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Maternal inbreeding reduces parental care in the zebra finch, *Taeniopygia guttata*

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Increased embryo mortality is the most commonly cited cause of reduced fitness in inbred organisms. Reduced embryo survival may be the result of reduced parental expenditure by inbred individuals and here we tested the hypothesis that inbreeding results in impaired incubation behaviour in captive zebra finches. We compared incubation attentiveness of inbred female zebra finches (derived from full-sibling mating) with that of control females (derived from unrelated parents) and found a statistically significant inbreeding depression of 17% in incubation attentiveness. This shows that inbreeding can significantly influence parental behaviour. Despite a reduction in the amount of time inbred females spent incubating, their partners were able to compensate for the reduced incubation attentiveness. Incubation temperature also did not differ between inbred and control females. To test for the effect of incubation behaviour, we fostered eggs laid by control females to either inbred or control females at the end of laying. Eggs that were incubated by inbred females had an 8.5% lower hatching success than eggs incubated by control females and, although based on a relatively small sample and not statistically significant, the magnitude of the difference was consistent with differences in hatching success observed in the wild under relatively benign environmental conditions. Thus, under more challenging environmental conditions usually encountered in the wild, the reduced incubation attentiveness of inbred females could provide one proximate explanation for the consistent finding of decreased hatching success with increasing maternal inbreeding in birds.

Inbreeding depression is the deleterious effect on fitness resulting from mating between relatives. Under natural conditions inbreeding depression can be biologically significant and it is important to consider it in evolutionary and conservation biology (Crnokrak & Roff, 1999; Keller & Waller, 2002). Severe inbreeding can ultimately lead to the extinction of small isolated populations (Saccheri, Kuussaari, Kankare, Vikman, & Hanski, 1998). Inbreeding increases genetic homozygosity and inbreeding depression is thought to occur mainly because of the unmasking of rare deleterious alleles, although reduced heterozygous advantage may also contribute (Charlesworth & Willis, 2009). Inbreeding may affect fitness in two ways (Margulis, 1998; Mattey, Strutt, & Smiseth, 2013). First, a mating between relatives can affect traits expressed by the offspring and lead to a reduction in fitness of the inbred offspring themselves (offspring inbreeding). Second, in species in which offspring depend on parental care, fitness of outbred offspring can also be reduced by some behavioural or physiological deficits in inbred parents (parental or intergenerational inbreeding). Although there are some examples of intergenerational inbreeding in which inbreeding of parents can have a detrimental effect on offspring fitness in birds, mammals, fish and insects (Jimenez, Hughes, Alaks, Graham, & Lacy, 1994; Keller, 1998; Mattey et al., 2013; van Noordwijk & Scharloo, 1981; Richardson, Komdeur, & Burke, 2004; Slate, Kruuk, Marshall, Pemberton, & Clutton-Brock, 2000; Szulkin, Garant, McCleery, & Sheldon, 2007) many studies of inbreeding depression confound offspring deficiencies with deficiencies in parental behaviour and physiology (Margulis, 1998) and the intergenerational effects have so far received very little attention (Mattey et al., 2013, but see Margulis, 1998). Careful consideration of the way in which inbreeding influences reproductive behaviour and physiology is essential, however, to assess the impact of inbreeding on populations (Margulis, 1998; Mattey et al., 2013).

A consistently reported deleterious effect of parental inbreeding is the reduced survival of embryos of inbred females as observed in birds, mammals, fish and insects, even if mothers are paired with an unrelated mate such that offspring heterozygosity is not reduced (e.g. Cordero, Aparicio, & Veiga, 2004; Farkas et al., 2007; Keller,

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1998; Margulis & Altmann, 1997; Marr, Arcese, Hochachka, Reid, & Keller, 2006; Mattey et al., 2013; Moura, Polastre, & Wechsler, 2000; van Noordwijk & Scharloo, 1981; Pulkkinen, Van Der Lende, Groen, Kaal, & Zonderland, 1998; Sittmann, Abplanalp, & Fraser, 1966; Su, Liljedahl, & Gall, 1996). The underlying causes of maternally mediated intergenerational inbreeding effects on embryo viability have not been identified, but could include deficiencies in the behaviour and physiology of the parents that detrimentally influence the inbred parent's capacity to raise young. Effects of inbreeding on embryo viability have been studied particularly in birds in which reduced egg quality (caused by reduced provisioning of the egg), reduced parental care in supporting development or a combination of the two can contribute to the poorer hatching success of inbred parents (for references see above). Inbred female birds can produce smaller eggs than control females (Sewalem, Johansson, Wilhelmson, & Lillpers, 1999; Sittmann et al., 1966; Wetzel, Stewart, & Westneat, 2012) and offspring from smaller eggs can have a reduced fitness (reviewed in Kirst, 2011; Williams, 1994). Avian parents must also actively maintain favourable conditions for optimal embryo development (Webb, 1987) which can be expensive in terms of both energy and time (reviewed in Reid, Monaghan, & Nager, 2002; Tinbergen & Williams, 2002). Thus incubation expenditure can be influenced by the parent's energy balance, body condition and food availability (Bryan & Bryant, 1999; Eikenaar, Berg, & Komdeur, 2003; Gorman & Nager, 2003; Reid et al., 2002). As inbred individuals may be in poorer condition (Jimenez et al., 1994; Knaepkens et al., 2002) or have less energy available to spend on other than self-maintenance activities (Ketola & Kotiaho, 2009) compared with outbred individuals, their capacity to expend resources on incubation expenditure could be lower than in outbred individuals. Low parental incubation expenditure can result in slower embryo development, birth defects due to homozygosity of deleterious alleles, increased bacterial growth in the egg and even embryo mortality (Cook, Beissinger, Toranzos, & Arendt, 2005; Tinbergen & Williams, 2002; Webb, 1987). The effects of parental inbreeding on parental care such as incubation expenditure have, however, so far been largely neglected (Mattey et al., 2013; but see Margulis, 1998).

In this study, we explored the relationships between parental inbreeding, parental behaviour and offspring viability in zebra finch females in a captive population. We compared incubation behaviour and incubation success between inbred and control females. To separate the effects of parental and offspring inbreeding we cross-fostered eggs between pairs with inbred and control females. We predicted that inbred females paired to unrelated, outbred males would spend less time incubating eggs (lower incubation attentiveness) and/or maintain eggs at a lower temperature than in pairs with control females. Because in zebra finches both parents incubate (Zann & Rossetto, 1991), we also tested whether males compensated for any reduction in their inbred partner's incubation expenditure. To assess the consequences of being incubated by an inbred mother on embryo survival and development, we compared hatching success and hatching mass of offspring produced by control females and incubated by either inbred or control females.

METHODS

Animals and Housing

Zebra finches used in this study were from the stock population kept at the University of Glasgow. Nonbreeding birds were kept at a density of six birds per cage (0.4 × 1.2 m and 0.4 m high, made of metal with a mesh front). Birds had access to four perches, two high and two low, of which at least half were flexible and tapered. Cage floors were covered with absorbent paper and hemp core (Law et al., 2010). All birds received a basic diet consisting of ad libitum water, grit and mixed dry seed, supplemented with vitamins, calcium and fresh organic greens once per week (for details see Hill, Lindström, & Nager, 2011). The room temperature was held at 23 ± 1 °C, relative humidity at 30–50% and photoperiod at 12:12 h light:dark using full-spectrum daylight fluorescent tubes (Arcadia Bird Lamp FB36, Redhill, U.K.). Birds were provided opportunities to bath twice a week.

Breeding of Experimental Birds

In the winter 2008/2009 we bred inbred and control zebra finch females from our stock of several hundred individuals with known pedigree since 2006; this stock is regularly replenished with birds from other populations in order to maintain genetic diversity. Inbreeding in captive zebra finches has been shown to be low and similar to that found in many wild avian populations (Forstmeier, Segelbacher, Mueller, & Kempenaers, 2007). To obtain inbred birds, we paired brothers with sisters; control birds were obtained by pairing males and females from our stock population that did not share any grandparents and supplemented with 10 females from another breeding stock brought into the stock just prior to this experiment that were assumed to be unrelated to any stock bird. We created 21 pairs of unrelated birds and 19 brother–sister pairs. Pairs of unrelated and related mates were randomly assigned to breeding cages (0.4 × 0.6 m and 0.4 m high) within the same bird room and experienced the same housing conditions and diet as described above for the stock birds. Cardboard nestboxes were attached to the outside of the cage and coconut fibres were provided ad libitum for nest building. Pairs were allowed to produce offspring between December 2008 and May 2009 and raised up to three broods in this period; females used in the inbreeding experiment come from either a first, second or very rarely a third brood of their parents and this was considered as the factor 'brood number' in subsequent analyses. The proportions of offspring that were derived from first, second or third broods were similar between the two groups (Fisher's exact test: $P = 0.550$). The probability of producing viable offspring (at least one chick that survived to independence at 35 days) was similar between control and inbred pairs (16 of 21 and 17 of 19 pairs produced viable offspring, respectively; Fisher's exact test: $P = 0.412$). All control and inbred birds were thus bred at the same time and under identical conditions. When they became independent at around 5 weeks of age they were separated from their parents and housed in cages (0.4 × 1.2 m and 0.4 m high) in same-sex groups of six birds until they were used in the inbreeding experiment.

Inbreeding Experiment

The daughters of the brother–sister pairs then became the inbred females (inbreeding coefficient $f = 0.25$), while those from the unrelated pairs became the control birds ($f = 0$, assuming no inbreeding in the stock population). Because these experimental birds were produced over an extended period and we wanted them to breed at a similar age, the experimental breeding rounds were conducted at two time points (July 2009 and October 2009), hereafter referred to as the first and second replicate, respectively. Based on a median inbreeding depression of 12% for life history traits (DeRose & Roff, 1999) and the observed distribution of incubation attentiveness of females in our population (mean ± SD = 62 ± 9%, Gorman & Nager, 2003; Hill et al., 2011) we calculated an expected Cohen's effect size of 0.82 (Nakagawa & Cuthill, 2007). For this effect size a sample size of 57 individuals would give a statistical power of 85% at $P = 0.05$ and we therefore decided to pair up 16 inbred and 16 control females in each of the two replicate breeding rounds

(giving a total sample of 64 females) in order to balance statistical power with logistical and welfare considerations. Each female bred in only one of the replicate breeding rounds. The age at pairing did not differ between inbred (median = 6.4 months, interquartile range (IQR) = 6.2–6.7) and control females (median = 6.3 months, IQR = 6.1–6.8; Mann–Whitney test: $W = 230.0$, $N_{control} = 15$, $N_{inbred} = 14$, $P = 0.395$). However, females in the second replicate were half a month older (median = 6.8 months, IQR = 6.4–7.5) than in the first replicate (median = 6.3 months, IQR = 6.1–6.3; Mann–Whitney test: $W = 3.25$, $N_{first\ replicate} = 14$, $N_{second\ replicate} = 15$, $P < 0.01$). Each female was paired with an unrelated male from the stock with whom they did not share any parents and grandparents. At pairing each female was weighed to the nearest 0.1 g on an electronic balance and her tarsus length measured to the nearest 0.1 mm using callipers. All biometric measurements were taken by the same observer (E.P.). The median age of the males did not differ between inbred and control pairs (inbred group: median = 6.3 months, IQR = 6.2–27.6; control group: median = 6.3 months, IQR = 6.3–28.7; Mann–Whitney test: $W = 222$, $N_{control} = 15$, $N_{inbred} = 14$, $P = 0.113$). In each replicate, the first clutch of each pair was removed at clutch completion for another experiment not presented here (Pooley, 2013). Birds were then allowed to lay a replacement clutch which they then incubated. During laying, nests were checked every day and the order in which the egg was laid was marked on each freshly laid egg with a permanent marker. The two replicate breeding rounds were carried out at similar room temperatures (mean ± SE: first replicate: 24.1 ± 1.83 °C; second replicate: 23.2 ± 1.13 °C), but the effective photoperiod was likely to differ between the two replicates as, despite the presence of blackout curtains, natural light probably extended the hours of light in the first replicate.

Housing conditions and the basic diet were the same as for the breeding of the stock birds. However, breeding birds were also supplied with ca. 8 g of soaked seed scattered on the floor of the cage as well as ad libitum millet seed the birds had to pick from a spray hanging from the cage ceiling that could only be reached by flying (Law et al., 2010). The use of scatter feeding and hanging millet-sprays increased the birds' foraging effort (Law & Nager, n.d.) , which makes meeting the demands of incubation more challenging and makes it more likely to see differences between inbred and control birds that may exist in more challenging natural environments.

Incubation Attentiveness

We recorded the incubation behaviour using small infrared-sensitive cameras inside the nestbox connected to a screen where up to four nests were watched simultaneously (Hill et al., 2011). All observations were also stored digitally. We observed 15 control females (derived from 10 different families) and 14 inbred females (from nine different families) in mid-incubation when female incubation attentiveness is highest (Gorman & Nager, 2003) and reduced ability and/or willingness to incubate of inbred females is most likely to show an effect on their incubation attentiveness. Experimental manipulation of the body condition of female zebra finches affected their incubation behaviour in mid-incubation but not at other stages (Gorman & Nager, 2003) and the difference in incubation behaviour between the two groups affected their offspring's fecundity (Gorman & Nager, 2004). Two recordings of 120 min each were made. The first was done 5 ± 1 days and the second 7 ± 1 days after clutch completion, respectively; timing of observations did not differ between inbred and control pairs (Mann–Whitney tests: $W \geq 178$, $N_{control} = 15$, $N_{inbred} = 14$, $P \geq 0.154$). All incubation observations were made in the middle part of the day, starting around 1200 hours, and the time did not differ between inbred and control pairs in either the first or the second observation (Mann–Whitney tests: $W \geq 223$, $N_{control} = 15$, $N_{inbred} = 14$, $P \geq 0.585$).

Recording of behaviour commenced after 15 min of habituation to the camera, by which time at least one parent had always resumed incubation. We recorded which parent resumed incubation after the disturbance of placing the nest camera. Incubation attentiveness was recorded as the time spent on the eggs with birds recorded as incubating only if they were sitting on the eggs, not if they were merely present in the nestbox. We used instantaneous scan sampling at 1 min intervals to score whether the females incubated and calculated incubation attentiveness as the number of minutes spent incubating out of a possible 120 min. We validated this approach using data from a previous study in which incubation attentiveness was estimated from continuous observation records of 2–3 h (Gorman, Arnold, & Nager, 2005); these data included records from 12 zebra finch nests in which incubation attentiveness of individual females was 9.6–92.8%. By resampling the continuous records at 1 min intervals (Martin & Bateson, 2007), we found that attentiveness estimates calculated from scan samples every minute correlated highly with incubation attentiveness derived from continuous recordings ($r = 0.99$, $N = 12$, $P < 0.001$) and the incubation attentiveness calculated from the two methods did not differ (mean difference = 0.4 ± 0.35%; $t_{11} = 1.03$, $P = 0.323$). Female incubation attentiveness recorded for the same female over 120 min each on 2 different days was repeatable ($r = 0.52$, SE = 0.138, $N = 29$, $F_{28,29} = 3.21$, $P = 0.001$, repeatability calculated after Lessells and Boag (1987) and SE after Becker (1984)). For repeatabilities exceeding 0.5 a minimum sample of two per individual is sufficient (Dingemanse & Dochtermann, 2013), and thus the sampling design is effective in representing real incubation attentiveness. We also recorded the total incubation attentiveness of the pair, i.e. the proportion of time that the eggs were incubated by either parent. In addition the length and number of complete bouts of female incubation were recorded for each 2 h observation.

Incubation Temperature

Incubation temperatures were obtained for nine inbred (derived from seven different families) and 10 control females (derived from eight different families). We recorded incubation temperature as the temperature of one dummy egg following the same methodology as described in Gorman, Arnold, et al. (2005). For the measurement of incubation temperature, the dummy egg containing the thermistor replaced one egg of the clutch (which was placed in an incubator during the period of temperature recording) so that parents still incubated the correct number of eggs. The incubation temperature was recorded every 2 s for up to 45 min. While recording temperature, we observed female incubation attentiveness through the nest camera and only temperature recordings when the female incubated alone were included in the analyses. Once the female returned to the nest and resumed incubation, it took up to 15 min of incubation for the egg temperature to reach an asymptote, the steady-state incubation (determined by visual inspection of the graph of temperature against time). We then calculated a mean steady-state incubation temperature at the asymptote for 30 min or until the female ended her incubation bout but for at least 15 min of steady-state incubation. The recording duration of steady-state incubation temperature averaged 28.2 (±SD = 3.67) min ($N = 19$ females) and did not differ between inbred and control groups (Mann–Whitney test: $W = 99$, $N_{control} = 10$, $N_{inbred} = 9$, $P = 0.962$). At the end of recording, the dummy egg was removed and the real egg returned to the nest. One temperature measurement was made per female during the middle part of the day between 3 and 8 days after clutch completion and

the timing of this measurement did not differ between control and inbred females, with respect either to day of incubation (Mann–Whitney test: $W = 104.5$, $N_{control} = 10$, $N_{inbred} = 9$, $P = 0.744$) or to time of day ($W = 77.5$, $N_{control} = 10$, $N_{inbred} = 9$, $P = 0.327$). On the recording dates, mean ambient temperature was significantly higher in the first than the second replicate (first: 22.22 ± 0.44 (SD) °C; second: 21.25 ± 0.35 °C; $t_{17} = 5.33$, $P < 0.001$). Ambient temperature did not, however, differ between inbred (21.61 ± 0.74 °C) and control groups (21.80 ± 0.54 °C; $t_{17} = 0.64$, $P = 0.530$).

Hatching Success and Hatching Mass

We also investigated the consequences of parental incubation on embryo survival (reflected as hatching success) and growth (reflected as hatching mass). Both embryo survival and growth will depend on parental incubation performance as well as egg production (Deeming, 2002). Inbreeding has been shown to affect egg production in several species (Sewalem et al., 1999; Sittmann et al. 1966; Wetzel et al., 2012) and affected the size and composition of eggs in this experiment independent of the female's body size (Pooley, 2013). To allow us to separate the effects of parental incubation performance on embryo growth and survival from possible effects of parental inbreeding on egg production, we cross-fostered eggs among nests of control and inbred females so that all cross-fostered nests consisted of similar numbers of eggs laid by an inbred and a control female. This was achieved by fostering eggs among groups of four nests that were initiated within 2 days from each other, with two inbred and two control females per group (Fig. 1). Within these groups of four nests, half of the eggs in each nest were exchanged with eggs from clutches of the same inbreeding status (i.e. inbred–inbred or control–control) and half the eggs were exchanged with eggs from clutches of the opposite inbreeding status (i.e. inbred–control or control–inbred) at the start of incubation. The design ensured that no pair incubated any of their own eggs and always incubated the same number of eggs as they had laid.

We compared hatching success and hatching mass between fostered eggs laid by control females only (to standardize egg quality with respect to parental inbreeding) and incubated by pairs containing either an inbred or a control female. Eggs were labelled in the order they were laid (zebra finches lay one egg per day, Zann, 1996). Around hatching, nests were checked hourly during the day; chicks hatch several hours apart, which allowed us to identify which chick came from which egg. Chicks were weighed to the nearest 0.001 g within 12 h of hatching. Any eggs that were destroyed by the parents or failed to hatch after 18 days of incubation were removed from the nest. Since eggs were cross-fostered among nests at the start of incubation, any differences in hatching success and hatching mass between eggs incubated by inbred and control females should be caused by differences in the incubation environment provided by the foster pair. As the inbred female was mated with an unrelated male, the heterozygosity in the offspring should have been regenerated (Frankham, Ballou, & Briscoe, 2002) and any effects on hatching success and hatching mass should be due to inbreeding in the incubating female.

Ethical Note

Brothers and sisters were paired following approval by the U.K. Home Office (Project licence number PPL60/3849) in adherence to the legal requirements of the U.K. All the birds were monitored throughout the experiment by a Named Animal Care and Welfare Officer and a Veterinarian of the Biological Service of the University of Glasgow. Birds in the experiment were provided with foraging enrichment (scatter feeding and hanging millet spray, Law et al., 2010). All birds were kept beyond this experiment and took part in a subsequent breeding experiment up to 3 years of age.

Statistical Analysis

Of the 32 inbred and 32 control females that were paired up, 24 inbred and 26 control females produced a first clutch and, of these,

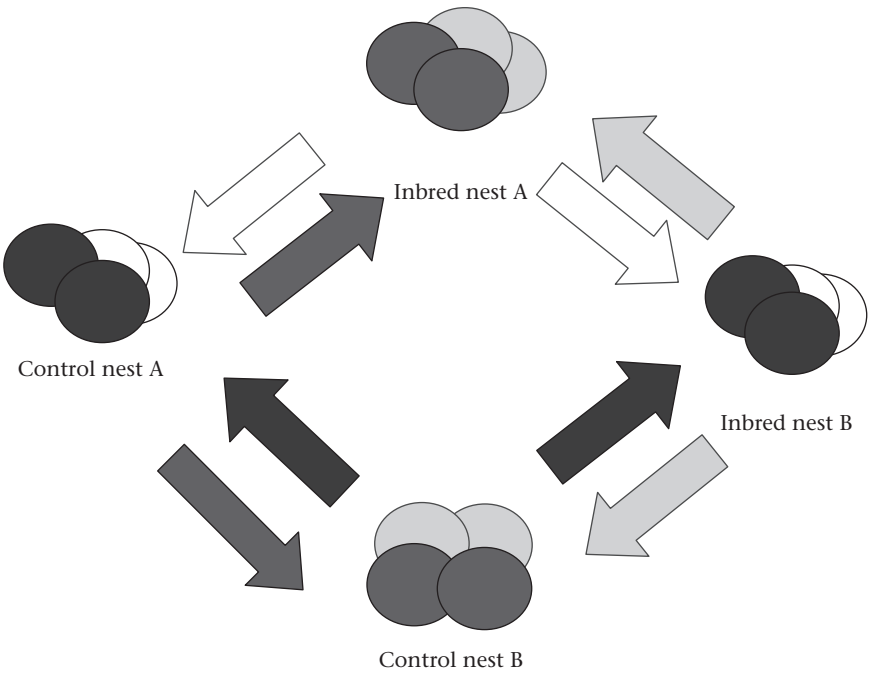

Figure 1. Schematic diagram of the cross-fostering design at the egg stage. □, ■: Eggs of inbred mothers; ■ and ■: eggs of control mothers. Eggs of each nest were divided among two nests of similar laying dates (i.e. within 2 days), one belonging to the same inbreeding status and one from the opposite inbreeding status group.

14 inbred females and 15 control females produced and incubated a second clutch. Neither the likelihood of producing a first clutch (chi-square test: $\chi^2_1 = 0.37$, $P = 0.543$) nor the likelihood of producing and incubating a second clutch (chi-square test: $\chi^2_1 = 0.06$, $P = 0.806$) differed between inbred and control females.

We calculated inbreeding depression in two ways. First, we calculated the coefficient of inbreeding depression (δ) as the percentage change in clutch size, incubation attentiveness and temperature, hatching success and mass between control and inbred females separately for each trait ($100\times((\text{trait}_{\text{inbred}} - \text{trait}_{\text{control}})/\text{trait}_{\text{control}})$). As a second method we also estimated inbreeding depression with Cohen's effect size *d*, calculated as the difference in trait value between inbred and control females divided by the pooled standard deviation of the samples. Effect size gives a standardized measure of inbreeding depression that can be more readily compared across different traits. Inbreeding depression was calculated between control birds (inbreeding coefficient assumed to be 0) and inbred birds from a brother–sister pair (inbreeding coefficient = 0.25) and thus comparable with data on inbreeding depression from other studies that conventionally estimate inbreeding depression as a change in phenotype over a change in inbreeding coefficient of 0.25 (e.g. DeRose & Roff, 1999).

Data were first checked for meeting the assumptions of parametric statistics, i.e. that residuals were normally distributed and showed homogeneity of variance. If assumptions were not met data were transformed in order to meet these assumptions. Female incubation attentiveness was transformed to the power of 1.5 (Hill et al., 2011). If this was not possible, alternative error distributions using generalized linear models (where data were over or under dispersed) or nonparametric tests were used. Contingency tables were analysed using chi-square tests where expected values were greater than 5 and Fisher's exact tests where expected values were less than 5 (Bailey, 1995).

The effect of maternal inbreeding on clutch size and incubation behaviour was analysed using a general linear mixed model (GLMM) including inbreeding status, replicate and the brood number the experimental birds were derived from as fixed factors and family of origin as a random factor since some families were represented by two or three sisters. Models were fitted in the nlme package of R2.11.1 (R Core Development Team, 2008). For incubation behaviour we also added clutch size and day of incubation as covariates. We included the female's clutch size as a covariate because larger clutches are energetically more expensive to incubate (Biebach, 1984; De Heij, Van der Graaf, Hafner, & Tinbergen, 2007). Incubation day (day 0 is the day of clutch completion) was included as a covariate since patterns of incubation attentiveness can vary over the incubation period in this species (Gorman & Nager, 2003). In the cases where we used two observations per female (female incubation attentiveness, bout length and number of bouts), we included female nested within family of origin as another random factor. There was no difference in female incubation attentiveness between birds derived from females from the Glasgow stock population and females added to the population (linear mixed model with female and family of origin as random factor, origin of parents: $t_{13} = 0.34$, $P = 0.739$) and this factor was therefore not included. Owing to the smaller sample sizes for incubation temperature (one record for each of 19 females), we first explored whether incubation temperature varied with replicate, brood number, clutch size and day of incubation using univariate general linear models (GLMs), with family of origin as a random factor. Incubation temperature was not associated with replicate, brood number and clutch size but it declined with increasing day of incubation (univariate GLMs; replicate: $t_{17} = 0.35$, $P = 0.731$; clutch size: $t_{17} = 0.54$, $P = 0.598$; brood number: $t_{17} = 1.84$, $P = 0.084$; incubation day: $t_{17} = 4.48$, $P < 0.001$). Hence when analysing for an effect of inbreeding on incubation temperature we only added day of incubation as covariate, and inbreeding status and brood number as fixed factors. It was not possible to transform total incubation attentiveness to meet the assumption of normality as data were highly right skewed with the majority (24/30) of pairs having 100% total incubation attentiveness. Therefore to analyse the difference in total incubation attentiveness between pairs with inbred and control females, total incubation attentiveness of a pair was pooled across all 4 h of observation (i.e. both of the 2 h observations) and compared between inbred and control pairs using a Mann–Whitney test.

We analysed hatching success and mass using mixed models including inbreeding status of the foster mother (all eggs were laid by control females) and replicate as fixed factors, clutch size and egg order as covariates and the identities of biological and foster mothers as crossed random factors. Egg order was included because hatching mass has been found to vary with egg order in zebra finches (Gorman, Orr, Adam, & Nager, 2005). Models were fitted in the lmer function in the package lme4 of R2.11.1 (R Core Development Team, 2008); in the case of hatching success we used a binary response variable for each individual egg (0 = unhatched, 1 = hatched). Brood number was not included in models of chick mass and survival as including all of the variables in this model led to false convergences when running the models; further analysis showed that inclusion of brood number caused false convergences in some of the models even in univariate analysis (i.e. when it was the only explanatory variable).

Because replicate (whether the experimental birds bred in the first round in summer or the second round in winter) and brood number (whether the experimental bird originated from a first, second or third brood) are correlated, as the majority of females (12/13) in the first replicate were from first broods whereas the majority of females in the second replicate (11/15) were from the second or third broods, there is potentially a multicollinearity between the replicate and brood number. We therefore tested the variance inflation factor (VIF) in any final models that included both replicate and brood number using the package car of R2.11.1 (R Core Development Team, 2008). In all cases VIF $\leq$ 1.94 whereas only VIF > 10 are regarded as a sign of severe collinearity (reviewed in O'Brien, 2007) and so collinearity is unlikely to be a problem in this study.

In all full models we included all possible two-way interactions. We then simplified full models using a backward stepwise elimination of nonsignificant variables starting with the least significant interaction term and then nonsignificant main effects. Tables show all main effects (*P* values of nonsignificant terms correspond to the last step they were included in the model) and only statistically significant interaction terms. All tests are two tailed and $P < 0.05$ is considered to be significant. Unless otherwise stated, reported values are mean ± SE.

RESULTS

There was no inbreeding depression in tarsus length ($t_{27} = 1.61$, $P = 0.118$) and body weight at pairing ($t_{27} = 1.23$, $P = 0.229$; Table 1). Body condition at pairing (body mass as response variable and tarsus length as a covariate) did not differ between inbred ($N = 14$) and control females ($N = 15$; GLMM: inbreeding status: $t_{25} = 0.62$, $P = 0.541$; brood number: $t_{26} = 1.21$, $P = 0.281$; replicate: $t_{24} = 0.42$, $P = 0.685$; tarsus length: $t_{27} = 2.34$, $P = 0.044$; all interactions: $P > 0.05$).

Clutch size showed no inbreeding depression (Table 1) and did not differ between inbred and control females (Table 2), but clutches were significantly larger in the second replicate (4.9 ± 0.25 eggs, $N = 15$) than in the first (3.9 ± 0.27 eggs, $N = 14$; Table 2).

Table 1
Summary of inbreeding depression effects

Trait	Inbred	Control	Inbreeding depression	
			δ (%)	Cohen's *d*
Tarsus length (mm)	14.2±0.20	14.6±0.15	−2.41	−0.54
Body weight (g)	16.7±0.53	17.7±0.61	−5.77	−0.47
Clutch size (eggs)	4.4±0.33	4.2±0.22	5.48	0.23
Female attentiveness (%)	54±5	65±4	−16.9	−0.64
Total attentiveness (%)	99.1±2	99.6±2	−0.50	−0.27
Female incubation temperature (°C)	36.42±0.24	36.03±0.29	1.08	0.47
Hatching success (%)	76±10	83±9	−8.52	−0.19
Hatching mass (g)	0.88±0.03	0.86±0.03	2.33	0.11

The table shows mean ± SE trait values of inbred females originating from a brother–sister pairing (inbreeding coefficient $f = 0.25$, $N = 14$) and control females originating from pairings of unrelated partners (inbreeding coefficient $f = 0$, $N = 15$). Inbreeding depression was calculated as the coefficient of inbreeding depression $\delta \times 100$ (%) and as Cohen's effect size *d*, calculated as the mean difference in trait value divided by the pooled standard deviation of the two groups (see Methods).

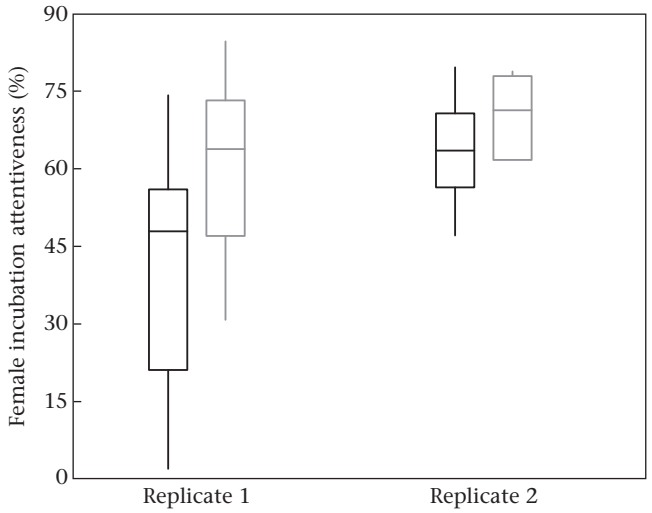

Figure 2. Incubation attentiveness in inbred (black boxes) and control (grey boxes) females in the first (six inbred and eight control females) and second replicates (eight inbred and seven control females) expressed as a percentage of total incubation time by pairs. Bottom and top hinges of the box plots represent first and third quartiles, respectively, the middle line represents the median and the ends of the whiskers represent the lowest and highest values still within 1.5 times the interquartile range of the lower and upper quartiles, respectively. There were no outliers beyond 1.5 times the interquartile range.

Inbred and control females were equally likely to return first to the nest and resume incubation (inbred females ($N = 14$): 28.6% first on both observations and 42.9% on one observation; control females ($N = 15$): 26.7% and 40%, respectively [including cases where both parents returned together, which happened in 10.3% ($N = 58$) of observations]; Fisher's exact test: $P = 0.947$). Inbred females had on average a 16.9% lower incubation attentiveness than control females (Table 1, Fig. 2), an effect that did not differ between replicate 1 (25.0% reduction of incubation attentiveness in inbred females) and replicate 2 (11.3%; inbreeding status * replicate interaction: $t = 1.43$, $P = 0.196$). Female incubation attentiveness was significantly higher in the second replicate than in the first (Table 2). Brood number was another significant factor (Table 2) with females originating from first broods having a higher incubation attentiveness than females originating from subsequent broods. After we controlled for the effects of replicate and brood number, the percentage decrease in incubation attentiveness due to inbreeding rose to 30.9% (Table 2). Inbred females did not have longer incubation bouts (34.9 ± 4.45 min, $N = 14$) than control females (30.4 ± 2.48 min, $N = 15$; GLMM: inbreeding status: $t = 0.05$, $P = 0.963$; brood number: $t = 0.03$, $P = 0.980$; replicate: $t = 0.28$, $P = 0.789$; clutch size: $t = 0.94$, $P = 0.370$; incubation day: $t = 1.86$, $P = 0.071$). Similarly, most females had two bouts per 2 h of observation and there was no statistically significant difference in number of incubation bouts in 2 h between inbred and control females (GLMM with Poisson distribution: inbreeding status: $z = 0.61$, $P = 0.541$; brood number: $z = 0.29$, $P = 0.771$; replicate: $z = 1.76$, $P = 0.079$; clutch size: $z < 0.01$, $P > 0.99$; incubation day: $z = 0.37$, $P = 0.714$). The total incubation attentiveness by both parents together was very high (Table 1) and did not differ between inbred and control pairs (Mann–Whitney test: $W = 180$, $N_{control} = 15$, $N_{inbred} = 14$, $P = 0.198$). Incubation temperature was similar in inbred and control females (Table 1) but was lower the later in the incubation period the measurement was taken (Table 2).

Hatching success of control eggs was 8.5% lower if they had been incubated by inbred mothers than by control mothers (Table 1), but this difference was not statistically significant (Table 3). Hatching success was also not associated with replicate, clutch size and relative egg order (Table 3). Hatching masses of offspring that hatched from eggs incubated by inbred and control females were similar (Table 1). While there was no effect of maternal inbreeding, replicate, clutch size and egg order affected hatching mass although in a complex pattern (Table 3). There was an interaction between replicate and egg order, with hatching mass increasing with egg order in the first replicate ($t = 2.24$, $N = 14$, $P = 0.031$) but not the second replicate. Hatching mass was also related to clutch size depending on replicate with no relationship between clutch size and hatching mass in the first replicate ($t = 0.04$, $N = 13$, $P = 0.559$),

Table 2
General linear mixed models of factors influencing reproductive traits

	Variable	*t*	*P*	σ^2 (%)	Estimate (SE)
Clutch size	Inbreeding	0.15	0.881		
	Replicate	*2.99*	*0.015*		*1.06 (0.36)*
	Brood number	0.24	0.815		
	Random effect				
	Family			1.7	
Female incubation attentiveness (%)	*Inbreeding*	*2.72*	*0.015*		*−30.9 (15.9)*
	Replicate	*3.32*	*0.011*		*41.1 (15.9)*
	Brood number	*2.30*	*0.050*		*−31.7 (18.2)*
	Day of incubation	0.57	0.571		
	Random effects				
	Female identity			9.0	
	Family			13.7	
Incubation temperature (°C/day)	Inbreeding	0.70	0.401		
	Day of incubation	*4.39*	*0.022*		*−0.43 (0.10)*
	Brood number	0.97	0.349		
	Random effect				
	Family			36.6	

General linear mixed models of clutch size, female incubation attentiveness (transformed to the power of 1.5) and female incubation temperature. Explanatory variables were maternal inbreeding, replicate (first and second), brood number (experimental female originated from a first versus subsequent broods) and day of incubation and clutch size as covariates for the two incubation traits. The table shows all main effects that were included in the full model and either eliminated if nonsignificant or retained in the final model if statistically significant (shown in italics). All interactions were statistically nonsignificant. Parameter estimates (and associated SEs) were derived from the final models and the estimates are relative to the control group for inbreeding, replicate 1 for replicate and first brood for brood number. In all models the identity of the parents from which the tested females derived (family of origin) was included as a random factor. In addition, in the model of female incubation attentiveness where there were two observations for each female, female identity was also added as a random factor nested within family (see Methods for further details).

Table 3
General linear mixed models of factors influencing embryo survival and growth

	Variable	*Z*/*t*	*P*	σ^2 (%)	Estimate (SE)
Hatching success	Inbreeding status	0.19	0.598		
	Replicate	0.04	0.972		
	Clutch size	0.60	0.548		
	Egg order	0.57	0.569		
	Random effects				
	Foster nest			46.9	
	Nest of origin			8.4	
Hatching mass	Inbreeding status	0.33	0.782		
	Replicate	0.93	0.663		0.27 (0.29)
	Clutch size	2.44	0.133		0.13 (0.05)
	Egg order	1.82	0.218		−0.14 (0.11)
	*Replicate * Clutch size*	*1.82*	*0.043*		*0.12 (0.06)*
	*Replicate * Egg order*	*2.42*	*0.013*		*0.32 (0.13)*
	Random effects				
	Foster nest			39.6	
	Nest of origin			44.8	

Generalized linear mixed model of hatching success (test statistic *Z*) and general linear mixed model of hatching mass (test statistic *t*) of offspring from eggs produced by pairs of unrelated birds and that were fostered to pairs with an inbred female and control pairs at the start of incubation. Explanatory variables were maternal inbreeding and replicate (first and second) as factors, and clutch size and egg order (relative to the number of eggs laid) as covariates. Identity of the biological mother ($N = 15$ different families) and identity of the foster mother ($N = 29$) were included as crossed random factors in both models. The table shows all main effects that were tested (significant variables in italics) and statistically significant ($P < 0.05$) interaction terms. *P* values for the hatching mass analysis were obtained by comparing models using the Anova function in R2.11.1. For 'replicate' the estimates are relative to replicate 1.

but hatching mass increased with clutch size in the second replicate ($t = 2.44$, $N = 12$, $P = 0.018$).

DISCUSSION

We found a significant inbreeding depression in the incubation attentiveness of captive female zebra finches that were derived from pairings between brothers and sisters compared with outbred control birds. This result provides a direct estimate of a considerable inbreeding depression in parental care behaviour. Despite the reduced incubation attentiveness of inbred females, total incubation attentiveness did not differ between pairs with inbred and control females. We suggest that this resulted from the male partners being able to fully compensate for their female's reduced incubation attentiveness. The temperature at which females maintained the eggs while incubating (steady-state incubation temperature) also did not differ between inbred and control females, suggesting that only the time available for incubation, but not ability to maintain egg temperature, was affected in inbred females. We found no statistically significant intergenerational effect of inbreeding in the female on embryo growth and survival in the benign captive environment, but, as we discuss later, it could be sufficient to result in significant fitness costs in the wild.

Inbreeding attentiveness also differed between the two experimental replicates and between brood numbers (whether the female hatched from a first, second or third brood). Females derived from second and third broods had lower incubation attentiveness than females derived from first broods, perhaps because of poorer rearing conditions provided by their parents that had already raised one or two previous broods (Burley, Price, & Zann, 1992), and poor rearing environment can have long-term consequences for the offspring's reproductive expenditure (Gorman & Nager, 2004; Naguib & Gil, 2005; Tschirren, Rutstein, Postma, Mariette, & Griffith, 2009). Incubation attentiveness of control females in the first replicate (64%) had been similar to that of females in unmanipulated pairs at mid-incubation, when we measured incubation behaviour, in earlier studies in our population (58% in Gorman & Nager (2003); 54% in Gorman, Arnold, et al. (2005); 66% in Hill et al. (2011)) and other captive populations of domesticated zebra finches (65% in El-Wailly (1966)). Gilby, Mainwaring, and Griffith (2013) have reported female incubation attentiveness of 64% in domesticated females during egg laying. In the second replicate (October) control females, however, had higher incubation attentiveness (71%). Similarly high female incubation attentiveness had been reported in some groups of birds (for example early laying females with attractive males: 74% in Gorman, Arnold, et al. (2005)). The reason for the higher female incubation attentiveness in replicate 2 is not known. Differences in food availability (Eikenaar et al., 2003), female body condition (Gorman & Nager, 2003), social environment (Gorman, Arnold, et al., 2005) and brood number (this study) can affect female incubation behaviour, and any of these factors could explain variation in female incubation attentiveness between studies and, although standardized as best as possible, between replicates within our study. In addition, females breeding in replicate 2 were older and bred in autumn rather than in summer. There is no information on how age affects incubation behaviour and the age difference is small (0.5 months). Although zebra finches are opportunistic breeders and are able to breed throughout the year (Zann, 1996), they still show some seasonal breeding patterns (Perfito, Zann, Bentley, & Hau, 2007). Even under apparently constant laboratory conditions, zebra finches can produce different clutch sizes in different seasons (Williamson, Gilbert, Rutstein, Pariser, & Graves, 2008). We found larger clutches in autumn (replicate 2) than in summer, which is the opposite pattern from Williamson et al. (2008). Our observations could indicate that incubation behaviour, like clutch size, varies between seasons or that the larger clutches in replicate 2 necessitated higher incubation attentiveness.

Although the effect of inbreeding on female incubation attentiveness appeared to be greater in the first than the second replicate, there was no statistically significant difference in the effects of inbreeding between replicates. Inbreeding depression is known to be greater in more stressful environmental conditions (Armbruster & Reed, 2005). Thus the higher female incubation attentiveness in replicate 2 may indicate less stressful conditions with the consequence of a possibly lower inbreeding depression in that replicate. Furthermore, the female contribution to incubation is the product of the behavioural interaction between male and female. The reduced incubation attentiveness of inbred females could therefore be a result of these females being less able or willing to invest in incubation and/or males responding to differences between inbred and control females and altering their incubation behaviour. The current data cannot distinguish between these possibilities and any differences in male response between replicates may also contribute to variation in female incubation attentiveness and inbreeding depression.

The precise level of inbreeding in our zebra finch population is not known, and if there is some background inbreeding, the true inbreeding coefficient of inbred females might be lower than $f = 0.25$ and higher than $f = 0$ for control females. Yet the background inbreeding levels of most captive zebra finches are small and similar to those in many wild avian populations (Forstmeier et al., 2007), so that the difference in inbreeding coefficient between treatments is likely to be close to 0.25. For incubation attentiveness, our study estimates a coefficient of inbreeding depression δ of 17% at full-sibling mating (although it may in fact be substantially higher as the GLM estimated a decrease of $30.9 \pm 15.9\%$ when statistically controlling for replicate and brood number). Our estimate of inbreeding depression for incubation attentiveness is comparable to the relatively high levels of inbreeding depression observed in life history traits (median $\delta = 11.8\%$) and higher than the inbreeding

depression in morphological traits (median $\delta = 2.2\%$) reported across 54 animal species by DeRose and Roff (1999). Life history traits are particularly susceptible to inbreeding depression as they are likely to be under strong selection, have a high ratio of dominance to additive variation and represent a wide mutational target owing to the large numbers of loci influencing such traits (Merilä & Sheldon, 1999). A study on the effects of inbreeding on parental care in oldfield mice, *Peromyscus polionotus*, found that the time parents spent in contact with their pups and building nests was reduced when the male was inbred, but not when the female was inbred (Margulis, 1998). In contrast, we found a reduction in incubation attentiveness when the female was inbred; all males were outbred in our study. The underlying mechanism of the effect of inbreeding on parental behaviour is, however, unclear. Inbred and control female zebra finches were not differentially affected by the disturbance caused by the nest camera as there was no difference between inbred and control females in the likelihood of resuming breeding after placement of a nest camera. The parental behaviour of inbred females may be more likely to be affected by physiological differences between inbred and control individuals. First, low incubation attentiveness in zebra finches can be associated with poor maternal condition (Gorman & Nager, 2003). Indeed, in female zebra finches inbreeding has been shown to be associated with reduced skeletal size and fat scores, a standard metric of clavicular and abdominal fat (Bolund, Martin, Kempenaers, & Forstmeier, 2010). In our population, however, at the start of breeding when birds were paired, inbred females were in similar body condition to control females. During a demanding activity, such as reproduction, body condition of inbred individuals may also deteriorate faster than in control individuals (Jimenez et al., 1994). We have no data on body mass of our birds during incubation and it would be interesting in future studies to investigate whether body mass dynamics during incubation vary with inbreeding status. Second, inbred individuals may have a higher resting metabolic rate (Ketola & Kotiaho, 2009). If so, and an inbred individual does not have a higher maximum metabolic rate (Ketola & Kotiaho, 2009), then inbred females will have less energy available for activities other than self-maintenance compared with control individuals. Indeed, we found that inbred female zebra finches in our population also had higher resting metabolic rates than control females (Pooley, 2013). Given that the length of incubation bouts of inbred females were not compromised in our study, we suggest that any physiological differences between inbred and control females will have most likely affected the rate at which inbred females replenished their body reserves in the recesses between incubation bouts and thus how quickly they returned to the nest to resume incubation, which then has implications for their partners.

If one parent reduces its parental expenditure, as did the inbred females in our study, then theoretical models of division of labour between parents predict that its partner should show partial compensation (reviewed in Houston, Székely, & McNamara (2005), Harrison, Barta, Cuthill, & Székely (2009)), with a consequent overall reduction in the total amount of parental care. In our study, the total amount of time that eggs were incubated by either the male or female parent (total attentiveness), however, did not differ between pairs with inbred and control females. This suggests that the males fully compensated for the reduction in their partner's incubation attentiveness. A few other studies have also demonstrated full compensation (Mrowka, 1982; Osorno & Székely, 2004; Sanz, Kranenbarg, & Tinbergen, 2000). Full compensation can occur if the risk of total breeding failure increases with a decline in parental care (Jones, Ruxton, & Monaghan, 2002). For zebra finches it has been shown that low total incubation attentiveness can negatively affect embryo viability (Gorman, Arnold, et al., 2005) and thus there would be selection for the partner to respond to reduced attentiveness by their partner. Whether full compensation in parental expenditure in response to an inbred partner's reduced parental care also occurs in any species under the more challenging conditions in the wild, compared with the relatively benign laboratory conditions examined here, remains to be demonstrated. If full compensation is absent in the wild, it is possible that the total parental expenditure may be reduced when one partner is inbred. On the other hand, the inbred parent may have to maintain a high parental expenditure if their partner does not compensate, in which case other aspects of parental expenditure may still be compromised owing to trade-offs between the different traits. Further observations in wild populations will be required.

We did not find any evidence that, in addition to reduced female incubation attentiveness, the quality of incubation was affected by inbreeding. There was no difference in the temperature at which inbred and control females maintained their eggs when incubating. Because we measured the temperature of dummy eggs, and there is no heat component from metabolically active embryos, this probably represents the temperature applied to the eggs by the incubating parent. Owing to logistical constraints, these data could only be obtained from a subsample of individuals, and thus the statistical power is low. However, the egg temperature was certainly not lower but, if anything, slightly higher in inbred than control females, and this might have resulted from the former's higher metabolic rate (Pooley, 2013). Since embryos contribute increasingly to their thermal environment as they develop (Turner, 2002), parents may reduce their heat output as incubation progresses in order to maintain a constant thermal environment of the embryo. That may be why incubation temperature declined with progressing incubation.

Several studies have shown an intergenerational effect of inbreeding in parents on hatching success in birds (Keller, 1998; van Noordwijk & Scharloo, 1981; Szulkin et al., 2007) and insects (e.g. burying beetles, *Nicrophorus vespilloides* in Mattey et al. (2013)). It had been suggested that inbred parents may invest less in parental care during early embryo development, either in egg formation or in incubation, than noninbred females (Richardson et al., 2004). The reduced female incubation attentiveness could represent a suboptimal incubation routine that can detrimentally affect fitness (reviewed in DuRant, Hopkins, Hepp, & Walters (2013)). Our experimental results of control eggs being incubated by inbred or control females showed no statistically significant effects of inbreeding status on hatching success and hatchling mass. Interestingly, the study on inbreeding in oldfield mice in captivity (Margulis, 1998) also found no effect of inbreeding in parents on offspring fitness. This was because only female parental care, which was the main contributor to total parental care, influenced offspring fitness, but maternal care was not compromised when the female was inbred. In zebra finches, females also did the majority of incubation and their incubation attentiveness was compromised when they were inbred. None the less, eggs incubated by inbred female zebra finches had an 8.5% lower, although not statistically significant, hatching success than eggs incubated by control mothers entirely due to the effect of maternal inbreeding as all eggs were from control females. Other studies have reported a coefficient of inbreeding depression for hatching success for mothers with $f = 0.25$ between 3 and 93% (median value = 17%, Keller, 1998; Marr et al., 2006; van Noordwijk & Scharloo, 1981; Sittmann et al., 1966), all of which combined the effects of embryo and maternal inbreeding. The statistical power of our test with a sample of 29 nests was too small to detect a median coefficient of inbreeding depression. Our observed difference, however, was larger than in a captive population of Japanese quails, *Coturnix japonica* (3%, Sittmann et al., 1966) and a wild population of song sparrows, *Melospiza melodia*, under benign conditions (3%, Marr et al., 2006,

years without rain). The lower inbreeding depression in hatching success found in captive populations and wild populations under benign conditions compared with wild populations under harsher conditions may be consistent with the idea that inbreeding depression is greater in more stressful environmental conditions (Armbruster & Reed, 2005). In our experimental study we only looked at the effect of maternal inbreeding on hatching success through differences in incubation attentiveness alone, but the effects observed in field studies are the result of cumulative deleterious effects of parental inbreeding on several life history stages. Parental inbreeding may also negatively affect the size of eggs (Pooley, 2013; Sewalem et al., 1999; Sittmann et al., 1966; Wetzel et al., 2012) and smaller eggs can have lower hatching success (Krist, 2011). Other aspects of egg quality may also be affected by inbreeding but have not received any attention. Estimates from descriptive studies of inbreeding depression in hatching success thus probably reflect a combination of inbreeding depression effects on incubation behaviour and egg formation. This might explain the lower inbreeding depression in hatching success in our study (8.5%, looking only at incubation behaviour) compared with the median inbreeding depression in hatching success reported above (17%), where the cumulative effects of maternal inbreeding on both egg formation and incubation behaviour would presumably have a greater effect on hatching success than one of these mechanisms acting alone. Hence the effects of maternal inbreeding are expected to be more substantial under the harsher conditions of the wild and when combined with the effect of maternal inbreeding on other reproductive traits. Moreover, offspring that experienced poor incubation conditions may also suffer from reduced reproductive output as adults (Gorman & Nager, 2004). Thus, an inbreeding depression of 8.5% is high compared with many other traits (DeRose & Roff, 1999) and may be biologically significant in combination with effects at other life history stages and in more challenging environments in the wild.

Hatching mass was influenced by interactive effects of replicate, egg order and clutch size. Zebra finch offspring size is expected to increase with egg order possibly to attenuate the effects of hatching asynchrony by making later hatched chicks better able to compete with their larger siblings (reviewed in Griffith & Buchanan (2010)). Hatching mass indeed increased with egg order but only in the first (summer) replicate and not in the second (autumn) replicate. Replicates 1 and 2 compared two different groups of experimental females, but were derived from the same pool of breeding birds and any differences between the two groups could explain the difference in the relationship between hatching mass and egg order between replicates. A similar difference in the relationship between egg order and hatching mass was found between seasons by Williamson et al. (2008) and thus our finding could be due to a seasonal change. The replicates also differed in clutch size, and in replicate 2, when clutches were larger, not increasing offspring size with egg order may reduce the effect of competition from the youngest chicks (reviewed in Griffith & Buchanan (2010)). Hatching mass was also positively associated with clutch size in the autumn but not in the summer and the reason for this is unknown.

We measured incubation behaviour only in mid-incubation and it is possible that female incubation attentiveness may not have differed between inbred and control females at other incubation stages. Previous work has shown that, in captive zebra finches, female incubation behaviour changes with incubation stage, but is consistent within individuals across incubation stages (Gorman & Nager, 2003). A difference in incubation behaviour in mid-incubation may mean that inbred females increased their incubation attentiveness with increasing embryo age more slowly than control females, as it did for females in poor condition compared with females in good condition (Gorman & Nager, 2003), which still leaves a difference in incubation behaviour at one particular incubation stage. We know very little about age-specific effects of unfavourable incubation conditions on the embryo (Webb, 1987), and whether differences in incubation behaviour at one particular stage affect the incubation outcome. The potentially best overall measure of the quality of incubation is hatching success, and the fact that hatching success did not appear to differ between treatment groups might indicate that overall incubation quality did not differ with inbreeding status. However, a difference in incubation behaviour only in mid-incubation between zebra finch females in good and poor condition, although not affecting hatching success (Gorman & Nager, 2003), did affect offspring fecundity (Gorman & Nager, 2004). Differences in incubation behaviour during laying and early incubation can also lead to differences in hatching asynchrony (Gilby et al., 2013), which could possibly affect the outcome of the breeding event. Future studies should look at additional incubation stages and also consider fitness measures beyond hatching success.

In conclusion, this study shows an inbreeding depression in the level of parental care in incubating zebra finches. Inbred females had lower incubation attentiveness than control females at the stage of incubation when their incubation expenditure is highest (Gorman & Nager, 2003), but this was fully compensated by an increase in male incubation attentiveness. The exact mechanism underlying the difference in female incubation attentiveness between control and inbred females is unknown. Differences in incubation strategies between control and inbred individuals can possibly have fitness consequences. We found no effect on hatching success. Previous studies that showed higher embryo mortality in inbred parents (e.g. Cordero et al., 2004; Farkas et al., 2007; Margulis & Altmann, 1997; Marr et al., 2006; Mattey et al., 2013; Moura et al., 2000; Keller, 1998; van Noordwijk & Scharloo, 1981; Pulkkinen et al., 1998; Sittmann et al., 1966; Su et al., 1996) have confounded the effects of several aspects of parental expenditure such as egg formation and offspring provisioning that may also detrimentally influence fitness of inbred mothers, but these traits remain to be fully investigated. As the reduced incubation attentiveness by females is completely compensated by an increase in male incubation effort, it is also possible that the male's increased incubation effort is traded off against their reproductive effort later in the same breeding attempt and/or in subsequent breeding attempts compromising the future reproductive success of the partnership. Thus if even small inbreeding depressions apply to other stages of parental care (egg formation, offspring provisioning, male contribution to subsequent stages), the resulting cumulative deleterious effects of parental inbreeding across all stages of parental care could be substantial (Frankel & Soulé, 1981; Frankham et al., 2002) and may contribute to a reduction in fitness of inbred mothers; inbreeding depression in parental care traits thus needs to be considered in understanding inbreeding depression effects.

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