PARENTAL INVESTMENT AND SONG LEARNING IN ZEBRA FINCHES (TAENIOPYGIA GUTTATA)

by

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Abstract

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In order to understand the effects of parental investment on learning, we conducted a series of experiments using zebra finches. The aspects of parental investment studied included deposition of maternally-derived hormones (MDH) into eggs, feeding and attention to chicks. Learning was assessed by song copying (mean accuracy, sequential matching and percent similarity to the father's song). We compared digit ratios, a marker for the amount of MDH chicks experienced in eggs, and song copying ability. Our data suggests that maternally-derived testosterone negatively affected the ability to sequentially match notes. To determine if parents were preferentially feeding chicks by hatch order, we weighed chicks at key developmental points prior to fledging. We found that chicks that hatched early were heavier than those who hatched late. Additionally, weight at day 10 was positively correlated with song learning. Acoustic cues are one obvious way that parents might differentiate chicks by hatch order. Therefore, we assessed the begging calls of 10 day old chicks. We found that early-hatched chicks begged at lower amplitudes than late-hatched chicks. We then conducted a playback experiment in which begging calls of 10 day old early- and late-hatched chicks were

presented to breeding adults. Adults were more attentive during the early-hatched chicks' playbacks. To determine if attention, in the form of clumping or perching closely together, affected song learning, we observed family groups when chicks were beginning song acquisition (day 25). We found that while clumped with their mates, mothers clumped with first-hatched more than second- or third- hatched chicks. Moreover, clumping behavior was positively correlated with the percent similarity of song copying. Clumping with first-hatched sons may be a way for mothers to give additional access to the father and thereby enhance son's song learning. Finally, we used a multiple linear regression, combining all three forms of parental investment to determine which were more important in song learning. We found that digit ratio 2:3 was positively correlated with sequential matching, nestling weight positively correlated with mean accuracy and clumping with the mother positively correlated with the percent similarity score.

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Thank you all for your support. I am eternally grateful. I will end with a few phrases from the sound that I feel has been playing as the soundtrack for this part of my life:

The years start coming and they don’t stop coming

Fed to the rules and hit the ground running

Didn’t make sense not to live for fun

Your brain gets smarter but your head gets dumb

So much to do

So much to see

So what’s wrong with taking the back streets?

You’ll never know if you don’t go

You’ll never shine if you don’t glow

All that glitters is Gold

-Smashmouth

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Chapter 1

Introduction to bird song learning

Introduction

It is a commonly held belief that the more parents invest in their young, the more successful the young will be as adults. However, the effects of parental investment on learning in humans have been the topic of much debate in recent years (Pinker, 2002; Harris, 1998; Levitt & Dubner, 2005). The findings are difficult to interpret in that what constitutes "success" in humans is also a matter of debate (Levitt & Dubner, 2005). Additionally, the long life span makes for long and expensive studies and participants often drop out before the end. Also, as in any human study, some variables are impossible to control. Zebra finches, *Taeniopygia guttata*, provide an opportunity to study the effects of parental investment on the success of young in a relatively short time while controlling many of the variables that might otherwise confound the findings. Here we chose to look at three possible mechanisms of parental investment-hormones, food and attention and one trait that potentially leads to reproductive success-song learning. This is not to say that there are not other forms of parental investment or other traits that lead to reproductive success.

Zebra finches are also an ideal animal model because in this species the brain areas involved in learning are clearly defined and discreet. In addition, the effects of hormones, nutrition and behavior on the development of these brain areas and the subsequent effects on learning have been the subject of numerous studies. For zebra finches, song learning is sexually dimorphic, restricted to a critical period and influenced by both hormones and environmental factors. Only male zebra finches have the ability to sing as females lack the necessary brain structures (Nottebohm & Arnold, 1976; McDougal-Shackleton & Ball, 1999). Song learning and ultimately song quality are essential to the fitness of males as song is one of the factors that influence female mate choice (Catchpole & Slater, 1995; Searcy & Yasukawa, 1996, Williams et al 1993; Tchernichovski & Nottebohm, 1998; Tomaszycki & Adkins-Regan, 2006; Holveck & Riebel, 2007). Typically, males learn the song of their father but may learn part or all of the song of another male in the colony (Immelmann 1969; Williams, 1990).

We followed Sossinka and Bohner's (1980) and Price's (1979) descriptions of typical zebra finch songs. A song motif begins with one to several introductory notes followed by a series of distinct syllables. The sequence of syllables within a motif is relatively rigid. Typically the motif is repeated about 5 times to create a song bout. The song bout can be made longer by having more syllables within a motif or more repeats of the motif within the song bout. Males who are poor learners tend to improvise notes and have shorter songs (Tchernichovski & Nottebohm, 1998; Holveck & Riebel, 2007). Females prefer to mate with males with long songs that have few intermotif silences or improvised notes (Williams, 1990; Holveck & Riebel, 2007). Females may use song as a marker for male quality because song reflects the quality of the post-hatchling environment as well as the parenting skills of the potential mate (Nowicki et al., 1998).

Factors that have been indicated to affect song quality include the allocation of maternally-derived hormones (MDH) to the yolk (Forstmeier et al 2004), nutrition (Nowicki et al., 1998; Spencer et al., 2003; Buchanan et al. 2004; Fisher et al. 2006) and attention from the tutor (Williams 1990). These three factors may be considered aspects of parental investment. This study investigates effects of variations in parental investment on song learning in zebra finches. The long range goal is to understand parental/offspring relationship effects on learning. The central hypothesis of this thesis is that increased parental investment in the form of maternally-derived hormones, increased nutrition from both parents and attention from the father leads to more precise song copying in zebra finches. Specifically, I address the following hypotheses: 1). digit ratio, a marker for prenatal exposure to hormones, correlates with song learning differences within a clutch, 2). parents differentially feed chicks by hatch order which leads to differences in song learning, 3). attention by the father in the form of clumping behavior correlates with song learning by sons.

Maternal Effects

According to the adaptive maternal effect hypothesis, mothers can change the physiology of offspring to be more adaptive to the environment that she is experiencing during the time of egg formation (Mousseau & Fox, 1998). For birds, this is accomplished through the deposition of MDH into the yolks of developing eggs. These hormones reflect the mother's circulating hormone levels and are influenced by the physiological state of the mother at or just prior to ovulation (Schwabl, 1996a; Gil et al., 1999; Gil et al., 2004; Groothius & Schwabl, 2002; Reeed & Vleck, 2001; Whittingham & Schwabl, 2002; Tschirren et al., 2004). MDH can have both short- and long-term effects on phenotype in several ways. In the short term, MDH can alter hatching time (Sockman & Schwabl, 2000), development of the hatching and begging muscles (Lipar & Ketterson, 2000; Lipar, 2001) increase begging behavior (Schwabl, 1993; Eising & Groothius, 2003; von Engelhardt et al., 2006), and alter nestling morbidity (Groothius et al., 2005; Müller et al., 2005; Navara et al., 2006; Rutkowska et al., 2007) and mortality rates (Sockman & Schwabl, 2000; Groothius et al, 2005). Suggested long-term consequences include both organizational and activational effects. For example, yolk hormones might change the responsiveness of hormone receptors in the brain that later affect adult birds (Partecke & Schwabl, 2008). Exposure to elevated testosterone in the egg might also cause a change in the production of testosterone later in life by modifying the hypothalamus-pituitary-gonadal axis (Daisley et al., 2005). Finally, differences in development, as a result of variation in yolk MDH levels, might alter the reproductive status of adult birds (Schwabl, 1996a).

The source of MDH is debatable but is most likely provided by the ovaries, specifically, the theca interna and the granulose cells (Porter et al., 1989; Gomaz et al., 1998; Kato et al., 1995; see Groothius et al., 2005 for a review). For many asynchronous layers, the deposition of MDH varies with the position of developing eggs within the fallopian tube and/or the timing of egg laying. For asynchronous layers, eggs are laid at a rate of one per day or one every other day. Therefore, maternal condition may change enough over the laying period so that last-laid eggs receive more (or less) of a particular hormone (Lipar et al. 1999; Sockman & Schwabl, 2000; Royle et al. 2001; French et al. 2001; Eising et al. 2001; Gil et al, 1999). It has been reported that zebra finches, who are both asynchronous layers and hatchers, show a pattern of decreasing testosterone across a clutch (Gil et al., 1999; Williams et al, 2005 but see Ward et al., 2001; Gilbert et al., 2005). The adaptive significance of such a trend may be that late-hatched chicks incur some advantage that allows them to better compete with their older siblings. For example, low levels of MDH are correlated with increased immunocompetence and growth rates and decreased oxidative stress and chick mortality (Folstad & Karter, 1992; von Schantz et al., 1999; DaSilva, 1999; Sockman & Schwabl, 2000; Saino et al., 2006a). For zebra finches, who typically lay a clutch of 4-5 eggs at a rate of one egg every day or every other day, the difference in age between first- and last-hatched siblings could be as much as 10 days. The differences in both size and development put late-hatched chicks at a clear disadvantage. Tchernichovski & Nottebohm (1998), however, found that as adults, last-hatched chicks produced the best copies of their fathers' songs and induced ovulation faster in mates than their early-hatched siblings. They suggested that this is the result of receiving less maternally-derived testosterone but could also result from differential allocation of attention by the father.

Unfortunately, in order to measure the hormone levels in egg yolks, one must remove the entire yolk. This leaves the egg inviable and therefore, most studies of the organizational/activational effects of maternally-derived hormones have been correlational (Burley & Foster, 2004; Forstmeier, 2005). One way to assess the effects of prenatal hormones on song learning is to examine the digit ratio of adult birds. In humans and other vertebrates, the ratio of digits 2 and 4 reflects the prenatal hormonal environment (vom Saal, 1989; Clark & Galef, 1998; Williams, et al., 2000; Manning, 2002). Digit 2 lengthens in response to estrogen and digit 4 lengthens in response to androgen, specifically, testosterone. Recent studies in birds, including zebra finches, have been promising but show much variability. One study found that digit ratio decreased with hatch order (Burley & Foster, 2004), while another found no hatch order effects (Forstmeier, 2005). Both studies, however, showed that digit ratio 2:4 correlated with adult behavior. We measured the digit ratios 2:3, 2:4 and 3:4 of adult zebra finches in order to examine the possibility of maternally-derived hormones affecting song learning. In comparison to both Burley's and Forstmeier's studies, we included in our analysis the independent variables clutch size and the number of male siblings a bird had. These variables have been shown to co-vary with maternally-derived hormones and to affect song learning (Tchernichovski et al., 1999; Gilbert et al., 2005).

Feeding by Both Parents

Nutrition early in development can also alter song learning in zebra finches. Poor nutrition and stress during the early nestling period has been shown to compromise song learning ability in several species (Nowicki et al., 1998; Spencer et al., 2003; Buchanan et al. 2004; Fisher et al. 2006). In some avian species, females reject males who have been nutritionally stressed during the nestling phase (Spencer et al., 2003; Nowicki et al., 2002). Poor nutrition may result from poor parenting but may also reflect differential feeding by parents. In many asynchronous hatchers, parents adopt strategies for increasing their inclusive fitness such as altering the sex ratio of clutches and/or provisioning resources to chicks based on hatching order. To determine if zebra finch chicks in our colony were being fed differentially by hatch order, we weighed chicks at 5 day intervals between hatch and independence (day 35). We also weighed chicks at fledge and at sexual maturity (day 90). We then assessed the relationship between song copying ability with weight at these key developmental stages.

If parents are preferentially feeding chicks by hatch order, one obvious cue for distinguishing chicks is their begging calls. To determine if there were discernible differences in the begging calls of chicks, we recorded the begging calls of 47 chicks when they were 10-11 days old. We classified these chicks as either early-hatched (EH) or late-hatched (LH) by where they were in the hatching order. EH chicks were those that hatched first or second. LH were those that hatched third, fourth or fifth. We then analyzed the calls to determine if there were fundamental differences in the calls as the result of hatch order. To test our hypothesis that adult zebra finches use begging calls to distinguish chicks by hatch order, we played recordings of EH and LH chicks to adults with active nests (breeders) and to those that had never bred nor raised chicks (naïves) and quantified their behavior.

Attention from the Father

Male chicks typically learn to imitate the song of their father or a foster male who has fed them (Williams, 1990). The filial bond is key to tutor choice (Roper & Zann, 2006). Additionally, fledglings prefer to copy the song of a paired male over that of an unpaired male (Williams, 1990, Roper & Zann, 2006). Song Elements from other males in close proximately to the nest may be added to the fledgling's repertoire as well. In our experiment, chicks were housed with their parents until independence (day 35). Although they could hear other birds in the room they had limited visual contact. When chicks reached independence, they were moved to an adjacent cage where they could see and hear parents but had limited physical contact. With this method, we expected chicks to copy their father's song with few elements from other males in the room. We expected there to be variation in song copying ability within a clutch and predicted that the chick who had spent the most time in close proximately to the father (clumped) would have the best copy of the father's song. We therefore observed clumping behavior at the start of song acquisition (days 25-30) between chicks and their parents. We then assessed the relationship between song quality and previous time spent clumping with one or both parents.

Chapter 2

Digit Ratio and Song Learning in Zebra Finches

Maternally-derived hormones (MDH) in yolk are often allocated unequally among chicks in a clutch. In some asynchronous layers, the deposition of these hormones varies according to laying order (Lipar et al. 1999, Sockman & Schwabl, 2000, Royle et al. 2001, French et al. 2001, Eising et al. 2001, Gil et al, 1999). Researchers have argued that the differences in hormone levels may enhance competition among siblings and alter survival rates of chicks by hatch order and/or sex (Rutkowska & Cichon, 2006; Badyaev et al., 2002; Krebs et al., 2002; Müller et al., 2002; Cordero et al., 2000). To date, MDH have been shown to affect begging rates (Godsave et al., 2002; Buchanan et al., 2007), growth rates (Tobler et al., 2007; Buchanan et al., 2007; Saino et al., 2006a), metabolic rates (Tobler et al., 2007), potential for song learning (Ward et al., 2001), song rates (Forstmeier et al., 2004), mate preference (Burley & Foster, 2004; Forstmeier et al., 2004), future reproductive success (von Engelhardt, 2006; Groothius et al., 2005; Romano et al., 2005) and mortality rates (Sockman & Schwabl, 2000; Navara et al., 2005). Accurate quantification of MDH requires removal of the entire yolk as hormones are distributed unevenly. Thus, data on the effects of MDH and the above- mentioned traits are correlational or based on results from treatment with exogenously-administered hormones.

Digit ratio, the relative length of one digit to another, may provide an alternative to measuring MDH directly. Digit ratio is widely accepted as a marker of prenatal hormone exposure in humans (Manning, 2002) and other mammals (Brown et al, 2002; Leoni et al., 2005; Roney et al., 2004). The genes responsible for growth of digits and gonads (HOX) are highly conserved in vertebrates. Digit 2 lengthens in response to increased estrogen levels and digit 4 lengthens in response to increased androgen levels early in development (Manning, 2002). The effect of hormone exposure on digit 3 is unknown but the hair pattern on the dorsal part of the finger suggests that androgens promote the growth of this digit as well (Manning, 2002).

A low 2:4 digit ratio, considered a masculinized ratio, is the result of decreased estrogen, increased androgen, or a combination of the two (Manning, 2002). Numerous studies have linked low 2:4 digit ratios with increased levels of male-typical behaviors and conditions in humans. For example, low 2:4 digit ratios in men correlate with high levels of aggression, heterosexuality, sports ability, autism, and reduced verbal fluency (Manning, 2001, 2002; Williams et al., 2000; Lippa 2003, Bailey et al., 2005). The number of older, male siblings a man has is also negatively correlated with 2:4 digit ratio while the number of older, female siblings is positively correlated with this ratio (Saino et al., 2006b). Similarly, females with a male twin have a masculinized 2:4 digit ratio (van Anders et al., 2006). Low digit ratios 2:3 and 3:4 have been associated with attention deficit/hyperactivity disorder, also considered a male-typical condition, in both men and women (Stevens et al., 2007; McFadden, 2002).

Hind limb digit ratios do not show such dramatic results, but nonetheless sexual dimorphisms have been demonstrated in both humans (Manning, 2002) and mice (Brown et al., 2002). Pheasant hind limb digit ratios were affected by the addition of both exogenous testosterone (Romano et al., 2005) and estradiol to yolk (Saino et al., 2006c). Recently, two studies examined the relationship between digit ratio 2:4 and hatch order in zebra finches, however, they produced contradictory results (Burley & Foster, 2004; Forstmeier, 2005). This has lead some to question the validity of digit ratio as a marker for prenatal exposure to hormones in birds (Forstmeier, 2005; Garamszegi et al., 2007). In this study we used a different technique for digit measurement and included in the analysis some variables that have been shown to influence MDH deposition in an attempt to validate digit ratio as a marker for prenatal exposure to hormones.

Testosterone levels in zebra finch egg yolks typically decreased across a clutch (Gil et al., 1999; Rustein et al. 2005 but see Ward et al, 2001 and Gilbert et al., 2005). Lower testosterone levels may leave late-hatched chicks at a disadvantage as testosterone has been correlated with shortened embryo development, vigorous begging rates and higher social status as adults (Eising et al., 2001; Lipar & Ketterson, 2000; Schwabl, 1993, 1996a). Nonetheless, late-hatched chicks produced the best songs within a clutch and induced ovulation faster than those with less complete song copies (Tchernichovski & Nottebohm, 1998). Additionally, chicks with a high digit ratio 2:4 sing at higher rates (Forstmeier, 2005). Females prefer males with high song rates which suggests that chicks exposed to the least maternally-derived testosterone produce the most attractive songs. We examined the relationship of three digit ratios (2:3, 2:4 and 3:4) with song learning while considering the influence of hatch order and two other variables (clutch size and number of male siblings), that covary with MDH (Williams, 2001; Rutkowska et al., 2005) and also have been suggested to influence song learning (Tchernbichovski & Nottebohm, 1998; Gilbert et al., 2005)

Methods

Animals

19 pairs of zebra finches were housed at Hunter College, City University of New York. Each breeding pair occupied a separate cage but could see and hear other zebra

finches. Stainless-steel breeding cages measured 56 X 56 X 56cm with a 13 X 12 X 12.5cm nest box centered high on the rear wall. The nest box had a 6 X 13cm opening on the side facing the cage and a clear Plexiglas top. Adults were fed a vitamin-supplemented commercial finch seed mix, grit, water and cuttlebone *ad libitum*. The diet was supplemented with fresh greens, oranges, and hard-boiled eggs. The animal rooms were maintained at 24° ± 2°C with over 50% relative humidity and a 14:10 light: dark cycle for optimal breeding.

Nests were checked daily, and eggs were marked to indicate laying order. Chicks were marked with acrylic paint on the day they hatched to indicate hatching order. Banding replaced the acrylic paint at day 9 post hatch. Clutch size varied between 2 and 6. Typically a clutch had one hatch per day, but occasionally two chicks would hatch on the same day. These clutches were removed from analysis. Additionally, when chicks' hatching order did not match their laying order, the entire clutch was removed from analysis. Chicks remained with their parents in the home cage until they reached 35 days of age. 35-day-old chicks were then sexed by examining plumage and placed in an adjacent cage where they could see and hear their parents but had limited physical contact. At sexual maturity (day 90), birds were removed from the breeding room. Females were placed in an all female aviary. Males were moved to the recording room.

Digit Ratio

We photographed the right foot of chicks when they were between 90-110 days old. Studies in humans have shown that the right foot is affected more by prenatal hormones than the left (Manning, 2002). Although no left/right differences have been found in zebra finches (Forstmeier 2005), we chose to adhere to this protocol. The length of digits 2, 3 and 4 were calculated with SigmaScan Pro 4 (SPSS, Inc., Chicago, Illinois). This program converts pixels to measurements and eliminates errors caused by differences in the distance from each bird to the camera. This method was less stressful for the birds than alternative techniques and proved to be an accurate and relatively easy method for measuring toes. Each photo was calibrated and measured twice (3 months apart). Due to injury and/or malformations of toes, sample sizes were not equal. For digits 2 and 4 the sample size was 121 (65 males and 56 females). For digit 3 the sample size was 106 (56 males and 50 females).

Songs

After digit measurements were taken, males were housed in individual sound-attenuated chambers with a model of a female zebra finch. Food and water were provided *ad libitum*. A microphone (Earthworks SRO, Milford, New Hampshire) was mounted on the ceiling, in the center of the chamber. Fathers were recorded in the same way, when they were between clutches. Songs were recorded and analyzed with Sound Analysis Pro (http://ofer.sci.ccny.cuny.edu). Sound Analysis compares two songs by measuring the sequential match, % similarity, mean accuracy as well as calculating an overall match. Briefly, sequential match is a measure of the temporal timing of the notes in a song. The % similarity is the percentage of the adult's sounds included in the young male's song. Mean accuracy is the average local similarity score across the songs. The overall score is the product of these three measurements (see Figure 1 for examples).

Results:

We used Snedecor's formula for coefficient of variation to examine variation between the two measurements of toes. The variation was under 2%. We used a stepwise multiple linear regression to test if digit ratio was influenced by sex, hatch order, clutch size and/or the number of male siblings. Because we were running 3 regresssions on the same independent variables, and the dependent variables were correlated, we used a Sidak procedure to lower the α-level to 0.017, thereby decreasing chances of a type I error (Uitenbroak, 1977; Saino et al., 2006c).

We found no differences between males and females for any of the digit ratios (all $p>0.2$), and therefore this variable was removed from further analysis. For digit ratio 2:3, hatch order (adjusted $r^2=0.023$, $p=0.099$) and number of male siblings (adjusted $r^2<0.001$, $p=0.95$) were also removed in a stepwise manner. With the removal of these variables, the resulting model was significant ($r^2 = 0.055$, $p = 0.010$). Clutch size was the only variable that significantly correlated with digit ratio 2:3 ($r^2=0.065$, $p=0.0097$, Figure 2).

Digit ratio 2:4 increased with hatch order ($r^2=0.052$, $p=0.011$, Fig 3). Clutch size and number of male siblings were not significant contributors to the results and were removed in a stepwise manner (clutch size $r^2<0.001$, $p=0.83$; number of male siblings $r^2= 0.03$, $p=0.07$). The resulting model was significant ($r^2= 0.044$, $p=0.011$).

There was a positive correlation between digit ratio 3:4 and the number of male siblings ($r^2=0.054$, $p=0.016$, Figure 4). Hatch order and clutch size did not significantly contribute to the model and were removed (hatch $r^2=0.00$, $p=0.43$, clutch size $r^2=0.014$, $p=0.24$). The resulting model was significant after the removal of these variables ($r^2=0.047$, $p=0.016$).

We then looked at the length of individual digits to understand the relationship between the ratios and the independent variables. Sex, hatch order and number of male siblings a chick had were not significantly correlated with any of the digit lengths ($p>0.07$ for all). Clutch size, however, was negatively correlated with the length of all three digits (digit 2: r^2=0.076, p<0.001, digit 3: r^2=0.13, p<0.001, digit 4: r^2=0.098, p<0.001).

We used multiple linear regressions to assess the relationship between digit ratio and song learning with the covariates hatch order, number of male siblings and clutch size. The α-level was lowered to 0.017 using a Sidak adjustment. Of the 56 males who had measurements for all three digits, 32 were recorded. Digit ratios 2:4 and 3:4 were not correlated with any of the parameters of song quality we measured. Sequential match, however, was positively correlated with digit ratio 2:3 (Table 1). We then looked at the individual digits and their relationships to sequential match. For this analysis we did not need to use a Sidak adjustment because only one variable was measured. Digit 3 increased in size as sequential match score decreased (r^2=0.17, p=0.0183). Digit 2 and 4 lengths were not significantly correlated with the sequential match score (p>0.4 for both). With these variables removed, the resulting model was significant ($F_{1,31}$=15.57, r^2=0.209, p<0.001.

Discussion:

The zebra finch population in this study showed no sexual dimorphisms in digit ratios. However, we did find a relationship between clutch size and digit ratio 2:3, hatch order and digit ratio 2:4 and the number of male siblings a chick had and digit ratio 3:4. Of the two studies of digit ratio in zebra finches published so far, a sexual dimorphism in digit ratio was found in one (Burley & Foster, 2004) but not the other (Forstmeier, 2006). Ring-necked pheasant data also showed inconsistency in sexual dimorphism of digit ratio (Saino et al., 2006c; Romano et al., 2005). Differences in housing conditions and the resulting differences in stress have been suggested as a reason for the contradictory findings between laboratories (Forstmeier, 2005; Romano et al., 2005). This is possible since females may produce more testosterone in response to stress (Hasselquist & Kempenaers, 2002; Rutkowska & Cichon, 2002; Müller et al., 2002; Rustein et al.,. 2005; Gil et al., 1999, 2004; Whittingham & Schwabl, 2002) and circulating levels of maternal testosterone are known to be correlated with the levels in yolk (Schwabl, 1996b). Genetic differences in both finch and pheasant populations have also been suggested as a possible reason for contradictory results between studies of digit ratios (Forstmeier, 2005; Saino et al., 2006c). This is consistent with findings in human and mouse studies in which ethnic (human) and strain (mouse) differences in digit ratios are greater than sexual differences (Bailey et al., 2005; McFadden et al., 2005). Unlike mice, zebra finches tend to be random bred. In fact, depending on the season, finches obtained from a local vendor may be purchased from breeders from different regions within the United States. While we agree that genetic and housing factors are likely to cause variation between studies, we suggest other factors that are known to affect MDH should also be considered when examining digit ratio as a marker for prenatal exposure to hormones. Specifically, clutch size and the number of male siblings a chick has should be considered when assessing digit ratio as a marker for MDH in eggs as these variables are associated with testosterone and estradiol levels of laying females (Williams, 2001; Viega et al., 2004; Rutkoska et al., 2005).

In Burley and Foster's study (2004), clutch size was considered in the analysis but not the number of male siblings a chick had. In Forstmeier's study (2005), chicks were cross fostered and although he controlled clutch size and the number of male chicks in the foster nest, he did not report the clutch size and number of male siblings chicks had in their clutches of origin. This may be an important point as Williams (2001) found that females given the estrogen blocker tamoxifen during egg formation produced larger clutches than controls.

Williams, Ames, Kiparissis and Wayne-Edwards (2005) also found that levels of estradiol deposited in yolk were positively correlated with levels in maternal circulation. Taken together, the results of these two studies suggest that chicks from large clutches hatched from eggs with less estradiol than chicks from small clutches. Our finding that chicks from large clutches had shorter digit 2s than chicks from small clutches supports this finding and is consistent with the premise that digit ratio is a marker for MDH. Circulating maternal testosterone, which is positively correlated with yolk testosterone levels (Schwabl, 1996b), was also shown to be negatively correlated with clutch size (Rutkoska et al., 2005). Again, our finding of a negative correlation between length of digit 4 and clutch size is consistent with the idea that this digit is influenced by testosterone as in humans, primates and mice. Digit 3 length was also negatively correlated with clutch size, suggesting that testosterone may increase the growth of this digit as well. This differs from Romano, Rubolino, Martinelli, Bonisoli, Alquati, and Saino's findings (2005) in which testosterone injected into the eggs of female ring-necked pheasant inhibited the growth of digit 3 and increased the 2:3 digit ratio. For humans and nonhuman primates testosterone appears to cause the growth of digit 3 in the same way that it does digit 4 (Manning, 2002). At this time, we have no explanation for our findings except to suggest that there may be species differences in controlling the growth of this digit.

Circulating maternal testosterone (and therefore high testosterone in yolks) positively correlates with the number of male chicks produced in a clutch (Rutkowska et al., 2005; Viega et al., 2004). We found a positive correlation between digit ratio 3:4 and the number of male siblings a chick had. Both of these digits are influenced by testosterone in mammals and ring neck pheasants (Manning, 2002; Romano et al., 2005). These results could be a consequence of high testosterone causing digit 3 to grow at a greater rate than digit 4, resulting in a greater 3:4 ratio with an increase in number of male siblings. However, since neither digit increased significantly with the number of male siblings nor do we have a direct measure of testosterone, we can not be sure that this is the case. Additionally, other maternally-derived hormones may affect these digits.

The validity of digit ratio as a marker for MDH levels in yolk came under scrutiny when a correlation between hatch order and digit ratio 2:4 found in one study (Burley & Foster, 2004) was not replicated by Forstemeir (2005). These authors assumed that testosterone levels consistently decrease across a clutch, however, since Gil, Graves Hazon and Wells (1999) first proposed this trend in zebra finches, two other studies have contradicted these findings. Ward, Nordeen and Nordeen (2001) found no trend in yolk testosterone levels and hatch order while Gilbert, Rustein and Hazon (2005) only found a trend in eggs with male chicks. Ward suggested that differences in housing conditions caused these differences but also reported high intra-clutch variability of yolk testosterone levels. That is, some clutches showed a trend for decreasing testosterone (31%), some for increasing (25%) and some (44%) showed no trend. There are many differences between these studies, so it is difficult to determine which methodological differences might be responsible for the varying results. Perhaps the deposition of testosterone in eggs is too variable between clutches for hatch order alone to be an indicator of testosterone levels. In the current study, we found digit ratio 2:4 increased with hatch order, suggesting that testosterone decreased across clutches. As we did not measure testosterone levels in these eggs, we can not know if indeed this was the case. Ideally, a study in which base line hormone levels of both breeding females and the yolks of several clutches bookending the clutch whose digit ratios are studied would be needed to allow better assessment of the relationship between maternal and yolk hormone levels and chick digit ratios. Then a study in which hormones are added to eggs early in incubation would provide additional evidence about how hormones in yolk affect digit ratios and whether digit ratios can be used as a marker for MDH in this species. In addition, variables such as housing conditions, clutch size and sex ratios should be controlled. Recently, Forstmeier, Rochester and Millam (2008) reported that estradiol injections of 5 day old chicks had no effect on 2:4 digit ratios. Since human digit ratios are determined at 13 weeks gestation (Manning et al., 2000), it is likely that zebra finch digit ratios would be influenced by hormones earlier in development than 5 days post hatch. While this study is interesting, we can not draw any conclusions about digit ratio and the effects of MDH from it.

The relationship between digit ratio 2:3 and the sequential match score is interesting but leaves us with more questions. Are both 2:3 digit ratio and the ability to sequentially match the notes of the tutor influenced by the same hormones? The length of digit 3, but not digit 2 was negatively correlated with sequential match, which suggests that testosterone and not estrogen levels in the egg have some relationship with song learning later in life. Could a bird with low digit ratio 2:3 still produce a song with a high sequential match if given a post hatching environment that promotes song learning? Clearly, further studies are needed to understand the relationship between prenatal hormone exposure, digit ratio and song learning.

Chapter 3

Hatch Order and Weight Gain in Zebra Finch Chicks

Birds that live in unstable environments may adopt strategies for increasing their indirect fitness such as altering the sex ratio of clutches and/or provisioning resources to chicks based on hatching order or sex. One adaptive strategy for such birds is to allocate food to the chick with the greatest chance of surviving and/or to the chick in which they have already invested the most (Lack 1947; Kilner 1995; Krebs et al. 1999). In zebra finches, mortality rates drop as chicks mature (Zann 1996), so feeding early-hatched (EH) chicks should increase the chances of the parents' passing on their genes. In most altricial birds studied, males and females differ in how they allocate food to chicks. Typically, females preferentially fed the smallest and males fed the largest chicks (Best 1977; Bengtsson & Ryden. 1981; Stamps et al. 1985; Gottlander 1987; Lifjeld et al. 1992; Leonard & Horn, 1996). One hypothesis for the behavioral differences between males and females is that females invest more in the brood and therefore may attempt to increase the chances of survival of late-hatched (LH), smaller chicks (Slavsvold et al., 1995). Additionally, males may invest more in early-hatched, larger chicks because extra-pair copulations typically occur at the end of the female's ovulatory period. Thus late-hatched chicks have a greater chance of being sired by another male (Gottlander, 1987; and see Slagsvold, 1997 for a review of other hypotheses). Exceptions to this rule include red-winged blackbirds (Westneat et al.1995) and European robins (Harper, 1985) in which males preferentially feed the smallest chicks and females feed the largest chicks. In species in which reproductive effort is relatively equal, males and females may not differ from each other but may still preferentially feed chicks by hatch order (Fredrick 1987; Price & Yadenberg,. 1995;

Weatherhead & McRae, 1990; Kopachena & Falls, 1991; Malcarne et al. 1994). In some cases, it may be that older chicks are better able to position themselves next to the parents during feeding as a result of their size and greater development, so that chicks and not parents decide who is fed. For starlings, it is both the position of the chicks and the intensity of their begging that parents use to allocate food (Kacelnik et al. 1995). Yellow-headed blackbirds, on the other hand, use both begging and relative offspring size in determining how food is allocated (Price & Yadenberg, 1995). In other birds feeding is based on intensity of begging alone (Redondo & Castor, 1992).

We have observed variability in the weights of zebra finch chicks at key developmental stages although food is provided to the parents *ad libitum*. We therefore predicted that zebra finches can differentiate chicks by hatch order and are preferentially feeding early-hatched chicks. To test if zebra finch chicks differed in development by hatch order, we weighed chicks at five-day intervals, from the day of hatch to independence (day 35), at fledging and at sexual maturity (day 90).

By creating a feeding hierarchy, parents introduce the potential for variance in song learning among chicks (Nowicki et al. 2002; Spencer et al., 2003). Song is considered an honest indicator of male quality for songbirds, and it has been proposed as a marker for the quality of a male's parents and his nestling environment (Buchanan & Catchpole, 2000; Møller et al., 2000). The nutritional stress hypothesis, proposed by Nowicki et al (2002) suggests that one reason for a male to produce a poor song is that he suffered from poor nutrition as a result of having been raised by an inattentive father and/or experienced food shortages early in development when key brain structures needed for song learning and production were developing. The result is a male who has decreased immunocompetence, an unattractive song and may himself be an inattentive father. Overcrowding in the nest may be one reason for poor nutrition as parents fail to keep up with the demands of the brood (Stearns, 1992). Male chicks from large broods may therefore be compromised in their song learning ability. The number of male siblings a chick has may also affect song quality as it has been shown to negatively correlate with song copying ability (Gil et al., 2006; Tchnernichovski & Nottebohm, 1998). Therefore, we considered the influence of hatch order, clutch size, number of male siblings and chick weight during the nestling period on song learning. We predicted that weight would be positively correlated with song copying ability but that clutch size and numbers of male siblings would be negatively correlated with song learning

Methods:

Study Animals

Fourteen pairs of breeding zebra finches were housed at Hunter College, City University of New York. Each breeding pair occupied a separate cage but could see and hear other zebra finches. Stainless-steel breeding cages measured 56 X 56 X 56cm with a 13 X 12 X 12.5cm nest box centered high on the rear wall. The nest box had a 6 X 13cm opening on the side facing the cage and a clear Plexiglas top. Adults were fed a vitamin-supplemented commercial finch seed mix, grit, water and cuttlebone *ad libitum*. The diet was supplemented with fresh greens, oranges, and hard-boiled chicken eggs. The animal room was maintained at 24° $\pm$ 2°C with over 50% relative humidity and a 14:10 light:dark cycle to encourage optimal breeding.

Nests were checked daily, and eggs were marked to indicate laying order. Chicks were marked with acrylic paint on the day they hatched to indicate hatching order. Banding replaced the acrylic paint at day 9 post hatch. Clutch size varied between 2 and 6. Typically a clutch had one hatch per day, but occasionally two chicks hatched on the same day. All chicks from these clutches were removed from analysis. When hatching order did not match laying order all of the chicks from that clutch were also removed from analysis. Zebra finch chicks are typically less than 1 gram at hatch, therefore, we used a scale with a resolution of 0.001g so that even small differences between groups could be detected. Chicks were weighed at 0, 5, 10, 15, 20, 25, 30, 35 and 90 days of age and at fledge. Fledging was defined as the first day that the chick flew out of the nest box upon daily inspection. Chicks remained with their parents in the home cage until they reached 35 days of age. 35-day-old chicks were then sexed by examining plumage and placed in an adjacent cage where they could see and hear their parents but had limited physical contact. When the chicks reached sexual maturity (day 90), they were removed from the breeding room. Females were placed in a same sex aviary and males were placed in a sound- attenuated recording chamber in another room.

Song recording:

The sound-attenuated chamber was fitted with a model of a female zebra finch, two perches and a microphone (Earthworks SRO, Milford, New Hampshire) mounted on the ceiling, in the center of the chamber. Food and water were provided *ad libitum*. Fathers were recorded in the same way, when they were between clutches. Songs were recorded and analyzed with Sound Analysis Pro (http://ofer.sci.ccny.cuny.edu). Sound Analysis compares the songs of father and son by measuring the sequential match, % similarity, mean accuracy and overall score. Briefly, the sequential match is a measure of the similarity of temporal timing of the notes in the two songs. The % similarity is the percentage of the father's sounds included in the son's song. Mean accuracy is the average local similarity scores across the song. The overall score is the product of these three measurements.

Data Analysis:

Hatch positions 4, 5 and 6 had very small sample sizes, and there were no differences in weight between chicks in these positions on any of the developmental days. Therefore, we combined the data from these chicks and called the group hatch order 4+. Furthermore, birds were grouped as early-hatched (EH) if they were in positions 1 and 2 or late-hatched (LH) if they were in positions 3 and 4+. This grouping is justified because we found no differences in weight between chicks in hatch order positions 1 and 2, nor 3 and 4+ on any of the days the birds were weighed. On the days on which we found a hatch order effect on weight (days 5 and 10), a Kruskal-Wallis analysis of weight by hatch order showed that there were significant differences between chicks 1 and 3 (both days $p<0.05$), chicks 1 and 4+ (both days $p<0.01$), chicks 2 and 3 (day 5 $p<0.01$, day 10 $p<0.05$) and chicks 2 and 4+ (day 5 $p<0.01$, day 10 $p<0.05$). Weight data were then analyzed with multiple linear regression in SPSS (version 11.5). The between-subject factors were sex, clutch size, number of male siblings, and hatch order. Weight was the within-subject factor. A backward stepwise procedure was used to remove insignificant terms (Sokal & Rolf, 1995). Song analysis was done by performing separate multiple linear regressions on the four parameters used in Sound Analysis Pro with weight at day 10, hatch order, clutch size and number of number of male siblings as the between-subject factors. Day 10 was chosen because this is when we saw the most significant differences in weight between EH and LH chicks. At day 10, chicks are completely dependent on the parents for food. Chicks also have their eyes open at this stage which may enhance competition between siblings for feeding opportunities. To reduce the possibility of a Type I error when multiple tests are conducted on the same data set, we used Sidak's procedure to lower the α to 0.042 (Uitenbroak, 1977).

Results:

Weight gain

On day 0, the day of hatching, none of the independent variables contributed significantly to chick weight. On day 5, clutch size and hatch order significantly contributed to chick weight. Not surprisingly, chicks from large clutches (more than 3 chicks) produced chicks that weighed less on day 5 than chicks from small clutches. EH chicks (positions 1 and 2) were heavier than LH chicks (positions 3 and 4+) (Figure 5). The difference in weight between EH and LH chicks became even greater by day 10. On day 10, the number of male siblings also significantly contributed to chick weight. The more male siblings a chick had, the less it weighed. Exceptions to this were chicks that had no male siblings. These chicks were, on average, lighter than chicks who had one male sibling but heavier than those that had 2 or more male siblings ($X_{o\ siblings} \pm SEM=8.928 \pm 2.38$g; $X_{1\ male\ sibling} \pm SEM=9.232 \pm 1.76$g; $X_{2\ male\ siblings} \pm SEM=7.516 \pm 1.31$g; $X_{3\ male\ siblings} \pm SEM=7.18 \pm 1.09$g; $X_{4\ male\ siblings} \pm SEM=6.93 \pm 0.13$g). Of the chicks that had no male siblings, 7 were females and therefore came from all female clutches. The average weight ($\pm$SEM) of these chicks was 8.04 ± 2.9g. This was not significantly different from the mean weight of the 6 males that had no male siblings (9.97 ± 1.05g; Mann-Whitney U=20, p=0.95).

On day 15 and the day of fledging, none of the variables significantly contributed to the weight of chicks. The fledge date average was 16.63 but ranged from day 13 to day 22. Clutch size contributed to fledge date with those from large clutches fledging later ($p<0.001$, $r^2=0.1417$). Hatch order and the number of male siblings a chick had did not contribute to fledge date. On day 20, chick sex significantly affected chick weight. Males were heavier than females on this day ($X_{male} \pm SEM=12.58+1.11g$, $X_{females} \pm SEM=11.61 \pm 1.58g$). On days 25, 30 and 35, clutch size again significantly affected chick weight. As before, the larger clutches produced smaller chicks. On day 90, none of the variables affected chick weight. The results of the weight data are summarized in Table 2.

The weight of a chick on day 10 was positively correlated with the mean accuracy of his adult song and its sequential match (Table 3). Hatch order, clutch size and the number of male siblings did not affect these parameters of song learning and were therefore removed in a stepwise manner. Overall song score and the % similarity score were not influenced by any of the variables we tested.

Discussion

Our data demonstrate that prior to fledging; hatch order contributed to chick weight with EH chicks weighing more than LH chicks. Preferential feeding by parents is one possible explanation for the hatch order effect on weight. We can not rule out metabolic differences correlated with hatch order or latent effects of maternally-derived hormones deposited in yolk. However one would expect these differences to be apparent on day 0. All chicks, regardless of sex, hatch order, clutch size or number of male siblings started off at roughly the same weight on the day they hatched. This suggests that differences in weight resulted from rearing environment post-hatch. After the first five days of being fed by parents, we found that EH chicks were significantly heavier than LH chicks. By developmental day 10, the differences were even greater. LH chicks began to catch up to EH chicks by the time they were 15 days old, which coincided with the fledging of their EHed siblings. Fledglings continued to beg from parents but also fed independently. Fledglings returned to the nest often, but in their absence, the LH chicks may have gotten additional food from their parents. Once LH chicks fledged, they could supplement parental feeding by feeding themselves. This would explain why there were no hatch order effects on weight between EH and LH chicks from day 20 on. It does not appear that parents were preferentially feeding chicks by sex as we found a sex effect only on day 20, several days after fledging. This is the period just prior to the start of song acquisition. The filial bond is fundamental to accurate song copying (Roper & Zann, 2006) and there may be an increase in competition between male chicks as they vie for the father's attention. Zebra finch chicks copy the song of the male that has fed them and they have had the most interaction with (Williams, 1990). Perhaps female chicks have less access to the father during this period and therefore receive less food.

Not surprisingly, clutch size had the greatest influence on chick weights, significantly influencing weights on 4 of the 10 days that chicks were weighed. Our results are consistent with previous studies of clutch size manipulation that have shown inverse correlations between clutch size and chick weights (Saino et al.,1997; Hõrak et al., 1999; Metcalf & Monaghan, 2001, Fargallo et al., 2002; Naguib et al., 2004; Gil et al., 2006). Chicks from large clutches have been shown to have reduced breeding success, presumably as a result of decreased nutritional status during development, although prolonged stress can not be ruled out. Females raised in large clutches delayed breeding, had decreased immunocompetence, produced chicks that were smaller than chicks from mothers raised in small clutches (Naguib et al. 2004, 2006; Alonso-Alvarez et al. 2006) and had decreased reproductive success overall (Naguib et al. 2006). In our study, clutch size negatively correlated with chick weights on day 5 but also on days 25, 30 and 35 when chicks could feed themselves as well as be fed by their parents. Although we provided ample food at several locations in each cage, there may have been competition between chicks for preferred feeding areas.

The number of male siblings has been shown to be negatively correlated with song copying accuracy. It is thought that the abundance of developing song models among siblings interferes with accurate song acquisition (Volman & Khanna, 1995; Jones et al., 1996; Tchernichovski & Nottebohm, 1998; Gil et al., 2006). Although we have no evidence that the number of male siblings a chick had influenced song learning, our data may add some additional insight to the song model abundance hypothesis. The number of male siblings was negatively correlated with chick weights on day 10. At this time the chicks are more mobile and have their eyes open (Zann, 1986). Competition among members of the clutch may be highest during this period and may increase with the number of male chicks in the nest. Competition requires greater energy expenditure resulting in decreased condition of all the chicks which may result in a later decreased capacity for song learning.

Allocating food preferentially to chicks by hatch order has immediate consequences on morbidity and mortality rates as well as long-term effects on breeding success (Birkhead et al. 1999; Nowicki et al. 2000; Spencer et al. 2003, Blount et al. 2003). Immediate consequences of poor nutrition include abnormal plumage (Birkhead et al. 1999), elevated corticosterone levels (Birkhead et al. 1999; Blount et al. 2003) and inability to learn (Buchanan et al. 2004; Fisher et al. 2006). Even after a period of rapid growth when food becomes available, the detrimental effects of early nutritional deprivation persist (Blount et al. 2003; Gross 2006; Fisher et al., 2006). Previous studies (Hasselquist et al. 1996; Nowicki et al. 1998, 2002; Buchanan et al. 2000, 2003; Spencer et al. 2003) have found that poor nutrition in the early developmental period resulted in poor song quality in adulthood.

Although we did not deprive chicks of food, our data supports the nutritional stress hypothesis in that the weight of chicks on day 10 positively correlated with two of the measures of song learning, mean accuracy and sequential match.

During the nestling period, zebra finches develop key neuroanatomical connections essential for song learning. In many oscine species, females prefer to mate with males who have long or complex songs (Clayton & Prove. 1989; Hasselquist et al. 1996; Collins, 1999; Gentner & Hulse, 2000). Males who have not copied the song of their father or another male accurately tend to improvise notes which are higher in frequency and have more upsweeps. They also include more intermotif silences and have less stereotypy of the note order in their songs (Williams et al., 1993; Holveck & Riebel, 2007). Females prefer songs which are more stereotyped, and have low proportions of intermotif silences (Holveck & Riebel, 2007). Males who produced poor song copies have decreased breeding success and take longer to induce ovulation in their mates (Williams et al., 1993; Tchernichovsky & Nottebohm, 1998; Tomaszycki & Adkins-Regan, 2006). Females who feed EH chicks preferentially are therefore not only increasing that chick’s survival but probably increasing its later breeding success. In allocating more food to EH chicks, males are hedging their bets that they are increasing the survival rates and breeding success of their own progeny rather than the offspring of another male. In this way both male and female zebra finches may act to increase their own fitness.

Chapter 4

Zebra Finch Begging Call Differences and Adult Responsiveness

One adaptive strategy for birds that live in an unstable environment is to feed the chick that they have invested the most in and/or has the greatest chance of surviving (Lack 1947; Kilner 1995; Krebs et al. 1999). Provisioning food by hatch order and/or sex is one way in which to do this and increase the inclusive fitness of the parents. Males that experience poor nutrition as nestlings are not able to accurately learn their father's song and consequently develop unattractive songs as adults (Nowicki et al. 1998; Nowicki et al. 2002; Spencer et al., 2003). These males take longer to pair bond, if at all, and longer to induce ovulation in their mates. Therefore, by creating a feeding hierarchy among chicks, parents are introducing nutritional differences which may lead to variation in overall health as well as creating the potential for differences in reproductive success among offspring (Williams et al., 1993; Tchernichovski & Nottebohm, 1998; Tomaszycki & Adkins-Regan, 2006).

For altricial birds**,** females typically prefer to feed the smallest and males the largest chicks (Best 1977; Bengtsson & Rydan, 1981; Stamps et al. 1985; Gottlander 1987; Lifjeld et al. 1992; Leonard & Horn, 1996; but see Harper, 1985 and Westneat et al., 1995). This may be because females invest more in the brood and therefore may want to increase the chances of survival of late-hatched (LH), smaller chicks (Slavsvold, 1997). Males, on the other hand, may invest more in early-hatched (EH), larger chicks because extra-pair copulations typically occur at the end of the ovulatory period. Thus LH chicks have a greater chance of being sired by another male (Gottlander 1987; and see Slagsvold 1997 for a review of other hypotheses). When the reproductive effort is relatively equal, as in zebra finches, males and females may not differ from each other but may still preferentially feed chicks by hatch order (Fredrick 1987; Price & Yadenberg, 1995; Weatherhead & McRae, 1990; Kopachena & Falls, 1991; Malcarne et al., 1994). Mortality rates of early-hatched (EH) zebra finch chicks are lower than those of late-hatched (LH) chicks **(**Zann 1996**)**. In our laboratory, we have observed variances in the weights of zebra finch chicks at key developmental stages although food is provided to the parents *ad libitum***.** EH chicks were heavier than LH chicks but there were no differences between male and females (Chapter 3). This suggests that parents are feeding EH chicks more, although we can not rule out metabolic differences as a result of hatch order.

If parents are allocating food by hatch order, there must be a mechanism that allows them to differentiate chicks. For asynchronous hatchers parents may simply be keying in on the size differences of chicks (Price & Yadenberg., 1995). Differences in size and development could also lead to behavioral differences among siblings. For example, older chicks may be better able to position themselves next to the parents during feeding (Kacelnik et al. 1995) or have more salient begging calls (Redondo & Castro, 1992; Kacelnik et al., 1995).

To determine if there were hatch order/sex differences in the begging calls of EH and LH chicks, we recorded and analyzed the calls of 48 chicks. To test our hypothesis that adult zebra finches use begging calls to distinguish chicks by hatch order, we played recordings of EH and LH chicks to adults with active nests (breeders) and to those that had never bred nor raised chicks (naïves) and quantified their behavior.

Methods:

Study Animals

Fourteen pairs of breeding and 22 naïve zebra finches (12 females and 10 males) were housed at Hunter College, City University of New York. Each breeding pair occupied a separate cage but could see and hear other zebra finches. Stainless-steel breeding cages measured 56 X 56 X 56cm with a 13 X 12 X 12.5cm nest box centered high on the rear wall. The nest box had a 6 X 13cm opening on the side facing the cage and a clear Plexiglas top. Naïve birds were housed in same-sex aviaries until testing. Naïve birds had never been housed with the opposite sex and therefore had never bred nor raised young. Adults were fed a vitamin-supplemented commercial finch seed mix, grit, water and cuttlebone *ad libitum*. The diet was supplemented with fresh greens and oranges, and breeders also received hard-boiled eggs. The animal rooms were maintained at 24° $\pm$ 2°C with over 50% relative humidity and a 14:10 light:dark cycle for optimal breeding.

Nests were checked daily, and chicks were marked with acrylic paint on the day they hatched to indicate hatching order. Banding replaced the acrylic paint at day 9 post hatch. Clutch size varied between 2 and 6. Typically a clutch had one hatch per day, but occasionally two chicks hatched on the same day. All chicks from these clutches were removed from analysis. Additionally, when a clutch contained chicks whose laying order did not match their hatching order the entire clutch was removed from analysis. Chicks remained with their parents in the home cage until they reached 35 days of age. On day 35, chicks were sexed by examining plumage and placed in an adjacent cage with siblings where they could see and hear parents but had limited physical contact.

Recordings:

We used GoldWave, version 5.14 (www.GoldWave.com) to record begging from 48 chicks (27 males, 21 females). All recordings were made one hour after lights came on but before greens and hard-boiled egg were offered. We have observed that parents typically feed chicks after these food items are offered. Begging calls were elicited when the chicks were 10-11 days old by waving a female bird model overhead in a sound-attenuated chamber. Each chick was approximately six inches directly below a microphone (Earthworks SRO, Milford, New Hampshire) as the model was passed overhead. The first 15 seconds of begging were then analyzed with Sound Analysis Pro (http://ofer.sci.ccny.cuny.edu). Specifically we compared entropy, amplitude, pitch, and frequency modulation of the begging calls of male versus female chicks and early- versus late-hatched chicks. We also compared the number of begging syllables within 15 seconds of the first call. A previous study (Chapter 3) on weight and hatch order found that chicks in position 1 and 2 weighed significantly more than chicks in positions 3, 4, 5, and 6 at several points during development. Therefore we grouped those in position 1 and 2 as EH and those in positions 3, 4, 5 and 6 as LH. From these recordings, two first- hatched male chick (EH) recordings and two fourth-hatched male chick recordings (LH) were chosen to test adult birds' ability to discriminate hatch order by begging calls. These exemplars fell within the average range of calls for EH and LH chicks, respectively. The amplitudes of the EH recordings were 35 and 41 dB. The amplitudes of LH recordings were 48 and 50 dB. Each of these recordings was made into a 15 minute loop of 15 seconds of begging followed by 15 seconds of silence. The adults tested were unrelated to the chicks used to make these recordings.

Data Analysis:

Begging calls were analyzed with a mixed model linear regression in SPSS version 11.5. Entropy, amplitude, pitch, and frequency modulation of the begging calls and number of syllables within the first 15 seconds of begging acted as the within factors in separate regression analyses. The between-subject factors were clutch identity, sex, clutch size, number of male siblings, and hatch order (EH or LH) in all regression models. A backward stepwise procedure was used to remove insignificant terms. We also calculated the coefficient of variation between EH and LH chick calls (Sokal & Rolf, 1995).

Behavioral testing:

Testing for breeders began when their first-hatched chick was 14 days old. One parent remained with the chicks in the home cage while the other parent was tested. The test bird was moved to another room and placed in a cage that was identical to the home cage except that a speaker (Kenwood KFC 1377, Kenwood USA Corporation, Long Beach, California) was mounted on top of the nestbox. After one half hour of acclimation, a playback of either EH- or LH-begging calls was presented. After a 5-minute rest period the second recording was played. Although all begging calls were recorded under identical conditions, those of LH chicks proved to be louder. The recordings were played back at the same setting at which they were recorded, preserving the amplitude differences. Following testing, the test bird was returned to the home cage, and the other parent tested. The order of testing was counterbalanced across subjects. Naïve birds were tested for hatch order recognition the same way and were returned to the aviary afterwards. Behaviors during playbacks were scored using instantaneous point sampling at 15-second intervals for 15 minutes. The following categories were recorded; scan, tail flick, change perch, change direction on perch, and fly. Scan was a fast movement of the head as the bird looked around the cage. Scan and tail flick are behaviors that indicate attention and alertness (Ryan et al, 1996). We chose change perch, change direction on perch and fly because they indicate an increase in activity and may, along with scan be an indicator that the birds are searching for the source of the begging calls. The cage was divided into three areas (top, center and floor) so that we could determine cage space usage. Muller and Smith (1978) found that finches began eating seeds in response to playbacks of chicks begging. Therefore, *a priori*, we expected breeding finches to enter the areas of the cage that had food during testing. We also expected naïves to remain in the top area. Top consisted of the two top perches and the nestbox. Center consisted of the two bottom perches located in the lower half of the cage, and a small feeder. Floor consisted of the floor which was sprinkled with some seed and grit and provided access to a large feeder, cuttlebone, and a waterer.

Data analysis:

The frequency of each of the behaviors during the playbacks was summed. Data were analyzed using repeated-measures 2-way ANOVAs, using a 2 X 2 repeated-measures design, with sex and breeding status as the between-subject variables and frequency of a behavior as the within-subject variable. This was followed by planned pair-wise comparisons using Bonferroni posthoc tests and one way ANOVAs. P-values < 0.05 were considered statistically significant. Cage usage was analyzed in a similar manner with number of visits in each area as the within-subject variable and sex and breeding status as the between-subject variables.

Results:

Begging calls:

Entropy, frequency modulation, and the pitch of begging calls as well as the number of calls within the first 15 seconds of the start of begging were not affected by any of the variables we tested (Table 4). There was, however, a hatch order effect on the amplitude of begging calls ($p=0.019$) with EH chicks begging at significantly lower amplitudes than LH chicks (Figure 6). The amplitude of calls of EH chicks also varied less than that of LH chicks ($p=0.016$).

Playback results:

Overall, naïves were less active during the playbacks. Specifically, naïves did not change perch, change direction on the perch, fly, scan or tail flick as often as breeders. The interaction between breeding status and playback was not significant for any of the behaviors.

Both breeding status and playback type had significant effects on the frequency of changing perch (breeding status $p=0.0002$, $F_{1,52}=16.32$; playback $p=0.0039$, $F_{1,52}=9.11$). Breeders changed perch more than naives during both playbacks (Kruskal Wallis $H4=29.31$, $p<0.0001$; Dunns posthoc test EH $p<0.001$; LH $p<0.01$). A Bonferroni post test showed that breeders changed perch significantly more when they listened to EH than to LH playbacks ($p<0.01$). Playback type did not affect the frequency of perch changing among naïve birds (Figure 7).

Both breeding status and playback type contributed significantly to the frequency of changing direction on the perch (breeding status $p<0.001$, $F_{1,52}=13.85$; playback type $p=0.0014$, $F_{1,52}=11.43$). Breeders changed direction on the perch more often than naïves

(Kruskal-Wallis H4=22.29, p<0.0001; Dunns posthoc test EH p<0.01, LH p<0.01) during both playbacks. Breeders changed direction on the perch more often when listening to EH playbacks than LH playbacks (Bonferroni post test p<0.001). Playback type did not affect the frequency of changing direction on the perch among naïve birds (Figure 7).

The frequency of flying was affected by both breeding status and playback type (breeding status p=0.0171, $F_{1,52}$=4.33; playback type p=0.0425, $F_{1,52}$=6.07). Breeders flew more than naives during both playbacks (Kruskal-Wallis H4=12.85, p=0.005) but when the two tests were examined separately, only during the LH playback was the difference significant (Dunns posthoc p<0.05). Breeders flew more during EH playbacks than LH playbacks. Playback type did not affect the frequency of flying among naïve birds (Figure 7).

Breeding status did not contribute significantly to the frequency of tail flick but playback type did (status p=0.22, $F_{1,52}$=1.52; playback type p=0.025, $F_{1,52}$=5.30). Breeders tail flicked more than naïves during both playbacks but the differences were significant for the EH playbacks only (Kruskal Wallis H4=8.202, p<0.05; Dunns posthoc EH p<0.05, Figure 7). Breeders tailed flicked more during the EH playbacks than the LH playbacks (Bonferroni post test p<0.05).

Both breeding status and playback type significantly affected the frequency of scanning (breeding status p=0.0004, $F_{1,52}$=14.132; playback type p=0.048, $F_{1,52}$=4.109). Breeders scanned more than naïves (Kruskal Wallis H4=14.15, p=0.0027; Dunns posthoc EH p<0.05, LH p<0.05). Breeders scanned more during the EH playbacks than the LH playbacks (Bonferroni posthoc p<0.05). The interaction between sex and breeding status was significant for the LH playback only (p=0.012, $F_{1,52}$=6.804). Posthoc analysis showed that breeding females scanned more than breeding males and naïve females during this playback

(Kruskal Wallis H8=20.7, p=0.004; Dunns posthoc for breeding females and breeding males p<0.05; breeding females and naïve females p<0.001) (Figure 8).

Breeders entered all cage sections during the playbacks, while most naïve birds stayed off the floor. The interaction between breeding status and playback did not significantly affect the frequency of birds entering the top and center sections. (breeding status*playback top section p=0.19, $F_{1,52}$=0.19; center section p=0.55, $F_{1,52}$=37). Breeding status and playback order did not affect the frequency of entering the top section (breeding status p=0.085, $F_{1,52}$=3.08, playback p=0.94, $F_{1,52}$=0.01) nor the center section (breeding status p=0.56, $F_{1,52}$=0.79; playbacks p=0.56, $F_{1,52}$=0.34). Since only one naïve bird entered the floor area, the difference between groups in the frequency of entering the floor area was significant. The interaction between breeding status and playback was significant (p=0.03, $F_{1,52}$=5.17) as was breeding status (p=0.009, $F_{1,52}$=7.35). Breeders entered the floor section more than naïves (Kruskal Wallis H4=19.54, p=0.0002). Breeders also visited the floor more often during the EH playbacks than the LH playbacks (Bonferroni p<0.05). The results are summarized in Figure 9.

Discussion:

LH chicks begged at higher amplitudes and their call amplitudes were more variable than those in the calls of EH chicks. LH chicks may be increasing the amplitude of their calls as their nutritional needs are insufficiently met. Begging intensity is negatively correlated with body condition in many species (Iacovides & Evens, 1998; Boncoraglio et al. 2006) including zebra finches (Von Englehardt et al. 2006). Typically, begging intensity is calculated by the rate of calling and the duration of the bout, however, amplitude can also convey information about the state of the chick (Leonard & Horn, 1999). Begging can attract predators (Haskell, 1999) and rallying for a feeding position can lead to injuries (Roulin et al., 2000). For these reasons, we would expect satiated chicks to decrease begging while hungrier siblings increase begging.

In many species, begging is an honest signal that is acknowledged by parents (Henderson 1975; Smith & Montgomerie, 1991; Godfrey 1991; Kilner 1995; Price & Yadenberg, 1995; Cotton et al. 1996; Leonard & Horn, 1999). Parents may recognize differences in begging intensity, however, but still continue to preferentially feed chicks by hatch order (Price & Yadenberg, 1995; Leonard et al. 1997: Hinde & Kilner, 2007). The increase in overall activity and frequency of the behaviors scan and tail flick during the playbacks of EH chicks suggests that parents can differentiate begging calls by hatch order and are responding accordingly. In this group both males and females responded more to EH playbacks suggesting that they are using the same strategy in allocating food.

Muller and Smith (1978) found that zebra finches will begin to eat in response to hearing the playbacks of chicks begging. We have also observed that finches will feed their own chicks when they hear the begging calls of other chicks in the colony (unpublished data). Overall, breeders responded to EH playbacks by becoming more alert and active, and entering the lower section and floor where they had access to food, and in some cases eating the food provided. We did not see many instances of adults eating seed but in contrast to Muller and Smith's study, we did not have live young in the test cage during the playbacks. Naïves on the other hand were either not motivated and/or did not have the experience to locate food in response to begging. Differences between breeders and naïves may result in part from the fact that breeders were tested when they had active nests. They were, therefore, both hormonally and experientially primed to respond to begging while naïve birds were not.

Allocating food preferentially to chicks by hatch order has immediate consequences on morbidity and mortality rates as well as long-term effects on breeding success (Birkhead et al. 1999; Nowicki et al. 2000; Spencer et al. 2003, Blount et al. 2003). Immediate consequences of poor nutrition also include abnormal plumage (Birkhead et al. 1999), elevated corticosterone levels (Birkhead et al. 1999; Blount et al. 2003) and inability to learn (Buchanan et al. 2004; Fisher et al. 2006). Even after a period of rapid growth when food becomes available, the detrimental effects of early nutritional deprivation persist (Blount et al. 2003; Gross 2006).

During the nestling period, zebra finches develop key neuroanatomical connections essential for song learning. In many oscine species, females prefer to mate with males who have long or complex songs (Clayton & Prove, 1989; Hasselquist et al. 1996; Collins, 1999; Gentner & Hulse, 2000). Females who feed EH chicks preferentially are therefore not only increasing that chick's survival but probably increasing its later breeding success. In allocating more food to EH chicks, males are hedging their bets that they are increasing the survival rate and breeding success of their own progeny rather than the offspring of another male. In this way both male and female zebra finches are acting to increase their own fitness.

Chapter 5

Attention from the Father

(Clumping behavior and song learning in zebra finches)

Song learning in zebra finches is restricted to a sensitive period which typically begins around day 25 and ends at day 55 (Immelmann, 1969; Roper & Zann, 2006). In the absence of a tutor, males sing a song incorporating improvised notes, long calls, and long intermotif silences. This type of song is unattractive to females and results in lower reproductive success for males (Williams, 1990; Tchernichovski & Nottebohm, 1998, Tchernichovski et al., 1999; Holveck & Riebel, 2007). Some males who have had access to a good song model will nonetheless produce poor copies of the song. Various reasons have been proposed for this including poor nutrition (Hasselquist et al. 1996; Nowicki et al. 1998, 2002; Buchanan & Catchpole, 2000; Buchanan et al., 2003; Spencer et al. 2003), stress (Buchanan et al., 2003), and model abundance (Tchernichovski & Nottebohm, 1998; Tchernichovski et al., 1999). Males who have not copied the song of their father or another male accurately tend to improvise notes which are higher in frequency and have more upsweeps. They also include in their song more intermotif silences and have less stereotypy of the note order (Williams et al., 1993; Holveck & Riebel, 2007). Males who have produced a poor song copy have decreased breeding success and take longer to induce ovulation in their mates (Williams et al., 1993; Tchernichovsky & Nottebohm, 1998; Tomaszycki & Adkins-Regan, 2006).

In a non-territorial, colonial species such as zebra finches, males have many potential tutors from whom to choose but typically choose the male that raised them (Clayton, 1987;

Roper & Zann, 2006). Thus the filial bond is important is song learning (Williams, 1990; Roper & Zann, 2006), however, several physiological and behavioral factors have also been suggested to be influential in tutor choice. For example, certain song characteristics correlate with the degree of copying (Clayton, 1987). Mann and Slater (1995) found that morphology influences tutor preference in that young males given the choice between two unrelated males choose to copy the one with the same color morphology as the male that raised them. Males also choose the colony member who has shown the most aggression towards them (Clayton, 1987; Jones, 1994; Baptista & Petrinovich, 1984; Pyane, 1981, Williams, 1990), however this may be because of proximity rather than aggression. That is, spending time next to an adult male would increase the chances of aggressive encounters. Social interactions which bring the young in close proximity to possible tutors include begging, allogrooming and clumping. All of these have been implicated in influencing tutor choice (Mann & Slater, 1995; Immelmann 1969; Eales, 1987; Williams, 1990). These behaviors are more likely to occur between fathers and their young than between unrelated birds (Houx & ten Cate., 1998; Roper & Zann, 2006) resulting in young males copying the songs of their fathers. Likewise, many studies in other song birds have shown that social interactions with an adult male make his song more likely to be copied than those of other potential tutors (Nicholai, 1959; Immelmann, 1969; Dietrich, 1980; Payne, 1981; Todt et al., 1979; Waser & Marler, 1977).

Tchernichovski and Nottebohm (1998) reported that song learning among siblings was unequal with the last-hatched chick having a more complete copy of the father's song. We have found, however, that weight of a nestling was a better predictor of song copying ability than hatch order. That is, the more a chick weighed at day 10 the better his song copying later in life. Arguably, early-hatched chicks tended to weigh more than their late-hatched siblings on this developmental day but this was not always the case. The differences in both weight and song learning may result from parents attending to chicks preferentially. That is, parents may increase their indirect fitness by interacting more frequently (feeding, allogrooming and clumping) with the chick that has the best chance of passing on their genes. For zebra finches, this is usually the first-hatched chick (Zann, 1996). We, therefore, observed social interactions between parents and chicks at the start of song acquisition (between days 25-30). We then compared the songs of fathers and sons to determine song copying accuracy.

Methods:

Study Animals:

Six pairs of breeding zebra finches were housed at Hunter College, City University of New York. Each breeding pair occupied a separate cage but could see and hear other zebra finches. Stainless-steel breeding cages measured 56 X 56 X 56cm with a 13 X 12 X 12.5cm nest box centered high on the rear wall. The nest box had a 6 X 13cm opening on the side facing the cage and a clear Plexiglas top. Two perches were positioned in the top section of the cage and two in the middle of the cage. A small feeder was attached to the cage wall next to one of the perches in the middle section. A large feeder, cuttlebone and waterer were located near the cage floor. Birds were fed a vitamin-supplemented commercial finch seed mix, grit, water and cuttlebone *ad libitum*. The diet was supplemented with fresh greens and oranges, and hard-boiled chicken eggs. The animal rooms were maintained at 24° $\pm$ 2°C with over 50% relative humidity and a 14:10 light:dark cycle for optimal breeding.

Nests were checked daily, and chicks were marked with acrylic paint on the day they hatched to indicate hatching order. Banding replaced the acrylic paint at day 9 post hatch. Each chick was banded with one black and one orange band on one or both legs to indicate hatching order. Laying order matched hatching order for all chicks. All clutches had 3 chicks. The sex ratio of the clutches observed was as follows: 2 clutches were 3:0 males:female, 3 clutches were 2:1 male:female and 1 clutch was 1:2 male:female.

Behavioral observations

Observations were done at midday and lasted for one hour. Each clutch was observed over 3 days when the oldest chick was between 25 and 30 days old. Behaviors were scored using instantaneous point sampling at 30-second intervals. Although we noted all behaviors, we were specifically interested in behaviors that involved social interactions. These included clumping, begging behavior, fighting, allopreening and feeding together on the floor or at the feed dish. Clumping is defined as perching immediately next to another bird or being in the nestbox together (Zann, 1996). We also noted time spent alone (no other bird on the same perch or in the nestbox). All behaviors except clumping occurred so infrequently that statistical analysis was not possible.

Song analysis:

When chicks were 35 days old, they were removed from the home cage and placed in an adjacent cage with siblings. They could hear and see parents but had little physical contact. Other zebra finches in the colony could be heard but not seen. When chicks were 90 days old they were removed from the breeding room. Females were placed in a same sex aviary. Males were placed in individual cages inside sound-attenuated chambers in a separate room. Each chamber was fitted with a model of a female zebra finch, two perches and a microphone

(Earthworks SRO, Milford, New Hampshire) mounted on the ceiling, in the center of the chamber. Food and water were provided *ad libitum*. Fathers were recorded in the same way, when they were between clutches.

Songs were recorded and analyzed with Sound Analysis Pro (http://ofer.sci.ccny.cuny.edu). Sound Analysis compares the songs of father and son by measuring the sequential match, % similarity, mean accuracy and overall score. Briefly, the sequential match is a measure of the temporal timing of the notes in a song. The % similarity is the percentage of the father's sounds included in the son's song. Mean accuracy is the average local similarity scores across the song. The overall score is the product of these three measurements.

Data analysis:

To account for variability in parent/chick clumping across adults, we took the frequency of parent/chick clumping with each chick divided by the frequency of the parent clumping with at least one chick. We then looked at the frequency of parent/chick clumping behavior that occurred at the same time as mate clumping (mate/parent/chick clumping). That is, how often was the parent clumped with a chick on one side and the mate on the other side? Data were analyzed using one-way ANOVAs. This was followed by planned pair wise comparisons using Dunns posthoc tests. p-values < 0.05 were considered statistically significant. To determine if there was a sex effect on clumping behavior between parents and offspring we used the Mann-Whitney U test (Sokal & Rolf, 1995).

Song learning and clumping behavior were evaluated by separate multiple linear regressions for both parent/chick clumping and parent/chick/mate clumping with the four song analysis parameters (score, % similarity, sequential match, mean accuracy).

Results:

Parents spent the majority of the observation period clumped with each other ($X \pm SEM = 45.58 \pm 5.76$). Fathers clumped with sons and daughters relatively equally ($X_{sons} \pm SEM. = 38.62 \pm 12.84$, $X_{females} + SEM = 33.37 \pm 11.59$, Mann Whitney $U=25$, $p=0.49$) (Fig 10). Hatch order also did not affect clumping between fathers and their offspring (Kruskal Wallis=0.082, $p=0.95$). Likewise, there was no hatch order effect when fathers were clumped with both a chick and their mate (Kruskal Wallis=0.184, $p=0.911$) (Figure 11).

Mothers did not differ in the time spent clumping with sons and daughters ($X_{sons} \pm SEM. = 39.58 \pm 13.18$, $X_{females} + SEM = 35.51 \pm 7.68$, Mann-Whitney $U= 28$, $p=0.6934$) (Fig 10). Mothers did, however, clump with chicks according to their hatch order (Kruskal Wallis=8.573, $p=0.0138$). Dunns posthoc analysis showed that mothers clumped more with first-hatched chicks than either second- or third-hatched chicks (Dunns $p<0.05$ for 1 and 2 and 1and 3, $p>0.05$ for 2 and 3). There was also a hatch order effect for mate/mother/chick clumping (Kruskal Wallis= 11.37. $p=0.0034$, Dunns posthoc analysis $p<0.05$ for 1 and 2, 1 and 3, and 2 and 3) (Figure 11).

None of the parameters of song learning were correlated with either father/chick clumping or mate/father/chick clumping (Figure 12). Both song score and % similarity were positively correlated with mother/chick clumping. Mate/Mother/chick clumping was also positively correlated with these parameters. Mean accuracy and sequential match were not correlated with mother/chick nor mate/mother/chick clumping.

Discussion:

Clumping between fathers and chicks was not affected by chick sex or hatch order. Father/chick clumping was also not correlated with any of the parameters of song learning.

Interestingly, clumping was more prevalent between mothers and first-hatched chicks. Only one of the 6 first-hatched chicks was female. Therefore, we can not ascertain if mothers preferred first-hatched sons over first-hatched daughters. No overall sex effect for mother/chick clumping was observed. When mothers clumped with their offspring and their mates, the offspring was more likely to be a first-hatched chick than a second-or third-hatched.

Overall song score and % song similarity were positively correlated with mother/chick clumping and mother/chick clumping when the mother was also clumped with her mate. In the wild, keeping in close proximity with siblings and parents may help young to find food and later to defend roosting sites (Stamps et al., 1990). Although in the lab, finches have unlimited access to food, clumping next to the mother may allow more parental feedings. Additionally, mother/chick clumping may provide a young male access to his singing father without the risk of aggressive attacks. Male zebra finches have been shown to prefer to copy the song of an unrelated male who is paired to their mother rather than an unrelated male who is unpaired (Mann & Slater, 1994; Roper & Zann, 2006). Roper and Zann (2006) found that fathers fed, allogroomed, and clumped in the nest box with their chicks more than unrelated males did. Consequently, these chicks copied the song of their father more than those of unrelated males in the aviary. Other studies of tutor choice have shown that chicks actively listen to singing males (Houx & ten Cate, 1998, pers obs.) and clump next to models when presented with a playback to learn from (Adret, 1993). Chicks may be picking up subtle cues such as head or wing movements that accompany song and make being in close proximity important in song learning (Williams, 1990; Mann et al., 1991; Houx & ten Cate, 1999; West & King, 1988). The observations occurred during a period when parents typically start to prepare for the next breeding cycle. Part of this process includes singing by the male to his mate (Zann, 1996). Perhaps, clumping next to the mother who is clumped next to the singing father gives a chick the best visual and acoustic access to the song model.

Because we used instantaneous point sampling, we do not know if it was the chicks or the mothers who initiated clumping. If chicks are initiating mother/chick clumping it may be that first-hatched chicks are stronger and more aggressive than their siblings. On the other hand, it may be that mothers actively seek out first-hatched chicks to give them access to a good song model as mates typically follow females to a perch. By preferentially allocating clumping to first-hatched chicks, she may be increasing her indirect fitness as these chicks have lower mortality rates than late-hatched chicks (Zann, 1996). Presumably the father has an attractive song since the mother has already successfully bred with him. Therefore, this would be a good model to present to the son that has the best chance of surviving and passing on her genes.

Chapter 6

Summary

The results of this study provide a step towards understanding the effects of parental investment in zebra finches and may, as previous zebra finch studies on learning have, provide greater insight into learning in other mammals, including humans. As in human studies, however, these variables are closely related and may skew our understanding of how learning can be enhanced through parental investment. For example, chicks from eggs that received more maternally-derived testosterone may be better able to position themselves during begging so that they receive more food than siblings who received less maternally-derived testosterone. Or mothers may clump next to sons who have some physiological trait as a result of increased exposure to testosterone in the egg. Additionally, other variables explored here are also closely related. For example, if a chick has a high number of male siblings he would also come from a large clutch. One way to understand how these variables interact and which are more important in song learning is to run a multiple linear regression with digit ratio, weight of chicks at day 10, hatch order, number of male siblings, clutch size and clumping behavior as the independent variables and song leaning (score, sequential match, mean accuracy and percent similarity) as the dependent variable. When we did this we found that for each of the parameters of song learning investigated here there was a different form of parental investment influencing the outcome. There was a positive correlation with the overall song copying score and the frequency of clumping with the mother, while she was also clumped with the father (r^2-0.533, $p<0.05$). This form of parental investment was also positively correlated with percent similarity of sons' and fathers' songs (r2=0.462, $p<0.05$). Mean accuracy of copying, on the other hand, was related to the weight of the chick at day 10 ($r2=0.298$, $p<0.05$). Although we did not find a hatch order effect, in both cases (% similarity and mean accuracy) parents appear to be handicapping late-hatched chicks. That is late-hatched chicks were less likely to be clumped next to the mother, and they tended to weigh less than their older siblings. The playing field may be leveled somewhat in that late-hatched chicks may have an advantage in sequentially matching the notes of their father's song. The positive correlation between 2:3 digit ratio and sequential matching of the father's song ($r2=0.145$, $p<0.05$) suggests that increased testosterone decreases a chick's capacity for this aspect of song learning. The positive correlation between 2:4 digit ratio and hatch order suggests that testosterone is decreasing with hatch order. This is consistent with the current hypothesis regarding maternally-derived testosterone. If this is the case, or at least a strong trend, then the last-hatched son would show better sequential matching than first-hatched sons. We did not find a hatch order effect on sequential match (or any of the other scores) but this may be because there can be variability between and across clutches in our birds as was the case in Ward, Nordeen & Nordeen's study (2001). The results of our research have given us insight into the effects of parental investment but also leave many questions unanswered. We hope that this research may serve as a foundation for future work studies of parental investment and make the following suggestions:

Maternally-derived hormones

At this point, there is not enough information on digit ratio or the pattern of distribution of maternally-derived hormones to draw any conclusions. However, our data suggests that parental investment in the form of increased maternally-derived testosterone and estradiol do not enhance song learning in zebra finches. While our data is intriguing, there are several questions that still need to be answered. For example, the effect of other MDHs, such as progesterone and corticosterone, were not investigated here but could be another source of parental investment. There are several directions that further studies could take in an attempt to understand the relationship between MDHs and song learning. First, as we mentioned in Chapter 2, a full understanding of the extent of MDH contribution to the egg is needed. Determining the baseline hormone levels of breeding females and the levels of these hormones in the yolks of eggs across several clutches is clearly needed to understand trends and fluctuations that may occur in this species. In between these clutches one could allow a clutch to develop and subsequently, analyze the digit ratio and song learning relationship of these chicks. Then a study in which hormones are added to eggs early in incubation would provide additional evidence about how hormones in yolk affect digit ratios and whether digit ratios can be used as a marker for MDH in this species. Second, chicks who have received hormones during incubation could be tutored with exemplars of songs so that a comparison of song learning ability could be determined. Third, a cross-fostering study of untreated chicks would provide insight as to how much of sequential matching ability is the product of MDH and how much is due to environmental effects after hatching. For example, experimental clutches that consist of unrelated chicks of the same age and hatch order could be created. Later, the chicks could be tested as adults and compared for song learning ability. Combined these data would allow for a better understanding of the effects of MDH on song learning. Lastly, the ultimate goal of parental investment is to create successful offspring. A negative correlation between digit ratio and female choosiness has been shown (Burley & Foster, 2004; Forstmeier, 2005) but data on male reproductive activity and success rates is lacking. Ideally this could be done with a wild population in which both digit ratio and fecundity were known for a large sample of males. In the laboratory, this could be done on a smaller scale in an aviary where females had free choice of males with other sexual traits controlled (i.e. beak and plumage color and song complexity).

Feeding:

Feeding behavior by both parents is a more apparent form of parental investment that leads to better song learning. Chicks that were heavier at day 10 produced songs as adults that had a higher mean accuracy in copying the father's song than chicks that were lighter at this time. Moreover, chicks that hatched first or second in the clutch tended to be heavier than chicks that hatched later. Because parents responded more to the begging calls of EH chicks, we suggest that parents can differentiate chicks by hatch order and are choosing to preferentially feed early-hatched chicks over late-hatched chicks. This leads to a learning hierarchy within a clutch. While we did not determine if both parents were preferentially feeding EH chicks our playback data suggests this is the case. Video monitoring of feeds in the nest would help in determining this. Additionally, a cross fostering study would further our understanding of preferential feeding by hatch order. For example, would parents feed chicks equally if all of the chicks in a clutch were either EH or LH foster chicks of the same age? We could have also supplemented LH chicks to see if the differences in amplitude of begging calls resulted from hunger or physiological/neurological differences that related to hatch order.

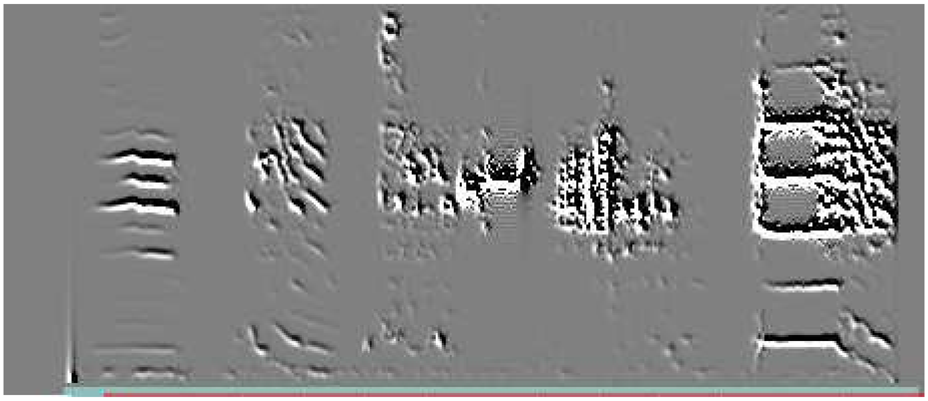
Attention:

The song learning hierarchy is furthered by clumping behavior with mothers. We had expected the clumping behavior of fathers to influence song learning but instead found that fathers clumped equally with all chicks. In Tchernichovski & Nottebohm's study (1998) mothers were removed from the cage and the last-hatched chick had the best copy of the father's song. Perhaps in the absence of the mother, the first-hatched son loses his advantage. In retrospect, we could have changed some of our methods to yield a better understanding of the effect of clumping behavior on song learning. For example, using continuous sampling would have allowed us to determine who initiated clumping behavior and might have allowed us to record more social interactions. Additionally, a larger sample size with a more even sex ratio would also have helped in understanding the adaptive significance of clumping with the mother. Finally, a cross fostering study in which all of the chicks were the same age but of different hatch orders might help in understanding why EH chicks were clumping with their mother more than siblings. It could be that being the oldest gave them a higher social status or that they were more interested in the singing father.

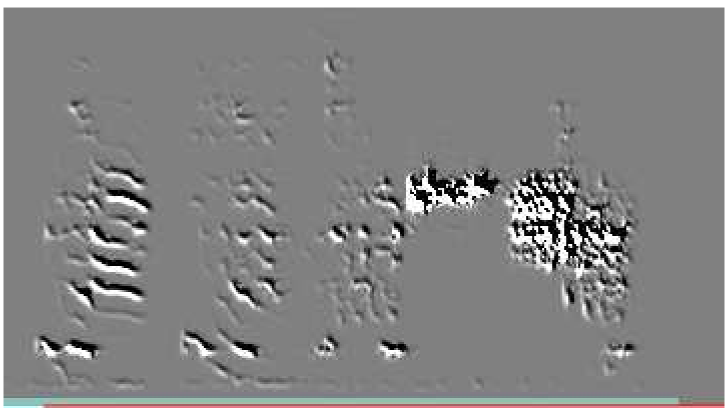
Concluding remarks:

Our goal in this study was to determine if parental investment in the form of MDH, feeding and attention resulted in increased song learning for male chicks. Our data show that for feeding and attention (from the mother) this is the case. Moreover, parents appear to be differentiating chicks by hatch order and allocating more resources to early-hatched chicks. In this way, parents are investing in the chick that has the best chance of surviving and are thereby increasing their own indirect fitness. On the other hand, the playing field may have been leveled somewhat by maternally-derived testosterone. We hope that the results of this research will serve as starting point for future research of parental investment in both song birds and other mammals, including humans.

Father



Son X



Son Y

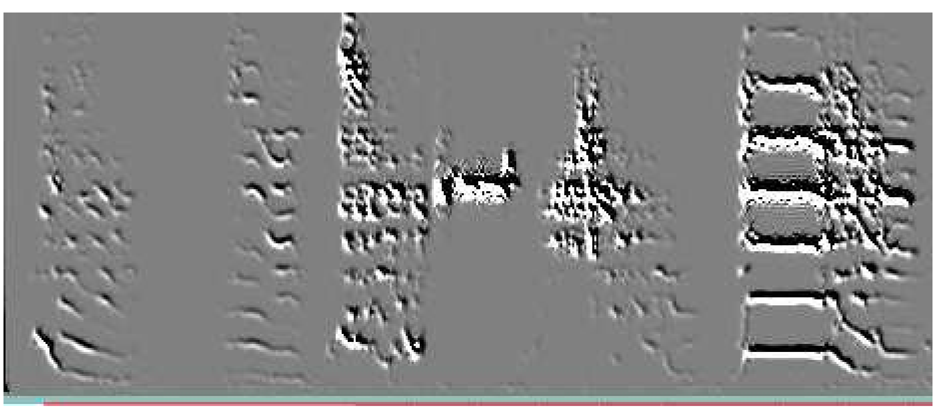
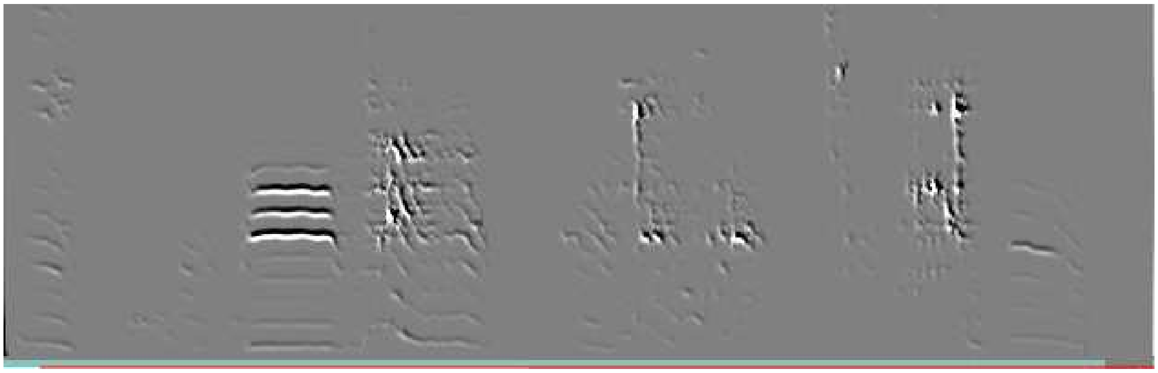
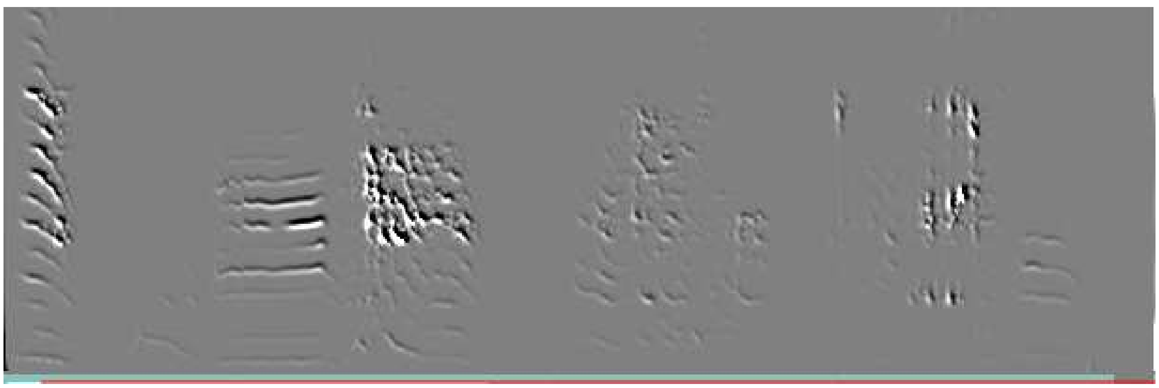


Figure 1A: Comparison of the sequential match of the songs of two brothers to their father's songs. Son X has a sequential match score of 40 while the son Y has a sequential match score of 80.

Father



Son X



Son Y

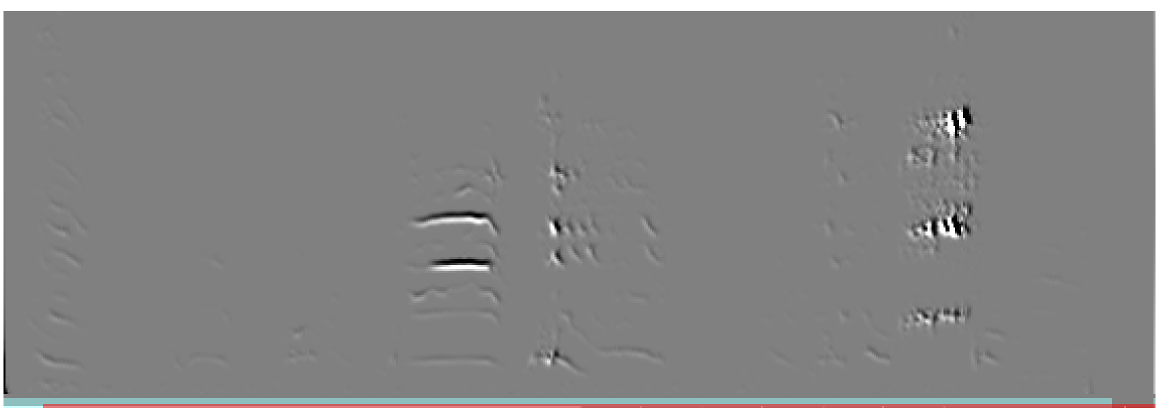
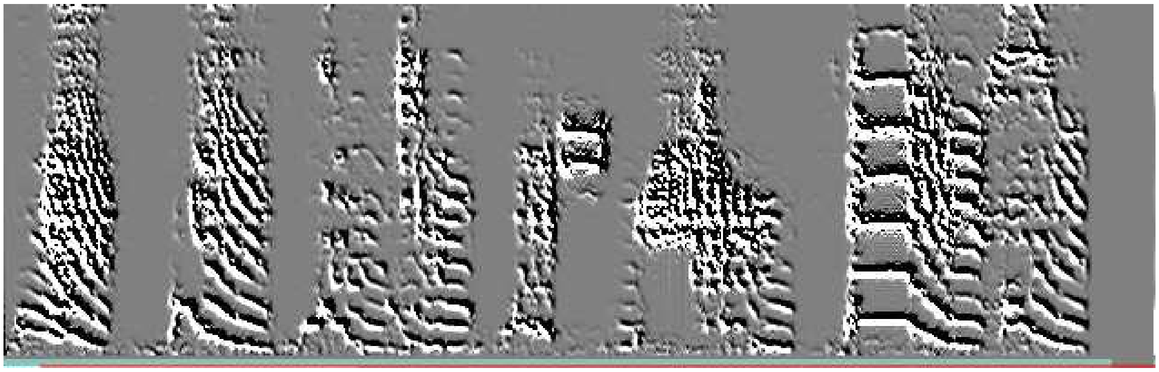
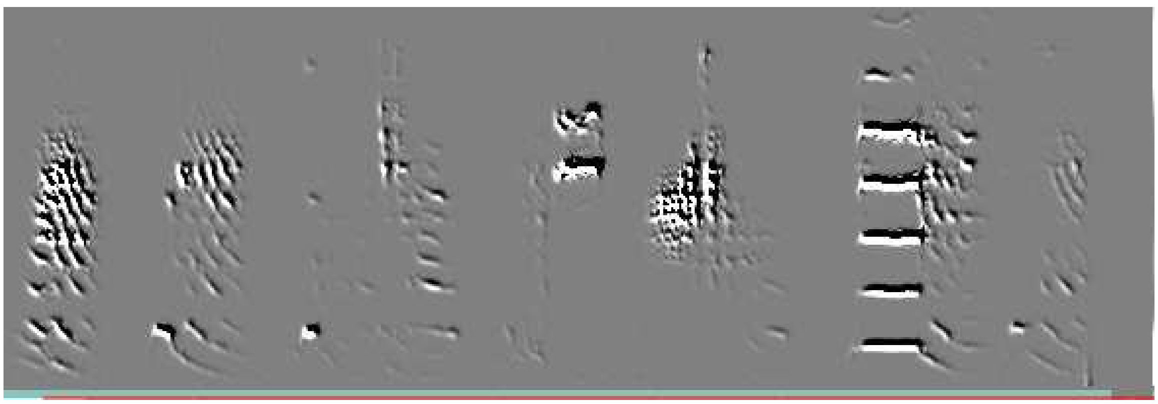


Figure 1B: Comparison of the mean accuracy of the songs of two brothers to their father's songs. Son X has a mean accuracy score of 68 while the son Y has a mean accuracy of 80.

Father



Son X



Son Y

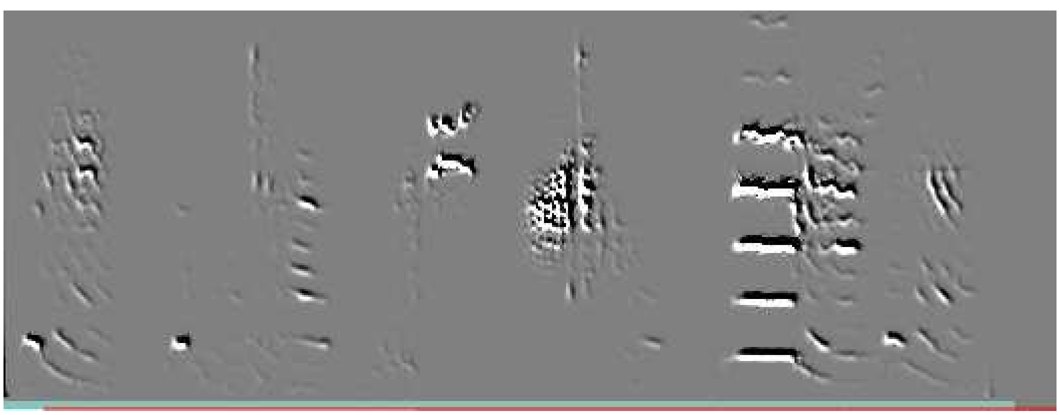


Figure 1C: Comparison of the % similarity of the songs of two brothers to their father's songs. Son X has a % similarity score of 95 while the son Y has a mean accuracy of 73.

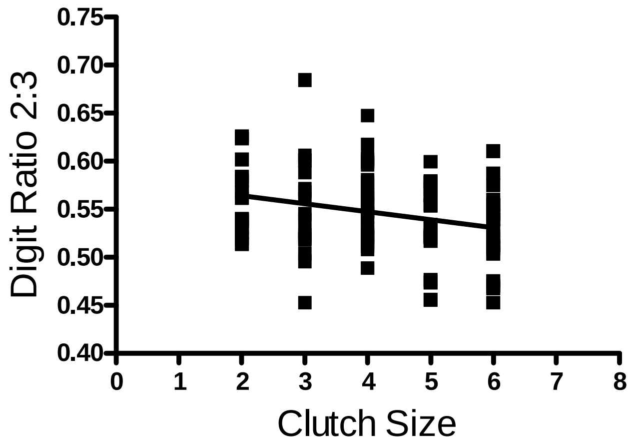


Figure 2. Digit ratio 2:3 as a function of clutch size.

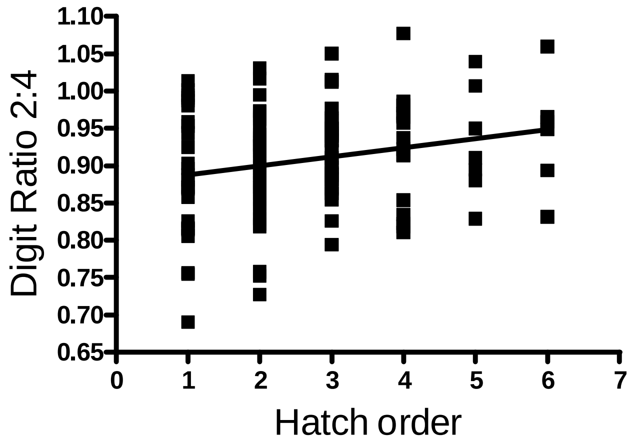


Figure 3. Digit ratio 2:4 as a function of hatch order.

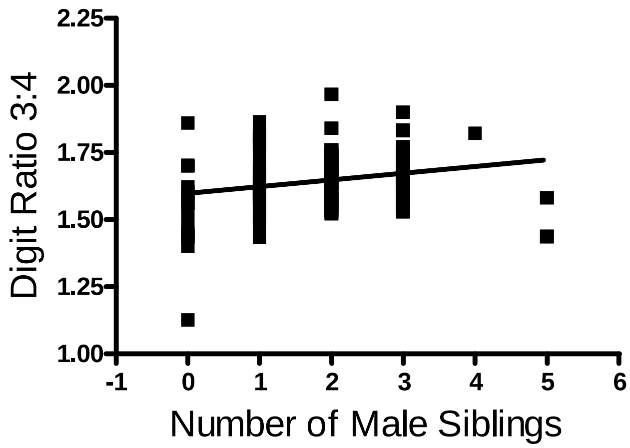


Figure 4. Digit ratio as a function of the number of male siblings a chick has.

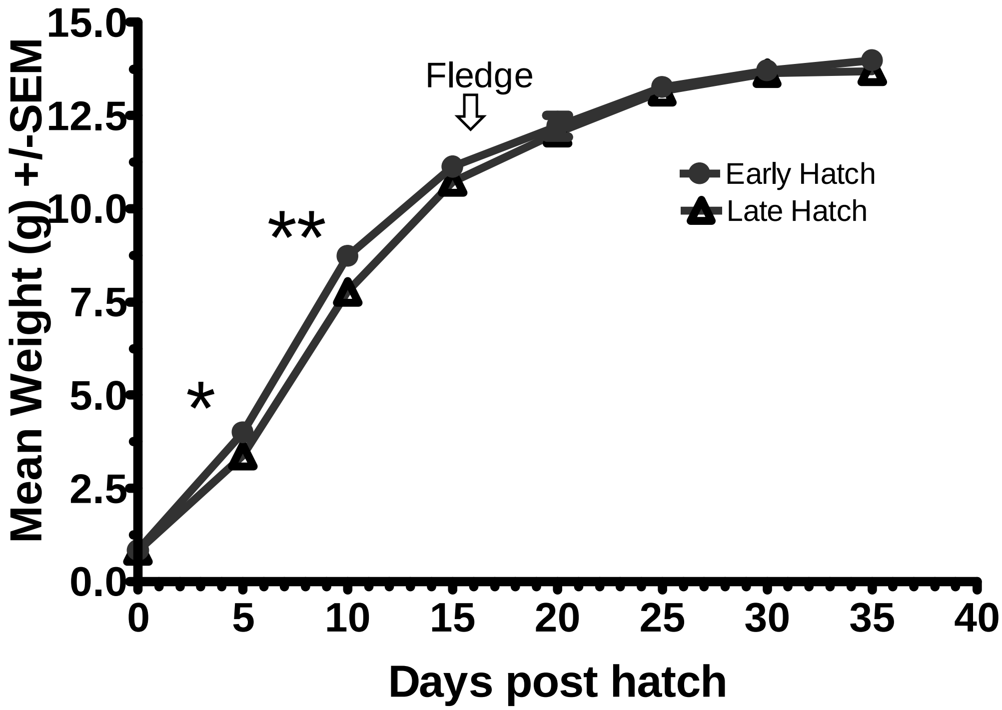


Figure 5: Mean weight of early-hatched chicks (positions 1 and 2) and late-hatched chicks (positions 3 and 4, 5 and 6) at 5 day intervals starting at hatch. Day 5 Mann Whitney U=360.5, $p<0.05$; day 10 Mann-Whitney U=951.5, $p<0.01$.

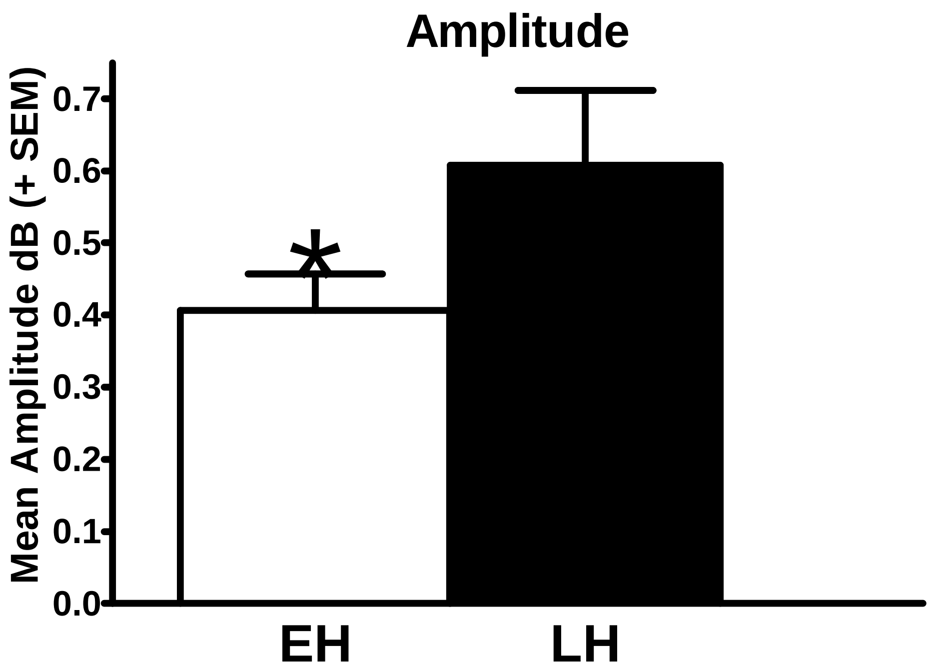


Figure 6. Mean amplitude of begging calls of 10-11 day old chicks. EH- early hatched chicks, LH=late-hatched chicks. *p<0.05.

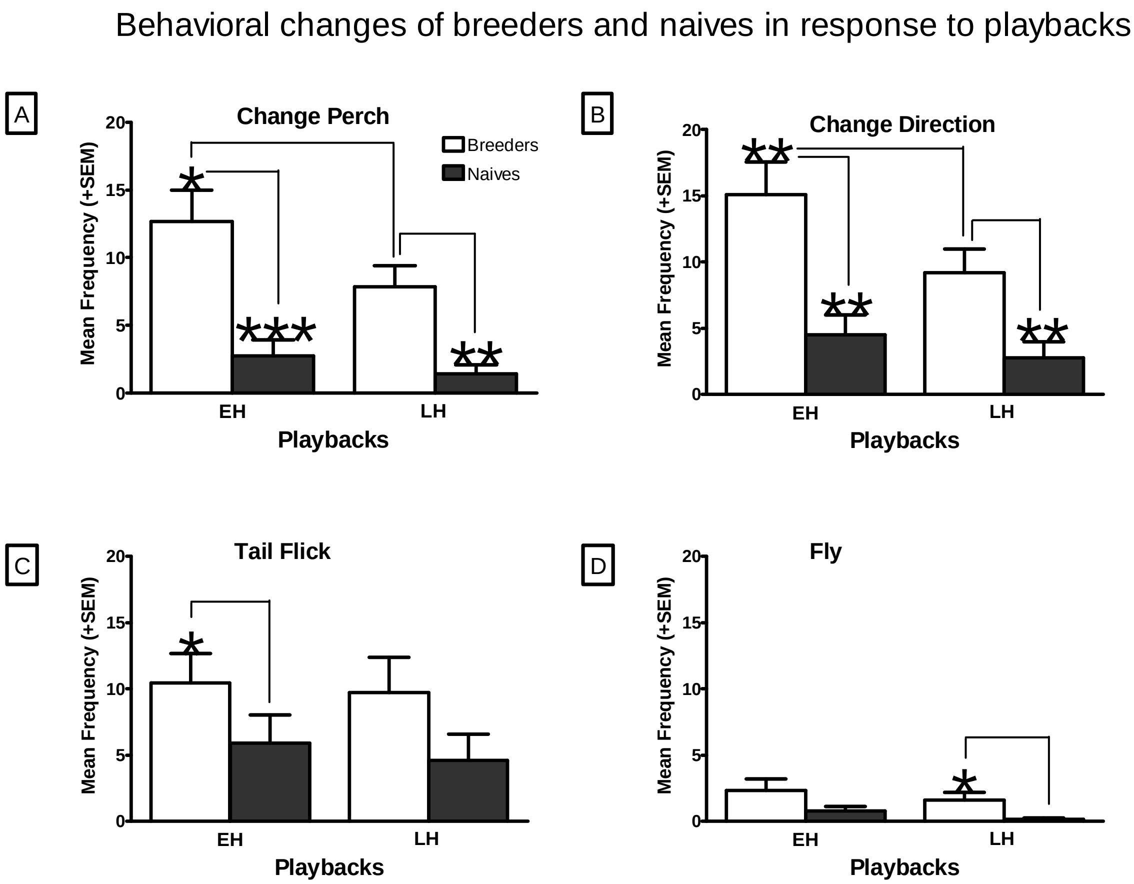


Figure 7: Mean frequency of the behaviors change perch, change direction, flying, and tail flicking of breeders and naives in response to playbacks of early-hatched (EH) and late-hatched (LH) chicks. *P<0.05. ** P<0.01, *** P<0.001.

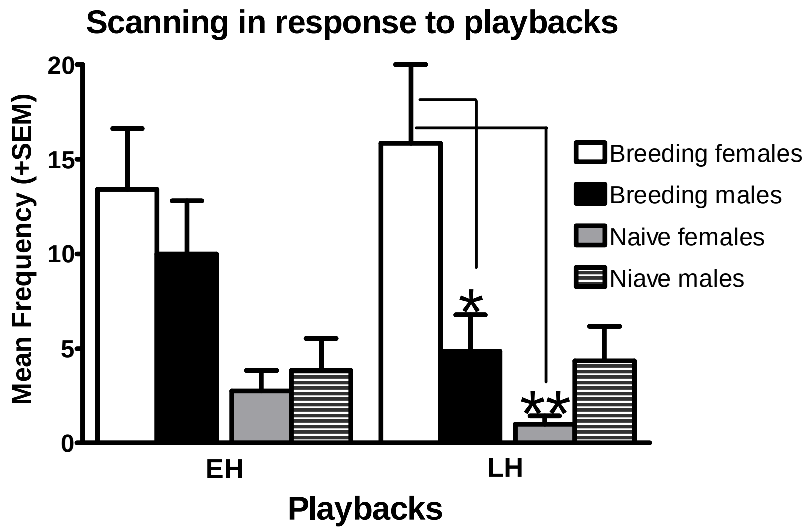


Figure 8: Mean frequency of male and female breeders and naives scanning in response to early-hatched (EH) and late-hatched (LH) begging call playbacks.

Entries Into Sections of the Cage

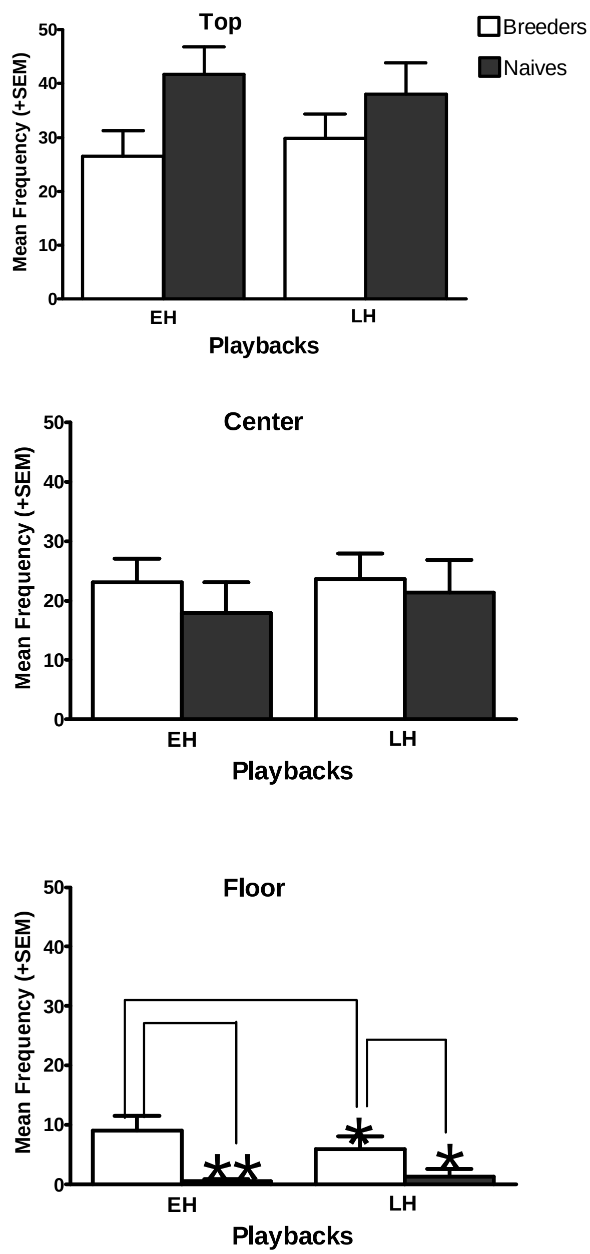


Figure 9: Mean frequency of breeders and naïves entering cage sections top, center and floor in during playbacks of early-hatched (EH) and late-hatched (LH) chicks begging calls. * P<0.05. ** P<0.01.

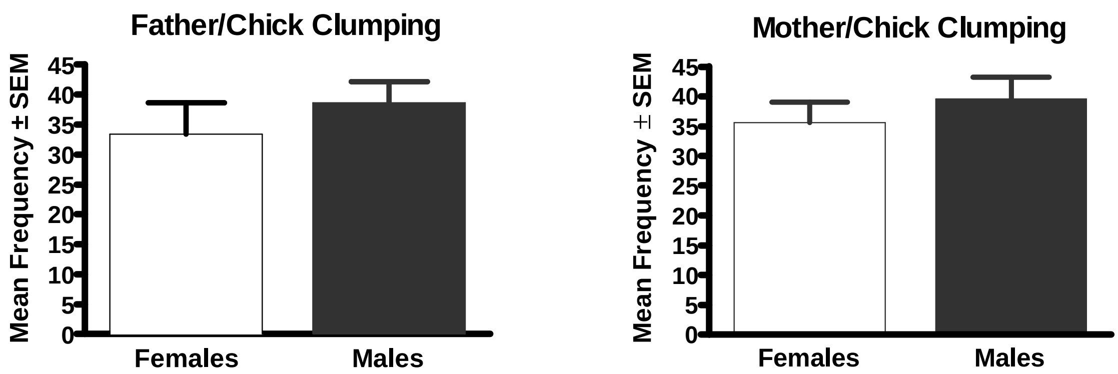


Figure 10. Mean frequency of parents clumping with chicks by sex.

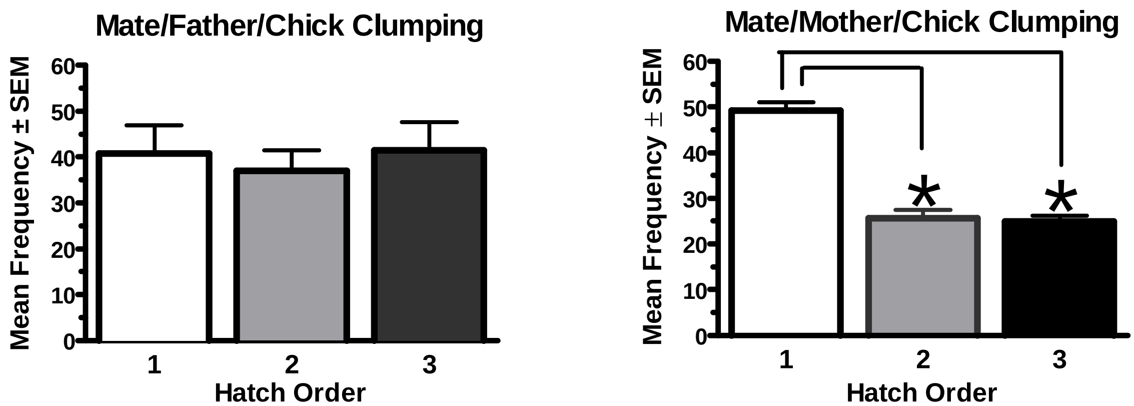


Figure 11: Mean frequency of parents clumping behavior with mate on one side and chick on the other in relation to hatch order. *=p<0.05

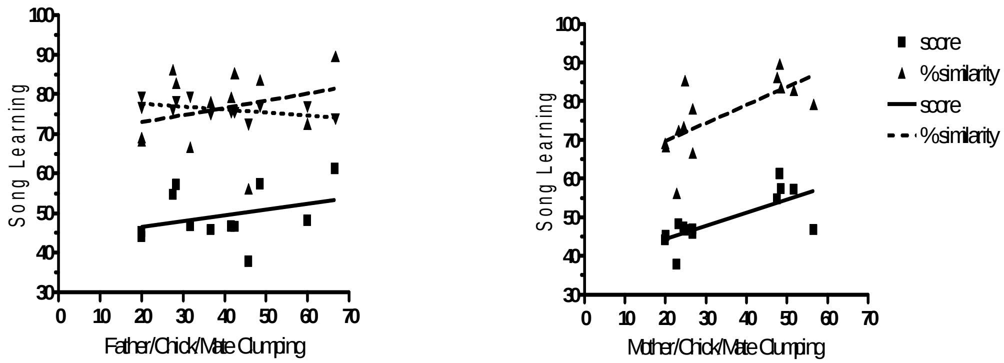


Figure12: Song learning of chicks in relation to the frequency of clumping with a parent while the parent is also clumped with the mate.

Digit ratio	Score	% Similarity	Mean Accuracy	Sequential Match
2:3	p=0.84 r^2=0.00	p=0.94 r^2=0.00	p=0.70 r^2=0.00	***p=0.0065* r^2=0.22
2:4	p=0.26 r^2=0.03	p=0.78 r^2=0.00	p=0.66 r^2=0.00	p=0.57 r^2=0.01
3:4	p=0.99 r^2=0.00	p=0.65 r^2=0.00	p=0.88 r^2=0.00	p=0.22 r^2=0.05

Table 1. Multiple linear regressions of digit ratios of males and song parameters. N=32 for all ratios. *=significance after Sidak's adjustment. Digit ratio 2:3 model $F_{1,31}$=8.565, p=0.006, adjusted r^2=0.196. Digit ratio 2:4 model $F_{1,31=}$0.998, p=0.423, r^2=0.00. Digit ratio3:4 model $F_{1,31}$=0.388, p=0.815, r^2=-0.086.

Days Post Hatch	Hatch Order	Clutch Size	# of Male Siblings	Sex	Adjusted r^2	ANOVA, F_{df}
0	p=0.579	p=0.982	p=0.891	p=0.345	0.069	p=0.852, $F_{4,37}=0.34$
5	*p=0.023	**p=0.005	p=0.475	p=0.727	0.410	p<0.001 $F_{2,40}=15.58$
10	***p<0.001	p=0.360	**p=0.003	p=0.591	0.3	p=<0.001 $F_{2,69}=16.24$
15	p=0.211	p=0.841	p=0.321	p=0.952	0.011	p=0.503 $F_{4,56}=1.01$
20	p=0.959	p=0.999	p=0.982	**p=0.016	0.097	p=0.016 $F_{1,48}=6.27$
25	p=0.727	*p=0.04	p=0.562	p=0.219	0.056	p=0.04, $F_{1,56}=4.40$
30	p=0.292	*p=0.027	p=0.224	p=0.534	0.076	p=0.027, $F_{1,50}=5.21$
35	p=0.357	***p=0.001	p=0.543	p=0.243	0.184	p=0.001, $F_{1,51}=12.70$
90	p=0.964	p=0.08	p=0.559	p=0.771	0.020	p=0.263 $F_{4,65}=1.35$
Fledge	p=0.219	p=0.465	p=0.370	p=0.844	0.018	p=0.340 $F_{4,33}=1.14$

Table 2: Multiple linear regression results for factors affecting the weights of chicks at key developmental days. Adjusted r^2 is after stepwise removal of non-contributing variables.

	%Similarity	Mean Accuracy	Sequential Match	Overall Score
Weight (g) day 10	p=0.079	**p=0.029*	**p=0.03*	p=0.73
Hatch Order	p=0.336	p=0.157	p=0.189	p=0.783
Clutch Size	p=0.908	p=0.481	p=0.063	p=0.689
# of Male Siblings	p=0.958	p=0.545	p=0.96	p=0.456
ANOVA $F_{df,}$ Adj r^2	p=0.659, $F_{4,16}$=0.614 r^2-0.133	p=0.018, $F_{4,16}$=4.085 r^2=-0.505	p=0.153, $F_{4,16}$=1.94 r^2=0.158	p=0.885, $F_{4,16}$=0.282 r^2=-0.168

Table 3: Multiple linear regression for factors affecting song learning. Adjusted r^2 is after stepwise removal of non-contributing variables. *=p<0.042 after Sidak's adjustment.

Parameters of begging	Hatch Order	Clutch ID	Clutch Size	# of Male Siblings	Sex	Adjusted R^2	ANOVA, F_{df}
Amp	*****_p=0.019_	p=0.992	p=0.941	p=0.729	p=0.988	0.182	p=0.019 $F_{1,40}$=5.977
Var Amp	*_p=0.016_	p=0.633	p=0.831	p=0.691	p=0.977	0.040	p=0.040 $F_{6,35}$=1.284
Entr	p=0.112	p=0.988	p=0.141	p=0.778	p=0.946	0.037	p=0.299 $F_{6,35}$=1.263
Var Entr	p=0.331	p=0.451	p=0.053	p=0.592	p=0.896	0.137	p=0.08 $F_{6,35}$=2.083
Freq Mod	p=0.584	p=0.940	p=0.055	p=0.383	p=0.432	-0.009	p=0.479 $F_{6,35}$=0.941
VFM	p=0.375	p=0.248	p=0.716	p=0.545	p=0.563	0.045	p=0.275 $F_{6,35}$=1.318
Pitch	p=0.999	p=0.909	p=0.816	p=0.072	p=0.190	0.52	p=0.230 $F_{5,36}$=1.451
Var Pitch	p=0.328	p=0.557	p=0.565	p=0.198	p=0.778	-0.044	p=0.643 $F_{6,35}$=0.711
# Calls/15	p=0.219	p=0.320	p=0.574	p=0.602	p=0.568	-0.043	p=0.653 $F_{5,36}$=0.664

Table 4: Multiple linear regression of the acoustic characteristics of begging calls of EH and LH old chicks recorded at days 10-11. *p<0.05.
Amp=amplitude, Var amp=variable amplitude, Entr=entropy, Var Entr= variable entropy, Freq Mod=frequency modulation, VFM= variable frequency modulation, Var pitch= variable pitch, #calls/15= the number of calls within the 15 seconds of the start of begging.

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