Pure

Scotland's Rural College

Female but not male zebra finches adjust heat output in response to increased incubation demand

Hill, DL; Lindstrom, J; McCafferty, DJ; Nager, RG

Link to publication

Female but not male zebra finches adjust heat output in response to increased incubation demand

Davina L. Hill[1*], Jan Lindström[1], Dominic J. McCafferty[1] and Ruedi G. Nager[1]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK

[*]Author for correspondence and present address: Animal and Veterinary Sciences, Roslin Institute Building, SRUC, Easter Bush, Midlothian, EH25 9RG, UK (davina.hill@sruc.ac.uk)

1 **SUMMARY**

2 In many incubating birds heat transfer from parent to egg is facilitated by the brood

3 patch, an area of ventral abdominal skin that becomes highly vascularised, swells and

4 loses its down feathers around the time of laying. Only the female develops a brood patch

5 in most passerine species, but males of some species can incubate and maintain the eggs

6 at similar temperatures to females even without a brood patch. Here we used a novel

7 application of infra-red thermography (IRT) to examine sex differences in parental care

8 from a physiological perspective. Using incubating male and female zebra finches

9 (*Taeniopygia guttata*), a species in which the male lacks a brood patch, we measured the

10 surface temperature of the ventral plumage overlying the abdomen and a reference area

11 that does not contact the eggs (thorax) twice per pair. In half of the pairs clutch size was

12 experimentally enlarged between the two sets of measurements to increase incubation

13 demand. We found that the temperature differential between abdomen and thorax

14 plumage was greater in females than in males, and that abdomen plumage was warmer

15 after clutch enlargement than before it in females but not in males. These findings are

16 consistent with morphological sex differences in brood patch development and suggest

17 that male and female zebra finches differ in the way they regulate abdomen versus

18 general body surface temperature in response to variation in incubation demand.

19

22

Introduction

23

24 Incubating birds must keep their eggs within the narrow range of temperature and

25 humidity that favours optimal embryonic development by transferring heat from their

26 body to the eggs (DuRant et al., 2013; Rahn and Ar, 1974; Webb, 1987). They can

27 regulate heat transfer behaviourally by adjusting their body position and the duration and

28 tightness of contact with the eggs (e.g. Drent et al., 1970; Gorman et al., 2005; White and

29 Kinney, 1974) and physiologically by increasing their metabolic rate (de Heij et al., 2007;

30 Nord et al., 2010; Vleck, 1981) or output of blood flow to the brood patch (Midtgard et

31 al., 1985). The brood patch is typically a defeathered, swollen and highly vascularised

32 area of ventral abdominal skin that develops under hormonal control around the time of

33 egg-laying and incubation in many bird species (Bailey, 1952; Jones, 1971; Lea and

34 Klandorf, 2002). As well as facilitating heat transfer during contact incubation, the brood

35 patch contains sensory receptors that enable incubating birds to detect suboptimal egg

36 temperatures (Drent et al., 1970; Lea and Klandorf, 2002; White and Kinney, 1974).

37 Even in biparental incubators, where both males and females contribute to

38 warming the eggs, brood patch development can differ between the sexes. In most

39 passerines only the female develops a brood patch (Lea and Klandorf, 2002). Although

40 we might expect the sex with the more developed brood patch to maintain higher steady-

41 state incubation temperatures or re-warm cold eggs more rapidly, empirical evidence of

42 this is mixed. Females warm eggs more rapidly (Kleindorfer et al., 1995) or to a higher

43 temperature (Voss et al., 2008) than males in many passerine species, but in others the

44 males warm eggs to a similar or even higher temperature than the females, even in

45 species in which males lack a brood patch (Auer et al., 2007; Zann and Rossetto, 1991).

46 While these studies focussed on the temperature of the egg, few have compared sex

47 differences in heat output at the parental body surface itself (for exceptions see Bartlett et

48 al. (2005) and Deeming and Du Feu (2008) in passerines, and Massaro et al. (2006) in

49 yellow-eyed penguins, *Megadyptes antipodes*). Such measurements are useful because

50 they enable heat output from the parents to be studied independently of potentially

51 confounding behavioural effects on egg temperature, which might also differ between the

52 sexes.

53   Here we examined sex differences in heat output from incubating zebra finches,

54 *Taeniopygia guttata* (Vieillot, 1817). During the day, free-living males and females

55 invest an equal share of time in incubation (Zann and Rossetto, 1991), whereas females in

56 captive domesticated populations spend more time incubating than males (Burley, 1988;

57 Gorman and Nager, 2003; Hill et al., 2011), and females incubate alone at night in the

58 wild and in captivity (Zann and Rossetto, 1991). Females develop the morphological

59 characteristics of a brood patch (e.g. skin colour change and oedema formation) before

60 the clutch is complete, although without the degree of vascularisation seen in most other

61 passerine species (Zann and Rossetto, 1991; Zann, 1996). These characteristics do not

62 develop in male zebra finches. The apterium is relatively bare throughout the year in both

63 sexes, and the female loses the few down feathers she has during laying.

64   Based upon these morphological observations we hypothesised that incubating

65 females will emit more heat from the ventral abdomen than males, and we measured the

66 temperature of the ventral plumage using infra-red thermography (IRT). IRT uses known

67 properties of an object's surface and simple physical laws to determine the object's

68 surface temperature from the infra-red radiation it emits (Speakman and Ward, 1998). We

69 compared plumage temperature in males and females at two ventral sites: one over the

70 area where the brood patch occurs in females (abdomen) and another away from the

71 brood patch (thorax) to give an approximation of general body surface temperature.

72   Incubating a large clutch requires greater energy expenditure than a small clutch

73 (Biebach, 1984; de Heij et al., 2007; Nord et al., 2010) and so we experimentally

74 enlarged clutch size between measurement days to test whether birds would respond to

75 the increased demands of keeping eggs warm by increasing heat output. We expected to

76 see a greater increase in abdominal heat output in response to clutch enlargement in

77 females than in males due to the presence of the brood patch in females, which enhances

78 blood flow and sensory perception in the region in contact with the eggs. The results of

79 this experiment will provide a better understanding of how parent birds physiologically

80 adjust expenditure to variation in incubation demand.

81

82

83   **Results**

84 Male and female zebra finches incubating natural clutch sizes did not differ in the

85 thickness of plumage on the ventral surface (β = 0.31, Credible Interval (CI) = -0.57 to

86 1.20, P = 0.488, N = 66 measurements from 33 individuals and 17 pairs on incubation

87 day 6; Table 1). Thorax plumage was thicker than abdomen plumage (β = 1.22, CI = 0.32

88 to 2.08, P <0.007; Table 1) and the effects of body part (thorax or abdomen) on plumage

89 thickness did not depend on the bird's sex (β = -0.72, CI = -2.38 to 1.03, P = 0.407).

90 Abdomen plumage thickness measurements were repeatable between incubation days 6

91 and 8 in females ($F_{15,16}$ = 7.31, r = 0.75±0.11, P < 0.001) and males ($F_{15,16}$ = 7.43, r =

92 0.75±0.11, P <0.001); thorax plumage thickness was repeatable in males ($F_{15,16}$ = 5.34, r

93 = 0.67±0.14, P < 0.001) but not in females ($F_{15,16}$ = 0.97, r = -0.05±0.25, P = 0.523).

94   Thorax plumage temperature did not differ between the sexes and was not

95 associated with thorax plumage thickness or clutch size in birds incubating natural clutch

96 sizes (incubation day 6; Table 1, Table 2A). Abdomen plumage temperature, by contrast,

97 decreased with abdomen plumage thickness and the relationship between thorax plumage

98 temperature and abdomen plumage temperature differed between the sexes (Table 2B): in

99 females, the abdomen plumage was warmer than the thorax plumage (β = -1.42, CI = -

100 2.43 to -0.48, P = 0.007; Table 1), but there was no difference between thorax and

101 abdomen plumage temperature in males (β = -0.42, CI = -1.62 to 0.74, P = 0.473).

102   Abdomen and thorax plumage temperatures did not differ between incubation

103 days 6 and 8 in control birds. There was a non-significant trend towards warmer

104 abdomens in control females (32.5±0.53ºC; Table 3) than in control males (31.7±0.61ºC),

105 but the sexes did not differ in thorax plumage temperature (females: 30.5±0.35ºC, males:

106 30.9±47ºC; β = 0.01, CI = -0.02 to 0.05, P = 0.510). Abdomen plumage temperature was

107 repeatable between incubation days 6 and 8 in control females ($F_{7,8}$ = 9.98, r =

108 0.82±0.12, P = 0.002) but not significantly so in control males ($F_{7,8}$ = 3.46, r =

109 0.55±0.25, P = 0.052). Thorax plumage temperature was not repeatable in control males

110 ($F_{7,8}$ = 2.32, r = 0.34±0.32, P = 0.131) or females ($F_{7,8}$ = 1.30, r = 0.07±0.36, P = 0.361).

111   Thorax plumage temperature was warmer in treatment group females than in

112 treatment males on incubation days 6 and 8, but was not influenced by the clutch size

113 enlargement or an interaction between sex and clutch enlargement (Table 4A; Fig. 1).

114 However, the effects of incubating an enlarged compared with a control clutch on

115   abdomen plumage temperature differed between the sexes (Table 4B): female abdomens

116   were warmer after the clutch size enlargement than before it, but male abdomen plumage

117   temperature did not change (Fig. 1). This result was qualitatively similar when the ventral

118   temperature differential (abdomen plumage temperature minus thorax plumage

119   temperature) was used as a response variable (Linear Mixed effects Model controlling for

120   plumage thickness, individual identity and pair identity: sex × clutch enlargement β = -

121   1.13, CI = -2.14 to -0.15, P = 0.026).

122

123

124   **Discussion**

125   We examined sex differences during incubation by comparing the ventral heat output of

126   male and female zebra finches using infra-red thermography. The plumage of females

127   incubating natural clutch sizes was warmer at the abdomen than the thorax, which we

128   used as a proxy for general body surface temperature, but the two areas did not

129   significantly differ in temperature in males. Similarly, in incubating female house

130   sparrows, *Passer domesticus*, the abdomen was warmer than a control area (the back) in

131   females but not in males (Bartlett et al., 2005). Moreover, female zebra finches appeared

132   to respond to the challenge of incubating experimentally enlarged clutch sizes by

133   increasing heat output from the abdomen (adjusted for general body temperature) relative

134   to their own output before the clutch size manipulation. By contrast, we observed no

135   change in heat output in males.

136   A sex difference in plumage temperature could be due to males and females

137   generating different amounts of heat, differing in insulation in layers above the heat-

138   generating tissue (plumage, skin and subcutaneous tissue) or both. Plumage thickness is

139   the main contributor to insulation in several bird species (McCafferty et al., 1997), but we

140   found no sex difference in plumage thickness in our population, suggesting that the

141   differences measured here are due to differences in the output of generated heat. These

142   results are consistent with differences between male and female zebra finches in brood

143   patch morphology (Zann and Rossetto, 1991; Zann, 1996) and suggest that the sexes

144   differ in their ability or willingness to increase abdomen temperature above general body

145 temperature in response to variation in incubation demand. To our knowledge this has not

146 been demonstrated previously.

147   It is worth emphasising that we did not measure brood patch skin temperature but

148 the temperature of the contour feathers overlying the egg-contact region. The aim of this

149 was to minimise variability between measurements and disturbance to the birds

150 associated with instrument attachment and handling. The difference between the

151 abdomen temperatures presented here (32.5±0.33°C for females on incubation day 6) and

152 the higher temperatures reported elsewhere for brood patch skin (41.2±0.11°C, mean ±

153 SE for 24 passerine species, mainly measured in females (Deeming, 2008)) highlights the

154 excellent insulating capacity of the plumage, even during incubation when the down

155 feathers have been lost. In addition, we found that abdomen plumage temperatures

156 decreased as plumage thickness increased, in agreement with studies of mounted

157 specimens of passerines, quails and owls (McCafferty et al. 1997; Walsberg, 1988). The

158 exact gradient of heat loss from the skin to the surface of the plumage is likely to be more

159 complex than is currently understood, and may depend on the type, quality, number and

160 placement of feathers overlying the skin (Wolf & Walsberg, 2000). Although plumage

161 temperature measurements are not a substitute for direct measurements of brood patch

162 temperature, they are valuable in studies such as this where the aim is to detect relative

163 changes in heat output. In particular they are likely to improve the precision of within-

164 individual studies where the greatest source of variability is due to measurement error.

165   While females incubating enlarged clutches on incubation day 8 had warmer

166 abdomen plumage than on day 6, there was no change in abdomen heat output in control

167 birds incubating unmanipulated clutch sizes. The response in the former group of females

168 is therefore most likely due to clutch size enlargement rather than changes over the

169 incubation period. Females may respond to increased incubation demand by directing

170 warm blood to arterioles that lie close to the brood patch surface and that typically

171 increase in musculature as part of brood patch development (Midtgard et al., 1985;

172 Peterson, 1955). This might occur to even a greater extent in females of other passerine

173 species whose brood patches exhibit more pronounced vascularisation than in zebra

174 finches.

175   Treatment male zebra finches, unlike females, did not respond to the clutch size

176   enlargement by increasing their abdomen temperature. While this could imply a reduced

177   ability or willingness to transfer heat in incubating males compared with females, Zann

178   and Rossetto (1991) did not observe a sex difference in steady-state incubation

179   temperature or the rate of re-warming cool eggs in this species and speculated that male

180   zebra finches increase heat transfer to the eggs by increasing metabolic rate. Our findings

181   do not support this idea because male thorax temperature was not higher than female

182   thorax temperature and did not increase in response to clutch enlargement. Indeed,

183   females allocated to the treatment group had warmer thoraxes than males and there was

184   no sex difference in thorax temperature in control birds. Nevertheless, metabolic rate has

185   rarely been measured in incubating males and it would be valuable to compare the

186   metabolic rates of incubating males and females directly.

187   A new question raised by this study is whether the difference in temperature

188   between the abdomen and thorax in females exists only in incubating birds. As the

189   temperature of the abdomen plumage relative to the thorax changed with incubation

190   demand we believe that it is reasonable to suggest that at least some of these differences

191   are related to incubation. A better understanding of this issue would provide information

192   on brood patch function and could be tested by comparing abdomen and thorax

193   measurements taken during incubation with those from the same females before the

194   brood patch develops or after it regresses. If the female has a true brood patch, we would

195   predict that the abdomen would be warmer in incubating than in non-incubating females

196   and that there would be no difference between incubating and non-incubating females in

197   thorax temperature nor between abdomen and thorax temperature in non-incubating

198   females.

199   Maintaining a brood patch is likely to be costly in terms of increased heat loss

200   (Haftorn and Reinertsen, 1985), and we would expect such costs to be offset by an

201   associated fitness benefit, such as an increased ability to keep the eggs at favourable

202   conditions for optimal embryo development and survival. Lower egg temperatures in

203   males than females during steady-state incubation have indeed been reported in some

204   species of biparentally incubating passerine (Reid et al., 2002; Voss et al., 2008).

205   However, no sex differences in steady state incubation were found in other species,

206   including zebra finches, as noted above, in spite of the absence of a brood patch in males

207   (Kleindorfer et al., 1995; Zann and Rossetto, 1991), and in chestnut-vented tit-babblers,

208   *Parisoma subcaeruleum*, eggs were warmer when incubated by males than by females

209   (Auer et al., 2007). Males have been observed to re-warm clutches after an incubation

210   break more slowly than females in some species (Kleindorfer et al., 1995; Voss et al.,

211   2008), while in others males re-warmed clutches more quickly than females (Reid et al.,

212   2002), and no clear difference between the sexes was seen in others, including zebra

213   finches (Auer et al., 2007; Hill, 2009). These conflicting results may seem to suggest that

214   the brood patch is not associated with improved performance during incubation.

215   However, we need to know more about sex differences in the costs of incubation and how

216   the brood patch might moderate these costs before we can draw a conclusion. The

217   presence of a brood patch might reduce the risk of tissue damage due to the protective

218   thickening of the epidermal skin (Jones, 1971), allow individuals to sustain longer or

219   more frequent incubation bouts or expend less effort to achieve the same thermal output

220   (Auer et al., 2007) or enable them to detect non-optimal egg temperatures through the

221   sensory receptors it contains (Drent et al., 1970; White and Kinney, 1974).

222   In our study, up-regulation of heat transfer to the brood patch in females after the

223   clutch size manipulation could be a tactile response to the increased number of eggs or a

224   thermal response to a decrease in mean egg temperature. Mean egg surface temperature

225   (both sexes pooled) was inversely related to natural variation in clutch size in our

226   population (Hill, 2009), and so we would expect the clutch enlargement in the present

227   study to produce a similar decrease in egg temperature. However, males might fail to

228   perceive such changes in temperature or clutch size without a brood patch. They do,

229   however, seem able to respond to variation in clutch size by adjusting the amount of time

230   spent incubating. Male European starlings, *Sturnus vulgaris*, increased incubation

231   attentiveness following clutch size enlargement and decreased it when clutch size was

232   reduced (Komdeur et al., 2002) and incubation attentiveness was positively related to

233   natural clutch size in male zebra finches (Hill et al., 2011). These results suggest that the

234   absence of a brood patch does not impair a male's ability to detect changes in clutch size,

235   although what cues they use is unknown.

236   Sex differences in the regulation of heat transfer to offspring have also been

237   recorded in humans. Studies of 'kangaroo care', where a newborn human infant is placed

238   in skin-to-skin contact upon the parental breast, show that mothers adjust their breast

239   temperature in response to their infants' immediate thermal needs whereas fathers

240   maintain a high heat output that can cause infants to become overheated and even

241   hyperthermic (Chiu et al., 2005; Ludington-Hoe et al., 1992; Ludington-Hoe et al., 2006).

242   These findings, in combination with our own, point to interesting differences between

243   male and female parents in the modulation of offspring temperature, and suggest that

244   such differences may be more widespread in endotherms than is currently recognized.

245   Our results suggest that males and females respond differently to the demands of

246   incubation. Understanding sex differences in the effectiveness of parental care has

247   implications for our understanding of sex role divergence and for interpreting empirical

248   studies of sexual conflict over parental effort. It might be maladaptive for males to

249   increase their incubation effort to levels shown by females because they are less certain

250   of their relatedness to the offspring (Queller, 1997; Trivers, 1972) and can potentially

251   obtain greater fitness benefits from seeking extra-pair copulations (EPCs) than attending

252   to the eggs or offspring, depending on the availability of receptive females (Bateman,

253   1948; Kokko and Jennions, 2008; Magrath and Komdeur, 2003). Perhaps for this reason

254   the complex morphological adaptations associated with the brood patch have not evolved

255   to the same extent or have not been conserved in male passerines. The sex difference we

256   observed in the birds' response to the clutch size enlargement could reflect the outcome

257   of a conflict that has been resolved over evolutionary time or differences in willingness to

258   respond (even if individuals are capable of doing so) measured over ecological time. In

259   practice it may not be possible to determine whether males are unable or unwilling to

260   adjust abdominal temperature, and in any case sex differences in ability and willingness

261   are likely to have arisen as a consequence of the same evolutionary pressures.

262   Nevertheless, by contributing to incubation, males play an important role in relieving the

263   female in times of energetic stress (Kleindorfer et al., 1995; Smith and Montgomerie,

264   1992) and in reducing the substantial energetic demands of re-warming cold eggs after

265   the female returns from a foraging bout (Vleck, 1981; Voss et al., 2008).

266

267

Materials and methods

Subjects and maintenance

270 This study was carried out on domesticated zebra finches bred at the University of

271 Glasgow and conforms to the 'Guidelines for the treatment of animals in behavioural

272 research and teaching' (Buchanan et al., 2012). Prior to pairing, the sexes were kept

273 separately at a density of six individuals per cage ($122 \times 41 \times 45$ cm length $\times$ width $\times$

274 height) under a 'short day' light regime ($10\frac{1}{2}$ h L: $13\frac{1}{2}$ h D) using daylight-spectrum

275 fluorescent tubes (Arcadia Bird Lamp FB36) with a gradual change in light at dawn and

276 dusk. Birds received *ad libitum* mixed seed consisting of canary millets (Foreign Finch

277 Mixture: Galloway MacLeod, UK), oyster grit, cuttlefish bone and water. Egg biscuit

278 protein with vitamin (Daily Essentials 2: The Birdcare company, Nailsworth, UK) and

279 calcium (Calcivet: The Birdcare company) supplements were provided three times a

280 week and fresh spinach leaves twice a week.

281 Males and females without previous breeding experience were size matched

282 according to tarsus length. To stimulate breeding, we increased daylight hours to 12 h L:

283 12 h D one week before birds were paired and maintained this 'long day' regime until the

284 end of the experiment. Each pair was kept in a breeding cage ($60 \times 40 \times 50$ cm length $\times$

285 width $\times$ height) with access to a nestbox and coconut fibre nesting material. Nestboxes

286 were checked daily from pairing, and lay date (the date that the first egg of a clutch was

287 laid) and clutch size were recorded. All pairs that laid in the nestbox were included in the

288 study.

289

Experimental design

291 We compared ventral plumage temperature in male and female zebra finches incubating

292 unmanipulated ('control', $N = 9$ pairs) and experimentally enlarged clutch sizes where we

293 increased incubation effort by adding two dummy eggs (see below) to a pair's natural

294 clutch size ('treatment', $N = 10$). Each pair was alternately allocated to control or

295 treatment group according to lay date. Control and treatment birds did not differ in the

296 number of eggs they laid (control: 4.8 ± 0.52 eggs (mean $\pm$ SE); treatment: 5.4 ± 0.54

297 eggs; $F_{1,17} = 0.68$, $P = 0.422$) or tarsus length (control males: 13.9 ± 0.16 mm; treatment

298 males: 14.2 ± 0.15 mm; $F_{1,17} = 1.84$, $P = 0.193$; control females: 14.3 ± 0.21 mm;

299 treatment females: 14.5 ± 0.17 mm (one measurement missing); $F_{1,16} = 0.69$, $P = 0.780$).

300   We assumed that zebra finches begin incubating ('day 0') on the fourth day of

301 laying in clutches of five eggs or more, and on the final day of laying in smaller clutches;

302 the incubation period spans 11-15 (median 14) days using these criteria (Zann and

303 Rossetto, 1991). On day 2 of incubation, we replaced all eggs with an equivalent number

304 of dummy eggs made of Fimo modelling clay (Eberhard Faber, Neumarkt, Germany).

305 Fimo eggs have similar thermal properties to fresh zebra finch eggs (Gorman, 2005) and

306 do not bring about changes in incubation behaviour compared to natural eggs (Gorman et

307 al., 2005). At dusk on incubation day 7, we added two additional Fimo eggs to the

308 clutches of the 10 treatment pairs; the 9 control pairs incubated unmanipulated clutch

309 sizes throughout. This design enabled us to test for sex differences in the ventral heat

310 output of incubating birds both within and between experimental groups.

311

312   *Temperature measurements*

313 We used infra-red thermography (IRT) to measure temperature at the surface of the

314 ventral plumage. IRT is a non-invasive, non-contact technique that can provide quick and

315 accurate measurements of avian skin and plumage temperatures (McCafferty, 2013). We

316 simultaneously measured the surface temperature of the undisturbed contour feathers

317 overlying two regions on the ventral side of the birds: the area that comes into contact

318 with the eggs, corresponding to the brood patch in females (hereafter abdomen

319 temperature) and a control area anterior to the brood patch that does not contact the eggs

320 (hereafter thorax temperature). This allowed us to test whether the output of heat from the

321 abdomen is regulated independently of the rest of the body. We measured plumage rather

322 than skin temperature to remove potential biases and variability associated with parting

323 the contour feathers by hand to expose the brood patch and thorax skin, which could

324 influence temperature and handling time. The insulating properties of plumage increase

325 with plumage thickness in several species of terrestrial birds (McCafferty et al., 1997;

326 Walsberg, 1988) and so we measured plumage thickness at the abdomen and thorax (see

327 below) to account for this variation.

328   IRT images were taken using the ThermaCam^{TM} E300 (FLIR Systems,

329   Burlington, Canada) on incubation days 6 and 8, that is, just before and after treatment

330   pairs experienced the clutch size manipulation (incubation day 7) and when nest

331   attentiveness reaches its maximum (Gorman and Nager, 2003). We gently displaced each

332   incubating bird with a tap to the nestbox and caught the bird without touching its

333   underside. The bird was then held with its ventral surface at a distance of 0.20 m from the

334   camera, which was supported on a fixed tripod. We took three replicate images of the

335   bird's ventral surface and recorded the time elapsed between the displacement of the bird

336   from the nest and the image being taken ('image latency', mean 65.8 ± 3.97 s, $N = 70$

337   images from 38 birds and 19 pairs; all reported values are based on the single best-

338   resolved of the three replicate images). Some individuals were not observed incubating

339   on the designated days, and so thermal images are missing for day 6 for two treatment

340   pairs and one control female and on day 8 for the partner of the latter female. Neither

341   abdomen plumage temperature ($\beta = -0.96$, Credible Interval (CI) = -2.53 to 0.65, $P = $

342   0.227 controlling for pair identity and plumage thickness) nor thorax plumage

343   temperature ($\beta = -0.001$, CI = -0.004 to 0.001, $P = 0.211$ controlling for pair identity;

344   plumage thickness was not significant) was associated with image latency ($N = 33$

345   individuals from 17 pairs on day 6 of incubation). Immediately after taking thermal

346   images on incubation days 6 and 8, we measured plumage thickness at the thorax and

347   abdomen to the nearest 0.5 mm by pushing a discarded primary feather, marked along the

348   shaft at 1 mm intervals, through the plumage down to the skin.

349   We used the software ThermaCAM^{TM} QuickReport 1.1 (FLIR Systems, 2007a) to

350   visualise the digital images and ThermaCAM^{TM} Reporter 8.3 (FLIR Systems, 2007b) to

351   analyse the best resolved of the three replicates taken for each individual and incubation

352   day. The radiation measured by the camera is a function of the object's surface

353   temperature and emissivity, ambient temperature and absorption and scattering by

354   atmospheric humidity. We set the surface emissivity value to 0.98 for the bird's plumage

355   (Hammel, 1956) and temperature and relative humidity to the mean values recorded in

356   the room during the measurement period ($22.2 \pm <0.01$ °C and 13.6 ± 0.04 %,

357   respectively). To standardise the size and positioning of measurement areas between

358   images we placed a sagittal line along the image of the bird's ventral surface (Fig. 2)

359 consisting of three transects that were scaled to the length of the bird's leg ring, which

360 was visible on all images. This was done by tracing a straight digital line along the length

361 of the ring (Fig. 2, Li1) with the polygon tool in ThermaCAM^{TM} Reporter 8.3, noting the

362 ring's length in pixels, and then producing three transects of the same pixel length (Fig. 2,

363 Li2, Li3, Li4). The first transect (Li2 = abdomen) was extended by a factor of three, since

364 the brood patch was at least three times the length of the ring, and placed along the

365 sagittal plane on the region of the thermal image corresponding to the plumage over the

366 brood patch in females. Li3 and Li4 were placed at the apex of Li2 and Li3, respectively

367 to standardise the distance between the two measurement areas (Li2 = abdomen and Li4

368 = thorax). We then calculated the mean pixel temperature along Li2 (abdomen plumage

369 temperature) and Li4 (thorax plumage temperature).

370

371 *Statistical analysis*

372 All data were analysed in R version 3.0.1 (R Development Core Team, 2013). Thorax

373 plumage temperature measurements were normalised by log transformation when used as

374 a response variable and abdomen plumage temperature measurements were squared to

375 allow parametric tests to be carried out.

376 We tested whether the thickness of plumage overlying the abdomen and the

377 thorax differs between the sexes and the body part in birds incubating unmanipulated

378 clutch sizes (incubation day 6). We fitted a general Linear Mixed effects Model (LMM)

379 with plumage thickness as the response variable, sex and body part (abdomen or thorax)

380 as fixed factors and individual and pair identity as random factors. We included the

381 interaction between sex and body part to test whether a difference in plumage thickness

382 between the two parts of the body depends on the bird's sex. We calculated the within-

383 individual repeatability (*r*) of plumage thickness between incubation days 6 and 8

384 (following Lessells and Boag, 1987) and its standard error (Becker, 1984) to allow us to

385 assess measurement precision.

386 To see whether males and females incubating unmanipulated clutch sizes differed

387 in heat output, we fitted LMMs to data from incubation day 6. This first involved testing

388 whether the sexes differed in general body temperature using thorax plumage temperature

389 as the response variable, and then whether males and females differentially regulate

390   abdomen temperature relative to general body temperature by fitting thorax plumage

391   temperature and the interaction between sex and thorax plumage temperature as fixed

392   effects. We included pair identity as a random factor, sex as a fixed factor and clutch size

393   and plumage thickness measured on incubation day 6 as covariates in both models.

394   Variance inflation factors calculated by the car package (Fox and Weisberg, 2012) were

395   <1.23 in both models.

396   If females have a true brood patch but males do not, we should expect only

397   females to maintain brood patch temperature above the temperature of the rest of the

398   trunk. To test whether this is likely to be the case, we ran two separate LMMs, one for

399   each sex, with thorax and abdomen plumage temperature (which were normally

400   distributed when pooled) on incubation day 6 as a single response variable, pair identity

401   as a random factor, body part as a fixed factor and plumage thickness on day 6 as a

402   covariate.

403   To see whether ventral heat output changed between incubation days 6 and 8 in

404   unmanipulated birds, we compared temperature measurements between the two days in

405   control birds using separate LMMs for thorax and abdomen plumage temperature. In both

406   models we fitted plumage thickness, sex, day of incubation and the interaction between

407   sex and day of incubation as fixed effects and individual and pair identity as random

408   effects. Where abdomen plumage temperature was the response variable, we also

409   controlled for thorax plumage temperature.

410   There was no effect among control birds of incubation day on either abdomen or

411   thorax plumage temperature (see Results). Consequently, differences in temperature

412   between incubation days in the treatment group are likely to be related to the clutch size

413   manipulation. We therefore compared treatment group individuals before (day 6) and

414   after (day 8) the clutch size enlargement. We tested for an effect of sex and clutch

415   enlargement as well as the interaction between the two on abdomen and thorax plumage

416   temperature in LMMs to determine whether the sexes respond differently to the challenge

417   of incubating an enlarged clutch.

418   We fitted LMMs by Restricted Maximum Likelihood using the lme4 package

419   (Bates et al., 2013). We removed interaction terms $P \geq 0.05$ in order of least significance

420   and then non-significant main effects to reach the final model. Significance is denoted by

421   a two-tailed *P*-value < 0.05 or a Credible Interval (CI) that does not include zero. We

422   present the intercept (constant) for all models containing significant fixed effects.

423   Random effects were fitted with random intercepts only, and were controlled for even

424   when not statistically significant. For all fixed effects tested we present the model

425   coefficient *β* with CIs calculated at the 95 % confidence level using Markov chain Monte

426   Carlo (MCMC) sampling with the chain length fixed at 10,000 and *P*-values based upon

427   the posterior probability distribution. These estimates were calculated using the pvals

428   function from the languageR library (Baayen, 2013). Means are presented ± SE.

References

Auer, S. K., Bassar, R. D. and Martin, T. E. (2007). Biparental incubation in the chestnut-vented tit-babbler *Parisoma subcaeruleum*: mates devote equal time, but males keep eggs warmer. *J. Avian Biol.* **38**, 278-283.

Baayen, R. H. (2013). languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics". Version 1.4. http: //cran.r-project.org/web/packages/languageR/

Bailey, R. E. (1952). The incubation patch of passerine birds. *Condor* **54**, 121-136.

Bartlett, T. L., Mock, D. W. and Schwagmeyer, P. L. (2005). Division of labor: Incubation and biparental care in house sparrows (*Passer domesticus*). *Auk* **122**, 835-842.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349-368.

Bates, D., Maechler, M. and Bolker, B. (2013). Linear mixed-effects models using S4 classes: R package, Version 0.999999-2. http://cran.r-project.org/web/packages/lme4/index.html

Becker, W. A. (1984). A manual of quantitative genetics. Pullman, Washington: Academic Enterprises.

Biebach, H. (1984). Effect of clutch size and time of day on the energy expenditure of incubating Starlings (*Sturnus vulgaris*). *Physiol. Zool.* **57**, 26-31.

Buchanan, K., Burt de Perera, T., Carere, C., Carter, T., Hailey, A., Hubrecht, R., Jennings, D., Metcalfe, N., Pitcher, T., Peron, F. et al. (2012). Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* **83**, 301-309.

Burley, N. (1988). The differential allocation hypothesis - an experimental test. *Am. Nat.* **132**, 611-628.

Chiu, S. H., Anderson, G. C. and Burkhammer, M. D. (2005). Newborn temperature during skin-to-skin breastfeeding in couples having breastfeeding difficulties. *Birth-Iss. Perinat. C.* **32**, 115-121.

de Heij, M. E., van der Graaf, A. J., Hafner, D. and Tinbergen, J. M. (2007). Metabolic rate of nocturnal incubation in female great tits, *Parus major*, in relation to clutch size measured in a natural environment. *J. Expt. Bio.* **210**, 2006-2012.

Deeming, D. C. (2008). Avian brood patch temperature: relationships with female body mass, incubation period, developmental maturity and phylogeny. *J. Therm. Biol.* **33**, 345-354.

Deeming, D. C. and Du Feu, C. R. (2008). Measurement of brood patch temperature of British passerines using an infrared thermometer. *Bird Study* **55**, 139-143.

Drent, R. H., Joustra, T. and Postuma, K. (1970). The effect of egg temperature on incubation behaviour in the herring gull. *Behaviour* **17**, 237-261.

DuRant, S. E., Hopkins, W. A., Hepp, G. R. and Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol. Rev.* **88**, 499-509.

FLIR Systems. (2007a). ThermaCAM QuickReport Version 1.1 Burlington, Canada.

FLIR Systems. (2007b). ThermaCAM Reporter 8.3 Professional. Burlington, Canada.

Fox, J. and Weisberg, S. (2012). car: Companion to Applied Regression. Version 2.0-18. http://cran.r-project.org/web/packages/car/index.html

Gorman, H. E. (2005). Maternal Effects in Avian Incubation, Ph. D. Glasgow: University of Glasgow.

Gorman, H. E., Arnold, K. E. and Nager, R. G. (2005). Incubation effort in relation to male attractiveness in zebra finches *Taeniopygia guttata*. *J. Avian Biol.* **36**, 413-420.

Gorman, H. E. and Nager, R. G. (2003). State-dependent incubation behaviour in the zebra finch. *Anim. Behav.* **65**, 745-754.

Haftorn, S. and Reinertsen, R. E. (1985). The effect of temperature and clutch size on the energetic cost of incubation in a free-living blue tit (*Parus caeruleus*). *Auk* **102**, 470-478.

Hammel, H. T. (1956). Infrared emissivities of some Arctic fauna. *J. Mammal.* **37**, 375-378.

Hill, D. L. (2009). Sexual conflict and division of labour during incubation. *Division of Ecology and Evolutionary Biology*, Ph.D, Glasgow: University of Glasgow.

Hill, D. L., Lindström, J. and Nager, R. G. (2011). Carry-over effects of male extra-pair copulation opportunity on biparental effort in zebra finches. *Behav. Ecol. Sociobiol.* **65**, 2049-2059.

Jones, R. E. (1971). The incubation patch of birds. *Biol. Rev. Camb. Philos.* **46**, 315-339.

Kleindorfer, S., Fessl, B. and Hoi, H. (1995). More is not always better: Male incubation in two *Acrocephalus* warblers. *Behaviour* **132**, 607-625.

Kokko, H. and Jennions, M. D. (2008). Parental investment, sexual selection and sex ratios. *J. Evol. Biol.* **21**, 919-948.

Komdeur, J., Wiersma, P. and Magrath, M. (2002). Paternal care and male mate-attraction effort in the European starling is adjusted to clutch size. *Proc. R. Soc. Lond. B.* **269**, 1253-1261.

Lea, R. W. and Klandorf, H. (2002). The brood patch. In *Avian Incubation: Behaviour, Environment, and Evolution*, (ed. D. C. Deeming), pp. 100-118. New York: Oxford University Press.

Lessells, C. M. and Boag, P. T. (1987). Unrepeatable repeatabilities - a common mistake. *Auk* **104**, 116-121.

Ludington-Hoe, S. M., Hashemi, M. S., Argote, L. A., Medellin, G. and Rey, H. (1992). Selected physiological measures and behavior during paternal skin contact with Colombian preterm infants. *J. Dev. Physiol.* **18**, 223-232.

Ludington-Hoe, S. M., Lewis, T., Morgan, K., Cong, X. M., Anderson, L. and Reese, S. (2006). Breast and infant temperatures with twins during shared kangaroo care. *J. Obst. Gyn. Neo.* **35**, 223-231.

Massaro, M., Davis, L. S. and Davidson, R. S. (2006). Plasticity of brood patch development and its influence on incubation periods in the yellow-eyed penguin *Megadyptes antipodes*: an experimental approach. *J. Avian Biol.* **37**, 497-506.

Magrath, M. J. L. and Komdeur, J. (2003). Is male care compromised by additional mating opportunity? *Trends Ecol. Evol.* **18**, 424-430.

McCafferty, D. J. (2013). Applications of thermal imaging in avian science. *Ibis* **155**, 4-15.

McCafferty, D. J., Moncrieff, J. B. and Taylor, I. R. (1997). The effect of wind speed and wetting on thermal resistance of the barn owl (*Tyto alba*). I: Total heat loss, boundary layer and total resistance. *J. Therm. Biol.* **22**, 253-264.

Midtgard, U., Sejrsen, P. and Johansen, K. (1985). Blood-flow in the brood patch of bantam hens - Evidence of cold vasodilation. *J. Comp. Physiol. B.* **155**, 703-709.

Nord, A., Sandell, M. I. and Nilsson, J. (2010). Female zebra finches compromise clutch temperature in energetically demanding incubation conditions. *Funct. Ecol.* **24**, 1031-1036.

Petersen, A. J. The breeding cycle in the Bank Swallow. *Wilson Bull.* 67.4 (1955): 235-286.

Queller, D. C. (1997). Why do females care more than males? *P. R. Soc. B.* **264**, 1555-1557.

R Development Core Team. (2013). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rahn, H. and Ar, A. (1974). The avian egg: incubation time and water loss. *Condor* **76**, 147-152.

Reid, J. M., Monaghan, P. and Ruxton, G. D. (2002). Males matter: The occurrence and consequences of male incubation in starlings (*Sturnus vulgaris*). *Behav. Ecol. Sociobiol.* **51**, 255-261.

Smith, H. G. and Montgomerie, R. (1992). Male incubation in barn swallows - the influence of nest temperature and sexual selection. *Condor* **94**, 750-759.

Speakman, J. R. and Ward, S. (1998). Infrared thermography: principles and applications. *Zool-Anal. Complex Sy* **101**, 224-232.

Trivers, R. L. (1972). Parental investment and sexual selection. In *Sexual selection and the descent of man*, (ed. B. Campbell), pp. 136-179. Chicago: Aldine Atherton.

Vleck, C. M. (1981). Energetic cost of incubation in the zebra finch. *Condor* **83**, 229-237.

Voss, M. A., Rutter, M. A., Zimmerman, N. G. and Moll, K. M. (2008). Adaptive value of thermally inefficient male incubation in barn swallows (*Hirundo rustica*). *Auk* **125**, 637-642.

Walsberg, G. E. (1988). Heat-flow through avian plumages - The relative importance of conduction, convection, and radiation. *J. Therm. Biol.* **13**, 89-92.

Webb, D. R. (1987). Thermal tolerance of avian embryos - a review. *Condor* **89**, 874-898.

White, F. N. and Kinney, J. L. (1974). Avian incubation. *Science* **186**, 107-115.

Wolf, B. O. and Walsberg, G. E. (2000). The role of the plumage in heat transfer processes of birds. *Am. Zool.* **40**, 575-584.

Zann, R. and Rossetto, M. (1991). Zebra finch incubation - brood patch, egg temperature and thermal-properties of the nest. *Emu* **91**, 107-120.

Zann, R. A. (1996). The zebra finch: a synthesis of field and laboratory studies. New York: Oxford University Press.

Fig 1. Abdomen and thorax plumage temperature (means + 1 SE) in incubating male and female zebra finches before (incubation day 6, $N = 8$ pairs) and after (incubation day 8, $N = 10$ pairs) a clutch enlargement manipulation. There was a clutch enlargement $\times$ sex interaction on abdomen plumage temperature (see Table 4) whereby females but not males increased abdomen plumage temperature following clutch enlargement. Thorax plumage temperature was warmer in females than in males, but was not influenced by the clutch size enlargement nor an interaction between sex and clutch enlargement.

Fig 2. Sample thermal image of the ventral surface of a male on day 6 of incubation illustrating the procedure used for measuring plumage temperature along a transect following the sagittal plane of the ventral surface, and (inset) a digital reference image of the same bird. We standardised the relative size and positioning of transects across all thermal images using the length of the bird's leg ring (Li1) as a scale measured in pixels. Three lines were generated from Li1: Li2 was placed on the abdomen, Li3 was placed at the apex of Li2, and Li4 was placed on the thorax at the apex of Li3; all were arranged along the same ventral transect. We then calculated the mean pixel temperature along Li2 (abdomen plumage temperature) and Li4 (thorax plumage temperature). The values accompanying Li2 and Li4 in the figure are mean temperatures along the two lines.

Table 1 Mean ± SE plumage thickness and plumage temperature in 17 male and 16 female zebra finches incubating natural clutch sizes on day 6 of incubation.

	Plumage thickness (mm)		Plumage temperature (°C)	
	Thorax	**Abdomen**	**Thorax**	**Abdomen**
Male	5.3±0.48	4.4±0.52	30.9±0.43	31.7±0.59
Female	5.3±0.42	3.7±0.31	31.1±0.39	32.5±0.33

Table 2. The effects of a bird's sex and other variables on (A) thorax and (B) abdomen plumage temperature on day 6 of incubation (33 measurements from 17 pairs of zebra finches; see Table 1). Coefficients (β and Credible Interval) are estimated using General Linear Mixed effects Models controlling for pair identity (random intercepts). *P*-values are based on the posterior probability distribution (see Methods). Significant fixed effects are shown in bold, non-significant fixed effects were removed from the models.

2A) thorax plumage temperature

	β	Credible Interval	*P*
Plumage thickness	-0.01	-0.020 to 0.002	0.104
Natural clutch size	0.004	-0.01 to 0.02	0.572
Sex[a]	-0.01	-0.04 to 0.03	0.718

2B) abdomen plumage temperature

	β	Credible Interval	*P*
Constant	344.46	-412.46 to 1133.34	0.371
Plumage thickness	-20.90	-37.04 to -4.25	**0.016**
Thorax plumage temperature	25.46	1.50 to 50.02	**0.039**
Sex[a]	-1242.54	-2269.04 to -280.42	**0.015**
Sex $\times$ thorax plumage temperature	39.37	7.53 to 71.43	**0.016**
Natural clutch size	-0.88	-18.73 to 16.44	0.922

[a] 'female' is the reference sex

Table 3. The effects of incubation day (6 *vs* 8) and other variables on (A) thorax and (B) abdomen plumage temperature in control pairs (34 measurements from 18 individuals from 9 pairs) of zebra finches. Coefficients (β and Credible Interval) were estimated using General Linear Mixed effects Models controlling for pair and individual identities (random intercepts). Significant fixed effects are shown in bold, non-significant fixed effects were removed from the models.

3A) thorax plumage temperature

	β	Credible Interval	*P*
Plumage thickness	-0.01	-0.015 to 0.003	0.177
Sex[a]	0.01	-0.02 to 0.05	0.510
Day of incubation[b]	-0.01	-0.05 to 0.02	0.493
Sex × day of incubation	0.02	-0.05 to 0.09	0.576

3B) abdomen plumage temperature

	β	Credible Interval	*P*
Constant	-41.43	-722.14 to 612.66	0.896
Plumage thickness	-21.67	-41.45 to -0.20	**0.039**
Thorax plumage temperature	38.10	16.96 to 58.59	**<0.001**
Sex[a]	-54.17	-115.62 to 3.45	0.075
Day of incubation[b]	7.12	-51.13 to 64.25	0.808
Sex × day of incubation	-33.94	-152.09 to 78.23	0.536

[a] 'female' is the reference sex

[b] 'day 6' is the reference day of incubation

Table 4. The effects of sex and clutch size enlargement on (A) thorax and (B) abdomen plumage temperature on days 6 and 8 of incubation (repeated measures) in 10 treatment pairs (36 measurements; see Fig. 1). Coefficients (β and Credible Interval) were estimated using General Linear Mixed effects Models controlling for pair and individual identities (random intercepts). Significant fixed effects are shown in bold, non-significant effects that were not components of a significant interaction were removed from the models.

4A) thorax plumage temperature

	β	Credible Interval	P
Constant	3.46	3.44 to 3.49	**< 0.001**
Sex[a]	-0.04	-0.08 to -0.001	**0.047**
Clutch enlargement[b]	0.01	-0.03 to 0.04	0.719
Plumage thickness	0.0002	-0.01 to 0.01	0.962
Sex × clutch enlargement	0.01	-0.07 to 0.08	0.762

4B) abdomen plumage temperature

	β	Credible Interval	P
Constant	-769.75	-1115.08 to -411.72	**<0.001**
Plumage thickness	-24.04	-39.17 to -9.23	**0.003**
Thorax plumage temperature	60.66	50.39 to 71.62	**<0.001**
Sex[a]	11.54	-39.01 to 64.53	0.657
Clutch enlargement[b]	46.51	-1.28 to 93.25	0.056
Sex × clutch enlargement	-74.92	-139.36 to -3.71	**0.031**

[a] 'female' is the reference sex

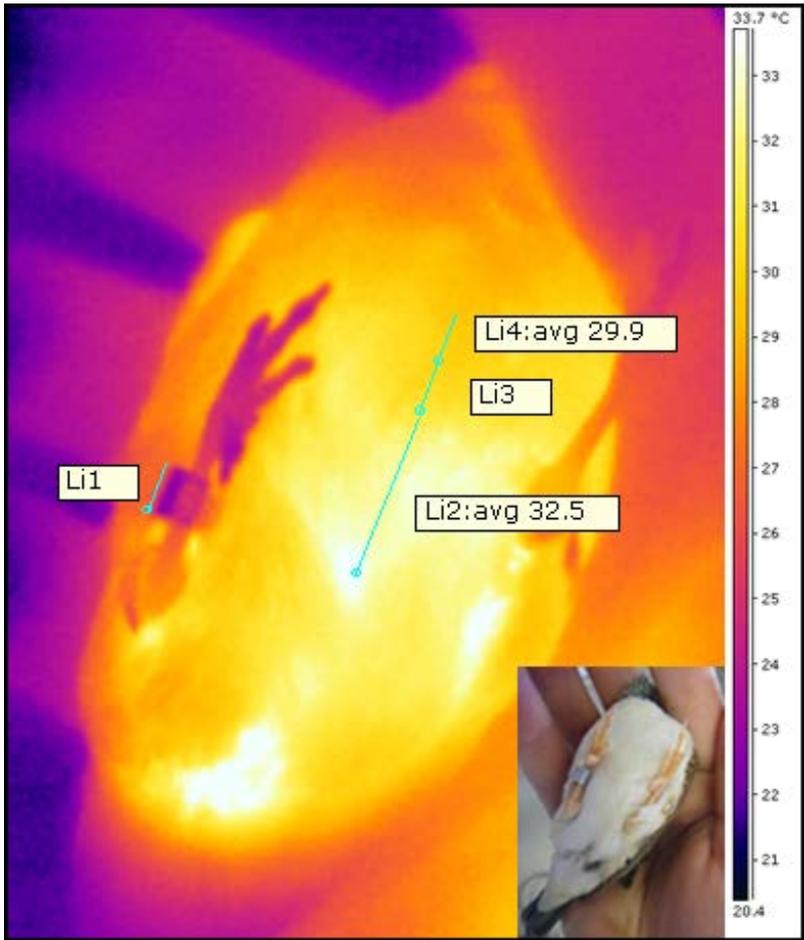[b] 'pre-treatment' is the reference stage of the experiment

Fig. 2