EATING LIKE A BIRD:
LONG TERM EFFECTS OF NUTRITIONAL STRESS

Mariam Honarmand
Ph.D. Thesis

Department of Animal Behaviour
Faculty of Biology
Bielefeld University
Germany

Supervision:

Prof. Dr. Marc Naguib
Netherlands Institute of Ecology (NIOO-KNAW)
P.O. Box 40
6666 ZG Heteren
The Netherlands

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Background

The interactions between environmental conditions and the expression of genes determine the phenotype of an organism. The period from conception to maturity is crucial for an individual's trajectory and especially the early pre- and postnatal environment has been shown to have profound impact (LINDSTROM 1999). Developing birds are in a vulnerable position when conditions fall off and individuals' trajectory can be severely affected in terms of growth, physiological properties, reproductive performance and survival (BIRKHEAD *et al.* 1999; BLOUNT *et al.* 2006; BOAG 1987; BRUMM *et al.* 2009; CRISCUOLO *et al.* 2008; KILNER 1998; NAGUIB and GIL 2005; NAGUIB *et al.* 2008; SPENCER *et al.* 2004). Yet, under natural conditions short periods of nutritional stress will be prevalent and environmental perturbations can be abrupt and are far from being reliably predictable.

Phenotypic plasticity is the adaptive response of competitive animals adjusting to environmental constraints or superabundance. It has been suggested that individuals, as a response to suboptimal conditions, are able to differentially allocate resources into growth of morphological structures according to their importance for ensuring survival and reproductive success (KITAYSKY 1999). Additionally a programming mechanism has been proposed*; the thrifty phenotype hypothesis* (BATESON *et al.* 2004; HALES and BARKER 2001; HALES and OZANNE 2003). Thereby providing a theoretical framework to explain how adaptive responses can lead to an altered postnatal metabolism which improves the chance of survival and reproduction on a short scale but bears consequence if there is a great mismatch between early and late developmental circumstances.

It is important to understand the mechanisms and factors underlying individual's ability to cope with environmental challenges. Such epigenetic influences can transfer into following generations with and even without altering genes (GOLDBERG *et al.* 2007) having important implications for sexual selection and the evolution of elaborate traits (DARWIN 1871).

The zebra finch (*Taeniopygia guttata*) as model species

Song birds have been a key model for studying effects of developmental stress as their chicks undergo an extremely fast development during the first weeks when they stay in the nest and are entirely dependent on the parents feeding regime (altricial). Contrary to mammals, birds have limited possibilities to buffer their young against harsh environmental conditions (altricial and precocial). As the egg membranes and the shell enclose the embryo all essential components for development have to be packed in albumen and yolk during egg formation. Egg resources are costly, and strongly influence nestling performance (GIL 2003; NAGER *et al.* 2000; WILLIAMS 1994). The zebra finch is an opportunistic breeder with biparental brood care, typically raising several broods during one rainy season (ZANN 1996). Survival rates are relatively low and successful reproduction for more than two breeding cycles is not the norm (ZANN 1996). Pairs form lifelong bonds and remain together during the non-breeding season. Therefore, harsh environmental conditions, such as droughts will likely affect both partners simultaneously. Zebra finches are easily bred and kept in captivity and they reach sexual maturity early. These are only a few aspects on a considerably long list, which made zebra finches become an ideal model for studying short and long term effects of developmental stress under controlled conditions. Zebra finches belong to the order of perching birds (*Passeriformes*), suborder of *Oscines*, the true song birds. Songs are only produced by the males even though females vocalize by species specific calls within the colony (ZANN 1996). Song is crucial for birds to survive and to reproduce and it is particularly interesting in regard to its underlying cultural component.

Vocal production and perception learning

There are only few mammalian vocal production learners (humans, pinnipeds, cetaceans and some chiroptera) in contrast to the thousands of bird species (parrots, perching birds, hummingbirds). Bird song is a complex acoustic signal with strikingly similar basic phenomenology to human speech during the process of learning (DOUPE and KUHL 1999). This rendered birds to become a very valuable model system for studying the underlying neural substrates (BRAINARD and DOUPE 2000). Vocal learning in songbirds is a two-step process. Song memorization (during the sensory phase) is followed by rehearsal and subsequent shaping of singing (during the sensorimotor phase) by referencing vocal feedback to the song memory (CATCHPOLE and SLATER 1995; DOUPE and KUHL 1999; KONISHI 1985). Both the memorization stage and the motor production or imitation stage have sensitive periods, which determine the timing when each type of learning is more likely to occur. In the zebra finch, both periods overlap (BRAINARD and DOUPE 2000; IMMELMANN 1969) (Fig.1).

Pronounced sex differences in song usage exist among species, but vocal learning in female songbirds has been so little studied that only a few years ago it was not certain whether sex differences apply as well to song learning (RIEBEL 2003). However, bird song is a communication system with a sender and a receiver and even though most research so far has focused on males, song usage in inter- and intra-sexual communication implies females to be as apt in learning and discriminating as males (RIEBEL 2003; RIEBEL *et al.* 2005). Receivers, may it be rivals which base their decision about starting a fight or may it be females who base their decision about choosing the singer as their mate, could use the song to assess the senders´ quality. Such perceptual fine tuning does require early exposure to song (NEUBAUER 1999; RIEBEL 2000; STURDY *et al.* 2001). However, it is not entirely clear whether such fine tuning requires learning and whether it leads toward narrowing or broadening females´ adult preferences (RIEBEL 2003). We still question what underlay and maintain the observed variation in female preferences (BADYAEV and QVARNSTROM 2002; JENNIONS and PETRIE 1997) and should be eager to understand more about developmental influences on female preferences (HOLVECK and RIEBEL 2006).

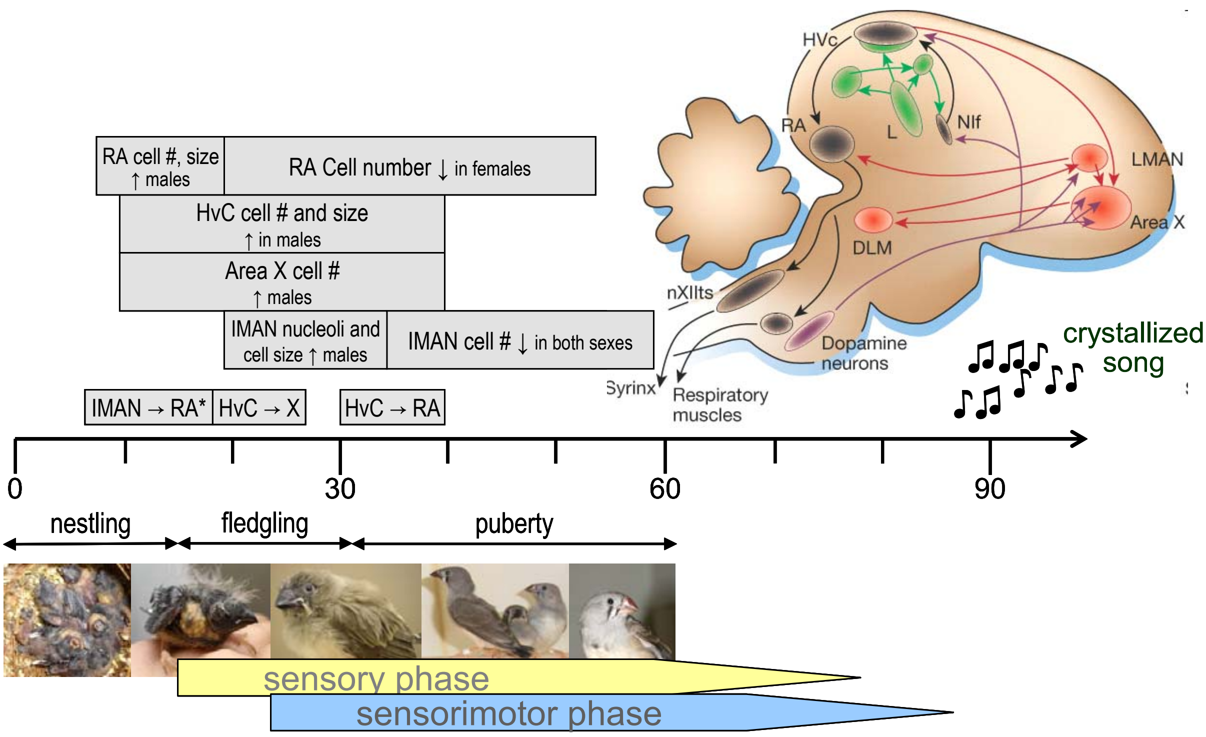

Fig. 1. Timelines for song learning and development of neural projections within the song system. Zebra finches develop rapidly, and their two phases of song learning overlap to a great extent. The initial vocalizations, or *subsong*, produced by young birds are variable and generic across individuals. Subsong gradually evolves into *plastic song*, which remains highly variable from one rendition to the next, but begins to incorporate recognizable elements of tutor songs. Plastic song is progressively refined until the bird *crystallizes* its stable adult song at ~100 days. The schematic sagittal drawing of the bird brain shows projections of major nuclei in the song system. The motor pathway (black) controls song production and includes the higher vocal centre (HVc) in the neostratum and the robust nucleus of the archistriatum (RA). RA projects to the tracheosyringeal portion of the hypoglossal nucleus (nXIIts), which controls the bird's syrinx and to nuclei involved in control of respiration during song. Additional nuclei afferent to HVc, including the nucleus interfacialis (NIf), are likely to be part of the motor pathway. HVc sends a second projection to the anterior forebrain pathway (AFP, red). The AFP includes Area X, the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (LMAN; a frontal cortex-like nucleus). LMAN sends a projection back into the motor pathway at the level of RA. Area X, LMAN, HVc and NIf receive midbrain dopamine input (purple). The Field L is the avian primary forebrain auditory area and projects to a complex network of higher auditory areas (green), including the caudomedial neostriatum and caudal portion of the ventral hyperstriatum (not shown). Auditory inputs likely enter the song system at the level of NIf and possibly HVc (after BRAINARD and DOUPE 2002, TOMASZYCKIE *et al* 2009.

Developmental influences on song

The handicap principle predicts elaborate traits to be costly in order to maintain reliability of signals (ZAHAVI 1975, 1977). Hence, a sexually selected trait should indicate the overall quality of an individual. Bird song is sexually selected and its variation is suggested to be largely caused by cultural inheritance. Early developmental constraints are hypothesized (*nutritional stress hypothesis*) to affect song learning, by impairment of the underlying neuronal structures (LEITNER 2007; NOWICKI *et al* 1998). A discrete network of interconnected nuclei, the song system, regulates song acquisition and production (Fig. 1). Additionally, the auditory song circuits appear to take part in song perception and discrimination both in males and in females (DEVOOGD 2004). The number, size, and connectivity of song-related neurons undergo dramatic changes during the periods of song learning (BOTTJER *et al.* 1986; KONISHI and AKUTAGAWA 1985; NORDEEN and NORDEEN 1988a; NORDEEN and NORDEEN 1988b), which in male zebra finches extends from 10 to 65 days post hatching (BRAINARD and DOUPE 2002; EALES 1985; IMMELMANN 1969). Not long ago, neurogenesis within the avian brain was thought to be limited to embryonic stages but nowadays, we consider even the adult brain to be considerably plastic, in terms of seasonal morphological changes and the birth of neurons (BRENOWITZ *et al.* 1997). There is a growing awareness that the brain is neither insensitive nor generally buffered against environmental influences. Neurogenesis and neuronal recruitment have been shown to be sensitive to experience, to environmental cues and particularly nutrient availability is a potential crucial factor (BAGNYUKOVA *et al.* 2008; CORNIOLA *et al.* 2008; INNIS 2008; MCGOWAN *et al.* 2008; PALMER *et al.* 2008; POGRIBNY *et al.* 2008). However neural costs remain a constant debate (GIL and GAHR 2002) and empirical evidence testing the nutritional stress hypothesis is ambiguous. A number of studies did show effects of early developmental constraints on male song characteristics (BRUMM *et al.* 2009; HOLVECK *et al.* 2008; SPENCER *et al.* 2003) even though there was no overall concordance about which parameters were affected. Furthermore, another study did not find an effect at all (GIL *et al* 2006). However, an influence in song parameters does not necessarily relate to impaired learning abilities, which is of course a fundamental prerequisite of the hypothesis (HOLVECK 2008). The sensitive phase for song learning goes well beyond the nestling and fledgling phase (Fig. 1) and this may bring important further implications. The second month in life bears a whole array of physiological and social challenges, which partly differ to those experienced during earlier periods. Until now nobody has addressed the question whether and to what extent challenges during later periods influence song learning. The period of early independence may be particularly revealing as birds have to master environmental and social constraints on their own. Selection pressure is high during that time in the wild and results in low survival rates (ZANN 1996). Traits that develop during this period will mirror the ability to cope with deleterious circumstances and the capacity to acquire resources. Social interactions between conspecifics are crucial in shaping various aspects of song learning: the timing of the sensitive phase for song learning, the accuracy of song copying and the selection of song tutors (BAPTISTA and PETRINOVICH 1984; EALES 1985; PAYNE 1981; SLATER and MANN 1990; TCHERNICHOVSKI and NOTTEBOHM 1998).

Aim and outline

The aim of this study was to assess the implications of dietary variation during important developmental phases on phenotypic plasticity and performance in zebra finches, *Taeniopygia guttata*. Within my study I conducted experiments for a comparative analysis between the nestling and fledgling phase as well as considering the second month (*puberty*) as important developmental period. The first month after hatching is very influential for an individuals' trajectory but it remains unclear whether shorter, biologically more meaningful periods of constraints during early development entail profound consequences. Nestlings and fledglings encounter a variety of physiological, social and behavioural challenges of which the magnitude appears to be very different between the two developmental phases. Furthermore, the period when secondary sexual characters develop, might also profoundly shape fitness relevant components. High selection pressure acts on this early period of independence, puberty, encompassing low survival rates in the wild. Traits that develop during this phase will mirror the capacity to acquire resources and the ability to cope with deleterious circumstances. During puberty, gonad maturation and the expression of secondary sexual ornaments coincides with the sensitive phase for song learning. The cultural transmission process of song learning exceeds beyond the first and even the second month in life and parallels the synchronized development of the underlying neuronal structures for song learning. Hence, this study clarifies the implications of different developmental phases on an individual's trajectory by considering short and long term effects and their consequences for shaping evolutionary processes.

In **chapter 2**, I tested the hypothesis that nestlings are more susceptible to nutritional constraints than fledglings. I conducted a breeding experiment in which nestling (until day 17) or fledgling (day 17 – 35) subjects were raised under different nutritional conditions, either on a low or a high quality diet. Three experimental treatment groups (low-high (LH), high-low (HL) and high-high (HH) received the diets in different orders including a swap at day 17 for two groups. Long and short term effects on biometry, physiology, male plumage ornaments and on survival probability were analyzed to ascertain whether proposed differential implications lead to variation in phenotypic plasticity.

In **chapter 3**, I tested the hypothesis that sexual attractiveness and reproductive performance in adulthood interacts with early nutritional constraints and assessed the magnitude of implications from epigenetic parental variation on the subsequent generation. After reaching sexual maturity I tested attractiveness of subjects from different nutritional backgrounds (LH, HL and HH; chapter 2) in a two way choice aviary. Reproductive competence was analyzed by conducting a subsequent breeding experiment in which subjects were mated to a partner from standard nutritional background. I tested offspring quality of mothers and fathers separately to account for sex-specific variation of parental effects and analyzed changes in offspring sex ratio from hatching until nutritional independence.

In **chapter 4**, I tested the hypothesis that song, a culturally transmitted, sexually selected trait, reflects the nutritional conditions experienced during early independence (*puberty)*, i.e. the time during which these traits develop. Subjects were exposed to qualitatively different nutritional treatments from day 35 until day 65 during song tutoring by an adult conspecific. After reaching sexual maturity I tested influences on song and auditory competence. Male song parameters and song learning abilities were analyzed by combining two different methodological approaches: visual comparisons of spectrograms and automated song similarity measurements via SAP Pro. I tested the hypothesis that interactions between nutritional deficits and receiver learning contributes to variation in female preference. Two different elegant, active choice paradigms allowed testing female song preferences by minimizing coincidental effects as social approach or potential side preferences. Due to its cultural transmittance process, song learning is greatly influenced by social stimuli which can greatly shape song learning, song output and perception learning. Therefore, a particular emphasis was laid on the influences of social circumstances during the experimental period.

In **chapter 5** the obtained results are summarized and discussed.

CHAPTER 2

FASTING IN NESTLINGS AND FLEDGLINGS: DIFFERENTIAL EFFECTS ON BIOMETRY AND PHYSIOLOGY

Mariam Honarmand[1], Wolfgang Goymann[2], Marc Naguib[1,3]

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[1] Department of Animal Behaviour, University Bielefeld, PO Box 100131,
33501 Bielefeld, Germany

[2] MPI für Ornithologie, Abteilung für Verhaltensneurobiologie, Eberhard-Gwinner-Str. Haus 6a, 82319 Seewiesen, Germany

[3] Department of Animal Population Biology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 40, 6666 ZG Heteren, The Netherlands

Author contributions:

MH & MN designed the experiments, WG took care of the corticosterone extractions, MH performed all experiments, analyzed the date, wrote the manuscript.

ABSTRACT

Phenotypic plasticity is the adaptive response of competitive animals adjusting to environmental constraints or superabundance. Changes in natural conditions can be abrupt and are far from being reliably predictable. Developing birds are in a vulnerable position when conditions fall off and individuals' trajectory can be severely affected in terms of growth, reproductive performance and survival. Yet, under natural conditions short periods of nutritional stress will be prevalent. Individuals may respond differently, depending on the period of development during which nutritional stress is experienced. Here, we investigated consequences of dietary restrictions during the nestling and fledgling period of zebra finches (*Taeniopygia guttata*) on biometry, physiology, the expression of sexually selected male ornaments and survival. As nestlings (day 0 - 17) or fledglings (day 17 – 35), subjects were raised under different nutritional conditions, either on a low or a high quality diet. Biometric measurements, blood samples to assess glucocorticoid titers and photographs to analyze male cheek patch expression were taken at different time points throughout development. The results revealed that early nutritional conditions affected nestlings' but not fledglings' morphology (except from body mass) and showed females to be more vulnerable than males. Nutritional fluctuations were physiologically stressful for growing chicks as baseline corticosterone titers were significantly elevated in dieting subjects. Even though nestlings showed substantial compensatory growth rates there were no long term consequences on morphology, male cheek patch expression or survival. Our results show that it is important to consider the entire trajectory of an individual to adequately assess phenotypic variation and its adaptive value. This study enlightens the mechanisms underlying phenotypic plasticity by considering the dynamic interactions among developmental, hormonal and environmental factors.

INTRODUCTION

A key period for vertebrates is the early development, when the young organism experiences a phase of substantial growth. At that early stage, biosynthetic and growth rate are both by far steeper than later in life and consequently a high energy and adequate nutrient intake is required (HARPER and SKINNER 1998). Among vertebrates, birds have been a key model for investigating effects of environmental conditions during early development (BATESON *et al.* 2004; BIRKHEAD *et al.* 1999). Unlike lactating mammals, birds cannot use own body resources to buffer their offspring against harsh nutritional conditions. Therefore an altricial hatchling, depending entirely on the feeding regime of its parents, can face serious problems when environmental conditions fluctuate or fall off. As a consequence, reproductive decisions and breeding should be timed with respect to peaks of food availability matching the period of offspring growth and development. However, optimal timing can be difficult, because fluctuating nutritional conditions, transient changes in climate, predator and competitor abundance are by far entirely predictable. Offspring may be confronted with poor conditions during important developmental periods. For instance, reproducing early in the season can entail fitness benefits for parents as they might be able to raise a second clutch or even their offspring might be able to reproduce within the same term (GUSTAFSSON and SUTHERLAND 1988; VERHULST *et al.* 1997; ZANN 1994). However, breeding too early may bear the risk of low initial and high subsequent food availability. Contrary, breeding late in the season likely results in the opposite scenario where a period of high food availability during the nestling phase is followed by a period of declining food abundance during the fledgling phase. Considering the changes in biometry and physiology nestlings compared to and fledglings undergo and additionally accounting for social and environmental aspects: these two phases differ substantially. Yet, little is known whether effects of nutritional shortage during these stages differ. In most experimental studies on birds, subjects were exposed to a stressor for a prolonged period such as one month post hatching via manipulating either brood size (DE KOGEL and PRIJS 1995; GIL *et al.* 2004; HOLVECK and RIEBEL 2006; VERHULST *et al.* 2006), nutritional conditions (BIRKHEAD *et al.* 1999) , or giving increased doses of corticosterone (SPENCER *et al.* 2003). For a growing individual, one month can be a certainly long period and shorter periods of constraints might in many cases be ecologically more relevant. It has been suggested that individuals, as a response to suboptimal conditions, are able to differentially allocate resources into growth of morphological structures according to their importance for ensuring survival and reproductive success (KITAYSKY *et al.* 1999). This adaptive strategy has been not only shown to bear significant fitness costs in terms of greater mortality (AIHIE SAYER *et al.* 2001; BIRKHEAD *et al.* 1999) but it might also be restricted to critical windows. Periods of brain growth or development of homoeothermy may be more vulnerable to variation in quantity or quality of food than e.g. periods of functional maturation of muscles and may therefore shape an individuals phenotype to a greater extent (BATESON *et al.* 2004). A few studies have shown that nutritional constraints during the nestling period bear long term effects for an individual (ARNOLD *et al.* 2007; BLOUNT *et al.* 2003a; BLOUNT *et al.* 2006); but only a recent study has investigated effects of dietary variation during the nestling and fledgling period (CRISCUOLO *et al.* 2008). CRISCUOLO *et al.* (2008) showed that dietary induced differences in growth trajectories had a long term effect on adult metabolic rate. Such long term effects may well be linked to immediate physiological stress responses during the period of food restriction. Their study demonstrates an individual's ability to compensate for unfavorable conditions at the same time suggesting catch-up growth to bear negative effects which may only show later in life (CRISCUOLO *et al.* 2008; FISHER *et al.* 2006; METCALFE and MONAGHAN 2001; MONAGHAN *et al.* 2008).

Resource limitations as a shortage of parentally provided food, impose energetic stress for a developing vertebrate (SOCKMAN and SCHWABL 2001) and can consequently elevate corticosterone secretion (DELAMORA *et al.*

1996; HARVEY *et al.* 1980; KITAYSKY *et al.* 1999). Subtly elevated corticosterone levels were shown to have a stimulatory effect on e.g. the immune response (reviewed in SAPOLSKY *et al.* 2000) and cognitive abilities (PRAVOSUDOV and CLAYTON 2001) but have on the other hand also been shown to negatively affect the immune system (SAPOLSKY 1992) and reproduction (WINGFIELD 1984; WINGFIELD *et al.* 1995). Despite such contradicting results about permissive and suppressive effects of glucocorticoids, it is well documented that their secretion following a stressor is essential for an adequate behavioural and physiological response (reviewed in (SAPOLSKY *et al.* 2000). Some studies suggest that nestling birds may indeed exhibit adequate adjustments in response to stressful situations (SCHWABL 1999; SIMS and HOLBERTON 2000) but experimental evidence for an up regulation in stress hormone titres as a response to nutritional conditions is missing in passerines. A demanding and stressful period, as catch-up growth, might consequently result in elevated corticosterone levels. Individuals should trade off short term advantages and long term cost of high corticosterone profiles as costs might be have to be paid much later in life. Therefore, it might be an adaptive strategy to circumvent high corticosterone profiles.

Here we studied whether zebra finch (*Taenopygia guttatta*) nestlings and fledglings are differentially susceptible to unfavourable conditions during their first month in life. To test the hypothesis that nestlings are more vulnerable than fledglings we exposed offspring to qualitatively different diets during a breeding experiment. Diets differed mainly in protein availability. We measured offspring biometry throughout development and determined basal corticosterone level, to assess physiological stress responses from nestlings and fledglings exposed to the different nutritional treatments. Moreover, we quantified male cheek patch expression to reveal possible differences in the timing or quality of moulting sexually selected ornaments. As delayed plumage maturation has been observed in a variety of species, we predicted males raised under constrained nutritional conditions to show less pronounced sexual plumage ornaments compared to males of a control group at the onset of maturity. A survival analysis analyzed potental long term costs.

METHODS

Subjects

85 pairs of zebra finches of wild Australian origin (about F8 generation of wild caught birds; for genetic data see FORSTMEIER (2007)) from the laboratory colony in Bielefeld were given the opportunity to breed under standardized conditions. Prior to the experiment and afterwards, all birds were kept in mixed groups of 10 to 30 individuals in large indoor aviaries (size 1 x 3 x 3.3 or 0.9 x1.8 x 1.9 m). Females were transferred one by one to their breeding cage (83 x 30 x 39.5 cm) and allowed to acclimatize for two days. Then males were randomly assigned to females controlling for relatedness for at least 2 generations. A nestbox (12.5 x 12 x 14 cm) and nesting material were provided from day 2 onwards. Pairs received a standard seed mix, millet and water ad libitum and additionally a mixture of germinated seeds and commercial egg food daily. Greens were provided once per week. Temperature in the three breeding rooms were held between 23° and 26°C in a light regime of 14:10 (light: dark) with half an hour dusk and dawn period, respectively. Breeding rooms were exclusively used for housing breeding pairs of this experiment. Nests were checked daily between 0900 and 1100 hours to mark newly hatched chicks by cutting their down feathers. Out of 69 pairs, 23 pairs did not reproduce (12 pairs did not lay eggs; 11 pairs produced no hatchling) and 17 did not produce surviving fledglings within 170 days from onset of breeding. Pairs that had not raised any offspring within 170 days from onset of breeding were removed from their breeding cage and either randomly re-paired with another partner (6 new pairs of which 9 pairs were successful) or transferred individually to their home indoor aviary (size 1 x 3 x 3.3 or 0.9 x 1.8 x 1.9 m). Some pairs do not breed in part due to

males failing to build a nest but the proportion of successful broods was comparable to previous experiments. In total 34 pairs sired 108 fledglings.

Experimental treatment and housing

All cages were assigned to one of two nutritional treatments once their oldest offspring was 3 days old. The assignment followed a predefined order to assure a random and equal distribution of subjects across treatments. Nutritional treatments differed mainly in protein availability and are subsequently called LQ for low quality food and HQ for high quality food, respectively. The LQ treatment consisted of a dried seed mix and water ad libitum, which was enriched with vitamins (Veyx-Pharma, BioWeyxin - Multi-Mulgat) two times weekly. The HQ diet additionally comprised millet, a mixture of germinated seeds and commercial egg food (Cédé) daily. Two times weekly grated salads, greens, fruits and vegetables were provided for birds on HQ diet. The minimum energy requirement for growing passerines is five times that of the adult birds. Young chicks nearly double their weight over 48 hours, and require a protein level of 15-20 % (as oppose to an adult protein requirement of 10-14 %) from which a significant part is dedicated to tissue growth (HARPER and SKINNER 1998). Thus, standard seed diets which may be sufficient for adult energy maintenance represent a low quality treatment for growing chicks. Offspring received the two diets in different order resulting in three different experimental groups: LH (low-high), HL (high-low) and HH (high-high) (Fig. 1). Offspring in the LH group received: LQ from day 3 until day 17 and HQ from day 17 until day 35. Offspring in the HL group received: HQ from day 3 until day 17 and LQ from day 17 until day 35. A HH group experienced HQ food conditions from day 3 until day 35. Despite the logical appeal we had to refrain from a fourth treatment group experiencing low quality nutrition throughout the first month to keep statistical power due to sample size on a sensible level. On day 20 (mean age of brood) the father was removed from the breeding cage. At nutritional independence (day 35 of the youngest of a brood), offspring were transferred to mixed tutor groups (cage: 49 x 98 x 35 cm) for controlled song learning where all received a regular diet (intermediate between LQ and HQ).

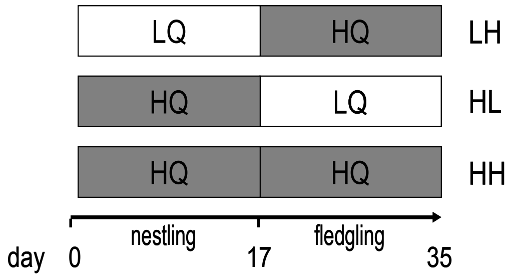

Fig. 1: Overview of nutritional treatments and time course. The three experimental groups received a low quality (LQ) or a high quality (HQ) rearing diet in different order during their first month post hatching. LH = low-high, HL = high-low and HH = high-high.

Biometric measurements and blood sampling

Biometric measurements and blood samples from offspring were taken at different time points throughout development: day0, d5, d10, d17, d35, d65, d280. Body mass was measured by using an electronic balance (Sartorius PT120 ± 0.01 g), wing length by measuring the maximum feather with a wing ruler (± 0.1 mm), and tarsus length was measured with a digital calliper slide (± 0.01 mm). Blood samples were taken at day 17 (mean age of a brood) and day 35 (youngest of a brood) from the brachial vein using heparinized capillary tubes within a time interval of 3 min for each individual. The sequence and duration of sampling for each individual was recorded. Samples were stored on ice immediately after sampling and centrifuged by ~6000g at 4°C for 10 min. Plasma was separated from the cellular fraction and stored by -70°C for hormone assays. Debris was dissolved in PBS+NaAc to inhibit conglomeration and stored at 4°C for DNA-sexing.

Corticosterone measurements

Corticosterone concentration was determined by direct radioimmunoassay (RIA, following (GOYMANN *et al.* 2006)). Plasma samples were extracted with dichloromethane (DCM) after overnight equilibration (4°C) of the plasma with 1500 dpm of tritiated corticosterone ([1,2,6,7-^{3}H], Perkin Elmer, Wellesley, MA, USA). The organic phase was then separated from the aqueous phase by plunging the extraction tubes into a methanol-dry ice bath (holding the tubes at an angle of approximately 60 and rotating them so that the water phase freezes to the side wall of the tube) and decanting the dichloromethane phase into a new vial. This extraction step was repeated twice to increase extraction efficiency. Then, the DCM phase was dried under a stream of nitrogen at 40°C, dried samples resuspended in phosphate buffered saline with 1 % gelatine (PBSG) and left overnight at 4°C to equilibrate. Then, an aliquot (80 µl) of the redissolved samples was transferred to scintillation vials, mixed with 4 ml scintillation fluid (Packard Ultima Gold) and counted to an accuracy of 2-3 % in a Beckman LS 6000 β-counter to estimate individual extraction recoveries. The remainder was stored at -40°C until RIA was conducted. Mean ± sd extraction efficiency for plasma corticosterone was 89.0 ± 1.2 %. For the RIA a standard curve was set up in duplicates by serial dilution of stock standard corticosterone ranging from 1.95 -1000 pg. Corticosterone antiserum (B3-163, Esoterix Endocrinology, Calabasas, CA, USA) was added to the standard curve, the controls and to duplicates of each sample (100µl). After 30 min corticosterone label (15000 dpm) was added and the assay incubated for 20 hours at 4°C. Then, bound and free fractions were separated at 4°C by

adding 0.5 ml dextran-coated charcoal (4 g charcoal (Sigma C-5510) in 1l PBSG assay buffer with 2 ml dextran solution). After 14 min incubation with charcoal samples were spun (3600 g, 10 min, 4°C) and supernatants decanted into scintillation vials at 4°C. After adding 4 ml scintillation liquid (Packard Ultima Gold) vials were counted. Standard curves and sample concentrations were calculated with Immunofit 3.0 (Beckman Inc. Fullerton, CA), using a four parameter logistic curve fit. The lower detection limits of the standard curves was determined as the first value outside the 95% confidence intervals for the zero standard (B_{max}) and was 5.9 pg/tube (day 17) and 7.3 pg/tube (day 35). Intra-assay coefficents of variation were 1.3% (day 17) and 3.5% (day 35). The inter-assay variation was 5.1%. If a sample concentration was below detection limit its value is set at the respective detection value.

There can be methodological reasons for a value below detection limit as for example low sample volumes can obscure extraction. At sampling day 35 a high proportion of samples was below detection limits which cannot be simply attributed to low sample volumes as there was no correlation between sample volume and corticosterone. As the high proportion of values below detection limits obscured data analysis a post-hoc test, excluding these data was performed (see statistical analysis).

Plumage measurements

To compare male cheek patch development, all subjects were photographed at day 65 (mean; range 60 – 73 days) against a black board with a digital camera (Canon Ixus digital 30 or Kodak LS753 Zoom Digital Camera). Photographs were taken under standard light conditions with the camera mounted at a standard distance from the subject. All files were transferred to a PC and imported into Adobe Photoshop 7.0 (Adobe Systems Inc. San Jose, CA, USA). To further standardize measurements of cheek patches, we calculated a ratio of cheek patch size and beak size. The pigmented cheek area and the shape of the beak were determined by using the "magic wand" tool, which allows selection of consistently coloured areas (LEADER and NOTTEBOHM 2006; NAGUIB and NEMITZ 2007). By using the "histogram" function (DALE 2000; MCGRAW and HILL 2004) we analyzed the number of pixels within the marked areas. For each picture the pixels of cheek patch and beak were taken three times and for each individual at least two different pictures were analyzed. Therefore ratios refer to mean beak pixels (total of 6 measurements) divided by mean patch pixels (total of 6 measurements).

Statistical analysis

Data were analyzed by using the free software R (R DEVELOPMENT CORE TEAM 2006). Linear mixed effect models (PINHEIRO *et al.* 2006) were calculated for each response variable (nestling body mass gain, body mass, wing length, tarsus length and corticosterone concentration) with fixed effects: treatment, sex and brood size. Predictions for random effects were made for the exact age at sampling and the nest. For the models with corticosterone concentration, the sequence of blood sampling and individual's sampling time [s] was additionally included as fixed effects. The model analyzing differences in growth patterns had fixed effects: treatment group, day of measurement and brood size and random effects: individual subjects nested in nest. The most parsimonious model was selected by stepwise deletion of nonsignificant terms from the maximum model, which allowed interactions between fixed effects, by using model comparisons with Akaike's Information Criterion (AIC). Data were transformed accordingly when models residuals violated normal distribution as tested with Shapiro-Wilk normality test (BOX and COX 1964; VENABLES and RIPLEY 2002).. However, if normality assumptions were not met after transformation non-parametric Kruskal-Wallis Chi^2-Test was used to analyze treatment effects on response variables (wing$_{day65}$, wing$_{day280}$, male cheek patch, body mass gain, CORT$_{day35}$) or Wilcoxon-Test (CORTshift from day 17 – day 35). Data of corticosterone concentrations at day35 were further analyszed with a post-hoc LME in which only samples within assay's detection limits were considered. Non-parametric Spearman-Rank Correlations (S, r_s) tested variables relationships. Variance in cheek patch expression between the treatment groups was testes with Fisher F-Test for variance homogeneity. Survival analysis (Kaplan-Meier) was performed when subjects were 1000 days old. All offspring reaching the minimum age of 4 days (treatment started at day 3) were considered either reaching the age of 1000 days post hatching (censorship) or not (exact age at death [days]). Sample size in the statistical tests differ in some cases, as for some individuals not all measurements were available.

RESULTS

At day of hatching (d0), offspring body mass did not differ between groups (LME; : $F_{2,30} = 0.08$, $p = 0.93$). Within the nine days post hatching subjects raised on a high quality (HQ) diet exhibited a significantly steeper growth pattern than did individuals, that fed on a low quality (LQ) food ($LME_{body\ mass}$: $F_{1,58} = 0.75$, $p = 0.39$; day: $F_{1,496} = 1479.34$, $p < 0.0001$; treat*day: $F_{1,496} = 13.99$, $p = 0.0002$; $LME_{mass\text{-}gain}$: $F_{1,58} = 15.21$, $p < 0.0001$; day: $F_{1,421} = 83.18$, $p < 0.0001$; treat*day: $F_{1,421} = 30.24$, $p < 0.0001$; Fig. 2).

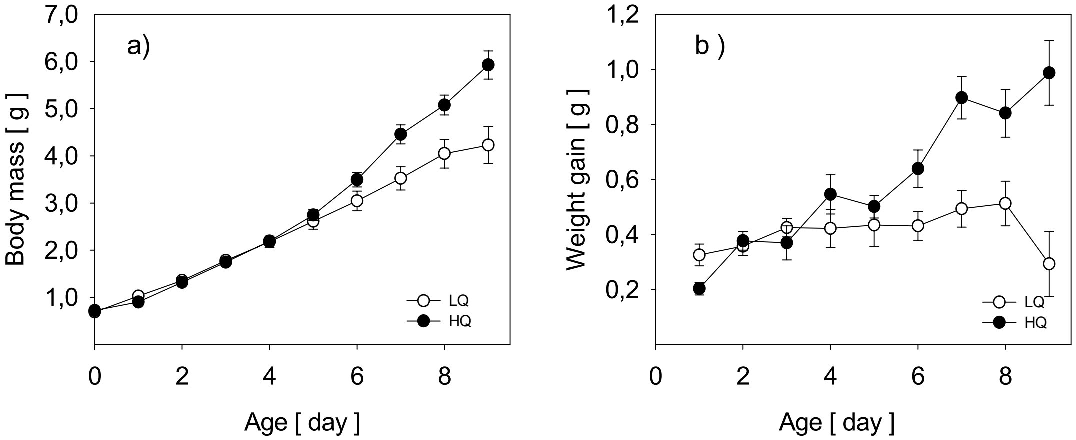

Fig.2: Nestling growth. Growth curves of zebra finch chicks which were reared either on a low quality (LQ) or a high quality (HQ) diet. Curves show **a)** body mass (mean ± s.e.) and **b)** body mass gain (mean ± s.e.).

At day 17 nutritional, treatment significantly affected all measured traits. Offspring raised under LQ conditions were lighter, had shorter wings and shorter tarsi than individuals which were raised on a HQ diet (Table 1, Fig.3a-c). There was a significant sex effect on tarsus) with females having shorter tarsi than males. This effect was independent of nutritional treatment.

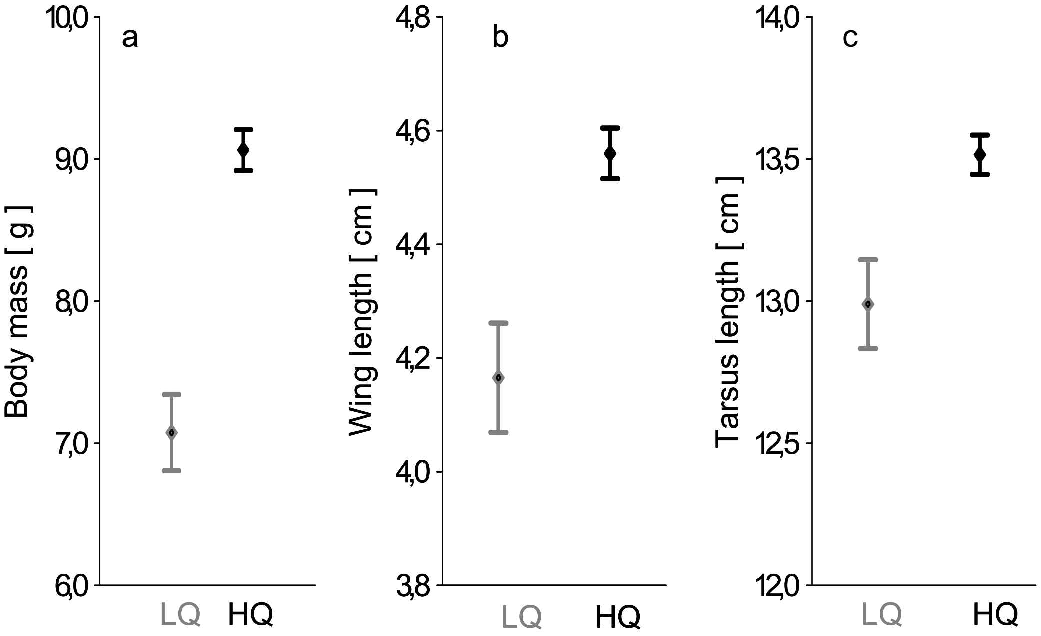

Fig. 3: Biometric measurements at day 17. Subjects were raised either on a low quality (LQ) or high quality (HQ) rearing diet. Depicted are means ± s.e. for biometric measuments **a)** body mass **b)** wing length and **c)** tarsus length.

Body mass was not different between groups at day 35 and females only tended to be lighter than males when feeding on LQ nutrition (Table 1). However, there remained a trend for individuals that experienced LQ conditions either as nestlings (LH) or as fledglings (HL) to weigh less than individuals in the HH group and the interaction between early dietary treatment and offspring sex was significant (posthoc LME: $F_{1,31} = 3.93$, $p = 0.056$; treat*sex: $F_{1,26} = 6.07$, $p = 0.021$; sex: $F_{1,26} = 3.41$, $p = 0.076$). Females had a lower mean body mass than males when experiencing LQ as nestlings (LH) or fledglings (HL). There was a significant treatment effect on catch-up

Table 1: Treatment effect on biometry (body mass, wing length, tarsus length). Tested with linear mixed effect models (LME) or Kruskal-Wallis Chi^2-Test (KW), when residuals did not meet normality assumptions. Upper case numbers indicate respective significant effects in the model.

	day 17 n = 106			**day 35** n = 96			**day 65** n = 95			**day 280** n = 97		
trait	**test**	**$F_{2,33}$**	**p**	**test**	**$F_{2,30}$**	**p**	**test**	**$F_{2,26}$**	**p**	**test**	**$F_{2,30}$**	**p**
body mass	lme	27.6	<0.001	lme	2.02	0.15 [2]	lme	1.57	0.225 [4]	lme	2.50	0.09
wing	lme	6.07	0.02	lme	4.54	0.02	KW	–	0.008	KW	–	0.88 [6]
tarsus	lme	6.87	0.01 [1]	lme	1.74	0.19 [3]	lme	0.44	0.65 [5]	lme	0.65	0.53

[1] sex: $F_{1,28}$ = 4.15, p = 0.051

[2] sex: $F_{1,25}$ = 3.37, p = 0.079; treat*sex: $F_{2,25}$ = 3.04, p = 0.066

[3] treat*sex: $F_{2,25}$ = 4.88, p = 0.016

[4] treat*sex: $F_{2,23}$ = 4.09, p = 0.030

[5] clutch size: $F_{4,26}$ = 2.90, p = 0.042

[6] sex: Chi_1 = 3.40, p = 0.064

growth, measured as body mass gain, from day 17 until 35 (Kruskal-Wallis: Chi^2 = 42.22, df = 2, p < 0.001; Fig. 4). Nutritional treatment neither affected skeletal nor wing growth in the HL group. The effects in the LH group on wing length that were present at day 17 persisted until day 35 whereas the effects on tarsus length were compensated for at day 35 (Table 1). Females had smaller tarsi than males when raised under LQ condition whereas females raised under HQ had longer tarsi than males.

At day 65 offspring which had experienced LQ diets either as nestlings (LH) or fledgling (HL) had compensated in body mass and tarsus length but the effect on wing length persisted (Table 1). The interaction between nutritional treatment and offspring sex was still significant, indicating that females, which fed on LQ nutrition, were lighter than males feeding on LQ nutrition whereas this pattern was not observed when chicks fed on HQ food. Treatment did not have an effect on body mass gain from nutritional independence until day 65 (Kruskal-Wallis: Chi^2 = 3.29, df = 2, p < 0.2 Fig. 4). As adults, at day 280, there were no main effects on biometric traits between subjects from the different treatment groups even though the interaction between nutritional treatment and offspring sex tended to be significant (Table 1). However, subjects that experienced a period of nutritional constraints during their first month in life, either as nestlings (LH) or fledglings (HL), were still lighter than subjects raised on HQ (posthoc LME: $F_{1,31}$ = 4.41, p = 0.044; sex: $F_{1,3}$ = 0.03, p = 0.88).

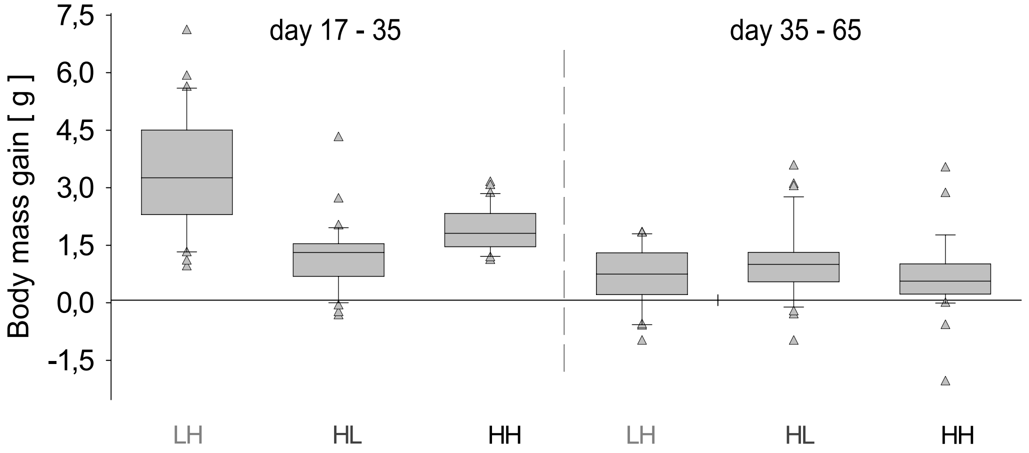

Fig. 4: Body mass gain from day 17 to 35 and from day 35 to 65. Shown are medians ± quartiles for change in body mass across the three experimental treatment groups.

Effects on basal corticosterone concentration

Nutritional treatment resulted in significantly higher basal corticosterone concentrations in the LQ group at day 17 with order of sampling being significant (LME: $F_{1,28} = 5.18$, $p = 0.031$, order of sampling: $F_{4,36} = 17.03$, p = < 0.001; Fig. 5). Variance in corticosterone concentration was to 33 % explained by sample volume at day 17 ($S = 39376.4$, $r_s = 0.34$, $p = 0.004$). At day 35 nutritional treatment did not show a significant effect on basal corticosterone (Kruskal-Wallis $Chi^2 = 3.00$, df = 2, $p = 0.22$; Fig. 6). In 68 % of the samples at day 35, the corticosterone concentration was below the detection limit (59% from LH, 55% from HL and 75% of the HH). This pattern can not be attributed to sample volume as there only tended to be a relationship with corticosterone concentration ($S = 38723.47$, $r_s = 0.23$, $p = 0.064$). Therefore a posthoc test was calculated with all samples within detection limits (LH = 9, HL = 10, HH = 6) showing a significant treatment effect on corticosterone basal level at day 35 (posthoc LME: $F_{2,16} = 3.85$, $p = 0.04$). None of the fixed factors was significant but all were retained in the final model as they explained a substantial part of the variation. There was an overall significant decrease in basal corticosterone concentration from day 17 to day 35 (Wilcoxon = 3993, $n_{day17} = 71$, $n_{day35} = 67$, $p < 0.001$; Fig. 5).

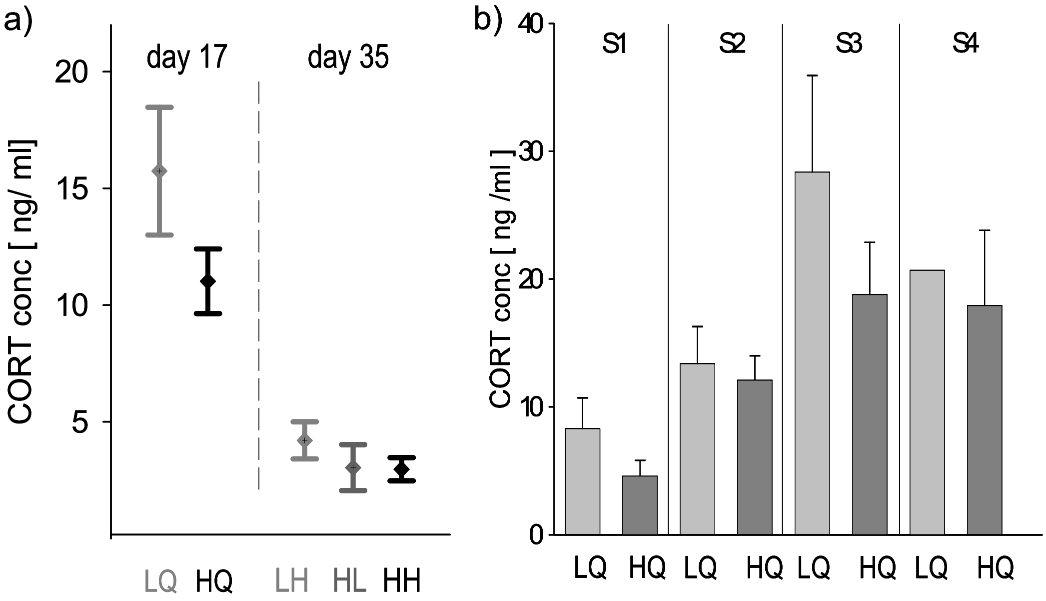

Fig. 5: Corticosterone concentrations. a) Mean corticosterone baseline titers ± s.e. at day 17 for subjects raised on low quality (LQ) or high quality (HQ) food and at day 35 when subjects experienced one of the three experimental treatments: low-high (LH), high-low (HL) or high-high (HH). **b)** Mean corticosterone baseline titers ± s.e. at day 17 across sampling sequence (S1-S4) for LQ and HQ subjects. As only one LQ subject was sampled at the forth position (S4) s.e. do not apply.

Effects on male plumage ornaments

Male cheek patch development did not differ significantly between subjects raised in different nutritional treatment groups (Kruskal-Wallis $Chi^2 = 2.32$, n = 44, df = 2, p= 0.31; Fig. 6). However, males raised under HH conditions tended to be more variable in their cheek patch size than males from the LH and HL group (Fishers-F = 2.97, $df_{num} = 11$, $df_{denom} = 14$, p = 0.059).

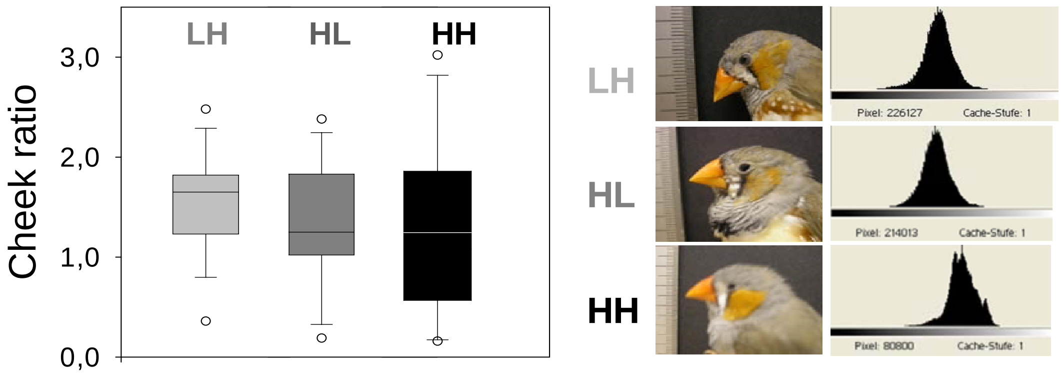

Fig. 6: Male cheek patch expression. a) Differences in cheek patch ratio between adult males raised either under low-high (LH), high-low (HL) or high-high (HH) treatments. **b)** Representation of an example for each treatment group: photo of a male and the pixel-histogram of the corresponding patch.

Effects on survival probability

A survival analysis showed that mean age at death was not significantly affected by nutritional treatment (Kruskal-Wallis Chi^2= 1.7, n = 128, df = 2, p = 0.44; Fig. 7). Within the first year, 16 LH, 12 HL and 9 HH birds died. Offspring's body mass at hatching and sex were no predictor for survival.

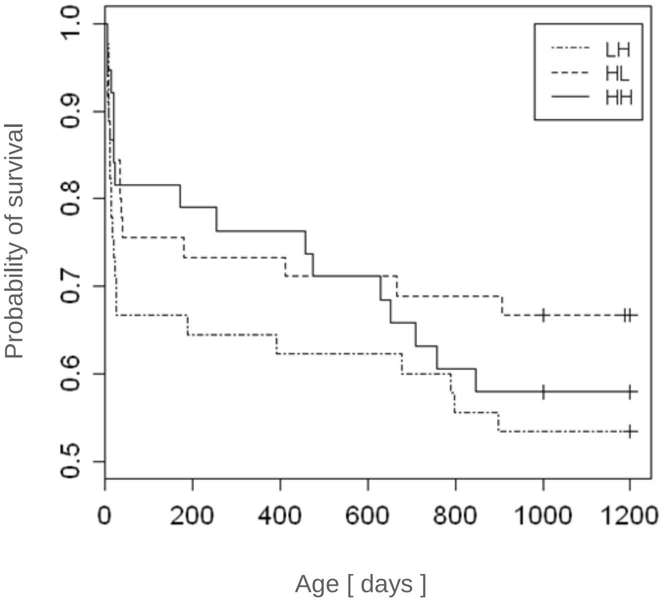

Fig. 7: Survival Analysis (Kaplan-Meier). Probability of survival for the three treatment groups: low-high (LH), high-low (HL) or high-high (HH) with censoring at day 1000.

DISCUSSION

The experiments revealed that zebra finches were more susceptible to unfavourable nutritional conditions as nestlings than they were as fledglings. A low quality (LQ) rearing diet during the nestling period (from day 17 – 35) significantly affected biometric traits and corticosterone profile. Contrary, fledgling growth and corticosterone profile at nutritional independence did not show such an interaction with nutritional treatment in the high-low (HL) group. Even though birds raised on low quality food as nestlings (LH) showed remarkable catch up growth in body mass and skeletal growth until nutritional independence we did not observe any long term consequences on male plumage ornaments or survivorship.

Experiencing low quality food as nestling compared to fledglings resulted in elevated basal levels of corticosterone and a steeper growth trajectory once conditions improved. Nestlings appear to have sufficient phenotypic plasticity to cope with such environmental stress. However, not only our results show females to be more vulnerable to fluctuations in diet quality (BRADBURY and BLAKEY 1998; KILNER 1998; MARTINS 2003; NAGUIB and GIL 2005; NAGUIB *et al.* 2006; RUTSTEIN *et al.* 2004b). Individuals raised on low quality food (LH, HL) were able to compensate for their bad start until adulthood, when subjects from the different treatment groups were not distinguishable. Our findings of differential responses by nestlings and fledglings to dietary constraints have implications for understanding adaptation of opportunistic breeders to fluctuations in food supply. Restricted periods of low food supply are common in natural situations and are more likely to occur than long term periods of low quality food, as applied by most studies. Therefore, to fully understand the scope and implications of phenotypic plasticity as a consequence of environmental challenges requires a broader range of studies varying the experimental stressor and its timing. The compensation in biometric and physiological traits, as shown here, nevertheless may carry subtle costs which might not be paid until later in life. Steeper growth acceleration by subjects which fed on low quality food as nestlings compared to subjects facing low quality nutrition as fledglings have indeed been

shown to carry physiological long term cost (CRISCUOLO *et al.* 2008; KRAUSE *et al.* 2009). CRISCUOLO *et al.* (2008) found an interaction between dietary induced growth acceleration and adult metabolic rate, which was measured after a long night (17 hours) of food deprivation. (KRAUSE *et al.* 2009) showed that even though resting metabolic rate was not affected, nutritional history resurfaced in body mass loss when subjects (same subjects as in our study) were challenged by a three hour food deprivation period. Such differences in adult metabolic rate and adult body mass loss might be causally linked to catch-up growth but both may also be linked to early physiological responses (VERHULST *et al.* 2006).

Our results reveal that dietary restrictions induced an elevation in corticosterone baseline levels at day 17 when nestlings were raised on LQ compared to HQ diet. Fledglings apparently were more robust and corticosterone basal levels at day 35 did not show a treatment dependent profile. However, a posthoc analysis revealed an effect at day 35. This is well in line with previous studies showing nutritional restrictions to impose physiological stress (KITAYSKY *et al.* 2001; PRAVOSUDOV and KITAYSKY 2006; SAINO *et al.* 2003) even though no such effect has been observed in a study with brood size manipulations (GIL *et al.* 2008). The positive relationship between order of sampling and circulating corticosterone in our study indicates a typical stress response due to the varying handling time for different positions within the sampling sequence (GIL *et al.* 2008; LOVE *et al.* 2003; SOCKMAN and SCHWABL 2001). Corticosterone is suggested to play an important role for adaptations to unpredictable environmental constraints (BLAS *et al.* 2006; KITAYSKY *et al.* 2003; KITAYSKY *et al.* 2001; MCNABB *et al.* 1998). However, additionally to the stress response the variation in corticosterone baseline titres appears to be associated with developmental status or age interacting with the nature of the stressor (SAINO *et al.* 2003) as the observed overall decrease in basal corticosterone between day 17 and day 35 suggests. A comparable pattern was recently reported in zebra finches, where corticosterone baseline and stress profile at day 16 and at the age of three month were compared (WADA 2008). Corticosterone concentrations between WADA (2008) and our experiment differ, which may be the result of the physiological differences between domesticated and wild type zebra finches (*Taeniopygia guttata*). Hence, differentiation between these distinct early developmental stages appears to be of emerging importance. Adrenal hormones regulate a multitude of homeostatic aspects and in a variety of species developmental transitions are accompanied or maybe even mediated by a shift in glucocorticoid levels (DUFTY *et al.* 2002). Corticosterone, the primary avian glucocorticoid, is elevated, compared to nestling and adult levels, during the fledgling period in a variety of bird species (captive canaries, *Serinus canaria*: (SCHWABL 1999); American kestrel, *Falco sparverius*: (SOCKMAN and SCHWABL 2001); pied flycatcher, *Ficedula hypoleuca*). Profiles around fledgling (at day 17), as shown here, might mirror the transition from nestling to fledgling. High corticosterone levels could not only ensure energy mobilization for enhanced motor activity but my also facilitate rapid learning and memory formation (PRAVOSUDOV and CLAYTON 2001). Even though LH nestlings showed a significantly higher corticosterone profile at day 17 compared to HH nestlings their levels had significantly decreased by day 35, when nutritional conditions had improved. Chronically high levels of corticosterone are known to potentially irreversibly damage the brain (KIM and YOON 1998) and it is argued that this might have comprehensive effects in the avian brain, given the substantial neurogenesis that can occur in adulthood in birds (NOTTEBOHM 2002).

‘Buying’ survival to reproduction at the expense of adaptations that are detrimental in old age is suggested to be a strategy with positive rather than negative evolutionary value in species with a short life expectancy (HALES and OZANNE 2003). However, our results do not indicate detrimental consequences resulting from catch-up growth or physiological challenges as probability of survival was not significantly affected by nutritional treatment, even though fewer individuals survived when they had received low quality food as nestlings (LH). Nevertheless, such costs might well be reflected in competition over access to resources in the wild or in breeding performance, a possibility that remains to be studied (Chapter 3).

Male cheek patches did not differ between treatment groups at day 65, showing that expression of these ornaments can be robust against a limited period of stress. Poor nutrition during the period of secondary sexual trait maturation has been shown to have a negative effect on the expression of sexually selected ornaments (NAGUIB and NEMITZ 2007) whereas poor nutrition during early development delivered contradicting results in either showing an interaction with the expression of sexually selected traits (BIRKHEAD *et al.* 1999; METCALFE and MONAGHAN 2001) or not (DE KOGEL and PRIJS 1995). The distinction between the nestling and fledgling phase is important and provides specific insights about how the maturation of sexually selected traits and the expression of ornaments that develop later in life, is linked to early developmental stages. Interestingly males raised under HQ condition tended to show more variability in patterns of plumage development after dietary treatments ended. Delayed plumage maturation (i.e. delayed acquisition of adult plumage by sexually mature birds) has been documented in many bird species (ROHWER and BUTCHER 1988; ROHWER *et al.* 1980; THOMPSON 1991). The moult constraint hypotheses (THOMPSON 1991) suggests that a delayed plumage maturation as a result of suboptimal environmental conditions might not necessarily be a strategy but rather the failure of subadults to overcome the costly production of ornamental displays (GUSTAFSSON *et al.* 1995; HILL 1996).

To conclude, our dietary treatment which significantly affected nestling biometry and stress hormone basal levels did not affect fledgling morphology except from body mass. The nestling phase appeared to be more susceptible to stress than the fledgling phase and effects are more pronounced in females than in males. Dietary restrictions in early development entail physiological stress for nestlings and likely for fledglings, an important link which was missing until now. Nevertheless a relatively short time period of adverse conditions as simulated in our experiments did not show persisting phenotypic effects and individuals appeared to have evolved mechanisms compensating for their bad start after reaching adulthood. Our results show that it is important to consider the entire trajectory of an individual to adequately assess phenotypic variation and its adaptive value to assess the mechanisms underlying phenotypic plasticity.

CHAPTER 3

FASTING IN NESTLINGS AND FLEDGLINGS: IMPLICATIONS ON ATTRACTIVENESS AND FECUNDITY

Mariam Honarmand[1], Eike Tobias Krause[1], Marc Naguib[1,2]

Manuscript

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[1]Department of Animal Behaviour, University Bielefeld, PO Box 100131,
33501 Bielefeld, Germany

[2] Department of Animal Population Biology, Netherlands Institute of Ecology
(NIOO-KNAW), P.O. Box 40, 6666 ZG Heteren, The Netherlands

Author contributions:

MH & MN designed the experiments, ETK performed the second breeding experiment, MH performed all other experiments, analyzed the date, wrote the manuscript.

ABSTRACT

An individual's phenotype is determined by the interplay between its genes and the environment in which these genes are expressed. The numerous influences an organism experiences throughout life and especially the conditions during early development can have profound and long lasting effects on an individual's performance. Early developmental constraints have the potential to even project into the following generation. Yet, the question arises whether stress experienced during distinct developmental phases contributes to variation in the expression of traits within and across generations. We conducted a two-step breeding experiment on zebra finches (*Taeniopygia guttata*) in which offspring were exposed to food regimes varying in quality during the nestling and fledgling period during early post-embryonic development. We show that despite short term effects on biometry and physiology there was no effect on adult attractiveness as tested in a two way choice aviary. In a subsequent breeding experiment adult offspring from the first breeding experiment were paired randomly to determine their reproductive success. This second breeding experiment revealed a sex specific transgenerational effect on offspring quality. Offspring from mothers raised under low quality nutrition were lighter compared to chicks from mothers raised on a high quality diet whereas paternal dietary history did not show any interaction with offspring quality. Offspring sex ratio was even at hatching but became strongly female biased due to lower male chick survival, when parents fed on low-quality nutrition as nestlings. Such long term and sex-specific transgenerational effects of dietary restrictions emphasize the importance of the two distinct early developmental phases for the expression of environmentally determined fitness components.

INTRODUCTION

Reproduction, with its complex costs and benefits, is one of the most important trade-offs in life history theory (PARKER and SMITH 1990; STEARNS 1989). Reproductive strategies are the result of balancing an individual's costs and benefits with the goal of optimizing an individual's success (WILLIAMS 1966). An important part which is tightly associated with parents' lifetime fitness is the quality and quantity of offspring. Investment in egg resources and parental care are costly and can both reduce future reproductive success of parents. Parents are predicted to adjust their reproductive decisions according to their own and their mate's quality by considering internal and external circumstances for direct benefits such as superior parental care (HOELZER 1989) or indirect benefits such as inheritance of good genes for *Fisherian traits* or viability (ANDERSSON 1982; FISHER 1930 ; HAMILTON and ZUK 1982; ZUK 2002). Even though an individual's current condition, as judged from its phenotype, appears to be reasonably well, the hidden developmental history may tell a completely different story. Each organism is a result of the interplay between its genes and the environment in which these genes are expressed. Conditions experienced during early development can have profound and long lasting effects on an individual's entire performance (biometry: DE KOGEL (1997); NAGUIB *et al.* (2004); physiology: BIRKHEAD *et al.* (1999), VERHULST *et al.* (2006); behaviour: (PENKE *et al.* (2001), SPENCER et al. (2003) reproduction: NAGUIB and GIL (2005), NAGUIB *et al.* (2006); survival: AIHIE SAYER *et al.* (2001), BIRKHEAD *et al.* (1999) and have been shown to project into the following generation (NAGUIB and GIL 2005). Phenotypic plasticity is the extent to which an organism can alter its morphological, physiological, behavioural and developmental responses to environmental conditions. Consequently, early developmental conditions can be the proximate cause of phenotypic plasticity and may ultimately have an impact on evolutionary processes.

Birds have been a key model for studying effects of early environmental constraints. Altricial birds develop within one month from a dependant hatchling to a fully grown and nutritionally independent juvenile (ZANN 1996). Previous studies have focused on the first month post hatching by experimentally manipulating either brood size or diet quality. As one month is certainly a long period for a developing bird and as parental mistiming of reproduction is likely to affect only offspring's nestling or fledgling phase, the question remains whether shorter, biologically more meaningful periods, have the potential to not only alter adult attractiveness and fecundity but to project into the following generation.

Individuals which experienced suboptimal conditions have been shown to be less attractive to prospective mates (DE KOGEL and PRIJS 1996; NAGUIB and NEMITZ 2007), which is likely to affect their reproductive success. Mate choice is a complex process which presumably involves multiple cues. In monogamous species with biparental care and mutual mate choice both sexes are considered to choose a mate according to direct and indirect fitness benefits (ANDERSSON 1994). Female mate choice and preference have received increasing attention and there is multiple evidence that females choose their mate according to behavioural, physiological and morphological characteristics (ANDERSSON 1994; HILL 1991; JORDAN and BRUFORD 1998; OLSSON *et al.* 2003). Yet, little is known about how females weigh different characteristics and whether there is a certain hierarchy in which male traits are taken into account. There is some evidence that females only pay detailed attention to condition dependent cues (IWASA and POMIANKOWSKI 1994; MOLLER and POMIANKOWSKI 1993). Male plumage characteristics and male song are secondary sexual traits which are considered to be such indicators of a male's quality (HILL 1991; NOWICKI *et al.* 2002) and previous studies showed effects of male nutritional history on cheek patch size and on female preferences in zebra finches (NAGUIB and NEMITZ 2007). Despite the wealth of theoretical and empirical studies of reproductive investment showing that females are generally the choosier sex (ANDERSSON 1994), it is widely accepted that in monogamous species, in which both sexes provide parental care, males should be choosy as well (AMUNDSEN 2000; JONES *et al.* 2001b; KOKKO and JOHNSTONE 2002). However, there are considerably fewer studies of male mate choice in birds (AMUNDSEN 2000; JONES *et al.* 2001b). In species were males participate in brood care they should be choosy about the quality of their mate to maximize their reproductive success. Indeed there is evidence that males can direct courtship towards more fecund females (MONAGHAN *et al.* 1996). Hence it remains to be shown whether in the absence of female-female competition a male will discriminate between females with different early developmental background, which previously has bee shown to bear reproductive consequences (NAGUIB and GIL 2005).

Parental effects represent the outcome of interactions between parents and offspring and do therefore influence an offspring's trajectory (MOUSSEAU and FOX 1998). However, even the environmental conditions parents experience during their early development have the potential to span into the subsequent generation (NAGUIB and GIL 2005). One important component of parental investment is sex ratio adjustment. There is evidence that sex ratio adjustment can be an adaptive strategy for parents such as when one sex has higher variance in its reproductive success than the other (TRIVERS and WILLARD 1973) or when relative attractiveness of adults is variable (BURLEY 1981). If fitness returns from investment in sons and daughters differ from each other, then unequal allocation in the two sexes will evolve (CHARNOV 1982). Several studies of avian species have shown sex ratio skews in relation to resource abundance whereas poor food availability was shown to favour production of female offspring, which are less costly to raise (APPLEBY *et al.* 1997; BRADBURY and BLAKEY 1998; PATTERSON *et al.* 1980; WIEBE and BORTOLOTTI 1992). Along this line, high-quality parents in polygynous species, where the reproductive success of males is more variable than that of females, a male-biased offspring sex ratio is predicted (TRIVERS and WILLARD 1973). Therefore, quality of parent and offspring are assumed to be correlated. Avian sex-ratio variation has received great attention in the literature but causal mechanisms remain poorly understood (KRACKOW 1995; PIKE and PETRIE 2003; VON ENGELHARDT 2004). To understand how evolutionary processes can be influenced by epigenetic plasticity and by selection pressure on adaptive strategies we need to redirect our focus of attention from a simple gene-centred view (ULLER 2008).

In this study we tested attractiveness and fecundity of zebra finches (*Taeniopygia guttatta*) which were raised under three different experimental treatments during their first month post hatching. Subjects received a high quality diet either during their nestling phase (HL = high-low), their fledgling phase (LH = low-high) or throughout the whole first month (HH = high-high). For the remainder of time during the first month they fed on a low quality diet. Treatment effects on offspring biometry, physiology and foraging behaviour have been shown previously (Chapter 2,, KRAUSE *et al.* (2009)). As adults, we tested male and female attractiveness in a mate choice experiment by giving opposite sex individuals (other then the experimental subjects) the opportunity to choose between two subjects raised under different treatments. In a subsequent breeding experiment, subjects were paired randomly to opposite sex mates to test their reproductive success. We compared male and female reproductive success separately. Identifying factors responsible for sex ratio biases through a correlative approach across individuals is a commonly used method for identifying relationships between variables and sex ratio variation, we had a particular focus on adjustments of sex ratios (after hatching).

TWO WAY MATE CHOICE EXPERIMENT

METHODS

Subjects and Husbandry

The experiment was conducted on wild type zebra finches from Australian origin (about F9 generation; for genetic data see FORSTMEIER (2007)) at the University of Bielefeld in May and June 2006. In the following it will be distinguished between subjects to choose (choosers), which make an active choice in the aviary (see fig with set up) and subjects to test (subjects), which are presented to choosers in the here described experiment. **Choosers** were 38 females and 44 males which were randomly chosen from the Bielefeld zebra finch colony. **Subjects** were adult offspring originating from a breeding experiment conducted in 2005 (44 males, 46 females) which were raised in one of three different nutritional treatments. Subjects stayed with their genetic parents in their natal brood size. Experimental groups differed in diet quality. Group LH (low-high) received low quality food (LQ) as nestlings from day 3 until day 17 and high quality food (HQ) as fledglings from day 17 until day 35. Group HL (high-low) received the food regime vice versa with HQ from day 1 until day 17 und LQ from day 17 until day 35. As a positive reference for normal development a control group experienced HQ food conditions during the whole first month (HH, high-high). LQ food consisted of the regular seed mix and water ad libitum. Twice per week, water was enriched with vitamins in both groups. Groups in the HQ treatment received additionally to the seed mix millet and a mixture of germinated seeds plus commercial egg food (CéDé, Evergern, Belgium) daily. Furthermore, salads, greens, fruits and vegetable were provided three times weekly. By the time of nutritional independence (day 35 of the youngest of a brood) until day 65 offspring were assigned to mixed sex tutor groups (in total 10 groups) to learn their song from an adult male. Groups consisted of a male tutor, an adult female and 6 - 11 tutees, which originated from different nutritional groups. All received an intermediate diet between LQ and HQ and stayed together until they were at least 6 month old. At day65 the tutor and the female were removed.

Housing prior to the experiment

Two weeks prior to the experiment all subjects were individually transferred to cages (83 x 30 x 39.5 cm) situated in the experimental room. Cages contained 4 - 6 subjects of the same sex. All subjects could vocally interact with each other at any time but could not visually interact with individuals outside their cage. After the experiment females were transferred to an indoor aviary while males stayed in the cages until their song was recorded (not part of this experiment).

Mate-Choice Experiment

Each of the 82 subjects to choose were tested with a randomly choosen unique subject dyad of the opposite sex. With a total of 82 dyads (44 female, 38 male) all three possible treatment combinations were tested in random order (Table A1, A2; Appendix). Dyads and assignment to choosers were controlled for genetic background and song tutoring, hence combinations with relatives and same song tutors were prevented. Subjects had 20min to acclimatize inside the test cages of the choice aviary (Fig. 1), then a chooser was put inside the start box and the observer hid in an observation shed equipped with a small one way mirror. A 20 min observation period started when choosers' start box was opened via a string. Noldus Observer (Basic 5.0) was used to record exact time spent in front of the cages and to sample behaviour (singing, courting) as events in 10 sec intervals. Choosers and subjects had access to ad libitum food and water at any time during the experiment. The mate choice aviary was situated in the same experimental room as described above.

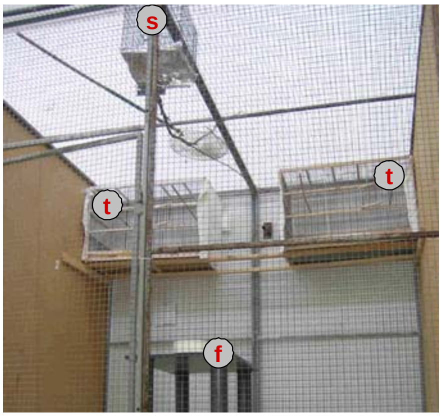

Fig. 1: Mate choice set-up. Choosers flew freely within choice aviry after start box (**s**) was opened. **f** = food & water, **t** = test cage with subjects.

Cheek patch measures

Cheek ratio, an indicator of plumage development was analyzed (Chapter 2) and taken into the analysis as an attractiveness measure. Cheek value is the mean number of pixels per cheek patch.

Statistical analysis

All data were analyzed by using the free software R (R DEVELOPMENT CORE TEAM 2006). Data were tested for normal distribution with Shapiro-Wilk normality test. Treatment effects of normally distributed data with homogenous variance were tested with paired t-test whereas data which violated assumptions were analyzed with paired Wilcoxon-test. Female and male preferences were analyzed with Spearman rank correlation regarding courtship behaviour, male singing and male cheek patch ratio at day 65. Individual preferences were tested for departure from 50 % chance level by G-test. Expected values were calculated as individual time spent in front either cage during the 20 min experiment divided by two. Hence, analysis was performed exclusively for choice-time whereas *e.g.* time spent feeding was not considered. Differences in proportions of significant choices (psc) across experiments were tested with X^2-test on Contingency tables.

RESULTS

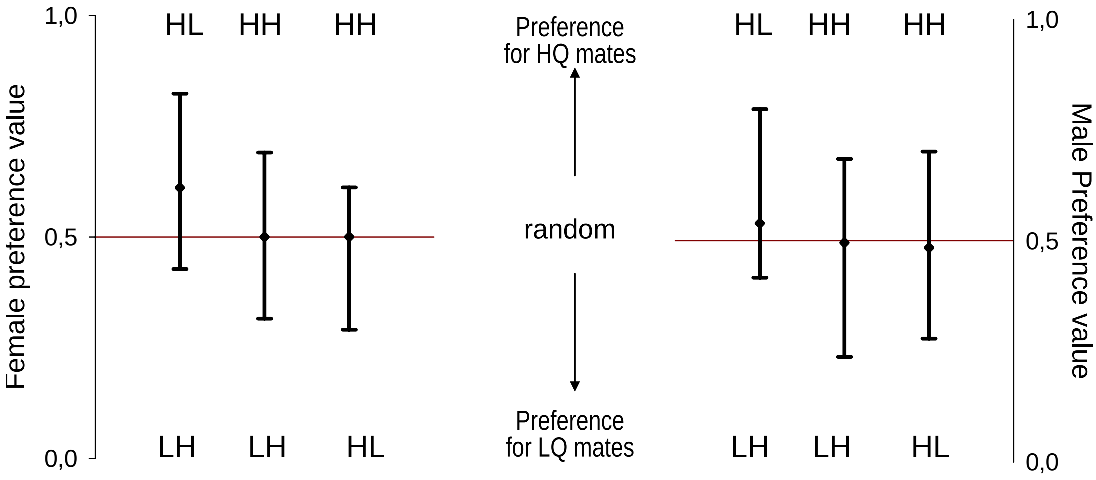

Fig. 2: Preference ratios. Female and male preference values for HQ mates in respective dyads (mean ± s.e.). The line depicts 50 % chance level. Early nutritional treatments in which subjects were raised during their first month post hatching: LH (low-high), HL (high-low) and HH (high-high).

Dietary restrictions had no effect on male or female attractiveness (Fig. 2). Neither females nor males raised under one of the three nutritional conditions (LH = low-high, HL = high-low and HH = high-high) were differently preferred by the opposite sex (Table 1a, b).

In total, 48 % males and 61 % females made a choice (G-Test against 50 % chance level; all $G_{df=1} > 3.88$, all $p < 0.05$). The proportion of choosing birds exhibiting a significant preference (psc) was different across experiments, even though this pattern was not significant (psc females: $Chi^2 = 0.139$, df = 2, p = 0.93, Table 1a; psc males: $Chi^2 = 0.251$, df = 2, p = 0.88, Table 1b). Courtship behaviour was not different across experimental groups in both sexes. Male singing behaviour did not show a treatment dependant profile (Table 1a, b). An individual's preference for a mate was significantly correlated with the mate's courtship behaviour (Fig. 3 & 4; Table 2) and female choosers courted significantly more in front of their preferred mate (Fig. 3; Table 2). Interestingly there was neither a correlation between female's preference for a male and its singing behaviour nor was females preference correlated with male's cheek patch ratio (Fig. 3 a-c; Table 2).

Table 1: Mate choice experiment. Results of the three mate choice experiments for each sex. Data were analyzed via paired t-test (t) or paired Wilcoxon test (V) considering normality assumptions respectively. **a)** Female choosers preference, **b)** Male choosers preference. psc = proportion of choosing birds, which exhibited a significant preference (regardless for which of the two subjects).

a) Female choosers			**Female preference for HQ males**				**Male behaviour**			
Test	**n**	**psc [%]**	**Time**	**p**	**Courtship**	**p**	**Singing**	**p**	**Courtship**	**p**
LH vs. HL	30	53,33	V = 38	0.38	V = 47	0.753	V = 66	0.75	$t_{14} = -0.37$	0.72
LH vs. HH	24	66,67	V = 39	0.63	V = 44	0.724	$t_{11} = 0.39$	0.71	V = 39.5	0.59
HL vs. HH	22	63,64	V = 39	0.64	V = 41	0.504	$t_{10} = 0.30$	0.77	$t_{10} = -0.62$	0.55

b) Male choosers			**Male preference for HQ males**				**Female behaviour**			
Test	**n**	**psc [%]**	**Time**	**p**	**Courtship**	**p**	**Singing**	**p**	**Courtship**	**p**
LH vs. HL	28	57,14	V = 35	0.30	V = 24	0.26	V = 32	0.61	V = 37.5	0.94
LH vs. HH	28	42,86	$t_{13} = -0.48$	0.64	V = 32.5	0.22	V = 27.5	0.39	V = 39.5	0.70
HL vs. HH	32	43,75	V = 75	0.74	V = 49	0.85	V = 20	0.27	V = 61	0.98

Males sang and courted significantly more in front of their preferred female (Fig. 3 d-f; Table 2).

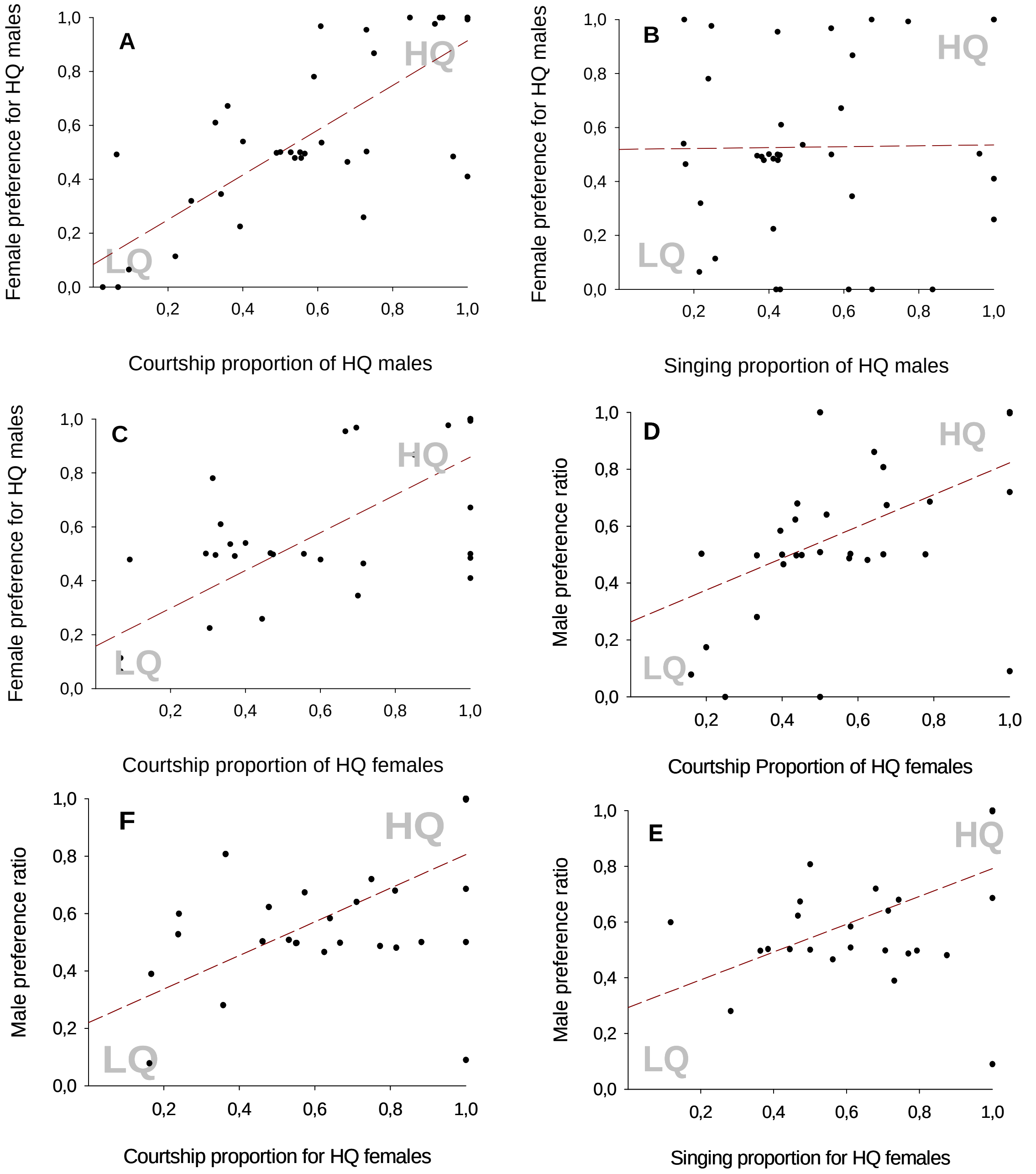

Fig. 3: Relationship between preference values and singing/ courtship behaviour. Female preference for high quality (HQ) males correlated with **a)** courtship proportion of HQ male, **b)** singing proportion of HQ male, **c)** female courtship proportion. Male preference for HQ females correlated with **d)** courtship proportion of HQ female, **e)** male singing proportion for HQ female, **f)** male courtship proportion for HQ female. A value in the top right corner depicts a high preference for the HQ mate, correlated with a high proportion of the respective behaviour. A value in the bottom left corner depicts a high preference for the low quality (LQ) mate, which exhibited a high proportion of the respective behaviour.

Table 2: Correlation analysis. Correlation between an individual's preference (pref. value) for the high quality mate and the behavioural variables courtship, singing and in case of females as well male cheek ratio.

	Male						Female	
Correlation	**Courtship**	**p**	**Singing**	**p**	**Cheek ratio**	**p**	**Courtship**	**p**
Male pref. value	$r_s = 0.69$	< 0.001	$r_s = 0.60$	< 0.001	—		r = 0.60	< 0.001
Female pref.value	S = 2506	< 0.001	$r_s = -0.006$	0.97	$r_s = 0.04$	0.81	$r_s = 0.74$	< 0.001

FECUNDITY TEST

METHODS

Subjects and Husbandry

Subjects were 41 males and 42 females from above described breeding experiment. As part of a larger experiment (total no of 137 breeding pairs) performed by E.T. Krause in autumn 2008 at Bielefeld University, subjects were paired randomly to opposite sex individuals from the colony controlling for relatedness (at least to great-grandparents). Biometric measurements and cheek patch size were taken before onset of the experiment. All pairs received high quality food until their first chick was 3 days old. Then, breeding pairs and offspring were assigned alternately to one of two nutritional treatments (LQ = low quality, HQ =high quality). LQ diet comprised an ad libitum seed mix and once a week supplementation of egg food (CéDé, Evergern, Belgium). HQ diet comprised as well ad libitum seed mix which was supplemented daily with egg food and germinated seeds and comprised greens three times weekly. Subjects from both treatments received fresh water daily which was supplemented with vitamins (Veyx-Pharma, BioWeyxin - Multi-Mulgat) twice weekly. Nutritional treatments ended when the oldest chick in a nest was 35 days. Thereafter, all subjects received an intermediate diet. Nests were checked daily between 9:00 and 11:00 for nest building, egg laying, chick hatching and cases of death.

Statistical analysis

All data were analyzed by using the free software R 2.8.1 (R DEVELOPMENT CORE TEAM 2008). All data and model's residuals were tested with Shapiro-Wilk for normality. If assumptions were violated either non-parametric tests were performed or for calculating linear mixed effects models data were transformed accordingly. Treament effects were analyzed for all latencies (to build a nest, lay the first egg, until the first hatchling, until the first fledgling, until the first independent chick), numbers of eggs, hatchlings, fledglings and independent chicks with Kruskal Wallis X^2-test. To analyze whether there is a difference in the probability of the distinct measures (nest, eggs, hatchlings, fledglings, independent chicks) X^2-test on Contingency tables were performed. Sex ratio departure from an equal 50 % sex ratio was analyzed across groups with a replicated G-Test. Linear mixed effect models for offspring hatching mass were calculated with parental treatment group, parental fledgling mass (fledgling mass was shown to be a predictor for female fecundity and attractiveness in KILNER (1998)), parental breeding mass, fathers' cheek patch size, no. of clutch and offspring sex as fixed effects (PINHEIRO *et al.* 2006). As nutritional treatment started not before day 3 it could not have influenced body mass as hatching. Predictions for random effects were made for natal cage and parents' exact age at onset of breeding. The most parsimonious model was selected by stepwise deletion of non-significant terms using model comparisons via Akaike's Information Criterion (AIC).

RESULTS

Female Fecundity

Females raised under different nutritional conditions during their first month were equally likely to lay eggs (F-test, p = 1), to produce viable = 0.451) and to nurture offspring until independence (F-test, p = 0.148). There tended to be a lower proportion of females raised under LH conditions to reproduce successfully compared to the other treatment groups (Friedman Chi ^{2} = 5.2, df = 2, p = 0.074; Fig. 5).

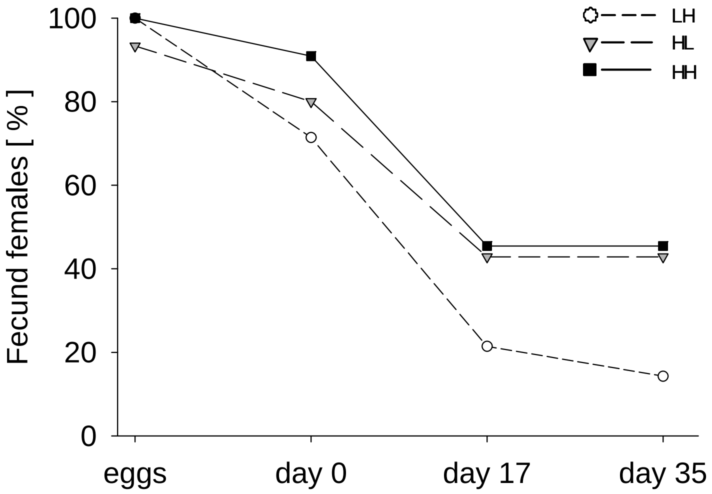

Fig. 5: Proportion of reproducing females. Proportion of breeding females, which successfully reproduced. Proportions are shown for egg laying, hatching (day 0), fledgling (day 17) and independent offspring (day 35) across groups. Early nutritional treatment groups in which mothers were raised during their first month post hatching: LH (low-high), HL (high-low) and HH (high-high).

There was no effect of mother's former nutritional treatment on the latency to lay the first egg, the latency until the first offspring hatching, the latency until the first fledgling, the latency until the first offspring was independent, the number of eggs, the number of hatchlings, the number of fledglings, the number of offspring to reach independence (all Kruskal-Wallis Chi ^{2} > 0.84, df = 2, p > p = 0.31). Despite the lack of statistical significance regarding the difference in offspring numbers between parental treatment groups, it is important to mention that in the LH group, which received low quality nutrition as nestling, only half as much females raised offspring until independence than in the other two treatment groups (HL and HH).

Offspring sex ratio was significantly uneven at fledgling, when mothers experienced dietary restrictions as nestlings (LH) whereas it was even for the remainder of time and treatment groups (replicated G-Test: G_{total} = 21.77, df = 6, p = 0.001; $G_{pooledl}$ = 21.32, df = 2, p < 0.001; $G_{heterogeneity}$ = 0.45, df

= 4, p = 0.98; Fig.6). This effect was due to male chicks showing lower survival until fledgling.

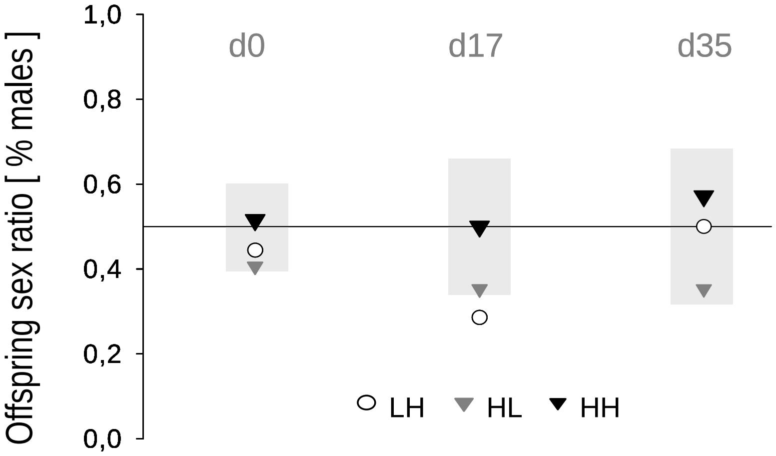

Fig. 6: Change in sex ratio. Offspring sex ratio (% males) at hatching (d0), fledgling (d17) and nutritional independence (d35) for the maternal groups: LH (low-high), HL (high-low) and HH (high-high). Greyshades indicate the region of non-significant departure from 50 % equal sex ratio (G-Test).

Offspring body mass was affected by mother's early nutritional treatment as offspring from LH and HL mothers were significantly lighter than offspring from HH females (lme: $F_{2,28}$ = 8.76, p = 0.001; Fig. 7a). There was an influential trend of mothers' fledgling weight (weight: $F_{1,28}$ = 3.83, p = 0.060). Contrary there was no effect of father's early nutritional treatment on offspring weight (lme: $F_{2,19}$ = 0.353, p = 0.71; Fig. 7b). However, the father's fledgling weight, father's cheek patch size and the interaction between father's early nutritional treatment group and weight at fledgling had a significant effect on offspring body mass (fathers fledgling weight: $F_{1,19}$ = 10.97, p = 0.004; cheek: $F_{1,19}$ = 7.27, p = 0.01; diet * weight: $F_{2,19}$ = 5.43, p = 0.014).

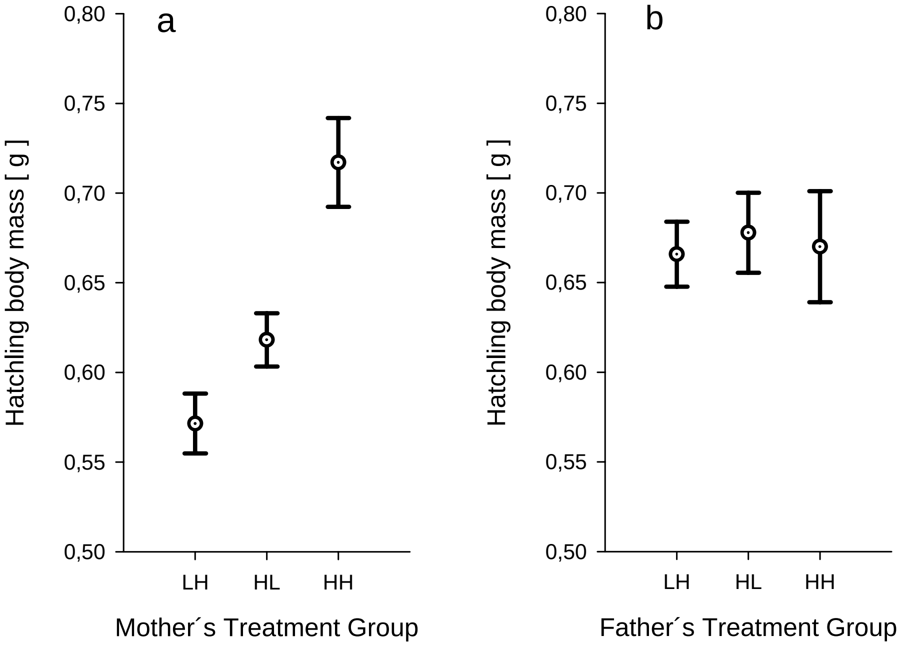

Fig. 7: Hatchling mass. Offspring body mass (mean ± s.e.) from a) mothers from different early dietary treatment groups or b) fathers from different early dietary treatment groups (LH =low-high, HL = high-low, HH = high-high).

Male fecundity

Males raised under different nutritional conditions during their first month were equally likely to build a nest, to have eggs laid, to have viable hatchlings, to raise hatchlings until fledging and to nurture offspring until independence (all F-test, $p > 0.91$). The change in successfully reproducing males over the time course was not differentially affected by nutritional treatment (Friedman Chi^2 = 3.5, df = 2, $p = 0.17$; Fig. 8). There was no effect of fathers' early dietary treatments on difference in the latency to build a nest, in the latency to lay the first egg, the latency until the first hatchling, the latency until the first chick reached fledgling day, the latency until the first offspring reached independence, the number of eggs, hatching success, hatchlings, fledglings or offspring to reach independence (all Kruskal-Wallis Chi^2 > 1.31, df = 2, $p > 0.065$).

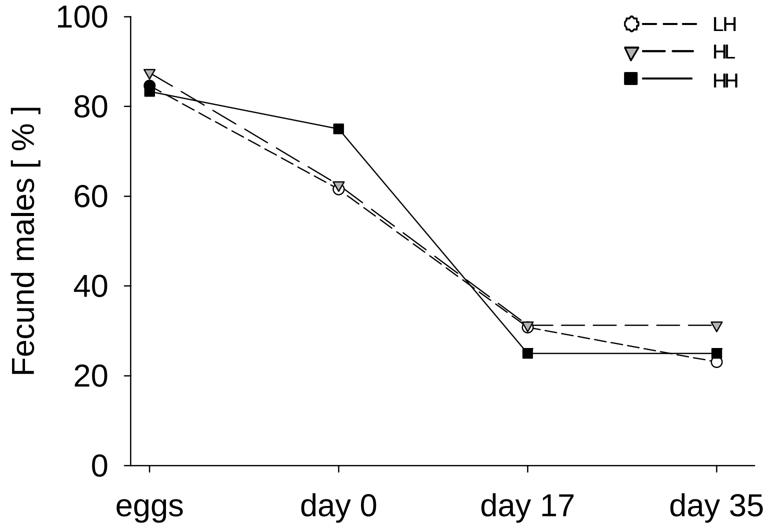

Fig. 8: Proportion of reproducing males. Proportion of males which reproduced successfully. Proportions are shown for egg laying, hatchlings (day 0), fledglings (day 17) and independent offspring (day 35) across treatment groups. Early nutritional treatments groups in which fathers were raised during their first month post hatching: LH (low-high), HL (high-low) and HH (high-high).

Offspring sex ratio departed significantly from equilibrium across time points but there was no overall treatment effect (replicated G-Test: G_{total} = 14.27, df = 6, p = 0.027; G_{pooled} = 11.59, df = 2, p = 0.003; $G_{heterogeneity}$ = 3.84, df = 4, p = 0.43; Fig. 9). Even though offspring hatching ratio was even it fell dramatically until fledgling day in the LH group. Offspring sex ratio (at fledgling and independence) was significantly female biased if fathers were raised under LH conditions. In the LH group a high proportion of male nestlings died. In clutches sired by HL fathers nestling and fledgling mortality was male biased leading to a slightly female biased sex ratio at independence. The HH fathers had an offspring ratio which was slightly male biased due to higher female nestling mortality, whereas this trend was reversed until independence due to higher male fledgling mortality.

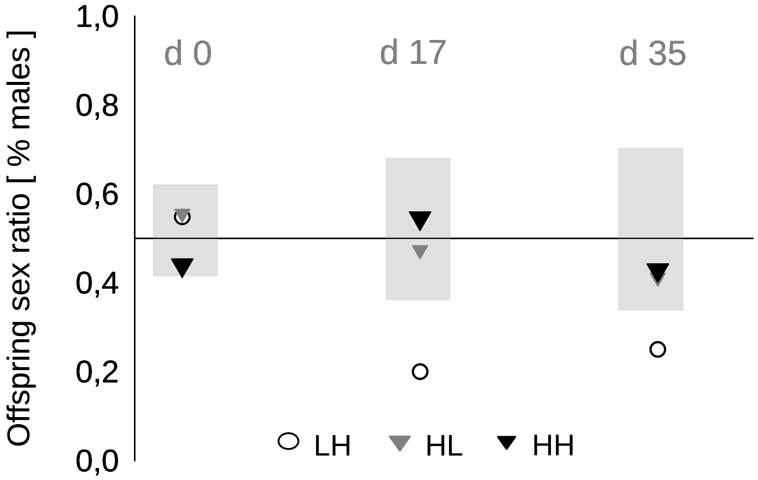

Fig. 9: Change in sex ratio. Offspring sex ratio (% males) at hatching (d0), fledgling (d17) and nutritional independence (d35) for the paternal groups: LH (low-high), HL (high-low) and HH (high-high). Greyshades indicate the region of non-significant departure from 50 % equal sex ratio (G-Test).

DISCUSSION

Nutritional treatment during early development did neither affect male or female attractiveness in adulthood nor frequencies of courtship or singing behaviour. Choosers courted more in front of the preferred individual. However, female's preference for a male was not correlated with male singing behaviour. Nutritional condition in which females where raised did not have an effect on the number of offspring or the latency to produce viable offspring. However, offspring from LH and HL mothers were significantly lighter than offspring from HH mothers whereas no effect of father's treatment could be found on offspring hatching mass suggesting that females not males had to trade off between offspring quantity and quality.

Mate choice

Female and male attractiveness was not influenced by early dietary treatments. The preference of choosing individuals was not related to the early nutritional history of the subjects. Subjects' courtship or singing behaviour (if subjects were male) did not have an effect on choosers' choices. Subjects from the three different nutritional groups (LH = low-high, HL = high-low and HH = high-high) did not show differential courtship or singing behaviour. Choosing birds exhibited more courtship behaviour in front of their preferred mate, irrespective whether subjects were male or female. Whether a male is attractive or not has been shown to be linked to its developmental history (DE KOGEL and PRIJS 1996; NAGUIB and NEMITZ 2007). However, the comparison of our results to previous studies suggests that the period in which constraints are encountered and their duration seems to be of fundamental importance (BLOUNT *et al.* 2003a; DE KOGEL and PRIJS 1996; NAGUIB *et al.* 2008a; NAGUIB and NEMITZ 2007; NAGUIB *et al.* 2008b)). NAGUIB *et al* (2008) did not find an effect on male attractiveness as a consequence of brood size manipulations and neither did BLOUNT *et al* (2003a), who experimentally manipulated nutritional quality during a 15 day period post hatching. Contrary studies in which experimental stressors endured the whole month post hatching, either via manipulation of brood size (DE KOGEL and PRIJS 1996) or nutritional constraints during the second month in life (NAGUIB and NEMITZ 2007), did show effects on male attractiveness. The conditions during the period in which secondary sexual traits develop appear to be crucial for an individual's attractiveness. Subjects in our experiment did compensate for their developmental history; i.e. resource limitations occurred early post hatching and were restricted to a biologically meaningful timescale.

Choosiness was neither different across treatment groups nor between the sexes. This study includes attractiveness tests for both sexes, showing that choosing males did not discriminate between females from different nutritional backgrounds. The proportion of males exhibiting a significant preference was lower than the proportion of females showing a significant preference. Especially in a species with biparental brood care as the zebra finch, mutual mate choice is expected even though male choice may be cryptic (ENGQVIST and SAUER 2001; KOKKO and JOHNSTONE 2002).

However, there was a difference in the proportion of individuals exhibiting a significant preference between the sexes. This could be the consequence of the differential costs both sexes have to pay when mated to a low quality partner. Females should be choosier, considering their reproductive input, which favors shaping the other sex towards more competition and the development of elaborate traits (ANDERSSON 1994).

Fecundity

Conditions experienced during early development projected into the subsequent generation as mothers, which were raised on a low quality diet as nestlings (LH; low-high) or fledglings (HL; high-low) had lighter hatchlings compared to mothers, which experienced high quality food throughout their first month post hatching (HH; high-high). Fathers' cheek patch size and father's weight at fledgling were significant predictors for offspring body mass. However, the effects on offspring quality were sex specific as father's offspring did not exhibit a paternal treatment effect on body mass at hatching.

Females are predicted to adjust their reproductive investment in relation to their mate's attractiveness (SHELDON 2000). The differential allocation hypothesis (DAH) postulates females to invest more, considering e.g. number or size of eggs and changes in yolk composition, when paired to an attractive male (BURLEY 1988; GIL *et al.* 1999; PETRIE and WILLIAMS 1993). However, results are ambiguous and other studies have reported a pattern where females invested more in reproduction, when paired to a low quality mate (BOLUND *et al.* 2009; GOWATY *et al.* 2003; NAVARA *et al.* 2006; RUTSTEIN *et al.* 2004a). This pattern can not be explained by only considering different aspects of

male attractiveness; even though this is crucial. Male attractiveness and male parental quality are not necessarily linked by a positive relationship and females need to trade off potential indirect versus potential direct benefits to optimize their reproductive output. For example has the experimental manipulation via colored rings lead to the conclusions that red banded males are more attractive to females (BURLEY 1986; BURLEY *et al.* 1982). BURLEY (1981, 1986) showed red ringed zebra finch males (= more attractive) males to provide less parental care compared to green banded males (= less attractive) whereas an opposite pattern was observed in the house finch *Carpodacus mexicanus* (NAVARA *et al.* 2006). The Compensation Hypothesis (CH) predicts females to increase reproductive effort when mated to a male of low quality in order to make the best out of a bad situation (BLUHM and GOWATY 2004; GOWATY *et al.* 2003). In line with that, our results can be discussed by considering BOLUND *et al* (2009), who suggested that rearing success depends on male identity as they found female zebra finches to invest more resources in their eggs (larger eggs and more orange yolks) when paired to a male of lower sexual attractiveness (established via prior EPC's). Additional evidence comes from female house finches which deposited more yolk androgens in their eggs when paired to a male of low sexual attractiveness (established via colour measurements followed PCA; NAVARA *et al.* (2006). Females seem to be able to compensate for low quality males by topping up egg resources to ensure offspring an acceptable start through increased maternal investment. Hence, the lack of an effect of fathers' early nutritional treatment on offspring quality could be the result of females differentially allocating resources to the eggs and therefore compensating for low quality mates (CA).

Limited resource supply, such as. antioxidant levels, have been suggested to decrease sperm quality, which is particularly susceptible to oxidative damage (BLOUNT *et al.* 2001; PETERS *et al.* 2004). A previous study on zebra finches could not support such an effect of LQ (BIRKHEAD *et al.* 1998b). However, as measurements of egg composition had to be excluded, it remains speculative and could well be an explanation that father's reproductive quality was not affected by early nutritional history. In that case CA would be not applicable. In fact, a recent article in which a state-based optimality approach investigated interactions between mate quality and female energetic resource levels suggested a limited scope for the predictions of the CA, which is only explained in specifically defined scenarios (HARRIS and ULLER 2009).

Results of our study are partly in line with a study of (BLOUNT *et al.* 2006). In their experiment females and males were paired assortatively according to the nutritional treatment they had received during their first two weeks post hatching (either low or standard quality food). They found pairs which were raised on low quality nutrition to initiate egg laying significantly later, to exhibit more laying breaks and having a lower laying rate within a clutch compared to pairs which were raised on standard quality diet. They did not find an effect on egg mass, clutch size or yolk concentrations of lipophilic antioxidants. Our results do not indicate an effect of parental nutritional history on reproductive quantity as clutch size or the latency to produce viable offspring was not different between parental treatment groups. This was true for females' and males' progeny. Breeding pairs in our experiment were not mated assortatively which may be the reason for the lack of effects on latency to initiate a clutch. The adaptive adjustment of clutch size is still a constant debate. Studies so far have led to contradictory results which, like our results, either have not found an effect (BLOUNT 2004; GILBERT *et al.* 2006; RUTSTEIN *et al.* 2004a; VON ENGELHARDT 2004) or an effect which was weak (BALZER and WILLIAMS 1998; HAYWOOD and PERRINS 1992). Summarizing, it appears that partners complement each others adjustments. Hence, negotiation concerning inter-sexual compensatory strategies could well be a plausible explanation. As timing of laying eggs can be important for improving individual fitness, breeding pairs do best if they only delay laying when environmental and internal state suggest to (BLOUNT *et al.* 2006). Zebra finches are opportunistic breeders and may improve the conditions for their offspring by timing egg laying and offspring hatching to environmental conditions and peaks of food availability. Additionally they may be able to raise a second clutch in the same term if conditions stay acceptable.

Life-history theory predicts individuals to invest in reproduction as to maximize their lifetime fitness (WILLIAMS 1966). Parental effort in current reproduction may affect the parent's future reproduction (intra-individual trade-off) and/ or the fitness of their offspring (inter-generational trade-off, reviewed in STEARNS 1989). This leads to an important trade off between offspring number and offspring quality, as egg production and parental care until nutritional independence, especially in altricial species, are considered to be very costly (MONAGHAN and NAGER 1997). Zebra finches are monogamous and both partners participate in brood care whilst their young are nutritionally dependant (ZANN 1996).

Early nutritional history did not have a statistically significant effect on the proportion of successfully reproducing females or males. Even though we would like to stress that only 20 % of the LH females were able to raise offspring until independence compared to 45 % of the HL and HH females. This has important consequences on the composition of the gene pool and shows that even though there is a lack of statistical significance, the effect is still of biological importance. In this experiment a low percentage of genes within the gene pool stems from individuals which experienced low quality nutrition as nestling. The variance in traits and not the traits themselves are the crucial elements which underlay sexual selection as driving evolutionary force. If nutritional history impairs females possibilities to leave the most genes in the following generation their variation in traits will be lost and diversity of the gene pool shrinks as a consequence of environmental conditions.

Sex ratios

Offspring sex ratios of breeding pairs, in which one of the parents experienced a period of dietary restriction as nestlings (day 17 – 35), were significantly female biased at fledgling (LH mothers and LH fathers) and at independence (LH fathers). This was due to lower survival rates of male progeny as offspring sex ratios were reasonably even at hatching across groups. The underlying reasons may be manifold and it remains speculative whether the causes lay in different parental investments during nestling or fledgling phase, sibling competition or differences in begging intensity as a consequence of differential egg resource compositions. In general, sex ratio manipulation and sex-biased mortality are two sides of a coin, which both determe populations' sex ratios.

If offspring sex ratios are representative for the division of resources between sons and daughters, then male-biased sex ratios are expected when the reproductive value of male offspring exceeds that of females and vice versa (ODDIE and REIM 2002). Females are the sex with lower reproductive variance and in unpredictable or adverse environmental conditions parents retrieve higher fitness benefits from investing in female offspring (TRIVERS and WILLARD 1973). At first sight, this seems to contradict the evidence that females show reduced fecundity as adults if they fledge at low body mass and have reduced nestling survival when food supply is low (ARNOLD *et al.* 2007; DE KOGEL 1997; KILNER 1998; MARTINS 2003). However, it increases parents reproductive output, to rather invest in a low quality female than in a low quality male. Whilst a high quality male will out-reproduce its high quality sister, a low quality sister will out-reproduce a low quality brother, who is unlikely to be competitive enough within the breeding population (TRIVERS and WILLARD 1973).

Sex-biased mortality possibly involves a differential effect of egg quality on male and female offspring (CUNNINGHAM and RUSSELL 2000; GIL *et al.* 1999; PETRIE *et al.* 2001; RUTKOWSKA and CICHON 2002) and differential effects of sex and size composition of broods on fledging sex ratio (ODDIE and REIM 2002). Egg resources are costly to produce and do indeed differentially influence female and male offspring (GIL 2003; NAGER *et al.* 2000; WILLIAMS 1994). Deposits of egg androgens have been shown to vary in regard to male attractiveness and these variances influence only female and not male begging intensity (GIL *et al.* 1999; VON ENGELHARDT *et al.* 2006; VON ENGELHARDT *et al.* 2004). Differential parental allocation into egg resources or into brood care could explain the observed lower male nestling survival. However, there need to be further analysis which should combine detailed analysis of egg compounds and further observations on parental feeding and offspring begging behaviour.

Measuring, analyzing and comparing offspring sex ratios, which can well be an indicator and the result of sex allocation, are only one step towards fully answering questions about the potential mechanisms. Measuring sex allocation explicitly is somewhat harder to pursue. Evolutionary, individual-based computer simulations have suggested a rather weak selection pressure on sex ratios, which may be counteracted by constraints and costs of manipulation (FAWCETT *et al.* 2007). This might be an explanation for the mixed evidence for adaptive sex allocation in birds (CASSEY and BLACKBURN 2004; EWEN *et al.* 2004; FAWCETT *et al.* 2007; KOMDEUR and PEN 2002; WEST and SHELDON 2002). Birds' life histories are very complex. Additionally, accounting for their physiology does complicate our understanding of sex allocation theory which is well modeled and understood in invertebrates (KOMDEUR and PEN 2002).

Dietary effects during early development had profound but only transient effects on biometry and physiology (Chapter 3). Short periods of food limitations are likely to occur in the wild and it appears to be a good strategy to outweigh phenotypic deficits. However, compensation is limited and tightly interconnected with the developmental period, the strength and the duration of the encountered constraints (BLOUNT *et al.* 2003a; NAGUIB and NEMITZ 2007). Parental history was reflected in different traits between the sexes when looking at measures of fitness and fecundity. Contrary to males, females only partly compensated for their nutritional history, which is in line with considerations about their increase vulnerability (BRADBURY and BLAKEY 1998; DE KOGEL 1997; KILNER 1998; NAGUIB and GIL 2005; NAGUIB *et al.* 2006), for their early developmental constraints.

CHAPTER 4

CONDITONS DURING PUBERTY AFFECT MALE SONG LEARNING AND FEMALE PREFERENCES

EVIDENCE FOR PEER LEARNING

Mariam Honarmand[1], Katharina Riebel[2], Marc Naguib[1,3]

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[1]Department of Animal Behaviour, University Bielefeld, P.O. Box 100131,
33501 Bielefeld, Germany

[2]Behavioural Biology, Institute of Evolutionary & Ecological Sciences, Leiden
University, P.O. Box 9516, 2300 RA Leiden, The Netherlands

[3] Department of Animal Population Biology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 40, 6666 ZG Heteren, The Netherland

Author contributions:

MH, KR & MN designed the experiments, MH performed the experiments, analyzed the data and wrote the manuscript.

ABSTRACT

The period from conception to maturity is crucial for an individual's trajectory. The wealth of studies on the effects of early developmental stress contrasts our little knowledge about how environmental perturbations during later developmental periods affect the expression of fitness relevant traits. The developmental period in which secondary sexual characters develop is of particular interest, as these traits can reflect the ability to acquire resources and to cope with environmental constraints after parental care has ceased. In zebra finches (*Taeniopygia guttata*), this is the second month in life: the period of song learning which coincides with moult into adult plumage and gonadal development. We expected the month following nutritional independence, when songs are acquired and when the underlying neural pathways controlling song learning and song production learning continue to develop, to be of fundamental importance. To test this hypothesis, subjects were exposed to qualitatively different nutritional treatments from day 35 until day 65 during song tutoring. After reaching sexual maturity female song preferences were tested via operant conditioning set ups. Males' song was analyzed via two different approaches (visual comparison & song similarity analysis via SAP Pro) to assess interactions between diets and cultural transmission. Additionally the relationship between female preferences and male song parameters was analyzed. Neither male song production learning nor female preferences were shown to be affected by dietary restrictions. Social environment was shown to be of fundamental importance as females preferred the songs of tutees with which they have been tutored over songs from unfamiliar-tutees and males showed higher motif similarity to another tutee than to their tutor. This provides not only concrete evidence for female song acquisition learning but proves the relevance of social cues in shaping the cultural transmission process of song learning in both sexes. Like in juvenile male birds the social circumstances can favour peer learning. For the first time important conclusions on the interactions between nutritional constraints during the

period of song learning and the cultural transmission process of male song learning and female song recognition learning were drawn.

INTRODUCTION

Acoustic signaling is widespread within the animal kingdom, but there are only few species who actually need to learn their production. The number of mammalian vocal production learners, which are humans, pinnipeds (dolphins, purpoises and whales), and chiroptera (bats), is easily outbid by the thousands of bird species (belonging to orders of hummingbirds, perching birds and parrots) which learn to produce their songs. Human speech and birdsong are both complex acoustic signals which exhibit a strikingly similar basic phenomenology during the process of learning (DOUPE and KUHL 1999). In humans and song birds it all begins with an experience-dependent memorization of sensory inputs followed by the adjustment of vocal outputs (CATCHPOLE and SLATER 1995; KONISHI 1985). The numerous parallels between human speech and bird song rendered birds to become a valuable model system to study the underlying neuronal substrates of such complex behaviour. The neural pathways for controlling song production and song learning develop early in life and have been considerably well studied in song birds (BRAINARD and DOUPE 2002). Vocal learning in songbirds is a two-step process. Song memorization (during the sensory phase) is followed by rehearsal and subsequent shaping of singing (during the sensorimotor phase) occurs by referencing vocal feedback to the song memory. During song learning changes within the brain can be observed at several loci, which suggests that there is not one particular site where motor learning takes place or where the template of song resides (MARGOLIASH 1997; YU and MARGOLIASH 1996).

Sexual selection theory predicts bird song, a complex sexually selected signal, to be costly to produce in order to reflect both direct and indirect qualities of the singer (GRAFEN 1990; ZAHAVI 1975). The *nutritional stress hypothesis* suggests only individuals faring well during the nestling and fledgling phase to be able to invest their resources in brain development necessary to optimize song learning (NOWICKI *et al.* 1998). Learned features of song thus become indicators of male quality, which maintain their reliability by the developmental costs. Effects on repertoire complexity depend on normal song development and may lead to variation in the development of brain areas associated with song learning (CATCHPOLE and SLATER 1995; NOWICKI *et al.* 1998; NOWICKI *et al.* 2002). Available evidence indicates, however, that repertoire size and song complexity are not learned traits *per se* (BRENOWITZ *et al.* 1995; KROODSMA *et al.* 1997; NOWICKI *et al.* 1998). By contrast, the ability to reproduce song elements or other acoustic features that are species- or population-typical clearly depends on song learning, as it is only by accurate copying that males can incorporate these details into their song (CATCHPOLE and SLATER 1995). How well males can copy the song they are exposed to when young, may be an accessible measure of their song-learning abilities. In zebra finches (*Taeniopygia guttata*), experimental studies testing the nutritional stress hypothesis led to inconsistent results on whether or not early nutritional stress does constrain song learning and which of the song parameters may be affected (BIRKHEAD *et al.* 1998b; BRUMM *et al.* 2009; GIL *et al.* 2006; HOLVECK *et al.* 2008; NOWICKI *et al.* 2002; SPENCER *et al.* 2003; ZANN and CASH 2008). One reason for this puzzling picture may be the inconsistencies in methodological approaches and the differences in the considered song parameters. Certainly, research on the interaction between developmental effects on male song learning is only one side of the coin when considering bird song as a communication system with a sender and a receiver. Song is sexually dimorphic in many species and in some species females never sing. Zebra finch females do not sing, although they frequently produce other vocalizations that develop independently of social learning processes (RIEBEL 2003; ZANN 1996). However, females may have to learn to recognize male song and increasing evidence points out the implications of the cultural transmittance process on both sexes (RIEBEL 2003; RIEBEL *et al.* 2002). Senders and receivers are predicted to have different evolutionary interests and females will likely face costs if their suboptimal mate choice is a consequence of poor song recognition. Hence, testing the nutritional stress hypothesis should not end by looking at song production learning but should broaden its view on female song learning as interactions between production and perception learning are uncoupled in females. The comparison of interactions between early developmental constraints on male song learning and on female preferences provides a unique opportunity to shed light on the implications on mate choice as important evolutionary force (ANDERSSON 1994; SEARCY and YASUKAWA 1996).

Every behaviour is the result of the interaction between an individual's genes, the conditions during embryonic development and the subsequent environment an individual lives in. The period of song learning and song preference acquisition starts in zebra finches at about day 20, when chicks have already experienced a period of rapid growth (during the nestling stage), and does not end until birds are fully developed by day 90 (BRAINARD and DOUPE 2002; ZANN 1996). After reaching nutritional independence at day 35 parental care is ceased and offspring's well being from that point onwards is the result of its own abilities and the conditions it encounters.

A discrete network of interconnected nuclei, the song system, regulates song acquisition and production (see Box, next page). Additionally, the auditory song circuits appear to take part in song perception and discrimination both in males and in females (DEVOOGD 2004). The number, size, and connectivity of song-related neurons undergo dramatic changes during the periods of song learning (BOTTJER *et al.* 1986; KONISHI and AKUTAGAWA 1985; NORDEEN and NORDEEN 1988a; NORDEEN and NORDEEN 1988b), which in male zebra finches extends from 10 to 65 days

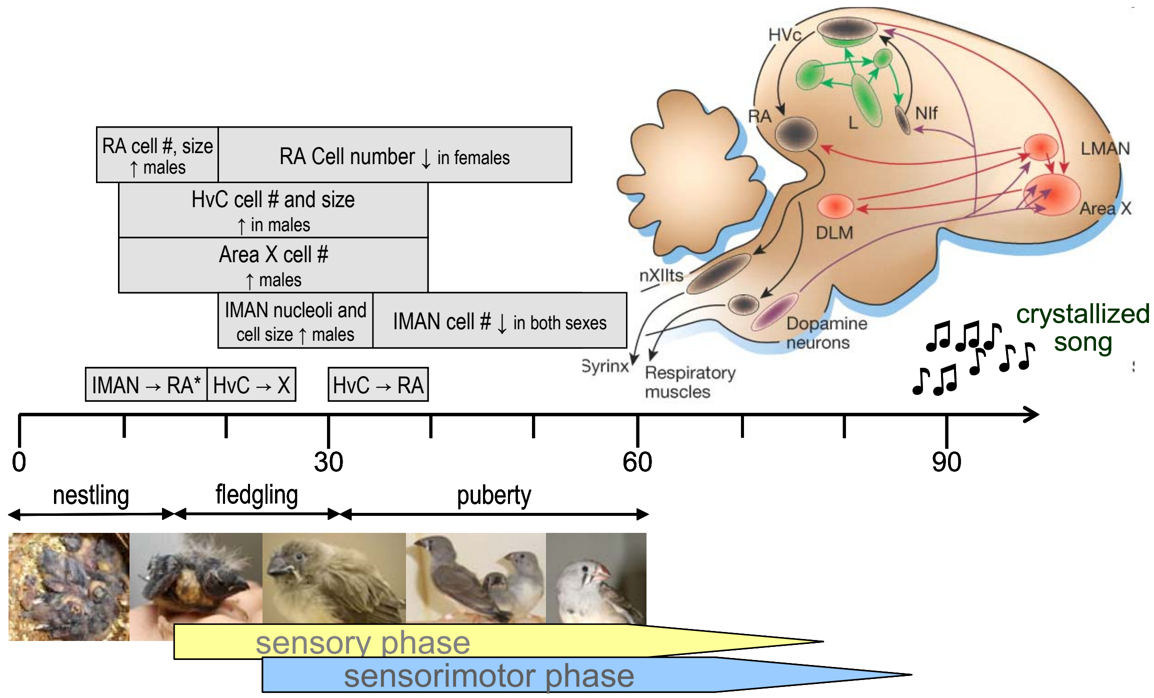


Fig. 1. Timeline for song learning and neural projections within the song system. Zebra finches develop rapidly, and their two phases of song learning overlap. The initial vocalizations, or *subsong*, produced by young birds are variable. Subsong gradually evolves into *plastic song*, which remains highly variable from one rendition to the next, but begins to incorporate recognizable elements of tutor songs. Plastic song is refined until the bird *crystallizes* its stable adult song at ~100 days. The schematic sagittal drawing of the bird brain shows projections of major nuclei in the song system. A more detailled description is given in chapter 1 (after BRAINARD and DOUPE 2002, TOMASZYCKI *et al* 2009)

post hatching (BRAINARD and DOUPE 2002; EALES 1985; IMMELMANN 1969). Not long ago, neurogenesis within the avian brain was thought to be limited to embryonic stages but nowadays, we consider even the adult brain to be considerably plastic, in terms of the birth of neurons and seasonal morphological changes (BRENOWITZ *et al.* 1997). There is a growing awareness that the brain is neither insensitive nor generally buffered against environmental influences. Neurogenesis and neuronal recruitment have been shown to be sensitive to experience and to environmental cues and nutrient availability can be a particularly crucial factor (BAGNYUKOVA *et al.* 2008; CORNIOLA *et al.* 2008; INNIS 2008; MCGOWAN *et al.* 2008; PALMER *et al.* 2008; POGRIBNY *et al.* 2008). Male song and female song recognition learning are likely to occur in conjunction with development of neuronal pathways and song control nuclei which have been shown to exhibit profound sex specific expression patterns (TOMASZYCKI *et al.* 2009) Therefore impacts of nutritional stress during that phase are particularly important to assess in both sexes. To test the hypothesis that nutritional conditions during the second month of life affect male song learning and female song preferences, we conducted experiments on zebra finches, which had experienced different nutritional conditions during this period (Chapter 2). Females preferences and implications on male song were analyzed. We compared two different methodological approaches to capture different aspects of variation in male song learning capacities: a visual observer comparison of spectrograms and an automated song recognition procedure via SAP Pro (TCHERNICHOVSKI *et al.* 2000). Particular emphasis was laid on testing whether social factors attributed to females preference learning and to males song acquisition. Additionally we tested whether there is an interaction between nutritional history and female's preference for song complexity.

Subjects, housing and experimental treatment

The breeding experiment took place in 2004 on non-domesticated zebra finches (about F7 generation of wild caught birds; for genetic data see FORSTMEIER 2007) at the University of Bielefeld, Germany. For breeding 52 pairs were kept in individual cages (83 x 30 x 40 cm) which were supplied daily with dried and germinated seeds, hen's egg and fresh water enriched with vitamins. Each cage was provided with coconut fibres as nesting material and a wooden nest box (12.5 x 12 x 14 cm). Breeding rooms had a temperature of 25°C and a light : dark regime of 16:8 hours. Nests were checked daily for egg laying and for newly hatched chicks. In total, 21 pairs bred successfully and reared 48 male and 38 female offspring. When offspring, which will be called subjects in the following, were 35 days old (time of nutritional independence), they were transferred to mixed-sex aviaries (dimensions 200 x 80 x 100 cm). Subjects remained in these song tutor groups (6 – 11 tutees per tutor group) with two different nutritional treatments (3 – 6 tutees per treatment) until day 60, i.e. until secondary sexual characters were fully developed and males had learned their song (ZANN 1996). In both treatments subjects were supplied daily with a mixture of dried seeds and fresh water (plus additional vitamins three times weekly). The high quality treatment group (HQ) was supplied with germinated seeds, egg food and hens' egg whereas the low quality treatment group (LQ) did not receive additional supply. Same sex siblings were assigned to different treatments (Table A2; Appendix). One adult male tutored two different groups synchronously while sitting in a cage (45 x 24 x 31 cm) attached to the two-compartment aviary (Fig. 2). In total 9 identical set-ups situated in the same room were used. For zebra finch song learning visual interactions can be crucial (HOUX and TEN CATE 1999b; MANN *et al.* 1991) and juveniles are not expected to copy from males they can not see (EALES 1989). Tutor was either an unfamiliar male (for 75 tutees) or the father (for 11 tutees; Table A2, Appendix). At day 60, experimental treatments and song tutoring ended and subjects were transferred to mixed sex aviaries where they received an intermediate diet consisting of standard seed mix supplemented by germinated seeds.

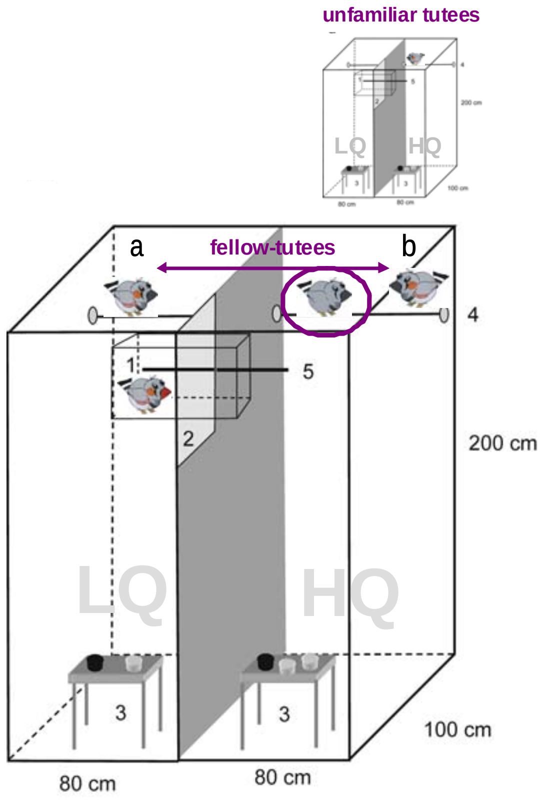


Fig. 2. Setup for song tutoring and nutritional manipulations. Tutoring aviaries consisted of two adjacent compartments/ groups. A tutor cage was centred at the front. Front = mesh wire, other parts = plywood (1). One perch was in the rear (4) and one perch adjacent to the tutor (5). A mesh window (2) enabled offspring to interact visually and vocally with the tutor, even while he was sitting in front of the other group. The mesh enabled interactions between the groups in which diets differed (3): one side fed on a low quality (LQ) and the other a high quality (HQ) diet. Fellow-tutees = tutees tutored by the same tutor; unfamiliar-tutee = tutee from a different tutor group. Fellow-tutees can be **(b)** of the same or **(a)** the other nutritional treatment group (after NAGUIB and NEMITZ 2007).

MALE SONG

Zebra finch song is highly complex and stereotyped and has to be learned from an adult conspecific during the sensory phase for song learning (~20 – 90 days post hatch; Fig.1). To analyze effects of the experimental dietary restrictions during puberty (day 35 – 65) on song learning, male song was recorded and analyzed. Following (SOSSINKA and BOHNER 1980), we call an individual's distinct syllable sequence *motif*. A *song* consists of a series of introductory syllables followed by several renditions of the motif (range 1-10 for non-directed songs).

Recordings:

Males directed song towards a female was recorded with a K64 Sennheiser microphone and a digital recorder (Marantz professional PMD670) in a sound attenuated chamber. Individuals were kept in adjacent small wire mesh cages (39 x 21 x 28,5 cm) ensuring visual interaction with food and water ad libitum. Recordings were made with one female at a time but in total 21 different females from the Bielefeld colony were used. In total 46 males (31 male offspring and 9 song tutors from the experiment described above, as well as 6 adult males from second breeding stock in Bielefeld) were recorded for analysis or further processing (see female preferences). There are considerable constraints in recording directed song of non-domesticated zebra finches. 1) males do not always sing easily within the sound chambers and 2) female call interference can affect sound analysis and experimental processing. Elements are delivered in a considerably fixed but not exclusively stereotyped sequential order (STURDY *et al.* 1999). Due to low singing performance of a variety of males and/or interference of female calls it was not possible to select a choice of motifs for subsequent analysis. Therefore, the intra-individual variation between slight variants of a male´s motif could not be taken into account within the analysis. Introductory elements are highly similar between individuals and are sung to a variable number before the first motif (HOLVECK *et al.* 2008). By comparing subsequent renditions one *core motif* per male was chosen (never the first or last within a bout; the variant most prevalent /recording) from 90 min recording.

Song rate

Song rate was analyzed from the first 5 minutes of song within each recording, calculated as the number of motifs/ per seconds.

Visual inspection

Song motifs were converted into 22100 kHz sampling rate with 512 accuracy performing anti-aliasing filtering and normalized (75 %, remove offset). Spectrograms of motifs (N = 256; F = 100, Hamming, Bw = 224Hz) were generated (Avisoft SASLab Pro 3.5. R. Specht, Berlin, Germany) to perform visual inspection by three different observers (MH, MN and RS; all experienced with spectral analyses). Observer reliability was calculated for all measured traits (Fig. A1, Table A1, Appendix) via Kendall´s coefficient of concordance.

Table 1: Abbreviations and definitions of the eight song structure parameters which were analyzed via visual inspection of spectrograms.

Label	Song parameter	Definition
ne	Number of elements	Total number of elements
rep	Repertoire	Number of unique elements in motif
et	Element types	Number of unique elements in motif / ne
m	Match	Number of elements found in tutee and tutor motif
um	Unmatch	Number of elements solely found in tutee motif
ls	Learning score	Matches / tutor repertoire *100
ss	Similarity score	Matches / tutee repertoire *100
ca	Copying accuracy	Sum copying score / matches

Automated analysis with SAP Pro

Song parameter measurements

The software SAP Pro (TCHERNICHOVSKI *et al.* 2000) is a valuable tool for analyzing animal vocalisations. Sound files can be loaded directly into the program, which will extract a variety of song parameters. Even in an automated song comparison procedure one of the most difficult user decisions is to set the parameters and their thresholds to detect vocalization and segment the sound to syllable units. Little is known about their excact perception in zebra finches (FRANZ and GOLLER 2002). There is no entirely objective criterion for separating syllables and it always entails substantial variation caused by human decision (JONES *et al.* 2001a). Therefore, I decided to assess SAP Pro song parameters as scores for each of the whole motifs. The measured parameters were: sound duration, element rate = number of elements (calculated by visual inspection)/ sound duration, mean frequency, FM = frequency modulation, amplitude, AM = amplitude modulation, pitch, goodness of pitch, harmonicity (for definitions and graphical representation see Table A1, Appendix). The parameters which were extracted either by visual inspection or an automated procedure were only comparable to a limited part. The visual comparison could not account for singing performance whereas the SAP Pro extracted parameters did only account for song complexitiy to a limited extent.

Motif comparisons

SAP Pro provides an overall similarity score which is a combination of three different measures: similarity, accuracy and mean sequential match. Mean sequential match is by default set to be an arbitrary 50 % weight of non-sequential sections. However, sequential mismatches might have different meanings and the user has to decide how to weight non-sequential sections. To work with an objective measure of resemblance between song motifs and for excluding observer biases I decided to work only with the measures for similarity and accuracy, refraining from analyzing a lower, overall similarity score. **SAP-similarity** is the percentage of overall significant similarity of final sections between two song phrases. Final sections are computed across intervals of 70ms: this similarity estimate is asymmetric. **SAP-accuracy** is the average fine scale accuracy or matching of the acoustic features across final sections (but see

SAP Pro manual for further details). To analyze the dietary treatment effect on tutees' song copying from their tutor and to analyze the effect of social parameters on song learning all song motifs (32 motifs from 32 tutees and 9 motifs from 9 tutors) were compared with each other (for methodological details concerning the motif comparisons see Box). The asymmetrie between motifs and whether or not one song was the template (tutor), has important implications, which made it essential to consider the sequence for comparisons. I calculated means from the two possible ways to compare two motifs for all possible dyads ($2n^2 = 2*1681$). Further analysis was performed with the means for SAP-similarity and SAP-accuracy score which was obtained for each motif-motif comparison. To obtain more information about the specific parameters which account most to tutor and tutees resemblance and to test whether there is a treatment effect the relative difference (RD) between tutor and tutees song parameter measurements was analyzed.

With SAP Pro (TCHERNICHOVSKI *et al* 2000) one can compare two different song motifs and calculate their Euclidean distances via two different methods:1) time-course and 2) mean-value. The time course approach is good for detecting similarity between two sequences of features that show similar curves of feature values. It compares all possible pairs of intervals between two sounds and can therefore detect the rare pairs of intervals where the match between all (or most) frames is high. Euclidean distance across mean values achieves exactly the opposite as dependency between neighbouring intervals is high and high similarity between distributions regardless of the short scale differences are detected. Similarity values were calculated for every tutor - tutee motif combination via both methods (time value and mean value) and the results were checked for correlation (similarity: r 0.93, $p < 0.001$; accuracy: r 0.88, $p < 0.001$; sequential match: r 0.62, $p < 0.001$). Due to this high correlation and the practical application, motif comparisons between all possible combinations of subjects were performed via the time-value method. The time value-method is enabled by the SAP Pro similarity batch which allows automated motif comparisons on a large data set.

Statistical Analysis

Data were tested for normal distribution. Normally distributed data were tested with parametric statistics. If data did not meet normality assumptions after transformation non-parametric statistics were applied. Model's residuals were tested for normal distribution. Treatment effect on song rate was analyzed with linear mixed effects models. The maximal model was fitted with fixed effects: ratio of males within a tutor group and female with whom recordings were obtained, and random effect: tutor group. Observers' reliability on the parameter values obtained via the visual inspection of spectrograms were analyzed with Kendall's W. In cases of significant concordance between observers, the respective parameter estimate of MH was analyzed separately for an effect of nutritional treatment (t-test). Correlation between variables was analyzed with Spearman-Rank Correlations (S, r_s) or Pearson's-R (R, r) respectively. Two principle component analysis (PCA) were performed on the SAP Pro extracted song parameters to minimize multiple testing. With PCA 2 tutees' individual song parameter values were reduced whereas PCA 1 combined the relative differences (RDs) between song structure parameters between tutor and tutee (value of tutor – value of tutee / the sum of both values). To ensure that the scores of the PCAs (orthogonal rotation: varimax with Kaiser normalization) were uncorrelated the Anderson-Rubin method was applied (Field 2000, Anderson & Rubin 1956). Linear mixed effects models (LME) were used to analyze effects of nutritional treatment on each of the 8 different PCs with first fitting the maximum model which allowed interaction between fixed effects: dietary treatment, number of males in tutor group, tutor's repertoire size and controlled for tutor group as random effect. Treatment effects on tutor-tutee motif similarity were calculated with t-test (t) or Wilcoxon-Test (W). I analyzed whether tutees showed highest similarity with their tutor or another tutee with a binomial test and tested for a treatment effect on this distribution with G-Test (against 50 % chance level). To analyze the similarity and accuracy values with a lme by considering implications of the four different classes: tutor, tutee from own nutritional treatment within the same tutor group, tutee from other nutritional treatment group within the same tutor group and a randomly picked motif (fixed effects: diet, tutor group; random effect: tutee identity). Final models were obtained by using Aikaike's Information Criterion (AIC) to select the most parsimonius model. Statistical analysis and models were run in R 2.8.1 (PINHEIRO *et al.* 2006; R DEVELOPMENT CORE TEAM 2008) with exception of PCA which was run using SPSS 16.0.1.

RESULTS

Song rate was not different between high quality (HQ) or low quality (LQ) males, even though there was a trend for LQ males to sing at a higher rate (LME: $F_{1,21} = 3.34$, $p = 0.082$, Fig. 3).

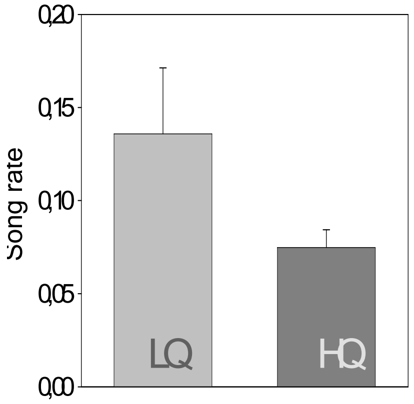


Fig. 3: Treatment effect on song rate. Song rate´s raw means ± s.e. (no. motifs/ s) for males which fed on low (LQ) or high quality (HQ)

Visual inspection

The three observers showed significant concordance in all measured traits, except for copying accuracy (Table 2). Subsequently, the values obtained by MH were analyzed. As observers disagreed in copying accuracy those values were excluded from the analysis. There was no treatment effect on any of the song characteristics (Table 2). The presence of males can be a crucial social factor which influences song learning in zebra finches. The number of males in a tutor group can influence male’s ability to produce adequate copies of their tutor’s song (GIL *et al.* 2006; HOLVECK *et al.* 2008; TCHERNICHOVSKI and NOTTEBOHM 1998). In our experiment, number of tutees and proportion of males within groups did not differ between treatment groups (Table 2) and there was no effect on male song learning. There were no significant correlations between number of males and tutee similarity score ($S = 5935.28$, $r_s = -0.09$, $p = 0.63$), between the number of males and learning score ($S = 4880.97$, $r_s = 0.11$, $p = 0.57$; Fig. 4a), the number of males and tutee repertoire size (S = 4727.44, r_s = 0.13, p = 0.47) and interestingly repertoire sizes did not correlate between tutor and tutee (R_{30} = 0.74, r = 0.13, p = 0.47; Fig. 4b)

Table 2: Visual motif comparisons. Definition for each parameter, observer's concordance (Kendall's W) and result of the statistical analysis (t-test) for a treatment effect on the song structure parameters.

Definition	Parameter	Kendalls W		test_{df}	p
total number of elements in tutee motif	number of elements	W = 0.93	p< 0.001	t_{30}	0.58
number of unique elements in tutee motif	repertoire	W = 0.91	p< 0.001	t_{30}	0.80
tutee repertoire/ number of elements	element types	W = 0.98	p< 0.001	t_{30}	0.53
mean duration of three song motifs [s]	motif duration	-		t_{30}	0.08[1]
number of elements/ motif duration	element rate	-		t_{30}	0.15
number of elements found in tutee and tutor motif	matches	W = 0.91	p< 0.001	t_{30}	0.73
number of elements only found in tutee motif	unmatches	W = 0.87	p< 0.001	t_{30}	0.81
no of matches/ tutor repertoire	learning score	W = 0.90	p< 0.001	t_{30}	0.71
no of matches/ number of elements	song similarity	W = 0.92	p< 0.001	t_{30}	0.96
no of matches/ tutee repertoire	new song similarity	W = 0.90	p< 0.001	t_{30}	0.82
sum copying score/ matches	copying accuracy	W = 0.44	p = 0.118	-	-
total number of tutees within tutor group	number of tutees	-		t_{30}	0.85
number of males in tutor group/ number of tutees	sex ratio (% males)	-		t_{30}	0.71

[1] excluding one tutee which had an extremely short motif resulted in t_{30}, p = 0.15

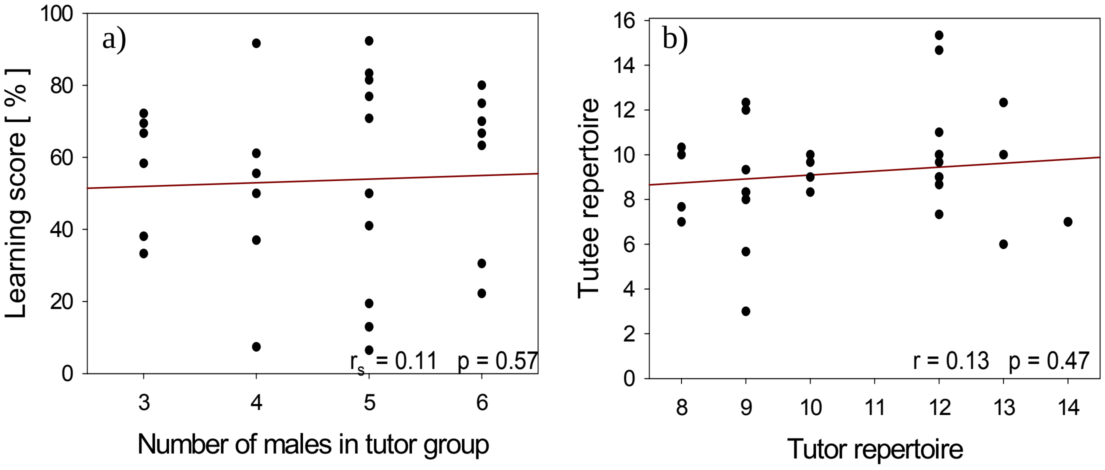


Fig. 4. Group composition effect on song learning. Correlation of **a)** number of males in tutorgroup with learning score (number of elements found in tutor and tutee motif / sum of elements in both motifs) and **b)** tutor repertoire size (number of unique elements within motif) correlated with tutee repertoire size.

Automated analysis via SAP Pro

Song parameters

PCA on SAP Pro song parameters led to four principal components (PCs) after rotation (Measure of adequacy: Kaiser Meyer Olkin = 0.507; Bartlett sphericity: Chi $^2_{66}$ = 314.42, $p < 0.001$; Table. 3). Initial eigenvalues > 1.0 and rotation converged in 7 iterations. There was no treatment effect on tutees' combined song traits (LME$_{PC1}$: $F_{1,211}$ = 0.106, p = 0.75; LME$_{PC2}$: $F_{1,21}$ = 0.05, p = 0.83; LME$_{PC3}$: $F_{1,21}$ = 0.80, p = 0.38; LME$_{PC4}$: $F_{1,21}$ = 0.03, p = 0.86; Fig. 5).

Table 3: Combined song parameters. Rotated matrices of the four components (PC1 - 4) and percentages of explained variation for the two PCAs. Analysis based on individual SAP Pro song parameters of tutees´ motif (PCA 1) and on relative differences (RD) between tutor and tutees SAP Pro parameters (PCA 2). Values in bold indicate highest contribution to a particular component.

	PC 1	**PC 2**	**PC 3**	**PC 4**
PCA 1 on individual SAP parameters of tutees´ motif				
duration	-0.238	0.029	-0.251	**0.853**
mean amplitude	0.494	0.276	**-0.651**	0.325
mean AM^2	0.438	0.467	**-0.650**	-0.060
mean FM	0.304	**-0.813**	-0.218	0.042
mean entropy	0.600	**0.606**	0.227	-0.278
mean pitch	**-0.891**	0.287	0.013	0.039
mean pitch goodness	**-0.894**	0.025	0.019	0.055
variance_pitch	**0.803**	-0.251	0.327	0.180
variance entropy	**0.839**	0.201	0.162	-0.039
variance mean freq	0.540	-0.159	0.470	**0.528**
variance FM	0.047	**0.690**	0.399	0.329
variance pitch goodness	**-0.832**	0.103	0.199	0.127
% Explained variance	**34.80**	**18.48**	**15.93**	**12.58**
PCA 2 on relative differences of SAP parameters between tutor and tutee´s motif				
RD duration	0.435	-0.164	**-0.651**	0.265
RD mean amplitude	0.256	-0.180	**0.785**	0.046
RD mean AM^2	0.589	0.002	**0.642**	-0.025
RD mean freq	-0.186	-0.066	-0.035	**0.774**
RD mean FM	0.424	**-0.573**	-0.064	0.342
RD pitch goodness	-0.087	**0.919**	-0.053	0.207
RD variance mean freq	-0.199	0.019	**0.729**	0.275
RD variance entropy	**0.880**	-0.192	0.068	-0.048
RD variance FM	**0.772**	-0.083	-0.080	-0.056
RD variance pitch	0.130	0.137	0.176	**0.862**
RD variance pitch goodness	-0.084	**0.945**	-0.035	-0.033
% Explained variance	**20.52**	**19.94**	**18.50**	**15.06**

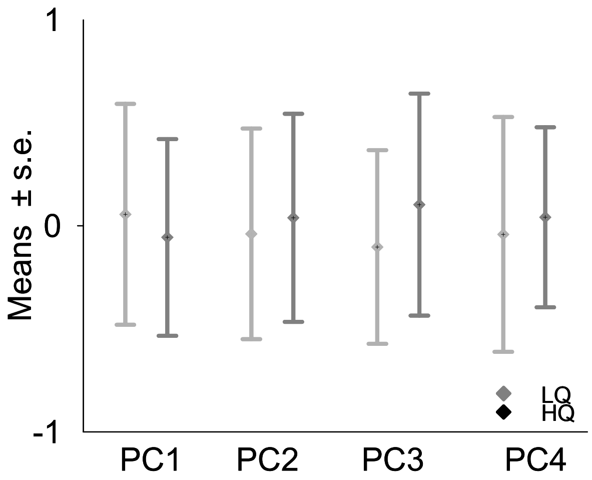


Fig. 5: Treatment effect on combined song parameters. Mean of rotated PCs (1-4) based on individual SAP Pro song parameters of tutees' motif. LQ = low quality, HQ = high quality.

Motif comparisons: Tutee versus tutor

Dietary treatment did not show an effect on resemblance between tutee and tutor motif (SAP-similarity: W = 118.5, p = 0.97; SAP-accuracy: t_{29} = 1.169, p = 0.25). The proportion of males within a tutor group did not significantly affect SAP Pro measured song similarity (SAP-similarity: S = 6355.82, p = 0.125, r_s = -0.28; SAP-accuracy: S = 4591.40, p = 0.70, r_s = 0.074).

Table 4: Treatment and social effects on combined song parameters. Dietary treatment (LQ, HQ) effect on number of males in a tutor group and tutor's repertoire on the four extracted components (PC1 - 4) was tested. Non significant terms were retained in the model when they explained a significant part of the variation (AIC). n/a = ns removed terms.

	Fixed Factor in Each Linear Mixed Effects Model								
Y	**Diet**		**Number of males**		**Tutor repertoire**		**Interaction Term**		
PC 1	$F_{1,21}$ = 3.15	p = 0.09	n/a		n/a		n/a		
PC 2	$F_{1,20}$ = 0.04	p = 0.85	$F_{1,20}$ = 0.60	p = 0.46	n/a		$F_{1,20}$ = 4.54	p = 0.045	1
PC 3	$F_{1,21}$ = 0.27	p = 0.61	$F_{1,6}$ = 0.30	p = 0.60	$F_{1,6}$ = 0.012	p = 0.92	$F_{1,6}$ = 3.96	p = 0.09	2
PC 4	$F_{1,21}$ = 3.51	p = 0.07	n/a		n/a		n/a		

Interaction terms: [1]males*tutor repertoire; [2]diet*number of males.

For a detailed analysis on relative differences (RD) between tutor and tutee motif, song parameters (SAP Pro) were combined via PCA. PCA extracted four principle components (Kaiser Meyer Olkin = 0.524; Bartlett test = Chi^2_{55} = 133.41, $p < 0.001$; Table 3). Initial eigenvalues > 1.0 and rotation converged in 5 iterations. RDs between tutee and tutor were not found to differ between treatment groups (Table 4; Fig. 6).

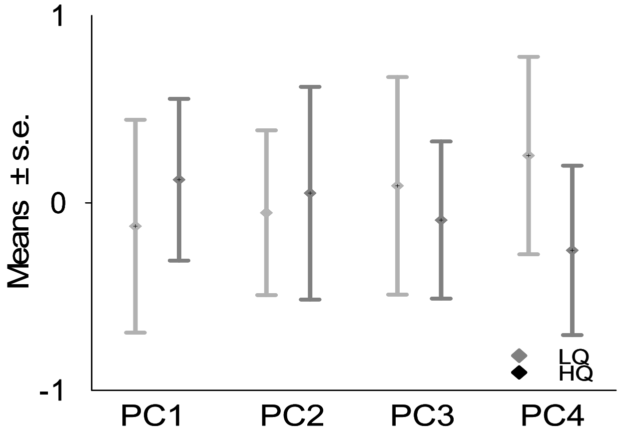


Fig. 6: Treatment effect on combined song parameters. Mean of rotated PCs (1-4) based on relative differences between SAP song parameters of tutor and tutees' motif. LQ = low quality, HQ = high quality

Motif comparisons: Tutee versus tutee

Six of the 31 tutees showed a higher motif resemblance to their tutor than to another tutee. Motifs of the remaining 25 tutees were more similar to another tutee than to their tutor. These differences were significant (binomial test: $k_{31} = 6$, $p < 0.01$; Fig. 7) and not affected by nutritional treatment (G-Test: all $G < 3.84$, all $p > 0.06$; Fig. 6). Tutees showed significantly lower SAP-accuracy when compared to a randomly picked motif than to their tutor or a tutee of their own group ($LME_{accuracy}$: $F_{3,63} = 5.73$, $p < 0.002$; Fig. 8) whereas no such effect was found on the SAP-similarity score, which was influenced by tutor identity ($LME_{similarity}$: $F_{3,79} = 1.12$, $p = 0.24$; tutor: $F_{9,21} = 2.78$, $p = 0.026$)

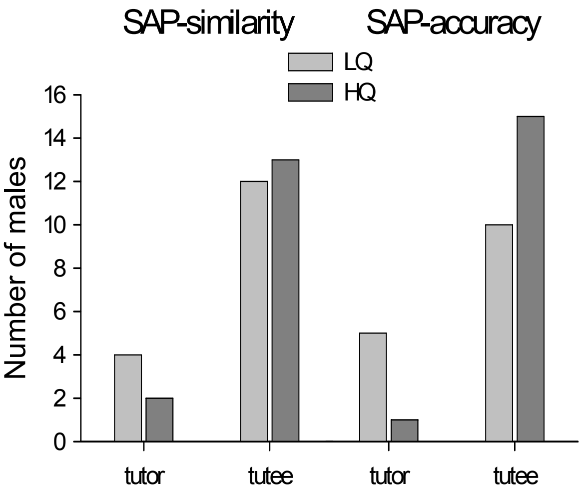


Fig. 7: Highest motif resemblance. Number of males which had the highest motif resemblance (SAP-similarity/ SAP-accuracy) to their tutor or another tutee. LQ = low quality, HQ = high quality.

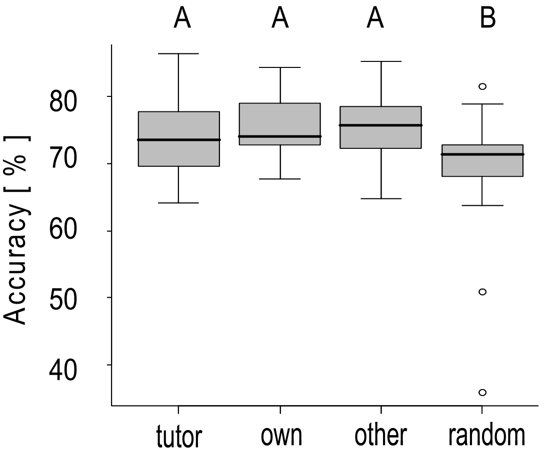


Fig. 8: Motif Comparisons. Tutee motif compared to its tutor, a tutee from his own diet, a tutee from the other diet within his tutorgroup and a randomly picked motif. Median SAP-accuracy ± IQR. Letters indicate significant differences (same letters = ns, different letters = $p < 0.05$).

FEMALE PREFERENCES

Three operant preference tests were conducted to analyze nutritional effects during the song learning phase (test 1, 2 and 3), preferences for song complexity (test 2) and effects of social circumstances (test 1, 3) on females song preferences. A detailed overview of subjects′ participation can be found in the Appendix (Table A2).

Preference test 1: tutor versus unfamiliar

Stimuli

Stimuli were created from 12 song motifs which were selected from recordings of 12 different males (see Male song, Recordings). Female subjects (n = 25) from eight different tutor groups were tested with a stimulus dyad containing one song of an unfamiliar male (either from another breeding stock in Bielefeld or the song tutor of another tutor group, which had no prior contact to subjects) and a song of their tutor. In two sets the unfamiliar male was the song tutor of another group, in three sets it was a male from the other colony. The song of any unfamiliar male was only used once in any of the 5 sets. Females which had been tutored by the same song tutor as juveniles received the same stimulus set. As I could not get a suitable recording from one of the tutors, his tutees could not be tested (Table A2 und A3, Appendix).

Transport and housing

Preference test was performed from May - August 2005 at Leiden University in the Netherlands. I transported females (n = 25) in wooden transport boxes (N = 2) via train. Each box had small openings to ensure air circulation and was subdivided in 2 compartments so that six birds sat together (in one case there were 7 birds per compartment). They had access to a seed-mix and fresh fruit for the whole trip, which lasted 7 hours (from the time point of catching in Bielefeld until cageing in Leiden). In Leiden, females were housed in one room (14:10 L:D) caged with the same females (3 - 4) with whom they had been transported previously. Water, seed mix, millet and cuttlefish were provided ad libitum.

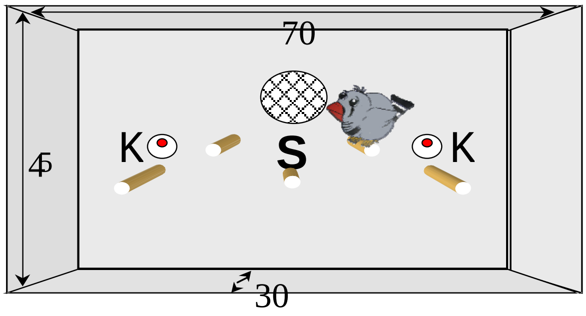


Fig. 9: Schematic front view of the operant test cage. Females could trigger playback by key pecking. Preference values were calculated as number of key pecks for each test song/ total number of key pecks. **S**:

Experimental set-up

The conditioning apparatus (Fig. 9; RIEBEL (2000)) was used in a sound attenuated chamber (8 identical set-ups). The cages were wire mesh with a wooden rear and were illuminated from the top. Five perches were spaced along the length of the cage (at 8, 23, 35, 47 and 62 cm), with perches 1, 3 and 5 at 23 cm height and perches 2 and 4 at 26 cm height. From the first and fifth perches, the birds could peck red response keys (d = 1cm) with a small red light-emitting diode (LED) in the centre (d = 2 mm), which triggered one playback of the song assigned to the key. Pecking keys were connected to a custom-built mini-computer (soundchip Oki MSM6388, Tokyo, Japan) which recorded time of key peck activation and controlled playbacks via a loudspeaker (Quart 250 or JBL Control 1, Northridge, CA, USA) placed behind an opening of 9 cm diameter in the back. Songs were played back at 70 dB (re 20 μPa peak amplitude at 30 cm, CEL-231 sound level meter, fast response setting (Lucas CEL Instruments, Hitchin, UK).

Training

Subjects (mean age 157 ± 42) were transferred individually to the experimental cage (38 ± 10 days after arriving in Leiden). Until the end of the day when first operant responses were recorded the pecking keys' LED lights were switched on. If no pecks were recorded within four days the LED lights were switched off and individuals received 20 min training sessions two times daily until operant responses were logged. Training started by switching LED's on (described in detail in HOUX and TEN CATE (1999)).

Data collection

Data were recorded the day after frequent operant responses were observed. The number of key pecks were registered by the control unit. To control for possible side preferences songs were daily swapped between keys. The first test lasted two or four days depending on whether both (n = 19) or one sensor (n = 2) were pecked. In 4 cases females did either not learn the task or the rate in initiating key pecks (motivation) was too low.

Transition to test 2

The second preference test followed without any days in between. After the last day of test 1 the stimulus dyads were exchanged (between 21:30 pm and 8:00 a.m of the following morning).

Preference test 2: long versus short

Stimuli

Stimuli were created from 12 song motifs selected from recordings of 12 different males (see Male song, recordings). Six long-short stimulus dyads were created (Avisoft SASLab Pro 3.5. R. Specht, Berlin, Germany). Motifs in a dyad differed by 50 - 80 % relative duration (means ± s.d.: short = 570 ± 91 ms, range 450 - 760 ms, long = 935 ± 114 ms, range 780 -1140 ms; mean difference 65 ± 12%). A standard pause of 180 ms was inserted after each motif before it was digitally multiplied into a 4-motif repetition song (mean duration songs: short = 2820 ± 364 ms, long = 3214 ± 466 ms). Females (n = 21) were tested with song from unfamiliar males (neither father nor tutor). Females from the same tutor group received identical stimulus dyads (Table A2, A3; Appendix).

Transport, housing, experimental procedure, data collection

All details were identical for test 1 and test 2 (see Preference test 2). Training was not necessary prior test 2 (see transition to test 2).

Preference test 3: fellow-tutee versus unfamiliar tutee

Stimuli

Three different song motifs of each male subject (n = 32) were chosen to estimate mean motif duration (mean ± s.d: 739.82 ± 173 ms, range 146 – 1167 ms). For the analysis of motif duration, female disturbances were negligible which made it possible to get three motifs of each male. For each male one motif of the three motifs was chosen randomly by generating random numbers (the motif with the highest random number was selected). In eleven cases the selected motif showed interferences by a female or other noise so that the motif with the next highest number was taken. In one case there was not a single appropriate motif due to female calls or noise. In this case a *synthetic* motif was created by *cutting & pasting* the undisturbed elements of two different original motifs. A standard pause of 180 ms was inserted after each motif before it was digitally multiplied into a 4-motif repetition. In total 22 songs from 22 males were created to form 11 stimulus dyads comprised of one song from each of the following two categories: **Fellow - tutee song:** the song of a male with whom the female sat together in a tutor group. In four cases the fellow-tutee was the female's brother. **Unfamiliar - tutee song:** the song of an unrelated subject who was tutored in a different tutor group. Visual and social interaction with this male was impossible but as all groups were kept within the same room vocal interaction was enabled if tutees happened to be tutored at the same time (depending on subjects age).

Motifs in stimulus dyads were allowed to differ up to 6 % in duration. Females (n =19) were tested for their song preferences. Three stimulus dyads were used once whereas the other eight were used twice so that two females received the same stimulus set. In doubled used sets the fellow-tutee song for one of the females served as the unfamiliar-tutee stimulus for the other female. Each of the two different stimulus songs served only once as fellow-tutee and once as unfamiliar tutee stimulus.

Housing prior to the experiment

Preference test 3 was performed in August 2006 at the University of Bielefeld as part of an undergraduate course. Two weeks prior to the experiments, female subjects were individually transferred to small same sex groups (82 x 30 x 38 cm; two-four females). After the experiments females were transferred to mixed sex aviaries.

Experimental set up

The operant conditioning apparatus (Fig. 9) was standing in a sound attenuated room. Two identical set ups were used in two rooms. The cages were wire mesh (70 x 37 x 43.5 cm). Two short and two long perches were spaced along the length of the cage at 30 cm height. A photo-electric switch connected the short perches to a computer device outside the room which counted and triggered playbacks via SAPII (Sound Analysis Pro II,

D. Swigger and O. Tchernichovski, CCNY, Dept. of Biology) when females perched. Songs were broadcasted via a loudspeaker centered in 85 cm distance to the switch (play back at 55 dB; re 20 µPa peak amplitude at 30 cm). Switches were not activatable during playback to infer overlapping of song. Lights were off from 10 p.m. to 6 a.m. but stimuli were retrievable and an emergency light prevented birds from sitting in the dark.

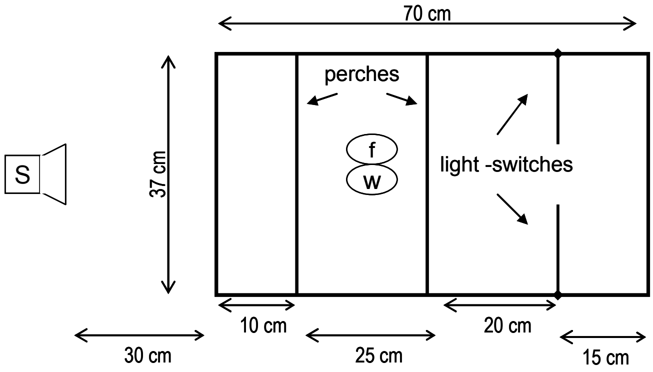


Fig. 10: Schematic aerial view of the operant test cage. S: speaker. Females could trigger playback by hopping on the small perches connected to light-switches. Preference values were calculated based on the number of perch hops for each test song (J. Wetzel).

Training

Females (mean ± s.d: age 938 days ± 37) were trained in the apparatus to habituate to the experimental procedure and to learn utilizing the photo-electric switches accordingly. In all training sessions the same stimulus set was used, which was different from the experimental stimulus dyads. Training lasted 2 days (± 3 s.d.) and was complete when songs from both sides were triggered for a total of ten times.

Data collection

When criterion for accomplishing training was reached the experimental stimuli were assigned to the photo electric switches. Number of perch hops (i.e. playbacks) were counted by the connected computer and after 100 songs have been triggered stimuli were swapped to control for side preferences. Due to a technical problem in some cases stimuli were swapped after 101 songs. For comparability the extra song was not taken into account. The experiment ended when after the stimuli swap another 100 songs were triggered.

Statistics

Female preference values were calculated as initiated playbacks for one stimulus / total playbacks for both stimuli. Data were tested for normal distribution and variance homogeneity with Shapiro-Wilk and Fishers F-Test respectively. Individual female preferences were analyzed by G-Test with Williams correction (against 50 % chance level). Differences between treatment groups were analyzed using t-test. Statistical analysis was run in R (R DEVELOPMENT CORE TEAM 2008).

RESULTS

Preference Test 1: tutor versus unfamiliar

Data for test 1 and 2 were retrieved from 21 females as two females did not learn the task at all and two others did not learn to peck both sensors frequently. Every individual female showed a significant preference for either of the songs (G Test as deviation from 0.5 with Williams correction; all sign. $p < 0.01$; Fig. 11). Most females preferred

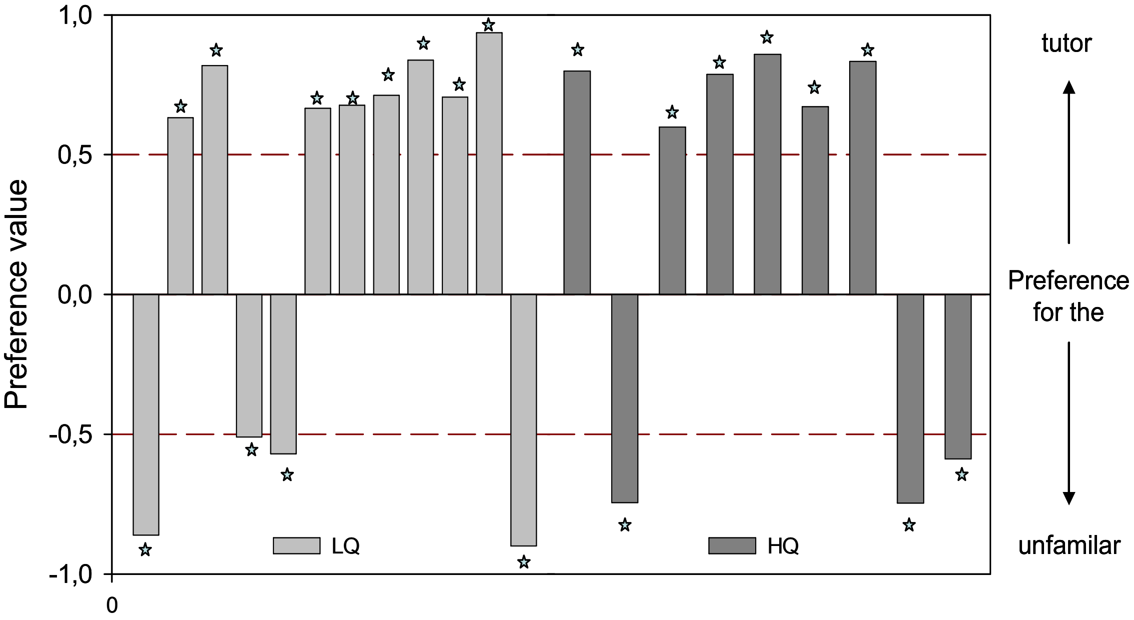


Fig. 11: Preference Test 1. Preference values for low (LQ) or high quality (HQ) females. Positive values indicate a preference for the tutor whereas negative values indicate a preference for the unfamilar. Stars indicate significant departure from 50 % chance (dashed line; G-Test all sig. $p < 0.01$).

their tutor's song but on a group level there was only a trend ($t_{20} = 1.95$, $p = 0.06$; Fig. 10). Female from different nutritional treatments did not differ in their preference strength ($t_{19} = 0.02$, $p = 0.98$; Fig. 12) or their pecking activity ($t_{19} = 0.07$, $p = 0.94$; Fig 13).

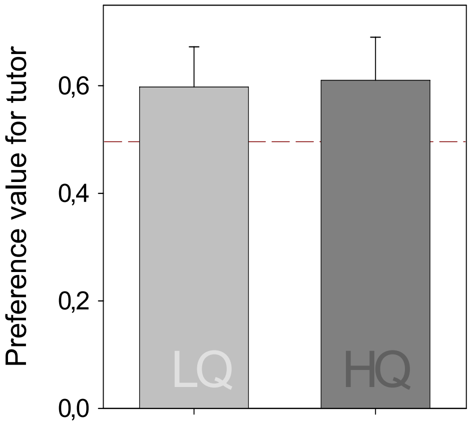


Fig. 12: Preference Test 1. Females (low quality (LQ) and high quality (HQ)) preference for the tutor (means ± s.e.). Dashed line depicts 50% chance level.

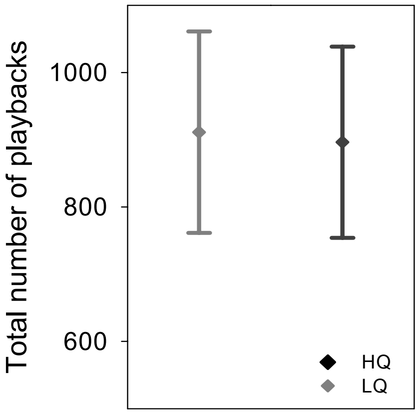


Fig. 13: Preference Test 1. Pecking activity (means ± s.e.) for females from the low quality (LQ) and high quality (HQ) group.

Preference test 2: short versus long

Most females showed a significant preference for one song ($t_{17} = 6.62$; $p < 0.0001$; Fig.14) but there was no significant preference for long songs ($t_{17} = 0.16$; $p = 0.87$). Preference values did not differ between females from treatments ($t_{19} = 0,2$; $p = 0.63$; Fig. 15). However, females from the high quality (HQ) group were pecking the keys more frequently ($t_{14} = 2.3$; $p<0.04$; Fig.16).

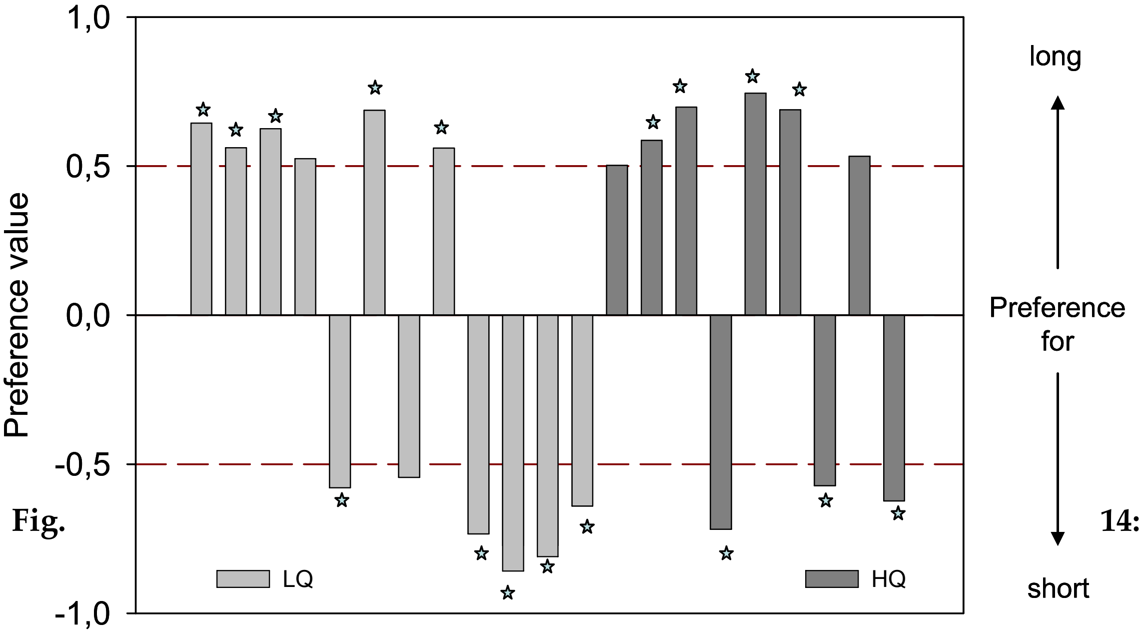


Fig. 14: Preference Test 2. Values for females from the low (LQ) or high quality (HQ) diet. Positive value = preference for the long song, negative value = preference for the short song. The dashed line depicts 50% chance level. Stars indicate significance (G-Test: deviation from 0.5; all sig. p < 0.01).

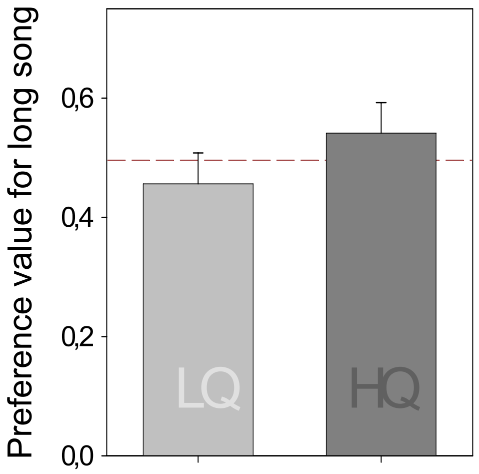


Fig. 15: Preference Test 2. Preference strength (long song) of low quality (LQ) and high quality (HQ) females. The dashed line depicts 50% chance

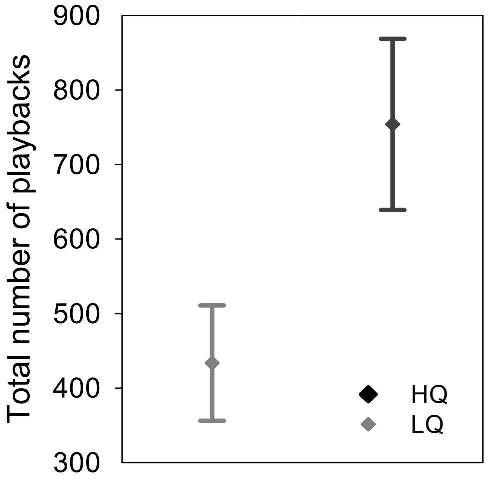


Fig. 16: Preference Test 2. Pecking activity (means ± s.e.) for females from the low quality (LQ) and high quality (HQ) group.

Females preference value for the long song did not correlate with the element rate of the long song ($S = 1176.03$, $p = 0.30$, $r_s = 0.27$; Fig. 17a). Interestingly, there was a high correlation between females preference value for the long song and the element rate of the respective short song within the dyad ($S = 636.73$, $p < 0.01$, $r_s = 0.59$; Fig. 17b). Only females from the low quality (LQ) diet showed a correlation between their preference for the long song and the element rate of the short song ($S = 99.71$, $p = 0.022$, $r_s = 0.65$; Fig.17b) whereas females from the high quality (HQ) group did not ($S = 59.2$, $p = 0.16$, $r_s = 0.51$; Fig.17b).

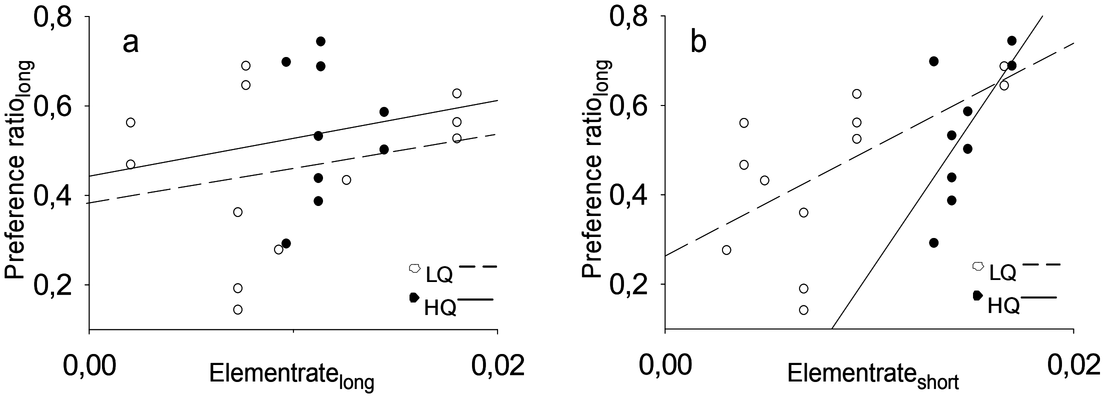


Fig. 17: Preference test 2. Correlation between female preference value for the long song and elementrates of **a)** the long song and **b)** the short song. Open circles = low quality (LQ), filled circles = high quality (HQ).

Preference test 3: fellow-tutee versus unfamiliar-tutee

Females significantly preferred the fellow-tutee song over an unfamiliar-tutee song ($t_{17} = 3.36$; $p<0.004$; Fig.18). Preference strength did not differ for males from different treatments ($t_{17} = 0.59$, $p = 0.57$; Fig. 19). Females from different nutritional treatments did not differ in their preference strength ($t_{17} = -1.63$, $p = 0.13$; Fig. 20).

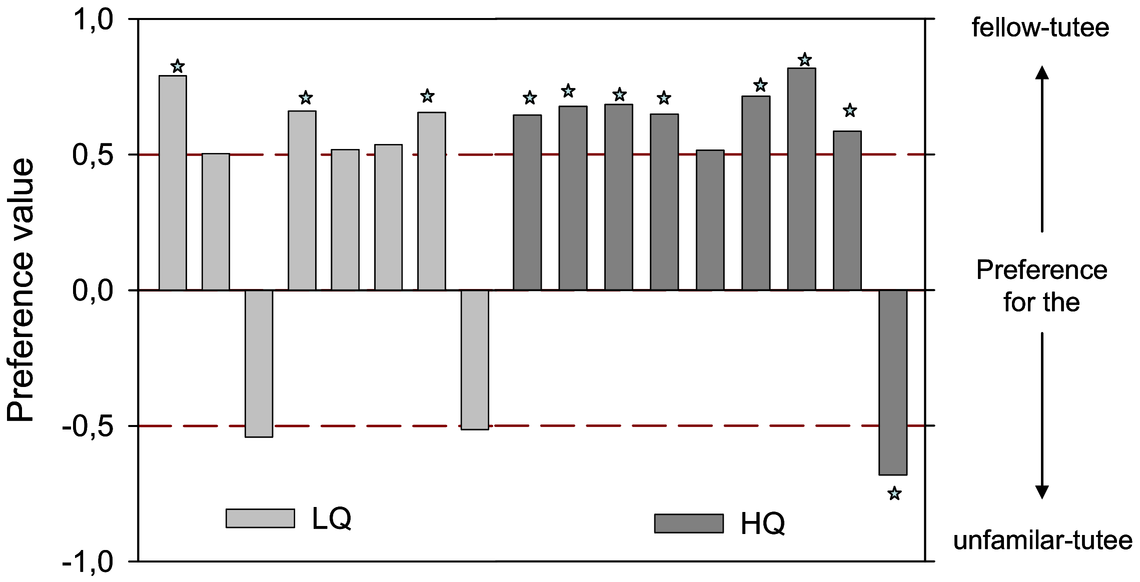


Fig. 18: Preference Test 3. Values for females from the low quality (LQ) or high quality (HQ) treatment. Positive value relates to a preference for the fellow-tutee song song whereas a, negative value is a preference for the unfamiliar tutee song. The dashed line depicts 50% chance level. Stars indicate significance (G-Test: deviation from 0.5; all sig. p < 0.01).

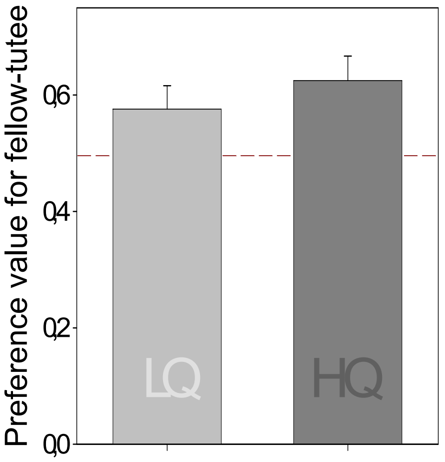


Fig. 19: Preference test 3. Preference strength (fellow-tutee) of low quality (LQ) and high quality (HQ) females. The dashed line depicts 50% chance

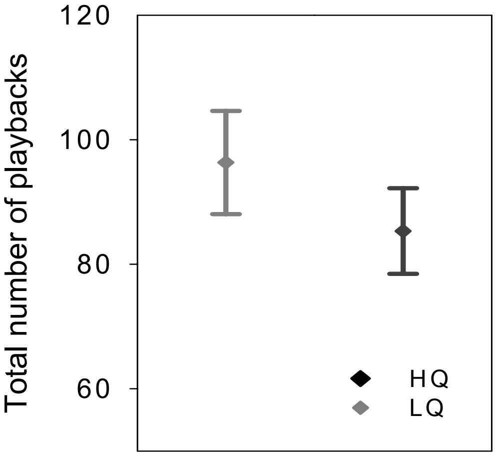


Fig. 20: Preference test 3. Pecking activity (means ± s.e.) for females from the low quality (LQ) and high quality (HQ) group.

Female song preferences in relation to male song parameters

Females preference value for the fellow-tutee (male tutee which had been tutored within the same tutor group) did not correlate with SAP-similarity between tutor and tutee (S = 174, p = 0.53, r_s = 0.21; Fig. 21a). There was a trend for females preference for the fellow-tutee to negatively correlate with SAP-accuracy (S = 354, p = 0.051, r_s = -0.61; Fig. 21b) indicating that females showed less preference for tutees with higher tutor resemblance. Contrary the preference for unfamiliar-tutees was correlated with their similarity to the female's tutor. The higher the unfamiliar-tutees' SAP-accuracy with females´ tutor was, the higher the female preference for the unfamiliar-tutees´ song.

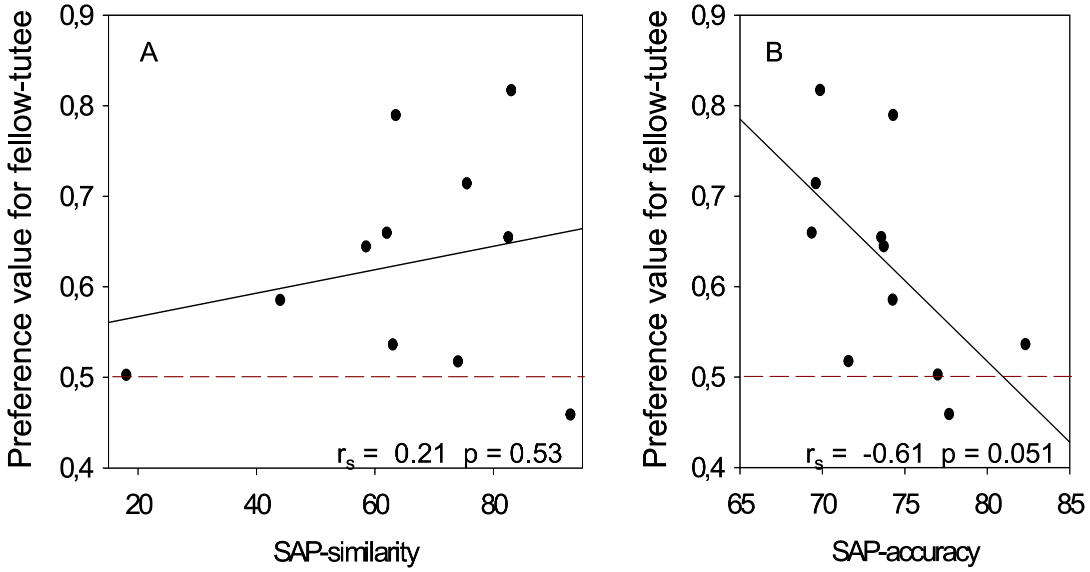


Fig. 21: Female preference value for the fellow-tutee in relation to fellow-tutees´ resemblance to tutor measured via SAP similarity parameters a) similarity and b) accuracy. The dashed line depicts 50 % chance level and a value above the line indicates a preference for the fellow-tutee whereas a value below the line translates to a preference for the unfamilar tutee.

Female preference value for the fellow-tutee (male tutee which had been tutored within the same tutor group) did not correlate with any of the four principle components (PC-1 – PC-4) combining the SAP Pro song parameter measurements (r_s = -0.12 – 0.57, all p > 0.05; Fig. 22 a-d).

However, there tended to be a positive relationship between preference value for the fellow-tutee and PC-4, on which motif duration and variance in mean frequency loaded most (rs = 0.57, p = 0.068; Fig. 22 d).

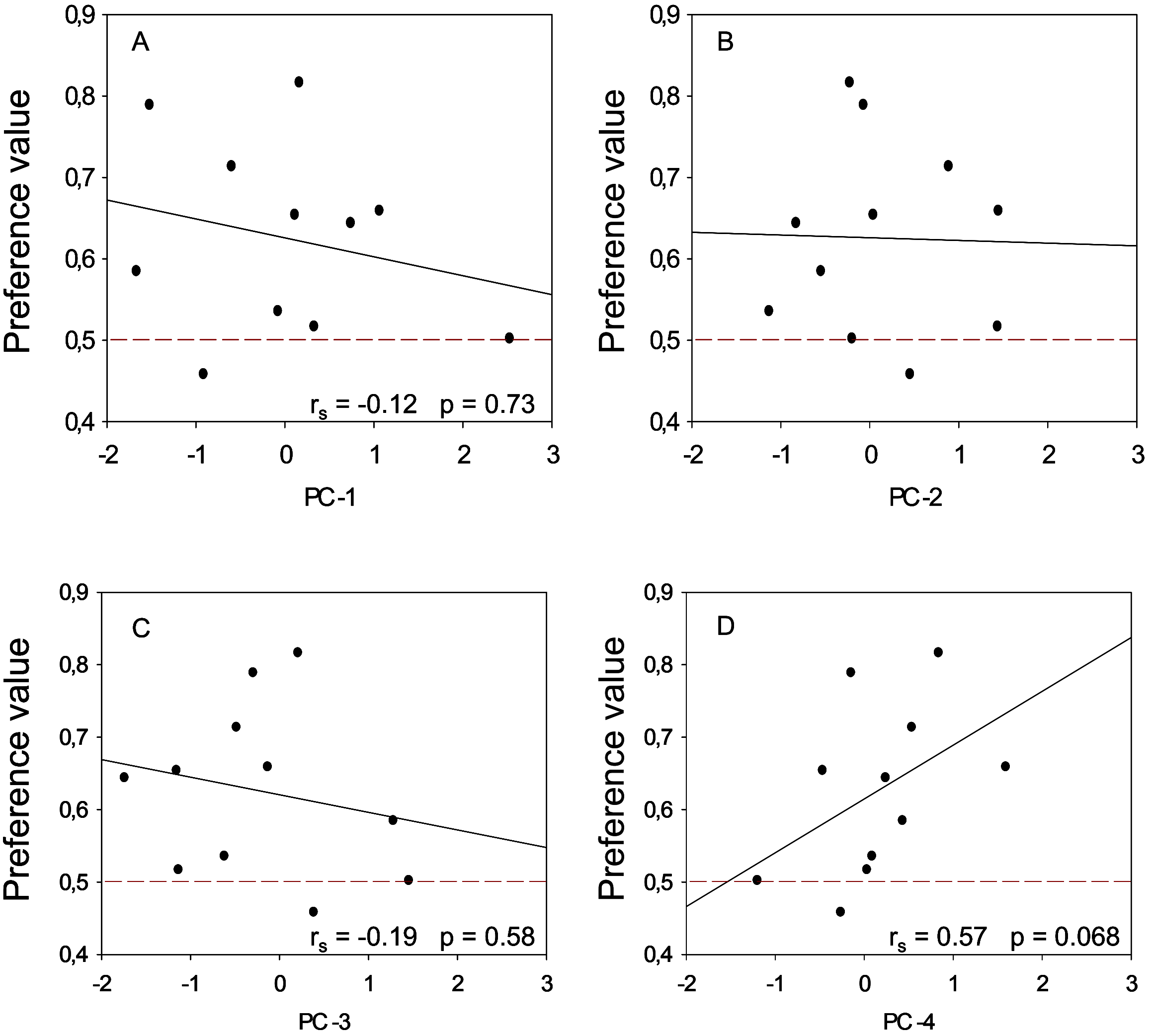


Fig. 22: Preference value for the fellow-tutee in relation to the four PCs (PCA-1). **a)** PC-1, **b)** PC-2, **c)** PC-3 and **d)** PC-4. A value above the dashed 50 % chance level indicates a preference for the fellow-tutee whereas a value below indicates a preference for the unfamiliar-tutee.

DISCUSSION

The results of this study could not support the hypothesis that nutritional constraints during puberty (day 35 - 60, a period important for song learning) lead to impaired male song copying or female preferences even though conclusions need to be drawn with care. Song rate tended to be higher in low quality males. Copying accuracy, as measured via two different methodological approaches and frequency related song parameters were not different between males of the low quality (LQ) or high quality (HQ) treatment. Tutees exhibited higher motif resemblance with another tutee than with their tutor and this effect was not evoked by dietary treatment. Female song preferences did not exhibit an interaction with nutritional history and there was no evidence that females differentiated between males from different nutritional backgrounds. Females only tended to prefer their tutor's over an unfamiliar song and they did not prefer more complex songs (longer). However, LQ-females preference for longer songs was correlated to the song rate of the short's song. Social environment had an impact on female recognition learning, as females preferred the songs of fellow-tutees over songs from unfamiliar-tutees whereas no such preference was expressed for their tutor's song. There was a negative correlation between the female's preference value for the fellow-tutee song and the similarity between tutor and fellow-tutee motif. Consequently, higher similarity between the unfamiliar-tutee's song and the song of the female's tutor was related to higher preference values for the unfamiliar-tutee. Female preferences did not seem to be affected with any of the SAP Pro measured song characteristics.

Male song

Dietary restrictions during puberty (d35 – 65) had no effect on male song learning. There were no differences in any of the structural or frequency dependant song parameters between males from the low quality (LQ) or high quality (HQ) treatment. However, song rate tended to be different between the groups with LQ males singing more than HQ males, which is in line with previous results, where males from larger broods were found to sing significantly more than males from small broods (TSCHIRREN *et al.* 2009). Previous studies tested the nutritional stress hypothesis, or in more general terms the developmental stress hypothesis, in relation to the period of early development, i.e. subjects encountered a period of suboptimal conditions during the first month post hatching (BRUMM *et al.* 2009; GIL *et al.* 2006; HOLVECK *et al.* 2008; NOWICKI *et al.* 2002; ZANN and CASH 2008). It is important to keep the methodological differences (developmental period) between their and our study in mind. This study provides evidence that nutritional constraints during puberty, a critical phase for song learning, do only show transient effects on individuals' trajectory (NAGUIB and NEMITZ 2007; NAGUIB and NEMITZ, *unpublished*) and that despite the ongoing development of the underlying neuronal pathways and nuclei of the song control system, no effects on song learning were observed. It appears that neither early nor juvenile developmental stress in zebra finches does affect song complexity, measured as song duration or repertoire size even though single studies yielded contrasting results (BRUMM *et al.* 2009; GIL *et al.* 2006; HOLVECK *et al.* 2008). However, there is empirical evidence for an effect on sequence copying accuracy and other performance related parameters (BRUMM *et al.* 2009; HOLVECK *et al.* 2008).

Males' learning score (matches per tutor repertoire) was 53 % in this study and therefore only partly comparable to previous results (BRUMM *et al.* 2009: ~83 %; CLAYTON 1987b: 53.3 ± 15.8 %, GIL *et al.* 2006: 86 %; RIEBEL and SMALLEGANGE 2003: 53 ± 10 %) whereas the similarity score (matches per tutee repertoire) was 64.2 %, which was considerably low compared to earlier studies (CLAYTON 1987b: 76 ± 20.4 %; GIL *et al.* 2006: 94 %;

TCHERNICHOVSKI and NOTTEBOHM 1998: 91 ± 3 %). Interestingly tutees showed the highest motif resemblance (SAP-accuracy) to another tutee, which may have resulted from the consequence of tutees being socially more available than the tutor. The experimental set-up used in this study did account for previously reported interactions between male singing behaviour and nutritional constraints (JOHNSON and RASHOTTE 2002; RASHOTTE *et al.* 2001). To prohibit such influences on tutor's singing behaviour and therefore give all tutees equal opportunities for learning, two different nutritional groups were tutored together by one tutor. The tutor resided in an adjacent full-wire-mesh cage and experienced no dietary restrictions. Mesh partitions have been used in several previous tutor-choice studies and enable vocal and visual but not social interactions between tutor and tutees (JONES *et al.* 2001a; MANN *et al.* 1991). Our results expand on previous suggestions that social interactions determine the extent to which tutees incorporate elements within their repertoire. JONES *et al* (2001a) kept one of five experimental groups (consisting of 2 - 5 male tutees) with their genetic parents until day 35.

with subsequent song tutoring (day 35 – 65) by an adult pair (no parents). The pair was separated by a mesh partition from tutees. At day 65 the pair was removed, hence, no male song model was present until day 120, when songs were recorded and peer groups were joined by an adult male (other than before tutor). JONES *et al* (2001a) showed tutees to exhibit a low percentage of shared syllables (similarity score) with their father (~ 18 %), a higher percentage with their first tutor (day 35 - 65; ~ 45 %) and the highest proportion of shared syllables with their fellow-tutees (~ 66 %). These results were as well observable in the other experimental groups, which experienced less stimulation by an adult tutor (~ 72 %, ~ 75 % respectively) whereas there were pronounced differences to the group in which juveniles were kept in isolation from day 35 – 65 (~ 31%) were observed. (TCHERNICHOVSKI and NOTTEBOHM 1998) suggested that imitation, when there is a model, takes precedence over song improvisation while at the same time model abundance affects the fidelity of imitation. In their study the number of male siblings influenced the accurateness of a song copied from a tutor, which led them postulate that certain rules govern the extent of imitation (TCHERNICHOVSKI and NOTTEBOHM 1998). The number of males did not appear to be a crucial factor in our study. However, our results expand on their idea about a set of rules governing imitation, which could underlay an hierarchical organisation. The amount and maybe as well the direction of social interactions influence the process of imitation.

Female preferences

We found no interaction between female song preferences and their nutritional history. Furthermore there was no evidence that females discriminated between males from different nutritional backgrounds despite apparent effects of dietary restrictions on male morphology at day 60 (NAGUIB and NEMITZ, unpublished) and on male attractiveness (NAGUIB and NEMITZ 2007). These results are in line with the observations on male song parameters and male learning abilities and suggest that nutritional constraints during puberty (from day 35 – 65), a phase crucial for song learning, have only temporary consequences.

The results of this study do not support the hypothesis that females per se prefer more complex songs even though complexity has been proposed to reliably reflect male's quality (reviewed in SEARCY 1992; CATCHPOLE and SLATER 1995; NOWICKI and SEARCY 2004; NOWICKI *et al.* 2002). There are indeed numerous studies documenting such a relationship for species with considerable variation in their repertoire size, from the zebra finch, *Taeniopygia guttata*, in which each male repeats one distinct motif, consisting of 3 to 14 different elements/ syllables, to form his song (CLAYTON and PRÖVE 1989), to species such as the song sparrow, *Melospiza melodia*, with 4 to 13 distinct songs (REID *et al.* 2004) starling, *Sturnus vulgaris*, whose repertoire can contain up to 68 different songs (MOUNTJOY and LEMON 1996) and finally the nightingale, *Luscinia megarhynchos*, where repertoires show pronounced inter-individual differences, can vary between first and second breeding season and reach on average 180 different song types per male (Kiefer *et al.* 2006; Kipper *et al.* 2006). In particular a female preference for longer versus shorter songs was reported (Clayton and Pröve 1989; Lambrechts and Dhondt 1986; Martin-Vivaldi *et al.* 1999; Neubauer 1999; Wasserman and Cigliano 1991). It was argued that preference for complexity could be the consequence of a general preference for greater heterogeneity of sound (Neubauer 1999) but males' song complexity can indeed explain a significant proportion of the variance in learning performance (Boogert *et al.* 2008). However, the two studies in which zebra finches preferred the longer song the stimuli durations were beyond the natural range (Clayton and Pröve 1989; Neubauer 1999). Our results and subsequent studies could not confirm this finding in zebra finches (Holveck and Riebel 2006; Leadbeater *et al.* 2005; Riebel *et al.* (in review).; Vyas *et al.* 2009). Two recent studies suggest differences in syllable repertoires to be important parameters for quality assessment (Holveck and Riebel 2007; Vyas *et al.* 2009). Taken together this may answer why females in our study did not prefer longer songs, even though song complexity could well be a reliable indicator mechanism (Airey *et al.* 2000; MacDougall-Shackleton *et al.* 1998; Tomaszycki and Adkins-Regan 2005). Experience with conspecifics, in a communication network, is essential for developing normal auditory perceptual abilities and shaping preferences (Neubauer 1999; Sturdy *et al.* 2001). An intriguing aspect of zebra finch song learning is the observable preference for the tutor song over the song of an unfamiliar male, which does persist into adulthood and has been observed both in males and females (Clayton 1988; Miller 1979; Riebel *et al.* 2002). At first glance this appears surprising and by far counterproductive if considering implications on female mate choice. However, in the wild, birds are exposed to numerous potential song tutors and thereby the tutor preference may be replaced with the notion of a preference for songs heard during the sensitive period (Clayton 1988; Riebel *et al.* 2002).

The songs heard during sensitive phases in ontogeny may form the template for the establishment of certain filters or the setting of specific thresholds. Certain hierarchical rules may govern template selection and females may store certain features from the song of their social mates as a reliable reference for later auditory input. The variation of the underlying "assessment-parameters" between the stored song template and presently perceived songs may determine whether or not it results in a preference for the song and whether this, as recently shown, relates to the preference for the singer (HOLVECK and RIEBEL 2007). Here, females exhibited an unexpected shift in their song preferences. Females preferred the song of a fellow-tutee (tutee within the same tutor group) over the song of an unfamiliar-tutee whereas contrary no such preference was observed for their song tutor. Contrary, to the variety of studies examining the importance of social factors as group composition (GIL *et al.* 2006), level of aggressiveness (CLAYTON 1987b), paternal care especially feeding behaviour (WILLIAMS 1990), mating status (MANN and SLATER 1994) on male song learning there has been considerably less effort has been put in testing effects on female perception learning (RIEBEL 2003; RIEBEL *et al.* 2005). This study for the first time shows that peer learning in zebra finch females is the consequence of a situation where (fellow-) tutees are more available and socially interactive than an adult song tutor. Zebra finches are gregarious, highly social birds which form flocks in the wild (ZANN 1996). According to their life style multiple cues are involved in tutor choice and the interactions between these have been shown to be very complex: some of them act together, such that they move preference in the same direction, while others conflict with one another (MANN and SLATER 1994). Taken together female's experience of a wide variety of external stimuli channel their preference in certain directions. This provides not only concrete evidence for song acquisition learning but proves the relevance of social cues in shaping the cultural transmission process of song learning, may it be female acquisition and or male song learning.

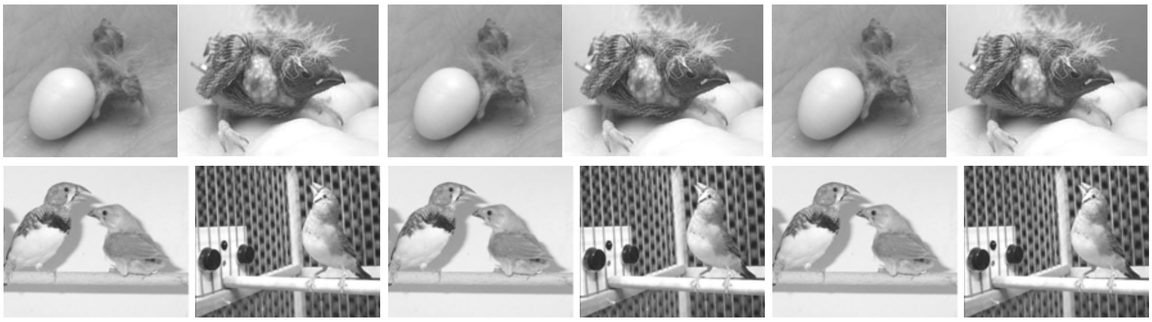
Female preferences in relation to male song parameters

There was a negative correlation between the female's preference value for the fellow-tutee song and the accurateness between tutor and fellow-tutee motif. Consequently, higher similarity between the unfamiliar-tutee's song and the song of the female's tutor was related to higher preference values for the unfamiliar-tutee. Female preferences did not seem to interact with any of the SAP Pro measured song characteristics.

It is a plausible strategy for females to assess relatedness with potential mates on the basis of song similarity to the tutor hereby optimizing out breeding (CLAYTON 1987a, b; GRANT 1984; MCGREGOR and KREBS 1982). The results are in line with this prediction as females did indeed show a bias for choosing socially familiar males with limited resemblance to tutor's motif (SAP-accuracy). Male song is a multidimensional signal and despite the extensive research on different aspects of structural and performance related parameters it remains fairly uncertain which cues females take into account when estimating a male's quality. So far there are inconsistent results. Our study does not find any of the considered song parameters to correlate with female preference (except the tendency in song duration). Further studies have to determine the underlying functional mechanisms of female's preference in relation to male song characteristics. At this point, we can not translate the adaptive significance of a learned song preference. However, bird song learning takes place on both sides, the sender and receiver side, which has particular implications for evaluating potential evolutionary consequences of receiver learning (PAYNE *et al.* 2000; RIEBEL 2000; RIEBEL and SLATER 1998).

For the first time the interactions between nutritional constraints during the period of song learning (d35 - 65) and the cultural transmission process of male song learning and female song recognition learning were tested. The limited social interactions with adult conspecifics during puberty resulted in peer learning in both sexes which has not been examined in females until now. Social conditions shaped female song preferences in adulthood. The results of this study shed light on the implications of a secondary selected trait, male song, on mate choice and its role in shaping evolutionary processes.

CHAPTER 5



GENERAL DISCUSSION

CONCLUSIONS

GENERAL DISCUSSION

This thesis investigated the implications of nutritional constraints during important developmental phases on phenotypic plasticity and performance in zebra finches, *Taeniopygia guttata*. I simulated suboptimal developmental conditions by altering diet quality during the first month in life, where I compared implications of nestling and fledgling fasting, and the subsequent second month (*puberty*). During puberty the emphasis was laid on implications on song learning, a culturally transmitted, sexual selected trait which neural substrates are known to develop in synchrony with song learning during that stage.

Dietary restrictions during the nestling or fledgling phase

The experiments in **chapter 2** and **chapter 3**, revealed that zebra finches were more susceptible to unfavourable nutritional conditions as nestlings than they were as fledglings. A low quality rearing diet during the nestling period (until day 17) significantly affected biometric traits and corticosterone profiles. Contrary, in fledglings (day 17 – 35) only body mass was affected, even though corticosterone profiles were increased at nutritional independence (day 35). Even though birds raised on low quality food as nestlings showed remarkable catch up growth in body mass and skeletal growth until nutritional independence I did not observe any long term consequences on male plumage ornaments, on male or female attractiveness or on survival rates. Interestingly, female preferences for a male were not correlated with his singing behaviour. Nutritional history was not reflected in the number of offspring or the latency to produce viable offspring. However, hatching mass mirrored maternal background whereas no such effect of paternal treatment was found. This suggests the sex specific trade off between offspring quantity and quality. Effects were more pronounced in females. Females deplete their body reserves for egg production which is a lot more demanding and costly than paternal investment until that stage is (NAGER *et al* 2000, reviewed in WILLIAMS 2005).

Effects on biometry & physiology

In zebra finches feather and skeletal growth rates normally reach their asymptote at about 17 days and about 23 days post hatching respectively (BOAG 1987; DE KOGEL 1997). Whereas the phase for skeletal growth was prolonged if individuals experienced nutritional restrictions during the nestling phase, feather growth did not show the same plasticity. This result is not surprising since fully protruded feathers do not retain any neural or vascular connection and are therefore precluded from growing. However, as all groups showed substantial growth rates from day 17 until day 35 it was surprising that fledgling wing growth was not affected. A change in diets did not lead to a refinement of wing growth rate in either direction as this was true in nestlings (LH) and in fledglings (HL). Subjects from the low-high (LH) group did not accelerate wing growth rates once conditions improved nor did they prolong the period of growth until mitigating earlier consequences. Many organisms seem to be capable of growing faster than average which suggests that growth rate is optimized rather than maximised, depending on the balance of associated fitness costs and benefits (METCALFE and MONAGHAN 2001; RICKLEFS *et al.* 1998). Offspring in this experiment were either faced with the scenario of a substantial improvement (LH) or decline (HL) in nutritional conditions, which mimicked breeding earlier or later than peaks of food abundance would recommend. There are a few possible explanations for the observed results. First, wing growth rate might underlay a certain stability once set and it may well be an adequate strategy to refrain from further adjustments. Second, there is the suggestion that key functions might be safeguarded, at the expense of others that have a lesser effect on fitness or a longer time window for compensation (SCHEW and RICKLEFS 1998). This might be an explanation for my results which are in line with previous findings. (ARNOLD *et al.* 2007; BRUMM *et al.* 2009). For a bird it is of great importance to develop adequate feathering for flight timely to fledgling.

Nestlings appeared to have sufficient phenotypic plasticity to cope with suboptimal conditions but there was substantial variation between the sexes. My results showed females to be more affected by dietary restrictions, which expands on previous suggestions that females are generally more vulnerable not only when conditions fall off (BRADBURY and BLAKEY 1998; KILNER 1998; MARTINS 2003; NAGUIB and GIL 2005; NAGUIB *et al.* 2006). The differential effects on biometry and catch-up growth, I have shown here, provide important insights towards understanding and further characterizing the range and limitations of predictive adaptive responses in such a widely used model system, the zebra finch. I showed variation in phenotypic plasticity to be different between the sexes and revealed its temporal limitations.

It is a commonly accepted fact that nutritional restrictions during early development profoundly affect a wide array of traits and can influence the whole trajectory of an individual (LINDSTROM 1999; METCALFE and MONAGHAN 2001). However, it is a constant debate within the scientific community about whether these impacts legitimate the term nutritional *stress* in the absence of physiological correlates (e.g. Marc Naguib, Katharina Riebel, Fritz Trillmich, personal communication). In general, I think it remains a matter of definition. Selye, the godfather of stress research, defined stress as the non-specific response of the body to any demand made on it (SELYE 1950). Additionally a number of previous studies have shown nutritional restrictions to be physiologically stressful (KITAYSKY *et al.* 2001; PRAVOSUDOV and KITAYSKY 2006; SAINO *et al.* 2003). This important link has been missing in the zebra finch and is now provided by my results. I show nutritional restrictions to result in elevated base line corticosterone levels in nestlings and fledglings, which may mirror increased metabolic regulation via glucose mobilization and fat depletion induced by corticosterone (SAPOLSKY *et al.* 2000). Very interesting was the down shift in baseline corticosterone profiles from fledgling day (day 17) to day of nutritional independence (day 35), which were not affected by nutritional treatments. A comparable pattern, even though concentrations between studies differed, was recently reported in zebra finches, where corticosterone baseline and stress profiles at day 16 and at the age of three month were analyzed (WADA *et al.* 2008). However, the extent to which nestling corticosterone levels vary over the course of development remains to be examined to ascertain an increase around fledgling day and not a decrease from potentially higher levels over the course of the nestling stage. Nevertheless, corticosterone profiles around fledgling (at day 17), as shown here, likely mirror the transition from nestling to fledgling. Adrenal hormones regulate a multitude of homeostatic aspects and in a variety of species developmental transitions are accompanied or maybe even mediated by a shift in glucocorticoid levels (DUFTY *et al.* 2002; WADA 2008). Corticosterone, the primary avian gluccocorticoid, is elevated prior to fledgling in a variety of bird species (captive canaries (*Serinus canaria*): (SCHWABL 1999); American kestrel (*Falco sparverius*): (HEATH 1997; SOCKMAN and SCHWABL 2001); pied flycatcher (*Ficedula hypoleuca*): KERN *et al.* (2001); thin-billed prion (*Pachyptila belcheri*) QUILLFELDT *et al.* (2007). High corticosterone levels could not only ensure energy mobilization for enhanced motor activity but my also facilitate rapid learning and memory formation (PRAVOSUDOV and CLAYTON 2001). To conclude, not only the stress response but as well variation in corticosterone baseline titres seem to be associated with developmental status or age of an individual, appears to interact with the nature of the stressor (SAINO *et al.* 2003). Hence, differentiation between the distinct developmental stages, nestling and fledgling, is important.

Effects on male plumage ornaments and attractiveness

The expression of male cheek patches appeared to be robust against a limited period of stress. Poor nutrition during the period of secondary sexual trait maturation has been shown to have a negative effect on the expression of male cheek patches (NAGUIB and NEMITZ 2007) whereas poor nutrition during early development delivered contradicting results in either showing an interaction with the expression of sexually selected ornaments (BIRKHEAD *et al.* 1998a; METCALFE and MONAGHAN 2001) or not (DE KOGEL and PRIJS 1996). Interestingly males raised under HQ condition tended to show more variability in patterns of plumage development after dietary treatments ended. It might be that HQ males are able to exploit the full potential of their genotype and observed variability reflects different abilities in expression patterns. Whereas LQ males, as a consequence of their nutritional history, have only a limited scope and can not fully benefit from variances in genetic potential.

Delayed plumage maturation (i.e. delayed acquisition of adult plumage by sexually mature birds) has been documented in many bird species (ROHWER and BUTCHER 1988; ROHWER *et al.* 1980; THOMPSON 1991). The *moult constraint hypotheses* (THOMPSON 1991) suggests delayed plumage maturation to be rather a consequence of suboptimal environmental conditions than a strategy (GUSTAFSSON *et al.* 1995; HILL 1996). If subadults fail to develop elaborate, species specific plumage displays they will likely face costs in terms of reduced reproductive success (GRAFEN 1990; WESTNEAT and BIRKHEAD 1998). Whether a male is attractive or not has been shown to be linked to its developmental history (DE KOGEL and PRIJS 1996; NAGUIB and NEMITZ 2007). However, I did not find an effect on adult attractiveness in either of the sexes. This is in line with NAGUIB *et al.* (2008), who did not find an effect on male attractiveness as a consequence of brood size manipulations and neither did BLOUNT *et al.* (2003b), who experimentally manipulated nutritional quality during a 15 day period post hatching. Contrary studies in which the experimental stressor endured the entire month post hatching (BIRKHEAD *et al.* 1998a; DE KOGEL and PRIJS 1996) or nutritional constraints during the second month in life (NAGUIB and NEMITZ 2007) did show effects on male attractiveness. My study included attractiveness tests for both sexes, showing that choosiness was neither different across treatment groups nor between sexes even though the proportion of males exhibiting a significant preference was lower than the proportion of females showing a significant preference. This could be the consequence of the differential costs both sexes have to pay when mated to a low quality partner. Theory suggests females to be choosier, considering their reproductive input, which favours shaping the other sex towards more competition and the development of elaborate traits (ANDERSSON 1994) even though especially in a species with biparental brood care, mutual mate choice is (ENGQVIST and SAUER 2001; KOKKO and JOHNSTONE 2002).

Effects on fecundity & survival

'Buying' survival to reproduction at the expense of adaptations that are detrimental in old age is suggested to be a strategy with positive rather than negative evolutionary value in species with a short life expectancy (HALES and OZANNE 2003). Despite the short life expectancy of zebra finches in the wild (ZANN 1996), my results do not show detrimental long term consequences, resulting from catch-up growth or physiological challenges, on survival. However, my hypothesis that such costs will be reflected in breeding performance was confirmed. Conditions experienced during early development projected into the subsequent generation as mothers, which were raised on a low quality diet as nestlings (LH) or fledglings (HL) had lighter hatchlings compared to mothers, which experienced high quality food throughout their first month post hatching (HH). The effects on offspring quality were sex specific as I found no paternal treatment effect on offspring mass at hatching. Several male traits which are related to reproductive performance have been shown to be not affected by dietary restrictions during early development (BIRKHEAD *et al.* 1999). Females are predicted to adjust their reproductive investment in relation to their mate's attractiveness (SHELDON 2000). The differential allocation hypothesis (DAH) postulates females to invest more, considering e.g. number or size of eggs and changes in yolk composition, when paired to an attractive male (Burley 1988; Petrie and Williams 1993; Gil et al. 1999). However, results are ambiguous and other studies have reported a pattern where females invested more in reproduction, when paired to a low quality mate (BOLUND *et al.* 2009; GOWATY *et al.* 2003; NAVARA *et al.* 2006; RUTSTEIN *et al.* 2004a). Considering different aspects of male attractiveness is of course crucial, but this alone can not explain such a complex pattern. Male attractiveness and male parental quality are not necessarily linked by a positive relationship and females need to trade off potential indirect versus potential direct benefits to optimize their reproductive output. (BURLEY 1981, 1986) showed red ringed zebra finch males (= more attractive) males to provide less parental care compared to green banded males (= less attractive) whereas an opposite pattern was observed in the house finch *Carpodacus mexicanus* (NAVARA *et al* 2006). (GOWATY *et al.* 2003) formulated the *Compensation Hypothesis* (CH) which predicts females to increase reproductive effort when mated to a male of low quality in order to make the best out of a bad situation. In my eyes the terminology of DAH and CH can be mistakable. If female differentiate between males by means of variation in quality and consequently invest more if paired to high quality males, the extent of their investment matches their partner's quality: high investment if paired to a high quality mate and low investment if mated with a low quality partner. I therefore suggest entitling this pattern: matched allocation (MA). If females increase reproductive effort when paired to low quality males, which is the opposite direction of the MA, they show compensatory allocation (CA). Hence, I think it would be helpful to consider the DAH as generic term for the two opposite predictions: compensatory allocation (CA) and matched allocation (MA). In the absence of an analysis of egg composites I can only speculate whether this effect was the result of females differentially allocating resources to the eggs and therefore compensating for low quality mates or the fact that father's reproductive quality was not affected by early nutritional history. In fact, the theory of CA is a relatively recent controversy and has been suggested a limited scope (HARRIS and ULLER 2009).

Life-history theory predicts parents to invest in reproduction as to maximize their lifetime fitness (WILLIAMS 1966). Individual effort in current reproduction may affect future reproduction (intra-individual trade-off) and/ or offspring fitness (inter-generational trade-off) (STEARNS 1989). This leads to an important trade off between offspring number and offspring quality, as egg production and parental care until nutritional independence, especially in altricial species, are considered to be very costly (GIL 2003; MONAGHAN *et al.* 1996). The adaptive adjustment of clutch size is an ongoing discussion. Studies so far have reported contradictory results and either, like in my study, have not found an effect on clutch size (BLOUNT 2004; GILBERT *et al.* 2006; RUTSTEIN *et al.* 2004a; VON ENGELHARDT 2004)or an effect which was weak (BALZER and WILLIAMS 1998; HAYWOOD and PERRINS 1992). Breeding pairs in my experiment were not mated assortatively which may be the reason for the lack of effects on latency to initiate a clutch. In a previous study pairs bred assortatively with regard to the nutritional treatment they had received during their first two weeks post hatching (either low or standard quality food; BLOUNT *et al.* (2006)). BLOUNT *et al.* (2006) found pairs which were raised on low quality nutrition to initiate laying significantly later, to exhibit more laying breaks and having a lower laying rate within a clutch compared to pairs which were raised on standard quality diet. As timing of laying can be important for improving individual fitness, breeding pairs do best if they only delay laying when environmental and internal state suggest to (BLOUNT *et al.* 2006). Zebra finches are opportunistic breeders and may improve the conditions for their offspring if they time egg laying and offspring hatching to environmental conditions and peaks of food availability.

I observed lower survival rates of male nestlings, when sired by a parent which experienced low quality food as nestling (LH). This consequently resulted in female biased sex ratios at fledgling. The underlying reasons may be manifold and it remains speculative whether the causes lay in differential parental investments during nestling or fledgling phase, sibling competition or differences in begging intensity as a consequence of differential egg resource compositions. In general sex ratio manipulation and sex-biased mortality are two sides of a coin, working together in determining populations' sex ratios. If offspring sex ratios are representative for the division of resources between sons and daughters, then male-biased sex ratios are expected when the reproductive value of male offspring exceeds that of females and vice versa (ODDIE and REIM 2002). In many species, females are the sex with lower reproductive variance and in unpredictable or adverse environmental conditions parents retrieve higher fitness benefits from investing in female offspring (TRIVERS and WILLARD 1973). At first sight, this seems to contradict with the results of females' susceptibility to adverse conditions and its implications on fecundity (ARNOLD *et al.* 2007; DE KOGEL 1997; KILNER 1998; MARTINS 2003) but whilst a high quality male is suggested to out-reproduce its high quality sister, a low quality sister will out-reproduce a low quality brother, who is unlikely to be competitive enough within his breeding population (TRIVERS and WILLARD 1973). It had been suggested that sex-biased mortality possibly involves a differential effect of egg quality on male and female offspring (CUNNINGHAM and RUSSELL 2000; GIL *et al.* 1999; PETRIE *et al.* 2001; RUTKOWSKA and CICHON 2002) and differential effects of sex and size composition of broods on fledging sex ratio (ODDIE and REIM 2002). Egg resources are costly to produce and do indeed differentially influence female and male offspring (GIL 2003; NAGER *et al.* 2000; WILLIAMS 1994). Deposits of egg androgens have been shown to vary with attractiveness of mates and these variances influence only female and not male begging intensity (VON ENGELHARDT 2004; VON ENGELHARDT *et al.* 2006). Differential allocation in egg resources or in parental care could explain the observed lower male nestling survival. However, there need to be further analysis which should encompass detailed analysis of egg compounds as well as further observations on parental feeding and offspring begging behaviour. Finally, I would like to add that evolutionary, individual-based computer simulations have suggested a very weak selection pressure on sex ratios, which may be counteracted by constraints and costs of manipulation (FAWCETT *et al.* 2007). This might be an explanation for the mixed evidence for adaptive sex allocation in birds (CASSEY and BLACKBURN 2004; EWEN *et al.* 2004; FAWCETT *et al.* 2007; KOMDEUR and PEN 2002; WEST and SHELDON 2002). Additionally, birds' life histories are very complex and accounting for their physiology does complicate our understanding of sex allocation theory which has been considerably well modeled and understood in invertebrates (KOMDEUR and PEN 2002).

The differential effects on biometry and catch growth, I have shown here, provide important insights towards understanding and further characterizing the range and limitations of predictive adaptive responses in such a widely used model system, the zebra finch. Even though dietary effects during early development had profound short term effects on biometry and were shown to be physiologically stressful (Chapter 2) subjects were able to partly compensate until adulthood (Chapter 2 & 3). Short biologically meaningful periods of food limitations are likely to occur in the wild and it appears to be a good strategy outweigh deficits. However, compensation appears to be limited and tightly interconnected to the developmental stage as well as to strength and duration of the encountered constraint. It is very interesting that early dietary restrictions resurfaced in adulthood when looking at measures of fecundity and particularly important to stress that the extent of phenotypic plasticity was shown to be different between the sexes (Chapter 2 & 3). It appears that partners can complement each others adjustments. Hence, negotiation concerning inter-sexual compensatory strategies could well be a plausible explanation but has to be further examined. My results are of emerging importance as considering modifications of programming by postnatal and adult responses are highly relevant to humans where in western societies, postnatal nutrition is plentiful or even excessive whereas the opposite is true for developing nations or immigrant populations (ARMITAGE *et al.* 2004).

Effects of dietary restrictions during the second month (puberty)

The results of **Chapter 4** can not corroborate the hypothesis that nutritional constraints during puberty (day 35 – 60) lead to impaired male song learning or female preferences. Copying accuracy, as measured via two different methodological approaches and frequency related song parameter measurements were not different between males of the low quality (LQ) or high quality (HQ) group. Tutees exhibited higher motif resemblance with another tutee than with their tutor and this effect was not evoked by dietary treatment. Female song preferences did not exhibit an interaction with nutritional history and there was no evidence that females differentiated between males from different nutritional backgrounds. Females only tended to prefer their tutor's over an unfamiliar song and they did not prefer more complex songs (longer). However, LQ-females preference for longer songs was correlated to the song rate of the short song. Social environment had an impact on female song learning as females preferred the songs of fellow-tutees over songs from unfamiliar-tutees. There was a negative correlation between the female's preference value for the fellow-tutee song and the similarity between tutor and fellow-tutee motif. Consequently, higher similarity between the unfamiliar-tutee's song and the song of the female's tutor was related to higher preference ratios for the unfamiliar-tutee. Female preferences did not seem to interact with any of the SAP-measured song characteristics.

My study provides evidence that nutritional constraints during puberty, a critical phase for song learning, did only show transient effects on individuals' trajectory (NAGUIB and NEMITZ, unpublished, NAGUIB *et al* 2007) and that despite the ongoing development of the underlying neuronal pathways and nuclei of the song control system, no effects on male song learning or female preferences were observed. Additionally there was no evidence that females discriminated between males from different nutritional backgrounds despite apparent effects of dietary restrictions on male morphology at day 60 (NAGUIB and NEMITZ, unpublished) and on male attractiveness (NAGUIB and NEMITZ 2007). Previous studies tested the nutritional stress hypothesis, or in more general terms the developmental stress hypothesis, in relation to the period of early development, i.e. subjects encountered a period of suboptimal conditions during the first month (NOWICKI *et al.* 2002; GIL *et al.* 2006; ZANN and CASH 2008; HOLVECK *et al.* 2008, BRUMM *et al.* 2009). It is important to consider the methodological differences in terms of experimental timing.

Interestingly male tutees showed highest motif resemblance (SAP-accuracy) to another tutee and female preferred the songs of fellow-tutees over their tutor's song, which I suggest to be a consequence of the particular social context in which tutees were more available than the tutor. The experimental set-up in this study accounted for previously reported interactions between male song rate and nutritional constraints (BRUMM *et al.* 2009; NAGUIB *et al.* 2008a). To give all tutees equal opportunities to learn from an adult conspecific, two different nutritional groups were tutored by the same tutor in an adjacent cage who did not experience dietary restrictions. Vocal and visual interactions between tutor and tutees were enabled at any time but social interactions limited to tutees within a treatment group. Hence, in a situation where (fellow-) tutees are more available and socially interactive than an adult song tutor, female preferences and male song similarities exhibited an unexpected shift. Zebra finches are gregarious, highly social birds which form flocks in the wild (ZANN 1996). A variety of studies examined the importance of social factors as group composition (GIL *et al.* 2006), level of aggressiveness (CLAYTON 1987b), paternal care and especially feeding behaviour (WILLIAMS 1990) anf mating status (MANN and SLATER 1994) on male song learning. Zebra finches are thought to prefer learning from live males over tape-tutoring and it has been shown that additional visual interactions can be crucial (HOUX and TEN CATE 1999, MANN 1991). It was argued that model imitation, if existent, takes precedence over song improvisation while at the same time model abundance affects the fidelity of imitation (TCHERNICHOVSKI and NOTTEBOHM 1998). In their study the number of male siblings influenced the accurateness of a song copied from a tutor, which led them postulate that certain rules govern the extent of imitation (TCHERNICHOVSKI and NOTTEBOHM 1998). Number of males did not appear to be a crucial factor in my study, contrasts existing empirical evidence for a sex-specific inhibition effect on song learning (GIL *et al.* 2006, HOLVECK *et al.* 2008; TCHERNICHOVSKI and NOTTEBOHM 1998). It is a plausible strategy for females to assess relatedness with potential mates on the basis of song similarity to the tutor hereby optimizing out breeding

(CLAYTON 1987a, b; GRANT 1984; MCGREGOR and KREBS 1982). My results are in line with this prediction as females did indeed show a bias for choosing socially familiar males with limited resemblance to tutor's motif (SAP-accuracy). Male song is a multidimensional signal and despite the extensive research on different aspects of the underlying parameters there is only inconsistent results on which cues females take into account when estimating a male's quality (RIEBEL 2003, breeding (CLAYTON 1987a, b; GRANT 1984; MCGREGOR and KREBS 1982). None of the considered song parameter measures was shown to correlate with female preference (except from the tendency in song duration). Therefore this study fails to determine the underlying functional mechanisms of female's preference in relation to male song characteristics. At this point, we can not translate the adaptive significance of a learned song preference. However, bird song learning takes place both on the sender and receiver side and this does have particular implications for evaluation potential evolutionary consequences of receiver learning (PAYNE *et al.* 2000; RIEBEL 2000; RIEBEL and SLATER 1998).

The results of this study do not support the hypothesis that females per se prefer more complex songs even though complexity has been proposed to reliably reflect male's quality (reviewed in SEARCY 1992; CATCHPOLE and SLATER 1995; NOWICKI and SEARCY 2004; NOWICKI *et al.* 2002). There are indeed numerous studies documenting such a relationship for species with considerable variation in their repertoire size, from the zebra finch, *Taeniopygia guttata*, in which each male repeats one distinct motif, consisting of 3 to 14 different elements/ syllables, to form his song (CLAYTON and PRÖVE 1989), to species such as the song sparrow, *Melospiza melodia*, with 4 to 13 distinct songs (REID *et al.* 2004) starling, *Sturnus vulgaris*, whose repertoire can contain up to 68 different songs (MOUNTJOY and LEMON 1996) and finally the nightingale, *Luscinia megarhynchos*, where repertoires show pronounced inter-individual differences, can vary between first and second breeding season and reach on average 180 different song types per male (KIEFER *et al.* 2006; KIPPER *et al.* 2006). In particular a female preference for longer versus shorter songs was reported (CLAYTON and PRÖVE 1989; LAMBRECHTS and DHONDT 1986; MARTIN-VIVALDI *et al.* 1999; NEUBAUER 1999; WASSERMAN and CIGLIANO 1991). It was argued that female preferences for complexity could be the consequence of a general preference for greater heterogeneity of sound (NEUBAUER 1999). Males' song complexity was shown to explain a significant proportion of the variance in learning performance (BOOGERT *et al.* 2008) whereas on the other hand it appears that neither early nor juvenile developmental stress in zebra finches does affect male song complexity, measured as song duration or repertoire size (BRUMM *et al* 2009, HOLVECK *et al* 2008, GIL *et al.* 2006, Chapter 4). However, single studies yielded contrasting results (Spencer et al, Buchanan). The question about why females in this study, if song complexity is a reliable indicator mechanism, did not prefer these attributes can not be answered sufficiently.

For the first time important conclusions on the interactions between nutritional constraints during puberty and the cultural transmission process of male song learning and female song recognition learning were drawn. Nutritional constraints during this stage had only transient consequences with no further implications on song learning. Female's experience channels their preference in certain directions and I showed social circumstances to be of comparable importance for both sexes. This provides concrete evidence for the relevance of social cues in shaping the cultural transmission process of song learning, may it be female acquisition and or male song learning. Hence, my results shed light on the implications of a secondary selected trait, male song, on mate choice and its evolutionary consequence.

CONCLUSIONS AND PERSPECTIVES

This thesis investigated the implications of dietary restrictions during important developmental phases on phenotypic plasticity and performance in zebra finches, *Taeniopygia guttata*. With a comparative experimental approach I analyzed differences in phenotypic plasticity as a consequence of nutritional variation during the nestling and fledgling phase. Furthermore, I took a closer look at implications of dietary restrictions during the second month (*puberty*) on a culturally transmitted, sexual selected trait; male song with a particular emphasize on female preferences.

Growth rates of zebra finch nestlings were severely affected and resulted in pronounced compensatory growth rather than prolonging the growth period itself. No such effect was observed for fledglings which appeared to be robust against nutritional constraints in terms of skeletal and feather growth. Dietary restrictions appeared to be physiological stressful for both nestling and fledglings and were mirrored in base line corticosterone titers. This important link between the apparent detrimental consequences of poor nutrition and the physiological response of the hypothalamic pituitary adrenal complex axes (HPA) not only justifies but requires the usage of the term nutritional stress when considering nutrient shortages during early development. I additionally provided evidence for the potential role of corticosterone to take part in mediating important developmental transitions. Despite physiological effects and pronounced compensatory growth I did not observe any long term effects on attractiveness or survival. I assume this to reflect the breeding ecology of zebra finches in the wild where they are likely to encounter short pitfalls of food supply which might have shaped the evolution of strategies for optimizing individual success. However, compensation was not complete and a sex-specific transgenerational effect on offspring quality and on the variation in offspring sex ratios indicated long term consequence for fasting nestlings of both sexes and female fledglings.

Neither male song learning nor female preferences showed an interaction with dietary restrictions during puberty. My suggestion about a mechanistic extension of the nutritional stress hypothesis had to be dismissed. Song learning in males and females appeared to be robust against nutritional limitations during puberty. However, male song production and female song recognition learning were significantly influenced by social stimuli. Interestingly male song motifs were found to be more similar between tutees than between tutor-tutee dyads. In line with this result I found females to show a pronounced preference for the song of a social mate but not for their tutor. I think this can be interpreted to be a direct consequence of the social implications from the experimental song tutoring set-up. For the first time, I was able to show peer learning to occur in both sexes as a consequence of social experience, a link which has been missing in females until now. Additionally, I found no further support for the common theory that females prefer longer songs. Taken together, I have shown that considerable variation between the extent to which individuals reflect nutritional limitations exists and that these distinct differences depend on the timing of constraint, on the duration of suboptimal conditions and on the modality of the trait showing interactions.

There are several directions in which future research may progress. Nutritional neuroscience is an important topic nowadays and we are just beginning to understand how specific nutrients shape molecular pathways in the brains via changes in membranes, receptors, trophic factors, and signaling cascades. Understanding the mechanisms and long-lasting consequences of alterations in nutrient availability to the developing and adult brain will be an important direction for shedding light on its implications on behavioural strategies and evolutionary forces.

Until now numerous speculations, hypothesis and a few empirical results exist concerning the cues by which females assess a male's quality via its song. Determining the structural song parameters underlying female's preferences would not only enlighten how quality assessment is maintained but additionally provide us with a powerful tool for further experimental alterations. It would allow elaborate analysis of variation between, what I suggest to be, complex interaction patterns for assessing the consequences of developmental influences. Additionally it would us bring closer to answering the multidimensional question about how such "attractive" features might relate to male quality.

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ABSTRACT

The entire period from conception until maturity is crucial for individual performance. Countless interactions between environmental conditions and the expression of genes determine the phenotype of an organism. Developing birds are in a vulnerable position as they exhibit steep early growth rates during a time when they are entirely dependent on parental feeding regimes. An individual's trajectory can be severely affected by adverse conditions, in terms of morphology, physiology and behaviour. However, short periods of stress will be prevalent under natural conditions and individuals may respond differently to fluctuations in food supply, predator abundance or climate changes. Responses may be directly linked to the nature and timing of the stressor. The first month after hatching has been shown to have pervasive effects on individual performances but it remains unclear whether shorter, biologically more meaningful periods entail profound consequences. Nestlings and fledglings encounter a variety of physiological, social and behavioural challenges of which the magnitude appears to be very different between the two stages. Furthermore, the period when secondary sexual characters develop, might also profoundly shape fitness relevant components. High selection pressure acts on these early periods and is reflected by low survival rates in the wild. Traits that develop during this phase will mirror the capacity to acquire resources and the ability to cope with deleterious circumstances.

This thesis investigated the implications of dietary restrictions during important developmental phases on phenotypic plasticity and performance in zebra finches, *Taeniopygia guttata*. An experimental approach assessed the consequences of dietary variation during the nestling and fledgling phase on biometric physiological, behavioural and fitness relevant traits. This study shows to which extent such epigenetic influences transfer into the following generation and whether these effects differ between the sexes. Furthermore, implications of dietary restrictions during the second month (*puberty*) on a culturally transmitted, sexually selected trait, such as male song were examined with a particular emphasis on female preferences.

Zebra finch nestlings were severely affected by qualitative food manipulations and exhibited pronounced compensatory growth whereas fledglings appeared to be robust against nutritional constraints in terms of skeletal and feather growth. Dietary restrictions appeared to be physiological stressful for both nestling and fledglings and were mirrored in base line corticosterone titres. This important link between the apparent detrimental consequences of poor nutrition and the physiological response of the hypothalamic pituitary adrenal complex axes (HPA) not only justifies but requires the usage of the term nutritional stress when considering nutrient shortages during early development. The results provide evidence for the potential role of corticosterone as mediator for developmental transitions. There was no long term effect on attractiveness or survival which may reflect the breeding ecology of zebra finches. In the wild, they are likely to encounter short pitfalls of food supply which might have shaped the evolution of strategies for optimizing individual success. However, compensation was not complete and a sex-specific transgenerational effect on offspring quality and on the variation in offspring sex ratios indicated long term consequence for fasting nestlings of both sexes and for female fledglings.

Neither male song learning nor female preferences showed an interaction with dietary restrictions during puberty. However, male song production and female song recognition learning were significantly influenced by social stimuli and for the first time a significant peer learning effect was analyzed and shown in males and in females. Song complexity is considered to reflect the singer's quality and should as a consequence translate into a female preference for longer songs. The results could not corroborate this hypothesis. Taken together, considerable variation exists between the extent to which individuals reflect nutritional history and these distinct differences depend on the timing and duration of the constraint and on the modality of the trait. Overall, these findings contribute to our understanding on the origin and evolutionary significance of individual differences. Selection will act on the individual phenotype and the results highlight the relevance of conditions during early development for the phenotypic plasticity.

ZUSAMMENFASSUNG

Jeder Organismus ist das Resultat vielfältiger Interaktionen zwischen der Umwelt und den Genen, welche gemeinsam zur Ausprägung des individuellen Phänotyps führen. Einflüsse während der frühen Ontogenese nehmen einen besonderen Stellenwert ein und können langfristige Konsequenzen mit sich bringen. Möchte man die Es ist jedoch wichtig, den gesamten Entwicklungszeitraum, von der Empfängnis bis zur Erreichung der sexuellen Reife, zu berücksichtigen um Auswirkungen von Strategien zum Umgang mit Stress verstehen zu können. Jungvögel sind sehr empfänglich für suboptimale Umweltbedingungen, da sie von ihren Eltern kaum gegen äußere Umstände abgepuffert werden können. Fluktuationen abiotischer und biotischer Umweltfaktoren kommen unter natürlichen Bedingungen häufig, meist unvorhersehbar und für ungewisse Dauern vor. Eine Vielzahl von experimentellen Studien an Singvögeln hat das Ausmaß negativer Bedingungen (Brutgrössen-, Nahrungs – oder hormonelle Manipulation) untersucht, doch es ist bislang unklar inwiefern kürzere, biologisch relevantere Restriktionsphasen während der unterschiedlichen Entwicklungsstadien, Nestling und Flüggling, langfristige Konsequenzen in Bezug auf Fitness und Reproduktion, mit sich bringen. Nestlinge und Flügglinge sehen sich mit einer Vielzahl von physiologischen, sozialen und verhaltensökologischen Herausforderungen konfrontiert, welche eine unterschiedliche Gewichtung innerhalb der Phasen erfahren.

Ein weiterer wichtiger Entwicklungsabschnitt von Singvögeln ist die Phase in der sich sekundäre Geschlechtsmerkmale ausbilden und der artspezifische Gesang erlernt wird. Während dieser Phase der frühen Unabhängigkeit herrscht ein hoher Selektionsdruck, der unter natürlichen Bedingungen in niedrigen Überlebensraten resultiert. Merkmale, die sich während dieser Zeit ausprägen, lassen Rückschlüsse auf das Potential und die Fähigkeiten der Ressourcenverteidigung von Individuen zu.

In dieser Arbeit wurden die durch Nahrungsrestriktionen hervorgerufenen Effekte, am Model des Zebrafinken, analysiert. In dieser Studie wurde mittels eines experimentellen Ansatzes Auswirkungen von Nestlings- und Flügglings-Stress, unter Berücksichtigung biometrischer, physiologischer und verhaltensrelevanter Aspekte, direkt verglichen. Das Ausmaß epigenetischer Einflüsse auf die nachfolgende Generation und auch die diesbezüglichen geschlechterspezifischen Unterschiede wurden untersucht. Des weiteren wurden Einflüsse während des zweiten Monats,

Pubertät, auf ein kulturell tradiertes, sexuell selektiertes Merkmal, den männlichen Gesang, untersucht. Ein besonderes Augenmerk wurde hierbei auf die Analyse von weiblichen Gesangspräferenzen gelegt.

Nahrungsrestriktionen während der Nesling-Phase führten zu ausgeprägtem Kompensationswachstum, welches bei Flügglingen nur in Bezug auf das Körpergewicht zeigte. Es konnte nachgewiesen werden, dass Nahrungsrestriktionen Auswirkungen auf den basalen Corticosteronspiegel mit sich ziehen. Dieser wichtige Aspekt der physiologischen Aktivierung der Hypothalamus-Hypophysen-Achse, als Antwort auf Nahrungsvariationen war bislang unbekannt in Zebrafinken und begründet die Verwendung des Termes Nahrungsstress. Es konnten keine Langzeitauswirklungen auf die Attraktivität oder Überlebensrate festgestellt werden, wobei die Ursachen hierfür in dem arteigenen Brutverhalten begründet liegen könnte. Unter natürlichen Bedingungen sind kurze Perioden von Nahrungsbeschränkungen vorherrschend und der damit verbundene Umgang könnte die Evolution individueller Optimierungsstrategien begünstigt haben. Dennoch war die Kompensation nicht allumfassend und geschlechterspezifische Auswirkungen spiegelten sich auch in der Qualität und im Geschlechterverhältnis der nachfolgenden Generation wider. Männliches Gesangslernen und weibliche Gesangspräferenzen wurden nicht durch Nahrungsrestriktionen während der Pubertät beeinflusst. Jedoch konnte gezeigt werden, dass soziale Einflüsse starken Einfluss auf das männliche Gesangslernen und weibliche Gesangspräferenzen nehmen und in sogenanntes *peer-learning* (Lernen von Mitschülern) resultieren. Entgegen der allgemeinen Hypothese, dass Gesangskomplexität als Qualitätsindikator dient und Weibchen längerem Gesang den Vorzug geben, konnte keinerlei Präferenz für längern Gesang ermittelt werden.

Zusammenfassend konnte gezeigt werde, dass es maßgebliche Unterschiede in der individuellen Ausprägung von Konsequenzen auf Nahrungsrestriktionen gibt und das diese Unterschiede vom Zeitpunkt und der Dauer des Stressors abhängen, sowie von der Modalität des untersuchten Merkmales. Dies Ergebnisse erweitern unser Verständnis in Bezug auf die Ursprünge und evolutionären Signifikanz individueller Unterschiede. Selektion agiert auf der Ebene des Phanötyps und die Resultate dieser Studie unterstreichen die Relevanz der frühkindlichen Entwicklungen auf die phänotypische Plastizität

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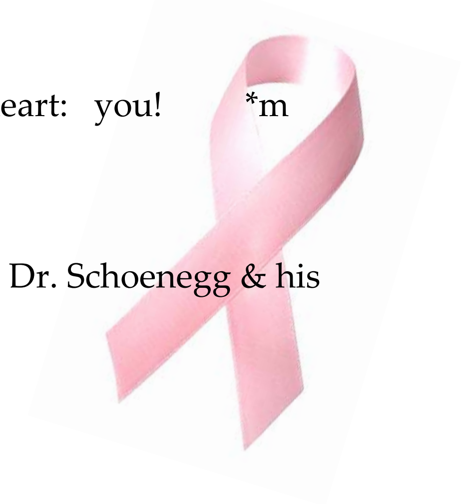
Even though some people consider it to be mutually exclusive: despite being a scientist, I know there is a lot more to life than unraveling nature's secrets. My close friends, my family and my son took great part in shaping me to become the person I am today. Therefore, I want to thank: Petra R. & Co, Lino, Luca & Co, Anne & Co, Judith, Susanne, Paula & Thijs, Rosemarie & Co, Markus & Co, Christian, Acki & Co, Heiko, Alina, Nina, Hossein, Marlen, Oma, Ulrike, Sybille & Co and Julia for being yourself, for letting me walk besides you and for being there for me when I needed you most.

I draw all my strength from the heart: you! *m



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…as well to the ones I forgot mentioning.

APPENDIX

Table A1: Labels and definitions of the 12 song structure parameters measuring song complexity or singing performance.

Song parameters	**Definition**
Sound duration	Duration of motif over which sound is present (ms), excluding silent gaps between elements
Element rate	Number of elements delivered per second, 'element number' / 'motif duration'
Mean frequency	Smooth estimate of the center of derivate power
Frequency modulation	Frequency modulation is based on time and frequency derivates across frequencies.
Amplitude	Amp = 10log10 x-baseline where Pf is the Power at any one frequency; baseline is set to 70dB
Amplitude modulation	Captures changes in the amplitude envelope of sounds.
Pitch	Pitch is a measure of the period of oscillation
Goodness of Pitch	
Harmonicity	Mean of the noisiness over the element duration; it measures the proportion of energy in the spectrum that falls within 50% of the phase cycle as would be expected if the signal was perfectly harmonic (a high value corresponds to a low noisiness)

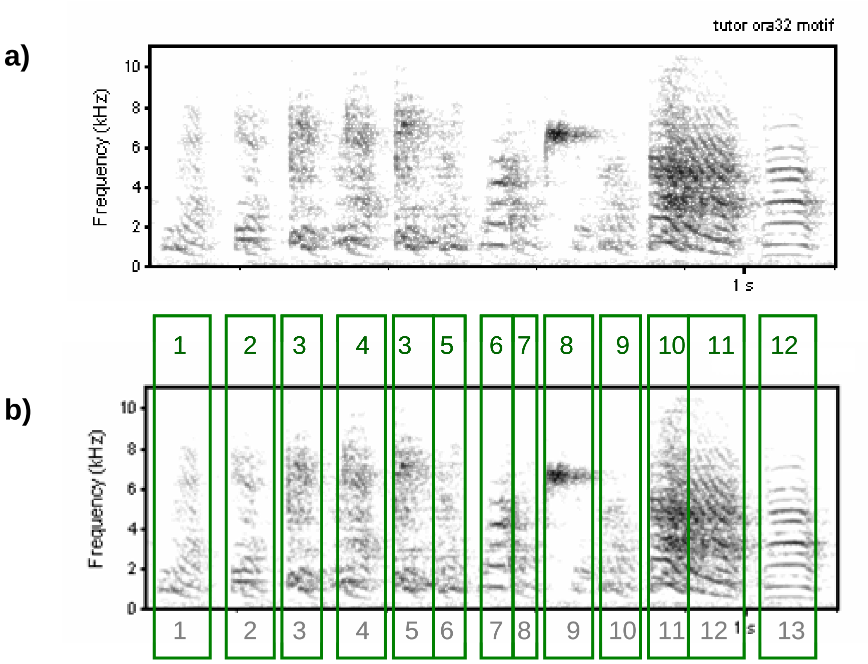

Fig. A1: Visual representation of the spectral parameter measures analyzed via visual comparisons of spectrograms). Parameter measures are represented exemplary for the song motif of tutor ora32. **a)** Spectogramm, **b)** Number of elements (grey) and repertoire (green).

Table A2: **Overview of song tutor group and participation in experimental tests**. Same sex siblings were kept within one tutor group in different dietary treatments (LQ = low quality, HQ = high quality). In 11 cases the father was as well song tutor (4 = male tutees, 7 = female tutees). The value within the colums for the female preference test (test1-3) indicated the stimulus set with which either females were tested or males´ song motif was used. A zero indicates that females did not learn the task and were excluded from the analysis. For stimulus sets see Table S3. Rec = song recording exists, VC = Visual comparison, SAP = automated song analysis via SAP Pro. † indicates death prior to experiments.

Group	Tutor	Tutee	Diet	Sex	Father	Mother	Rec.	VC	SAP	Test1	Test2	Test3	
1	ora032	w142	LQ	m	ora805	w062	√	√	√			XII	
1	ora032	w143	HQ	f	ora805	w062				1	b		
1	ora032	w144	HQ	m	ora805	w062	√	√	√			VII	
1	ora032	w145	LQ	f	ora032	w134							
1	ora032	w146	HQ	f	ora032	w134				1	b	VII	
1	ora032	w147	LQ	f	ora032	w134				1	b	XII	
1	ora032	w148	HQ	m	ora032	w134							
1	ora032	w152	LQ	f	w059	w065				1	b		
1	ora032	w153	LQ	f	w059	w065				1	b		
1	ora032	w154	HQ	f	w059	w065				1	b		
2	w109	w169	LQ	m	ora808	w137	√	√	√				
2	w109	w170	HQ	m	ora808	w137	√	√	√			II	
2	w109	w171	LQ	m	ora808	w137	√	√	√			I	
2	w109	w172	HQ	m	ora808	w137	√	√	√			X	
2	w109	w173	LQ	f	w109	w102				5	g	I	
2	w109	w174	HQ	f	w109	w102				0		II	
2	w109	w175	HQ	f	w109	w102							
2	w109	w176	LQ	m	w109	w102	√	√	√				
3	w229	w220	HQ	f	ora199	w021				0		XIV	
3	w229	w221	LQ	f	ora199	w021				4	e		
3	w229	w222	LQ	f	w131	w127				4	e		
3	w229	w223	HQ	f	w131	w127				4	e		
3	w229	w224	HQ	f	w131	w127				4	e		
3	w229	w225	LQ	f	w131	w127				4	e	VII	
3	w229	w226	LQ	m	w131	w127							
3	w229	w227	HQ	m	w080	w129	√	√	√			XIV	
3	w229	w228	LQ	m	w080	w129	√	√	√			VII	
4	w059	w149	HQ	m	ora808	w137	√	√	√			IX	
4	w059	w150	LQ	m	ora808	w137	√	√	√			II	
4	w059	w155	HQ	m	w110	w119	√	√	√			XIII	
4	w059	w156	HQ	f	w110	w119						IX	
4	w059	w158	LQ	f	ora055	w133							†
4	w059	w159	LQ	m	ora055	w133							
4	w059	w160	HQ	f	ora055	w133							
4	w059	w168	LQ	m	w110	w119	√	√	√				
5	ora814	w161	LQ	m	w131	w127							†
5	ora814	w162	HQ	f	w131	w127						IX	

Group	Tutor	Tutee	Diet	Sex	Father	Mother	Rec.	VC	SAP	Test1	Test2	Test3	
5	ora814	w163	LQ	f	ora814	w33						0	
5	ora814	w164	HQ	m	ora814	w033							
5	ora814	w165	LQ	m	ora814	w033	√	√	√				
5	ora814	w166	LQ	m	w080	w104	√	√	√			III	
5	ora814	w167	HQ	m	w080	w104	√	√	√			IX	
6	w110	w177	HQ	m	ora805	w062	√	√	√				
6	w110	w178	HQ	f	ora805	w062						I	
6	w110	w179	LQ	m	ora805	w062							
6	w110	w180	HQ	m	ora805	w062	√	√	√			I	
6	w110	w181	LQ	f	ora805	w062							†
6	w110	w184	LQ	m	w059	w065	√	√	√				
6	w110	w185	HQ	m	w059	w065							†
6	w110	w187	LQ	m	w059	w065							
7	ora814	w195	LQ	m	ora055	w133							
7	ora814	w196	HQ	m	ora055	w133	√	√	√			XII	
7	ora814	w197	LQ	f	ora055	w133				3	a	XII	
7	ora814	w198	LQ	f	ora055	w133				3	a	X	
7	ora814	w199	HQ	f	ora055	w133				3	a	VI	
7	ora814	w200	HQ	m	ora055	w133	√	√	√			VI	
7	ora814	w201	LQ	m	w110	w119	√	√	√				
7	ora814	w202	HQ	f	w110	w119				3	a		
7	ora814	w203	HQ	m	w110	w119							
7	ora814	w204	LQ	m	w110	w119							
7	ora814	w205	LQ	m	w110	w119	√	√	√			X	
8	ora060	w182	HQ	m	w126	w135							
8	ora060	w183	HQ	f	w126	w135				2	d	XIII	
8	ora060	w188	LQ	m	w092	w097	√	√	√			III	
8	ora060	w189	HQ	m	w092	w097	√	√	√			VIII	
8	ora060	w190	HQ	f	ora009	ora562				2	d	XIV	
8	ora060	w191	LQ	m	ora009	ora562							
8	ora060	w210	LQ	m	ora463	w081	√	√	√			VI	
8	ora060	w211	HQ	m	ora463	w081	√	√	√			XIV	
8	ora060	w212	LQ	f	ora463	w081				2	d	VI	
8	ora060	w219	LQ	f	w126	w135				0			
8	ora060	w230	LQ	f	ora009	ora562				2	d	III	
9	ora403	w207	LQ	m	ora810	ora596	√	√	√				
9	ora403	w208	HQ	m	ora810	ora596							
9	ora403	w209	LQ	m	ora810	ora596							†
9	ora403	w213	LQ	f	w136	w118				1	c		
9	ora403	w214	HQ	m	w136	w118	√	√	√			IV	
9	ora403	w215	LQ	m	w103	w115							†
9	ora403	w216	HQ	f	w103	w115				0		IV	
9	ora403	w218	HQ	m	w103	w115	√	√	√				
10	ora861	w231	HQ	f	w131	w127							
10	ora861	w232	HQ	m	w131	w127	√	√	√				
10	ora861	w233	LQ	f	w131	w127						IV	
10	ora861	w234	LQ	m	w131	w127							
10	ora861	w235	HQ	f	ora810	w120							
10	ora861	w236	LQ	m	ora810	w120	√	√	√			IV	

Table A3: **Overview of stimulus dyads used in the female preference tests (1 -3). ong tutor group**. Female tutees of one tutor group were all tested with the same stimulus dyad within one test.

Test	set	stimulus 1	stimulus 2
1	1	ora032	ora403
1	2	ora060	ora321
1	3	ora402	ora814
1	4	w059	w229
1	5	ora819	w109
2	a	ora321	ora819
2	b	w059	ora050
2	c	ora717	ora060
2	d	ora403	ora402
2	e	ora473	ora032
2	g	ora081	ora814
3	I	w180	w171
3	II	w150	w170
3	III	w188	w166
3	IV	w236	w214
3	VI	w200	w210
3	VII	w144	w228
3	VIIII	w167	w149
3	X	w172	w205
3	XII	w196	w142
3	XIII	w189	w155
3	XIV	w227	w211

MARIAM HONARMAND

Contact: m.honarmand@web.de
Nationality: Austria
Child: Lino Honarmand (7.November 2006)

1977	Birth
1989 - 1996	Secondary School, Abitur
1997 - 2003	MSc Biology (Diplom), Free University Berlin Thesis: „DNA Amplification in follicle cells of *Drosophila melanogaster* “
2005 - 2009	PhD in Behavioural Ecology, Bielefeld University Thesis: „Eating like a bird: Long term effects of nutritional stress”

PUBLICATIONS & CONFERENCE CONTRIBUTIONS

12.2005	ASAB Winter Meeting, UK
08.2006	Symposium on Birdsong in Behavioural & Neurobiological Research
08.2006	24th International Ornithology Conference 2006 (IOC),
09.2006	3rd European Conference on Behavioural Biology (ECBB)
09.2006	99. Meeting of the DZG
08.2008	Symposium on Vocal Communicatioin in Bird and Mammals (VoCom)
04.2009	Postgraduate workshop/ ASAB Easter Meeting

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Personal values are highly subjective
and depend on our experience.

However….it is not **what** we value in
life but **that** we value life!

FÜR EINEN KLEINEN MANN VOM MEER