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Testes asymmetry, condition and sexual selection in birds: an experimental test

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The functional significance of the marked directional asymmetry in testes size observed in many bird species is obscure. Møller suggested that (i) the smaller of the two testes serves a compensatory role and increases in size (and hence reduces asymmetry) when the larger one is defective in some way, and (ii) as a consequence, the degree of directional asymmetry in testes size reflects male quality and covaries positively with the expression of secondary sexual traits. We conducted an experimental test of these two hypotheses in the zebra finch, *Taeniopygia guttata*. Neither hypothesis was supported. First, there was no significant relationship between the size of the left testis and relative testes asymmetry. Second, we obtained no support for the hypothesis that the degree of directional asymmetry in testes mass covaried with condition. On the contrary, directional asymmetry in testes mass was significantly greater in birds whose condition was experimentally reduced, compared with control birds. Moreover, we found no significant relationships between testes asymmetry and secondary sexual traits. We conclude that directional asymmetry in testes size does not reflect male condition in the zebra finch.

Keywords:

1. INTRODUCTION

In many species of bird the left testis is larger than the right (Lake 1981). Møller (1994) proposed a functional explanation for this pattern. First, he assumed that the difference in size of the left and right testis arose because the right testis increased in size to compensate for any reduction in function of the left. Second, he proposed that as a consequence, the degree of directional asymmetry reflected male quality: high-quality individuals would have more asymmetric testes. Møller (1994) supported his hypothesis by finding that the degree of testes asymmetry was positively correlated with the development of secondary sexual traits in the barn swallow, *Hirundo rustica*, and house sparrow, *Passer domesticus*. However, two subsequent studies failed to find a significant positive relationship between testes asymmetry and the extent of secondary sexual traits in either the sedge warbler, *Acrocephalus schoenobaenus* (Birkhead *et al.* 1997), or the jungle fowl, *Gallus gallus* (Kimball *et al.* 1997).

Møller (1994) states that: 'The difference in size of the left and right testis has usually been interpreted as evidence for mainly the left testis being functional with the right testis having a compensatory role, i.e. the right testis would increase in size if the left testis became non-functional (Lake 1984).' However, Lake (1981 *sic*; P. E. Lake, personal communication) does not state this. Notwithstanding, if this compensation hypothesis is correct then relative asymmetry in testis mass should be positively correlated with the mass of the left testis. There are two other possibilities: (i) if the mass of the right testis is a constant proportion of that of the left, no relationship

between relative testes asymmetry and left testis mass is predicted; and (ii) if the difference in absolute mass between the left and right testis is fixed, a negative relationship between testes asymmetry and left testis mass will exist.

The studies of testes asymmetry conducted so far have been observational and have relied on natural variation among individuals to test the hypothesis that the degree of directional asymmetry in testes mass covaries with condition in male birds. Because many secondary sexual traits are condition-dependent (Andersson 1994), an alternative approach is to experimentally manipulate male condition to establish whether a change in the expression of secondary sexual traits is accompanied by a change in testes size asymmetry. The aim of this study therefore was to manipulate body condition and determine its effect on testes size and two secondary sexual traits in the zebra finch, *Taeniopygia guttata*. We test two hypotheses: (i) that the right testis increases in size to compensate for reduced function of the left, and (ii) that the degree of directional asymmetry in testes mass covaries with male quality. We predicted that a reduction in condition would result in a reduction in the expression of secondary sexual traits and in testes mass, and, if Møller's hypothesis is correct, a decrease in testes asymmetry.

2. METHODS

(a) *Study species*

The study was conducted on domesticated zebra finches, from an outbred colony established at the University of Sheffield since 1985. The secondary sexual traits important in female choice are reasonably well established in the zebra finch. For both social and extra-pair partners, female zebra finches prefer males with a

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relatively high song rate, a redder beak and more symmetrical chest band plumage (Immelmann 1959; Sonneman & Sjolander 1977; Ten Cate 1984; Ten Cate & Mug 1984; Burley & Cooper-smith 1987; Ratcliffe & Boag 1987; Houtman 1992; Feil & Curio 1993; Collins *et al.* 1994, 1996; Swaddle & Cuthill 1994). Beak colour is heritable (Price & Burley 1993; Price 1996; cf. Houtman (1990) cited in Collins *et al.* (1994)), and androgen- and condition-dependent (Cynx & Nottebohm 1992; in addition, see Burley *et al.* (1992) and Zann (1994, 1996)). Zebra finch song rate may also be heritable as Houtman (1992) found significant heritability in song rate in a small ($n=6$ offspring) cross-fostering study. Song rate is androgen-dependent (Arnold 1975; Harding *et al.* 1983) and condition-dependent (Birkhead *et al.* 1998).

(b) *Manipulation of body condition*

We established two groups of birds, comprising 31 pairs of brothers, with one randomly chosen brother in each group. This paired experimental design minimizes any effects due to family differences (Birkhead *et al.* 1993). However, we are aware that by using just two aviaries the experimental design includes an element of pseudoreplication. We considered dividing birds between several different aviaries, but felt that even if these were in the same room or same building they may not have been considered to be independent. By using captive birds we were able to control for the stage of the male's breeding cycle, which is a potentially confounding variable. In the wild, zebra finches are opportunistic breeders and do not show the predictable seasonal patterns of testes enlargement and regression seen in most temperate species. Instead, many zebra finch populations appear to maintain their testes in a state of year-round readiness for breeding (Davies 1977; Vleck & Priedkalns 1985; Zann 1996).

The two groups of birds were housed in two adjacent aviaries (2 m × 2 m × 1 m high) on an 11:13 h light:dark cycle, in auditory but not visual contact with each other, and in visual and auditory contact with other male and female zebra finches. All birds were between 12 and 24 months of age. We manipulated condition in one group of birds by recreating a situation that resembled conditions in the wild. In the experimental group, condition was manipulated using a combination of two techniques employed previously with the zebra finch: (i) reduction in the rate of food intake and (ii) additional exercise. Using the first technique, Lemon (1991) reduced the rate at which zebra finches obtained food (but not the total amount eaten each day) by providing seed mixed with husks (in a ratio of 1:3 by mass) and found that these birds had lower breeding success and survival compared with control birds. Using the second technique, Deerenberg *et al.* (1997) showed that additional exercise reduced body condition in zebra finches. In the present study, birds were made to undertake additional exercise by encouraging them to fly from one end of the aviary to the other for a total 3.64 km day⁻¹ for five consecutive days each week for 11 weeks. Video analysis during the tenth week revealed that experimental birds flew a mean of 3.86 km day⁻¹ (3.64 km exercise + 0.22 km voluntary flight), about 4.5 times the distance flown by birds in the control group (0.85 km day⁻¹), which were allowed to exercise at will and had *ad libitum* food, water and grit. The distance flown by the experimental group each day was within the range that zebra finches in the wild would normally fly (R. Zann, personal communication). The treatment of the experimental birds was designed to avoid any bias associated with captive birds having *ad libitum* food and as a result these are the main focus of the present study. We predicted that the treatment of the experimental birds would

have two effects: (i) reduce condition overall relative to the control birds, and (ii) increase the variance in condition. Both of these effects occurred (Birkhead *et al.* 1998).

Birds were maintained under these regimes for 11 weeks after which we compared the body mass and capture order of the experimental and control birds. The mass of birds was measured using an Ohaus LS200 electronic balance (accurate to the nearest 0.1 g) at the start of the experiment and after 6 and 11 weeks. Body mass was recorded at the same time of day to control for diurnal variation (Metcalf & Ure 1995). Body mass did not differ significantly between the two groups of birds at the start of the experiment ($p=0.476$; Birkhead *et al.* 1998). Capture order was the order in which an assistant, unaware of the experiment's objectives, was able to capture the birds from the aviary using a hand net. For the experimental birds (but not the control birds), the order in which birds were captured on two occasions 5 weeks apart was significantly repeatable (39%, $p<0.02$). This is probably a better index of condition than body mass, or the change in body mass over the study, as it probably most accurately reflects the ability of an individual to avoid a predator (Birkhead *et al.* 1998). We also measured two secondary sexual traits, beak colour and song rate, as in our previous studies (Birkhead & Fletcher 1995). Briefly, beak colour was scored subjectively on a scale of 1 (light orange) to 6 (dark red) using Munsell colour chips (see Burley & Cooper-smith 1987). Song rate was scored by presenting each male with two, different, randomly selected females on consecutive days, behind a wire screen, and recording the mean amount of song in the first five minutes (Birkhead & Fletcher 1995).

(c) *Testes asymmetry*

At the end of the study the birds were humanely killed by cervical dislocation and were dissected and the testes removed and measured. The mass of the left and right testes was recorded to the nearest milligram on a Mettler AE160 electronic balance. We measured testes asymmetry in two ways: (i) absolute asymmetry, the mass of the larger left (L) testis minus the mass of the right (R); and (ii) relative asymmetry, the difference in testis mass/0.5(L + R testes mass) (see Møller & Swaddle 1997, p. 24). We have shown in a previous study that directional asymmetry in testes size occurs in the zebra finch, with the left testis being larger than the right (Birkhead *et al.* 1993).

Using data from the experimental birds, we tested the hypothesis that the mass of the smaller (right) testis increases in size to compensate for some reduction in function in the left.

To test the hypothesis that the degree of testes asymmetry covaries with male condition our experimental design allowed us to make comparisons between and within the two groups of birds. Møller's (1994) hypothesis predicts that: (i) in a pair-wise comparison between groups, experimental birds will have less asymmetric testes, after controlling for size, than the control birds; and (ii) within the experimental group, significant positive relationships will exist between the expression of secondary sexual traits and the degree of testes asymmetry.

3. RESULTS

(a) *A compensatory role for the right testis?*

In both the control and the experimental groups of birds the left testis was larger than the right (table 1). There was no significant, positive relationship between the mass of the left testis and relative testes asymmetry in the experimental group ($r=0.264$, 29 d.f., $p>0.1$)

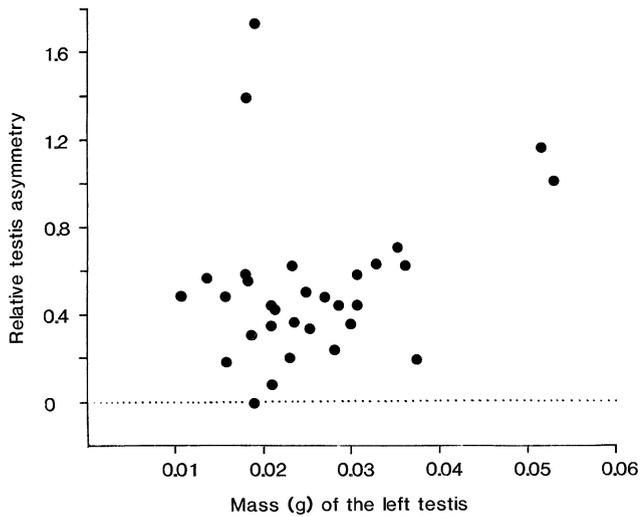


Figure 1. Relationship between the mass of the left testis and relative testes asymmetry in the zebra finch. The relationship is not significant ($r=0.264$, 29 d.f., $p>0.1$; $y=16.33x - 0.10$).

(figure 1). The lack of a significant relationship is consistent with the hypothesis that the mass of the right testis is a constant proportion of the left testis. There was therefore no evidence that the right testis serves a compensatory role.

(b) Comparison between groups

The mass of the experimental birds was significantly less ($p<0.02$) than that of the control birds by the end of the experiment (Birkhead *et al.* 1998). The experimental birds also had a significantly lower mean testes mass than control birds (table 1). Although there was no difference between the two groups in absolute testes asymmetry (table 1), after taking the reduced body mass of the experimental birds into account, by expressing the difference in testes mass as a proportion of total testes mass, relative testes asymmetry was significantly lower in the experimental birds than in the control birds (table 1). This result was contrary to that predicted by Møller's (1994) hypothesis.

(c) Comparison within experimental group

Using capture order as a measure of condition (see Birkhead *et al.* (1998) for details), within the experimental

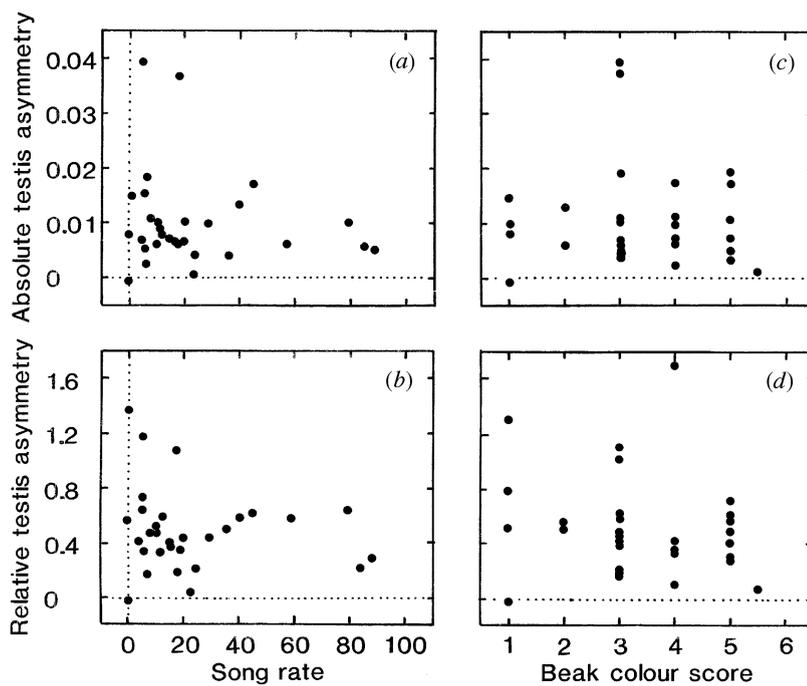


Figure 2. Relationships between the degree of expression of secondary sexual traits (beak colour and song rate) and testes asymmetry for 31 pairs of male zebra finches. (a) Song rate and absolute asymmetry ($r_s = -0.122$, $p>0.5$), (b) song rate and relative asymmetry ($r_s = -0.119$, $p>0.5$), (c) beak colour and absolute asymmetry ($r_s = 0.025$, $p>0.5$) and (d) beak colour and relative asymmetry ($r_s = -0.164$, $p>0.2$). None of these relationships is significant.

Table 1. Mean mass (g) \pm s.e. of testes and testes asymmetry in the zebra finch under control and experimental conditions

(All statistical comparisons are paired *t*-tests ($n=31$). Comparisons within groups: control left versus right, $t=6.91$, $p<0.0001$; experimental left versus right, $t=6.93$, $p<0.0001$.)

variable	control	experimental	<i>t</i>	<i>p</i>
left (L) testis mass	0.029 \pm 0.001	0.026 \pm 0.002	1.92	0.064
right (R) testis mass	0.020 \pm 0.001	0.015 \pm 0.001	4.92	<0.0001
total (L+R) testes mass	0.049 \pm 0.002	0.040 \pm 0.002	3.34	<0.003
absolute asymmetry	0.009 \pm 0.001	0.011 \pm 0.002	1.02	0.31
relative asymmetry	0.376 \pm 0.056	0.540 \pm 0.067	2.57	<0.02

birds there was no relationship between capture order and either absolute or relative testes asymmetry (absolute: $r_s=0.295$, $n=31$, $p=0.106$; relative: $r_s=0.006$, $n=31$, $p=0.979$). Nor was there any relationship between either of the two secondary sexual traits and testes asymmetry among the experimental birds (figure 2), despite the fact that both of these traits were correlated with the birds' condition, as measured by capture order (Birkhead *et al.* 1998). The lack of any relationships between condition or the expression of secondary sexual traits and testes asymmetry do not support Møller's (1994) hypothesis.

4. DISCUSSION

This study provides no evidence for either of Møller's (1994) hypotheses. There was no evidence that the right testis serves a compensatory role. Nor was there any evidence that directional asymmetry in testis mass reflects male quality. In fact we found evidence to the contrary: relative testes asymmetry was significantly greater in the group of birds in which condition was experimentally reduced and within this group testes asymmetry was independent of both secondary sexual traits.

In Møller's (1994) study there were significant relationships between testes asymmetry and secondary sexual traits, but for both species the r^2 values were relatively low (swallow: $r^2=0.12$, $p=0.021$, $n=44$; house sparrow: $r^2=0.09$, $p<0.001$, $n=175$). These results indicate that one reason why we might have failed to find an effect is that the power of our tests, with $n=31$ birds, was relatively low. However, the fact that the relationships in figure 2 are negative indicate that this is an unlikely explanation for our results. Moreover, in another independent study there was no significant relationship between absolute testes asymmetry and a secondary sexual trait (tail length) in the barn swallow ($r^2=0.067$, $p=0.269$, $n=20$) (T. R. Birkhead, A. P. Møller and N. Saino, unpublished data).

If Møller's (1994) hypothesis was correct another possible explanation for the increased testes asymmetry among the experimental birds in the present study is that the testes of these birds had regressed because conditions were unsuitable for breeding and the greater degree of testes asymmetry was merely a consequence of testicular regression. That is, for some unknown reason the left and right testes regressed at different rates. If that were the case we might expect the birds in the control group to show stronger relationships between testes asymmetry and secondary sexual traits. However, in the control birds all the relationships were negative, and whereas those with song rate were non-significant (absolute testes asymmetry, $r=-0.0038$, $p=0.841$; relative testes asymmetry, $r=-0.054$, $p=0.772$), those with beak colour were significant (absolute testes asymmetry, $r=-0.617$, $p<0.01$; relative testes asymmetry, $r=-0.505$, $p<0.01$). Thus in control birds, poorly developed beak colour was associated with a greater degree of testes asymmetry; again, a pattern opposite of that predicted by Møller's hypothesis.

Overall, our results provide no support for the hypothesis that the degree of testes asymmetry covaries with male quality in the zebra finch. Two other studies also found no evidence for this hypothesis (Birkhead *et al.* 1997; Kimball *et al.* 1997). If Møller's (1994) novel hypothesis is incorrect,

what does explain the asymmetry in avian testes size? One non-adaptive explanation is that the asymmetry in the male reproductive system is a by-product of selection for asymmetry in the female reproductive tract in birds (Stanley & Witschi 1940). In the majority of birds only the left ovary and oviduct are functional (Stanley 1935; Gilbert 1979; Lake 1981), and this has been proposed to be an adaptation for flight (Witschi 1935). However, one could equally well argue that the asymmetry in the female reproductive tract is a consequence of selection for asymmetry on the male reproductive system. Moreover, given that in some bird species there is no asymmetry in testes size (Friedmann 1927), this non-adaptive explanation hardly seems adequate to explain those cases where asymmetry does exist. A comparative study is much needed to describe the patterns that exist and to generate new hypotheses.

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REFERENCES

- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Arnold, A. 1975 The effects of castration and androgen replacement on song, courtship and aggression in zebra finches (*Poephila guttata*). *J. Exp. Zool.* **191**, 309–326.
- Birkhead, T. R. & Fletcher, F. 1995 Male phenotype and ejaculate quality in the zebra finch *Taeniopygia guttata*. *Proc. R. Soc. Lond. B* **262**, 329–334.
- Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1998 Sexual selection in the zebra finch *Taeniopygia guttata*: the effect of condition on sex traits and immune capacity. *Behav. Ecol. Sociobiol.* (Submitted.)
- Birkhead, T. R., Pellatt, E. J. & Fletcher, F. 1993 Selection and utilization of spermatozoa in the reproductive tract of the female zebra finch *Taeniopygia guttata*. *J. Reprod. Fert.* **99**, 593–600.
- Birkhead, T. R., Buchanan, K. L., Devoogd, T., Pellatt, E. J., Szekely, T. & Catchpole, C. K. 1997 Song, sperm quality and testes asymmetry in the sedge warbler *Acrocephalus schoenobaenus*. *Anim. Behav.* **53**, 965–971.
- Burley, N. T., Price, D. K. & Zann, R. A. 1992 Bill color, reproduction and condition effects in wild and domesticated zebra finches. *Auk* **109**, 13–23.
- Burley, N. T. & Coopersmith, C. B. 1987 Bill color preferences of zebra finches. *Ethology* **76**, 133–151.
- Collins, S. A., Hubbard, C. & Houtman, A. M. 1994 Female mate choice in the zebra finch—the effect of male beak colour and song. *Behav. Ecol. Sociobiol.* **35**, 21–25.
- Cynx, J. & Nottebohm, F. 1992 Testosterone facilitates some conspecific song discrimination in castrated zebra finches (*Taeniopygia guttata*). *Proc. Natn. Acad. Sci. USA* **89**, 1376–1378.
- Davies, S. J. J. F. 1977 The timing of breeding by the zebra finch *Taeniopygia guttata* at Mileura, Western Australia. *Ibis* **119**, 369–372.
- Deerenberg, C., Apanius, V., Daan, S. & Bos, N. 1997 Reproductive effort decreases antibody responsiveness. *Proc. R. Soc. Lond. B* **264**, 1021–1029.

- Feil, B. & Curio, E. 1993 Phenotypic traits and female choice in Timor zebra finches. *XXIII International Ethological Conference*, 244.
- Friedmann, H. 1927 Testicular asymmetry and sex ratio in birds. *Biol. Bull. Woods Hole* **52**, 197–207.
- Gilbert, A. B. 1979 Female genital organs. In *Form and function in birds* (ed. A. S. King & J. McLelland), pp. 237–360. New York: Academic Press.
- Harding, C. F., Sheridan, K. & Walters, M. J. 1983 Hormonal specificity and activation of sexual behavior in male zebra finches. *Hormones Behav.* **17**, 111–133.
- Houtman, A. M. 1992 Female zebra finches choose extra-pair copulations with genetically attractive males. *Proc. R. Soc. Lond. B* **249**, 3–6.
- Immelmann, K. 1959 Experimentelle untersuchungen uber die biologische bedeutung artsspezifischer merkmale beim zebra-finken (*Taeniopygia guttata* Gould). *Zoologische Jahrbucher Abteilung fur Systematik Okologie und Geographie die Tiere* **86**, 437–592.
- Kimball, R. T., Ligon, J. D. & Merola-Zwartes, M. 1997 Testicular asymmetry and secondary sexual characters in red jungle-fowl. *Auk* **114**, 221–228.
- Lake, P. E. 1981 Male genital organs. In *Form and function in birds* (ed. A. S. King & J. McLelland), pp. 1–61. London: Academic Press.
- Lemon, W. C. 1991 Fitness consequences of foraging behaviour in the zebra finch. *Nature* **352**, 153–155.
- Metcalf, N. B. & Ure, S. E. 1995 Diurnal variation in flight performance and hence predation risk in small birds. *Proc. R. Soc. Lond. B* **261**, 395–400.
- Møller, A. P. 1994 Directional selection on directional asymmetry: testes size and secondary sexual characters in birds. *Proc. R. Soc. Lond. B* **258**, 147–151.
- Møller, A. P. & Swaddle, J. P. 1997 *Asymmetry, developmental stability, and evolution*. Oxford University Press.
- Price, D. K. 1996 Sexual selection, selection load and quantitative genetics of zebra finch bill colour. *Proc. R. Soc. Lond. B* **263**, 217–221.
- Price, D. K. & Burley, N. T. 1993 Constraints on the evolution of attractive traits: genetic (co)variance of zebra finch bill colour. *Heredity* **71**, 405–412.
- Ratcliffe, L. M. & Boag, P. T. 1987 Effects of colour bands on male competition and sexual attractiveness in zebra finches (*Poephila guttata*). *Can. J. Zool.* **65**, 333–338.
- Sonneman, P. & Sjolander, D. 1977 Effects of cross-fostering on the sexual behaviour of the female zebra finch, *Taeniopygia guttata*. *Z. Tierpsychol.* **45**, 337–348.
- Stanley, A. J. 1937 Sexual dimorphism in North American hawks. *J. Morph.* **61**, 321–349.
- Stanley, A. J. & Witschi, E. 1940 Germ cell migration in relation to asymmetry in the sex glands of hawks. *Anat. Rec.* **76**, 329–342.
- Swaddle, J. P. & Cuthill, I. C. 1994 Female zebra finches prefer males with symmetric chest plumage. *Proc. R. Soc. Lond. B* **258**, 267–271.
- Ten Cate, C. 1984 Behavioural differences between zebra finch and Bengalese finch (foster) parents raising zebra finch offspring. *Behaviour* **81**, 152–172.
- Ten Cate, C. & Mug, G. 1984 The development of mate choice in zebra finch females. *Behaviour* **90**, 125–150.
- Vleck, C. M. & Priedkalns, J. 1985 Reproduction in zebra finches: hormone levels and effect of dehydration. *Condor* **87**, 37–46.
- Witschi, E. 1935 The origin of asymmetry in the reproductive system of birds. *Am. J. Anat.* **56**, 119–114.
- Zann, R. A. 1994 Effects of band color on survivorship, body condition and reproductive effort of free-living Australian zebra finches. *Auk* **111**, 131–142.
- Zann, R. A. 1996 *The zebra finch: a synthesis of field and laboratory studies*. Oxford University Press.

