JOURNAL OF AVIAN BIOLOGY

Article

Barred buttonquail males outlive females

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Sex differences in lifespan can vary considerably across species. Variance in lifespan depends on the progression of the mortality rate with age. Males are usually thought to have a shorter lifespan than females, which can be explained by sexual selection acting on secondary sexual traits that affect longevity. Such a bias in mortality between the sexes is also an indicator of the adult sex ratio. While there is evidence for this relationship from species with traditional sex-roles, little is known about the sex difference in lifespan of species that exhibit a reversal of sex-roles. Here we investigated sex differences in longevity and hatchling sex ratio in a captive population of barred buttonquails *Turnix suscitator*, a sex-role reversed species with a classical polyandrous mating system. We found that males lived on average 1.7 times longer than females. Further, sex ratio at hatching did not divert significantly from parity. Our data suggest that in sex-role reversed species selective forces act on females leading to a shorter lifespan.

Keywords: buttonquail, lifespan, longevity, sex-role reversal, *Turnix suscitator*

Introduction

Lifespan in vertebrates can have a considerable variation not only between species but also within species, especially according to sex (Carey and Judge 2000, Austad 2011). To understand the evolutionary mechanisms of sex differences in longevity, different hypotheses have been put forward. Williams (1957) assumed that the sex that is less affected by extrinsic mortality will be the longer-lived, more slowly aging, sex. Intense intrasexual competition among males for access to mates, as found in many polygynous vertebrates, is thought to be associated with a greater adult mortality rate and a more rapid decline in reproductive probability in males than in females. Therefore, selection pressures favouring longevity are weaker in males, resulting in a more rapid senescence (Williams 1957). This hypothesis was supported by the findings from a comparative study of long-lived polygynous and monogamous mammal and bird species (Clutton-Brock and Isvaran 2007). Males of polygynous species were found to be generally shorter-lived than females and the magnitude of the sex difference in life expectancy was closely associated with the magnitude of sex differences in the duration of effective breeding (Clutton-Brock and Isvaran 2007). Further, the unguarded X hypothesis, proposed by Trivers (1985), suggests that the reduced sex chromosome in the heterogametic sex makes these organisms more likely to express deleterious mutations arising from the X

1

(in mammals) or Z (in birds) chromosome because in the homogametic sex, such mutations will be compensated for by the second copy of that chromosome. These deleterious mutations are thought to reduce longevity. Therefore, it was predicted that in all mammals, males are short-lived, whereas in all birds, females will be short-lived. A recent meta-analysis conducted across many animal taxa found support for this hypothesis (Xirocostas et al. 2020).

An excellent model to test evolutionary hypotheses represent sex-role reversed species. Among birds, the vast majority of species engage in social monogamy and biparental care, in a small number of species uniparental care has evolved. In most of these cases the females are the care takers while the males seek additional mates. However, in approximately 1% of all avian species, called classically polyandrous species, sex-roles are reversed; females compete over males and/or resources (Emlen and Oring 1977). Beyond that, they are often much larger in body size and more conspicuously coloured than males. The males, on the other hand, have the sole responsibility for all parental care, including nestbuilding and incubation (Emlen and Oring 1977, Andersson 2005). Williams's hypothesis predicts that in polyandrous species the high level of competition among females should result in a higher adult mortality rate and consequently, females should be shorter-lived than males. Our current study on a captive population of barred buttonquails *Turnix suscitator* is one of the first to tackle this topic (Oring et al. 1991, Eberhart-Phillips et al. 2018).

Sex-role reversal has been identified mainly in non-passerine birds, such as in jacanas, sandpipers, phalaropes (English et al. 2014), coucals *Centropus grillii* (Goymann et al. 2015) and buttonquails (Ridley 1978). It has been suggested for sex-role reversed species, that mate attraction and male offspring care are the result of a male bias in the sex ratio at hatching (Jenni 1974). However, in several such species, there is also a strong male bias in adult sex ratio (Liker et al. 2013), which is explained by a higher mortality of the female, e.g. by female-biased predation (Donald 2007) and recently, by a sex difference in juvenile survival (Eberhart-Phillips et al. 2018).

Here we investigated sex differences in lifespan and hatchling sex ratio in a captive population of barred buttonquails *Turnix suscitator*, a sex-role reversed species of the family Turnicidae (order Charadriiformes). The family of buttonquails comprises 16 precocial species (Debus 1996) with a polyandrous mating system, of which three species have been studied in detail, the barred buttonquail (Starck 1991), the Kurrichane buttonquail *T. sylvatica lepurana* (Mörs 1915, Hoesch 1960) and the yellow-legged buttonquail *T. tanki* (Hilfiker 1983). Barred buttonquails exhibit sequential polyandry with sex-role reversal and reversed sexual dimorphism in size and plumage colouration. Females are larger than males and have a black throat and breast patch, which is lacking in males. Moreover, while females engage in territory defence and mate attraction, the latter through producing a booming vocalization, males are responsible for parental care including incubation (Starck 1991, Debus 1996).

Material and methods

Animals

Barred buttonquails were either obtained from breeders or raised at the Max Planck Institute for Ornithology, Germany. Birds obtained from breeders were wearing a closed aluminium ring showing the year of hatch. The exact hatching date was provided by the breeder. Birds that originated from our institute hatched artificially and were hand-raised because breeding success in our captive population was low. Therefore, at regular time intervals, eggs were collected and stored at 14°C until placed together in an incubator (J. Hemel Brutgeräte, Verl-Kaunitz, Germany) at 37.3°C and 69% humidity to synchronize hatching. Juveniles hatched after an average incubation time of 14 days and were placed together in a container equipped with a heat lamp and a plush duck to provide comfort. Buttonquails are nidifugous birds, which, after hatching, are very active but do not feed themselves until about day 10 posthatching. Under natural conditions, they need to be fed by the father. Artificially hatched chicks readily accept food soon after hatching. Chicks were fed several times throughout the day with ant eggs and buffalo worms until they had learned to feed by themselves. Sexual maturity was reached at about three months of age (Starck 1991). For individual recognition birds received a closed numbered aluminum ring and a coloured plastic ring.

Adult birds were kept in pairs in boxes of $1.25 \times 0.8 \times 0.6$ m together in a single room ($4 \times 3 \times 2$ m). A light-dark cycle was adjusted at 13:11 (L/D) in summer and 11:13 (L/D) in winter, thus simulating the photoperiod of their natural environment. The boxes were covered with a protective polyamide net and equipped with artificial plants as shelter and a heated sand bath. Birds were allowed to breed by providing grass and hay as nesting material. Food (buffalo worms, seeds, insect, fat and egg food) and water was provided ad libitum. Health status of animals was checked regularly by the animal caretakers. The local veterinarian took fecal samples of the birds as well as swabs from cloaca and throat at regular intervals in order to evaluate their health status.

Data collection

Data on longevity were obtained between March 2008 and March 2021 from 22 birds, comprising 11 males and 11 females. All other birds within our captive population (n = 38) were part of experimental studies that either involved hormonal manipulation or sacrifice and therefore could not be used for the present study. The 22 birds comprised of 10 birds that were hand-raised (four males, six females) and 12 birds obtained from breeders (seven males, five females). Of the 11 males, three are still alive and were included with their current age in the analysis to increase the sample size. No more birds are alive that could be added to the study. All birds included in the analysis presumably died a natural death, i.e.

not through accident or a diagnosed disease. However, we cannot rule out that some birds died due to a disease because no post-mortem examination was performed.

Sex determination

Data on the hatching sex ratio were obtained in 2009 and 2010 by means of artificial incubation as described above. Sex determination was done for in total 84 individuals from 28 broods. These included 64 embryos that were part of another study. Tissue samples from embryos and hatchlings were stored in queen's lysis buffer until analysis. Sex was determined by means of DNA sexing using the P2/P8 primers from Griffiths et al. (1998).

Statistical analysis

Data for lifespan were analysed by means of a Mann–Whitney U-test. To test whether the sex ratio deviated significantly from parity we analysed the data using a general linear model using the R package lme4: model = glm (sex ~ 1/brood.id, family = 'binomial'). The response variable was sex (1 = male, 0 = female), the fixed effect was the intercept and brood ID was included as random factor. All tests are two-tailed with a significance level of α = 0.05.

Results

Lifespan of barred buttonquails

Males had a median lifespan of 79 months (= 6.6 years) and a mean of 82 months (= 6.8 years) with a maximum lifespan of 156 months (= 13 years). Females had a lifespan of 48 months (= 4 years, median and mean value) with a maximum of 83 months (= 6.9 years). Therefore, males lived on average 1.7 times longer than females (U = 19.5, p = 0.0078, Fig. 1). The age distribution at death in male and female barred buttonquails in terms of age classes is shown in Table 1. A power analysis using G*Power ver. 3.1.9.6 (Faul et al. 2009) revealed a statistical power of 0.808. Parameters can be found in the supplementary materials.

Hatchling sex ratio

Artificial incubation of in total 84 eggs resulted in a male to female sex ratio of 44:40. Thus, the proportion of males was 52.4%. The fixed effect intercept did not significantly differ from 0 (Estimate = 0.095, SE = 0.219, z = 0.436, p = 0.663). Thus, the sex ratio did not deviate significantly from parity.

Discussion

Our results show that female barred buttonquails have a shorter lifespan than males. These data are in support of the Williams' hypothesis, which predicts that the sex with the more intense sexual selection should be shorter-lived due to
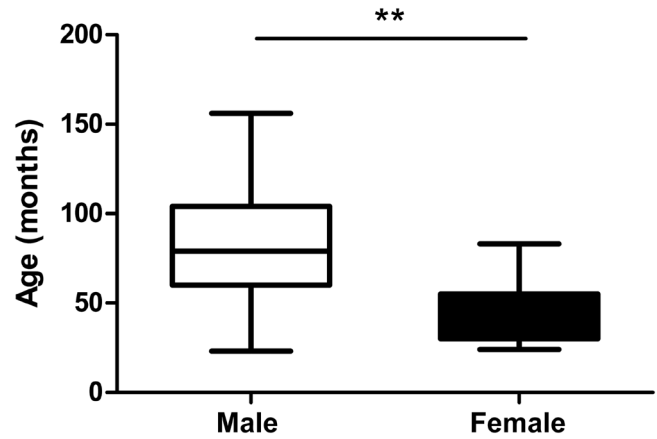

Figure 1. Male barred buttonquail lifespan is longer than that of females (n = 22, p = 0.0078). Bars represent medians with quartiles and range.

a higher mortality rate. Previously, the hypothesis was supported by comparative studies, which found that males are shorter-lived than females in polygynous but not in monogamous species (Clutton-Brock and Isvaran 2007, Tidière et al. 2015). Generally, it is thought that the degree of male bias in mortality rate covaries with the strength of sexual selection acting on males (Bonduriansky et al. 2008). Therefore, polygynous males are expected to experience a greater mortality rate due to intense male–male competition. However, in all of these species, sex roles are arranged 'traditionally'; i.e. males compete over females (Janicke et al. 2016). Our data, derived from studying the exceptional case, a species where the conventional sex roles are reversed, therefore, provide important evidence to support the link between sex differences in lifespan and the strength of sexual selection. In sex-role reversed species, females compete over males by using a similar behavioural repertoire as males in species with traditional sex-roles (Oring 1986). Female buttonquails perform territorial and courtship behaviours including booming, chasing and courtship feeding while males have the sole responsibility for all parental care, including nestbuilding

Table 1. Distribution of age classes at death in male (n = 11) and female (n = 11) barred buttonquails. Numbers in italics and brackets show the age of individuals that are still alive in our colony.

Age class (years)	Males	Females
1	1	
2		3
3		2
4		4
5	3 (1)	1
6	(1)	1
7	1	
8	2 (1)	
9		
10		
11		
12		
13	1	

and incubation (Trollope 1970, Debus 1996, Voigt 2016). Therefore, whereas males may adopt a life history strategy similar to females of species with traditional sex roles, intense female–female competition is likely to have an impact on adult female mortality rate.

Our results are in contrast with two previous studies on polyandrous snowy plovers *Charadrius nivosus* and spotted sandpipers *Actitis macularia*, which reported equal apparent survival or lifespan respectively between males and females. However, while we were able, in our captive population, to determine the exact age of all individuals, the results of the previous studies could potentially be confounded by dispersal (Oring et al. 1991, Eberhart-Phillips et al. 2018).

A remarkable fact is that Charadriiform species have a 1.5 times higher lifespan potential compared to other similar sized non-passerines, which has been attributed to a high level of antioxidants (Goede 1993). Originally proposed by Harman (1956), the oxidative stress hypothesis of ageing states that age-related physiological decline occurs due to the accumulating tissue damage caused by free radicals generated by the oxygen-related metabolism. Upregulation of antioxidants via the action of estrogens, known to have antioxidant properties, has been postulated as one mechanism to explain sex differences in longevity (Vina et al. 2005). Other hormones, including those from the reproductive system are thought to have a direct or indirect influence on longevity (Brown-Borg 2007). In barred buttonquails, secondary sexual characteristics such as the black throat patch have been found to be strongly positively related to circulating testosterone levels in females (Muck and Goymann 2011). Moreover, a recent neuroendocrine study suggests that females possess a higher neural sensitivity to androgens, i.e. a higher number of androgen receptors, than males in several hypothalamic brain regions involved in reproductive behaviours (Voigt 2016). Maintaining higher numbers of androgen receptors in those areas would allow females to bind more testosterone compared to males. Testosterone has been shown to increase oxidative stress in birds (Alonso-Alvarez et al. 2007). A possible mechanistic explanation for the observed sex difference in longevity could be derived from the results of a recent study on captive male red-legged partridges *Alectoris rufa*. Experimentally elevated testosterone levels in these birds led to reduced survival by making them prone to infection, possibly via a reduced resistance to oxidative stress (Alonso-Alvarez et al. 2020). However, further studies are needed to identify the role of steroid hormones in modulating lifespan of male and female buttonquails.

In our captive colony, hatchling sex ratio did not significantly deviate from parity, which is in line with Fisher's theory of equal investment (Fisher 1930). Similar results were obtained from sex-role reversed red-necked phalaropes *Phalaropus lobatus*, spotted sandpipers *Actitis macularia* and black coucals *Centropus grillii*; Andersson et al. 2003, English et al. 2014, Goymann et al. 2015). In the majority of sex-role reversed species, adult sex ratio is, however, male-biased (Liker et al. 2013). Our result of a shorter lifespan in females supports the view that higher female mortality

contributes to a male-biased adult sex ratio. Similarly, in polyandrous black coucals, Goymann et al. (2015) found in a field study in Tanzania an equal sex ratio at hatching, but a strongly male-biased adult sex ratio, indicating higher female mortality. Alternatively, sex-specific juvenile survival could lead to biased adult sex ratios, as was found in facultatively polyandrous snowy plovers *Charadrius nivosus* (Eberhart-Phillips et al. 2017). In our study, we found no evidence for a sex difference in juvenile survival.

Most information on the mating system of buttonquail comes from studies on captive birds, as in the wild, this species lives a rather secretive life (Debus 1996). Moreover, while some species of the family Turnicidae are threatened, the Andalusian buttonquail *Turnix sylvatica sylvatica* was recently declared extinct (source: <www.magornitho.org>). Therefore, data on longevity are important in order to understand their biology and to develop conservation programs.

Author contributions

Stefan Leitner: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing – original draft (equal). **Roswitha Brighton**: Conceptualization (supporting); Investigation (equal); Methodology (supporting). **Cornelia Voigt**: Data curation (equal); Methodology (equal); Resources (equal); Writing – original draft (equal).

Transparent Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/jav.02722>.

Data availability statement

Data are available in Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.pg4f4qrkp> (Leitner et al. 2021).

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