THE ROYAL SOCIETY
biology letters

Diet quality and resource allocation in the zebra finch

A. N. Rutstein*, **P. J. B. Slater and J. A. Graves**

School of Biology, University of St Andrews, St Andrews KY16 9TS, UK

* *Author for correspondence (ar27@st-andrews.ac.uk).*

Recd 30.10.03; *Accptd* 03.12.03; *Published online* 20.02.04

We investigated the effect of diet quality on resource allocation in zebra finches (*Taeniopygia guttata*) by providing females with a high-quality (HQ) or low-quality (LQ) diet for six weeks prior to pairing, and continuing these diets during egg laying and chick rearing. Diet treatments were then reversed and the experiment repeated. When females laid on the HQ diet, egg mass increased with laying order, but the reverse was true on the LQ diet. Females laid significantly more male eggs on the LQ diet compared with on the HQ diet. In addition, female eggs were more frequent at the end of the clutch when on the HQ diet and at the beginning of the clutch when on the LQ diet. These differences in the primary sex ratio are in line with predictions from sex allocation theory, since in this species females are more vulnerable to nutritional stress than males.

Keywords: zebra finch; *Taeniopygia guttata*; diet quality; sex ratio; egg mass

1. INTRODUCTION

When resources are plentiful, females should make relatively large reproductive investments, as reflected by heavier and higher-quality clutches (Pilz *et al.* 2003). According to sex allocation theory, under such conditions, females should also produce offspring of the sex with the greatest variance in reproductive success (Trivers & Willard 1973; Appleby *et al.* 1997). Conversely, when conditions are harsh, females should produce offspring of the sex with the lowest variance in reproductive success (Nager *et al.* 1999).

Zebra finches (*Taeniopygia guttata*) are socially monogamous, with low rates of extra-pair paternity in the wild (Birkhead *et al.* 1990). Although there is no discernible size dimorphism in this species, females are more vulnerable to nutritional stress than males (Bradbury & Blakey 1998; Kilner 1998; Martins 2004), and compared with males, female fecundity is more dependent on the resources received as nestlings (Haywood & Perrins 1992). Therefore, it would seem that being of lower quality has stronger negative effects on the lifetime reproductive success of females than of males.

Although a number of laboratory studies have manipulated maternal diet to examine the effects on offspring sex ratios in this species, two such studies measured the hatching sex ratio (Bradbury & Blakey 1998; Kilner 1998), which may not be an accurate reflection of the sex ratio at laying. In the case of two more recent studies, diets were switched at egg laying (Rutkowska & Cichon 2002; Arnold *et al.* 2003). However, interpretation of these data is problematic. Females respond to a variety of cues, of which the constancy of the environment may be of particular importance, and is therefore likely to affect the fitness returns of adjusting the sex ratio.

We investigated whether females adopted different strategies in relation to diet quality, with a particular focus on primary sex ratio adjustment. So that females would perceive the environment as relatively stable, they received their experimental diets for a considerable period prior to breeding, and diets were continued during egg laying and chick rearing.

2. METHODS

(a) *Experimental design*

Thirty-four females were randomly assigned to either a high-quality (HQ) or low-quality (LQ) diet (see electronic Appendix A). Females were kept on these diets in single-sex groups for a period of six weeks and then they were paired randomly with males (with whom they had not previously bred). Pairs were housed in individual breeding cages, equipped with nest-boxes. For the six weeks prior to pairing, all males had been caged in a separate room, on the standard laboratory diet of foreign finch mix, supplemented with spinach and egg biscuit once a week. Once paired, males were kept on the same diet as their mate, and these diets were maintained during chick rearing.

Nests were checked daily for eggs, which were weighed and numbered. Thirteen days after the first egg had been laid, nests were checked for hatchlings. To ascertain hatching order, nests were inspected four times a day, and chicks were individually marked with coloured Tippex correction fluid on their right or left leg.

Pairs were then left together to lay second clutches to obtain additional data on the sex ratio at laying. Each egg was weighed and numbered. Once an embryo was visible (after 2–3 days), the egg was removed and replaced with a dummy egg. The embryo was extracted and stored at $-20\ ^\circ\mathrm{C}$ in 96% alcohol for sexing.

After second clutches were complete, pairs were separated and transferred to single-sex groups. Birds were given a two-week rest period (during which they all received the standard laboratory diet) before diet treatments were reversed. Thereafter, females that were previously on the HQ diet received the LQ diet, and vice versa. Males remained on the standard laboratory diet. After six weeks, all birds were re-paired with the same mate as in the first round, and the experiment was repeated.

(b) *Sexing*

Offspring that survived to sexual maturity were sexed from plumage characteristics. DNA was extracted from fertile second-clutch eggs, from chicks that died prior to sexual maturity, and from unhatched, fertile eggs in first clutches, using the Puregene extraction kit (Gentra Systems, Minneapolis, MN, USA). Molecular sexing was carried out using methodology and primers described in electronic Appendix A.

(c) *Statistical analysis*

Egg mass data were analysed using repeated-measures general linear models (GLMs) in SAS (SAS Institute 1998). Diet was entered as a fixed factor and female identity as a random factor, with eggs within the clutch as a repeated measure. Sex ratio (number of males/total number of sexed eggs) was analysed at the brood level by fitting a generalized linear model, using the GLIMMIX macro in SAS. The error distribution was binomial with a logit link, weighted by brood size. Sex and mortality were analysed in relation to position in the laying order and treatment using logistic regression. To avoid small sample sizes in some categories (to which binomial data are particularly sensitive) eggs were assigned to the first, middle or last third of the clutch rather than by egg number (see electronic Appendix A). In the analyses of sex ratio and mortality, diet was entered as a fixed factor and female identity as a random factor.

3. RESULTS

(a) *Clutch and egg size*

Twenty females laid clutches when fed on both the HQ and LQ diets (12 females laid on the HQ diet and eight on the LQ diet in round 1). Six females did not lay a

DOI 10.1098/rsbl.2003.0154

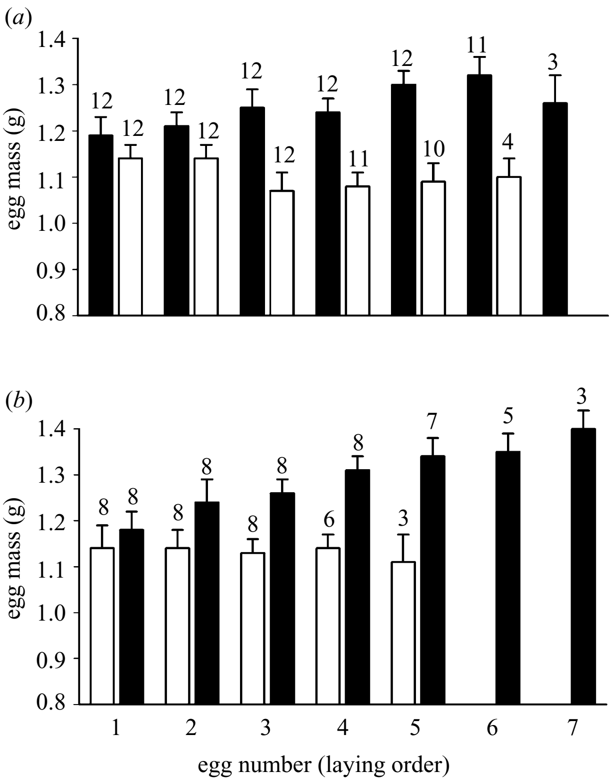

Figure 1. Mass of eggs (mean ± s.e.) in relation to laying order. Data are paired for females that laid (*a*) on the HQ diet in round 1 and on the LQ diet in round 2 ($n = 12$) and (*b*) on the LQ diet in round 1 and on the HQ diet in round 2 ($n = 8$). The filled bars represent the eggs produced when females were fed on the HQ diet and the open bars represent the eggs produced when females were fed on the LQ diet. Numbers of eggs are given above bars.

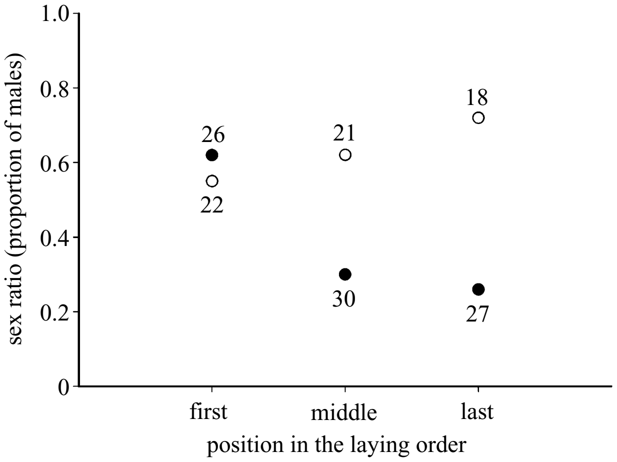

Figure 2. Sex ratio (proportion of males) at laying in relation to relative position in the clutch (first, middle or last) for first-clutch eggs, for females that laid on both the HQ and LQ diets. Filled circles represent the eggs produced when females were fed on the HQ diet and open circles represent the eggs produced when females were fed on the LQ diet. The numbers of eggs that were sexed are indicated.

clutch on either diet, five laid only on the HQ diet and three laid only on the LQ diet.

For females that laid on both diets ($n = 20$), clutches were significantly larger on the HQ diet compared with on the LQ diet (HQ diet: median (interquartile range) six eggs (6–7); LQ diet: five eggs (4–5); Wilcoxon signed-ranks test, $W = 171.0$, $p < 0.001$) and mean egg mass per clutch was significantly heavier (HQ diet: (mean ± s.e.) 1.26 ± 0.02 g; LQ diet: 1.12 ± 0.02 g; paired *t*-test, $t = 8.23$, $p < 0.001$). In a paired, repeated-measures GLM, there was a significant interaction between diet and egg number with respect to egg mass ($F_{1,25} = 21.27$, $p = 0.0001$; figure 1). On the HQ diet, egg mass increased significantly with egg number ($F_{1,101} = 16.72$, $p < 0.0001$), but on the LQ diet egg mass tended to decrease with egg number ($F_{1,89} = 3.39$, $p = 0.07$). There was no effect of order of treatment on egg mass ($F_{1,36} = 0.32$, $p = 0.58$). There was also no difference in the mass of male and female eggs in either diet treatment ($F_{1,95} = 0.25$, $p = 0.56$).

(b) *Sex ratios*

The number of male and female chicks produced on each diet is summarized in table 1. The percentage of eggs that were successfully sexed on the HQ and LQ diets were 220 out of 237 eggs (93%) and 102 out of 116 eggs (88%), respectively ($G_{\mathrm{adj}\,1} = 2.19$, $p > 0.1$). When the data were pooled for each diet from both breeding rounds, the sex ratios were as follows: at laying (including second clutches): HQ diet: 0.46 ($G_{\mathrm{adj}\,1} = 1.47$, $p > 0.2$); LQ diet: 0.60 ($G_{\mathrm{adj}\,1} = 3.93$, $p < 0.05$); at hatching: HQ diet: 0.42 ($G_{\mathrm{adj}\,1} = 2.61$, $p > 0.1$); LQ diet: 0.65 ($G_{\mathrm{adj}\,1} = 3.95$, $p < 0.05$); at fledging: HQ diet: 0.41 ($G_{\mathrm{adj}\,1} = 2.85$, $p > 0.05$); LQ diet: 0.66 ($G_{\mathrm{adj}\,1} = 2.79$, $p > 0.05$).

Because chicks within a brood may not be independent, paired analyses on the clutch sex ratio at laying were carried out for 14 females that laid fertile eggs on both diets. The clutch sex ratio was significantly more male-biased on the LQ diet than on the HQ diet (HQ diet: (mean ± s.e.) 0.39 ± 0.04; LQ diet: 0.63 ± 0.05; $F_{1,13} = 14.51$, $p = 0.002$). This was also the case when second-clutch eggs were included in the analysis (HQ diet: 0.41 ± 0.04; LQ diet: 0.66 ± 0.04; $F_{1,19} = 16.69$, $p = 0.0006$). There was no effect of order of treatment ($F_{1,12} = 1.46$, $p = 0.25$) and there was no difference between the mean clutch sex ratio of first and second clutches ($F_{1,18} = 0.04$, $p = 0.85$).

Egg sex was analysed in relation to position in the laying order and experimental treatment, revealing a significant interaction between diet and laying order ($F_{1,114} = 6.42$, $p = 0.01$; figure 2). The sex ratio decreased with egg number on the HQ diet, i.e. became progressively more female-biased ($F_{1,68} = 6.43$, $p = 0.01$), and tended to increase with position in the laying order on the LQ diet, although this did not approach significance ($F_{1,46} = 1.25$, $p = 0.27$). When the first and second clutches were combined, the interaction between diet and laying order remained significant ($F_{1,191} = 5.77$, $p = 0.02$).

At hatching and fledging paired data were available for 13 and 10 females, respectively. Again, the brood sex ratio was significantly more male-biased on the LQ diet, both at hatching (HQ diet: 0.34 ± 0.06; LQ diet: 0.64 ± 0.08; $F_{1,12} = 11.50$, $p = 0.005$) and at fledging (HQ diet: 0.35 ± 0.07; LQ diet: 0.61 ± 0.12; $F_{1,9} = 5.84$, $p = 0.04$).

(c) *Mortality*

There was a significant interaction between diet and embryo sex with respect to embryo mortality ($F_{1,115} = 4.03$, $p = 0.047$). On the HQ diet, male embryo mortality (18.2% (6 out of 33)) tended to be higher than that of females (8% (4 out of 50), $F_{1,68} = 1.92$, $p = 0.17$). On the LQ diet, female embryo mortality (37.5% (9 out of 24)) tended to be higher than that of males (26.3% (10 out of 38), $F_{1,47} = 2.32$, $p = 0.13$). There was no effect of egg number ($F_{1,113} = 0.15$, $p = 0.70$) or egg mass ($F_{1,114} = 0.71$, $p = 0.40$) on embryo mortality on either diet.

There was no sex-biased post-hatching mortality on either diet ($F_{1,82} = 0.18$, $p = 0.67$). The only significant predictor of post-hatching mortality was egg mass ($F_{1,83} = 17.53$, $p < 0.0001$). Chicks that died before fledging came from significantly lighter eggs, both on the HQ diet ($F_{1,52} = 8.19$, $p = 0.006$) and the LQ diet ($F_{1,30} = 9.39$, $p = 0.005$).

4. DISCUSSION

Females laid heavier clutches on the HQ diet, and egg mass increased with laying order. On the LQ diet, however, egg mass tended to decrease with laying order. Such differences in egg mass within clutches are in line with predictions of brood-survival and brood-reduction strategies, respectively (Slagsvold *et al.* 1984). Support for the existence of such adaptive strategies is further strengthened by the observation that post-hatching mortality was negatively correlated with egg mass, when controlling for hatching order. Within-clutch differences in egg mass have also been reported in relation to maternal age in red-winged blackbirds (*Agelaius phoeniceus*) and were similarly found to influence chick mortality (Blank & Nolan 1983).

As predicted, the sex ratio at laying differed between the two diets. Females laid a significantly higher proportion of male eggs on the LQ diet compared with on the HQ diet. A male bias under poor dietary conditions was also found by Kilner (1998) and Bradbury & Blakey (1998), although they sexed chicks at hatching, by which time there may have already been substantial mortality. A recent study found no overall bias in the sex ratio at laying in relation to diet quality (Arnold *et al.* 2003). However, an important difference between their study and ours was the duration of the diets. In our study, diets were maintained during egg laying whereas Arnold *et al.* (2003) stopped the experimental diets prior to pairing and all females laid on the same diet. A change of diet could affect sex ratio adjustment because it will influence the female's predictions of the rearing diet. It is probable that sex ratio adjustment is brought about in response to a variety of cues, including maternal condition, the quality of the eggs and the expected rearing environment of the chicks. The strong result in our study may be because all three cues were operating in the same direction.

Although there was no difference in the mass of male and female eggs on either diet, there was a significant difference in the position in the laying order of male and female eggs. Female eggs were laid later in the laying order on the HQ diet and the reverse tended to be true on the LQ diet. Kilner (1998) found that females hatched earlier than males under both restricted and abundant food regimes, and suggested that females were more sensitive

Table 1. The number of offspring of each sex at laying, hatching and fledging for females that laid eggs on the HQ and LQ diets.
(Sex ratio = males/(males + females). All females in round 2 had also laid clutches in round 1. Numbers in brackets include second clutches that were removed for molecular sexing when females had already reared their first brood to independence. $G_{\text{adj 1}}$ values are goodness of fit against the Mendelian expectation of equal numbers of both sexes ($^*p < 0.05$). M, males; F, females.)

	laying				hatching				fledging			
	number of clutches	M/F	sex ratio	$G_{\text{adj 1}}$	number of broods	M/F	sex ratio	$G_{\text{adj 1}}$	number of broods	M/F	sex ratio	$G_{\text{adj 1}}$
round 1												
HQ diet	14 (26)	36/47 (69/92)	0.43 (0.43)	1.45 (3.29)	14	30/42	0.42	2.00	14	23/38	0.38	3.70
LQ diet	5	7/6	0.54	0.07	4	4/4	0.50	0	4	4/4	0.50	0
round 2												
HQ diet	7 (12)	16/19 (32/27)	0.46 (0.54)	0.25 (0.42)	7	11/15	0.42	0.61	5	9/9	0.50	0
LQ diet	10 (20)	33/17 (54/35)	0.66 (0.61)	5.16* (4.06*)	9	24/11	0.69	4.88*	7	15/6	0.71	3.89*

to resource availability than males, and so should be in the 'favoured' position. She did not report any changes in egg mass with laying order. In our experiment, because egg mass increased with laying order on the HQ diet, and because heavier eggs had lower post-hatching mortality, the 'favoured' position on the HQ diet would be later (rather than earlier) in the clutch.

In conclusion, we have demonstrated that female zebra finches show very different reproductive strategies on LQ and HQ diets, with respect to egg mass and laying order, and differential sex allocation. Females produced more of the sex that has the highest fitness returns under these conditions, and in addition, were able to optimize within-clutch resource allocation.

Acknowledgements

The authors thank Lucy Gilbert for valuable advice. They also thank BBSRC for funding this work.

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