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RESEARCH ARTICLE

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

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ABSTRACT

In many incubating birds, heat transfer from parent to egg is facilitated by the brood patch, an area of ventral abdominal skin that becomes highly vascularised, swells and loses its down feathers around the time of laying. Only the female develops a brood patch in most passerine species, but males of some species can incubate and maintain the eggs at similar temperatures to females even without a brood patch. Here we used a novel application of infrared thermography to examine sex differences in parental care from a physiological perspective. Using incubating male and female zebra finches (*Taeniopygia guttata*), a species in which the male lacks a brood patch, we measured the surface temperature of the ventral plumage overlying the abdomen and a reference area that does not contact the eggs (thorax) twice per pair. In half of the pairs, clutch size was experimentally enlarged between the two sets of measurements to increase incubation demand. We found that the temperature differential between abdomen and thorax plumage was greater in females than in males, and that abdomen plumage was warmer after clutch enlargement than before in females but not in males. These findings are consistent with morphological sex differences in brood patch development and suggest that male and female zebra finches differ in the way they regulate abdomen versus general body surface temperature in response to variation in incubation demand.

KEY WORDS: Brood patch, Clutch size manipulation, Infrared thermography, IRT, parental care, *Taeniopygia guttata*

INTRODUCTION

Incubating birds must keep their eggs within the narrow range of temperature and humidity that favours optimal embryonic development by transferring heat from their body to the eggs (DuRant et al., 2013; Rahn and Ar, 1974; Webb, 1987). They can regulate heat transfer behaviourally by adjusting their body position and the duration and tightness of contact with the eggs (e.g. Drent et al., 1970; Gorman et al., 2005; White and Kinney, 1974) and physiologically by increasing their metabolic rate (de Heij et al., 2007; Nord et al., 2010; Vleck, 1981) or output of blood flow to the brood patch (Midtgard et al., 1985). The brood patch is typically a defeathered, swollen and highly vascularised area of ventral abdominal skin that develops under hormonal control around the time of egg-laying and incubation in many bird species (Bailey, 1952; Jones, 1971; Lea and Klandorf, 2002). As well as facilitating heat transfer during contact incubation, the brood patch contains sensory receptors that enable incubating birds to detect suboptimal egg temperatures (Drent et al., 1970; Lea and Klandorf, 2002; White and Kinney, 1974).

Even in biparental incubators, where both males and females contribute to warming the eggs, brood patch development can differ between the sexes. In most passerines, only the female develops a brood patch (Lea and Klandorf, 2002). Although we might expect the sex with the more developed brood patch to maintain higher steady-state incubation temperatures or re-warm cold eggs more rapidly, empirical evidence of this is mixed. Females warm eggs more rapidly (Kleindorfer et al., 1995) or to a higher temperature (Voss et al., 2008) than males in many passerine species, but in others the males warm eggs to a similar or even higher temperature than the females, even in species in which the males lack a brood patch (Auer et al., 2007; Zann and Rossetto, 1991). While these studies focused on the temperature of the egg, few have compared sex differences in heat output at the parental body surface itself [for exceptions, see Bartlett et al. (Bartlett et al., 2005) and Deeming and Du Feu (Deeming and Du Feu, 2008) in passerines, and Massaro et al. (Massaro et al., 2006) in yellow-eyed penguins, *Megadyptes antipodes*]. Such measurements are useful because they enable heat output from the parents to be studied independently of potentially confounding behavioural effects on egg temperature, which might also differ between the sexes.

Here we examined sex differences in heat output from incubating zebra finches, *Taeniopygia guttata* (Vieillot 1817). During the day, free-living males and females invest an equal share of time in incubation (Zann and Rossetto, 1991), whereas females in captive domesticated populations spend more time incubating than males (Burley, 1988; Gorman and Nager, 2003; Hill et al., 2011), and females incubate alone at night in the wild and in captivity (Zann and Rossetto, 1991). Females develop the morphological characteristics of a brood patch (e.g. skin colour change and oedema formation) before the clutch is complete, although without the degree of vascularisation seen in most other passerine species (Zann and Rossetto, 1991; Zann, 1996). These characteristics do not develop in male zebra finches. The apterium is relatively bare throughout the year in both sexes, and the female loses the few down feathers she has during laying.

Based upon these morphological observations, we hypothesised that incubating females will emit more heat from the ventral abdomen than males, and we measured the temperature of the ventral plumage using infrared thermography (IRT). IRT uses known properties of an object's surface and simple physical laws to determine the object's surface temperature from the infrared radiation it emits (Speakman and Ward, 1998). We compared plumage temperature in males and females at two ventral sites: one over the area where the brood patch occurs in females (abdomen) and another away from the brood patch (thorax) to give an approximation of general body surface temperature.

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Table 1. Mean ± s.e.m. plumage thickness and 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

Incubating a large clutch requires greater energy expenditure than a small clutch (Biebach, 1984; de Heij et al., 2007; Nord et al., 2010), and so we experimentally enlarged clutch size between measurement days to test whether birds would respond to the increased demands of keeping eggs warm by increasing heat output. We expected to see a greater increase in abdominal heat output in response to clutch enlargement in females than in males due to the presence of the brood patch in females, which enhances blood flow and sensory perception in the region in contact with the eggs. The results of this experiment will provide a better understanding of how parent birds physiologically adjust expenditure to variation in incubation demand.

RESULTS

Male and female zebra finches incubating natural clutch sizes did not differ in the thickness of plumage on the ventral surface [β=0.31, credible interval (CI)=–0.57 to 1.20, P=0.488, N=66 measurements from 33 individuals and 17 pairs on incubation day 6; Table 1]. Thorax plumage was thicker than abdomen plumage (β=1.22, CI=0.32 to 2.08, P<0.007; Table 1), and the effects of body part (thorax or abdomen) on plumage thickness did not depend on the bird's sex (β=–0.72, CI=–2.38 to 1.03, P=0.407). Abdomen plumage thickness measurements were repeatable between incubation days 6 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=0.75±0.11, P<0.001); thorax plumage thickness was repeatable in males ($F_{15,16}$=5.34, r=0.67±0.14, P<0.001) but not in females ($F_{15,16}$=0.97, r=–0.05±0.25, P=0.523).

Thorax plumage temperature did not differ between the sexes and was not associated with thorax plumage thickness or clutch size in birds incubating natural clutch sizes (incubation day 6; Tables 1, 2). Abdomen plumage temperature, by contrast, decreased with abdomen plumage thickness and the relationship between thorax plumage temperature and abdomen plumage temperature differed between the sexes (Table 2): in females, the abdomen plumage was warmer than the thorax plumage (β=–1.42, CI=–2.43 to −0.48, P=0.007; Table 1), but there was no difference between thorax and abdomen plumage temperature in males (β=–0.42, CI=–1.62 to 0.74, P=0.473).

Abdomen and thorax plumage temperatures did not differ between incubation days 6 and 8 in control birds. There was a non-significant trend towards warmer abdomens in control females (32.5±0.53°C; Table 3) than in control males (31.7±0.61°C), but the sexes did not differ in thorax plumage temperature (females: 30.5±0.35°C, males: 30.9±47°C; β=0.01, CI=–0.02 to 0.05, P=0.510). Abdomen plumage temperature was repeatable between incubation days 6 and 8 in control females ($F_{7,8}$=9.98, r=0.82±0.12, P=0.002) but not significantly so in control males ($F_{7,8}$=3.46, r=0.55±0.25, P=0.052). Thorax plumage temperature was not repeatable in control males ($F_{7,8}$=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).

Thorax plumage temperature was warmer in treatment group females than in treatment males on incubation days 6 and 8, but was not influenced by the clutch size enlargement or an interaction between sex and clutch enlargement (Table 4, Fig. 1). However, the effects of incubating an enlarged compared with a control clutch on abdomen plumage temperature differed between the sexes (Table 4): female abdomens were warmer after the clutch size enlargement than before it, but male abdomen plumage temperature did not change (Fig. 1). This result was qualitatively similar when the ventral temperature differential (abdomen plumage temperature minus thorax plumage temperature) was used as a response variable (linear mixed effects model controlling for plumage thickness, individual identity and pair identity: sex × clutch enlargement β=–1.13, CI=–2.14 to −0.15, P=0.026).

DISCUSSION

We examined sex differences during incubation by comparing the ventral heat output of male and female zebra finches using IRT. The plumage of females incubating natural clutch sizes was warmer at the abdomen than the thorax, which we used as a proxy for general body surface temperature, but the two areas did not significantly differ in temperature in males. Similarly, in incubating female house sparrows, *Passer domesticus*, the abdomen was warmer than a control area (the back) in females but not in males (Bartlett et al., 2005). Moreover, female zebra finches appeared to respond to the challenge of incubating experimentally enlarged clutch sizes by increasing heat output from the abdomen (adjusted for general body

Table 2. The effects of a bird's sex and other variables on thorax and abdomen plumage temperature on day 6 of incubation (33 measurements from 17 pairs of zebra finches; see Table 1)

	β	Credible interval	P
Thorax plumage temperature			
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
Abdomen plumage temperature			
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 × 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.

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 Materials and methods). Significant fixed effects are shown in bold; non-significant fixed effects were removed from the models.

Table 3. The effects of incubation day (6 versus 8) and other variables on thorax and abdomen plumage temperature in control pairs (34 measurements from 18 individuals from nine pairs) of zebra finches

	β	Credible interval	*P*
Thorax plumage temperature			
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
Abdomen plumage temperature			
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.
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.

temperature) relative to their own output before the clutch size manipulation. By contrast, we observed no change in heat output in males.

A sex difference in plumage temperature could be due to males and females generating different amounts of heat, differing in insulation in layers above the heat-generating tissue (plumage, skin and subcutaneous tissue) or both. Plumage thickness is the main contributor to insulation in several bird species (McCafferty et al., 1997), but we found no sex difference in plumage thickness in our population, suggesting that the differences measured here are due to differences in the output of generated heat. These results are consistent with differences between male and female zebra finches in brood patch morphology (Zann and Rossetto, 1991; Zann, 1996) and suggest that the sexes differ in their ability or willingness to increase abdomen temperature above general body temperature in response to variation in incubation demand. To our knowledge this has not been demonstrated previously.

It is worth emphasising that we did not measure brood patch skin temperature but the temperature of the contour feathers overlying the egg-contact region. The aim of this was to minimise variability between measurements and disturbance to the birds associated with instrument attachment and handling. The difference between the abdomen temperatures presented here (32.5±0.33°C for females on incubation day 6) and the higher temperatures reported elsewhere for brood patch skin [41.2±0.11°C, mean ± s.e.m. for 24 passerine species, mainly measured in females (Deeming, 2008)] highlights the excellent insulating capacity of the plumage, even during incubation, when the down feathers have been lost. In addition, we found that abdomen plumage temperatures decreased as plumage thickness increased, in agreement with studies of mounted specimens of passerines, quails and owls (McCafferty et al., 1997; Walsberg, 1988). The exact gradient of heat loss from the skin to the surface of the plumage is likely to be more complex than is currently understood, and may depend on the type, quality, number and placement of feathers overlying the skin (Wolf and Walsberg, 2000). Although plumage temperature measurements are not a substitute for direct measurements of brood patch temperature, they are valuable in studies such as this where the aim is to detect relative changes in heat output. In particular they are likely to improve the precision of within-individual studies where the greatest source of variability is due to measurement error.

While females incubating enlarged clutches on incubation day 8 had warmer abdomen plumage than on day 6, there was no change in abdomen heat output in control birds incubating unmanipulated

Table 4. The effects of sex and clutch size enlargement on thorax and abdomen plumage temperature on days 6 and 8 of incubation (repeated measures) in 10 treatment pairs (36 measurements; see Fig. 1)

	β	Credible interval	*P*
Thorax plumage temperature			
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
Abdomen plumage temperature			
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.
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.

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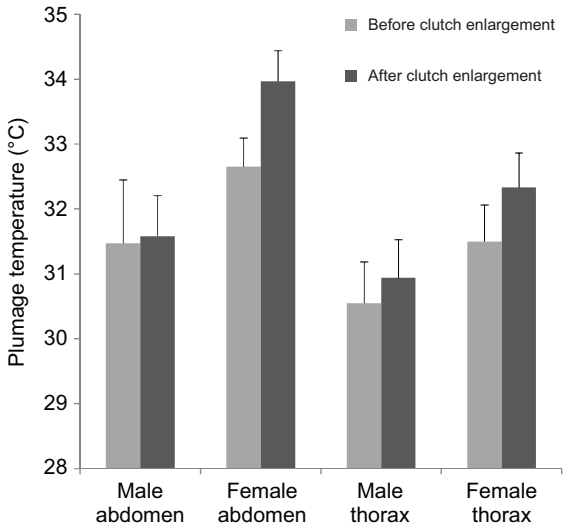

Fig. 1. Abdomen and thorax plumage temperature (means + 1 s.e.m.) in incubating male and female zebra finches before (incubation day 6, *N*=8 pairs) and after (incubation day 8, *N*=10 pairs) a clutch enlargement manipulation. There was a clutch enlargement × sex interaction on abdomen plumage temperature (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 or an interaction between sex and clutch enlargement.

clutch sizes. The response in the former group of females is therefore most likely due to clutch size enlargement rather than changes over the incubation period. Females may respond to increased incubation demand by directing warm blood to arterioles that lie close to the brood patch surface and that typically increase in musculature as part of brood patch development (Midtgard et al., 1985; Petersen, 1955). This might occur to even a greater extent in females of other passerine species whose brood patches exhibit more pronounced vascularisation than in zebra finches.

Treatment male zebra finches, unlike females, did not respond to the clutch size enlargement by increasing their abdomen temperature. While this could imply a reduced ability or willingness to transfer heat in incubating males compared with females, Zann and Rossetto did not observe a sex difference in steady-state incubation temperature or the rate of re-warming cool eggs in this species, and speculated that male zebra finches increase heat transfer to the eggs by increasing metabolic rate (Zann and Rossetto, 1991). Our findings do not support this idea because male thorax temperature was not higher than female thorax temperature and did not increase in response to clutch enlargement. Indeed, females allocated to the treatment group had warmer thoraxes than males and there was no sex difference in thorax temperature in control birds. Nevertheless, metabolic rate has rarely been measured in incubating males and it would be valuable to compare the metabolic rates of incubating males and females directly.

A new question raised by this study is whether the difference in temperature between the abdomen and thorax in females exists only in incubating birds. As the temperature of the abdomen plumage relative to the thorax changed with incubation demand, we believe that it is reasonable to suggest that at least some of these differences are related to incubation. A better understanding of this issue would provide information on brood patch function and could be tested by comparing abdomen and thorax measurements taken during incubation with those from the same females before the brood patch develops or after it regresses. If the female has a true brood patch, we would predict that the abdomen would be warmer in incubating than in non-incubating females and that there would be no difference between incubating and non-incubating females in thorax temperature or between abdomen and thorax temperature in non-incubating females.

Maintaining a brood patch is likely to be costly in terms of increased heat loss (Haftorn and Reinertsen, 1985), and we would expect such costs to be offset by an associated fitness benefit, such as an increased ability to keep the eggs at favourable conditions for optimal embryo development and survival. Lower egg temperatures in males than females during steady-state incubation have indeed been reported in some species of biparentally incubating passerines (Reid et al., 2002; Voss et al., 2008). However, no sex differences in steady-state incubation were found in other species, including zebra finches, as noted above, in spite of the absence of a brood patch in males (Kleindorfer et al., 1995; Zann and Rossetto, 1991), and in chestnut-vented tit-babblers, *Parisoma subcaeruleum*, eggs were warmer when incubated by males than by females (Auer et al., 2007). Males have been observed to re-warm clutches after an incubation break more slowly than females in some species (Kleindorfer et al., 1995; Voss et al., 2008), while in others males re-warmed clutches more quickly than females (Reid et al., 2002), and no clear difference between the sexes was seen in others, including zebra finches (Auer et al., 2007; Hill, 2009). These conflicting results seem to suggest that the brood patch is not associated with improved performance during incubation. However, we need to know more about sex differences in the costs of incubation and how the brood patch might moderate these costs before we can draw a conclusion. The presence of a brood patch might reduce the risk of tissue damage because of the protective thickening of the epidermal skin (Jones, 1971), allow individuals to sustain longer or more frequent incubation bouts or expend less effort to achieve the same thermal output (Auer et al., 2007), or enable them to detect non-optimal egg temperatures through the sensory receptors it contains (Drent et al., 1970; White and Kinney, 1974).

In our study, upregulation of heat transfer to the brood patch in females after the clutch size manipulation could be a tactile response to the increased number of eggs or a thermal response to a decrease in mean egg temperature. Mean egg surface temperature (both sexes pooled) was inversely related to natural variation in clutch size in our population (Hill, 2009), and so we would expect the clutch enlargement in the present study to produce a similar decrease in egg temperature. However, males might fail to perceive such changes in temperature or clutch size without a brood patch. They do, however, seem able to respond to variation in clutch size by adjusting the amount of time spent incubating. Male European starlings, *Sturnus vulgaris*, increased incubation attentiveness following clutch size enlargement and decreased it when clutch size was reduced (Komdeur et al., 2002), and incubation attentiveness was positively related to natural clutch size in male zebra finches (Hill et al., 2011). These results suggest that the absence of a brood patch does not impair a male's ability to detect changes in clutch size, although what cues they use is unknown.

Sex differences in the regulation of heat transfer to offspring have also been recorded in humans. Studies of 'kangaroo care', where a newborn human infant is placed in skin-to-skin contact upon the parental breast, show that mothers adjust their breast temperature in response to their infants' immediate thermal needs whereas fathers maintain a high heat output that can cause infants to become overheated and even hyperthermic (Chiu et al., 2005; Ludington-

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Hoe et al., 1992; Ludington-Hoe et al., 2006). These findings, in combination with our own, point to interesting differences between male and female parents in the modulation of offspring temperature, and suggest that such differences may be more widespread in endotherms than is currently recognized.

Our results suggest that males and females respond differently to the demands of incubation. Understanding sex differences in the effectiveness of parental care has implications for our understanding of sex role divergence and for interpreting empirical studies of sexual conflict over parental effort. It might be maladaptive for males to increase their incubation effort to levels shown by females because they are less certain of their relatedness to the offspring (Queller, 1997; Trivers, 1972) and can potentially obtain greater fitness benefits from seeking extra-pair copulations than attending to the eggs or offspring, depending on the availability of receptive females (Bateman, 1948; Kokko and Jennions, 2008; Magrath and Komdeur, 2003). Perhaps for this reason the complex morphological adaptations associated with the brood patch have not evolved to the same extent or have not been conserved in male passerines. The sex difference we observed in the birds' response to the clutch size enlargement could reflect the outcome of a conflict that has been resolved over evolutionary time or differences in willingness to respond (even if individuals are capable of doing so) measured over ecological time. In practice it may not be possible to determine whether males are unable or unwilling to adjust abdominal temperature, and in any case sex differences in ability and willingness are likely to have arisen as a consequence of the same evolutionary pressures. Nevertheless, by contributing to incubation, males play an important role in relieving the female in times of energetic stress (Kleindorfer et al., 1995; Smith and Montgomerie, 1992) and in reducing the substantial energetic demands of re-warming cold eggs after the female returns from a foraging bout (Vleck, 1981; Voss et al., 2008).

MATERIALS AND METHODS

Subjects and maintenance

This study was carried out on domesticated zebra finches bred at the University of Glasgow and conforms to the 'Guidelines for the treatment of animals in behavioural research and teaching' (Buchanan et al., 2012). Prior to pairing, the sexes were kept separately at a density of six individuals per cage (122×41×45 cm length × width × height) under a 'short day' light regime (10.5 h:13.5 h light:dark) using daylight-spectrum fluorescent tubes (Arcadia Bird Lamp FB36) with a gradual change in light at dawn and dusk. Birds received *ad libitum* mixed seed consisting of canary millets (Foreign Finch Mixture; Galloway MacLeod, UK), oyster grit, cuttlefish bone and water. Egg biscuit protein with vitamin (Daily Essentials 2; The Birdcare Company, Nailsworth, UK) and calcium (Calcivet; The Birdcare Company) supplements were provided three times a week and fresh spinach leaves twice a week.

Males and females without previous breeding experience were size matched according to tarsus length. To stimulate breeding, we increased daylight hours to 12 h:12 h light:dark 1 week before birds were paired and maintained this 'long day' regime until the end of the experiment. Each pair was kept in a breeding cage (60×40×50 cm length × width × height) with access to a nestbox and coconut fibre nesting material. Nestboxes were checked daily from pairing, and lay date (the date that the first egg of a clutch was laid) and clutch size were recorded. All pairs that laid in the nestbox were included in the study.

Experimental design

We compared ventral plumage temperature in male and female zebra finches incubating unmanipulated ('control', N=9 pairs) and experimentally enlarged clutch sizes where we increased incubation effort by adding two dummy eggs (see below) to a pair's natural clutch size ('treatment', N=10). Each pair was alternately allocated to control or treatment group according to lay date. Control and treatment birds did not differ in the number of eggs they laid (control: 4.8±0.52 eggs, mean ± s.e.m.; treatment: 5.4±0.54 eggs; $F_{1,17}$=0.68, P=0.422) or tarsus length (control males: 13.9±0.16 mm; treatment males: 14.2±0.15 mm; $F_{1,17}$=1.84, P=0.193; control females: 14.3±0.21 mm; treatment females: 14.5±0.17 mm, one measurement missing; $F_{1,16}$=0.69, P=0.780).

We assumed that zebra finches begin incubating ('day 0') on the fourth day of laying in clutches of five eggs or more, and on the final day of laying in smaller clutches; the incubation period spans 11–15 days (median 14 days) using these criteria (Zann and Rossetto, 1991). On day 2 of incubation, we replaced all eggs with an equivalent number of dummy eggs made of Fimo modelling clay (Eberhard Faber, Neumarkt, Germany). Fimo eggs have similar thermal properties to fresh zebra finch eggs (Gorman, 2005) and do not bring about changes in incubation behaviour compared with natural eggs (Gorman et al., 2005). At dusk on incubation day 7, we added two additional Fimo eggs to the clutches of the 10 treatment pairs; the nine control pairs incubated unmanipulated clutch sizes throughout. This design enabled us to test for sex differences in the ventral heat output of incubating birds both within and between experimental groups.

Temperature measurements

We used IRT to measure temperature at the surface of the ventral plumage. IRT is a non-invasive, non-contact technique that can provide quick and accurate measurements of avian skin and plumage temperatures (McCafferty, 2013). We simultaneously measured the surface temperature of the undisturbed contour feathers overlying two regions on the ventral side of the birds: the area that comes into contact with the eggs, corresponding to the brood patch in females (hereafter abdomen temperature), and a control area anterior to the brood patch that does not contact the eggs (hereafter thorax temperature). This allowed us to test whether the output of heat from the abdomen is regulated independently of the rest of the body. We measured plumage rather than skin temperature to remove potential biases and variability associated with parting the contour feathers by hand to expose the brood patch and thorax skin, which could influence temperature and handling time. The insulating properties of plumage increase with plumage thickness in several species of terrestrial birds (McCafferty et al., 1997; Walsberg, 1988) and so we measured plumage thickness at the abdomen and thorax (see below) to account for this variation.

IRT images were taken using the ThermaCam™ E300 (FLIR Systems, Burlington, Canada) on incubation days 6 and 8, that is, just before and after treatment pairs experienced the clutch size manipulation (incubation day 7) and when nest attentiveness reaches its maximum (Gorman and Nager, 2003). We gently displaced each incubating bird with a tap to the nestbox and caught the bird without touching its underside. The bird was then held with its ventral surface at a distance of 0.20 m from the camera, which was supported on a fixed tripod. We took three replicate images of the bird's ventral surface and recorded the time elapsed between the displacement of the bird from the nest and the image being taken ('image latency', mean 65.8±3.97 s, N=70 images from 38 birds and 19 pairs; all reported values are based on the single best-resolved of the three replicate images). Some individuals were not observed incubating on the designated days, and so thermal images are missing for day 6 for two treatment pairs and one control female and on day 8 for the partner of the latter female. Neither abdomen plumage temperature (β=–0.96, CI=–2.53 to 0.65, P=0.227 controlling for pair identity and plumage thickness) nor thorax plumage temperature (β=–0.001, CI=–0.004 to 0.001, P=0.211 controlling for pair identity; plumage thickness was not significant) was associated with image latency (N=33 individuals from 17 pairs on day 6 of incubation). Immediately after taking thermal images on incubation days 6 and 8, we measured plumage thickness at the thorax and abdomen to the nearest 0.5 mm by pushing a discarded primary feather, marked along the shaft at 1 mm intervals, through the plumage down to the skin.

We used the software ThermaCAM™ QuickReport 1.1 (FLIR Systems, 2007a) to visualise the digital images and ThermaCAM™ Reporter 8.3 (FLIR Systems, 2007b) to analyse the best resolved of the three replicates taken for each individual and incubation day. The radiation measured by the camera is a function of the object's surface temperature and emissivity,

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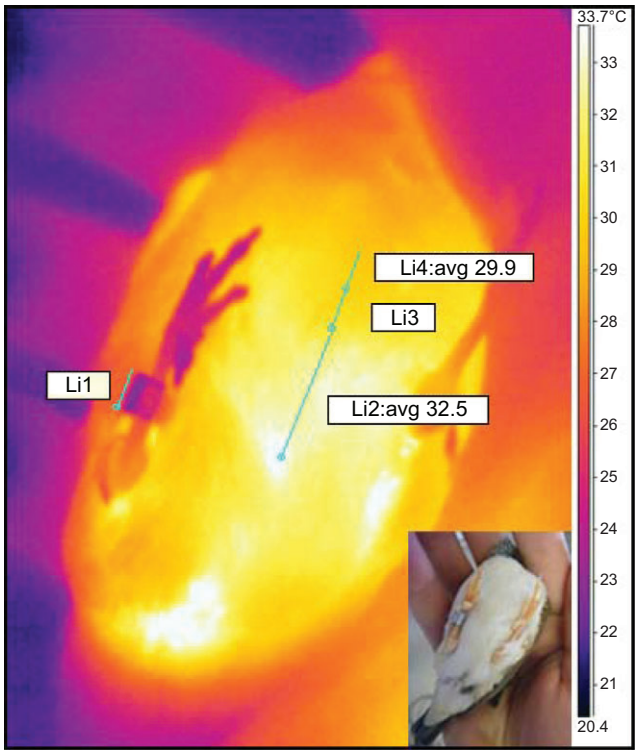

Fig. 2. Sample thermal image of the ventral surface of a male on day 6 of incubation illustrating the procedure used for measuring plumage temperature along a transect following the sagittal plane of the ventral surface, and (inset) a digital reference image of the same bird. We standardised the relative 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.

ambient temperature, and absorption and scattering by atmospheric humidity. We set the surface emissivity value to 0.98 for the bird's plumage (Hammel, 1956) and temperature and relative humidity to the mean values recorded in the room during the measurement period (22.2±<0.01°C and 13.6±0.04%, respectively). To standardise the size and positioning of measurement areas between images, we placed a sagittal line along the image of the bird's ventral surface (Fig. 2) consisting of three transects that were scaled to the length of the bird's leg ring, which was visible on all images. This was done by tracing a straight digital line along the length of the ring (Fig. 2, Li1) with the polygon tool in ThermaCAM™ Reporter 8.3, noting the ring's length in pixels, and then producing three transects of the same pixel length (Fig. 2, Li2, Li3, Li4). The first transect (Li2=abdomen) was extended by a factor of three, as the brood patch was at least three times the length of the ring, and placed along the sagittal plane on the region of the thermal image corresponding to the plumage over the brood patch in females. Li3 and Li4 were placed at the apex of Li2 and Li3, respectively, to standardise the distance between the two measurement areas (Li2=abdomen and Li4=thorax). We then calculated the mean pixel temperature along Li2 (abdomen plumage temperature) and Li4 (thorax plumage temperature).

Statistical analysis

All data were analysed in R version 3.0.1 (R Development Core Team, 2013). Thorax plumage temperature measurements were normalised by log transformation when used as a response variable and abdomen plumage temperature measurements were squared to allow parametric tests to be carried out.

We tested whether the thickness of plumage overlying the abdomen and the thorax differs between the sexes and the body part in birds incubating unmanipulated clutch sizes (incubation day 6). We fitted a general linear mixed-effects model (LMM) with plumage thickness as the response variable, sex and body part (abdomen or thorax) as fixed factors and individual and pair identity as random factors. We included the interaction between sex and body part to test whether a difference in plumage thickness between the two parts of the body depends on the bird's sex. We calculated the within-individual repeatability (r) of plumage thickness between incubation days 6 and 8 (following Lessells and Boag, 1987) and its standard error (Becker, 1984) to allow us to assess measurement precision.

To see whether males and females incubating unmanipulated clutch sizes differed in heat output, we fitted LMMs to data from incubation day 6. This first involved testing whether the sexes differed in general body temperature using thorax plumage temperature as the response variable, and then whether males and females differentially regulate abdomen temperature relative to general body temperature by fitting thorax plumage temperature and the interaction between sex and thorax plumage temperature as fixed effects. We included pair identity as a random factor, sex as a fixed factor and clutch size and plumage thickness measured on incubation day 6 as covariates in both models. Variance inflation factors calculated by the car package (Fox and Weisberg, 2012) were <1.23 in both models.

If females have a true brood patch but males do not, we should expect only females to maintain brood patch temperature above the temperature of the rest of the trunk. To test whether this is likely to be the case, we ran two separate LMMs, one for each sex, with thorax and abdomen plumage temperature (which were normally distributed when pooled) on incubation day 6 as a single response variable, pair identity as a random factor, body part as a fixed factor and plumage thickness on day 6 as a covariate.

To see whether ventral heat output changed between incubation days 6 and 8 in unmanipulated birds, we compared temperature measurements between the two days in control birds using separate LMMs for thorax and abdomen plumage temperature. In both models we fitted plumage thickness, sex, day of incubation and the interaction between sex and day of incubation as fixed effects and individual and pair identity as random effects. Where abdomen plumage temperature was the response variable, we also controlled for thorax plumage temperature.

There was no effect among control birds of incubation day on either abdomen or thorax plumage temperature (see Results). Consequently, differences in temperature between incubation days in the treatment group are likely to be related to the clutch size manipulation. We therefore compared treatment group individuals before (day 6) and after (day 8) the clutch size enlargement. We tested for an effect of sex and clutch enlargement as well as the interaction between the two on abdomen and thorax plumage temperature in LMMs to determine whether the sexes respond differently to the challenge of incubating an enlarged clutch.

We fitted LMMs by restricted maximum likelihood using the lme4 package (Bates et al., 2013). We removed interaction terms $P \geq 0.05$ in order of least significance and then non-significant main effects to reach the final model. Significance is denoted by a two-tailed P-value <0.05 or a CI that does not include zero. We present the intercept (constant) for all models containing significant fixed effects. Random effects were fitted with random intercepts only, and were controlled for even when not statistically significant. For all fixed effects tested, we present the model coefficient β with CIs calculated at the 95% confidence level using Markov chain Monte Carlo sampling with the chain length fixed at 10,000 and P-values based upon the posterior probability distribution. These estimates were calculated using the pvals function from the languageR library (Baayen, 2013). Means are presented ± s.e.m.

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Competing interests
The authors declare no competing financial interests.

Author contributions
All authors conceived and designed the experiment. D.L.H. carried out the experiment and the thermal and statistical analyses, and wrote the manuscript. R.G.N., J.L. and D.J.M. commented on the manuscript.

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