140 mm long x 93 mm wide x 120-180mm high) were mounted on steel poles, 1.0-1.85 m above ground level (Griffith et al., 2008). These nest boxes were distributed in five 'colonies', each consisting of 20-24 nest boxes located in close proximity to one another, or in smaller groups of one or three.

5.2.2 Nestling survival and parent morphology

Clutch size, egg volume and hatching success rates were recorded. The volume of each egg was calculated as $V = 0.57LB^2$, where L = length and B = maximum breadth of the egg (Hoyt, 1979). Nestlings were banded at day 12 and care was taken not to disturb the nest after this date as doing so can provoke premature fledging (Zann, 1996). Survival to day 12 was therefore used as a proxy for fledging success (fledging usually occurring at 16-18 days, Zann, 1996). As part of another study, taking place concurrently, some chicks were cross-fostered to other nests in order to manipulate brood size. Nestlings from each brood were cross-fostered at 2 ± 1 days after hatching, to up to three other nests with chick age differences of no more than one day. Experimentally manipulated broods consisted of two to seven chicks. A few broods were not cross-fostered due the unavailability of any other broods of suitable age.

Attempts were made to catch the parent birds from each nest using nest box traps. When caught, adults were banded (details in Griffith et al., 2010) and a number of morphological measures were taken, including: mass, tarsus length (measured from the right tarsus) and wing cord length (measured from the right wing). Females caught on the nest box during the rearing period were assumed to be the mate of the male observed at the box. This could be confirmed in many cases by observation of the coloured leg bands.

5.2.3 Song recording and analysis

The songs of male parents were recorded opportunistically at the nest boxes, using a Marantz PMD670 solid state recorder (Marantz, Kanagawa, Japan) and a Sennheiser MKE 2-P condenser tie-clip microphone (Sennheiser electronic GmbH, Wedemark, Germany). Recordings had a sampling rate of 44.1 kHz at 16-bit resolution. The microphone, attached to a 20 m cable was attached to the base of the pole on which a nest box was mounted and I was located in a hide 15-20 m from the nest box. In total, recordings were obtained of the songs of 48 males whose identity could be verified either from their coloured leg band combination or, in the case of unbanded birds (24 males), whose behaviour confirmed ownership of the nest box (e.g. nest building or feeding chicks). Raven 1.2 sound analysis software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) was used to analyze all song recordings (Hann weighting, 1024 point DFT size, 140 Hz filter bandwidth). The spectrogram of each male's recording was examined by eye to determine the composition of the stereotyped song phrase and accurate measures of seven song parameters (summarised in Table 5.1) were made from five phrases selected at random. Fewer than five complete phrases were recorded for 12 males (the smallest number of phrases recorded was two), and in these cases all the recorded phrases were measured.

Table 5.1: Definitions of the seven song structure parameters.

Parameter	Definition
Syllable number	The total number of syllables that make up the song phrase, excluding introductory syllables
Unique syllables	The number of different syllable types in the song phrase
Phrase length (s)	Duration of the song phrase
Peak frequency (kHz)	The frequency of maximum power in the phrase
Maximum frequency (kHz)	The highest frequency reached during the phrase
Frequency bandwidth (kHz)	The difference between the minimum and maximum frequency of the syllable with the greatest frequency range
Proportion unique	Proportion of unique syllables per song (= Unique syllables / Syllable number)

Table 5.2: Correlation matrix of seven song parameters measured from recordings of 48 adult male zebra finches. Each cell shows the Pearson correlation coefficient (*r*) with the associated *P* value in brackets. Significant *P* values are indicated in bold type.

Variable	Unique syllables	Phrase length	Peak frequency	Maximum frequency	Frequency bandwidth	Proportion unique
Syllable number	0.694 **(<0.001)**	0.844 **(<0.001)**	0.097 (0.513)	0.282 (0.052)	0.289 **(0.047)**	-0.607 **(<0.001)**
Unique syllables		0.526 **(<0.001)**	0.207 (0.159)	0.365 **(0.011)**	0.367 **(0.010)**	0.112 (0.448)
Phrase length			-0.036 (0.806)	0.374 **(0.009)**	0.385 **(0.007)**	-0.602 **(<0.001)**
Peak frequency				0.112 (0.448)	0.082 (0.582)	0.055 (0.711)
Maximum frequency					0.997 **(<0.001)**	-0.036 (0.810)
Frequency bandwidth						-0.041 (0.780)

5.2.4 Statistical analysis

All analyses were carried out using Minitab 15 statistical software (Minitab Inc., Pennsylvania, USA). A number of the measures of song structure were correlated strongly with one another (Table 5.2) and consequently were non-orthogonal. Principal components analysis (PCA) was used to transform the data and extract components that explained more of the variation in song structure between males than any single song parameter. Three principal components had eigenvalues greater than one, and together they explained 85% of the variance (Table 5.3). The first principal component, loading evenly on all song parameters (with the exception of peak frequency, which had little influence, and the proportion of unique syllables in the song phrase which loaded negatively), explained 46% of the variance in male song structure. PC2 loaded mainly on maximum frequency, frequency bandwidth and the proportion of unique syllables in the song phrase, while PC3 appeared to be mostly determined by the negative influence of peak frequency and the number of unique syllables, as well as loading positively on maximum frequency and frequency bandwidth.

Table 5.3: Rotated component matrices, eigenvalues and percentages of explained variance of principal components analyses on seven song structure parameters.

Parameter	PC1	PC2	PC3
Syllable number	0.48	-0.32	-0.19
Unique syllables	0.39	0.11	-0.49
Phrase length	0.48	-0.29	0.05
Peak frequency	0.08	0.19	-0.68
Maximum frequency	0.39	0.48	0.29
Frequency bandwidth	0.40	0.47	0.31
Proportion unique	-0.26	0.55	-0.27
Eigenvalue	3.21	1.65	1.11
% Explained variance (%)	45.9	23.6	15.9
Cumulative explained variance (%)	45.9	69.5	85.4

I was able to determine whether the males I recorded used nest boxes in colonies, small groups or solitary boxes in 42 cases out of 48. Since the sample size for solitary and small group males was small (N = 7 and 4, respectively), these were combined. In order to test whether male song structure is predictive of whether a male chose to breed in a colony or as part of a small group, I performed a MANOVA in which the first three principal components of song structure were the dependent variables. There was a single predictor, indentifying the singer as a colonial or group male.

Mass, tarsus length and wing length measurements were obtained for 24 males. The same measurements were obtained for the mates of 17 of the males for which I had recordings. PCA on these morphological measures yielded a single principal component with an eigenvalue exceeding one. This component explained 45% of the variance and was used as a single measure of male and female morphology. I investigated whether a male's song structure predicted either his own morphology or that of his mate using two regressions in which the principal component of morphology was entered as the dependent variable and the first three song structure components constituted the predictors. There were 13 pairs for which both the male's and female's morphological data were obtained and a separate regression was used to test whether the male's morphology predicted that of his mate.

Breeding attempts were made by 42 of the males I recorded. I took three main measures of reproductive success: clutch size, mean volume of the eggs in each clutch, and number of offspring surviving to day 12 post-hatching. Eight breeding attempts failed at the egg stage due to predation or parental abandonment. Consequently, only nests in which at least at least one egg hatched were used in the analysis of chick survival. Due to a brood size manipulation experiment running concurrently, not all nestlings were reared by their genetic parents. Because of this, I used two different measures of nestling survival: the number of genetic offspring of each male that survived to day 12, regardless of which nest they were reared in; and the number of nestlings in each male's nest that survived to day 12, regardless of whether they were the genetic offspring of that male. Note that although I use the term genetic offspring to refer to nestlings originating in a male's nest, no attempt was made to establish paternity for each chick. Nonetheless, extra-pair paternity accounts for only a very small percentage of zebra finch offspring in the wild (Birkhead et al., 1990; Griffith et al., 2010), so is unlikely to be a significant factor in this analysis. Each of these variables was tested in a multiple regression with the three principal components of song structure as predictors. A correlation between survival and the initial brood size is to be expected. To control for this, brood size was used as a covariate in the analysis of genetic offspring survival and the number of nestlings in the nest after cross-fostering was a covariate in the analysis of survival within the nest. I also performed a regression in which a single measure of song complexity, syllable number, was used as a predictor of the number of genetic offspring surviving to day 12, in which clutch size was included as a covariate.

Table 5.4: Descriptive statistics of song structure parameters (see Table 5.1 for definitions) of adult 48 male zebra finches.

Parameter	Mean	S.D.	Range
Syllable number	6.58	2.03	3-13
Unique syllables	5.13	1.20	3-8
Phrase length (s)	0.92	0.28	0.29-1.66
Peak frequency (kHz)	4.15	1.19	1.12-7.24
Maximum frequency (kHz)	12.97	4.90	6.50-21.21
Frequency bandwidth (kHz)	11.98	4.93	5.29-20.57
Proportion unique	0.81	0.15	0.38-1.00

5.3 Results

Descriptive statistics for the seven song structure parameters of zebra finch songs in the study population are presented in Table 5.4. There was no difference between males breeding in colonies and those in small groups or solitary nest boxes in any of the three principal components of song structure (Fig. 5.1; $F_{3,38} = 0.38$, $P = 0.766$).

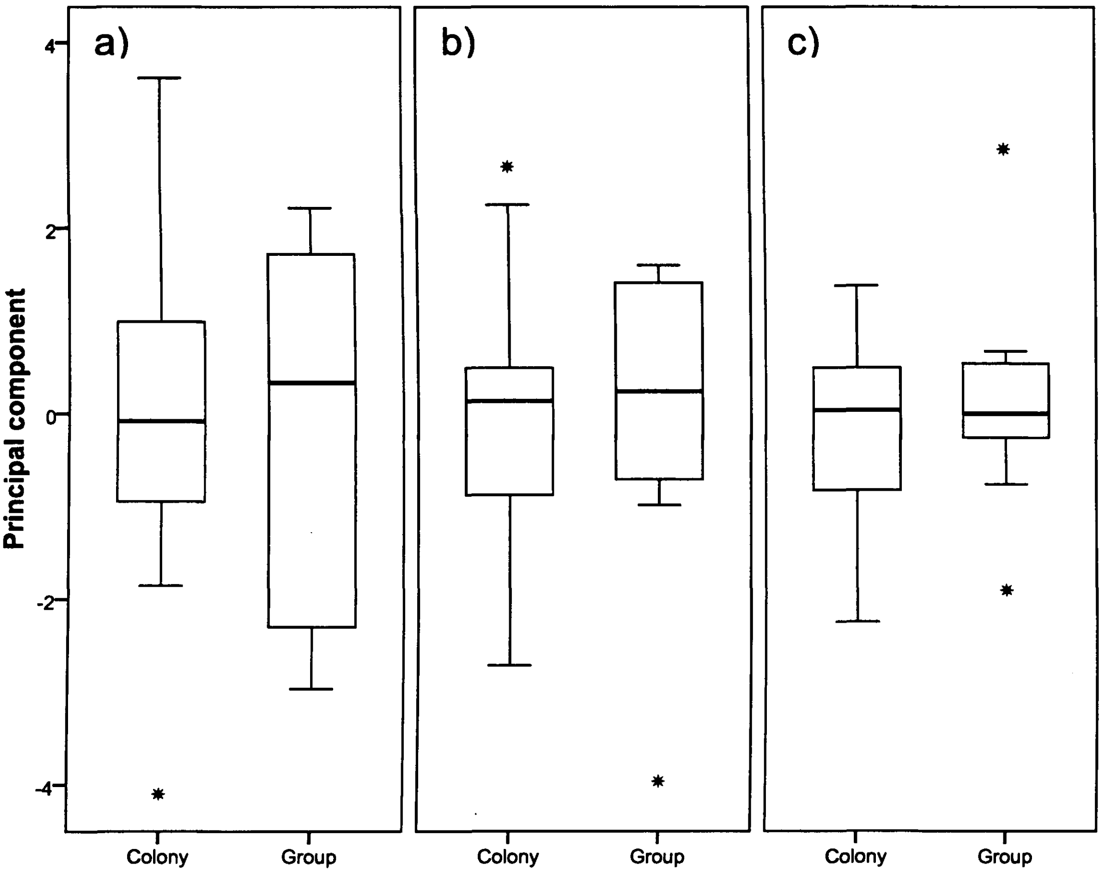

Figure 5.1: Boxplots of three principal components of the song structure of male zebra finches, breeding in colonies and small groups. a) – c) represent the values of the PC1, PC2 and PC3, respectively.

Song structure predicted neither a male's own morphology nor that of his mate (Table 5.5). Furthermore, a male's morphology did not predict that of his mate ($F_{1,11}$ = 0.63, P = 0.444). Male song did not predict the number of eggs laid in a clutch or the mean volume of those eggs (Table 5.5). Although male song did not predict clutch size, there was a significant positive relationship between the number of genetic offspring surviving to day 12 post-hatching and two measures of the father's song, PC1 and PC3, when clutch size was controlled for (Fig. 5.2; Table 5.5). Together, song structure and clutch size predicted nearly 38% of the variance in the number of surviving chicks produced (Table 5.5). The number of surviving chicks varied significantly with clutch size alone ($F_{1,32}$ = 4.93, P = 0.034), but clutch size explained only 13.4% of the variance. There was a trend toward syllable number predicting the number of genetic offspring surviving to day 12, when clutch size was controlled for, but this fell short of statistical significance ($F_{1,31}$ = 3.8, P = 0.060, slope of regression equation = 0.21). Survival within the nest, however, did not vary significantly with male song (Table 5.5).

Table 5.5: Results of regressions of seven measures of male and female morphology, and reproductive success, on the principal components of male song structure. Note that R^2 values refer to the variance accounted for by all predictors, rather than to individual predictor variables. Significant *P* values are indicated in bold type.

Dependent variable	Predictor	*F*	d.f.	*P*	Slope	R^2 (%)
Male morphology	Song PC1	1.60	1,20	0.221	0.18	8.4
	Song PC2	<0.01	1,20	0.951	0.01	
	Song PC3	0.13	1,20	0.726	-0.08	
Female morphology	Song PC1	0.02	1,13	0.900	-0.02	8.9
	Song PC2	0.43	1,13	0.522	0.18	
	Song PC3	0.66	1,13	0.432	0.30	
Clutch size	Song PC1	0.04	1,38	0.849	-0.02	1.3
	Song PC2	0.05	1,38	0.820	-0.04	
	Song PC3	0.43	1,38	0.518	-0.13	
Mean egg volume (mm^3)	Song PC1	0.09	1,28	0.762	-2.30	4.4
	Song PC2	1.07	1,28	0.310	11.66	
	Song PC3	<0.01	1,28	0.976	-0.39	
Number of genetic offspring surviving	Song PC1	4.22	1,29	**0.049**	0.25	37.6
	Song PC2	0.47	1,29	0.498	-0.12	
	Song PC3	6.26	1,29	**0.018**	0.54	
	Clutch size	6.13	1,29	**0.019**	0.52	
Number of nestlings surviving in nest	Song PC1	2.64	1,29	0.115	0.29	23.4
	Song PC2	0.18	1,29	0.677	0.11	
	Song PC3	0.55	1,29	0.465	0.26	
	Nestling number (after cross-fostering)	4.10	1,29	0.052	0.77	

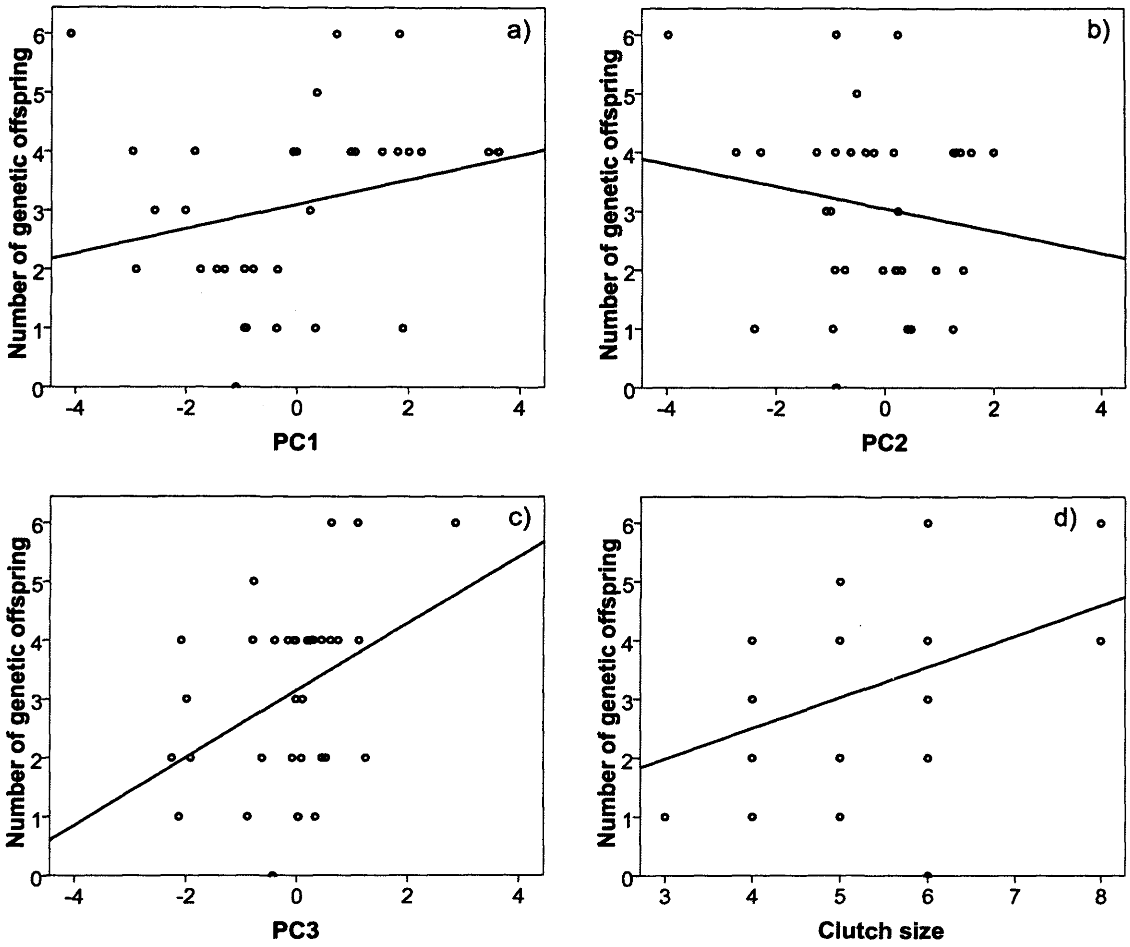

Figure 5.2: Regressions of the number of genetic offspring surviving to 12 days post-hatching on a) – c) three principal components of their father's song structure, and d) clutch size.

5.4 Discussion

This study is the first to describe the song structure of male zebra finches in the wild population at Fowlers Gap, New South Wales, and the first to test whether song structure predicts male reproductive success or morphology in any wild population of zebra finches. I found that two of the first three principal components of song structure predicted the number of offspring surviving to day 12 post-hatching, when initial clutch size was controlled for. Song structure and clutch size accounted for about 38% of the variance in offspring survival, considerably more than could be explained by variation in clutch size alone. This relationship between song and reproductive success adds support to the hypothesis that the structure of zebra finch song has been subject to sexual selection. A similar positive relationship between repertoire size and annual reproductive success has previously been demonstrated in great reed warblers (Catchpole, 1986), song sparrows (Hiebert et al., 1989; Reid et al., 2005), European starlings (Eens et al., 1991), and willow warblers (Gil and Slater, 2000).

A relationship between song and reproductive success might imply that males with more attractive songs provide direct or indirect benefits to the female (Andersson, 1994) or that females invest more in their offspring if their mate has an attractive song. Boogert et al. (2008) found that male zebra finches whose song consisted of a greater number of syllables learned a novel foraging task faster than those with less complex songs, implying that song complexity may act as a signal of a male's ability to find and exploit sources of food. Song repertoire or singing rate have been found to indicate male parental care in several species (red-winged blackbird, Yasukawa et al., 1980; stonechat, *Saxicola torquata*, Greig-Smith, 1982; sedge warbler, Buchanan and Catchpole, 2000; whitethroats, *Sylvia communis*, Halupka and Borowiec, 2006), although this may be confounded by an association between song and territory quality. The most obvious direct benefit provided by male songbirds from many species is territory quality (Andersson, 1994), but zebra finches are not territorial (except in the immediate vicinity of the nest, Zann, 1996), so obtaining good genes for their offspring may be of more importance than direct benefits for zebra finch females. During this study, the breeding population was subject to a partial cross-fostering manipulation and I found that there was a relationship between song structure and offspring survival across all offspring, regardless of whether they were raised in their natal nest or a foster nest. By contrast, song structure was not predictive of nestling survival within each nest (a mixture of genetic and foster offspring), suggesting that the correlation between song and nestling survival did not reflect direct benefits provided by the male or increased parental effort by his mate. My results most likely indicate either that song predicts genetic quality and that females obtain genetic benefits for their offspring by mating with males that produce attractive songs, or that females invest more resources in their eggs when mated with attractive males.

Females might manipulate clutch size, egg size or the concentration of hormones within the egg yolk. Egg volume is probably related to offspring survival in the zebra finch (Rutkowska and Cichon, 2005; Bolund et al., 2009), and Bolund et al. (2009), found that females laid larger eggs when paired with low quality rather than high quality males. I found no relationship between male song structure and either the number of eggs in a clutch or the mean egg volume, implying that females did not manipulate these traits in response to the attractiveness of their partner's song. Female zebra finches have been found to lay eggs with higher testosterone levels when the attractiveness of their mates was artificially enhanced using coloured leg bands (Gil et al., 1999), but no data were gathered on hormone levels within the eggs in this study. It would be interesting to see future studies address the possibility that females might manipulate hormone levels in their eggs in response to their partner's song structure. In the absence of any data regarding maternal effects on egg hormone levels, it appears perhaps more likely that the relationship between male song structure and nestling survival reflects paternal good genes effects than maternal investment in the eggs.

My analysis assumed that all nestlings originating in a male's nest were his genetic offspring, but it should be noted that no attempt was made to confirm paternity of the nestlings using DNA fingerprinting. Extra-pair paternity in wild zebra finches has been found to be as low as 1.7% (Birkhead et al., 1990), and Griffith et al. (2010) found that only 2.4% of nestlings in the Fowlers Gap colony were extra-pair offspring, suggesting that it is unlikely to be a significant source of bias in the present study. Having said that, there is evidence that conspecific brood parasitism may occur in 17.5% to 21% of broods in both wild and domesticated zebra finch populations (Griffith et al., 2010; Schielzeth and Bolund, 2010), and 5.4% of all nestlings studied at Fowlers Gap were unrelated to either 'parent' (Griffith et al., 2010). Birkhead et al. (1990) found that up to 11% of offspring in a different wild population were the product of conspecific brood parasitism. Brood parasitism could well constitute a source of noise in my data and future studies would benefit from establishing paternity via genetic methods.

Although the partial cross-fostering of nestlings between nests allowed me to partially distinguish whether male song structure predicted direct or indirect benefits for offspring survival, it should be noted that it introduced a number of potential confounds that could not be controlled for in my analysis. Brood sizes were manipulated which is likely to affect both the quality of parental care and sibling interactions. Foster parents are likely to vary in their parental care as well as in their song structure. Unfortunately, due to the essentially opportunistic way in which I obtained song recordings, I did not have data on both the father's and foster father's song for enough nestlings to able to address the relative contributions of each to offspring survival rates. Another factor that may have affected reproductive success in this study is the fact that the zebra finches were breeding in nest boxes. Griffith et al. (2008) found that nest predation in the population breeding in nest boxes at Fowlers Gap was significantly lower than reported in previous studies and that reproductive success was greater for pairs breeding in the nest boxes than those in natural nests. The effects of these various factors, unquantifiable in this study, mean that my estimates of the relationship between song structure and reproductive success may not be typical of wild zebra finch populations.

Previous studies investigating the effects of song on reproductive success have used date of pairing, the number of offspring that survive to breed themselves, or lifetime reproductive success as measures of male success. It was not possible to collect data on the date of pairing because the majority of birds in this study were only caught and ringed after breeding had begun (and thus could only then be identified). Zebra finches in wild populations have very high dispersal rates (e.g. Zann, 1996, reported that only 22% of adults in a colony were hatched there), making it impractical to collect data on post-fledging offspring survival or lifetime reproductive success. Previous studies on several species (great reed warbler, Catchpole, 1986; European starling, Eens et al., 1991) have found a relationship between song repertoire and annual reproductive success, comparable to my findings. However, two studies on the great tit that found no effect of repertoire on annual reproduction, found that repertoire size did predict lifetime reproductive success (McGregor et al., 1981; Lambrechts and Dhondt, 1986), perhaps because males with larger repertoires survived longer. Male song sparrows with larger song repertoires enjoy both greater annual and lifetime reproductive success (Hiebert et al., 1989; Reid et al., 2005). If there are comparable effects of song structure on survival or lifetime reproductive success in zebra finches, song structure may in fact predict even greater fitness benefits than those suggested by this study.

Song performance traits such as song rate have previously been shown to predict reproductive success in some species (e.g. barn swallow, *Hirundo rustica*, Møller et al., 1998). In this study, I found that male zebra finches only sang infrequent and short bouts of song at the nest boxes making it impossible to obtain recordings of sufficient volume of song to derive any meaningful measure of song rate. However, song rate is unlikely to be an important trait in determining female choice in the zebra finch since females' initial preferences in mate choice trials, made at a stage before singing rate could be reliably assessed, have been shown to predict their overall preferences (Collins, 1994; Rutstein et al., 2007). It seems likely then, that any information available to females regarding male quality will be signaled by song structure rather than performance, in this species.

A male's song structure did not predict the principal component used as a measure of his morphology, in this study. Several studies in other songbird species have found a relationship between song repertoire size and male condition (pied flycatcher, *Ficedula hypoleuca*, Lampe and Espmark, 1994; European starling, Mountjoy and Lemon, 1996; song sparrow, Pfaff et al., 2007) or male size *(nightingale, Luscinia megarhynchos*, Kipper et al., 2006). No such relationship between song and morphology was observed in several other species, despite song repertoire being predictive of reproductive success (great tit, McGregor et al., 1981; great reed warbler, Catchpole, 1986; great tit, Lambrechts and Dhondt, 1986; European starling, Eens et al., 1991). Working on domesticated zebra finches, Holveck and Riebel (2007) reported a relationship between certain measures of song performance and male mass and tarsus length, but these results were not replicated by Forstmeier et al. (2009). Nutritional stress and artificially raised corticosterone levels have been shown to have detrimental effects on song complexity, but not on adult mass (Spencer et al., 2003; Zann and Cash, 2008), providing further evidence that song structure is not a reliable signal of male morphology or condition in the zebra finch. I also found that neither a male's morphology nor his song structure predicted the morphology of his mate. Male preferences have been observed in zebra finches under laboratory conditions (Burley et al., 1982; Burley and

Coopersmith, 1987), including preferences for females that were heavier and in better condition (Wynn and Price, 1993), but it is not known whether assortative mating for morphology or other measures of quality occurs in the wild. My results suggest that this is not the case. However, I was able to obtain morphology data for just 17 females whose mate's song I had recorded, and I had both male and female morphology data for only 13 pairs. In addition, it is possible that a male's song structure might predict other aspects of his mate's quality that were not assessed in this study.

The nest boxes at the Gap Hills site belonged either to large colonies (of 20-24 boxes) or existed as single boxes or small groups, at a distance from other nest boxes. Small numbers of finches, breeding at a distance from the main colony have been reported in several natural populations (Zann, 1996), but Zann considered all pairs to form a single social group, despite the relative dispersal of their nests. Nest predation appears to be the major factor affecting nesting sociality in zebra finches (Zann, 1996), so pairs nesting in small groups or single boxes may be at more risk than those within the colonies. On the other hand, there may be benefits to nesting apart from the colonies such as reduced competition for food or reduced risk of brood parasitism. Immelmann reported that the first nests in a new breeding colony were built as far apart from each other as possible (see Zann, 1996), and there is some evidence that breeding pairs prefer to not to nest in a shared bush with others, defending their bush against intruders (Kikkawa, 1980), although Zann (1994) could not confirm this. I found no difference in song structure between males occupying the solitary or small group nest boxes and those nesting in the colonies, indicating that if there are any consistent differences in quality or behaviour between birds using the two types of nest box, they are not signaled in male song.

It should be noted that, unlike many previous studies to investigate the relationship between song and reproductive success in the field, I have not used a single measure of song complexity as a predictive variable. Rather, I used principal components analysis to describe the variation across a number of song structure parameters, capturing more of the variation between individual songs than could be achieved using any single parameter. Despite the widespread use of zebra finches as a model to investigate song and female choice, it remains unclear exactly what song traits are attractive to females or what is the relative importance of individual song traits in female choice (reviewed in Riebel, 2009). In light of this, my approach using PCs rather than individual traits might identify information signaled by song structure as a whole, regarding male quality, which is accessible to female zebra finches but not apparent in a single, reductive measure of song complexity. Having said that, it is important not to conflate these principal components of song structure with song complexity or what is attractive to females. In this study, none of the PCs were heavily weighted toward a single song parameter and, while individual variation in PCs 1 and 3 appears to explain some of the variation between males in reproductive success, it is unclear whether individuals with a greater number of surviving offspring have more complex or attractive songs. For this reason, I repeated the analysis on the number of genetic offspring surviving to day 12 post-hatching, using syllable number, a single measure of song complexity, as a predictor. Although falling short of statistical significance there was a trend toward males with a greater number of syllables in their song phrase having a greater number of surviving offspring, indicating that song complexity may indeed play a role in signaling male quality.

Overall, this study provides the first evidence that song structure in the zebra finch predicts reproductive success in a wild population. It is not clear what mediates this relationship but my results suggest that neither differences in parental care nor maternal investment in the eggs are responsible. The most likely explanation appears to be that song structure reflects variation in heritable quality and may signal that a male carries good genes for offspring viability.

Chapter 6: Discussion

6.1 Evidence for and against the developmental stress hypothesis

The developmental stress hypothesis (Nowicki et al., 1998a; Buchanan et al., 2003) proposed that bird song could act as an honest signal of male quality, due to detrimental effects of early stress on the development of song control regions in the brain. This suggestion was intuitively appealing and several early studies provided empirical support (e.g. Nowicki et al., 2002a; Buchanan et al., 2003; Spencer et al., 2003), but more than a decade on from its proposal the evidence appears decidedly mixed. Although there is evidence to suggest that developmental stress has deleterious effects on neural development, there is less agreement regarding whether it has the potential to affect male song and attractiveness (see Table 6.1).

6.1.1 Effects of developmental stress on the brain

With the exception of the birds studied in chapter 4 of this thesis, which I will argue did not experience deleterious levels of stress, all but one of the experiments that have investigated the effects of developmental stress on neural development, thus far, have found that HVC volume was reduced in male birds that experienced stress (Table 6.1). Only one of these studies (Nowicki et al., 2002a) found evidence for an effect of stress on other song control regions or the size of the telencephalon or entire brain. These studies (Nowicki et al., 2002a; Buchanan et al., 2004; Spencer et al., 2005a; MacDonald et al., 2006) provide fairly convincing evidence that developmental stress does have a detrimental effect on the neural development of male songbirds, as predicted by the developmental stress hypothesis, and also that stress selectively affects the HVC, perhaps the most important brain region for the learning and control of complex song.

Table 6.1: Studies that have investigated the effect of developmental stress on male neural development, song or attractiveness. Key: + denotes a positive effect of stress treatment; —, a negative effect of stress treatment; n/e, no effect of stress treatment detected.

Notes: [i]Repertoire of song types; [ii]Repertoire of syllable types; [iii]Total syllable number in phrase; [iv]Female preferences for song recordings in an operant choice test; [v]Female preferences for live males in choice chamber tests; [vi]Female responsiveness to male courtship in free flight aviary. [a]Brain nuclei measured when birds still juveniles; [b]Stressor applied to wild caught fledglings, not to nestlings; [c]Fledglings given different quality diet after nutritional independence, rather than as nestlings. [1]Non-significant trend toward reduced telencephalon volume; [2]Maximum frequency; [3]Song complexity (linear combination of 6 song parameters); [4]Phrase length; [5]Peak frequency; [6]Length of song bout; [7]Song rate; [8]Consistency of sound duration during song phrase; [9]Consistency of phrase length; [10]Reduced syntax learning, but no effect on proportion of syllables learned.

Stressor	Study species	HVC volume	Other song nuclei	Overall brain size	Song repertoire	Other aspects of song	Song learning	Male attractiveness	Reference
Reduced food volume	Swamp sparrow	—	—	—	n/e [i]		—		Nowicki et al. (2002a)
Reduced food volume	Song sparrow	— [a]	n/e	n/e [1]					MacDonald et al. (2006)
Seed mixed with husk / raised corticosterone	Zebra finch	—	n/e	n/e	— [ii, iii]	— [2, 4]		— [iv]	Spencer et al. (2003); Buchanan et al. (2004); Spencer et al. (2005b)
Seed mixed with husk	Zebra finch				n/e [ii]	— [3], + [4, 5]	n/e		Zann & Cash (2008)
Seed mixed with husk	Zebra finch				n/e [ii, iii]	n/e [4]	—		Brumm et al. (2009)
Seed mixed with husk	Zebra finch	n/e		n/e	+ [iii]	+ [5], n/e [2, 4]			Chapter 4, this thesis

Table 6.1, continued

Stressor	Study species	HVC volume	Other song nuclei	Overall brain size	Song repertoire	Other aspects of song	Song learning	Male attractiveness	Reference
Unpredictable food supply[b]	European starling				—[i]	—[6, 7]			Buchanan et al. (2003); Spencer et al. (2004)
Reduced food quality	Zebra finch							n/e[v]	Blount et al. (2003)
Reduced food quality[c]	Zebra finch							—[v]	Naguib & Nemitz (2007)
Blood parasite infection	Canary	—	n/e	n/e	—[ii]				Spencer et al. (2005a)
Brood size manipulation	Zebra finch	n/e	n/e	n/e	n/e[ii]	—[7], n/e[4]	n/e	n/e[v]	Gil et al. (2006); Naguib et al. (2008)
Brood size manipulation	Zebra finch					—[7]		—[v]	De Kogel & Prijs (1996)
Brood size manipulation	Zebra finch				n/e[ii, iii]	—[8, 9], n/e[4]	—[10]		Holveck et al. (2008)
Brood size manipulation	Zebra finch					+[7]			Tschirren et al. (2009)
Brood size manipulation	Blue tit					—[6]			Dreiss et al. (2006)
Natural variation in nestling growth rates	Zebra Finch				n/e[ii]	n/e[4, 7]		n/e[v, vi]	Bolund et al. (2010b)

6.1.2 Effects of developmental stress on song

Despite this apparent effect of stress on HVC development, it is not clear whether male song quality is affected by developmental stress, or in what way. Only one study has found that stress led to reduced syllable number in zebra finches, *Taeniopygia guttata* (Spencer et al., 2003), whereas several have found syllable number to be unaffected (Gil et al., 2006; Holveck et al., 2008; Zann and Cash, 2008; Brumm et al., 2009; see table 6.1). Reduced syllable or song repertoires have, however, been reported for two other species (European starling, *Sturnus vulgaris*, Spencer et al., 2004; canary, *Serinus canaria*, Spencer et al., 2005a). Interestingly, the majority of studies to address the predictions of the developmental stress hypothesis to date have done so using species with very small song repertoires, or repertoires of just one song phrase, such as zebra finches and song sparrows. It is possible that early condition might have more drastic effects on song complexity in species that have evolved larger repertoires (e.g. blue tit, *Parus caerulus*, Doutrelant et al., 2000; Dreiss et al., 2006). Detrimental effects of developmental stress have been observed on a number of other traits associated with male song, from differences in the rate at which males produce song, or the length of their song bouts, to differences in the frequencies produced or even in the consistency of the length of the song phrase (de Kogel and Prijs, 1996; Spencer et al., 2003, 2004; Dreiss et al., 2006; Holveck et al., 2008; Naguib et al., 2008; Zann and Cash, 2008; see Table 6.1). There is, however, little consensus between studies on exactly what attributes of song are affected by stress.

A major barrier to our understanding the effects of the early environment on male song is caused by the fact that no two studies have measured the same set of song traits (Table 6.1). For example, Holveck et al. (2008) found that male zebra finches reared in enlarged broods were less consistent in the duration of sound produced in a song phrase and that there was a non-significant trend toward phrase length being less consistent as well. To my knowledge Holveck et al. (2008) are the only authors to have measured these subtle variations in performance in the context of the developmental stress hypothesis, so there is no way of knowing whether singing consistency might have been affected by other manipulations of early condition. Equally, I am unaware of any studies testing whether females attend to subtle differences in singing consistency, so it is not clear whether any effect of stress on consistency could have biologically meaningful effects on male attractiveness. There is some evidence from other species, however, that singing consistency could act as a more reliable signal of male quality than repertoire size (great tit, Parus major, *Lambrechts and Dhondt*, 1986; chestnut-sided warbler, *Dendroica pensylvanica*, Byers, 2007). Byers (2007) found that consistency of the peak frequency reached in song, as well as temporal consistency, predicted male extra pair paternity, but the effects of early condition on this song parameter have yet to be investigated.

6.1.3 Effects of developmental stress on song learning

At first glance, those studies that have looked at the accuracy of song learning in stressed and control males appear also to report very mixed results, with some finding reduced learning accuracy in stressed males and others finding no effect of stress (Table 6.1). However, this discrepancy is probably explained, at least in part, by differences in the way learning accuracy has been measured. Three studies measured the proportion of syllables in a male's song that could be identified as having been copied from his tutor (Gil et al., 2006; Holveck et al., 2008; Brumm et al., 2009), and none found a difference between stressed and control males. On the other hand Nowicki et al. (2002a) found that individual syllables in the songs of stressed swamp sparrows were less similar in structure to the syllables in their tutors' songs from which they were learned than those of controls, while Holveck et al. (2008) found that stressed males were less accurate in copying the order in which syllables were sung, compared to controls. Sound Analysis Pro (SAP) software was specifically designed to quantify the accuracy of song learning in zebra finches (Tchernichovski et al., 2004), and calculates a single measure of similarity based on all three measures of learning described above: proportion of shared syllable types, fine scale similarities between syllables, and accuracy of syntax matching. Two studies using SAP disagreed about whether there was a significant difference between stressed and control males in this learning score (Zann and Cash, 2008; Brumm et al., 2009), but Brumm et al. (2009), who found a lower score in males reared under nutritional stress, found that the effect of stress on learning was mainly attributable to differences in syntax learning rather than the other two components of the similarity score. Overall it seems likely that developmental stress does impact negatively on males' syntax learning and potentially also on the accuracy with which individual syllables are copied from their tutor, but there is no evidence to suggest that stressed males copy fewer syllables from their tutor.

1.6.4 Effects of developmental stress on male attractiveness

It seems highly plausible that deleterious effects of developmental stress on the development of the HVC could limit the accuracy of syntax learning in male birds. Again, though, the fitness consequences of such an effect are unclear. In the absence of other stress effects on song structure or performance, it is difficult to see how females could detect differences between males in learning accuracy without knowledge of the tutor song. One of the key predictions of the developmental stress hypothesis is that males that coped better with stress would have greater reproductive success, but it seems unlikely that this could result from differences in female choice, if learning accuracy is the only aspect of male song that reflects developmental conditions. Spencer et al.

(2005b), found that female zebra finches preferred the songs of control males over stressed ones, but theirs was also one of the few studies to find an effect of stress on syllable repertoire size, a parameter known to affect female preference (Neubauer, 1999; Holveck and Riebel, 2007; Vyas et al., 2009; and see chapter 3). Several other studies have found that female zebra finches did not prefer males reared under control conditions to those reared in large broods or on low quality diets (Blount et al., 2003; Naguib et al., 2008). Those that did find a difference in female preference did not measure male song structure or learning (de Kogel and Prijs, 1996; Naguib and Nemitz, 2007), so it is unknown whether these preferences could have reflected differences in male song. Holveck and Riebel (2010), found that female zebra finches reared in large broods preferred the songs of males from large broods, while those from small broods preferred the songs of males from small broods. The stimulus songs from their experiment were recorded from male subjects that had previously been found to differ in singing consistency and syntax learning (Holveck et al., 2008), implying that females may well be able to perceive, and base their mating preferences on, these subtle differences in song.

1.6.5 Summary

The current state of the literature suggests that stressors experienced in early development can influence HVC development, which in turn might cause subtle differences in the learning and final structure of male song. It is still unclear whether developmental stress can lead to major differences in song structure, in repertoire size for example, as originally predicted by the developmental stress hypothesis, or whether these differences will lead to differences in female choice behaviour that could drive the evolution of complex songs, but I contend that it would be premature to conclude from the current state of the literature that no such process occurs. The current, confused nature of the evidence for and against the developmental stress hypothesis is unlikely to become clearer unless future studies address the differences in methodology that characterise previous studies, in at least three important areas: the nature and degree of experimental stressors, and the model species that are used.

6.2 Could methodological differences explain the lack of consensus?

6.2.1 Degree of stress

Perhaps the most commonly observed effect of sub-optimum developmental conditions on birds is reduced nestling growth rate, although stressed birds are often able to compensate for a poor start and reach similar adult size and mass to controls (Boag, 1987; de Kogel, 1997; Nowicki et al., 2002a; Spencer et al., 2003; Naguib et al., 2004; Arnold et al., 2007; Zann and Cash, 2008; Brumm et al., 2009). Although this distinctive pattern was found in the female zebra finches used as subjects in chapters 2 and 3 of this thesis, it was not apparent in males reared as part of the same experiment (see chapter 4). Since there is evidence that female zebra finches are more susceptible to developmental stress than males (de Kogel, 1997; Martins, 2004; Arnold et al., 2007), this finding is likely to indicate that the experimental manipulation was sufficient to induce deleterious effects on female development but not to affect males. Perhaps this explains why the control and experimental males in this experiment did not differ in HVC size or song complexity (see chapter 4), when similar stressors have previously been found to affect these traits (Spencer et al., 2003; Buchanan et al., 2004).

This raises the question of whether some of the differences between the results of other studies can be attributed to differences in the degree of stress experienced by the subjects. It is plausible that different amounts of stress could affect song in different ways, with mild stress having only small, subtle effects on song learning or production but more extreme levels of stress leading to larger scale differences in song structure, such as repertoire size. Because experimental protocols differ between studies, it is impossible to compare the results of different experiments without a reliable measure of the degree of stress that was experienced by the subjects. Although the majority of studies have reported that their stress treatment affected nestling mass, and sometimes also body size as measured by wing or tarsus length, only Zann and Cash (2008), and this thesis (chapters 2 and 4), have reported effect sizes. If future studies would report the size of any effects of stress on nestling growth, it would go a long way toward improving our understanding of the extent to which differences in the effects of developmental stress on neural development or song are the result of different levels of stress.

A more direct measurement of an individual's stress response might also prove useful. Circulating corticosterone levels are the most commonly used measure of stress in avian studies but Spencer et al. (2005a) found no effect of experimental malaria infection on corticosterone levels in canaries, even though the parasitised birds showed a reduced immune response and both reduced HVC volume and song complexity. Likewise, Pravosudov and Kitaysky (2006) found that the effects of nutritional restriction on basal corticosterone levels were transitory, which would limit the usefulness of corticosterone titres for comparisons between studies because it might not always be possible or practical to take blood samples at exactly the same stage during the treatment. It has been suggested that the production of heat shock proteins might present an alternative method of measuring stress levels (Merino et al., 1998).

6.2.2 Type of stressor

It is possible that not just the degree of stress, but also the type of stressor applied, might determine how song and brains develop. The effects of several different stressors on neural and song development have been investigated (Table 6.1), but the majority of studies have used either nutritional restriction or brood size manipulation as experimental stressors. Spencer et al. (2003; Buchanan et al., 2004) demonstrated that artificially raised corticosterone levels had similar effects to nutritional stress, suggesting a common mechanism through which developmental conditions could affect male song quality, but it is possible that different stressors might differ in their effects. Most of the evidence that developmental stress has a deleterious effect on HVC volumes or song repertoire sizes has come from nutritional stress studies (see Table 6.1). Although brood manipulation studies have found effects of brood enlargement on several, mostly performance related, aspects of male song (Table 6.1), the few to have looked at neural development or song complexity have found no effect (Gil et al., 2006; Holveck et al., 2008).

Brood size manipulations have been found to affect juvenile growth, condition, survival and immune response (de Kogel and Prijs, 1996; de Kogel, 1997; Naguib et al., 2004; Verhulst et al., 2006; Tschirren et al., 2009), and are generally supposed to induce nutritional stress in the nestlings because parents cannot fully compensate their levels of food provisioning when broods are enlarged (Stearns, 1992; Gil et al., 2006). However, provisioning is not the only aspect of nestling experience that might be affected by brood size manipulation. Nestling competition is greater in larger broods (Neuenschwander et al., 2003), and sensory experience, such as the amount of song to which nestlings are exposed, and the song templates they acquire for learning, is likely to be affected as well. These differences might affect the development of male sexual traits and future studies would benefit greatly from investigating whether such differences might account for some of the discrepancies in the results of previous studies of developmental stress.

6.2.3 Model species

Finally, species-specific differences in the response to stress must also be considered. By far the majority of studies, including this thesis, have used the zebra finch as a model species. Zebra finches make an appealing study species for a number of reasons, including the ease of keeping them in captivity, their short generation time and, not least, the sheer amount that is known about them from previous studies (Griffith and Buchanan, 2010). There are, however, several aspects of zebra finch biology that suggest they might be less than ideal as a model species for studying the effects of developmental stress on sexual selection. Male zebra finches are apparently more robust than females to the effects of stress, as mentioned above (de Kogel, 1997; Martins, 2004; Arnold et al., 2007), and there is even evidence that zebra finches manipulate their primary sex ratios to produce more males when food availability is low (Kilner, 1998). This implies that producing male biased broods can increase parents' fitness in unfavourable conditions, so any detrimental effects of stress on male attractiveness are likely to be outweighed by the more robust growth and lower mortality rates of male offspring. This in turn suggests that male zebra finches may be comparatively robust in the face of developmental stress, and thus not the best species in which to test the predictions of the developmental stress hypothesis.

Male zebra finches also produce extremely simple songs, with just one song phrase consisting of only 3-14 syllables (Zann, 1993). As originally proposed, the developmental stress hypothesis seeks to explain how complex song repertoires may have arisen (Nowicki et al., 1998a; Buchanan et al., 2003), but the relatively simple song structure of the zebra finch suggests that historically they have not been under strong selection for complex song. If song repertoires do function as honest signals of early condition, more drastic effects of developmental stress on song might be predicted in species with relatively more complex songs. Both canaries and European starlings subjected to early stress produced less complex song in adulthood (Buchanan et al., 2003; Spencer et al., 2005a). Early stress has been shown to affect HVC development in the song sparrow, *Melospiza melodia* (MacDonald et al., 2006), and song rate in the blue tit (Dreiss et al., 2006), two species with more complex songs, but no controlled experiments have yet looked at the effect of stress on song complexity in these or other repertoire producing species. Observational studies of wild birds have suggested that song repertoires are correlated with other traits that are reliable proxies for developmental conditions or immune function in great reed warblers, *Acrocephalus arundinaceus* (Nowicki et al., 2000), blue tits (Doutrelant et al., 2000), and song sparrows (MacDougall-Shackleton et al., 2009). If these results are borne out in controlled laboratory experiments, effects of early stress on song complexity might turn out to be more common than the current state of the literature suggests, and zebra finches may well end up being viewed as an anomaly.

6.3 Does developmental stress affect female choice?

One of the major aims of this thesis was to investigate whether early environmental conditions affect female choice behaviour. The developmental stress hypothesis suggests that complex male songs evolved as a signal of developmental condition through the action of female choice, but the same stressors that are predicted to regulate male attractiveness have the potential also to act on females. The HVC is thought to play an important role in female song perception (Brenowitz, 1991; Del Negro et al., 1998; Del Negro et al., 2000; Halle et al., 2002), so stress might affect females' ability to evaluate song at a mechanistic level. Another possibility is that poor quality females could suffer higher costs from choosiness than high quality females and so be less discriminating, or expend less effort in sampling potential mates (Fawcett and

Johnstone, 2003a; Cotton et al., 2006). Four studies have now addressed these questions explicitly (Riebel et al., 2009; Holveck and Riebel, 2010; Woodgate et al., 2010, chapter 2; Woodgate et al., in press, chapter 3).

6.3.1 Perception of male signals

Perhaps the most important finding to emerge from these four studies is that none have found results consistent with the hypothesis that female song perception is affected by stress. Riebel et al. (2009) found that there was no difference between female zebra finches from large or small broods in either the direction or strength of their preferences when choosing between their tutor's song and the song of an unfamiliar male. As in previous experiments (Riebel, 2000; Riebel et al., 2002), females were found to prefer songs to which they had been exposed during the sensitive phase for song learning, showing that even females from experimentally enlarged broods retained the ability to recognise their tutor's song. In this project, I did not test female preferences for familiar songs but, in chapter 3, I found that both females from control and nutritional stress treatments preferred more complex songs, demonstrating that females from the stress treatment were able to discriminate between songs on the basis of their structure. Finally, Holveck and Riebel (2010) found that females showed assortative preferences for males originating from similar broods sizes to themselves, confirming their ability to discriminate between songs, even though the only known structural differences between the two groups of stimulus songs were subtle differences in temporal consistency or the accuracy of syntax learning (Holveck and Riebel, 2010). These results do not prove that there was no detrimental effect of stress treatment on perceptual ability, but they do provide strong evidence that any effect of early condition on auditory perception is not large enough to prevent females making choices based on naturally occurring levels of variation between male songs. Note that, while the simplified songs I used as stimuli in chapter 2 were created by digital editing, rather than being naturally occurring songs, they were designed to mimic the levels of song complexity previously found in nutritionally stressed males (as in Spencer et al., 2003).

6.3.2 Direction of preference

Even in the absence of a direct effect of stress on song perception, developmental conditions could influence the strength or direction of female preferences. Holveck and Riebel (2010) reported a very striking effect of brood size manipulation on the direction of preference, with female zebra finches preferring the songs of males raised in similar sized broods to themselves. Assortative mating by phenotypic quality might be expected to occur if low quality females face greater costs from the search for a high quality mate or are likely be outperformed by higher quality females in competition for mates (Fawcett and Johnstone, 2003a; Hardling and Kokko, 2005), and Holveck and Riebel (2010) suggest this might explain their results. Once again it is possible that differences in the nature or severity of the experimental stressor might underly some of the different results reported by different experiments (see section 6.2, above). Holveck and Riebel (2010) tested the preferences of females from a brood size manipulation experiment for the songs of males from the same experiment. Riebel et al. (2009) also tested the preferences of females reared in a similar brood manipulation experiment but offered them the choice of songs from unmanipulated males, and found no such evidence for an effect of brood size on female preference, even though the stimulus songs differed in phrase length. This raises the question of whether the females tested by Holveck and Riebel (2010) were expressing preferences for males of similar quality to themselves or for song characteristics that convey information about brood size.

I found no effect of nutritional stress on the direction of female preferences when choosing between song recordings in operant tests (Woodgate et al., in press, see chapter 3). These stimulus songs had been edited to differ in syllable number but were otherwise identical. In the only experiment to date, testing the effects of developmental stress on female preferences for live, unmanipulated males (Woodgate et al., 2010, see chapter 2), I likewise found no evidence that stressed and control females differed in the direction of their preferences. The assortative preferences found by Holveck and Riebel (2010) were not apparent when females were offered a choice between the songs of familiar males, implying that learned preferences are strong enough to override any effect of developmental conditions on song preference.

6.3.3 Choosiness

Females are predicted to be less choosy when the costs of choice are greater, but more choosy when the benefits of choosing the best male are great (Cotton et al., 2006). Both stressed and control females, tested in chapter 3, expressed a preference for more complex songs and there was no difference between the groups in the strength of this preference. Females from small and large broods did not differ in preference strength, either when choosing between familiar or unfamiliar songs recorded from males originating in small or large broods (Holveck and Riebel, 2010), nor when choosing between their tutor's or an unfamiliar song (Riebel et al., 2009). However, when Riebel et al. (2009) offered females from experimentally enlarged broods a choice between two unfamiliar male songs they expressed significantly weaker preferences than those from smaller broods. In mate choice tests I found that females reared under conditions of nutritional stress were less active and made fewer sampling visits to the stimulus males than controls, indicating reduced investment in mate sampling and reduced choosiness (Woodgate et al., 2010, chapter 2).

The discrepancy between these results might be attributable to differences either in the costs of choice, or in the potential benefits of choosiness, between the studies. Choices for live males in the mate choice apparatus used in chapter 2 are likely to carry greater costs than in operant song preference tests, both in terms of the energetic requirements of moving between viewing arms and perhaps also the cognitive challenge of relocating the position of a favourite male. If this is the case, low quality females might benefit from reducing choosiness in the live male tests but, in song preference tests, could still expect to maximise their fitness through choosiness. Some evidence in favour of this interpretation comes from the fact that the difference in activity rates between stressed and control females that I observed in mate choice tests was not apparent in later, no-choice tests (Woodgate et al., 2010, chapter 2), although it should be noted that there are a number of potential alternative explanations (see section 2.4).

The benefits of choosiness may also have differed between studies. Stressed females appeared less choosy when offered a choice between unfamiliar, unmanipulated males (Riebel et al., 2009; Woodgate et al., 2010, chapter 2), perhaps indicating that the benefits of choosiness were relatively small, and did not outweigh the costs, for low quality females. When offered a choice between male songs that were known to differ in structure and might therefore signal differences in male quality, all females expressed strong preferences (Holveck and Riebel, 2010; Woodgate et al., in press, chapter 3), as did they when one of the stimulus songs was one for which they had a learned preference (Riebel et al., 2009). In these cases the perceived benefits of choice were perhaps great enough that even low quality females could enhance their fitness by expressing strong preferences.

6.2.4 Summary

To summarise, the only studies to date that have addressed the effects of developmental stress on female choice (Riebel et al., 2009; Holveck and Riebel, 2010; Woodgate et al., 2010, chapter 2; Woodgate et al., in press, chapter 3) suggest it is unlikely that developmental condition has any biologically relevant effect on females' ability to discriminate between male songs. Developmental stress may, however, cause context dependent differences in the strength or even the direction of song preferences. Even these relatively subtle effects have the potential to have profound consequences for mating decisions in natural populations. For example, differences in the way females sample potential mates could still affect the outcome of mate choice even where females do not differ in preference since females that sample few potential mates will show less bias in their mate choice than those that choose between many (Wagner, 1998).

6.4 Potential areas of interest for future research

I have argued above that future studies testing the predictions of the developmental stress hypothesis would benefit from considering and quantifying differences in both the nature and magnitude of the stressor they apply, and from considering a broader range of model species. In this section I will suggest several further areas of research that might prove to be of interest.

6.4.1 Does male song predict phenotypic quality?

In the research reported in chapter 4, I attempted to quantify the relative influences of genetic and environmental factors on male neural development and song structure, in order to determine whether these traits might signal phenotypic or genetic quality. When genotype-by-environment interactions are involved in determining phenotype, the magnitude of a male trait developed in the face of an environmental stressor might be used in mate choice as an indicator of genetic quality (Kokko and Heubel, 2008). I found that small but significant stress treatment by genotype interactions played a role in the expression of several song traits, indicating that females could obtain information about male genotypic quality by attending to these traits. Females choosing males with more complex songs might thus be choosing males with a resistant genotype to developmental stress (see chapter 4). The majority of the variance in song structure, however, and virtually all the variance in HVC volume, was attributable to environmental, rather than genetic, effects. In this study, nutritional stress did not have deleterious effects on neural development or song structure, contrary to predictions, perhaps because the stressor was insufficiently severe (see chapter 4). Nonetheless, if these relative contributions of genotype and environment to neural and song development are similar in individuals that have experienced greater levels of stress, then male song structure is likely to largely reflect the magnitude of stress an individual has experienced, rather than his genotypic quality. Thus females choosing males on the basis of song structure are likely to be selecting a male with a higher phenotypic, as opposed to genetic, quality.

Previous studies have shown that developmental stress has long term effects on various aspects of fitness including morphology, fecundity, quality of offspring, antioxidant defenses and potentially longevity (Birkhead et al., 1999; Metcalfe and Monaghan, 2001; Blount et al., 2003; Metcalfe and Monaghan, 2003; Arnold et al., 2007), so female choice for males on the basis of their developmental environment is likely to enhance fitness. However, none of the studies to date that have tested the effects of developmental stress on song or neural development have also investigated whether there is a direct link between male song and phenotypic quality. Future studies might test whether differences in song structure between stressed and control males also predict differences in male quality that females might benefit from obtaining information on. For example, Boogert et al. (2008) found that the song complexity of male zebra finches was correlated with their learning ability in a novel foraging task, and cognitive ability, particularly foraging ability, is likely to play a large role in both individual survival (Sol et al., 2007), and parenting ability. It is not currently known, however, if this apparent relationship between song and learning ability reflects differences in developmental stress, nor whether similar relationships exist in other songbird species.

6.4.2 What are the effects of developmental stress in wild populations?

Although a link between repertoire size and various proxies for developmental stress has been reported by field studies on several songbird species (Doutrelant et al., 2000; Nowicki et al., 2000; MacDougall-Shackleton et al., 2009), the majority of studies testing the developmental stress hypothesis have been performed under laboratory conditions. One interesting aspect of laboratory studies of developmental stress is that control birds are usually raised under conditions that may be unusually benign, with *ad libitum* food supply, no predation risk, predictable temperature and predictable photoperiod. Indeed, the predictability and mildness of the laboratory environment might mean that even birds subjected to experimental stressors experience less stress than their wild counterparts. If this is the case, most studies might be comparing two groups of comparatively high quality males, in which case one might expect to see greater effects of stress in wild birds, reared in less favourable and predictable environments. It will be interesting to see whether future studies find effects of stress on song and brain development, and on mating or reproductive success, in wild populations. In chapter 5, I showed for the first time in the zebra finch, that song structure may predict male reproductive success. Song repertoire size has previously been found to predict reproductive success in several other species (McGregor et al., 1981; Catchpole, 1986; Lambrechts and Dhondt, 1986; Hiebert et al., 1989; Eens et al., 1991; Hasselquist et al., 1996; Gil and Slater, 2000; Reid et al., 2005). Future studies may wish to address whether these differences in reproductive success reflect differences in males' ability to withstand developmental stress.

6.4.3 Does developmental stress affect neural development in females?

As discussed above (section 6.3), the ability of females to perceive male songs is probably robust to the effects of developmental stress, but there is some evidence that females originating from different developmental backgrounds might differ in choosiness or even in which males they prefer. Future studies might investigate the mechanism underlying these differences and, in particular, whether they result from the effects of stress on neural development. There is mounting evidence that developmental stress has deleterious effects on neural development in male songbirds (Nowicki et al., 2002a; Buchanan et al., 2004; Spencer et al., 2005a), and MacDonald et al. (2006) found that the development of the HVC in juvenile female song sparrows was impaired by nutritional stress, but no studies have yet looked at the effects of stress on the brain of adult females.

6.5 Conclusions

The developmental stress hypothesis has raised many questions since its original suggestion, as a mechanistic solution to explain the evolution of song complexity as a handicap. The research reported in this thesis has addressed several of these questions and shed light on several related areas of interest: the effects of stress on female mate choice behavior; what information may be signaled by song, regarding a male's genetic and phenotypic quality; and whether male song structure predicts reproductive success in the zebra finch, the most widely used model species for research into the effects of developmental stress. My results suggest that early stress might have long lasting context dependent effects on the way females sample potential mates (chapter 2), but that it apparently does not reduce female preference for complex song and is unlikely to affect females' ability to perceive male sexual signals (chapter 3). Male song structure in the zebra finch is largely determined by environmental factors so probably functions largely as a signal of phenotypic quality, but there may be potential for syllable number to signal information about genetic quality, mediated by developmental stress (chapter 4). I have also found the first evidence that song structure may predict male reproductive success in a wild population of zebra finches (chapter 5). Many questions still remain, regarding the long term effects of developmental stress on sexual selection. In particular, female preferences remain poorly understood. The need remains to develop a clearer understanding of the importance of early conditions for the formation and expression of female preferences, and the evolution of secondary sexual traits.

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