Title: Postnatal nutrition influences male attractiveness and promotes plasticity in male mating preferences

Short title: Nutrition and plasticity in mate choice

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ABSTRACT

Poor early life nutrition could reduce adult reproductive success by negatively affecting traits linked to sexual attractiveness such as song complexity. If so, this might favour strategic mate choice, allowing males with less complex songs to tailor their mating tactics to maximize the reproductive benefits. However, this possibility has been ignored in theoretical and empirical studies. By manipulating the micronutrient content of the diet (e.g. low or high) during the postnatal period of male zebra finches, we show for the first time: (1) that males reared on a poor (low) micronutrient diet had less complex songs as adults, (2) that these males, in contrast to the high micronutrient diet group, were more selective in their mating strategies, discriminating against those females most likely to reduce their clutch size when paired with males having less complex songs and (3) that by following different mating strategies, males reared on the contrasting diets obtained similar reproductive benefits. These results suggest that early life dietary conditions can induce multiple and long-lasting effects on male and female reproductive traits. Moreover, the results seem to reflect a previously unreported case of adaptive plasticity in mate choice in response to a nutritionally-mediated reduction in sexual attractiveness.

KEYWORDS: differential allocation, fertility, mate choice, song, *Taeniopygia guttata*

INTRODUCTION

Mate choice generally has profound fitness consequences (Andersson 1994), which has led to the evolution of mating preferences expressed by one or both sexes in organisms where some form of direct gametic transfer occurs (Andersson 1994; Jennions and Petrie 1997). Studies on sexual selection have focused mainly on female mate preferences since their lower potential reproductive rate means that they are expected to be the more choosy sex (Trivers 1972; Andersson 1994; Jennions and Petrie 1997). However, theoreticians have long realized that whenever males exhibit some postcopulatory parental investment, male mate choice and preferences can also occur (Parker 1983; Andersson 1994; Amundsen 2000). Nonetheless, the causes underlying variation in male mating preferences are still poorly understood (Edward and Chapman 2011).

Ornaments and armaments (e.g. weapons) occurring in females have been traditionally considered as a correlated response to selection for the trait in males (Lande 1980). However, empirical evidence indicates that showy female traits can also evolve as a result of male mate choice. Indeed, previous studies of the factors affecting male mate choice and preferences have focused on female ornaments, with males often preferring more ornamented females as mates (see e.g. Hill 1993; Amundsen, Forsgren et al. 1997; Amundsen and Forsgren 2003 but see Cuervo et al. 2016). In addition, other factors such as females' potential fecundity (Monaghan, Metcalfe et al. 1996), the level of exposure to predators (Magurran and Seghers 1990) or the number of ready-to-mate females (e.g. Operational sex ratio (OSR); Emlen and Oring 1977) can also influence the direction of male mate preferences (Jennions and Petrie 1997). Surprisingly, the influence of the early life conditions that males have experienced on their future mate preferences and reproductive strategies has been largely ignored so far (but see Holveck, Geberzahn et al. 2011), even though it is well established that early life conditions can have long-term effects on the phenotype, including secondary sexual traits, and on life history trajectories including longevity. A low availability of food or a poor diet (i.e. low vitamin and mineral content) during early life can have detrimental effects on adult traits involved in male sexual attractiveness such as song complexity in birds (Nowicki, Searcy et al. 2002) or body size in mammals (Metcalfe and Monaghan 2001), and consequently might influence a male's mating preferences. Males exposed to early life nutritional stress would be expected to adjust their courtship and mating

preferences in line with their probability of success or their potential reproductive outcome, so preferring less discriminating females that are more likely to accept them as mates (Crino, Prather et al. 2014).

Male attractiveness and quality can influence offspring viability (Sheldon 2000; Andersen 2003), and therefore it has been hypothesized that females should invest more in reproduction when they are paired with a mate of high rather than of low phenotypic quality (e.g. differential allocation hypothesis, DA; Burley 1986; Sheldon 2000). Indeed, empirical evidence indicates such differential reproductive investment in relation to male attractiveness and quality is a widespread female strategy (reviewed by Sheldon 2000), often involving a strategic change in female reproductive investment (Sheldon 2000 and references therein). However, the opposite scenario has also been hypothesized, that is an increase in female reproductive investment toward less preferred males (e.g. reproductive compensation hypothesis; RC; Gowaty, Anderson et al. 2007). Although the RC hypothesis has also received empirical support (i.e. Gowaty, Anderson et al. 2007; Bolund, Schielzeth et al. 2009), a recent theoretical model indicates that differential allocation (DA) is likely to be a more common and optimal strategy for females (Harris and Uller 2009). Since differential reproductive investment has the potential to affect male reproductive success (Sheldon 2000; Gowaty, Anderson et al. 2007), it might be expected that less preferred males will discriminate among females with respect to their likely level of reproductive investment in order to maximize their own fitness.

The degree of female reproductive investment, however, is likely to vary with the expected fitness gains that her investment confers. Females with reduced potential longevity might, therefore, be selected to increase their investment in reproduction regardless of male quality, since their number of future breeding opportunities is reduced (Pianka and Parker 1975; Clutton-Brock 1984). Early life dietary conditions could play an important role here. For example, a diet poor in micronutrients (i.e. vitamins and minerals) early in life can result in increased cell senescence (Badás, Martínez et al. 2015) and reduced future survival (Saino, Caprioli et al. 2011; Noguera, Kim et al. 2012). However, to what extent early life dietary conditions can affect the degree of female reproductive investment, and whether such variation might drive male mate preferences, is still unknown.

In this study, we examined the effects of early life nutrition on adult male songbird song complexity (as a proxy of sexual attractiveness; Riebel 2009), mating preferences and the response of female partners in terms of their reproductive investment. We experimentally manipulated the intake of dietary micronutrients (i.e. dietary antioxidants and minerals) in the early life of zebra finches *Taeniopygia guttata*. In birds, including zebra finches, an inadequate early supply of dietary micronutrient induces different types of physiological stress (i.e. oxidative stress or higher glucocorticoids level; Surai 2002; Mahmoud, Edens et al. 2004; Surai 2006; Metcalfe and Alonso-Alvarez 2010) and premature cellular senescence (Noguera, Metcalfe et al. 2015a) which can lead to reduced longevity (Alonso-Alvarez, Bertrand et al. 2006; Saino, Caprioli et al. 2011; Noguera, Kim et al. 2012). We therefore assessed whether variation in early life dietary micronutrients influences subsequent male song characteristics by quantifying adult song structure, an important sexual trait involved in mate choice in this species; male song motif duration and complexity (number of different elements or syllables in a motif) are considered honest signals of male quality (Spencer, Buchanan et al. 2004; Boogert, Giraldeau et al. 2008), with females preferring males showing more complex songs (Riebel 2009). Moreover, some previous studies suggest that song complexity and duration may act as a reliable signal of developmental stress in songbirds, including zebra finches (Nowicki, Searcy et al. 2002; Riebel 2009 and references therein). We predicted that the exposure to a diet low in micronutrients would result in males producing less complex songs (Nowicki, Searcy et al. 2002). Secondly, we evaluated male mating preferences by quantifying their courting behaviour in standardized conditions when presented with adult females that differed in their early life diet. Finally, we paired both high and low-micronutrient diet males (that is males with potentially attractive and unattractive songs) with a partner of the same and the opposite dietary treatment to themselves. We predicted that females reared on a high micronutrient diet would change their investment pattern according to male´s early diet. That is, investing more in reproduction (i.e. shorter latency to start laying after pairing, larger clutch size or egg mass) when mated with a male reared on the high rather than the low micronutrient diet (i.e. DA hypothesis). We expected that the degree of differential allocation would be less marked (or absent) in females reared on the low-micronutrient diet. We predicted that male mate preferences should, therefore, reflect the patterns of female reproductive investment, with both types of

males preferring those females that provided them with the highest reproductive investment when mated with them.

MATERIAL AND METHODS

Animals and housing and nutritional treatments

The experimental subjects were domesticated zebra finches that had the availability of dietary micronutrients (e.g. antioxidant vitamins and essential minerals) modified during their full postnatal growth period (i.e. between hatching and 90 days of age; a detailed description of the dietary treatments and the housing conditions of the birds is provided in Noguera, Metcalfe et al. 2015c). The experimental birds were the offspring of randomly mated domesticated adult zebra finches from our outbred zebra finch population (≈ 1500 birds) at the University of Glasgow. Briefly, one day after hatching, 56 zebra finch chicks (from 16 different families) were randomly assigned to either a 'low micronutrient' (hereafter 'low' or 'L') or 'high micronutrient' diet (hereafter 'high' or 'H'). Chicks in the low (L) micronutrient diet were fed with a special seed mix composed by Proso and Finger millet in a ratio 1:1. We previously showed that this special diet had a lower micronutrient content as the diet normally used in captive conditions for this species. The chicks assigned to the high (H) micronutrient diet received the same seed mix than birds in the low micronutrient diet but supplemented with a commercial micronutrient supplement (Magic Antistress Mix/Performax, Feed-Food Ltd, UK; a full description of the micronutrient content of the experimental diets and the commercial micronutrient supplement is provided in Noguera, Monaghan & Metcalfe, 2015b). The micronutrient intake of the experimental birds was always within the natural range of variation for this species in aviary conditions (Noguera, Metcalfe et al. 2015c). At 90 days, when birds became sexually mature, the dietary treatments ceased and all birds were fed with a standard aviary diet (Johnson & Jeff, UK) and maintained in single-sex groups.

Song recording and analyses

Song development in zebra finches occurs during the first few months of life and by 90 days of age male song is crystallized and shows no further major changes (Riebel 2009). To assess whether the

early dietary treatment affected adult male song traits, song was assessed once between 5 and 8 months of age in a song attenuated chamber (80 x 80 x 80 cm), using Audacity v2.0.6 software (http://audacity.sourceforge.net/) via an Eagle G160 Omnidirectional microphone (600Ω) and laptop computer (Sound Blaster Audigy Fx 5.1 soundcard). The age at which the song of each male was recorded did not differ between experimental groups (see ESM). The directed song of each bird was recorded after the introduction of an adult non-experimental female into the chamber. The recordings were then analyzed using Sound Analysis Pro 2011 (Tchernichovski, Nottebohm et al. 2000; freely available at http://soundanalysispro.com/), with the analysis being conducted blind to the male's dietary treatment. Five randomly-selected motifs for each male were analyzed and the following information calculated based on spectrograms and waveforms: motif duration (ms), total number of syllables per motif and number of different syllables per motif (see Fig. S1). These song parameters were significantly repeatable among individuals (see ESM) and have been shown to be highly consistent over time in the zebra finch (see e.g. Nordeen and Nordeen 1992). Moreover, previous studies have shown that these male song parameters are related to male phenotypic quality and female preferences in this and other bird species (Spencer, Buchanan et al. 2003; Riebel 2009). The mean values for each of these parameters across all 5 of a male's motifs were used in the statistical analyses (see ESM for further details).

Male preference tests

The mating preferences of the experimental male zebra finches (n=24; 11 H and 13 L) were assessed at 8 months of age (mean 260 ± 2.19 days, range 239-270 days) in a dichotomous choice arena similar to that used by Collins et al. (1994), using trios of one L or H male and two adult females (one female from each dietary group; L or H). All males were weighed (±0.01 g) just before the preference tests and all tests were carried out between 9:00-13:00 hr. To control for any initial difference in bill coloration between experimental groups of males which might potentially alter females' behavior during the preference tests (Zann and Bamford 1996), bill color was also measured on a scale of 1 (light orange) to 9 (dark red) using the standard color chips previously described in Blount et al. (2003). The dichotomous chamber choice arena consisted of a large cage (140 x 50 x 50 cm) with three different

compartments separated by wire mesh dividers. The male's (central) compartment had 3 equally separated plastic perches: one placed in the middle of the male compartment and the other two placed 5 cm from the mesh dividers that created the two female compartments (on the right and left the side of the male compartment). To ensure that the male could have a good view of the females, the female compartments had only one plastic perch placed 15 cm from the nearest perch in the male's area. Because the majority of the experimental females had to be used for the breeding trials (see below), for the mate preference tests we were limited to use four different (extra) pairs of females (i.e. stimulus pairs); there was no consistent difference between the H and L female in each pair in age, body mass, bill color or plumage phenotype (all wild morph), and the females in each pair had separate genetic and rearing parents (a summary of the females' phenotypic traits is provided in Table S1 in the ESM). Similarly, in each mate choice test, the male was genetically unrelated to (and had previously never been housed with) the two females to which he was exposed. Each pair of females was used the same number of times for each of the two treatment groups of males (i.e. L and H), but the spatial position of the two females (left or right arena) was alternated between consecutive tests within each experimental group of males to control for any possible effect of female position on male preference (Rutstein, Brazill-Boast et al. 2007). The average time between consecutive tests using the same set of females in the same day was 57.50 min (range 15-90 min). To reduce the stress caused by being in a novel environment and to minimize the auditory isolation from conspecifics, which might affect the males' sexual behavior (i.e. singing behaviour; Adar, Lotem et al. 2008), all preference tests were carried out in auditory (but not visual) contact with conspecifics (10 males and 10 females). These non-experimental birds were kept in two separated cages placed approx. 2 m away from the dichotomous choice arenas. All preference tests were carried out under full spectrum artificial light (Bird Lamp, Arcadia, Croydon, UK) and food and water were provided *ad libitum* to all birds during each trial. Body mass, bill colour and age did not differ between the experimental groups of males (statistics reported in the ESM).

Trials started with the male being placed in the central area and given a 15 min period of acclimation. After this period, females were introduced into their compartments and the position and behaviour of the males was monitored for the following 90 mins. All observations were made by the

same observer from behind a screen placed approx. 1.5 m from the arena, and was scored blind with respect to male and female dietary treatment group. At each assessment (60 times in total), the male's position was recorded as (N) if he was on the central (neutral) perch, (L) if he was on the left perch and (R) if he was on the right perch of the test chamber. His behaviour at that moment was recorded as 'active' or 'passive', where 'active' was defined as facing the female or courting her (i.e. producing head bows, bill wipes, singing or doing hop-pivots (Zann and Bamford 1996), and all other options defined as 'passive'. The position recordings were used to estimate the relative time a male spent with each female (affiliation behaviour) and the recordings of active behaviour to assess the strength of the male mating preferences. Time spent associating with an individual has been validated as a measure of sexual attraction in zebra finches, as it is correlated with female mating preferences (Cate and Mug 1984) and predicts pair formation (Clayton 1990).

In order to confirm that the observed pattern of male preferences (see results) was consistent, and to check that it was not influenced or biased by differences in female behavior or feed-back processes as a consequence of all birds being in visual contact with each other (Zann and Bamford 1996), the males' mating preferences were assessed for a second time (6 months after the first assessment) using the same experimental conditions with the exception that this time opaque dividers separated the male and females (see Fig. S2 for a schematic representation of the experimental setup and Griggio and Hoi 2010 for similar experimental setup). This precluded any visual interaction between the females and prevented the male from simultaneously observing both females, and gave qualitatively the same results as the first test (see ESM and Fig. S3 for further details).

Breeding trials

After the male mate preference trials had been completed, the males were used in a breeding trial. Half of the males in each dietary treatment group were paired with a female of the same dietary treatment as themselves (matched early life conditions) and the other half with a female of the opposite treatment (mismatched early life conditions). Both male and female body mass was measured (± 0.01 g) on the day they were paired. All pairs were formed on the same day and were between unfamiliar and genetically unrelated birds. Note that none of these females had previously been in contact with their

mate. One male (High group) died before the breeding trial, so a total of 23 males (10 H, 13 L) were used in this part of the experiment. The experimental manipulation of this first reproductive event resulted in 10 mismatched and 13 matched pairs.

All breeding cages were equipped with an external nest-box and coconut fibre as nesting material. Commercial seed mix and water were provided *ad libitum*. Nest-boxes were inspected once daily between 7:00-10:00 h and any new egg was marked and weighed using an electronic balance (±0.01 g). Differential allocation studies in birds, including zebra finches, have shown that females may alter their primary reproductive effort (i.e. egg and clutch size) in response to male quality (Sheldon 2000 and reference therein). Because we wished to examine female differences in reproductive investment when mated with a male from the two dietary treatments, new laid eggs were switched for a dummy clay egg and all pairs were separated once the first clutch was completed. This allowed us to quickly breed the females for a second time (four weeks after the first reproductive event), thus avoiding any potential influence of female variation in age or condition on her reproductive investment (Williams 2012). During the second reproductive event, females were re-paired and allowed to produce a clutch for a second time, but this time with a male of the opposite dietary treatment to the one they had previously. As before, mates were both unrelated and unfamiliar. During the breeding trials one matched pair (H male/H female from the 1st reproductive event) and one mismatched pair (L male/H female from the 2nd reproductive event) did not reproduce and so were excluded from statistical analyses. In both breeding events we collected data on the latency (from date of pairing) to laying the first egg, clutch size and mean egg mass per clutch as measures of reproductive investment (Christians 2002; Holveck and Riebel 2010). Neither the age nor body mass differed between experimental groups or sexes on the day of pairing during the two reproductive events (statistics reported in the ESM).

Statistical analysis

Song parameters

The effects of the male dietary treatment on adult song traits (number of syllables in a motif, number of different syllables and motif duration) were analyzed using linear mixed models (LMM). The male's dietary treatment was included as a fixed factor and body mass as a covariate. The identity of the female

used to record the male's song (two levels: female 1 or 2) was also included as a fixed factor in all the above models to test whether the stimulus female had any influence on song traits. In these and all other analyses described below, we initially accounted for the non-independence of males sharing the same genetic or rearing background by initially including the identities of the genetic and rearing family as random factors. However, since only 2 males shared the same genetic or rearing origin these random effects were not significant ($p<0.05$) and so were excluded from the final models.

Male mate preferences

For each experimental male, we calculated the relative time showing affiliative behaviour and showing active choice (i.e. facing or courting) towards each female (i.e. number of observations/total number of observations). Then these variables were analyzed separately using LMM. The models included male and female dietary treatment and their interaction as fixed factors, and male identity and the identity of the stimulus set of females as random terms. Male body mass was also included as a covariate.

Female reproductive investment

The effect of male and female dietary treatments on latency to lay the first egg (breeding latency), clutch size and mean egg mass per clutch were analyzed using LMM or GLMM as appropriate. Male and female dietary treatments and the reproductive event (first or second) were included as fixed effects in the models, and female body mass as a covariate. The birds' identity was included in all models as a random factor to account for nonindependence of two successive reproductive events from the same bird. Two and three-way interactions between fixed factors were also tested.

All analyses were carried out using IBM SPSS 22. Models were fitted with Satterthwaite's approximation for degrees of freedom. To avoid inflating the type I error we did not apply model selection in any analyses, and so report results for full models after removing non-significant interactions as recommended by Whittingham et al. (2006). When needed, *post hoc* comparisons were performed using LSD *post hoc* tests. Data are presented as means ± standard error, and the significance level was set at $P = 0.05$.

RESULTS

Male song traits

The availability of micronutrients in the diet of male zebra finches during their first 90 days of age had no significant effect on the duration of adult song motifs (L males: 508.84 ± 84 ms; H males: 555 ± 85 ms; $F_{1,9}$=0.151, p=0.707) or the total number of syllables in a motif (L males: 5.26 ± 0.47 syllables; H males: 6.35 ± 0.40 syllables; $F_{1,9}$=2.594, p=0.142). Early life conditions did, however, have a significant effect on adult song complexity (L males: 4.33 ± 0.33 syllables; H males: 5.77±0.28 syllables; $F_{1,9}$=9.884, p=0.012); the songs of males that were reared on a diet low in essential micronutrients had, on average, 14% fewer different syllables in a motif compared to those reared on a high micronutrient diet (Fig. 1). Neither the identity of the stimulus female used during the song recordings nor the male body mass had a significant influence on the duration of song motifs (stimulus female: $F_{1,9}$=0.936, p=0.359; body mass: $F_{1,9}$=3.018, p=0.116), the total number of syllables (stimulus female: $F_{1,9}$=1.080, p=0.0.326; body mass: $F_{1,9}$=0.311, p=0.591) or song complexity (stimulus female: $F_{1,9}$=0.130, p=0.726; body mass: $F_{1,9}$=0.297, p=0.599).

Male mate preferences

The relative time spent in affiliative behaviour varied according to the male and female dietary treatment (male x female treatment: $F_{1,43}$=9.617, p=0.003; Fig. 2a). Thus, whereas males reared on a high micronutrient diet had no clear preference for females reared on either the low or high micronutrient diet (LSD *post hoc* test; H vs L: $F_{1,20}$=0.083, p=0.777; Fig. 2a), males reared on a low (L) diet spent significantly more time with the female that had been reared on that same (Low) diet (LSD *post hoc* test; H vs L: $F_{1,24}$=33.434, p<0.001 Fig. 2a). A similar pattern was observed in the relative time the males spent in active choice (male x female dietary treatment: $F_{1,22}$=5.519, p=0.028; Fig. 2b); the males from the low micronutrient diet spent more time being active next to the female that was reared on the low micronutrient diet (LSD *post hoc* test; H vs L: $F_{1,24}$=4.619, p=0.042). In contrast, males from the high micronutrient treatment behaved equally to the two females (LSD *post hoc* test; H vs L; $F_{1,20}$=0.564, p=0.461). Neither the time spent in affiliative behaviour ($F_{1,43}$=0.415, p=0.523) nor in active choice ($F_{1,20.43}$=3.754=0.067) was related to male body mass.

Female reproductive investment

On average, females started their clutch sooner after pairing when reproducing for the second time (reduction in latency between 1st and 2nd reproductive event, mean=2.2 days ± 0.3 SE; Table 1a), but the latency to start a clutch was unaffected by either of the male or female dietary treatments (Table 1). It was also unaffected by female body mass (Table 1a). Neither the rest of three- and two-way interactions were significant into the model (male treatment x female treatment x reproductive event: $F_{1,28.18}$=0.275, p=0.604; male treatment x female treatment: $F_{1,19.78}$=0.041, p=0.841; male treatment x reproductive event: $F_{1,28.51}$=0.722, p=0.403; female treatment x reproductive event: $F_{1,20.34}$=3.049, p=0.096).

The breeding trials showed, however, that the effect of a within-pair mismatch in dietary treatment on female fecundity (clutch size) depended on the dietary treatment of both the male and the female (see Table 1). Thus females paired with a high micronutrient diet male (H) produced similar clutch sizes whether their own early life dietary conditions were matched (i.e. also H) or mismatched (L) to those of the H male (LSD *post hoc* test: H vs L; $F_{1,20}$=0.856, p=0.368; Fig. 3). However, when paired with a low micronutrient diet male (L), the high (H) diet females had smaller clutch sizes (LSD *post hoc* test: $F_{1,20}$=3.885, p=0.063) whereas the low diet females showed no evidence of this (LSD *post hoc* test: $F_{1,20}$=1.315, p=0.265). As a result, low micronutrient diet females laid on average a larger clutch size than high micronutrient diet females when both types of females were mated to an L male (LSD post hoc test: H vs L; $F_{1,23}$=6.375, p=0.019; Fig. 3). Such effects were independent of the order in which females were paired with the different types of males (reproductive event x male treatment x female treatment; $F_{1,24.97}$=0.675, p=0.419). Neither female body mass (Table 1) nor the rest of two-way interactions were significant into the model (male treatment x reproductive event: $F_{1,24.18}$=0.018, p=0.895; female treatment x reproductive event: $F_{1,15.79}$=0.790, p=0.387).

Mean egg mass was similar among experimental groups, reproductive events and was not influenced by female body mass (Table 1). The rest of three- and two-way interactions were no significant into the model (male treatment x female treatment x reproductive event: $F_{1,26.88}$=0.314,

p=0.580; male treatment x female treatment: $F_{1,18.41}$=3.460, p=0.079; male treatment x reproductive event: $F_{1,27.44}$=0.602, p=0.445; female treatment x reproductive event: $F_{1,17.91}$=3.055, p=0.098).

DISCUSSION

The results of this study show that a diet low in essential micronutrients in early life influenced adult male song complexity and male mate preferences in what seems to be a strategic fashion. Males reared on a low level of micronutrients produced less complex courtship songs as adults, which suggests that they may have suffered a reduction in sexual attractiveness. These males were themselves more selective in their mating tactics, showing a particular preference for females that had experienced the same (poor) early dietary conditions as themselves. In contrast, males reared on a diet high in micronutrients, which produced more complex songs, did not show a particular preference for females on the basis of their rearing diet. We also provide evidence that early life diet affected female willingness to invest in reproduction, an effect that differed according to the early diet received by the partner: whereas zebra finch females reared on a high micronutrient diet tended to produce larger clutch sizes when they reproduced with a male singing a more complex song (i.e. an H male), females reared on a low micronutrient diet invested similarly in egg production regardless of their mate's song complexity (i.e. similar clutch size with L and H males). As a result of this variation in female reproductive investment, low micronutrient diet males (L) obtained smaller clutches if their mate had the opposite diet than when the mate had also experienced a low (L) diet. These results suggest that by following a different mating strategy, males with a less complex song are able to achieve comparable reproductive benefits in terms of clutch size as males with more complex songs. Thus, the adaptive significance of the observed male mate choice seems to be determined by the effect of a male's early nutritional conditions on the degree of reproductive investment by his mate.

Our results provide support for the 'nutritional stress hypothesis' which states that stress during early development, in our case in the form of a diet low in micronutrients, can significantly reduce the complexity of the song produced by adult male birds (Nowicki, Searcy et al. 2002). In our experiment, the birds fed with a low micronutrient diet showed a significant reduction in the number of different syllables in a motif, which is in agreement with previous studies showing a negative effect of early food

deprivation (Buchanan, Spencer et al. 2003) and high stress level (Spencer, Buchanan et al. 2003) on adult male song complexity. Our results, however, contrast with other previous studies which did not find an effect of poor early conditions (via brood size manipulation) on song complexity (Gil, Naguib et al. 2006; Holveck, de Castro et al. 2008). This lack of consistency among studies might be explained by variation in the nature and duration of the experimental treatments, since different kinds of stressor are likely to influence different features of the song system (i.e. brain nuclei size, cell size, synaptic density or neurological properties) and lead to different effects on song traits (Nowicki, Searcy et al. 2002). Interestingly, our study is the first to show that a reduction in the micro- rather than a macro-nutrient intake can affect song complexity. In this regard, two different, but not exclusive, mechanisms might explain the effects on song complexity that we observed. On the one hand, it is thought that early nutritional stress can affect song complexity by impairing song learning during critical sensitive periods (Nowicki, Searcy et al. 2002). Stress-induced impairment of song learning has been related to a reduction in the adult size of the high vocal center (HVC), a telencephalic nucleus associated with song complexity in birds (Buchanan, Leitner et al. 2004). Micronutrient insufficiency or imbalance during development can compromise brain development and function (Ashworth and Antipatis 2001), and so our experimental treatments might have affected the development of the HVC which in turn, might have influenced the capability of our experimental birds to develop their song. On the other hand, it is known that the accumulation of oxidative damage in the brain can impair learning and reduce memory (Liu, Liu et al. 2003). Since dietary micronutrients are an important contributor to brain antioxidant defences (Ashworth and Antipatis 2001; Liu, Liu et al. 2003), our low micronutrient group of birds may have also experienced a higher level of oxidative damage in the brain and therefore, a reduced capacity for song learning. Irrespective of the mechanism, our results indicate that song complexity in adulthood can act as a reliable indicator of a male zebra finch's nutritional history.

It has been shown that female zebra finches invest more resources in terms of egg volume and carotenoid content when paired with lower quality males (Bolund, Schielzeth et al. 2009), which suggests that female reproductive compensation may take place in this species. In our study, the fact that L females laid more eggs than H females when paired with a male with less complex song (L) might, at first glance, also suggest reproductive compensation. However, for this hypothesis to be

correct, L females should have produced larger clutch sizes when paired to an L than an H male (Gowaty, Anderson et al. 2007; Gowaty 2008), which was not the case. Indeed, our results indicate that the differences in clutch sizes observed in the L group of males were more likely to be caused by the tendency of H females to reduce their clutch size when mated to a less preferred (attractive) male (L). Such reduction in clutch size better fits the differential allocation scenario (Sheldom 2000) and supports previous studies showing differential allocation in clutch size in this species (Balzer and Williams 1998).

Since song complexity is related to male attractiveness, and attractive males attain higher social status (Spencer, Buchanan et al. 2004) and have improved cognitive abilities (Boogert, Giraldeau et al. 2008), the H diet females could gain substantial direct and indirect fitness benefits from increasing their investment in reproduction when mated to a male with a more complex song (e.g. an H male) (Sheldon 2000). Moreover, even if more attractive males contribute less to parental care (Burley 1988), H female could benefit from increasing the investment in reproduction (e.g. clutch size, egg quality, etc.) as such strategy might help females to compensate for the reduction in paternal care. This begs the question as to why the low (L) diet group of females laid a similar size of clutch regardless of the dietary treatment of their mate. It is possible that they did not alter their reproductive investment simply because they were inherently of poorer quality (and hence condition) and so may not have been able to increase their clutch size any further when mated to a high diet male. However, this seems unlikely since female body mass on the day of pairing was similar between groups, suggesting that the low micronutrient diet group of females were not in poorer condition. Alternatively, an early diet low in essential micronutrients may have had some (unmeasured) negative effects on female cognitive abilities (Lucas, Morley et al. 2001; Bonaparte, Riffle-Yokoi et al. 2011) which in turn, may have potentially impaired her capacity to discriminate between males with more or less complex songs. Although plausible, a previous study where early life dietary conditions were experimentally altered via brood size manipulation does not support this possibility, as females reared in enlarged broods, and therefore facing poor early life dietary conditions were perfectly able to discriminate between high and low-quality males (Holveck and Riebel 2010). By contrast, recent studies in wild and captive bird populations indicate that a low availability of dietary micronutrients may accelerate cell senescence through different mechanisms such as

increasing the susceptibility to oxidative stress (Noguera, Kim et al. 2012) or reducing the length of telomeres in the chromosomes (Badás, Martínez et al. 2015; Noguera, Metcalfe et al. 2015a). Nestlings with increased levels of oxidative damage or relatively short telomeres have reduced adult survival (Noguera, Kim et al. 2012) and a shorter lifespan (Heidinger, Blount et al. 2012). Thus, supporting previous theoretical model predictions, an early diet low in micronutrients may have stimulated the females to invest heavily in reproduction irrespective of their partner's quality, in order to maximize their fitness given the possibility of a shortened lifespan (Pianka and Parker 1975; Clutton-Brock 1984; Stearns 1992).

The variation in female reproductive investment (fecundity) provides an adaptive explanation for the observed plasticity in male mate preferences, at least as long as chicks from larger broods do not have reduced fitness (i.e. survival). Although chicks from larger broods may have reduced survival when food availability is scarce (Zann and Bamford 1996), in captive conditions where food is not a limiting factor the effect of brood size on the rearing capacity of the zebra finch parents as well as on chick survival seems to be negligible (e.g. Reichert, Stier et al. 2014; Noguera 2017). Hence, our data suggest that by altering their mate preferences, males reared on poor (micronutrient) diets and with less complex songs could avoid being penalized by those females that are less willing to invest in reproduction when mated with them, thus maximizing their reproductive fitness. Conversely, selective mating would not have resulted in any detectable fitness benefit for males with more complex songs (e.g. H males) since the females laid the same size of clutch for those males regardless of female diet. At first sight, this result seems to conflict with traditional models of sexual selection which predict that attractive males should be more discriminating (Andersson 1994). However, it has been shown that when the reproductive benefits of being selective are negligible, as seems to be the case for the high micronutrient diet males in our experiment, discriminative mating is not always the best strategy (see i.e. Kokko and Härdling 2005).

The non-random preference pattern showed by our experimental males raises the question as to the underlying mechanism. Previous studies have shown that male zebra finches prefer females with more yellow-orange bill colouration (Burley and Coopersmith 1987) or more potential fecundity (Monaghan, Metcalfe et al. 1996). However, in the male preference tests, there were no consistent

differences between the H and L female in age, body mass, bill colour or plumage phenotype. Micronutritional differences during post-natal development, however, may have affected many aspects of a female's adult behavior, such as her posture, the level of activity or behavioral type (Noguera, Metcalfe et al. 2015b) and which may have influenced the probability of being preferred by a male (Laubu, Schweitzer et al. 2017). In addition, differences in the availability of diet micronutrients may have also influenced the expression of female UV signals that are not visible to humans (Mougeot, Martinez-Padilla et al. 2007) but that may have played an important role in the males' sexual preferences (Bennett, Cuthill et al. 1996).

The results of our male mate preference experiments contrast with those reported by Holveck et al. (2011), which showed an absence of male preference for females of different phenotypic quality (induced via brood size manipulation), even though the same authors previously reported better breeding performance when both members of a pair had similar phenotypic quality (Holveck and Riebel 2010). However, a lack of male mate preferences might have been caused by the environmental conditions in which male mate preferences were evaluated. In contrast to our study, Holveck et al. (2011) assessed male mate preferences in the presence of several competitor males which could have affected the perceived level of male-male competition and so suppressed the focal male's mating preferences (Candolin and Salesto 2009; Mautz and Jennions 2011).

In summary, we have shown that a poor start in life can reduce adult male courtship song complexity which in turn, may promote plasticity in male mate choice. The observed variation in male mate preferences is the result of a complex interplay between early nutrition-dependent male and female reproductive traits: by following different mating strategies, males with complex (H) and less complex (L) songs obtained similar reproductive benefits in terms of clutch size. The consequences of such nutrition-induced plasticity in male and female reproductive behaviour for sexual selection and the evolution of reproductive strategies deserve further empirical and theoretical investigation.

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Author contributions: JCN, NBM and PM conceived and designed the experiments. JCN performed the experiments and analyzed the data. JCN, NBM and PM wrote the manuscript.

Compliance with Ethical Standards

Ethical approval: The study was carried out with the permission of UK Home Office and all tests were subjected to local ethical review (Project License No. 60/4109).

***Competing interests*:** The authors declare that they have no competing interests.

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Table 1. Summary of the mixed models (LMM and GLMM) testing for the effects of early male and female dietary treatment and covariates on breeding latency (delay from pairing until the laying of the first egg), clutch size and mean egg mass. Nonsignificant interactions were removed from full models and significant terms are highlighted in bold.

Dependent variable	Variables	Parameter estimate	F or Z	$DF_{n,d}$	p-value
Breeding latency	Intercept	7.656			
	Male treatment (L)	0.797	1.456	1,21.46	0.241
	Female treatment (L)	-1.076	1.269	1,18.98	0.274
	Reproductive event (1^{st})	2.216	11.723	1,20.25	**0.003**
	Female body mass	-0.166	0.257	1,37.35	0.615
	Random factors				
	Female ID	2.791	1.561		0.119
Clutch size	Intercept	4.887			
	Male treatment (L)	-1.107	0.277	1,16.49	0.606
	Female treatment (L)	-0.727	0.159	1,15.27	0.696
	Reproductive event (1^{st})	-0.075	0.049	1,15.30	0.827
	Female body mass	-0.009	0.003	1,35.84	0.958
	Male treatment x female treatment	1.851	7.163	1,16.50	**0.016**
	Random factors				
	Female ID	0.734	1.253		0.210
Mean egg mass	Intercept	1.037			
	Male treatment (L)	-0.001	0.007	1,19.38	0.935
	Female treatment (L)	0.063	0.648	1,19.90	0.430
	Reproductive event (1^{st})	-0.014	0.631	1,19.64	0.436
	Female body mass	0.007	0.334	1,25.23	0.569
	Random factors				
	Female ID	0.329	2.994		**0.003**

Fig. 1. Box plots showing the effect of dietary micronutrient intake during the first 90 days of life on the complexity (number of different syllables in a motif) of a male zebra finch's song in adulthood.

Fig. 2. Box plots showing the relative time spent by males in (a) affiliative behavior and (b) active choice with the female reared under a low (white boxes) or a high (grey boxes) micronutrient diet in relation to the male's own diet during his early life. There is a significant male x female diet interaction (Table 1).

Fig. 3. Box plots showing the clutch size of females reared under a low (white boxes) or a high (grey boxes) micronutrient diet in relation to the micronutrient diet in early life of their male partner. There is a significant male x female diet interaction (Table 1).