



# Primary and secondary sex ratio manipulation by zebra finches

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## ABSTRACT

Wild zebra finches, *Taeniopygia guttata*, breed opportunistically when there is sufficient food available, often rapidly mobilizing their reproductive systems in response to an ephemeral boom in grass seed production. For females in captivity, fecundity, attractiveness to mates and survival to reproduction are all correlated with their fledging weight. By contrast, for males, only attractiveness is related to fledging weight; the relationship between fledging weight and male mortality is much weaker and that for male fecundity is unknown. Previous work thus suggests that how much food nestlings receive will have a profound impact on their reproductive success, and that this effect may be more marked for females than for males. I manipulated the food available to domesticated breeding zebra finches to test [Trivers & Willard's \(1973, \*Science\*, 179, 90–92\)](#) hypothesis of adaptive sexual investment. When food availability was restricted, clutch sex ratios were significantly more male biased than when food was available in excess. Within clutches, daughters hatched sooner than sons and first-hatched chicks fledged at higher weights than those that hatched last. Chick mortality was female biased when food availability was low but male biased when food availability was unrestricted. I compared the song output of brothers of differing weight at independence, but found no significant difference between them. These data suggest that zebra finches manipulate both their primary and secondary sex ratios in relation to food availability to invest adaptively in sons and daughters, and support [Trivers & Willard's](#) hypothesis.

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Avian clutch sex ratios deviate from parity in a number of species (e.g. [Dijkstra et al. 1990](#); [Appleby et al. 1997](#); but see [Clutton-Brock 1986](#); [Koenig & Dickinson 1996](#)), sometimes in a way that is clearly adaptive (e.g. [Ellegren et al. 1996](#); [Svensson & Nilsson 1996](#); [Komdeur et al. 1997](#); but see [Lessells et al. 1996](#)). Females may adjust the sex ratio of their clutch to reap direct future fitness gains (e.g. [Komdeur et al. 1997](#)) or in relation to the attractiveness or quality of their mate ([Ellegren et al. 1996](#); [Svensson & Nilsson 1996](#)). [Trivers & Willard \(1973\)](#) predicted that local deviations from the population sex ratio should also occur in direct relation to the quantity of resources, such as food, available to parents ([Trivers & Willard 1973](#); [Frank 1990](#)). These effects are expected in species where one sex has higher variance in reproductive success than the other, where the reproductive success of individuals is a function of the quantity of resources they received from their parents, and where parents differ in the quantity of resources they provide their offspring. When parents have few resources available, they should then bias their offspring's sex ratio towards the sex with lower variance in reproductive success, but when resource

levels are higher, they should bias it towards the sex with the higher variance. A variety of correlational evidence from birds is consistent with this idea (e.g. [Dijkstra et al. 1990](#); [Wiebe & Bortolotti 1992](#); [Appleby et al. 1997](#)). Moreover, in several species, sex-biased mortality of nestlings between hatching and fledging yields biased secondary sex ratios that are also consistent with the predictions of [Trivers & Willard](#) (e.g. [Blank & Nolan 1983](#); [Røskaft & Slagsvold 1985](#)). Whether such sex ratio biases at fledging simply reflect that one sex is more likely to die when food is scarce ([Clutton-Brock et al. 1985](#); [Weatherhead & Teather 1991](#)) or indeed are the outcome of direct parental manipulation is unclear. Thus, while previous studies have established the plausibility of [Trivers & Willard's](#) hypothesis in explaining deviant avian clutch sex ratios, the data are mainly correlational and the hypothesis has rarely been tested experimentally on birds. In this paper, I describe two experiments on captive zebra finches designed to test this hypothesis.

Wild zebra finches, *Taeniopygia guttata*, are small, nomadic, seed-eating passerines which inhabit the arid and semiarid zones of Australia and Indonesia ([Zann 1996](#)). They breed opportunistically in central Australia when there is sufficient food available ([Immelman 1965](#)), often rapidly mobilizing their reproductive systems in

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response to an ephemeral boom in grass seed production (Sossinka 1980). Mortality rates are extremely high, life expectancy at hatching is 51 days, so the birds are highly precocial, often breeding within 2–3 months of hatching (Zann 1996). Consequently, individuals have little time to sequester resources before attempting reproduction.

Zebra finches have been extensively studied in captivity, and several lines of evidence suggest that local deviations in offspring sex ratio, in relation to food availability during breeding, are likely to occur in this species. A systematic attempt to document the variance in male and female reproductive success in the wild across colonies encompassing the full demographic range of zebra finches would be extremely difficult (but see Zann 1996 for details of one colony), but a number of studies on captive birds have demonstrated that reproductive success is a function of the quantity of resources received in the nest from the parents, and hint that females may benefit more than males from additional resources.

For both sexes, growth rate as nestlings and fledging weight are strongly related to eventual adult weight (Boag 1987; Skagen 1988). Fecundity, attractiveness to mates and mortality all correlate with fledging weight, but the relationships differ between the sexes. Females typically plunder their body reserves of protein and lipid to manufacture eggs (Houston et al. 1995a, b) and their fecundity is strongly related to their weight at fledging (Haywood & Perrins 1992). As yet, nothing is known of the effect of male fledging weight on fecundity, although ejaculate quality is not related to phenotypic traits that are influenced by fledging weight (Birkhead & Fletcher 1995).

There is mutual mate choice in this species (Wynn & Price 1993) but female choice may be stronger than male choice (Zann 1996). Females choose males carefully through prolonged courtship rituals, soliciting copulations in relation to male beak colour (Burley & Coopersmith 1987) and song output (ten Cate & Mug 1984; Houtman 1992; Collins et al. 1994; K. Immelman, cited in Zann 1996). Beak brightness varies with environmental conditions experienced after fledging (Burley & Coopersmith 1987; de Kogel & Prijs 1996), while song rate is highly heritable (Houtman 1992) and is influenced by conditions during the nestling period (de Kogel & Prijs 1996). The relative importance of beak colour versus song rate in affecting male attractiveness is controversial (Collins & ten Cate 1996). Pair bonds are tight, and durable (Zann 1996), with males rarely gaining extrapair paternity in the wild (Birkhead et al. 1990). Males also select females with care, preferring those that are more fecund (Monaghan et al. 1996).

Finally, mortality rates are correlated with fledging weight. Females reared in experimentally enlarged broods leave the nest at lower weights and are more likely to die after independence than males reared in experimentally enlarged broods (de Kogel 1997). In short, the precocial, opportunistic life history of the zebra finch strongly suggests that the amount of resources received in the nest by offspring will have a profound impact on their reproductive success. Laboratory studies suggest that this effect may be more marked for females than for males.

A number of intriguing secondary sex ratio skews in zebra finches have been reported, both for wild and domesticated birds. Burley was the first to demonstrate that systematic deviations from unity could be generated in domesticated zebra finch secondary sex ratios. Using coloured leg rings to enhance or diminish individual attractiveness, whilst keeping food availability constant, she found that the secondary sex ratio was consistently biased towards the sex of the most attractive parent, whether male or female (Burley 1981, 1982, 1986). Subsequent work on wild populations suggests that secondary sex ratios may also be biased to match prevailing food availabilities (Burley et al. 1989), with a female-biased secondary sex ratio when food is abundant, although trends are not consistent between years (Zann 1996). Studies of domesticated and wild birds thus hint that zebra finches may be capable of biasing their primary sex ratios, perhaps preferring to produce daughters when food is abundant and sons when resources are more limited. I tested this hypothesis by manipulating the quantity of food available to captive breeding birds and monitoring the subsequent primary and secondary sex ratios, both between and within broods as a function of hatch order and egg number. I predicted that the primary and secondary sex ratios of broods should be female biased when food was abundant, but male biased when food was limited. Furthermore, since in captivity, first-hatched individuals attain heavier fledging weights (Boag 1987; Skagen 1988), I predicted that within broods females should tend to hatch first, and males last. By comparing the song output of brothers of low and high weights at independence, I also investigated the potential impact of parental resources on one component of male attractiveness. I predicted that within pairs of brothers, weight at independence should have a weak effect on song output, since this trait may be more heavily influenced by genetic quality (Houtman 1992).

## GENERAL METHODS

Zebra finches were housed and bred in captivity at the Sub-Department of Animal Behaviour at Madingley, Cambridgeshire, U.K., between March 1994 and August 1995. The breeding room was heated during winter to 15–20°C. The birds were kept on a 16:8 h light:dark regime. Further details are described in Kilner (1996). The colony was founded by previously unmated birds bought from five local members of the Zebra Finch Society. Adults were ringed with either two orange or two purple numbered leg bands, one on each leg. The sexes were kept in visual isolation, with two individuals of the same sex occupying each breeding cage, for 1 week before pairing. To control for any possible confounding effects of mate attractiveness on clutch sex ratios, I paired birds arbitrarily, the only criterion was that both individuals were not from the same breeder. The pairs bred in metal breeding cages (0.47 × 0.61 × 0.295 m) in visual isolation from other birds. The nestbox compartment hung on the side of the breeding cage, so that I could check nests with minimal disturbance to the adults.

## Food Availability

Adults were fed a 1:1 mixture of white and panicum millet and Nectarblend rearing mix, purchased from Haith's Seeds, Cleethorpes, U.K. Where food was available in ad libitum quantities, it was supplied in ceramic hamster bowls. In some treatments (described below) food was rationed using electronic hoppers, to reduce adult perception of food availability. Access to food was regulated via a door which required two pushes to open. Behind the door was 75% of the daily quantity of food supplied in the pottery bowls, which was still more than the zebra finches ever consumed. The hoppers were used to manipulate adult perception of food availability by making access to food more difficult, and by presenting adults with smaller, although still excess, quantities of food. In the week before pairing, I trained birds to use the hoppers by introducing them with doors held wide open with blu-tack. Every day I closed the door slightly by moving the position of the blu-tack, until at the end of the week, the birds were familiar with pushing the door to obtain food. I checked the hoppers at least twice per day to ensure they were working properly, and that they still contained seed. Water, grit and cuttlebone were supplied ad libitum in all treatments.

## Sex Ratio

I used two types of zebra finches, the 'normal' type which has grey plumage identical to wild birds, and the 'fawn' type which results from a sex-linked recessive mutation and generates brown rather than grey plumage (Birkhead et al. 1988). By pairing normal females with fawn males, I could sex all offspring at hatching. Sons were all phenotypically normal, and had dark skin, whereas daughters were phenotypically fawn with paler skin. I kept offspring until they reached sexual maturity and confirmed that sexes had been assigned correctly. After pairing, I checked boxes daily for the presence of eggs, which at some nests I numbered using a nontoxic, indelible pen. Thirteen days after the first egg was laid, I checked the nests every morning and evening for hatchlings. Where two chicks had hatched since the last inspection, hatch order could be assigned by the dampness of nestling down. Chicks were marked with coloured Tippex on one foot, renewed twice daily, until 8 days after hatching when they were identified with plastic leg rings. During the nestling period, until day 14, I inspected nests for any mortalities. Nestboxes were left undisturbed after this point until fledging to avoid premature departure of chicks from the nest. Nestlings fledged at 16–18 days. Offspring remained with their parents until 30 days after hatching, when they were weighed (to measure weight at independence) and removed from their parents' cage. The brood secondary sex ratio was measured at this point.

## Experiment 1: Food Availability and Brood Sex Ratios

I monitored the sex ratio of broods reared under two regimes of food availability. Twelve pairs of birds were

established on a food-restricted regime, in which food was rationed via the electronic hopper as described above. Each pair produced a brood and 10 of these pairs produced a second brood. Thirty days after the second brood had hatched, that is, on the day that these offspring were separated from their parents, I removed the electronic hoppers and supplied food in ad libitum quantities. Nine pairs subsequently produced a third brood. To control for the possibility that any effects of food availability on the clutch sex ratio might be confounded with the number of broods that the adults had previously reared, I allowed another group of 11 pairs of birds to rear their first brood with excess quantities of food available.

## Experiment 2: Weight at Independence and Male Song Output

I compared the song output of brothers from the same brood, but of differing weight at day 30. The difference in weight between brothers at this point was always at least 0.6 g, but the range in weights of males in each category overlapped ('heavy' males: 13.7–15.8 g; 'light' males: 13.1–14.4 g). I collected data using the following procedure. Test male and female zebra finches, which had never previously interacted, were housed separately by sex prior to the experiment. None of the males and females used in the experiment had bred, but all were at least 4 months old and had reached sexual maturity. The females were unrelated to the males. The experimental cage contained excess quantities of food and water and closely resembled the cages they had previously inhabited. At the start of each experiment, this cage was split in half by a removable wooden partition. I placed a female on one side of the cage and a male on the other. The side occupied by the female was alternated between experiments. The birds were left to settle for 30 min. I then removed the wooden partition so that the male was exposed to the female. I recorded all vocal output in the subsequent 30 min with a Sony tie-clip microphone, attached to the cage bars, and a Sony DATman tape recorder. I then reinserted the wooden partition to separate the male and the female. The male was removed, weighed, and replaced by his brother. I then repeated the procedure for the new male. The order in which 'heavy' and 'light' brothers were exposed to the female was alternated between experiments. The experiment was repeated for nine pairs of brothers with nine different females; five of these nine pairs of males were reared under conditions of restricted food.

Methodologies for measuring zebra finch song output vary between studies, but agree that it is the total amount of song produced that is of significance (ten Cate & Mug 1984; Houtman 1992; Collins et al. 1994). I assessed the total amount of song produced by counting (1) the number of songs produced by each male and (2) the total duration of all the songs, calculated as the sum of the duration of each song. Song structure is described in detail by Zann (1993). Each song was considered complete when followed by a silence lasting more than 1 s. I analysed tapes 'blind', using a Kay Sonograph machine, with no direct knowledge of the relative weights of the

brothers. Measures of song output by 'heavy' brothers were compared with those of 'light' brothers. I also assessed the effect of encountering the female first or second on song output.

### Statistics

I analysed data using parametric statistics unless the data were inappropriate for such analyses. In these cases I used nonparametric statistics (Siegel & Castellan 1988). All tests were two-tailed.

### Ethical Note

This study was designed to generate biases in the primary sex ratio and was not intended to affect chick health and survival. Throughout the experiment, I used a number of checks to ensure that food rationing with the hoppers did not threaten either adult or chick health and survival. Before the experiment, I decided to intervene if adults died, displayed symptoms of ill health or lost more than 20% more weight per breeding attempt than pairs rearing young with abundant food. None of these potential eventualities occurred. A previous study (Skagen 1988) had calculated the daily food consumption of zebra finches rearing young. By weighing seed each time it was changed, I calculated daily seed consumption by zebra finches in my experiments and it was always in excess of the quantities used by Skagen. To monitor the well-being of chicks reared when food was restricted, I weighed individuals on day 7 and compared their masses with those of offspring reared by zebra finches in Skagen's conditions of low food availability. Chicks reared in this study on low food availability were substantially heavier (this study:  $N=72$ ,  $\bar{X} \pm \text{SD}=6.39 \pm 1.26$  g; Skagen (1988):  $N=56$ ,  $4.03 \pm 1.11$  g). Finally, I decided before the experiment to intervene if chick mortality was substantially higher when food was rationed than when it was not, but again these circumstances never arose.

## RESULTS

### Experiment 1

#### *Effects on weight at independence*

To begin with, I compared seed consumption during the nestling period under the two regimes of food availability on days 1, 3, 6, 9 and 30 after hatching on day 0. Parents rearing first broods consumed more food when food was abundant ( $N=11$ ) than when it was restricted ( $N=12$ ; Mann-Whitney  $U$  tests: day 1:  $U=123$ ,  $P<0.001$ ; day 3:  $U=119$ ,  $P<0.01$ ; day 6:  $U=116$ ,  $P<0.01$ ; day 9:  $U=115$ ,  $P<0.01$ ; day 30:  $U=98$ ,  $P<0.05$ ). Next, I analysed the effects of hatch order and food availability on weight at independence, which is a known component of reproductive success. I examined the effect of hatch order (first or last in the brood), offspring sex and the food regime under which they were reared on offspring weight 30 days after hatching, using a factorial ANOVA, and restricting the analysis to first broods raised under each food

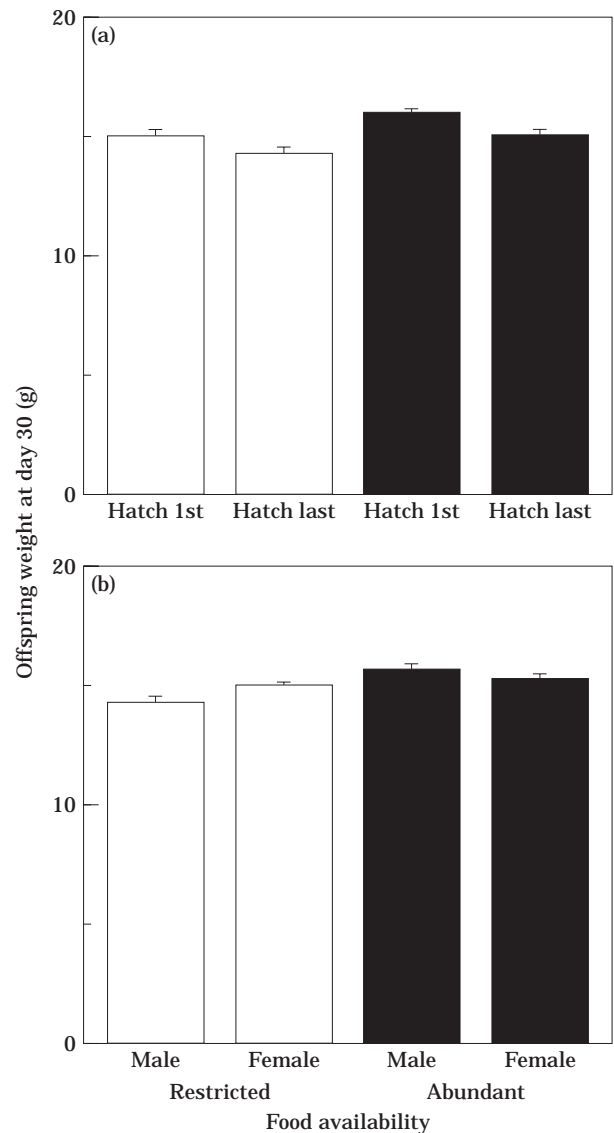


Figure 1. The effect of food availability to parents and (a) hatch order or (b) offspring sex on offspring weight at day 30.  $N=23$  broods, 46 chicks. Means and standard error bars are shown.

regime ( $N=23$  broods, 46 chicks). At 30 days after hatching, nestlings that hatched first were significantly heavier than those that hatched last ( $F_{1,38}=8.46$ ,  $P<0.01$ ; Fig. 1a) and those reared with abundant food were significantly heavier than those with restricted food ( $F_{1,38}=7.77$ ,  $P<0.01$ ; Fig. 1a). Offspring sex had no effect on weight at day 30 ( $F_{1,38}=0.002$ , NS). The only significant interaction term in the model was that between offspring sex and food regime ( $F_{1,38}=6.0$ ,  $P<0.05$ ; Fig. 1b). The difference in male weight between the two regimes of food availability was greater than the difference in equivalent female weight. With abundant food, males were heavier than females, but with restricted food females weighed more.

#### *Effects on clutch size and hatchability*

There was no evidence that the food availability treatments affected egg hatchability, or brood and clutch

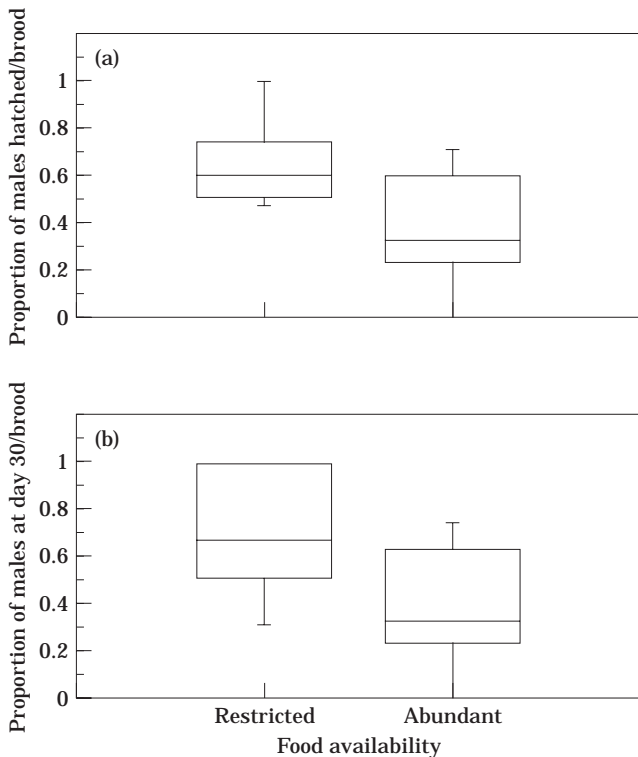


Figure 2. The effect of food availability to parents on (a) brood hatch sex ratio and (b) brood sex ratio 30 days after hatching.  $N=42$  broods from 23 pairs. Horizontal lines in the boxplots show 10th, 25th, 50th, 75th and 90th percentiles.

sizes. Of the eggs laid, 77.3% hatched (174 chicks from 225 eggs), which closely matches rates of hatching in wild colonies (reported as 83% in Zann 1996). Comparing 22 clutches in the restricted food treatment with 20 clutches in the abundant food treatment, egg hatchability was not influenced by food availability (Mann-Whitney  $U=264$ ,  $Z=-1.12$ , NS). Finally, neither clutch size (Mann-Whitney  $U=225$ ,  $Z=-0.13$ , NS), nor the number of young that hatched (Mann-Whitney  $U=265$ ,  $Z=-1.16$ , NS), was related to food availability.

#### Effects on primary and secondary sex ratios

Next, I analysed the effect of food availability on primary and secondary brood sex ratios, using Mann-Whitney  $U$  tests. When food was restricted, the primary sex ratio was higher (i.e. more male biased;  $\bar{X} \pm \text{SE} = 0.63 \pm 0.04$ ;  $N=22$ ) than when food was abundant ( $0.4 \pm 0.06$ ;  $N=20$ ;  $U=333$ ,  $Z=-2.87$ ,  $P<0.01$ ; Fig. 2a). The same was true of the secondary sex ratio (restricted food:  $0.67 \pm 0.06$ ; abundant food:  $0.39 \pm 0.06$ ;  $U=334$ ,  $Z=-2.89$ ,  $P<0.01$ ; Fig. 2b). This pattern of results held when the analysis was restricted to first broods in each food availability treatment. When food was restricted the primary sex ratio was higher than when food was abundant, although not significantly so ( $U=95.5$ ,  $Z=-1.85$ ,  $P<0.1$ ), as was the secondary sex ratio ( $U=102$ ,  $Z=-2.24$ ,  $P<0.05$ ).

I repeated the analysis, to investigate whether the same pairs of birds adjusted the primary and secondary sex

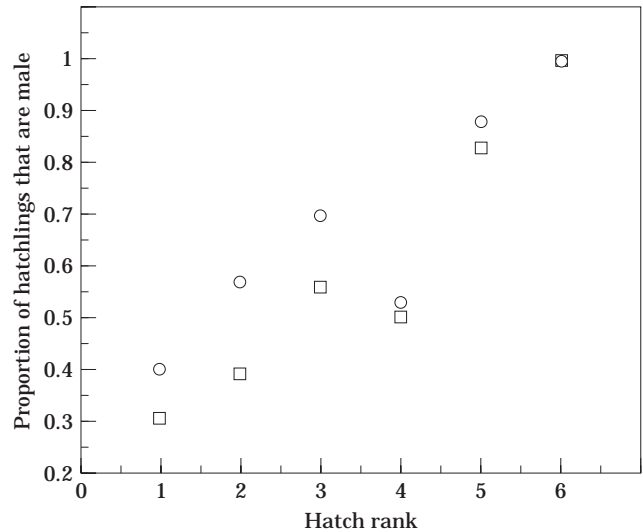


Figure 3. Sex ratio at hatching with respect to hatch order, for broods reared with abundant (□) or restricted (○) food. Data taken from 42 broods. Reading from left to right sample sizes (i.e. number of chicks) for each data point are as follows. Restricted food: 22, 21, 20, 15, 9, 4; abundant food: 20, 18, 16, 10, 6, 1.

ratios of their broods in relation to changes in food availability. Using a Friedman two-way ANOVA, I compared the sex ratios of three successive broods produced by the same parents ( $N=9$ ), where the first two broods had food restricted while the third had abundant food. The primary ( $\chi^2_2=6.06$ ,  $P<0.05$ ) and secondary ( $\chi^2_2=7.37$ ,  $P<0.05$ ) sex ratios were significantly lower (more female biased) when food was abundant than when it was restricted. To exclude the possibility that this effect was created by comparing successive broods of the same parents, rather than conditions of food availability alone, I compared the sex ratios of first broods ( $N=11$ ) and third broods ( $N=9$ ) of 20 pairs, all reared with abundant food. There was no difference in the sex ratios of these broods either at hatching ( $U=52.5$ ,  $Z=-0.23$ , NS) or at independence ( $U=50.5$ ,  $Z=-0.08$ , NS). I also compared the sex ratios of first broods ( $N=12$ ) and second broods ( $N=10$ ) of 12 pairs, reared with restricted food. Again I found no significant difference, either at hatching ( $U=80.5$ ,  $Z=-1.39$ , NS) or independence ( $U=67.5$ ,  $Z=-0.5$ , NS).

#### Relationship between hatch order and sex ratio

I examined the relationship between hatch rank (first hatched=1, second hatched=2, etc.) and sex ratio. First, I compared the median hatch rank of males and females using a Wilcoxon signed-ranks test, both for broods where food was restricted ( $N=22$  broods) and broods where food was abundant ( $N=20$  broods). Females hatched significantly earlier within the brood than males both when food was restricted ( $Z=-3.21$ ,  $P<0.01$ ) and when it was abundant ( $Z=-1.96$ ,  $P<0.05$ ; Fig. 3).

Next, I pooled the data from broods where food was restricted into one group, and the data from broods where food was abundant into another. For each of these two groups I calculated the proportion of young hatching first, second, third, etc. that were male. I analysed the

relationship between hatch rank and the proportion of males hatching using a Spearman rank correlation. The proportion of males hatching increased with increasing hatch order, almost to the point of significance when food was restricted ( $r_s=0.83$ ,  $N=6$ ,  $P<0.1$ ) and significantly when food was abundant ( $r_s=0.94$ ,  $N=6$ ,  $P<0.05$ ; Fig. 3). I compared the proportion of males hatching at each position in the two regimes of food availability using a Wilcoxon signed-ranks test. The proportion of males hatching at each rank was significantly lower when food was abundant ( $Z=-2.02$ ,  $N=6$ ,  $P<0.05$ ; Fig. 3).

I repeated these analyses, to investigate whether the same pairs of birds adjusted the proportion of males hatching at each rank in relation to changes in food availability. I used the data collected for the broods of nine parents where the first two broods were reared on restricted food while the third had abundant food. I pooled the data into three groups (first brood, second brood and third brood) and for each group calculated the proportion of males hatching at each rank. Using a Friedman two-way ANOVA, I compared the proportion of males hatching at each rank ( $N=6$ ) in each group: this proportion was significantly lower when food was abundant ( $\chi^2_2=6.0$ ,  $P<0.05$ ). Again, to exclude the possibility that this effect was created by comparing successive broods of the same parents, rather than conditions of food availability alone, I compared the proportions of males hatching at each rank in first broods with third broods all reared with abundant food. There was no difference between the two (Wilcoxon test:  $Z=-0.67$ ,  $N=6$ , NS). I also compared the proportion of males hatching at each rank in first broods reared with restricted and abundant food: again this proportion was lower when food was abundant, but the difference was not quite significant (Wilcoxon test:  $Z=-1.83$ ,  $N=6$ ,  $P<0.1$ ).

#### Relationship between egg number and sex ratio

I investigated whether the strong relationship between hatch order and sex ratio reflected the sequence in which eggs of each sex were produced. In total, data were collected on egg number and sex ratios for 16 of the 42 broods in the study (10 reared with abundant food, six with restricted food). In seven of these 16 samples, all the eggs in the clutch hatched. For all 16 broods, there was a strong positive relationship between laying order and hatch order (Spearman:  $r_s=0.83$ ,  $N=84$ ,  $P<0.001$ ). Female eggs were significantly more likely to be laid earlier within a clutch than male eggs (Mann-Whitney  $U=934.5$ ,  $Z=-2.29$ ,  $P<0.05$ ; Fig. 4), and the proportion of male eggs increased with egg number, although not significantly (Spearman:  $r_s=0.6$ ,  $N=7$ , NS; Fig. 4). These patterns persisted when the 10 broods reared with abundant food were considered in isolation. Within each clutch, female eggs were laid significantly sooner than male eggs (Mann-Whitney  $U=326.5$ ,  $Z=-2.03$ ,  $P<0.05$ ), and the proportion of male eggs increased with egg number, but again not significantly (Spearman:  $r_s=0.71$ ,  $N=6$ , NS). Equivalent trends were found for broods reared with restricted food but these were not statistically significant, probably as a result of the small sample size (Mann-Whitney  $U=155.5$ ,  $Z=-1.14$ , NS; Spearman:  $r_s=0.31$ ,

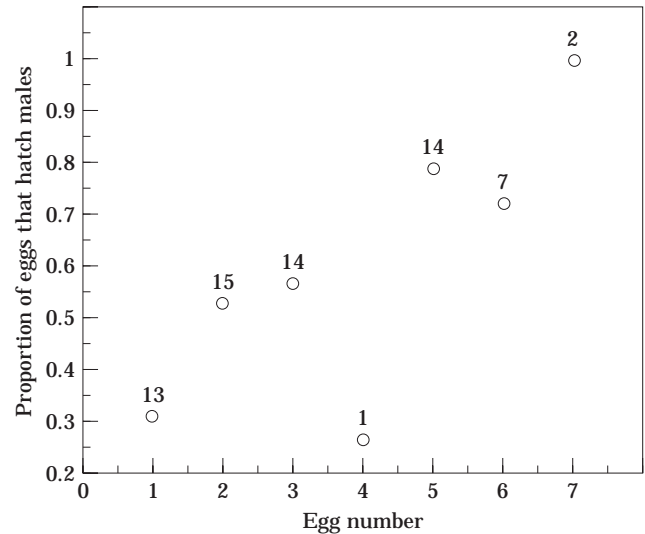


Figure 4. Sex ratio at hatching with respect to egg number. Numbers denote sample sizes for each egg number.

Table 1. Number of deaths, through brood reduction, of male and female chicks in relation to the food available to parents ( $N=17$ ); and timing of chick deaths, in relation to hatching, for broods reared with abundant or restricted food ( $N=14$ )

	Abundant food	Restricted food
Male	6	2
Female	1	8
Chicks dead before hatching complete	2	7
Last-hatched chicks dead	4	1

$N=6$ , NS). There was no relationship between egg number and the likelihood of it not hatching (Mann-Whitney  $U=1382$ ,  $Z=-0.32$ , NS).

#### Cases of brood reduction

Of the 174 chicks that hatched, 17 died before they were 8 days old. There was no indication that this was simply the result of restricted food: seven instances of brood reduction occurred when food was abundant and 10 when food was restricted ( $\chi^2_1=0.40$ , NS). Nor was there any indication of sex-biased mortality. Of the 17 chicks that died, nine were female and eight were male ( $\chi^2_1=0.23$ , NS). I looked for evidence of adaptive brood reduction by comparing the number of male and female chicks that died before fledging in the two food availability treatments. (The Fisher's exact test used assumed a conservative null hypothesis of equal proportions of male and female deaths in the two treatments.) Significantly more females than males died when food was restricted, and more males than females died when food was abundant (Fisher's exact test:  $P=0.015$ ; Table 1). In 15 broods only one chick died, but in one instance two females from the same brood died when food was restricted. Repeating the analysis but excluding data from this brood still yielded the same result; more males than females died when food

was abundant, while more females than males died when food was restricted (Fisher's exact test:  $P=0.0406$ ).

Why did these chicks die? One possibility is that parents manipulated the secondary sex ratio directly. Even if parents could not sex their young directly (but see [Burley 1986](#)), they might still penalize one sex selectively by using a simple rule of thumb based on the strong relationship between hatch order and sex ratio, and acting during hatching. Thus parents trying to starve males should target individuals that hatch last, whereas parents aiming to prune out females should penalize individuals that hatch sooner. I tested this hypothesis by examining the timings of chick deaths in relation to hatching, for broods reared under the two food availability regimes. I predicted that male-biased chick mortality should be the consequence of last-hatched chick deaths whereas more females would die if chick deaths occurred before all the eggs in the clutch had hatched. The mean hatching spread for zebra finch broods in this study  $\pm$  SE was  $70 \pm 5$  h. Of the 17 cases of brood reduction, 14 happened within 72 h of the first chick hatching. For these 14 cases, I compared the number of mortalities occurring before the last egg in the clutch had hatched with the number of deaths of the last-hatched chick, for broods reared with abundant and restricted food. Last-hatched chicks were significantly more likely to die when food was abundant, whereas more chicks died before the last egg in the clutch had hatched when food was restricted (Fisher's exact test:  $P=0.023$ ). These results may also account for the significant interaction between the effects of chick sex and food availability on offspring weight at day 30. Mean male weight at day 30 might have been higher when food was abundant because later hatched, lighter males died soon after hatching.

## Experiment 2

The pairs of brothers differed significantly both in their weight at independence (Wilcoxon:  $Z = -2.67$ ,  $N=9$ ,  $P<0.01$ ) and in their adult weight (Wilcoxon:  $Z = -2.67$ ,  $N=9$ ,  $P<0.01$ ). Adult weight was strongly influenced by weight at day 30 (Spearman:  $r_s=0.63$ ,  $N=18$ ,  $P<0.01$ ). However, there was no significant effect of weight at independence on my measures of male singing performance. There was no effect of weight at day 30 on either the number of songs produced (Wilcoxon:  $Z = -0.42$ ,  $N=9$ , NS; [Fig. 5](#)) or the total duration of the songs (Wilcoxon:  $Z = -1.007$ ,  $N=9$ , NS), when heavier and lighter brothers were compared. These data were not confounded by order effects. Males exposed to the female first did not produce more songs than males exposed second (Wilcoxon:  $Z = -0.28$ ,  $N=9$ , NS) nor was the total duration of their songs different (Wilcoxon:  $Z = -0.89$ ,  $N=9$ , NS).

Between-brood comparisons also showed no significant relationship between weight at independence and male singing performance, although strong positive trends were discernible ([Fig. 6](#)). When the data for all 18 males were pooled, there was no significant effect of adult weight on either the total duration of songs produced (Spearman:  $r_s=0.44$ ,  $N=18$ ,  $P<0.1$ ; [Fig. 6](#)), or the number of songs produced (Spearman:  $r_s=0.36$ ,  $N=18$ , NS).

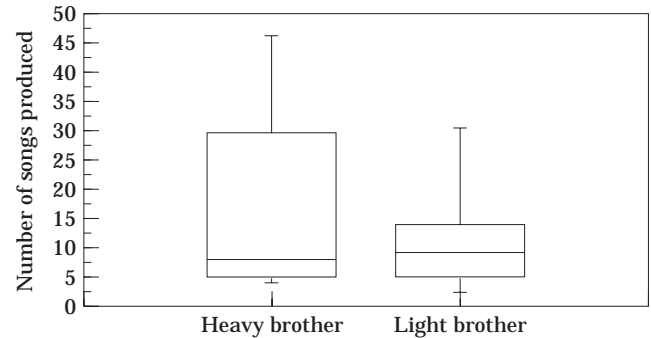


Figure 5. The influence of male weight, comparing nine pairs of brothers, on the number of songs produced in 30 min when each brother was introduced to a female. Horizontal lines in the boxplots show 10th, 25th, 50th, 75th and 90th percentiles.

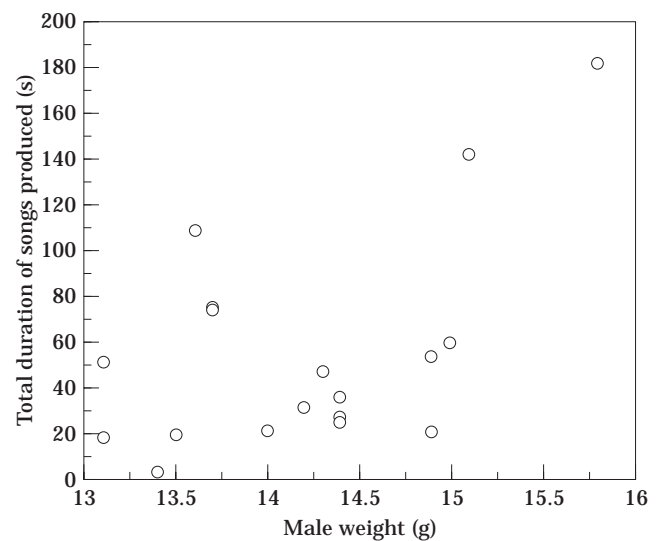


Figure 6. The influence of male weight on the total duration of song produced in 30 min when each male was introduced to a female ( $N=18$  males from nine broods).

## DISCUSSION

My results strongly support the predictions of [Trivers & Willard's \(1973\)](#) hypothesis, and are consistent with the unusual and condensed life history patterns of zebra finches summarized above. Three lines of evidence from this study indicate that zebra finches manipulate the primary and secondary sex ratios of their broods in relation to the resources offspring are likely to receive, to invest adaptively in their young. Within clutches, females were more likely to hatch earlier, from earlier-laid eggs, than later, from later-laid eggs, and hence fledged at a higher weight than if they had hatched last. Sex ratios at hatching were male biased when food was restricted, but female biased when food was abundant. Finally, selective brood reduction resulted in more female than male nestlings dying when food was restricted, but more male than female nestlings dying when food was abundant. While these sexual investment strategies seem adaptive, it is worth considering that the benefits of sex ratio manipulation may be different for wild birds. For

example, Zann (1996) pointed out that wild zebra finches hatch their young more synchronously than those in captive populations. The concomitant difficulties of assigning hatch order to nestlings in the wild mean that it is not known whether first-hatched individuals accrue more parental resources than their later hatched siblings. It is therefore not clear whether the practice of biasing the sex ratio with respect to hatch rank would be as beneficial in the wild as it appears to be in the laboratory. Even in captivity, it may be that the benefits of biasing sex ratio with respect to hatch rank differ between laboratory populations. For example, a different study on sex ratio variation within broods of captive zebra finches found the reverse pattern to that described here: males hatched sooner than females (Clotfelter 1996). A number of methodological differences in the two studies suggest two possibilities for the discrepancy in results.

The first is that zebra finches may have phenotypically plastic sexual investment strategies. In Clotfelter's study, pairs were bred together in an aviary (on an abundant food supply) which introduced a range of complexities not present in my study. There might thus have been sex ratio manipulation in relation to mate attractiveness (Burley 1986), or in anticipation of an extra-pair mating (see Sheldon & Ellegren 1996) or simply in relation to the success of individuals in competitive interactions for food (Ratcliffe & Boag 1987). Any of these factors acting either together or in isolation could have produced the overall male-biased sex ratio at fledging reported in Clotfelter's study and may also have been responsible for the difference in sex ratio with egg number. For example, females may produce more sons in first-laid eggs than daughters when paired with an attractive or high quality male (see Ellegren et al. 1996; Sheldon & Ellegren 1996; Svensson & Nilsson 1996), especially if nesting communally and if male attractiveness can be assessed only by comparison. This suggests a particularly interesting line for future work. Clearly female zebra finches can bias their brood sex ratios in relation to both food availability and mate attractiveness, but which has priority, and when?

Alternatively, close inspection of Clotfelter's data and mine may reveal that the differences are only superficial. A strange quirk of the sex ratio trends in relation to hatch order and egg number in my study was a dip in the sex ratio at hatch rank and egg number 4 (Figs 3 and 4). In fact, the sex ratio dipped so much at egg number 4 that more fourth-laid eggs tended to hatch females than first-laid eggs (Fig. 4). Despite this anomaly, females always had a lower median hatch rank than males in my study, because a high proportion of males hatched from fifth- and sixth-laid eggs. It is not clear from Clotfelter's analyses whether he found a dip in the sex ratio at egg number 4. In Clotfelter's study, brood sizes were smaller ( $\bar{X} \pm SD = 2.5 \pm 1.36$ ,  $N = 19$ ) than in my study ( $3.81 \pm 1.47$ ,  $N = 42$ ) so potentially any anomalous female-biased fourth egg could have had a greater impact on his comparisons of median hatch rank between the sexes.

Whatever the reason for the difference in results, Clotfelter's study and mine both illustrate that substantial deviations from parity occur within the egg-laying sequence in zebra finches. Trends in the sex ratio within

clutches are not uncommon (e.g. Howe 1976; Ryder 1983; Edmunds & Ankney 1987; Olsen & Cockburn 1991), which suggests a relatively simple underlying mechanism, although not one sufficiently simple to have been elucidated to date. Krackow (1995) speculated that the developmental pace of follicles after the first meiotic division may be linked to the sex chromosome they bear, which would result in nonrandom production of sons and daughters. However, if there really is phenotypic plasticity in the direction of the trend in the sex ratio with egg sequence, zebra finch mothers must intervene directly by somehow manipulating the outcome of the first meiotic division. Why the sex ratio should dip so dramatically at the fourth-laid egg remains a mystery both mechanistically and functionally. As a potential clue to the mechanism, it would be interesting to know if a dip in the sex ratio also occurred at eighth-laid eggs.

### Does Low Weight at Independence Affect Male Attractiveness?

The evidence that low weight at independence affects male attractiveness is mixed. Within broods, there was no evidence from experiment 2 that low weight at day 30 penalized males in their song output, but across broods there was a nonsignificant trend in this direction (Fig. 6). Previous work found that male weight at independence correlated with overall attractiveness to females (de Kogel & Prijs 1996), but suggested these effects could be transient since lighter males may simply take longer to reach the same level of attractiveness as heavier individuals (de Kogel 1997). The evidence so far suggests that male weight at independence may be correlated with male attractiveness in the short term, but in the longer term lighter males may accrue sufficient resources to 'catch up'. The cost to parents of producing sons that fledge at low weights may therefore vary with the likelihood of precocial breeding. Costs may be higher when the likelihood of sons breeding precocially is high, but lower when the chance of sons delaying reproduction is high, as happens at certain times of the year in Northern Victoria populations (Zann 1996).

### Selective Brood Reduction

Parents appeared selectively to abort investment in offspring of a particular sex within hours of hatching, thus honing their investment in each sex. Perhaps parents are incapable of producing perfect primary sex ratios and have to resort to more costly posthatching methods of investing adaptively in their surviving young. Certainly the anomalous female fourth-laid egg (Fig. 4) suggests that parents may be constrained in their ability to manipulate the clutch sex ratio. Alternatively, the apparently adaptive brood reduction described above may be an artefact of the two plumage morphs I used. Fawn nestlings (female) have paler mouth markings than normal nestlings (males) and previous studies have found that when food is limited, nestlings lacking mouth markings receive less food and grow more slowly than

nestmates with normal mouth markings; however, no such discrimination is seen when food is abundant (Immelman et al. 1977, cited in Zann 1996; Skagen 1988). While these studies may account for the higher number of female nestling deaths observed when food was restricted, they cannot explain why more male nestlings died when food was abundant. Furthermore, previous reports of brood reduction in zebra finches have hinted that it is a more sophisticated process than simple starvation of chicks in times of low food availability (cf. Lack 1947). For example, Skagen (1988) found that brood reduction was equally likely in two regimes of food availability, and Burley has observed parents removing live young from the nest (reported in Zann 1996). I found only one corpse of a missing chick, lying on the cage floor beneath the nest entrance. However, on three separate occasions, and at three different nests, I found a live chick on the cage floor under the nest hole. Assuming each time that the chick had been kicked out accidentally, I returned it to the nest. All three nestlings subsequently reached adulthood successfully. In retrospect, and particularly in the light of Burley's observations, it seems possible that these chicks might have been evicted deliberately. It would be interesting to identify the precise cues used by parents to practise brood reduction, and in particular to discover whether the ejection of live young is relatively commonplace. Burley (1986) suggested that parents might be capable of discriminating between sexes by using begging calls. In my study, however, the majority of deaths (14 of 17) happened within 72 h of hatching, before young had begun to vocalize during begging (Zann 1996). It seems more probable that in my colony parents used a simple rule of thumb, based on knowledge of the sex ratio in relation to hatch order, to target individuals of a particular sex. Even this strategy is not as simple as it first seems (and may be inappropriate in wild, synchronously hatched broods). What if sex ratio in relation to hatch sequence really is phenotypically plastic? How would the female know which sex had hatched first? Perhaps the female could be aware of her own phenotypic plasticity. Would she then have to be responsible for all brood reduction, with no action by the male? Further experiments are needed to address these issues.

In summary, zebra finches appear to be extraordinarily adept at manipulating their clutch and brood sex ratios. Perhaps their short reproductive life span, coupled with the unpredictability of breeding opportunities, has resulted in selection for accurate sexual investment strategies. Zebra finches may have only one breeding opportunity in their lifetime (Zann 1996), so the margin for error is very narrow. Previous studies on zebra finches have focused on the significance of mutual mate choice in affecting reproductive success and hence sexual investment strategies in relation to mate attractiveness (Burley 1981, 1982, 1986). My study supports Trivers & Willard's hypothesis by showing that food availability during reproduction also has a significant effect on clutch and brood sex ratios. Exactly how food availability and mate attractiveness interact to influence clutch and brood sex ratios remains to be discovered.

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