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Sexual Conflict and Division of Labour during Incubation

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

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Thesis abstract

Individuals have limited time and energy and so they face a trade-off between the resources they allocate to parental, mating and self-maintenance activities and between care of current and future offspring. Within a biparental system, models predict that an individual will do better if it can reduce its own level of investment and rely on its partner to compensate, but that the partner should not compensate completely or it will be exploited. The costs and benefits of care can differ between males and females, leading to sex-specific differences in optimum resource allocation to a given breeding attempt. Sexual conflict can occur over evolutionary or ecological time, as predicted by ‘sealed bid’ and ‘negotiation’ models of parental investment, respectively.

While researchers have traditionally used handicapping or mate removal to induce variation in paternal effort and then measure the female response, I achieved this by carrying out two experiments in which I separately manipulated (a) paternal certainty and (b) male extra-pair copulation (EPC) opportunity during incubation in a biparentally incubating passerine, the Zebra Finch, *Taeniopygia guttata*. Both experiments were based on a ‘crossover design’, which took place over two breeding attempts: in the first attempt half the pairs experienced a treatment (i.e. reduced paternal certainty or increased male EPC opportunities, depending on the experiment) while the remaining pairs were control; in the second breeding attempt the type of treatment was switched between groups. This allowed me to explore the effects of previous events on current effort. I also statistically controlled for male attractiveness, based on association times obtained from mate choice trials, as this varies between males, and females may respond differently to it.

In the first round of the experiments, when all subjects lacked breeding experience, treatment males reduced incubation effort relative to controls. However, when the treatments were switched, males continued to invest the same level of effort that they had shown previously, which suggests that individuals may use early life experiences to shape their later responses. Males and females responded to each other’s contributions over ecological time, and control and treatment pairs negotiated different patterns of effort. Contrary to predictions of most game theoretic models, treatment females compensated fully for the reduction in partner effort.

Since there can be a trade-off in investment between reproductive stages and attempts, I tested whether compensating females showed reduced reproductive output in a subsequent breeding attempt. In the second attempt, clutch size was negatively related to the amount

of time spent incubating the first clutch in females that had previously fledged a large brood. I also found that the number of offspring surviving to maturity increased with total incubation attentiveness. Together, these findings suggest that incubation attentiveness is an appropriate proxy for parental investment since it enhances offspring survival at a cost to the mother's future fitness.

Incubation effort can be measured in terms of temperature as well as time, and the sexes may differ in the quality of the incubation they provide for a given unit of time. In the Zebra Finch, the female alone possesses a brood patch, a bare area of thickened, loose ventral skin which facilitates heat transfer from parent to egg. I examined sex differences in incubation ability by comparing the ventral surface temperature of incubating males and females using infra-red thermography (IRT) under standard incubation conditions and also under more challenging conditions where the energetic costs of incubation had been experimentally increased by a clutch size manipulation. The ventral surface was warmer in females than males under standard conditions, and ventral temperature was higher than baseline body temperature in females but not males. Moreover, females increased ventral temperature after the clutch enlargement, whereas males showed no response. These results suggest that the sexes differ in their ability or willingness to incubate, presumably due to sex differences in parental certainty or mate availability.

Since the female has a warmer ventral surface and spends more time incubating than the male, I predicted that she would be able to re-warm cool eggs more quickly and incubate eggs to a higher temperature. I used IRT to measure egg surface temperature and a temperature probe inside a dummy egg to measure rates of re-warming. Following the clutch size enlargement, the variance in egg temperature increased with clutch size more rapidly in males than in females, but no sex differences were otherwise detected in clutch surface temperature or rate of re-warming. This suggests that there are differences in the quality of incubation provided by males and females for a given contribution of time, but that these are subtle.

In conclusion, variation in male and female incubation effort can be influenced by paternal certainty, male EPC opportunity, clutch size and the partner's behaviour and these effects can be observed over ecological time. The flexibility of an individual's response depended on the type of cue and the stage in an individual's reproductive life that the cue was presented. Variation in parental effort can influence offspring fitness, and females that invest more effort in one breeding attempt may be unable or unwilling to do so in a

subsequent attempt. Male and female parents can differ in their abilities to care and this can lead to differences in the amount and quality of care provided.

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Declaration

I declare that the work presented in this thesis is entirely my own unless otherwise cited or acknowledged. It has not been submitted for any other degree, in whole or in part.

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Chapter 1 - Sexual conflict and division of labour during incubation: General introduction

Sexual conflict over parental care

Sexual conflict is a conflict of interests caused by an asymmetry between the sexes in the costs and benefits of reproduction (Trivers 1972; Parker 1979; Arnqvist and Rowe 2005), and it can take place over evolutionary or ecological time. Pre-zygotic conflicts are those that occur prior to fertilisation, such as where optimal mating frequency or duration differ between the sexes. Post-zygotic conflicts occur following fertilization, and generally equate to conflict over the relative amount of effort each parent invests into offspring (Trivers 1972). Differences between the sexes in mate availability, paternal certainty and the intensity of sexual selection are thought to promote sex role divergence (Trivers 1972; Queller 1997; Kokko and Jennions 2008; Kunz and Hosken 2009), resulting in one sex, typically the male, being selected to invest more into mating competition (Berglund *et al.* 1996; Kokko *et al.* 2006), and the other, usually the female, investing more into parental care (Kokko and Jennions 2008). Parental care is costly in terms of time and energy, either of which could be reallocated to activities that enhance survival or residual reproductive fitness of the parents (Williams 1966; Trivers 1972; Clutton-Brock 1991). The sexes can differ in the costs and benefits of parental care relative to other activities and this can lead to differences between them in division of labour over care (Trivers 1972; Chase 1980; Parker *et al.* 2002).

Within biparental systems, where both parents play a role in the care of offspring, each parent will be at an advantage if it can reduce its own investment into the young and rely on its partner to make a greater contribution (Trivers 1972; Lessells 1999; Houston *et al.* 2005). The distinction between parental investment and parental effort is an important one: Trivers (1972) defined parental investment as ‘any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring’. Parental effort, the amount of time or energy that a parent contributes to care, does not necessarily enhance offspring survival at a cost to the parents’ future fitness but is used as a proxy for parental investment in the majority of studies (e.g. Kopisch *et al.* 2005; Kosztolanyi *et al.* 2009; Lendvai *et al.* 2009). One of the aims of this thesis is to address this issue by integrating a number of measures of parental effort over incubation, namely incubation attentiveness (time spent incubating), incubation temperature and the rate at which parents re-warm the eggs, with differences in the costs of incubation between the sexes and with the fitness consequences of variation in effort.

Avian systems are convenient for studying the costs of reproduction since the breeding cycle is partitioned into distinct phases, which include incubation of the eggs and nestling provisioning. Most empirical work has so far concentrated on sexual conflict over nestling provisioning (e.g. Kempenaers *et al.* 1998; Hinde and Kilner 2007), but the number of studies that use incubation to address these questions is expanding. The experimental work presented in this thesis will focus on division of labour between the sexes during incubation in a captive population of the Zebra Finch (*Taeniopygia guttata*), a socially monogamous biparentally incubating passerine. Here I summarise theoretical and empirical treatments of division of labour over both incubation and provisioning in birds, with emphasis on the optimal response of an individual to a reduction in its partner's parental investment.

The optimal response to a lazy partner

A key issue in the behavioural ecology of sexually reproducing species is the level of effort each parent should invest into the offspring. This problem can be approached as a game of two players, a male and a female, each of whom aims to maximise his or her respective lifetime fitness returns (Chase 1980; Houston and Davies 1985; McNamara *et al.* 1999). The outcome of this game depends upon the relative costs and benefits of investment to each partner (Parker 1979; Yaniv 2005), the behaviour of each partner (Houston and Davies 1985) and the behaviour of other members of the population (Webb *et al.* 1999). Each player will do better if it can minimise the level of effort it invests into a particular breeding attempt whilst relying on its mate to pay the costs by compensating (Trivers 1972; Lessells 1999; Houston *et al.* 2005). The risk that the offspring will die if the mate is unable or unwilling to compensate sufficiently can set a limit to this laziness (e.g. Jones *et al.* 2002).

Most game theoretic models predict that partial compensation for a reduction in a partner's investment is the most evolutionarily stable strategy (ESS) under biparental care (e.g. Chase 1980; Maynard Smith 1982; Houston and Davies 1985; Winkler 1987; Kacelnik and Cuthill 1990; McNamara *et al.* 1999) and that an individual will be exploited by its partner if it is willing to compensate for it completely. However, in species in which one parent cannot care alone and where even a small decrease in care endangers offspring survival, individuals may compensate completely to ensure that their partners' body condition does not fall so low that they can no longer care (Jones *et al.* 2002; see also Johnstone and Hinde 2006).

These optimal response models fall into two groups according to the timescale over which an investment decision is reached (McNamara *et al.* 1999). The earliest models, including those formulated by Chase (1980) and Houston & Davies (1985), were built upon the assumption that each individual shows a fixed level of investment throughout its reproductive life, which is the average best response given the average best response of its partner, and these have been termed ‘sealed envelope’ (Houston and McNamara 1999) or ‘sealed bid’ (Schwagmeyer *et al.* 2002) models. Any adjustments to investment occur as mutant strategies that invade the population over evolutionary time. These models predict that an individual will show a level of parental effort that is consistent within and between breeding attempts and is independent of its mate’s input (e.g. Schwagmeyer *et al.* 2002), although it may be influenced by other factors, such as clutch size or age of offspring (Moreno 1987; Carey 1990).

Subsequent theoretical work led to the development of ‘negotiation’ models in which each player is able to modify its level of effort sequentially in response to its partner’s effort and the environment over ecological time (McNamara *et al.* 1999). Since environmental and phenotypic variables, such as EPC (extra-pair copulation) opportunity or physical state, can change over ecological time and the information that would help individuals to make appropriate life history decisions is not always available (e.g. the partner’s parental ability at the time of pairing), negotiation would enable the partners to form a pattern of effort that is the best given the current situation and the current availability of information (McNamara *et al.* 1999; Houston *et al.* 2005; Johnstone and Hinde 2006).

Whether the response we see is fixed or plastic may depend on the degree of variability in the costs and benefits of parental care to that species, on the level of information available concerning these costs and benefits and on the ability of the species to assimilate this information (Johnstone and Hinde 2006; Hinde and Kilner 2007). These factors combine to determine where a particular species or individual lies on a ‘negotiation continuum’ between a fixed response at one extreme and a response that is sensitive and variable at the other (Hinde and Kilner 2007). Where certain aspects of the environment do not change or these changes cannot be assimilated over ecological time, evolution may reach a stable endpoint, and we see a sealed bid response (Houston *et al.* 2005).

Empirical tests of the response of an individual to reduced partner effort

A number of experimental approaches have been adopted to test the models described above and determine how individuals respond to a reduction in partner effort. The

majority of these have used handicapping (e.g. Paredes *et al.* 2005; Foster and Burley 2007) or mate removal (e.g. Markman *et al.* 1996; Lendvai *et al.* 2009) to induce a decline in effort and have then measured the partner's response. Others have attempted to redirect male effort from the current clutch or brood to additional mating attempts by providing supplementary nestboxes (e.g. Smith *et al.* 1995), manipulating male badge size (Nakagawa *et al.* 2007b) or implanting the male with testosterone (e.g. Mazuc *et al.* 2003; Schwagmeyer *et al.* 2005). Another study used a capture-restraint protocol to induce stress (Lendvai *et al.* 2009). These studies have reported a range of responses in the unmanipulated partner from full compensation (e.g. Wright and Cuthill 1990a) to no apparent response to the reduction in partner effort (e.g. Hegner and Wingfield 1987; Whittingham *et al.* 1994; Schwagmeyer *et al.* 2002). Here I focus my attention on the two most widely used of these approaches: mate removal and handicapping.

Handicapping manipulations aim to impose artificial asymmetries on the costs of parental care in one sex, and this is usually done by attaching small weights to individuals of that sex or clipping their feathers. When the manipulation succeeds in reducing the parental output of the handicapped individual, researchers can compare the parental effort of its partner to that of control partners. For example, Markman *et al.* (1995) added small weights to the tail feathers of the female Orange-Tufted Sunbird, *Nectarinia osea*. Handicapped females showed a reduction in the rate at which they provisioned nestlings and removed faecal sacs, and also spent less time at the nest, and their partners compensated partially for reductions in these measures of care. The ability of these birds to respond to a change that was induced in ecological time is consistent with predictions of negotiation models.

One criticism of handicapping studies is that the manipulation may bring about a change in the perceived quality or attractiveness of the handicapped individual through the eyes of its partner, and that the partner thus responds to this perceived change rather than to the change in the parental behaviour of its handicapped mate (Schwagmeyer *et al.* 2002; Hinde 2006). This may be one explanation for results described by Sanz *et al.* (2000) and Lozano and Lemon (1996) where the partners of feather-clipped female Great Tits, *Parus major*, and weighted male Yellow Warblers, *Dendroica petechia*, respectively, decreased their provisioning rates in response to a reduction in their partners' work-rates (see also Johnstone and Hinde 2006).

Mate removal studies simulate a situation where the focal individual has been temporarily, and researchers compare its parental effort to that of control individuals whose partners have not been removed. Harrison *et al.* (2009) found that the mean response to mate removal across 25 studies of provisioning behaviour was one of incomplete compensation and that widows increased effort significantly more than the partners of individuals experiencing other types of manipulation. Individuals increased incubation effort in response to removal or manipulation of their mate, but there was no difference in combined male and female incubation effort between treatments and controls, or between mate removal and other types of manipulation.

The greater increase in effort associated with mate removal compared to manipulations of partner provisioning suggests that individuals are capable of working harder than they do under biparental care (Harrison *et al.* 2009), which is what we expect to see when sexual conflict occurs over parental care, given that care is costly (Trivers 1972; Lessells 1999; McNamara *et al.* 2003; Houston *et al.* 2005). The response of females to a manipulated reduction in male provisioning rate was compared with their response to mate removal by Whittingham (1994) in the Tree Swallow, *Tachycineta bicolor*, and Lendvai *et al.* (2009) in the House Sparrow, *Passer domesticus*. Neither study found a change in female provisioning rate in response to the manipulated reduction in male provisioning by handicapping but there was a dramatic increase in female effort when males were removed. Females were only willing to provide their ‘best’ levels of effort once sexual conflict was eliminated (Lendvai *et al.* 2009), as predicted by McNamara *et al.* (2003). These findings suggest that the level of response that females show is context-dependent, and this is consistent with the predictions of negotiation models (McNamara *et al.* 1999).

Further support for this idea comes from a correlational study of Magnificent Frigatebirds, *Frigata magnificens*, a species in which females show full compensation for their partners after they have deserted (Osorno and Szekely 2004). The male contributes to nestling provisioning for a variable period after hatching, but deserts his mate before the chick becomes independent. The female responds to desertion by increasing her provisioning rate compared to her previous effort to such an extent that she overcompensates for her partner’s absence. Similarly, female Zebra Finches raising offspring alone provided greater *per capita* parental effort than females in biparental systems (Royle *et al.* 2002). In addition to this, male offspring resulting from single parent families were more sexually attractive than those raised by a pair. These results suggest that females in biparental systems can withhold care to avoid being exploited or to manipulate the male into working

harder than he would otherwise. As a consequence of sexual conflict, biparental systems do not function at full capacity and this can potentially influence offspring performance.

While mate removal studies can shed light on the behaviour of individuals when the constraints of sexual conflict are removed, they do not address the issue of how an individual should reconcile the need to provide an adequate level of parental care with its own need to avoid being exploited by a partner and maintain body reserves. A widowed parent faces a different set of decisions from the partner of a lazy individual: it must decide whether it has more to gain from abandoning the brood or struggling to raise it alone and paying the cost in terms of reduced body condition and subsequent reproductive output. It is also worth noting that an individual may be unable to do the job of two due to time and energy constraints (Drent and Daan 1980).

The minimum amount of work that an individual partner can do without its partner deserting has been termed the 'laziness threshold' (Jones *et al.* 2002). This laziness threshold lies within a buffer zone since neither individual has complete information regarding its partner's body condition and does not want to risk pushing it too far. In this way, an individual would benefit from being able to assess its partner's body condition accurately so that it could maintain its effort close to the laziness threshold. However, each individual would prefer its partner to perceive its body condition to be lower than it actually is in order to manipulate it to work harder. Information relating to body condition may be exchanged using visual displays or calls at changeover (Galeotti *et al.*, 1997) or perhaps during nest visits or while foraging (Hinde 2006).

The information hypothesis: an integration of cues

The level of care that a parent should provide is often unclear from its own perspective since complete information on the quality or need of the brood or clutch is not always available and the two partners may not be equally informed (Hinde 2006; Johnstone and Hinde 2006). Johnstone and Hinde (2006) showed that, where there is an asymmetry in the parents' levels of information, the less informed parent can infer brood need or quality from the level of effort shown by its partner and may then 'match' this change in parental care by adjusting its own effort in the same direction. We would expect the parental effort of the two partners to be positively related under matching, while compensation predicts a negative relationship. The partner's response will be based on an integration of both direct (partner's behaviour) and indirect (e.g. nestling begging signals, incubation temperature) cues.

Hinde (2006) found support for the information hypothesis (Johnstone & Hinde, 2006) in a study of the Great Tit, *Parus major*. Rather than attempting to reduce the effort of one partner, she achieved an increase in work rate by playing a recording of nestling begging calls to one parent from each nest. As predicted by the information hypothesis, the partners of manipulated birds also increased provisioning rates, albeit to a lesser extent, and this was observed in both sexes. This shows that both direct and indirect cues of partner effort are important to Great Tits while provisioning nestlings.

An asymmetry in information between partners may explain the complete compensation reported in a number of studies (e.g. Sanz *et al.* 2000). Handicapping techniques not only affect direct cues by reducing provisioning efficiency but work indirectly by inducing an increase in begging intensity (Hinde 2006), and this may incite both partners to work harder (Ottoosson *et al.* 1997; Burford *et al.* 1998; Kilner *et al.* 1999). In response to increased begging intensity, the handicapped partner may be able to increase its effort to a sufficient level to counteract the effects of the manipulation, while the unmanipulated partner also increases its effort in response to the same cues.

The Information Hypothesis may help to shed light on the various studies that have reported mixed responses within a single investigation. Paredes *et al.* (2005) attached data loggers to one partner in the Thick-Billed Murre, *Uria lomvia*, and observed full compensation to a reduction in the partner's provisioning effort. When the data loggers were removed, and manipulated individuals recovered their feeding rates to previous levels, their partners maintained the same high provisioning rates that they had shown previously. This also highlights the influence that previous events can have on current parental effort.

Consistency over parental care

The effects of previous events on current parental behaviour has been relatively understudied in the context of sexual conflict, although a number of authors have addressed the idea of within-individual consistency in parental behaviour (Schwagmeyer *et al.* 2002; Schwagmeyer and Mock 2003; Kopisch *et al.* 2005; Nakagawa *et al.* 2007a). The level of sensitivity that individuals show to changes in the environment and the consistency of their parental response has clear implications for the location of these species on the negotiation continuum.

Schwagmeyer *et al.* (2002) noted that the House Sparrow, *Passer domesticus*, showed a consistent level of parental care over ecological time and that individuals appeared to be insensitive to the behaviour of their mates, as predicted by sealed bid models. Schwagmeyer *et al.* (2002) suggested that individual quality might be a better predictor of an individual's parental effort than partner effort is in that species. Schwagmeyer and Mock (2003) compared the provisioning rate across broods in the same species to investigate the role of individual quality in parental care. They discovered that the within-individual behaviour of male House Sparrows was highly repeatable across broods, even when they were paired with different partners, but that it varied between males. Females, by contrast, showed little within-individual consistency and low levels of between-individual variability. Similar trends were observed by Nakagawa *et al.* (2007a) in the same species, and this was true across both incubation and provisioning. A female may use the level of care a male provides during incubation to predict how much he is likely to contribute to provisioning (Kopisch *et al.* 2005; Nakagawa *et al.* 2007a).

Variation in the costs of care

To my knowledge, Smith (1995), Schwagmeyer *et al.* (2002) and Paredes *et al.* (2005) are the only studies that have attempted to induce variation in the costs of parental care and explore the partner's response using a within-individuals approach. Other researchers have used a between-groups design in which they compare the response of the partners of manipulated individuals to control partners. As well as taking into account within-individual variation, the former approach allows the effects of recent experience to be explored. Both of these factors have important implications for our interpretation of the parental response and where individuals fall on the negotiation continuum.

Like many previous researchers, Schwagmeyer *et al.* (2002) and Paredes *et al.* (2005) both attempted to increase the costs of care by using a weighting handicap and studied the effects of this on nestling provisioning in wild populations. The costs of parental care can potentially be manipulated in a number of ways, such as by increasing male EPC opportunity so that males face a trade-off between parental and mating effort (see Magrath and Komdeur 2003 for review), or by challenging his paternal certainty so that he risks investing costly care in unrelated offspring (see Wright 1998 for review). The paternal response to manipulations of male EPC opportunity and paternal certainty has been explored previously, but not in the context of negotiation over biparental care. A possible exception to this is Smith (1995), who manipulated the opportunity for males to attract additional mates in a wild facultatively polygynous population of the European Starling,

Sturnus vulgaris, by providing monogamous males with additional nestboxes. Males with two nestboxes sang more than those with a single nestbox during the pre-laying and incubation periods, and spent less time incubating their initial partner's clutch during early incubation, but not during late incubation or provisioning. Females compensated for the reduced incubation effort of males that attracted a second mate so that total incubation effort, nestling body mass and fledging success were unchanged. These results demonstrate a direct trade-off in time between a male's mating and parental effort, but no fitness cost was detected in terms of reduced offspring performance.

Male phenotypic quality or attractiveness can influence the trade-off between mating, paternal and somatic effort (Magrath and Komdeur 2003). For example, male Bluethroats, *Luscinia svecica*, whose attractiveness was experimentally reduced, spent less time soliciting extra-pair mates, participated in fewer EPCs and increased nestling provisioning relative to control males (Johnsen *et al.* 1998), while experimental enhancement of the attractiveness of male Zebra Finches, *Taeniopygia guttata*, led to increased male mating effort and decreased parental effort (Burley 1988). These findings are consistent with the Differential Allocation Hypothesis (DAH), which predicts a reduction in parental investment among more attractive males and an increase in resource allocation by their partners because the offspring of attractive males are likely to be of greater residual reproductive value (Burley 1986; Sheldon 2000). A number of empirical studies at the levels of primary and secondary investment have offered support to the DAH (reviewed in Sheldon 2000).

However, the Compensatory Investment Hypothesis (CIH) predicts a negative relationship between male attractiveness and female investment (Saino *et al.* 2002; Gowaty *et al.* 2007; Bolund *et al.* 2009; Harris and Uller 2009). According to this hypothesis, females that are constrained to pair with less attractive males should be more willing to compensate for the inferior quality of their mates by increasing their own investment (negative differential allocation) into that breeding attempt. Whether females should show positive or negative differential allocation depends on the relative impact of parental investment on offspring quality, female condition and the number of times that they are likely to breed with partners of differing quality (Bolund *et al.* 2009; Harris and Uller 2009). Monogamous species or those that are unlikely to make a subsequent breeding attempt should be more willing to compensate for low quality partners whereas those that are likely to breed with a number of males of varying quality should allocate more resources to offspring produced by the more attractive of these males. In species that breed more than once the degree of

compensatory investment to a given breeding attempt is also influenced by the trade-off between current and future reproductive investment (Williams 1966). Regardless of the direction of the relationship between male attractiveness and female investment, females clearly respond to variation in male attractiveness in different ways and it remains a source of unexplained variation in many studies of parental effort.

Furthermore, few studies of parental effort have considered the relationship between male attractiveness, EPC opportunity and paternal certainty (Magrath and Komdeur 2003). These variables are particularly difficult to quantify in the field, where most work of this nature has been carried out. Attractive males are likely to achieve more EPCs and higher paternity of their own brood than less attractive males, and yet increased EPC opportunity and paternal certainty are predicted to affect paternal care in opposite directions (Wright 1998).

The costs and benefits of incubation

Avian incubation is a convenient stage for exploring sexual conflict over division of labour because it is costly in terms of energy and time that could be invested into other activities (for reviews see Reid *et al.* 2002a; Tinbergen and Williams 2002) but is essential for embryonic growth and development in many species (Webb 1987). (Clutton-Brock 1991; Dickens *et al.* 2008; Kunz and Hosken 2009) In contrast to offspring provisioning, intra-familial conflict over incubation involves two parties rather than three and parental contributions of time or energy can be measured at a single point in space (the nest rather than the foraging territory). Parental effort during incubation is less likely to be influenced by differences between offspring in quality and need than during provisioning because such differences are less apparent to the parent at the egg stage (although the female potentially has information on the composition of hormones and nutrients inside the egg, which can affect both offspring quality and need). Incubation takes place earlier in a pair's breeding cycle, when partners are still learning about each other's patterns of effort, and this may make it a more promising stage in which to find evidence of behavioural negotiation (Schwagmeyer *et al.* 2002). Partners may be able to evaluate each other's contributions more easily and accurately during incubation, potentially taking cues from each other's behaviour and the temperature of the eggs.

Variation in incubation behaviour can significantly influence the levels of temperature and humidity experienced by the embryo (Ricklefs and Smeraski 1983; Webb 1987; Reid *et al.* 2002b; Gorman *et al.* 2005b), and developmental abnormalities and hatching failure can

occur when these conditions deviate from a specific range (Reid *et al.* 2000a; Nilsson *et al.* 2008). Gorman *et al.* (2005b) manipulated body condition in the Zebra Finch using high and low protein diets, and cross-fostered the eggs of these birds between different diet groups. They found that embryo mortality was lower and hatching mass was greater when eggs were incubated by foster parents in better condition, and that the body condition of the biological parent had no effect. There was also a difference between male and female embryos in their sensitivity to the incubation environment. The effects of the incubation environment can continue to be felt much later in life, and female offspring who had been incubated by foster parents in good condition produced larger clutches over two years than those incubated by poorer condition birds (Gorman and Nager 2004). This influence of incubation environment on offspring fecundity suggests that incubation effort can be a good proxy for parental investment in the sense proposed by Trivers (1972).

A given unit of time spent incubating could differ in thermal value to the embryos or be more energetically costly to one sex if there are differences between males and females in incubation ability. A number of studies have described sex differences in incubation temperature and egg re-warming rates among socially monogamous passerines. The male Chestnut-vented Tit-babbler, *Parisoma subcaeruleum*, incubates at a higher temperature than the female, but rates of re-warming and nest attentiveness are equal between the sexes (Auer *et al.* 2007); males of the Reed Warbler, *Acrocephalus scirpaceus*, and the Moustached Warbler, *A. melanopogon*, heat the eggs more slowly than the female (Kleindorfer *et al.* 1995); eggs tended to cool following female to male changeover in North American Barn Swallows, *Hirundo rustica*, but at a significantly slower rate than when left unattended (Voss *et al.* 2008). The male re-warms the clutch more rapidly but to a slightly lower temperature than his partner in a facultatively polygynous population of the Starling, *Sturnus vulgaris*, (Reid *et al.* 2002b).

A difference in incubation ability between the sexes may be expected if they differ in brood patch development (Jones 1971). In most bird species, the brood patch is a bare area of highly vascularised ventral skin that facilitates heat transfer from parent to egg (Bailey 1952; Lea and Klandorf 2002) and is also involved in sensory perception of heat (White and Kinney 1974). Where a brood patch is present in the female only, we may expect her to incubate at a higher temperature or to re-warm the eggs more rapidly (Zann and Rossetto 1991), yet in some species the male is able to incubate equally well even where a brood patch is absent (Skutch 1976; Reid *et al.* 2002b; Auer *et al.* 2007). Given that the brood patch is likely to be costly to maintain in terms of increased heat loss (Haftorn and

Reinertsen 1985), it is clear that a number of questions remain to be answered concerning its adaptive benefit in males and females.

Thesis overview

The purpose of this project was to explore factors that influence variation in male and female incubation effort and its subsequent fitness consequences. I addressed this question using a domesticated population of the Zebra Finch, *Taeniopygia guttata castanotis* (Estrildae), a socially monogamous, sexually dimorphic passerine that forms permanent pair bonds but engages in EPCs in the wild and in captivity (Birkhead *et al.* 1988a; Birkhead *et al.* 1988b). It breeds readily in captivity and is easy to maintain, and these features have contributed to its status as a model organism in evolutionary biology (e.g. Zann 1996). Both parents contribute to incubation and nestling provisioning in the wild and in captivity, but the male spends less time incubating than the female overall (El-Wailly 1966; Delesalle 1986; Burley 1988; Gorman and Nager 2003).

I explored the male response to an increase in the costs of care by manipulating paternal certainty (**chapter two**) and male EPC opportunity (**chapter three**), respectively. In **chapter two**, paired female Zebra Finches were given the opportunity to mate with extra-pair males during their fertile period while their partners remained in visual contact through one-way glass. I tested whether females were willing to copulate with extra-pair males, whether pair males responded as if their paternity had been threatened, and subsequently whether cuckolded males decreased incubation effort into their partner's clutch. In **chapter three**, incubating males received EPC opportunities with extra-pair females. I observed whether males attempted to court the females and subsequently measured their incubation attentiveness towards their partner's clutch. Both experiments employed a 'crossover design', which took place over two breeding attempts: in the first attempt half the pairs experienced a treatment, the other half a control, and in the second breeding attempt the type of treatment was switched between groups. This allowed me to statistically control for within-individual variation and to explore the effects of previous experience on current effort. Male attractiveness was considered in both experiments, and attractiveness scores were obtained using a series of mate choice trials.

Since paternal effort varied in response to the manipulations in **chapters two and three**, I was able to explore the female response to the deficit in male care. The magnitude and direction of the slope of each parent's current response against its partner's can be used to explore negotiation (e.g. Schwagmeyer *et al.* 2002) and to distinguish compensation,

which predicts a negative slope, from matching, which predicts a positive one. Complete compensation, in its strictest sense, can only be said to occur when the offspring are unaffected (Harrison *et al.* 2009) so, in **chapter four**, I examined who pays the costs when a male trades off parental effort for mating effort. I measured the pre- and post-embryonic survival of the offspring and their size at adulthood, as well as female investment into the subsequent reproductive attempt. There should be a cost to the reduction in male effort if incubation attentiveness is a good proxy for parental investment. I also explored the role that the father's attractiveness may play on current and subsequent reproductive output.

The work described so far quantifies incubation effort as a function of time. In **chapter five**, I explored other potential measures of incubation effort that have received comparatively little attention, namely the ventral surface temperature of incubating birds, clutch temperature and rate of re-warming cold eggs. Since the female alone possesses a brood patch in the Zebra Finch (Zann and Rossetto 1991; Zann 1996), she may be better adapted to incubate the eggs or she might experience greater energetic costs due to heat loss. In **chapter five**, I compare ventral plumage surface temperature between males and females using infra-red thermography (IRT), and then how egg temperature and re-warming rate might differ between the sexes using IRT and temperature probes, respectively. A sex difference in incubation ability or the quality of care provided has implications for the way that incubation attentiveness is interpreted and for predictions of division of labour between the sexes. I also compared IRT measurements of egg surface temperature to those taken at the core of the egg using the conventional method of inserting a temperature probe into a dummy egg connected to a data logger. Finally, in **chapter six**, I discussed these findings in the context of our current understanding of sexual conflict and division of labour over parental care. Thus the work I present here is the product of a series of experiments in which I manipulated factors influencing variation in male and female incubation effort and test theoretical predictions of how partners should respond to each other's effort (**chapters two and three**), assessed the consequences of the actions taken by the parents (**chapter four**) and explored some of the physiological constraints to incubation behaviour (**chapter five**).

Chapter 2 - Negotiation over biparental care: The effects of variation in paternal certainty on male and female incubation effort

Abstract

Since parental care is costly, males that can care preferentially for related offspring are likely to obtain increased fitness benefits. However, empirical studies have reported conflicting trends in terms of the male response to variation in paternal certainty, and this remains a contentious issue. Most of these studies have failed to consider an individual's previous experience, its partner's effort, male attractiveness and clutch size. Taking these factors into account, I manipulated female extra-pair copulation (EPC) opportunity in a domesticated population of the Zebra Finch, *Taeniopygia guttata*, to investigate whether (a) paired females were willing to engage in EPCs with extra-pair males, (b) paired males copulated more frequently with their partners after the manipulation (compared to control and pre-manipulation observations) as a paternity guard and (c) males whose paternal certainty had been challenged spent less time incubating their partner's clutch. Association times recorded in two-way mate choice trials provided a proxy for male attractiveness. Half the females received an EPC opportunity with an 'extra-pair male' (treatment) during the fertile period of their first breeding attempt, while the other half did not (controls). Each pair bred together twice within a 'crossover design', which meant that females that had previously been controls received EPC opportunities in the second breeding attempt and *vice versa*. During the manipulation, females could be observed by their partner through a transparent divide covered with one-way film. Extra-pair males courted the females more persistently than pair males, yet females were willing to copulate only with their partners. Males whose paternal certainty had been challenged in the first breeding attempt reduced incubation effort relative to controls, and continued to do so in the second breeding attempt in spite of the treatments being switched. This suggests that individuals use early life experiences to shape their future behaviour, and this may explain the conflicting trends reported in previous studies where the subjects' history was unknown. Males and females responded to each other's contributions over ecological time, and females compensated for their partners fully. Male attractiveness did not influence male or female incubation effort, but females paired to less attractive males laid larger clutches, as predicted by the Compensatory Investment Hypothesis.

Introduction

Kin-selection models predict that individuals can increase their fitness by preferentially directing investment into relatives (Hamilton 1964; Trivers 1972; Maynard Smith 1977). Since birds are oviparous, either parent can be deceived into investing effort into unrelated young, but males face added uncertainty over paternity of the clutch if their partners engage in extra-pair copulations (EPCs). Parental care is costly to the care-giver (Trivers 1972; Clutton-Brock 1991), and so a male that is able to adjust his level of parental effort in response to his probability of paternity should benefit by directing care from another male's offspring into his own (Werren *et al.* 1980; Houston and Davies 1985; Winkler 1987). A number of empirical studies have investigated the effects of variation in paternity or paternal certainty on paternal effort (e.g. Sheldon and Ellegren 1998; Dickinson 2003; Suter *et al.* 2009), but the response of the male differs vastly between studies (reviewed in Wright 1998; Sheldon 2002) and sometimes even within species (e.g. Dixon *et al.* 1994; Bouwman *et al.* 2005).

Although these conflicting trends may reflect life-history differences between species or populations, some studies have been criticised for showing an over-simplistic approach to the question and failing to adopt an appropriate experimental design (Wright 1998). The ability of a male to adjust his parental behaviour according to his paternity depends upon the fulfilment of three assumptions (Westneat and Sherman 1993). There must be a difference in a male's actual level of paternity between broods, or there would be no selective pressure favouring behavioural adjustments over reproductive attempts. Second, the benefits of reducing parental care must outweigh the costs. For example, if a male has no chance of finding another mate, he will gain more from remaining with his current partner and raising a brood of which he has low paternity. Third, a male must be able to reliably estimate his own paternity (Kempnaers and Sheldon 1997). Discriminate cues, traits expressed in individual offspring that allow the father to identify them as his own (Westneat and Sherman 1993), might be selected against because it is in the best interests of foreign offspring, as well as their mother, to disguise the offspring's provenance and avoid discrimination (Beecher 1988; Kempnaers and Sheldon 1996). Since it is unlikely that a male can measure paternity directly and accurately, his relatedness to his partner's offspring is essentially reduced to a probability of paternity (Wright 1998). He may, however, be able to estimate this probability indirectly using 'indiscriminate cues' taken from the female's behaviour or the environment (Westneat and Sherman 1993; Kempnaers and Sheldon 1996), and this suggests that empirical studies should focus on a

male's confidence of paternity, rather than his actual paternity. In addition to this, Kempenaers and Sheldon (1998) point out that manipulating actual paternity does not guarantee that a male's perceived paternity has been affected.

Previous workers have attempted to manipulated paternal certainty in three ways: by denying the male access to his partner during her fertile period (e.g. Davies *et al.* 1992), by removing her during laying (e.g. Sheldon *et al.* 1997) or by manipulating the number of EPCs she receives (e.g. Møller 1988; Ewen and Armstrong 2000). However, it is difficult to be sure that a manipulation has succeeded in changing the male's perception of his paternity. A null response to the manipulation could indicate either that a male's perception of his paternity was not manipulated successfully or that he chose not to respond to his perceived low level of paternity (Wright, 1998). However, the success of the manipulation may be inferred by observing the behaviour of the pair after they have been reunited: a male might show aggression towards a promiscuous female or he may copulate with her upon her return to attempt to regain paternity (Kempenaers and Sheldon 1998).

The situation is complicated further within biparental systems where there can be conflict over the amount of care that each sex provides. The sexes can differ in their optimum allocation of resources to a given breeding attempt due to differences between males and females in the costs and benefits of care (Trivers 1972; Chase 1980; Parker *et al.* 2002). Each sex will do better if it can reduce its own parental investment and rely on its partner to compensate (Trivers 1972; Lessells 1999; Houston *et al.* 2005). Most theoretical models predict that the best response to reduction in a partner's level of effort is one of partial compensation in order to avoid being exploited (e.g. Chase 1980; Houston and Davies 1985; McNamara *et al.* 1999). Such models fall into two groups depending on the timescale over which the conflict is settled. 'Sealed bid' models such as Chase (1980) and Houston and Davies (1985) describe a conflict that takes place over evolutionary time and predict a level of parental effort that is consistent between breeding attempts and is not influenced by the partner's behaviour. In 'negotiation' models, by contrast, each partner can modify its level of effort sequentially over real time (McNamara *et al.* 1999). Although most negotiation models predict some degree of compensation, the Information Hypothesis shows that partners can 'match' each others' levels of investment when information on the need or quality of the offspring is incomplete (Johnstone and Hinde 2006). We would expect to see a positive relationship between the effort of one individual

and its partner where matching occurs, and a negative relationship where there is compensation.

Differences between individuals, as well as between the sexes, in the costs and benefits of care relative to other activities can lead to variation in parental effort (Kempnaers and Sheldon 1997). Male phenotypic quality or attractiveness may influence the relative amount of effort a male invests into mating, parental and self-maintenance behaviour (Magrath and Komdeur 2003). Females are more likely to seek EPCs with attractive males (Burley *et al.* 1996; Møller and Tegelstrom 1997; Johnsen *et al.* 1998), and have also been shown to adjust their parental effort in response to male attractiveness (e.g. Burley 1988; Gorman *et al.* 2005a; Bolund *et al.* 2009). The Differential Allocation Hypothesis (DAH) suggests that, given that there is a trade-off between current and future reproduction (Williams 1966), a female should invest more resources into the offspring of an attractive mate because his offspring will be more valuable (reviewed in Sheldon 2000), while the Compensatory Investment Hypothesis (CIH) predicts that females constrained to mate with less attractive males should show more parental effort to compensate for the low quality of their mates (Gowaty *et al.* 2007; Gowaty 2008). Whether females should show positive or negative reproductive allocation depends upon their likelihood of achieving future mating opportunities, the extent to which males differ in quality and the impact of parental care on offspring quality (Bolund *et al.* 2009; Harris and Uller 2009).

I manipulated variation in paternal certainty in a domesticated population of the Zebra Finch (*Taeniopygia guttata*) by allowing paired females to interact with extra-pair males during their fertile period. The Zebra Finch is a socially monogamous, sexually dimorphic passerine that forms permanent pair bonds but engages in EPCs in the wild and in captivity (Birkhead *et al.* 1988a; Birkhead *et al.* 1988b). Extra-pair paternity was estimated to account for 2.4 % of offspring in a wild population of the Zebra Finch (Birkhead *et al.* 1990), and 34 % of eggs or offspring in an aviary population (Bolund *et al.* 2009). Given that male Zebra Finches make a substantial contribution to parental care (e.g. Zann 1996) and that EPCs can result in extra-pair paternity (Birkhead *et al.* 1988b), I would expect males to be able to modify their levels of paternal care according to their chances of paternity or to employ paternity guards to reduce their chances of being cuckolded. Most previous investigations of the effects of variation in paternal certainty on parental effort have been field-based studies that have not considered differences in male attractiveness or variation in male and female EPC opportunity (Magrath and Komdeur 2003). Attractive males may be able to achieve more EPCs as well as higher paternity of their own brood,

yet increased EPC opportunity and paternal certainty are expected to have opposite effects on paternal care (Wright 1998). Here I measured the attractiveness of pair males relative to extra-pair males by recording the proportion of time that females spent associating with each male in spatial association trials. This is the most widely used means of quantifying female mate choice (Rutstein *et al.* 2007), and associations formed by Zebra Finches in choice chambers have been shown to reflect sexual preferences (Clayton 1990a; Clayton 1990b; Forstmeier 2007). I used a within-individual crossover design: each individual bred twice with the same partner, and females that had received EPC opportunities in the first breeding attempt acted as controls in the second attempt and *vice versa*. This allows a reduced sample size and increased analytical power (e.g. Bolund *et al.* 2009). The aim of this study was to test the following hypotheses: 1) The receptivity of the female to the extra-pair male depends on the relative attractiveness between him and her partner. 2) The pair male copulates more frequently with his partner upon her return in order to achieve last male sperm precedence. 3) Males whose paternal certainty has been challenged spend less time incubating their partner's clutch.

Methods

Subjects and maintenance

26 birds of each sex were bred at the University of Glasgow between September and December 2005 and sexes were housed in separate cages (41 x 122 cm and 45 cm high) in the same room at a density of six birds per cage between reaching sexual maturity and pairing. They were paired at 185.4 ± 3.77 days old (mean \pm SE, $n = 52$) and none had bred previously. Before and during the experiment, they were maintained at 46.0 ± 0.90 % relative humidity, an ambient temperature of 22.3 ± 0.07 °C and under a photoperiod of 14L:10D. Natural light was excluded from the room, which was illuminated overhead by daylight-spectrum fluorescent tubes (Arcadia Bird Lamp FB36). A standard diet of *ad libitum* mixed seed, consisting of red, pearl white, panicum, yellow, Japanese and Canadian canary millets (Foreign Finch Mixture, supplied by Haith's, Cleethorpes, Lincolnshire, U.K.), cuttlefish bone, oyster grit and water was administered to the birds daily, along with fresh spinach leaves, egg biscuit protein, vitamin supplements (Daily Essentials 2: The Birdcare Company, Nailsworth, U.K.) and fresh spinach leaves once a week, and a calcium supplement (Calcivet: The Birdcare Company, Nailsworth, U.K.) thrice weekly. The seed was mixed with hemp and empty husks to increase food handling

time and decrease net energy gain, thus increasing the potential trade off in time spent foraging and incubating (Lemon and Barth 1992; Lemon 1993).

Outline of the experiment

Each female Zebra Finch was given a choice between two potential mates (see Male attractiveness below) and was then paired randomly with one of them for the duration of the experiment. The amount of time a female spent associating with her partner during the trial was used as a proxy for his attractiveness. Breeding cages were separated into two halves by a transparent divide covered with one-way film. Pairs were housed on one side of the divide and the other side was empty. Pairs bred together twice within a crossover design in which half the pair males received challenges to their paternal certainty (treatment) during their first breeding attempt and the other half in their second breeding attempt. In the first attempt, both treatment and control females were removed from their partners during their fertile period to the other side of the divide for three hours. Half of the females received an EPC opportunity with an ‘extra-pair male’ during this period of separation, while the other half did not. The one-way film ensured that the females’ extra-pair behaviour could be observed by their partners but that the females were unable to see their partners. I recorded courtship behaviour between the female and extra-pair male to establish whether the extra-pair male attempted to copulate with her, and interactions between the female and her partner before and after the manipulation in order to infer whether the partner perceived the preceding manipulation as a threat to his paternity. In the second breeding attempt, males that were previously controls received challenges to their paternity (referred to hereafter as ‘CT’ birds) and *vice versa* (‘TC’). Male and female incubation behaviour was recorded to test the hypothesis that males with reduced paternal certainty spend less time incubating their partner’s clutch. Details are described below.

Extra-pair males

An additional fifty males that were unrelated to and unfamiliar with the other birds served as extra-pair males (breeding attempt 1: $n = 26$; attempt 2: $n = 24$; two pairs failed to produce a clutch in the first breeding attempt and were removed from the experiment). The extra-pair males used in the first breeding attempt hatched before October 2005, while those used in the second attempt hatched between November 2005 and February 2006. The extra-pair males were housed in single-sex cages at a density of six birds per cage in a separate room from the paired birds, but were otherwise maintained under the same conditions.

Male attractiveness

I recorded the amount of time that females spent associating with males in two sets of two-way spatial association trials. Females were paired randomly with one of the two males encountered in the first set of trials and the percentage of time a female spent associating with her partner was used as a proxy for his attractiveness (hereafter, male attractiveness₁) in analyses of incubation attentiveness. The second set of trials provided a measure of the pair male's attractiveness against the extra-pair male (male attractiveness₂), and this was used as a covariate in analyses of courtship behaviour.

Pair male association trials

Prior to pairing, 26 birds of each sex were grouped into 13 quads of unrelated individuals, each consisting of two males, matched for colour morph, and two females ($n = 1$ fawn and $n = 12$ wild type quads). Each female had the opportunity to choose between the two males from her quad within a choice chamber (figure 2.1) in a separate room from where the birds were housed. Lighting was supplemented by an additional fluorescent tube (Arcadia Bird Lamp FB36) placed behind the neutral zone.

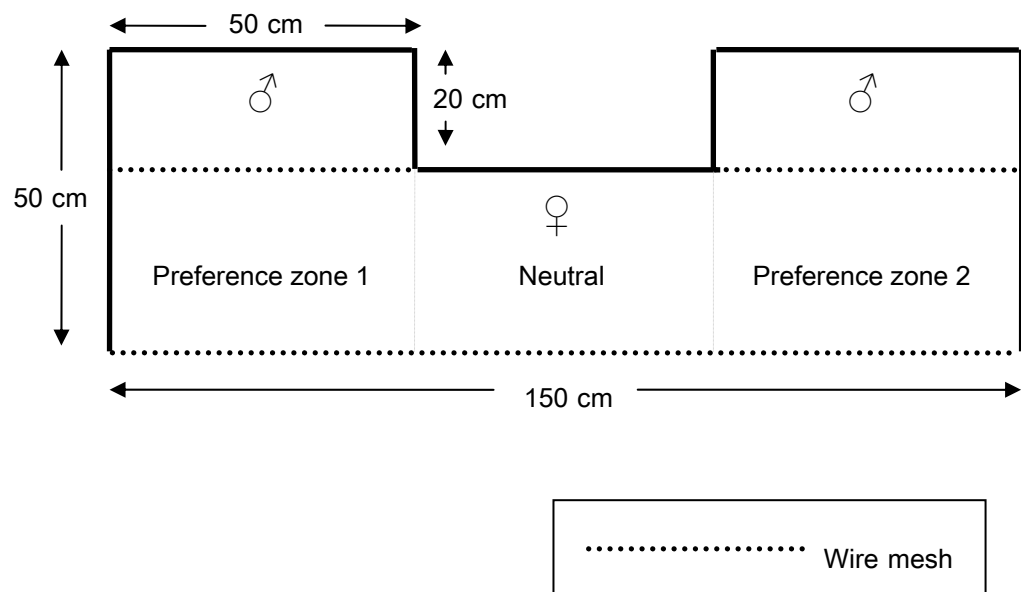


Figure 2.1 Mate choice chamber, modified after Jones *et al.* (2001). The two preference zones and the compartments housing the males each contained a pair of perches positioned at equal heights and distance from the chamber walls. The two male compartments and the neutral zone each contained a water font and a dish of mixed seed. The males were unable to interact with each other visually, although they may have interacted acoustically. Three choice chambers were stacked on top of each other to allow three trials to be performed simultaneously. The observer was stationed in a hide facing the neutral zone.

The two males of a quad were weighed to the nearest 0.01 g on an electronic balance and then released into their respective compartments and one of the females of their quad into the neutral zone. After 15 minutes' acclimatisation, I recorded which preference zone the female was occupying (1, 2 or neutral) at minute intervals for one hour. The two males were then switched between compartments to control for a potential female side bias. The birds experienced another five-minute acclimatisation period and then the second half of the trial began. Once the trial was complete, the female was replaced with the second female from her quad, and the process was repeated with the same two males. All females visited both left and right compartments during each pair of trials. Female preference was not side-biased (mixed model controlling for female and trial identities: right: 51.1 ± 3.96 %, $n = 26$ females; left 44.8 ± 4.03 %, $n = 26$; $P = 0.271$) and nor did a male's body mass influence female preference ($P = 0.375$). I summed the number of minute intervals each female spent with each male over the two sides and expressed this as a percentage of the total observation time (including time spent in the neutral zone, which averaged 4.1 ± 0.64 %). In this way, each male received an attractiveness₁ score from each of the two females in his quad. Preference for a particular male was not correlated between females ($r_p = 0.14$, $P = 0.505$, $n = 26$). I assigned each pair to one of two experimental groups (TC or CT) such that groups did not differ in pair male attractiveness score₁ nor tarsus length, and this was still true of the 22 pairs that made up the final sample (see statistical analysis for details) (male attractiveness: CT: 48.0 ± 6.66 %, $n = 10$; TC: 50.6 ± 6.12 %, $n = 12$; $F_{1,20} = 0.08$, $P = 0.774$; male tarsus length: CT: 15.1 ± 0.11 mm; treatment: 14.7 ± 0.38 mm; $F_{1,20} = 0.82$, $P = 0.377$; female tarsus length: TC: 15.3 ± 0.16 mm; treatment: 15.0 ± 0.21 mm; $F_{1,20} = 0.32$, $P = 0.264$). TC males would receive a challenge to their paternal certainty during the first breeding attempt, the others in the second. The 22 females varied in their strength of preference from 90 % of time with one male and 5.8 % with the other to 51.7 : 48.3 % (no preference).

Extra-pair vs. pair male association trials

The same methodology was used again prior to pairing to offer each female a choice between the male to which she had been assigned following pair male association trials and one of the 26 extra-pair males, selected at random, whom treatment females would encounter during the paternal certainty manipulation of the first breeding attempt. The procedure was repeated once more with the second set of extra-pair males in the period between the first breeding attempt ending and the second one beginning. Again, all females visited both stimulus males during each paired trial. Mean male attractiveness₂ scores for partners and extra-pair males were 57.3 ± 3.35 % ($n = 22$) and 38.2 ± 3.43 % (n

= 22), respectively. There was no difference between breeding attempts in the tendency for treatment females to prefer their partner over the extra-pair male (attempt 1 treatment: $n = 12$; attempt 2 treatment: $n = 10$; $t_{20} = 1.38$, $P = 0.182$).

Manipulation of paternal certainty

Twenty-six cages were separated into a 'pair side' and an 'extra-pair side' (each side 41 x 61 cm and 45 cm high) by a Perspex divide. At the start of each manipulation this was substituted for a second Perspex divide upon which was mounted one-way film (Regency Glazing, Glasgow). This allowed the pair male to observe any interactions that took place between his partner and the extra-pair male during the manipulation, but reduced his influence over their behaviour (Whittingham *et al.* 1993). The pair side contained coconut fibre nesting material (replenished once a week until the onset of incubation), food and water. A wooden nestbox could be accessed by the birds from the pair side. The cages were arranged in stacks of four facing the centre of the room so that pairs were visible to each other during the experimental period, but were unable to interact physically.

A small video camera was erected 40 cm from the cage and connected to a VCR and television monitor in the next room. The focal side of the cage was illuminated by a fluorescent tube during the trials to enhance the properties of the one-way film. To provide a baseline level of within-pair behaviour for comparison with post-manipulation behaviour, the pair was filmed from 12:00 to 15:00 on the day that the first egg was laid, which was termed day 0 by Birkhead *et al.* (1988b). The following morning, I switched the Perspex divide for the one-way film and supplied the extra-pair side with mixed seed and water. The extra-pair male was transferred to the extra-pair side for the manipulation and experienced five minutes' acclimatisation time. I then transferred the female to the extra-pair side, simulating a situation in which she leaves her partner's territory, and the observation began immediately. The manipulations would have ideally occurred the day before laying to increase the chances of the extra-pair male fathering the entire brood, but I was not able to predict the onset of laying, and so manipulations were performed at the earliest practical opportunity. The fertile period of the female Zebra Finch begins 11 days before the start of laying and ends 24 hours before the last egg is laid (Birkhead *et al.* 1989). Females continued to lay more than 24 hours after the manipulation had been carried out (control clutch size: 5.0 ± 0.24 eggs, $n = 22$; treatment: 5.0 ± 0.17 eggs, $n = 22$), and so the timing of the manipulation was biologically relevant in terms of fertilisation and egg formation, and extra-pair males had the potential to father at least some of the brood. Moreover, extra-pair courtship of paired females peaked on this day in a population of wild

Zebra Finches (Birkhead *et al.* 1988a) Extra-pair interactions were monitored by video camera for three hours (09:00 to 12:00); at noon the extra-pair male was removed and the female was returned to her partner. Controls proceeded in the same manner and for the same duration, except that no extra-pair male was present when the female was transferred to the extra-pair side of the cage. Control females were presented with an empty cage rather than an additional female in order to standardise the conditions experienced by all controls. For example, the behaviour of the focal female might be influenced by the phenotype of the additional female or by the type and duration of interactions occurring between them. In addition to this, an encounter with an additional female close to the nest during the focal female's laying period could represent an increased risk of brood parasitism, to which a reduction in incubation effort by the focal female might be an appropriate response. The camera was installed in front of the cage as in treatment manipulations. Immediately after the female was returned to her partner, I recorded the interactions between the pair for three hours (12:00 to 15:00) in order to infer whether the male's perception of paternity has been manipulated successfully. The last copulation a female receives before ovulating a particular egg has the highest chance of fertilising that egg (Birkhead *et al.* 1988b), so males may frequently copulate with their partners during their fertile period to ensure paternity (Butterfield 1970). I removed the divide following the post-manipulation recording to allow the pair to make use of the full cage for the rest of the breeding attempt.

Video analysis of within-pair and extra-pair behaviour was carried out by five observers who were 'blind' to the treatment group and whether they were watching within- or extra-pair behaviour. The observers recorded by continuous observation the number of successful and failed copulation attempts, the time at which copulations occurred (copulation latency) and the highest stage of courtship reached (1-4, see Zann (1996, pages 170-174)). There was no difference between breeding attempts in baseline levels of these behaviours (pairwise Wilcoxon signed rank test with continuity correction: $P \geq 0.378$) so data from both attempts were combined to test for observer differences. There were no differences between observers (Kruskal-Wallis rank sum test: $P \geq 0.220$) or between treatment groups (pairwise Wilcoxon signed rank test with continuity correction: $P \geq 0.407$) in baseline measurements of any of the four variables.

Each pair was allowed to complete incubation and raise chicks. I did not assess paternity of the offspring since the aim of the experiment was to manipulate paternal certainty rather than actual paternity, and manipulation of actual paternity does not guarantee that paternal

certainty has been manipulated (Kempnaers and Sheldon 1998). Fledglings were separated from their parents at 30 days of age, nestboxes were cleaned and supplied with new nesting material, and pairs were left to initiate a second breeding attempt with the same partner. Two treatment clutches from the first breeding attempt did not hatch and the offspring of three control pairs died within 24 hours of hatching. These five pairs were maintained on either side of a Perspex divide in the same cage until 30 days after the eggs were due to hatch to standardise the interval between breeding attempts. The remaining 17 pairs fledged at least one offspring. The interval between the manipulations of the first and second breeding attempt being carried out was 44 ± 0.46 (range 41-50) days ($n = 22$). The second breeding attempt proceeded as the first, except that the treatments were switched so that pairs that had previously been control were now treatment and *vice versa*.

Incubation data collection

Incubation begins (day zero) on the day that the fourth egg is laid in clutches of more than four, or on the final day of laying in smaller clutches (Zann and Rossetto 1991). Incubation data were collected in the morning (07:40-10:40) of days five and eight of incubation using a small infra-red video camera mounted above the nestbox. The female incubates at night (Zann and Rossetto 1991) and so her energy reserves are likely to be lowest and her need to forage greatest in the morning. This means that it is particularly important for the male to incubate at this time.

Incubation video analysis was performed blindly with regard to treatment group, observation day and breeding attempt (1 or 2) by a single observer (myself). For each observation, I recorded the incubation attentiveness (the amount of time spent incubating the eggs) and nest occupancy (the amount of time spent inside the nestbox) of each sex, distinguishable on the basis of plumage dimorphism, to the nearest second. A bird had to pass over the threshold of the nestbox to be scored as occupying it, and to cover the eggs with its ventral surface to be scored as incubating. The Zebra Finch achieves close contact between brood patch and eggs by undertaking a distinctive ‘shuffling’ movement at the onset of an incubation bout and at regular intervals throughout in which the feathers of the brood patch are parted. The first four minutes of each observation was excluded from these totals to allow birds to acclimatise to the camera (birds began incubating mean 308.6 ± 34.08 s after the recording began). Incubation attentiveness (s) is presented in figures and means as a percentage of the observation duration. ‘Shared’ incubation, when both partners frequently shuffle on the eggs and departure of one partner does not leave the whole clutch exposed, contributed to 7.0 ± 1.64 % ($n = 22$ pairs) of the total observation

time. Although this could differ from non-shared incubation in terms of energetic costs to the parent or heat transfer to the developing embryo, it involves an equal investment of time from each of them and so it was not excluded from time budget calculations. Due to shared incubation, a pair's combined attentiveness could exceed 100 %. Nest occupancy and incubation attentiveness were correlated in males on both days of the first breeding attempt ($r_s \geq 0.65$, $P \leq 0.002$), but not the second attempt ($r_s \leq 0.31$, $P \geq 0.227$). The two measures of incubation effort were correlated on all four days in females ($r_s \geq 0.52$, $P \leq 0.031$). I used incubation attentiveness rather than nest occupancy as my proxy for incubation effort since this reflects the component of incubation behaviour that is costly in terms of both time and energy.

Statistical analysis

Two CT pairs were not included in the analysis because one produced multiple clutches (totalling 17 eggs) after the treatment manipulation had taken place which meant that the timing of the manipulation was no longer accurate for this pair and the other pair failed to enter the nestbox on three of four observations of incubation. Observations of extra-pair behaviour, pre- and post-manipulation within-pair behaviour and incubation behaviour on days five and eight of both breeding attempts were available for the remaining 22 pairs (one recording of within-pair behaviour was not transcribed for 1 CT and 2 TC pairs due to a technical fault). Data were analysed using R version 2.9.2 (R Development Core Team 2009).

Within- and extra-pair behaviour analyses

The total number of copulation attempts, the number of successful copulation attempts and latency to copulate followed a Poisson distribution. Since no extra-pair male achieved a copulation with the paired females, I compared the total number of copulation attempts made by the extra-pair male to those (whether successful or not) made by the pair male before the manipulation to see whether extra-pair males differed in their willing to copulate with the female. The total number of attempts was used as a response variable in a generalized linear mixed models (GLMM) fit by the Laplace approximation, which includes a log link function appropriate for Poisson errors, and is found in the lme4 library (Bates and Maechler 2009). Female identity was fitted as a random factor (to control for repeated measures) with random intercepts only, the type of male (pair or extra-pair) was a fixed factor and pair male attractiveness₂ was a covariate. Here and in subsequent mixed models, I tested all possible two-way interactions between main effects and used backwards stepwise regression to reach the final model, removing interaction terms $P \geq$

0.05 in order of least significance and then main effects. This approach was deemed most appropriate for hypothesis testing within a controlled experiment. GLMMs are presented with parameter estimates (β), standard error, Z values and P -values. All main effects and significant interactions ($P < 0.05$) are reported. Means are presented \pm SE; mean copulation attempts are expressed as attempts hr^{-1} . All statistical tests are two-tailed.

The post-manipulation within-pair attempted copulation rate was used to infer whether the pair male behaved as if he perceived his paternity to have been challenged. I used the total number of copulation attempts as the response variable in a nested GLMM fitted by the Laplace approximation as before. The type of within-pair observation (pre- or post-manipulation) was included as a random factor nested within female identity; treatment and type of observation were fixed factors, and pair male attractiveness₂ was a covariate. If pair males responded to female removal as a challenge to their paternity regardless of whether the female had the opportunity to interact with the extra-pair male, I would expect males to copulate more frequently with their partners after the manipulation than before it and to find no effect of treatment. However, if males copulate with their partners more frequently in pairs where females were presented with extra-pair males during the periods of separation, I would expect to see an interaction between treatment and observation type. To test whether post-manipulation copulation latency is shorter in males whose partner received an EPC opportunity (treatment) compared to those whose partner did not (control), I first established whether treatment males were more likely to copulate with their partners than controls using a chi-square test with copulation success expressed as a binary variable (successful or not). Since controls and treatments did not differ in their probability of copulating ($P = 0.785$), I tested whether they differ in copulation latency using a Wilcoxon rank sum test with continuity correction on the subset of males that did copulate with their partner.

Incubation analyses

Male and female incubation attentiveness data were analysed using linear mixed effect models by the Restricted Maximum Likelihood method from the lme4 library. These models are appropriate for normal (or normalised) data and produce unbiased estimates of variance parameters (Crawley 2007). Individual identity was fitted as a random factor (random intercepts only) to control for repeated measures regardless of statistical significance. For each model I present estimates of the model coefficient (β), highest posterior density (HPD) intervals calculated at the 95 % level using Markov Chain Monte Carlo (MCMC) sampling with the chain length fixed at 10,000 (reported in brackets after

the coefficient), and P -values based upon the posterior probability distribution. I also present the variance between (individual identity) and within (residual variation) individuals as a percentage of the total variance. Significance is denoted by a HPD interval that does not include zero and P -values < 0.05 . These estimates were calculated using the `pvals` function from the `languageR` library (Baayen 2009).

I tested the hypothesis that males whose paternity had been challenged spent less time incubating their partner's clutch, by investigating the effect of treatment group, which was a fixed factor with two levels (control and treatment), on male incubation attentiveness. Since the treatments were switched in the second breeding attempt, breeding attempt (1 or 2) was a fixed factor in the model. An interaction between treatment and breeding attempt would indicate that the male's parental effort varied with respect to the sequence in which treatments were presented. I included the incubation attentiveness of the female from the same observation as a covariate to see how partners influence each other's effort. The day of incubation that the observation was made was also included in the model as attentiveness may differ over time (Gorman 2005). Incubation attentiveness can increase with clutch size since larger clutches are more valuable (Komdeur *et al.* 2002), but the energetic cost of incubating larger clutches is greater (Biebach 1981; Biebach 1984; Haftorn and Reinertsen 1985; de Heij *et al.* 2007) so clutch size was included as a covariate. The manipulation may also have a greater effect in smaller clutches since the male has potentially lost a larger proportion of paternity (Whittingham *et al.* 1993). Pair male attractiveness₁ was included as a covariate since more attractive males are predicted to show reduced investment in parental care (Burley 1988; Qvarnström 1997). Before running this main analysis, I carried out some preliminary mixed model analyses to test for collinearity between the response variables. Clutch size did not differ between treatment groups (β : -0.015 (-0.511 to 0.468), $P = 0.953$) but it did decrease as pair male attractiveness₁ increased (β : -0.017 (-0.030 to 0.005), $P = 0.007$) and was higher in the second breeding attempt than the first (attempt one: 4.6 ± 0.21 eggs, $n = 22$; attempt two: 5.4 ± 0.16 eggs, $n = 22$; β : 0.816 (0.335 to 1.294), $P = 0.001$). Pair male attractiveness₁ was measured on a single occasion so the same scores were used for both breeding attempts. To ensure that the main analysis was not confounded by these effects, I ran two general linear models with male and female incubation attentiveness as respective response variables and clutch size as a single covariate. Incubation attentiveness data (s) followed a right-skewed distribution for males and left-skewed for females, so I respectively used square-root and power of 1.5 transformations to normalise them. I then extracted the raw residuals from these models and used the corrected values of male incubation attentiveness

(response variable) and partner's attentiveness (explanatory variable) in the main analysis of male effort. I calculated the repeatability of incubation attentiveness across both days and breeding attempts for males and females separately using the formulae given in Lessells & Boag (1987) and in Becker (1984) for standard errors.

I examined the effect of male incubation effort on female effort by including male attentiveness (square-root transformed and regressed against clutch size) as a covariate in a separate general linear mixed model with female attentiveness (transformed to the power of 1.5 and regressed against clutch size) as the response variable. This allowed me to assess whether partners respond to each other's effort over ecological time (McNamara *et al.* 1999), and whether this response constitutes compensation or matching, based upon the slope of the relationship between the partners' effort (negative or positive, respectively). Since breeding attempt was involved in an interaction with treatment on male incubation attentiveness, analysis of the female response was restricted to the first attempt. Male attractiveness₁, treatment group and day of incubation were included in the model. I expected to find an interaction between treatment and partner's attentiveness if treatment and control females respond differently to their partner's effort. Next, I investigated the magnitude and direction of the slopes of the parents' effort for treatment and control pairs separately. Female incubation attentiveness (here untransformed) was the response variable, male attentiveness was a covariate and individual identity was a random factor in a general linear mixed model. I did not use the values corrected for clutch size here since the single explanatory variable meant that collinearity of explanatory variables was not a problem. The slope of the relationship between male and female effort is equal to the mean change in effort per partner unit of effort, and I used the HPD intervals from these regression analyses to establish where the female response lay on the continuum between matching (HPD intervals overlapping a line of slope = 1) and compensation (HPD intervals overlapping a line of slope = -1).

Next I tested whether there was a difference in combined (male + female) incubation attentiveness between treatment groups. I used combined incubation attentiveness (corrected for clutch size, as described earlier) as the response variable in a general linear mixed effect model, with pair identity as a random factor, and treatment and breeding attempt as fixed factors.

Since female incubation attentiveness was influenced by her partner's current effort, I explored temporal aspects of the negotiation process by testing whether male incubation

attentiveness in the first breeding attempt (hereafter, previous effort) explained female incubation attentiveness in the second breeding attempt (current effort) once current male attentiveness was statistically controlled for. To do this, I extracted the raw residuals of current female incubation attentiveness (transformed to the power of 1.5) regressed against current male attentiveness in a general linear model. These residuals were then used as the response variable in a general linear mixed model in which individual identity was a random factor and previous male attentiveness was a covariate. I used the same approach to determine whether current male effort explains current female effort once previous male effort is statistically controlled for. Investigating the effects of current and previous male effort separately avoids problems due to collinearity of the two explanatory variables.

Results

Extra-pair copulations

None of the 22 extra-pair males copulated successfully with the females during the three hour observations of extra-pair behaviour, while seven pair males achieved at least one copulation during pre-treatment observations (pre-treatment: 0.14 ± 0.05 copulations hr^{-1} , $n = 21$). Thus the number of females that copulated with extra-pair males was lower than expected from baseline observations of within-pair behaviour (χ^2 with Yates' continuity correction: 6.48, $df = 1$, $P = 0.011$). However, twenty extra-pair males did reach the first stage of courtship or higher (median: stage 2 over 3-hr observation, range: 0-3, $n = 22$), and eleven of them made at least one copulation attempt (mean: 0.47 ± 0.15 attempts hr^{-1} , $n = 22$). Extra-pair males attempted to copulate more frequently than pair males (pre-treatment pair baseline: 0.25 ± 0.05 attempts hr^{-1} , $n = 21$; β : 0.62 ± 0.32 , $Z = 1.97$, $P = 0.049$), and 56.3 % of all copulation attempts made by pair males during baseline observations resulted in a copulation. The number of copulation attempts was not affected by the partner's attractiveness₂ (β -0.02 ± 0.01 , $Z = 1.44$, $P = 0.151$) and there was no interaction between type of male and the partner's attractiveness₂ (β : -0.01 ± 0.02 , $Z = 0.67$, $P = 0.503$).

Within-pair pre- and post-manipulation behaviour

In order to assess whether pair males whose partners had received EPC opportunities (treatment) responded as if their paternity had been threatened, I compared the rate at which males attempted to copulate with their partner before and after the manipulation and between treatments and controls. Contrary to prediction, control males attempted to

copulate more frequently than treatment males (control: 0.39 ± 0.09 attempts hr^{-1} , $n = 22$; treatment: 0.21 ± 0.04 attempts hr^{-1} , $n = 22$; β : -0.55 ± 0.25 , $Z = 2.20$, $P = 0.028$). Males did not attempt to copulate more frequently after the manipulation than before it (pre-manipulation: 0.36 ± 0.09 attempts hr^{-1} , $n = 22$; post-manipulation: 0.25 ± 0.05 attempts hr^{-1} , $n = 22$; β : 0.29 ± 0.29 , $Z = 1.01$, $P = 0.314$) and attempted copulation rate was not influenced by the partner's attractiveness₂ (β : -0.01 ± 0.01 , $Z = 0.89$, $P = 0.376$), nor by any interaction between these variables ($P \geq 0.509$). A separate model showed that control males were not more likely to achieve copulations with their partners than treatment males were (control: 0.14 ± 0.04 copulations hr^{-1} , $n = 22$; treatment: 0.11 ± 0.03 copulations hr^{-1} , $n = 22$; β : -0.22 ± 0.37 , $Z = 0.58$, $P = 0.562$). Post-manipulation copulation latency did not differ between treatment and control males (control: 1555 ± 1406 s, $n = 6$; treatment: 600 ± 339 s, $n = 5$; $W = 15$, $P > 0.999$).

Effects of female EPC opportunity on male incubation attentiveness

Male incubation attentiveness was influenced by a number of factors (table 2.1). The effect of the manipulation on male attentiveness depended on the sequence in which it was presented (treatment x breeding attempt interaction): males whose paternity had been threatened in their first breeding attempt showed lower incubation attentiveness than controls in that attempt and both groups maintained a similar level of effort when the treatments were switched in the second attempt (figure 2.2). Male incubation attentiveness decreased with increasing female attentiveness, and attentiveness varied more within than between males. It was not influenced by male attractiveness or by the day in the incubation sequence. Incubation attentiveness was repeatable across days and breeding attempts in both males ($F_{21,60} = 3.40$, $r = 0.380 \pm 0.12$, $P < 0.001$) and females ($F_{21,60} = 2.34$, $r = 0.370 \pm 0.12$, $P < 0.001$).

Table 2.1 General linear mixed model showing the effects of female extra-pair copulation (EPC) opportunity (treatment) on male incubation attentiveness in 22 pairs over two breeding attempts each (attempt 1: $n = 10$ control and 12 treatment pairs; attempt 2: $n = 12$ control and 10 treatment pairs; $n = 88$ observations overall). Male and female incubation attentiveness data were transformed and corrected for clutch size (see methods). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for all main effects and the only significant interaction. All other interactions $P \geq 0.192$.

	β	HPD interval	P
<i>Fixed effects</i>			
Intercept	3.65	-1.3366 to 8.913	0.160
Partner's attentiveness	0	-0.0001 to 0	< 0.001
Treatment group ^a	-7.75	-14.6505 to -1.0820	0.022

Breeding attempt ^b	-5.83	-13.1473 to 0.9240	0.104
Treatment group x breeding attempt	13.66	3.5092 to 24.6440	0.011
<i>Main effects not in final model</i>			
Day of incubation	0.78	-0.7025 to 2.1555	0.275
Male attractiveness	0.05	-0.0850 to 0.1872	0.450
<i>Random effects</i>			<i>Variance (%)</i>
Individual identity	2.92	0 to 5.4935	25.8
Residual	10.13	8.3209 to 11.9749	74.2

^a 'control' is the reference group

^b '1' is the reference breeding attempt

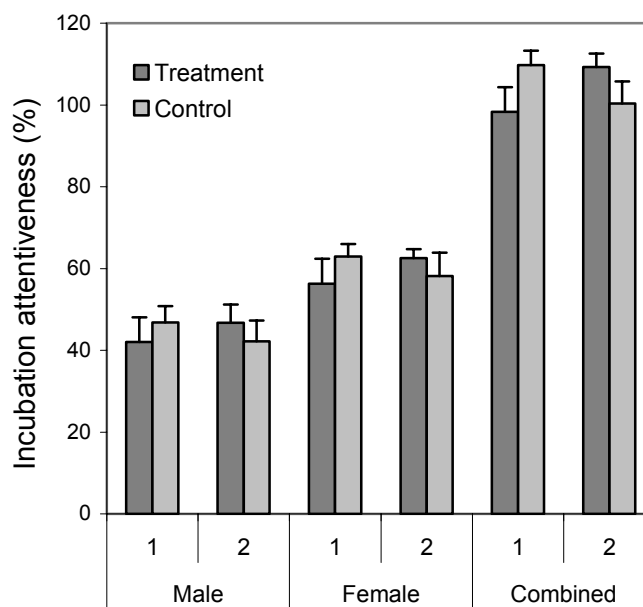


Figure 2.2 Male, female and combined male and female incubation attentiveness (means + 1 SE) for each breeding attempt (1 or 2) as a proportion of the total time available to each (breeding attempt 1: n = 10 control and 12 treatment pairs; breeding attempt 2: n = 12 control and 10 treatment pairs). There was a treatment group x breeding attempt interaction on male attentiveness (see table 2.1). Control pairs from the first breeding attempt became treatment pairs in the second breeding attempt and *vice versa*.

The female response: compensation or matching?

Female incubation attentiveness in the first breeding attempt was influenced by an interaction between the treatment and her partner's incubation effort (table 2.2, figure 2.2). It was not influenced by male attractiveness or by the day of incubation. There was a negative relationship between female and male incubation attentiveness among treatment pairs (β : -0.93 (-1.14 to -0.73), $P < 0.001$, $n = 12$). The HPD intervals include -1, which suggests that females compensated fully for their partners' reduced effort (figure 2.3). Male and female attentiveness also followed a negative trend in control pairs, but this was not statistically significant (β : -0.34 (-0.75 to 0.10), $P = 0.100$, $n = 10$).

Table 2.2 General linear mixed model showing the effects of female extra-pair copulation (EPC) opportunity and male incubation attentiveness on female incubation attentiveness in 22 pairs during their first breeding attempts (control $n = 10$; treatment $n = 12$; 44 observations overall). Male and female incubation attentiveness data were transformed and corrected for clutch size (see methods). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for all main effects and the only significant interaction. All other interactions $P \geq 0.157$.

	β	HPD interval	
<i>Fixed effects</i>			P
Intercept	54112	-19134 to 127007	0.145
Treatment group ^a	-112916	-209921 to -14834	0.024
Partner's attentiveness	-5617	-10969 to -16	0.047
Treatment x partner's attentiveness	-7331	-13962 to -768	0.032
<i>Main effects not in final model</i>			
Day of incubation	23464	-5768 to 54788	0.124
Male attractiveness	-379	-2887 to 1951	0.756
<i>Random effects</i>			Variance (%)
Individual identity	17231	0 to 56688	< 0.1
Residual	153310	119872 to 189112	> 99.9

^a 'control' is the reference group

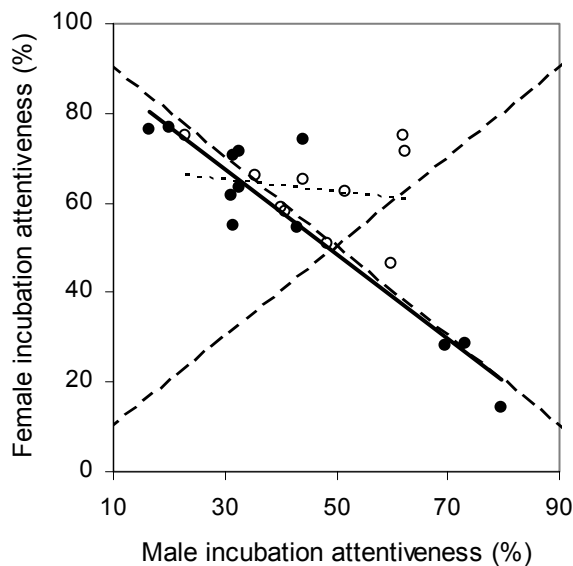


Figure 2.3 Regression analysis to assess the effects of male incubation attentiveness on female attentiveness in treatment (closed circles, bold line, $n = 12$) and control (open circles, dotted line, $n = 10$) pairs. The dashed line with the positive slope indicates where points would lie if male and female attentiveness were equal and combined attentiveness were 100 % (perfect matching). Where points fall above that line, females show higher attentiveness than their partners. The dashed line with the negative slope represents full compensation: points below it reflect partial compensation and points above it reflect overcompensation.

Each point represents the mean of 2 days. There was a negative relationship between male and female incubation attentiveness, but post-hoc analysis showed that this was only significant in treatment pairs..

Combined incubation attentiveness

Combined male and female attentiveness was influenced by an interaction between the treatment and the breeding attempt (treatment: β : -5.65 (-9.96 to -1.17), $P = 0.012$; breeding attempt: β : -4.28 (-8.81 to 0.30), $P = 0.072$; treatment x breeding attempt interaction: β : 9.84 (3.00 to 16.65), $P = 0.006$; figure 2.2). In the first breeding attempt, control pairs showed greater combined incubation attentiveness than treatment pairs, and continued to do so in the second breeding attempt when the treatments were switched.

Effects of a partner's previous effort on a female's current incubation attentiveness

Finally, I tested whether a female's current effort is determined by her partner's current effort or by the effort he showed in the previous breeding attempt. Female effort was not influenced by her partner's effort in a previous breeding attempt (table 2.2a), but there was a negative relationship between the partners' current incubation attentiveness (table 2.2b).

Table 2.3 General linear mixed models showing the effects of the partner's (a) previous and (b) current incubation attentiveness on the female's current attentiveness (transformed to the power of 1.5) in 22 pairs. Previous attentiveness refers to the first breeding attempt and current refers to the second. The residuals of current female attentiveness regressed against the partner's current effort were used as the response variable in a; the residuals of current female attentiveness regressed against the partner's previous effort was the response variable in b. Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for all main effects.

	<i>a) Effects of partner's previous effort</i>			<i>b) Effects of partner's current effort</i>		
	β	HPD interval	P	β	HPD interval	P
<i>Fixed effects</i>						
Intercept	74770	-58283 to 211485	0.276	236179	93854 to 377957	0.004
Partner's attentiveness	-16	-42 to 11	0.230	-51	-80 to -23	0.002
<i>Random effects</i>			<i>Variance</i>			<i>Variance</i>
			(%)			(%)
Individual identity	18658	0 to 60535	7.0	21567	21567 to 0	20.6
Residual	159426	124182 to 199629	93.0	159956	122396 to 197976	79.4

Discussion

I manipulated female EPC opportunity in the Zebra Finch to investigate the extent to which females were willing to copulate with extra-pair males during their fertile period, whether pair males responded as if their paternity had been threatened, and subsequently whether cuckolded males decreased incubation effort into their partner's clutch. Extra-pair males courted the females with more persistence than pair males, yet females were willing to copulate only with their partners. The post-manipulation copulation rate did not differ from baseline levels, although pair males copulated relatively frequently with their partners throughout the experiment. Males whose paternal certainty had been threatened in the first breeding attempt reduced incubation effort relative to controls, and continued to do so in the second breeding attempt in spite of the treatments being switched. A reduction in male incubation effort led to an increase in female effort within pairs. Although male and female incubation effort were repeatable across breeding attempts, previous male effort did not explain the variation in female effort once a male's current effort was statistically controlled for. Male attractiveness did not influence male or female incubation effort, but females paired to less attractive males laid larger clutches, as predicted by the Compensatory Investment Hypothesis (CIH). The design of this experiment improved upon previous studies of the effects of paternal certainty on parental effort by taking male attractiveness, clutch size, individuals' previous experience and their partner's current effort into consideration under standardised laboratory conditions and where male EPC opportunity did not differ between pairs.

Female control over extra-pair copulations

Extra-pair males courted the females more persistently than the females' partners did, yet females copulated only with their partners. This lack of receptivity towards extra-pair males supports the conclusions of Forstmeier (2004) and Burley *et al.* (1996) that copulations are under female control in the Zebra Finch. Female control over copulations has been observed in other species, such as the Blue Tit, *Cyanistes caeruleus* (Kempnaers *et al.* 1995) and the Yellow-Legged Gull, *Larus cachinnans*, in which females copulate with their mates in return for food (Velando 2004). In the wild, Zebra Finch sex ratios fluctuate widely and females that are constrained to pair with low quality males may make the best of a bad job by seeking extra-pair fertilisations (EPFs) (Zann 1996). Females are predicted to accept copulations from extra-pair males that are more attractive than their partners to gain increased indirect genetic benefits (Zahavi 1975; Fisher 2005), such as more viable or sexually attractive offspring (for review see Jennions and Petrie 2000), and

evidence of preference for male traits indicative of ‘good genes’ has been found in a number of species (e.g. Hoikkala *et al.* 1998; Forsman and Hagman 2006). In the current experiment however females did not appear to be seeking ‘good genes’ because even the extra-pair males that were initially considered more attractive than the pair males were rejected once pairing had occurred. A female’s receptivity to copulate with a given male may also be influenced by their genetic compatibility (Zeh and Zeh 1996), the benefits of producing a more genetic diversity brood as a result of having multiple fathers (Jennions and Petrie 2000), and the probability of acquiring injury or disease (Ihara 2002; Pitnick and García-González 2002). An additional cost to females of accepting EPCs is the possibility of being detected by the partner, especially if he withholds paternal care as a result (Suter *et al.* 2009). Although the pair male was not visible to the female during the manipulation, she may have been aware that he was close by, perhaps through vocal cues. Moreover, females could not choose the identity of the extra-pair male or the time and location of the EPC opportunity in the current experiment. Any one or a combination of these factors may have contributed to the females’ reluctance to accept EPCs.

Was the manipulation successful?

Although females did not copulate with extra-pair males, the reduction in parental effort in males whose partners experienced an EPC opportunity in their first breeding attempt suggests that males did perceive the manipulation as a threat to their paternity. They could observe their partners interacting with and in many cases being courted and mounted by the extra-pair male through the one-way window over a three-hour period. It is likely that males can identify threats to their paternity, since paired male Zebra Finches in an aviary population responded aggressively towards attempts to copulate with their partners, displacing the extra-pair male and chasing him around the aviary (Birkhead *et al.* 1989). Perhaps more importantly, the response described by Birkhead *et al.* (1989) shows that males behave aggressively to attempted copulations and not just actual copulations. However, that the manipulation raised doubts over paternity was not evident from the within-pair copulation rate in the current experiment. Last male sperm precedence occurs in the Zebra Finch, and a well-timed copulation can fertilise 50-80 % of the clutch (Birkhead *et al.* 1988b). Therefore, we would expect males to increase their paternal certainty by copulating with their partners after a period of separation. However, pair males did not attempt to copulate more often after they were reunited with their partners than they did before the manipulation, and controls actually made more copulation attempts than treatment males.

The within-pair copulation rate over all lay day observations was relatively low (0.13 ± 0.03 copulations hr^{-1} or 0.36 ± 0.09 (successful and unsuccessful) copulation attempts hr^{-1} , $n = 22$) compared to 2.1 ± 0.7 copulations hr^{-1} ($n = 29$) during the fertile period of the Crested Tit, *Parus cristatus* (Lens *et al.* 1996) or 4.4 attempts hr^{-1} three days before laying in the Chaffinch, *Fringella coelebs* (Sheldon 1994b). However, most copulations result in sperm transfer in the Zebra Finch (Birkhead *et al.* 1989) and so even a low within-pair copulation rate may serve as an effective guard against extra-pair paternity. It may be more efficient for a male to copulate intermittently with his partner throughout her fertile period than to restrict this to times of low paternal certainty, especially if paternity cannot be assessed accurately. In the Chaffinch, females often seek EPCs outside their territory, and these tend to take place in ground vegetation where they are less conspicuous (Sheldon 1994a). Paired male Chaffinches copulate frequently with fertile females and do not show a behavioural response to variation in female EPC opportunity (Sheldon 1994b).

Effects of the paternal certainty manipulation on incubation effort

Males whose paternity was threatened during their partner's fertile period of their first breeding attempt (TC males) showed a reduction in incubation attentiveness into that attempt relative to controls. This finding is consistent with a number of other studies that have investigated the effects of variation in paternity or paternal certainty on care (Dixon *et al.* 1994; Johnson and Burley 1998; Sheldon and Ellegren 1998). All males continued to provide parental care to some extent, and this is probably because males perceived their chances of fertilising at least some of the clutch to be high. A male is unlikely to be capable of distinguishing his own eggs or nestlings from those of an extra-pair male (Beecher 1988; Kempenaers and Sheldon 1996), and may therefore be unwilling to deprive his own offspring of care.

When the treatments were switched in the second breeding attempt, however, males continued to invest the same level of parental effort that they had shown previously, and incubation attentiveness was repeatable across breeding attempts. This suggests that males, all of whom lacked breeding experience prior to the experiment, were primed by their experiences in the first breeding attempt to invest a similar level of effort into the subsequent attempt. This would be useful response if an individual's experiences in its first breeding attempt serve as a good indicator of future conditions, for example, if paternity levels differ little between broods (Westneat and Sherman 1993). Therefore males may not respond to variation in paternal certainty *per se* but to an estimation of their probability of paternity that was made in early life. This may partly explain the failure of

some studies to observe a response to manipulations of variation in paternal certainty or to other factors that are predicted to influence parental effort. If individuals are indeed primed by early life experiences, it would be interesting to know how long these effects persist and whether they vary with context. For example, males may have responded appropriately to their partners' EPC opportunity in the second breeding attempt if they had been paired with a new female. In species which form temporary pair bonds, males may negotiate a distinct level of paternal effort with each new partner, especially if females vary in their EPF rate.

Consistency in parental effort within individuals is a prediction of sealed bid models of behaviour (e.g. Chase 1980; Houston and Davies 1985). However, a true sealed bid response predicts that partners do not respond to each other's current levels of effort, and this was not the case here. An individual's current incubation attentiveness was influenced by the partner's current effort in both males and females, as predicted by negotiation models (McNamara *et al.* 1999). Moreover, the variation in a female's current effort was not explained by her partner's previous effort once his current effort was taken into account. This differs from the results of Schwagmeyer *et al.* (2002) in the House Sparrow, *Passer domesticus*, who found that a male's previous (baseline) effort was a better predictor of the female's current effort than was the male's current effort. The relative influence of previous and current effort may differ between species or from one stage of parental care to the next: here I focussed on incubation effort, while Schwagmeyer *et al.* (2002) examined nestling provisioning behaviour, and indeed they suggest that incubation might be a more promising stage in which to find evidence of negotiation because partners can evaluate each other's effort more easily and accurately during incubation.

The reduction in male incubation effort in TC pairs led to an increase in female effort within pairs, as predicted by compensation models (Houston and Davies 1985; McNamara *et al.* 1999), but TC and CT pairs responded differently to each other's levels of effort: TC females compensated fully for the reduction in their partner's effort, while CT females overcompensated. Although incubation is energetically costly in captivity (Vleck 1981), the costs may be short-lived compared to the wild as a consequence of higher food availability and shorter foraging distances. The different strategies of incubation behaviour exhibited by TC and CT groups are likely to be due to differences between them in the perceived costs and benefits of incubation. TC males probably perceive incubation to be more costly than CT males due to reduced paternal certainty. However, an alternative interpretation of these results is that the manipulation directly influenced female incubation

effort in some way, and that this led to a reduction in female attentiveness and a compensatory increase in male effort. This could be the case if the manipulation altered the strength of the pair bond, for example. A further possibility is that the EPC opportunity was not perceived as such by females and that the results observed were due to treatment females being confined with an unfamiliar bird or to controls being maintained without social stimulation. However, it is unclear why these latter circumstances would lead to changes in parental effort in the directions observed here. Male attractiveness did not influence male or female incubation effort, but females paired to less attractive males laid larger clutches, as predicted by the Compensatory Investment Hypothesis (Gowaty 1997; Gowaty 2008; Harris and Uller 2009). Similarly, Bolund *et al.* (2009) found that female Zebra Finches produced larger eggs containing a higher carotenoid content when paired with less attractive males.

Conclusions

A number of studies have investigated the parental response of males to variation in paternal certainty, but have reported conflicting results. I found that inexperienced male Zebra Finches whose partners received an EPC opportunity during their fertile period spent less time incubating relative to controls. However, in the second breeding attempt, when the treatments were switched, males continued to show the same level of effort that they had invested previously. This suggests that individuals can be primed by their early life experiences to show similar behaviour in later life, and this may be a useful strategy if early life conditions serve as a good indicator of future events. This also emphasises the important role that previous experience can play in individual investment decisions, and may shed light on the conflicting patterns of paternal behaviour in response to variation in paternal certainty.

Chapter 3 - Negotiation over biparental care: The effects of male EPC opportunities on male and female incubation effort

Abstract

Parental care is costly, and each individual within a biparental system will do better if it can reduce its own level of effort and rely on its partner to compensate, yet the partner should be unwilling to compensate completely if it is to avoid being exploited. Whether such patterns of effort can be negotiated over ecological time and how these patterns vary under different contexts is poorly understood. As an alternative to handicapping or mate removal, I manipulated male extra-pair copulation (EPC) opportunity in a captive population of the Zebra Finch, *Taeniopygia guttata*, and recorded (a) the extent to which males with increased EPC opportunity incubated their partner's clutch, and (b) how female respond to their partner's effort. Male fitness is limited by the number of females he can fertilise, so a male who is already involved in parental care may do better by shifting his effort from parental care to seeking (EPCs) when receptive females are available. Male and female effort may also be influenced by their previous experience, clutch size and male attractiveness, and previous studies have frequently failed to take these into account. Here, association times recorded in two-way mate choice trials provided a proxy for male attractiveness. Females were paired randomly with one of the males for the duration of the experiment, and bred with him twice. Half the males received EPC opportunities with 'extra-pair females' (treatment) during incubation in the first breeding attempt, while the other half did not (controls). I used a 'crossover design', which meant that males that had previously been controls received EPC opportunities in the second breeding attempt and *vice versa*. Male and female incubation behaviour was recorded on two days on which EPC opportunities were not presented. In their first breeding attempt, males with increased EPC opportunities reduced incubation effort relative to controls, but when the treatments were switched in the second breeding attempt, males continued to invest the same level of effort that they had previously. This emphasises the importance of experience in shaping reproductive decisions and may explain conflicting results in studies where individual history is not known. Individuals responded to each other's contributions over ecological time, and control and treatment pairs negotiated different patterns of effort. Investment decisions are thus based on cues taken both from the environment and from the level of effort that the partner is willing or able to make.

Introduction

Sexual conflict over biparental care occurs due to differences between the sexes in the costs and benefits of care, and these can lead to sex-specific differences in optimum resource allocation to a given breeding attempt (Trivers 1972; Chase 1980; Parker *et al.* 2002). In many species, parental care is essential to enable offspring to grow and survive to sexual maturity, but it is also costly to the parent (Trivers 1972; Clutton-Brock 1991). This means that individuals must resolve a trade-off between the resources they invest into different activities, and between current and future offspring (Williams 1966; Trivers 1972; Magrath and Komdeur 2003). The optimal situation for a male is one in which he can minimise his parental effort, thus freeing up time and energy to invest in self-maintenance and alternative reproductive strategies, whilst relying on his partner to compensate (Trivers 1972; Lessells 1999; Houston *et al.* 2005). However the female may endanger her own survival or future reproduction if she compensates, so may be unwilling, or simply unable, to do so, and both partners will suffer if the offspring die. This sets a limit to how far a particular male should reduce his investment (Jones *et al.* 2002).

Theoretical models have traditionally predicted that a female should increase her own level of effort in response to a decrease in her partner's effort but that she should not offset the deficit fully or she will be exploited (e.g. Chase 1980; Houston and Davies 1985; McNamara *et al.* 1999); in other words, partial compensation is the most evolutionarily stable strategy (ESS) under biparental care (Maynard Smith 1982). Early models such as Chase (1980) and Houston and Davies (1985) were formulated as an evolutionary game of two players, male and female, who each bring to the game a fixed 'sealed bid' (Schwagmeyer *et al.* 2002) or 'sealed envelope' (Houston and McNamara 1999) of parental effort, which is the average best response given the average best response of its partner. These contributions are consistent between breeding attempts and are not influenced by the partner's response: the conflict takes place over evolutionary time. This theoretical framework was subsequently built upon by McNamara *et al.* (1999) to describe a situation in which each player can modify its level of effort sequentially over ecological time in response to its partner's behaviour. These models are referred to as 'negotiation' models and have explored how a parent can adjust effort according to its own and its partner's condition, the time in the season and the amount of information available to each (Barta *et al.* 2002; Jones *et al.* 2002; Johnstone and Hinde 2006).

The predictions of these models have been tested by a number of empirical studies in biparental birds (e.g. Hinde and Kilner 2007), fish (e.g. Steinegger and Taborsky 2007) and insects (e.g. Smiseth *et al.* 2006). Most experimental work has involved avian subjects and falls into two broad groups: mate removal (e.g. Royle *et al.* 2002) and handicapping (e.g. Beaulieu *et al.* 2009). In mate removal studies, researchers compare the parental response of the partners of removed birds to the response of birds whose mate was not removed, while handicapping manipulations serve to increase in the energetic costs of care with the aim of inducing individuals to decrease parental effort, and then the response of the manipulated partner is compared to the response of control partners. Alternative approaches to these have involved playback of offspring begging calls to one member of a pair to achieve an increase in its provisioning rate (Hinde 2006) or directing the male from parental to mating effort using testosterone implants (e.g. Hegner and Wingfield 1987; Mazuc *et al.* 2003; Schwagmeyer *et al.* 2005), male badge size manipulation (e.g. Nakagawa *et al.* 2007b) or provision of additional nestboxes (Smith *et al.* 1995).

While some authors have observed no apparent response to manipulations (e.g. Hegner and Wingfield 1987; Whittingham *et al.* 1994; Schwagmeyer *et al.* 2002), others have reported full compensation (e.g. Wright and Cuthill 1990a), partial compensation (e.g. Wright and Cuthill 1989; Markman *et al.* 1995) or even a ‘matching’ response involving a change in parental investment in the direction taken by the manipulated partner (e.g. Hinde 2006). The Information Hypothesis predicts that partners should ‘match’ each others’ levels of investment when information on the need or quality of the offspring is incomplete (Johnstone and Hinde 2006). We would expect to see a positive relationship between male and female parental effort under matching, while compensation predicts a negative relationship. Partial compensation was found to be the mean response to reduced partner effort over nestling provisioning where mate removal and partner manipulation studies were combined in a meta-analysis by Harrison *et al.* (2009). There was a non-significant trend among the partners of removed or manipulated birds to increase incubation effort, and combined parental effort was lower in treatment pairs than in controls.

Although behavioural negotiation may potentially occur at any stage of biparental care, Schwagmeyer *et al.* (2002) suggested that it most likely occurs during incubation when partners can evaluate each other’s effort more easily and accurately, potentially taking cues from incubation attentiveness and egg temperature. Parental effort is less likely to be influenced by differences between offspring in quality and need during incubation because these are less apparent to the parent (although the female potentially has information on

egg composition, which can affect both quality and need of offspring). Incubation is also an appropriate stage in which to study the conflict between mating and parental effort because males may have more opportunities for engaging in extra-pair copulations (EPCs) when their partners are confined to the nest for longer periods.

Since an individual has a finite amount of time and energy, and male fitness is limited by the number of females he can fertilise (Bateman 1948; but see Snyder and Gowaty 2007 for re-analysis), a father may do better by shifting his resource allocation from parental care to seeking extra-pair copulations when receptive females are available (Maynard Smith 1977; Westneat *et al.* 1990; Magrath and Komdeur 2003). The trade-off between paternal effort, mating effort and self-maintenance is influenced by individual and environmental variation, and previous studies have often failed to consider these (Magrath and Komdeur 2003; Stiver and Alonzo 2009). For example, a male might increase investment in larger clutches or broods since their relative value is greater (Komdeur *et al.* 2002), but they may be more costly to care for (Biebach 1981; Gustafsson *et al.* 1995). The costs of paternal care are also influenced by food availability (e.g. Dunn and Robertson 1992), ambient temperature (e.g. Weston and Elgar 2005), the level of effort invested in previous breeding attempts (e.g. Gustafsson *et al.* 1995) and the carer's relatedness to the offspring (Werren *et al.* 1980; Houston and Davies 1985; Winkler 1987), while the benefits of investing in mating effort depend on the availability of fertile females (Westneat 1988b; Magrath and Elgar 1997) and appropriate nest sites (Smith 1995). Male phenotypic quality or attractiveness is particularly important to consider because attractive males have more to gain from reallocating effort from parental care into seeking EPCs (Magrath and Komdeur 2003). However, good quality males may be able to maintain high levels of both parental and mating effort because they can afford to reduce their investment into somatic maintenance (Lessells 1991). The Differential Allocation Hypothesis (DAH) suggests that, given that there is a trade-off between current and future reproduction (Williams 1966), a female should invest more resources into the offspring of an attractive mate because his offspring will be more valuable (Burley 1986; Sheldon 2000), and many empirical studies have found support for this hypothesis (reviewed in Sheldon 2000). However, the Compensatory Investment Hypothesis (CIH) predicts that females constrained to pair with less attractive males should compensate for the low quality of their mates by increasing their parental effort (negative differential allocation) (Saino *et al.* 2002; Bolund *et al.* 2009). Whether females should show positive or negative differential allocation depends on their chances of obtaining further mating opportunities, their

energetic state and the relative impact of parental effort on offspring quality (Gowaty *et al.* 2007; Bolund *et al.* 2009; Harris and Uller 2009).

I manipulated male EPC opportunity in a domesticated population of the Zebra Finch (*Taeniopygia guttata*) by allowing incubating males to interact with ‘extra-pair females’. The quality of EPC opportunity offered was also manipulated by providing these unpaired females with either a high or low quality diet. While researchers have traditionally used handicapping or mate removal to induce variation in parental effort, manipulating male EPC opportunities during incubation might be a promising context in which to test how males adjust their patterns of effort and how their partners respond to variation in male parental effort. Handicapping may bring about a change in a female’s perception of the quality of its mate or their shared offspring, and thus it is hard to distinguish whether a change in female effort is a response to her partner’s effort or to the handicap itself (Sanz *et al.* 2000; Harrison *et al.* 2009). Most previous investigations of the relationship between mating and parental effort have been field-based studies that have not considered differences in male attractiveness, variation in EPC opportunity or paternal certainty (Magrath and Komdeur 2003). Attractive males are predicted to achieve more EPCs and higher paternity of their own brood than less attractive males, yet increased EPC opportunity and paternal certainty are predicted to have directionally opposite effects on paternal care (Wright 1998). In contrast, the experiment described here is laboratory-based and uses a crossover design in which each individual bred twice with the same partner and the type of treatment was switched after the first breeding attempt. This allowed the effects of previous experience to be taken into account, while using a reduced sample size and preserving analytical power (e.g. Bolund *et al.* 2009). The Zebra Finch is a socially monogamous, sexually dimorphic passerine that forms permanent pair bonds but engages in EPCs (Birkhead *et al.* 1988a). Breeding synchrony is low in wild Zebra Finch colonies (Birkhead *et al.* 1988a; Zann and Rossetto 1991) so some females are likely to be sexually receptive while the others are caring for young (Birkhead and Biggins 1987). Both parents contribute to incubation and nestling provisioning in the wild and in captivity (e.g. Zann 1996). The aim of this study was to test the following hypotheses: 1) Males with increased EPC opportunity spend less time incubating their partner’s clutch. 2) Individuals are able to respond to each other’s effort over ecological time, and females compensate partially for a reduction in male effort.

Methods

Subjects and maintenance

34 birds of each sex were bred at the University of Glasgow between January and June 2006 and sexes were housed separately at a density of six birds per cage (41 x 122 cm and 45 cm high) before pairing. They were 316 ± 5.45 days old (mean \pm SE, $n = 68$) when manipulations began and none had bred previously. Before and during the experiment, they were maintained at 38.3 ± 0.54 % relative humidity, an ambient temperature of 21.1 ± 0.11 °C and under a photoperiod of 14L:10D in a room without natural light. The room was illuminated overhead by daylight-spectrum fluorescent tubes (Arcadia Bird Lamp FB36). Birds received a ‘standard’ diet of *ad libitum* mixed seed, consisting of pearl white, yellow, panicum, red, Japanese and Canadian canary millets (Foreign Finch Mixture, supplied by Haith’s, Cleethorpes, Lincolnshire, U.K.), oyster grit, cuttlefish bone and water. They were also given egg biscuit protein, a vitamin supplement (Daily Essentials 2: The Birdcare Company, Nailsworth, U.K.) and fresh spinach leaves once a week, and a calcium supplement (Calcivet: The Birdcare Company, Nailsworth, U.K.) three times a week. Since birds may offset the costs of incubation more quickly in captivity, paired birds received seed that had been mixed with hemp and empty husks to increase food handling time and decrease net energy gain (Lemon and Barth 1992; Lemon 1993).

Outline of the experiment

Each female Zebra Finch encountered two potential mates during a series of mate choice trials (see Male attractiveness below) and was then paired randomly with one of them for the duration of the experiment. The amount of time she spent associating with her partner during the trial was used as a proxy for his attractiveness. During the incubation stage of the first breeding attempt, half the males received EPC opportunities with ‘extra-pair females’, while the other half did not encounter other birds during the brief periods of separation from their partners. The quality of EPC opportunity was manipulated by administering either a high or low quality diet to the extra-pair females. In the second breeding attempt, males that were previously controls received EPC opportunities (referred to hereafter as ‘CT’ birds) and *vice versa* (‘TC’). Male and female incubation behaviour was recorded on two days on which EPC opportunities were not presented. Details are described below.

Male attractiveness

I recorded the amount of time females spent associating with males in a series of two-way spatial association trials to provide a proxy for male attractiveness to be used as a covariate in mixed-model analysis of male and female incubation attentiveness. Behaviours characteristic of pair bond formation can occur within minutes of introducing unfamiliar male and female Zebra Finches (Silcox and Evans 1982; Zann 1996), and so much of the process of pair bond formation should be captured in the two-hour trials described here.

Thirty-four birds of each sex were grouped into 17 quads of unrelated individuals, each consisting of two males, matched for colour morph ($n = 2$ fawn and $n = 15$ wild type quads), and two females. Each female had the opportunity to choose between the two males from her quad within a choice chamber (figure 2.1) in a separate room from where the birds were housed. Lighting was as previously described and supplemented by a fluorescent tube (Arcadia Bird Lamp FB36) placed behind the neutral zone.

The two males of a quad were weighed to the nearest 0.01 g on an electronic balance and then released into their respective compartments and one of the females of their quad into the neutral zone. After 15 minutes' acclimatisation, I recorded which preference zone the female was occupying (1, 2 or neutral) at minute intervals for one hour. When the trial was complete, the two males were switched between compartments to control for a potential female side bias. The birds experienced an additional five-minute acclimatisation period and the trial was repeated. After the second trial, the female was replaced with the second female from her quad, and the process was repeated with the same two males. All females visited both left and right compartments during each pair of trials. Female preference was not side-biased (mixed model controlling for female and trial identities: right: 48.1 ± 4.15 %, $n = 34$ females; left 48.2 ± 4.01 %, $n = 34$; $P = 0.987$) and nor did a male's body mass influence female preference ($P = 0.434$). I summed the number of minute intervals each female spent with each male over the two sides and expressed this as a percentage of the total observation time (including time spent in the neutral zone, which averaged 3.7 ± 1.54 %), resulting in each male receiving a single 'attractiveness' score from each of the two females in his quad. The two females within a quad did not prefer the same male ($r_p = 0.03$, $P = 0.874$, $n = 34$).

I assigned each pair to one of two experimental groups (TC or CT) so that groups did not differ in male attractiveness score or tarsus length. This was still true of the 17 pairs that made up the final sample (see statistical analysis for details) (male attractiveness: CT: 52.5

± 8.73 %, $n = 8$; TC: 55.9 ± 8.37 %, $n = 9$; $F_{1,15} = 0.08$, $P = 0.783$; male tarsus length: CT: 14.2 ± 0.19 mm, $n = 8$; TC: 14.4 ± 0.20 mm, $n = 9$; $F_{1,15} = 0.22$, $P = 0.642$; female tarsus length: CT: 14.5 ± 0.11 mm, $n = 8$; TC: 14.6 ± 0.16 mm, $n = 9$; $F_{1,15} = 0.09$, $P = 0.762$). TC males would receive EPC opportunities during the first breeding attempt, CT in the second. Males varied in attractiveness from 8-97 % during their partners' initial assessments ($n = 17$).

Extra-pair females

To test how male courtship and incubation behaviour might differ according to the quality of EPC opportunity offered, each male was assigned to a group of extra-pair females who had received either a high protein (HP) or low protein (LP) diet treatment. Jones *et al.* (2001) found that male Zebra Finches spent more time associating with females who had received a HP diet for two weeks, irrespective of the females' body mass. Sixteen females served as extra-pair females and were evenly distributed between two cages. The females were unrelated to the paired birds, although they were raised at the same time and under the same conditions. They were weighed to the nearest 0.01 g on an electronic balance and measured to check that birds allocated to the two groups did not differ in body mass, size or age ($t_{14} \leq 1.16$; $P \geq 0.267$) before diet treatments began. Each group was randomly assigned to receive either a HP diet consisting of the standard diet (see subjects and maintenance) supplemented with micro-waved hen's egg four times a week or a LP diet of mixed seed and water in place of the standard diet. Diet treatments began eight weeks before the first manipulation and continued until manipulations were complete. The females within each diet treatment group were teamed into four dyads. The members of each dyad would accompany each other during manipulations, and each pair male would encounter all four dyads, each only once, from his assigned female diet group over his time as a treatment male.

Manipulation of EPC opportunities

Thirty-four cages were separated by an opaque divide into a 'pair side' and an 'extra-pair side' (each side 41 x 61 cm and 45 cm high) so that the behaviour occurring on one side of the cage was not visible from the other (although acoustic information could not be excluded). The pair side contained coconut fibre nesting material (which was replenished once a week until the onset of incubation), food and water. A wooden nestbox could be accessed by the birds from the pair side. The cages were arranged back to back so that the birds were unable to see other pairs, with the pair side alternating between left and right hand sides of the cage, and birds were paired at staggered intervals.

Before each manipulation, the extra-pair side was provided with mixed seed and water, and a small video camera was installed and connected to a JPEG 2000 DVR system and television monitor in the next room. Incubation begins (day zero) on the day that the fourth egg is laid in clutches of four or more, and on the final day of laying in smaller clutches (Zann and Rossetto 1991). At 11:00am (once incubation recordings were complete) on days three, five, seven and nine of incubation, a dyad of extra-pair females from the appropriate diet group was released into the extra-pair side (the day nine manipulation was part of a separate study presented in chapter four). After five minute's acclimatisation time, the male was transferred from the pair side to the extra-pair side and the observation began. I noted at minute intervals for 15 minutes whether the male was feeding, resting or courting. The Zebra Finch courtship sequence is described in Zann (1996; pages 170-174), and is divided into four stages. I recorded by continuous observation the number of successful and unsuccessful copulation attempts, the number of times the male wiped his bill against the perch and the highest stage of courtship reached. 'Bill wiping' is an easily recognisable behaviour that forms part of the first stage of courtship. The amount of time spent courting and the stage reached were used as proxies for courtship effort and success, respectively. After 15 minutes of observation, the male was returned to the pair side and the extra-pair females to their home cage. Controls proceeded in the same manner and for the same duration, except that no extra-pair females were present when the male was transferred to the extra-pair side of the cage. The pair female was unable to observe her partner's behaviour during control and treatment manipulations. Each treatment male encountered all four dyads within his assigned female diet group over the four treatment days.

Each pair was allowed to complete incubation and raise chicks. Fledglings were separated from their parents at 30 days of age and nestboxes were cleaned and supplied with new nesting material. Two clutches did not hatch (TC $n = 1$; CT $n = 1$) and the offspring of four pairs (TC $n = 1$; CT $n = 3$) died before reaching 5 days of age. To standardise the interval between breeding attempts, I separated males and females and maintained them on either side of a Perspex divide in the same cage until 30 days after the eggs had been due to hatch. The remaining 11 pairs fledged at least one offspring. Birds made a second breeding attempt with the same partner and the experiment proceeded as before, except that the treatments were switched so that pairs that had previously been control were now treatment and *vice versa*. The interval between the first manipulation of the initial breeding attempt and that of the second attempt was 47.2 ± 0.91 days (range: 42-55 days, n

= 17), depending on lay latency and the size of the second clutch. Allowing the birds to make two breeding attempts in short succession allowed me to investigate the effects of recent experience on subsequent incubation behaviour and also on female reproductive output (chapter four).

Incubation effort

To test whether males with increased EPC opportunities showed reduced incubation effort, I recorded incubation attentiveness on two days (six and eight) on which EPC opportunities were not presented. Since the female incubates at night (Zann and Rossetto 1991), her energy reserves are low by the morning and she has to leave the nest to forage. Male incubation is particularly important at this time, and so observations took place in the morning (07:40 and 10:40am). The evening before observations took place, I installed a small infra-red video camera above the nestbox and set the DVR system to record. This allowed birds to acclimatise to the camera overnight and reduced disturbance prior to recordings.

Video analysis was performed blindly with regard to treatment group, observation day and breeding attempt (one or two) by a single observer (myself). For each sex, distinguishable on the basis of plumage dimorphism, I recorded incubation attentiveness (the amount of time spent incubating the eggs) and nest occupancy (the amount of time spent inside the nestbox) to the nearest second. A bird had to pass over the threshold of the nestbox to be scored as occupying it, and to cover the eggs with its ventral surface to be scored as incubating. The Zebra Finch achieves close contact between brood patch and eggs by undertaking a distinctive ‘shuffling’ movement at the onset of an incubation bout and at regular intervals throughout in which the feathers of the brood patch are parted. Incubation attentiveness (s) and nest occupancy (s) was calculated for each sex over each observation period (presented in figures and means as a percentage of the observation duration). Periods in which the bird lost contact with the eggs (to maintain the nest, for example) were not included in calculations of incubation attentiveness. ‘Shared’ incubation, when both partners frequently shuffle on the eggs and departure of one partner does not leave the whole clutch exposed, contributed to 11.1 ± 3.33 % of total incubation time ($n = 17$ pairs). Although this could differ from non-shared incubation in terms of energetic costs to the parent or heat transfer to the developing embryo, it involves an equal investment of time from each of them so was included as such in time budget calculations. Due to shared incubation, a pair’s combined (male + female) attentiveness could exceed 100 %. Nest occupancy and incubation attentiveness were correlated in males on just one of the four

observation days (two days per breeding attempt) ($r_s = 0.66$, $P = 0.005$; otherwise $r_s \leq 0.40$, $P \geq 0.107$), whereas the two measures of incubation effort were correlated on all four days in females ($r_s \geq 0.86$, $P < 0.001$). I used incubation attentiveness rather than nest occupancy as my proxy for incubation effort since this reflects the component of incubation behaviour that is costly in terms of both time and energy. Time spent outside the nestbox (total observation duration minus nest occupancy) was used to examine differences in the time budgets of treatment and control birds in response to the EPC manipulation.

Statistical analysis

Of the 34 initial pairs, some pairs could not be used in the analyses because they did not produce a clutch in one (TC: $n = 1$) or both (TC $n = 4$; CT $n = 4$) breeding attempts, nested on the floor of the cage (TC: $n = 3$; CT: $n = 3$) or failed to incubate during either observation of the first attempt (TC: $n = 1$); the male of another pair (TC: $n = 1$) died before the second manipulation, leaving 17 pairs with complete data from both breeding attempts. Data were analysed using R version 2.9.0 (R Development Core Team 2009).

Courtship data was collected on days three, five and seven and nine of incubation when extra-pair females were presented to pair males for periods of fifteen minutes. I do not present courtship data from day nine since incubation behaviour data collection ended on day eight. Each of the five measures of courtship behaviour was respectively summed over the three observations of extra-pair behaviour, giving cumulative scores for the stage of courtship reached, the number of successful and unsuccessful copulation attempts, the number of male bill wipes and the amount of time the male spent courting, and these were used as the unit of analysis in tests to infer whether the male perceived the manipulations as EPC opportunities. Where means are given, the numbers of successful and unsuccessful copulations and bill wipes are presented as behaviours hr^{-1} , and time spent courting as a percentage. Median values of stage reached are presented since this is a categorical measure, and scores ranged from 0 to 12 once expressed cumulatively over the three observation periods. Since the measures were not normally distributed, associations between them were explored using non-parametric Spearman's rank correlations. The Wilcoxon rank sum test (with continuity correction) was used to test whether males spent more time (courtship effort) courting HP diet extra-pair females and reached a higher stage of courtship with them (courtship success).

Observations of incubation behaviour were available from days six and eight of both breeding attempts for all 17 pairs. Incubation attentiveness data (s) followed a right-skewed distribution for males and left-skewed for females, so I respectively used square-root and square transformations to normalise. Transformed data were analysed using linear mixed effect models by the Restricted Maximum Likelihood method from the lme4 library (Bates and Maechler 2009). All mixed models were fitted with individual identity (unless stated otherwise) as a random factor (random intercepts only) to control for repeated measures even when not statistically significant. These models are presented with estimates of the model coefficient (β), highest posterior density (HPD) intervals (reported in brackets after the coefficient) calculated at the 95 % level using Markov Chain Monte Carlo (MCMC) 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 2009). I also present the variance between (individual identity) and within (residual variation) individuals as a percentage of the total variance. Final models were reached using backwards stepwise elimination of non-significant ($P \geq 0.05$) terms, removing interactions and then main effects in order of least significance. This approach was deemed most appropriate for hypothesis testing within a controlled experiment. Statistical tests are two-tailed, all two-way interactions were tested and all main effects and significant interactions ($P < 0.05$) are reported. Significance is also denoted by HPD intervals that do not include zero. Means are presented \pm SE.

I tested the hypothesis that males with increased EPC opportunity spend less time incubating their partner's clutch, by investigating the effect of treatment group, which was a fixed factor with two levels, on male incubation attentiveness. Since the treatments were switched in the second breeding attempt, I included breeding attempt (one or two) as a fixed factor in the model. If males responded to the treatment regardless of the order in which treatments and controls are presented, I would see a main effect of treatment without an interaction between treatment and breeding attempt. An interaction between the two would indicate that male effort varies with respect to the sequence of treatments. The benefits of incubation are influenced by clutch size since larger clutches are more valuable (Westneat 1988a; Komdeur *et al.* 2002), but the energetic cost of incubating larger clutches is greater (Biebach 1981; Biebach 1984; Haftorn and Reinertsen 1985; de Heij *et al.* 2007) so clutch size was included as a covariate. Clutch size was not normally distributed so non-parametric statistics were used to test for differences in clutch size between treatment groups and breeding attempts, and to test for collinearity with male attractiveness. Clutch size did not differ between breeding attempts (paired Wilcoxon signed rank test with

continuity correction: attempt 1: 5.0 ± 0.31 eggs, $n = 17$; attempt 2: 4.6 ± 0.30 eggs, $n = 17$; $V = 52$, $P = 0.316$) or treatment groups (Wilcoxon rank sum test with continuity correction: attempt 1: control: 5.0 ± 0.57 eggs, $n = 8$; treatment 5.0 ± 0.33 eggs, $n = 9$; $W = 42$, $P = 0.571$; attempt 2: control: 4.7 ± 0.47 eggs, $n = 9$; treatment 4.6 ± 0.37 eggs, $n = 8$; $W = 39$, $P = 0.804$), and did not vary with male attractiveness (attempt 1: $r_s = -0.40$, $P = 0.114$; attempt 2: $r_s = -0.23$, $P = 0.376$). Male attractiveness was included as a covariate in the main analysis since more attractive males are predicted to show reduced parental effort (Burley 1988; Qvarnström 1997). I included the incubation attentiveness of the female from the same observation as a covariate since the effort of one partner may influence that of the other. I tested whether the number of manipulations a male received had a cumulative effect in reducing his attentiveness over time by including the day that the observation was made as a covariate in the model. I also calculated the repeatability of incubation effort across both days and breeding attempts for males and females separately using the formulae given in Lessells & Boag (1987) and in Becker (1984) for standard errors.

As well as testing for the effects of increased EPC opportunity on incubation effort, I tested whether treatment males also spent more time outside the nestbox. They may do this in order to seek EPCs (though none were presented while incubation behaviour was being recorded). Nest occupancy and incubation attentiveness were correlated in males on just one of the four observation days, so an effect of treatment on incubation attentiveness does not necessarily correspond to an effect on nest occupancy. I used a mixed model with time spent outside the nestbox as the response variable (square-root transformed to meet assumptions of normality). Treatment and breeding attempt were included as fixed factors, clutch size, male attractiveness, observation day and female nest occupancy as covariates and individual identity as a random factor.

To test the hypothesis that males that experience a higher quality of EPC opportunity spend less time incubating their partner's clutch, I included diet treatment as a fixed factor and time spent courting as a covariate in an analysis of treatment males only. Breeding attempt, male attractiveness and clutch size were also included in the model.

I examined the relationship between the partners' effort by running a further mixed model with female attentiveness as the response variable and male attentiveness during the same observation as a covariate. This allowed me to assess whether behavioural negotiation might occur: there will be a negative relationship between the partners' incubation effort if

they compensate for each other (McNamara *et al.* 1999), and a positive relationship if they match each other's response (Johnstone and Hinde 2006). Since breeding attempt was involved in an interaction with treatment on male incubation attentiveness, analysis of the female response was restricted to the first attempt. Male attractiveness, treatment group, clutch size and observation day were included in the model. I expected to find an interaction between treatment and partner's attentiveness if treatment and control females respond to their partner's effort in different ways. To separate the interacting effects of treatment and male effort, I analysed treatment and control pairs separately, with female attentiveness as the response variable (here untransformed), male attentiveness as a single covariate and pair identity as a random factor. The slope of the correlations between male and female attentiveness is equal to the mean change in attentiveness per partner unit of attentiveness, and I used the HPD intervals from these regression analyses to assess where the female response lay on the continuum between perfect matching (HPD intervals overlapping a line of slope = 1) and perfect compensation (HPD intervals overlapping a line of slope = -1). While traditional tests for compensation compare the response of the partners of manipulated individuals to the partners of controls, regression analysis uses a within-pair approach to examine the current effort of individuals to their partners in the context of behavioural negotiation. Finally, I tested whether combined (male + female) incubation attentiveness differed between treatment groups in a general linear mixed effect model, with pair identity as a random factor, treatment and breeding attempt as fixed factors and clutch size and male attractiveness as covariates. If females compensate completely for their partners, I would not expect to see a difference between treatment and control groups in combined incubation attentiveness.

Results

Courtship behaviour

All 17 pair males reached at least the first stage of courtship when presented with extra-pair females. Seven males copulated (mean: 1.4 ± 0.51 copulations hr^{-1} , range: 0-5, $n = 17$), while another five made at least one copulation attempt (mean: 5.1 ± 1.25 attempts hr^{-1} , range: 0-11, $n = 17$). All five courtship behaviours were positively correlated ($r_s \geq 0.54$, $P \leq 0.026$, $n = 17$), with the exception of the numbers of bill wipes and successful copulations ($r_s = 0.42$, $P = 0.095$, $n = 17$) and bill wipes and attempted copulations ($r_s = 0.46$, $P = 0.062$, $n = 17$). The quality of diet that the extra-pair females received had no effect on the amount of time the male spent courting them (HP mean: 22.5 ± 7.38 %, $n = 7$;

LP mean: $18.2 \pm 3.55\%$, $n = 10$; $W = 36$, $P = 0.961$) or the cumulative stage of courtship reached over the three observations (HP median: stage 7, range: 3-12, $n = 7$; LP median: stage 6, range: 2-12, $n = 10$; $W = 41.5$, $P = 0.556$).

Effects of male EPC opportunity on male incubation attentiveness

Male incubation attentiveness was influenced by a number of factors (table 3.1). The effect of EPC opportunity on male incubation attentiveness depended on the sequence of treatments (treatment group x breeding attempt interaction): males who had experienced EPC opportunities in their first breeding attempt showed lower attentiveness than controls in that attempt and both groups maintained a similar level of effort when treatments were switched in the second attempt (figure 3.1). Male incubation attentiveness decreased with increasing female attentiveness only when females were paired with a less preferred male (figure 3.2a) or when clutch size was large (figure 3.2b). Male incubation attentiveness was not influenced by the day of incubation. In fact it was repeatable across days and breeding attempts in both males ($_{16,50} = 4.15$, $r = 0.444 \pm 0.13$, $P < 0.001$) and females ($F_{16,50} = 1.96$, $r = 0.195 \pm 0.13$, $P = 0.036$).

Table 3.1 General linear mixed model showing the effects of increased male extra-pair copulation (EPC) opportunity (treatment) on male incubation attentiveness (square-root transformed) in 17 pairs over two breeding attempts each (attempt 1: $n = 8$ control and 9 treatment; attempt 2: $n = 9$ control and 8 treatment; $n = 68$ observations overall). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for all main effects and significant interactions. All other interactions $P \geq 0.085$.

	β	HPD interval	
<i>Fixed effects</i>			P
Intercept	-126.6895	-220.2862 to -29.2301	0.011
Treatment group ^a	-14.5905	-26.4440 to -3.4833	0.013
Breeding attempt ^b	-9.7824	-21.7157 to 1.8970	0.101
Partner's attentiveness	0.0200	0.0075 to 0.0331	0.003
Clutch size	28.8858	15.3801 to 41.4930	< 0.001
Male attractiveness	1.1020	0.1270 to 2.1153	0.033
Treatment group x breeding attempt	27.7211	9.5536 to 45.0292	0.005
Partner's attentiveness x male attractiveness	-0.0002	-0.0003 to 0	0.026
Partner's attentiveness x clutch size	-0.0027	-0.0044 to -0.0010	0.002
<i>Main effect not in final model</i>			
Day of incubation	0.5713	-3.0071 to 4.1151	0.746
<i>Random effects</i>			Variance (%)
Individual identity	4.2312	0 to 8.8019	32.2
Residual	14.9583	11.9863 to 18.1702	67.8

^a 'control' is the reference group

^b '1' is the reference breeding attempt

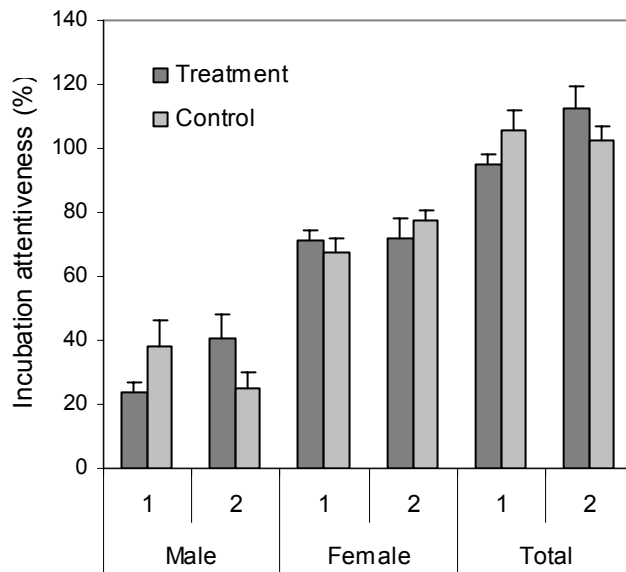


Figure 3.1 Male, female and combined male and female incubation attentiveness (means + 1 SE) for each breeding attempt (1 or 2) as a proportion of the total time available (breeding attempt 1: n = 8 control and 9 treatment pairs; breeding attempt 2: n = 9 control and 8 treatment pairs). There was a treatment group x breeding attempt interaction on male attentiveness (see table 3.1). Control pairs from the first breeding attempt became treatment pairs in the second breeding attempt and *vice versa*.

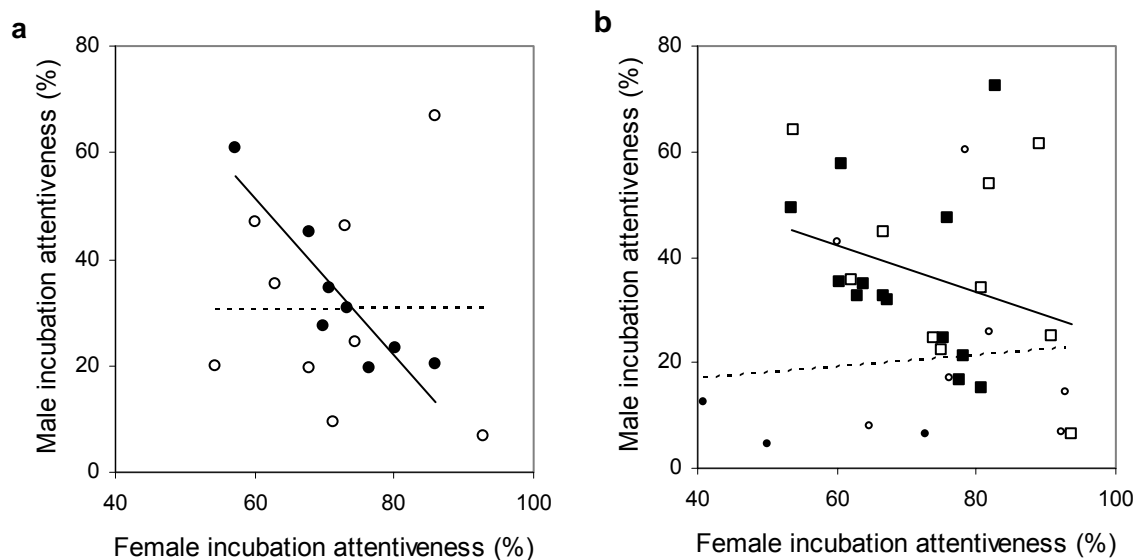


Figure 3.2 The interactions between (a) male attractiveness and female incubation attentiveness and (b) clutch size and female incubation attentiveness on male incubation attentiveness. (a): For purpose of illustration only, males with attractiveness scores $\geq 50\%$ or $< 50\%$ are termed 'preferred' (open symbols, dashed line, n = 9) and 'less preferred' (closed symbols, solid line, n = 8) respectively. Each point represents the mean of 2 observations over 2 breeding attempts. Male and female incubation attentiveness were negatively related when the female was paired with her less preferred partner, but there was no relationship between male and female incubation attentiveness when she was paired with her preferred partner. (b): Each

point represents mean attentiveness on days 6 and 8. Since individual pairs did not produce clutches of identical size in both breeding attempts, attentiveness is illustrated with closed symbols for the first attempt and open symbols for the second; lines of best fit summarise both attempts. Clutches ≥ 5 are represented by squares and the solid line (attempt 1: $n = 14$, attempt 2: $n = 10$); clutches ≤ 4 by circles and the dashed line (attempt 1: $n = 3$, attempt 2: $n = 7$). For purposes of illustration, I placed the cut-off point for clutch size at the mean of 5 eggs. Male incubation attentiveness was negatively related to female attentiveness in larger clutches but not in smaller clutches.

Males that received EPC opportunities in their first breeding attempt and incubated less in both breeding attempts could have spent more time seeking potential EPCs. TC males did indeed spend more time outside the nestbox than CT males (treatment: β : 25.68 (14.19 to 36.75), $P < 0.001$; breeding attempt: β : 18.89 (7.46 to 30.21), $P = 0.001$; treatment x breeding attempt interaction: β : -42.54 (-62.07 to -24.12), $P < 0.001$, figure 3.3).

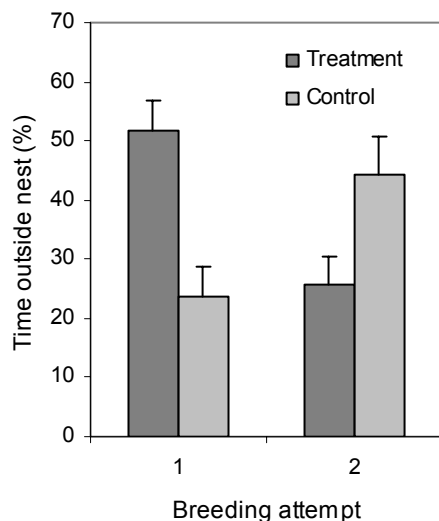


Figure 3.3 Time spent outside the nestbox by incubating males (means + 1 SE) for each breeding attempt (1 or 2) as a proportion of the total time available (breeding attempt 1: $n = 8$ control and 9 treatment pairs; breeding attempt 2: $n = 9$ control and 8 treatment pairs). There was a treatment group x breeding attempt interaction on time spent outside the nestbox (see text). Control pairs from the first breeding attempt became treatment pairs in the second breeding attempt and *vice versa*.

Although male courtship behaviour was not affected by extra-pair female diet quality, it is possible that males who had encountered females from the HP diet group incubated their partner's clutch less than males who had encountered LP diet females. There was no difference in incubation attentiveness between males that encountered the two groups of females (HP: $22.9 \pm 3.57\%$, $n = 7$; LP: $37.7 \pm 5.67\%$, $n = 10$; β : 9.777 (-1.595 to 21.23), $P = 0.096$), but the trend was in the predicted direction. There was no relationship between

the amount of time a male spent courting the extra-pair females and the time spent incubating his partner's clutch (β : 0.3918 (-0.2581 to 1.064), $P = 0.241$, $n = 17$).

The female response: compensation or matching?

Female incubation attentiveness was influenced by male EPC opportunity and male incubation attentiveness (table 3.2), and was greater than male attentiveness in all pairs (figure 3.4). It was not influenced by male attractiveness or by clutch size; nor did it differ between days. Female attentiveness was negatively related to male attentiveness in treatment pairs (β : -0.6118 (-1.065 to -0.123), $P = 0.014$, $n = 9$), which indicates that treatment females did not match the direction of their partners' response. The slope of the regression line < 1 , and this suggests that they compensated. No points pass beyond the full compensation line in treatment pairs, so no individual overcompensated (figure 3.4). Male and female attentiveness followed a positive but non-significant trend in control birds (β : 0.211 (-0.286 to 0.727), $P = 0.396$, $n = 8$).

Table 3.2 General linear mixed model showing the effects of male extra-pair copulation (EPC) opportunity and male incubation attentiveness on female incubation attentiveness (squared) in 17 females during their first breeding attempt (control $n = 8$; treatment $n = 9$). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for all main effects and the only significant interaction. All other interactions $P \geq 0.112$.

	β	HPD interval	
<i>Fixed effects</i>			P
Intercept	52412159	28686352 to 75707212	< 0.001
Treatment group ^a	35155026	1925010 to 68744550	0.038
Partner's attentiveness	1212	-3862 to 6037	0.616
Treatment x partner's attentiveness	-11442	-20971 to -1972	0.019
<i>Main effects not in final model</i>			
Day of incubation	4329216.8	-3559525 to 12140837	0.260
Clutch size	2024975	-6487316 to 11589107	0.638
Male attractiveness	24535.4	-356647 to 409295	0.891
<i>Random effects</i>			Variance (%)
Individual identity	2687377	0 to 9080533	< 0.1
Residual	23387123	17734863 to 29785847	> 99.9

^a 'control' is the reference group

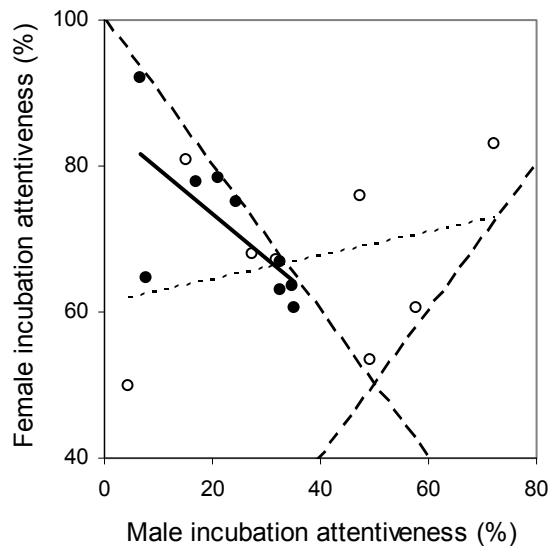


Figure 3.4 Regression analysis to examine the effects of male EPC opportunity and male incubation attentiveness on female attentiveness. The dashed line with the positive slope indicates where points would lie if male and female attentiveness were equal and total attentiveness were 100 % (perfect matching). Where points fall above that line, females show higher attentiveness than their partners. The dashed line with the negative slope represents full compensation: points below it reflect partial compensation and points above it reflect overcompensation. Each point represents the mean of 2 days. Male and female incubation attentiveness were negatively related in treatment pairs (closed circles, bold line, $n = 9$), but not in controls (open circles, dotted line, $n = 8$).

Combined male and female incubation attentiveness increased with clutch size (β : 685.7 (385.2 to 1008.0), $P < 0.001$), but did not differ between treatment groups (β : -131.8 (-881.7 to 628.4), $P = 0.726$) or breeding attempts (attempt 1: β : 150.1 (-589.2 to 929.7), $P = 0.697$; figure 3.1). It was not influenced by male attractiveness (β : 2.8 (-14.8 to 20.5), $P < 0.753$) or by any interactions between these variables ($P \geq 0.163$).

Discussion

I manipulated male EPC opportunity in the Zebra Finch to investigate the extent to which males courted extra-pair females and incubated their partner's clutch, and subsequently how male incubation attentiveness influenced female effort. Males appeared to perceive the manipulations as EPC opportunities since they all attempted to court the extra-pair females, and many of them attempted or even achieved copulations. Males with increased EPC opportunities reduced incubation attentiveness relative to controls in the first breeding attempt, but when the treatment was switched in the second attempt both groups maintained the same level of incubation effort that they had shown earlier. The quality of

EPC opportunity that males experienced, which was manipulated by offering different diet treatments to the extra-pair females, did not influence male incubation attentiveness. Males and females responded to each other's contributions over ecological time, and treatment females compensated fully for the reduction in male effort. Females paired to more attractive males did not spend more time incubating or lay larger clutches than those paired to less attractive males, as predicted by the Differential Allocation Hypothesis. The design of this experiment improved upon previous studies of the conflict between mating and parental effort by taking male attractiveness, clutch size, individuals' previous experience and their partners' current effort into consideration under standardised laboratory conditions.

The effect of increased EPC opportunity on male incubation attentiveness

Males with increased EPC opportunities were predicted to reduce incubation attentiveness towards their partner's clutch because parental care is costly to the care-giver (Trivers 1972; Clutton-Brock 1991) and males can maximise their lifetime fitness benefits by trading-off parental effort for mating effort when EPC opportunities are available (Magrath and Komdeur 2003). This may amount to a direct trade-off between time invested in mating and parental effort, to a change in a male's perception of the value of his partner's clutch relative to the fitness he could accrue elsewhere or to a reduction in the strength of the pair bond. Males that received EPC opportunities in the first breeding attempt of the current experiment spent more time outside the nestbox than controls, but I can only speculate that they did so in expectation of EPC opportunities since I did not monitor how that time was spent. Incubation attentiveness data was collected when a male had experienced two (day six) and subsequently three (day eight) extra-territorial forays to test the hypothesis that the number of manipulations he received had a cumulative effect in reducing his incubation attentiveness over time. Attentiveness did not vary between days, but this may suggest that the difference in the number of forays that males had experienced was not great enough for an effect to be found.

Males that had encountered extra-pair females from the high quality diet group did not spend more time courting them or reach a more advanced stage of courtship with them compared to females from the low quality diet group. Jones *et al.* (2001), working with the same population, found that male Zebra Finches spent more time associating in mate choice trials with females who had received a high protein diet for two weeks, in spite of there being no between-group differences in female body mass before or after the diet treatment. This suggests that males should have perceived a difference in attractiveness

between females from the two diet groups in the current experiment. The difference between the two sets of results could be due to a difference in sample size (Jones *et al.* (2001) used 32 males compared to my 17) or in methodology. The former study quantified relative preferences during mate choice trials whereas I used a between-groups approach in which males encountered extra-pair females from one diet group or the other. Under the conditions presented here we might not expect males to respond differently to the two groups of females because the benefits of fertilising even a low quality female probably outweigh the costs of courting her.

Within-individual consistency in incubation attentiveness and the role of experience

The consistency in incubation effort when the treatments were switched in the second breeding attempt with the same partner raises the possibility that the experiences that individuals receive in early life serve as a good indicator of future events and that they use these early life experiences to shape their future responses. This may be particularly true in short-lived species that form permanent pair bonds, such as the Zebra Finch. Most studies to date have investigated parental responses in individuals whose history is not known, and this may explain some of the conflicting trends between studies (Wright 1998). The consistency in levels of effort between the first two breeding attempts of a male's life in the current study suggests that the window for developing such rules may occur in the first breeding attempt of an individual's life.

A similar phenomenon has been observed with female investment in egg mass in the same species: females did not adjust their levels of investment in the second breeding attempt of a crossover design when paired with a male that was either more or less attractive than their previous partner (Rutstein *et al.* 2004). In the first round of their experiment, Rutstein *et al.* (2004) found that females mated to more attractive males laid heavier eggs, as predicted by the DAH. It is interesting to note that, although the effects described by Rutstein *et al.* carried over into the second round of the experiment in spite of a change of partner, all birds had breeding experience prior to the experiment and that those effects did not seem to carry over. Therefore, it remains to be seen how long the effects of early life experience might endure and whether they can be updated under certain circumstances. Such effects probably differ according to the longevity and mating system of the species in question, and there may also be differences between the sexes.

Sex differences in repeatability of parental effort was also investigated by Nakagawa *et al.* (2007a) who found high within- and between-year repeatability within males in the House

Sparrow, *Passer domesticus*, during nestling provisioning, and low to moderate repeatability in females during provisioning and in both sexes during incubation. They found an individual's incubation effort to be a good predictor of its subsequent provisioning rate in males, but not females, while Kopisch *et al.* (2005) found this effect in both sexes of a different population. Consistent male care during incubation may be a useful indicator of male provisioning effort and this information may help the female to prepare for the next stage of parental care (Kopisch *et al.* 2005; Nakagawa *et al.* 2007a).

Negotiation between partners over incubation attentiveness

I found a negative correlation between male and female incubation attentiveness in pairs in which the male had received increased EPC opportunities, and females appeared to compensate for their partner's effort, as predicted by most theoretical models (e.g. Chase 1980; Houston and Davies 1985; McNamara *et al.* 1999). A strong negative relationship between partners' incubation effort was also found by Nakagawa *et al.* (2007a). However, I found a weak positive relationship between the attentiveness of control males and females, as part of the interaction between treatment group and partner's attentiveness. A positive relationship between male and female effort is predicted by the Information Hypothesis (Johnstone and Hinde 2006), which suggests that partners should 'match' each other's levels of investment when information on the need or quality of the offspring is incomplete. I suggest that the direction of the response may also be influenced by the costs and benefits of parental care compared to mating effort. Incubation has been shown to be a costly activity even within a laboratory setting (Vleck 1981), but these costs are likely to be settled more readily in captivity due to higher food availability and shorter foraging distances. Males who were primed in their first breeding attempt to expect EPC opportunities probably perceived the higher potential for lost mating opportunities as an additional cost of incubation, while controls had no better option and so probably perceived the costs to be lower. However, the relationship between male and female incubation attentiveness was no longer significant when treatment pairs were excluded from the analysis, so support for the Information Hypothesis is not conclusive.

Schwagmeyer *et al.* (2002) investigated how partners respond to each other's provisioning behaviour in the House Sparrow, *Passer domesticus*, by attaching small weights to the tail feathers of either the male or the female. The manipulation induced a short-term reduction in the provisioning rate of handicapped males, but females did not appear to compensate for this, and Schwagmeyer *et al.* (2002) interpreted their findings as a 'sealed bid' response. They also used a regression analysis approach to conclude that the current food

delivery rates of birds paired with handicapped mates are not influenced by their partners' current contributions but are positively related to their own *pre*-manipulation food delivery rates and the pre-manipulation rates of their partners. This suggests that House Sparrows do not adjust their levels of effort sequentially in response to each other's contribution, as predicted by negotiation models (McNamara *et al.* 1999). However, Schwagmeyer *et al.* (2002) propose that partners could negotiate response rules in the early stages of reproduction, such as during incubation, and use these in subsequent stages. Lendvai *et al.* (2009) used a combination of mate removal and handicapping to investigate the female response to a decline in male incubation effort in the same species. They found that female Sparrows provisioned at a higher rate when the male was removed than if he were present but working at an experimentally lowered rate. They concluded that the response exhibited by females whose partner is present but working suboptimally compared to that of females whose partners have been removed suggests parental adjustment of effort is context-dependent and may involve behavioural negotiation.

Effects of clutch size and male attractiveness on incubation attentiveness

Clutch size influenced male but not female incubation attentiveness. Clutch size is under female control, and is therefore a component of her overall effort. The male, on the other hand, is unable to influence clutch size but he can respond to it through variation in incubation attentiveness. Males and females responded negatively to each other's attentiveness in pairs where the male was less attractive or where the female had produced a larger clutch. High quality females have been shown to produce larger clutch sizes (Slagsvold and Lifjeld 1990; Selman and Houston 1996), and it is feasible that males were more willing to exploit their partner or that females were more able or willing to compensate when female quality was high relative to other females (i.e. where they produced a large clutch size) or relative to the male (i.e. where they were paired to their less preferred mate). Therefore, males may allocate parental effort differentially in response to female quality. I found no support for the DAH or CIH in females, since male attractiveness did not influence female incubation attentiveness or clutch size. However, females can show differential allocation in a number of ways that were not measured in this study - for example, female Zebra Finches paired to more attractive males have been shown to invest more testosterone into eggs (Gil *et al.* 1999).

Conclusions

These findings have a number of implications for our understanding of the negotiation process and for our interpretation of empirical tests of these models. First, in the initial

breeding attempt, when all subjects lacked breeding experience, males with increased EPC opportunities responded by reducing incubation effort relative to controls. This shows that individuals are able to respond to environmental variation as predicted by negotiation models. Second, when the treatments were switched, males failed to respond appropriately and continued to invest the same level of effort that they had shown previously. This shows that the ability of individuals to respond to the environment is limited, and if their previous experience (i.e. the events of the first breeding attempt) were not known, these results would be incorrectly interpreted as a sealed bid response. Individuals may be primed by the experiences they receive during the early stages of reproduction and this may be a good rule of thumb if early life events serve to be good predictors of future conditions. This also emphasises the important influence that an individual's experience can have on its future reproductive decisions and may explain conflicting results found in studies where this is not known (Wright 1998). Third, males and females responded to each other's contributions over ecological time, which is also consistent with predictions of negotiation models. Control and treatment pairs negotiated different patterns of effort, and this suggests that investment decisions are based on cues taken from the environment (EPC opportunity) and from the level of effort that the partner is willing or able to make. However, contrary to predictions of most game theoretic models, females compensated fully for the reduction in their partner's effort. There is a trade-off in investment between different reproductive stages and attempts (Williams 1966), so these females are expected to pay the costs of compensating in terms of reduced subsequent reproductive output (chapter four). Individual reproductive decisions are thus complex and influenced by multiple factors: male EPC opportunity, male attractiveness, the partner's contribution, clutch size and previous experience.

Chapter 4 - The effects of reduced male incubation effort on offspring performance and subsequent female reproductive output: who pays?

Abstract

Who pays the costs when a male trades off parental effort for increased mating effort? A number of studies have described a negative relationship between paternal care and mating effort, but few of these have explored the fitness consequences of such a trade-off. Avian incubation is costly to the parent but essential for embryonic growth and survival, and so we expect the optimum levels of incubation attentiveness to differ between parents and offspring, and also between male and female parents when there are differences between them in the costs and benefits of parental care. In a previous experiment, I experimentally reduced male incubation attentiveness by presenting extra-pair copulation (EPC) opportunities to males in a captive population of the Zebra Finch, *Taeniopygia guttata*, and allowed them to breed twice with the same partner. Females compensated to varying extents for the deficit in their partners' care. Since there is a trade-off between different reproductive attempts, we would expect to see a reduction in subsequent female reproductive output or reduced offspring performance depending on the level of female compensation. I found that hatching success was lower in the nests of males that had experienced increased EPC opportunities during incubation, and was also influenced by the father's attractiveness, which was assessed through a series of mate choice trials. The treatment did not influence post-hatching growth for a given brood size, but survival of hatched offspring was greater in treatment nests. The primary sex ratio in the second breeding attempt was more male biased as the father's attractiveness increased, but only among offspring whose fathers had previously received EPC opportunities. The number of eggs a female laid in the second breeding attempt was influenced by her previous incubation effort and the number of offspring she had fledged. These results suggest that females who compensated for the deficit in their partner's effort paid the costs of doing this, and that incubation attentiveness is an appropriate proxy for parental investment since there can be a trade-off between female incubation attentiveness and subsequent reproductive output.

Introduction

Parental care benefits the offspring at the expense of the parents because the time and energy that parents invest into caring for current offspring must be traded off against investment into self-maintenance, mating effort and caring for future offspring (Williams 1966; Trivers 1972; Clutton-Brock 1991). Parental investment into care is thus a compromise between the optimum levels of care for parents and offspring, and in a biparental system this optimum is also likely to differ between males and females due to asymmetries between them in the costs and benefits of care to each sex (Trivers 1972; Chase 1980; Parker *et al.* 2002). This leads to sexual conflict over the relative contributions that males and females make to parental care, and each partner will do better if it can reduce its own investment and rely on its partner to compensate (Houston and Davies 1985; Lessells 1999; Arnqvist and Rowe 2005). Most theoretical models predict that to avoid being exploited an individual should be unwilling to compensate completely (e.g. Chase 1980; Houston and Davies 1985; McNamara *et al.* 1999).

Parental effort is the amount of time or energy that a parent contributes to care, and, unlike parental investment, it does not necessarily enhance offspring survival at a cost to the parents' future fitness. Parental effort is often used as a proxy for parental investment (e.g. Hinde and Kilner 2007; Kosztolanyi *et al.* 2009; Lendvai *et al.* 2009), but few manipulations of parental effort have assessed the fitness consequences of variation in effort to determine whether it reflects investment in the sense defined by Trivers (1972). Avian incubation is a good system in which to address this question because it is costly to the parent (reviewed in Reid *et al.* 2002a; Tinbergen and Williams 2002) but essential for embryonic growth and development (Webb 1987). Males contribute a lower and more variable proportion of time to incubation than females in most passerine species, and this is probably a function of reduced parental certainty (Møller and Birkhead 1993; Queller 1997) and the trade-off a male faces between care of his own offspring and the pursuit of extra-pair copulations (EPCs) (Westneat 1988a; Pinxten and Eens 1994; Whittingham *et al.* 1994; Magrath and Komdeur 2003). Time spent incubating, hereafter incubation attentiveness, can vary between individual males, as well as between the sexes, in response to the benefits of mating effort relative to parental effort for a particular male.

The benefits of investing in mating effort relative to parental effort can depend upon a male's phenotypic quality or attractiveness. Attractive males have more to gain from reallocating effort from parental care into seeking EPCs (Magrath and Komdeur 2003), and

a number of studies have found a negative relationship between male attractiveness and paternal effort (Badyaev and Hill 2002; Duckworth *et al.* 2003; Mitchell *et al.* 2007). This pattern of effort is predicted by the Differential Allocation Hypothesis (DAH), which also predicts that the female will invest more effort into offspring resulting from mating with attractive males since these offspring will be of greater residual reproductive value (Burley 1986; Sheldon 2000). Females with attractive partners have been shown to increase incubation (Gorman *et al.* 2005a) or provisioning effort (Burley 1988), or to increase primary reproductive effort by producing heavier eggs (Rutstein *et al.* 2004) or larger clutches (Lopez-Rull and Gil 2009). However, females that are constrained to breed with a less attractive male and whose future reproductive opportunities are limited might be more willing to compensate for a low quality partner (Gowaty *et al.* 2007; Gowaty 2008; Harris and Uller 2009), as predicted by the Compensatory Investment Hypothesis (CIH). Evidence of a negative relationship between male quality and female expenditure has been found in a few species (Saino *et al.* 2002; Navara *et al.* 2006; Bolund *et al.* 2009).

When males show a reduction in parental effort for which females are unable or unwilling to compensate, the current offspring are likely to experience suboptimal incubation conditions, and these can have detrimental effects on embryonic development and hatching success (Reid *et al.* 2002b; Gorman and Nager 2003; Gorman *et al.* 2005b; Nilsson *et al.* 2008) and even impair fecundity in later life (Gorman and Nager 2004). The sexes may differ in their sensitivity to suboptimal developmental conditions (for reviews see Sheldon 1998; Badyaev 2002), and the stage of development at which these effects are felt might also vary (Gorman *et al.* 2005b). Another means by which the sex ratio may differ from parity is through adaptive sex ratio manipulation (e.g. Arnold *et al.* 2003): the sex ratio may be adjusted in favour of the sex that has the greatest fitness returns under the conditions experienced by the mother (Trivers and Willard 1973) or in favour of sons when the mother has copulated with an attractive male (Burley 1981; Ellegren *et al.* 1996).

If females do respond by compensating for their partners, they may show reduced effort in later reproductive attempts by skewing the sex ratio in favour of the less vulnerable sex or by producing fewer or smaller eggs, and thus future offspring may also suffer as well as the female herself. Since there can be a trade-off between investment into incubation and subsequent reproductive stages (Heaney and Monaghan 1996; Reid *et al.* 2002c), females that compensate during incubation may be unable to sustain this effort at the provisioning stage. Therefore, even where we observe female compensation at one reproductive stage, there may still be a detrimental effect on the current offspring. Delesalle (1986) found that

female Zebra Finches, *Taeniopygia guttata*, only produced a second clutch if their partners contributed sufficiently to incubating the previous one. A reduction in female effort may reflect the costs of increased previous investment and the need to regain body mass or it might be a strategic decision to retaliate against a lazy partner (Dearborn 2001; Barta *et al.* 2002), and it is difficult to distinguish between the two. Parental body condition can influence both energetic and temporal currencies of cost because lost energy has to be regained through increased time spent in self-maintenance (Dearborn 2001; Cresswell *et al.* 2003).

In chapter three I manipulated male EPC opportunity in a domesticated population of the Zebra Finch by allowing incubating males to interact with extra-pair females (treatment). Increased male EPC opportunity was associated with decreased paternal incubation attentiveness in the first breeding attempt, and treatment males continued to show low attentiveness in the second attempt in spite of the treatments being switched; control males showed relatively high attentiveness in both attempts (chapter three). Treatment females compensated for their partners. Here I investigated the effects of the treatment on offspring performance and the reproductive effort of the mother in the next breeding attempt. If parental effort is a good proxy for investment, we would expect to see reduced subsequent reproductive output in females that compensated for the reduction in their partners' care or reduced offspring performance if they did not compensate sufficiently. The aim of this study was to test the following hypotheses: 1) Offspring from treatment nests experience reduced hatching success, reduced post-hatching growth and survival and a sex ratio biased towards the less vulnerable sex. Male nestlings tend to be less vulnerable to nutritional stress in the Zebra Finch (Kilner 1998; Martins 2004). 2) Females that compensated in the previous breeding attempt lay smaller subsequent clutches and bias the sex ratio towards males. 3) Incubation attentiveness is a good proxy for parental investment. I tested these hypotheses whilst statistically controlling for male attractiveness, as this varies between males, and females may respond differently to it.

Methods

Outline of the experiment

This study was based on an experimental manipulation of male EPC opportunity in 17 pairs of Zebra Finches during incubation, as detailed in chapter three. Briefly, each female Zebra Finch was given the chance to choose between two males in spatial association trials

and was paired at random with one of them for the duration of the experiment. The percentage of time a female spent associating with the male to whom she was assigned is referred to as the male attractiveness score. Each pair had the chance to produce two clutches and raise both broods to fledging. During the incubation stage of the first breeding attempt, half of the males received EPC opportunities with ‘extra-pair females’ ($n = 9$), while the other half did not encounter other birds whilst separated from their partners for the same duration ($n = 8$). In the second breeding attempt, males that were previously controls received EPC opportunities and *vice versa*. Male and female incubation attentiveness were measured on two days on which EPC opportunities were not presented. Since males in each group adjusted their effort according to the type of treatment they received in their initial breeding attempt, and maintained this level of effort when the treatments were switched, I describe pairs according to the type of treatment they originally received. Therefore, pairs in which the male received EPC opportunities in the first breeding attempt are hereafter termed ‘TC’, those that received the treatment in the second attempt are ‘CT’. For each nest and each breeding attempt, I recorded clutch size, egg volume, the proportion of eggs that hatched, offspring tarsus length at approximately 30 and 100 days, and the number of offspring that were still alive 1000 days after the first egg was laid. All eggs and offspring were sexed where possible.

Data collection

Seventeen pairs were allowed to incubate and raise offspring from two consecutive breeding attempts in which male EPC opportunity was manipulated during incubation (chapter three). One CT pair was excluded because it produced yolkless eggs in both breeding attempts, and the sample size hereafter is 16. After the first breeding attempt, fledglings were separated from their parents at 30 days of age, nestboxes were cleaned and new nesting material was provided. In order to standardise the interval between manipulations in chapter three, initiation of the second clutch was suspended in five pairs (TC $n = 2$, CT $n = 3$) that failed to fledge offspring in the first attempt by separating the members of each pair with a transparent divide until 30 days after the hatch date (mean separation period: 18.4 ± 1.47 days, $n = 5$). Nests were checked for eggs daily from pairing until clutch completion in the first breeding attempt and after the offspring from the first breeding attempt had fledged, and clutch size was noted. Upon clutch completion, eggs were measured along their length and breadth using callipers, and egg volume was calculated using Hoyt’s (1979) formula: $V = K_e \times LB^2$, where K_e is a volume coefficient of 0.5, L is the length of the egg and B is its breadth. At the first breeding attempt, there were no differences between treatment groups in clutch size (TC: 5.0 ± 0.33 eggs, $n = 9$; CT: 5.3

± 0.57 eggs, $n = 7$; $W = 41.5$, $P = 0.275$), lay latency, where birds were paired on day 0 (TC: 5.4 ± 0.65 days, $n = 9$; CT: 4.0 ± 0.84 days, $n = 7$; $W = 44.5$, $P = 0.177$) or mean egg volume (TC: 93.5 ± 2.42 mm³, $n = 9$; CT: 89.3 ± 1.97 mm³, $n = 7$; $t_{14} = 1.34$, $P = 0.202$).

Incubation begins ('day zero') on the fourth day of the laying sequence in clutches of five or more, and on the final day of laying in smaller clutches; eggs hatch 11-15 days later (Zann and Rossetto 1991). Towards the end of the incubation sequence, I checked nests for hatchlings once in the morning and again in the afternoon. The number of hatched and unhatched eggs and the date that nestlings hatched or died were noted. Prior to ringing, hatchlings were marked with coloured non-toxic pens to record their identity. Tarsus length was measured at approximately 30 and 100 days of age, and the exact age in days was used as a covariate in later analyses. Unhatched eggs were opened and examined for signs of embryonic development. Sex could be assigned to adult offspring by plumage dimorphism; embryos and offspring that died before sexual plumage had developed were frozen and molecularly sexed using methodology described in Arnold *et al.* (2003). After being separated from their parents at 30 days of age, offspring were maintained in single sex cages under the conditions described in chapter three (subjects and maintenance). On 17th October 2009, 1000 days after the first egg was laid, I determined which offspring and mothers were still living and calculated their age and the lifespan of those that had perished.

Statistical analysis

Life history data for 16 pairs, which produced a total of 158 eggs over two breeding attempts, were analysed using R version 2.9.2 (R Development Core Team 2009). Data are incomplete for 8.9 % of eggs because the eggs broke (embryo present) or disappeared during incubation ($n = 2$), failed to hatch but were not examined for embryonic development ($n = 5$) or because the chicks disappeared within 48 hours of hatching ($n = 7$). Eggs and nestlings that disappeared were presumably removed by the parents, although their bodies could not be recovered. There was no difference between treatment groups (β : 0.05 ± 0.64 , $Z = 0.08$, $P = 0.938$; GLMM with nest identity as a random factor) or breeding attempts (β : 0.71 ± 0.60 , $Z = 1.18$, $P = 0.238$) in the proportion of incomplete (unsexed) cases ($n = 16$ pairs), and there was no interaction between the two (β : -2.31 ± 1.43 , $Z = 1.62$, $P = 0.106$).

In all analyses I examined the effects of treatment ('TC' or 'CT'), a fixed factor, and male attractiveness (%), a covariate. A father's attractiveness may affect primary and secondary

parental effort (Burley 1988; Balzer and Williams 1998; Gorman *et al.* 2005a; Mitchell *et al.* 2007). Male attractiveness score did not differ between treatment groups (TC: 55.8 ± 8.49 , $n = 9$; CT: 48.3 ± 8.81 , $n = 7$; $t_{14} = 0.61$, $P = 0.554$). Breeding attempt (one or two) was included as a fixed factor in all analyses of offspring performance (i.e. where data from both attempts was used).

Using all 158 cases, I tested whether hatching success was influenced by treatment, breeding attempt and the father's attractiveness. I combined the number of hatched and unhatched eggs into a single variable using the 'cbind' function (base package), and used this as the response variable in a generalized linear mixed model (GLMM) fit by the Laplace approximation method from the lme4 library (Bates and Maechler 2009). The cbind function takes account of the number of values (in this case, eggs) that make up each ratio, and is an appropriate way of dealing with a non-normal error structure, non-constant variance and a response bounded by 1 and 0 (Crawley 2007). All mixed models were fitted with parental nest identity as a random factor (random intercepts only) to control for repeated measures. All main effects and two-way interactions between them were included in the initial model. The final model was reached using backwards stepwise elimination of non-significant ($P \geq 0.05$) terms, first removing interactions and then main effects in order of least significance. GLMMs are presented with parameter estimates (β) \pm 1 standard error, Z- and P-values for all main effects and significant interactions ($P < 0.05$) are reported, and all tests are two-tailed.

The treatment could influence hatching success via the total amount of time that the eggs are incubated (93.9 ± 1.86 %, $n = 16$) or the ratio of attentiveness between male and female if the sexes differ in the quality of care that they provide. I explored this issue by replacing 'treatment' in the model described above with male, female and total incubation attentiveness in three separate models. This allowed me to avoid problems due to colinearity between the explanatory variables. Male and female incubation attentiveness are expressed as the mean proportions of time spent incubating (%) over two three-hour observation periods, respectively. 'Shared' incubation, when both partners frequently settle on the eggs and when part of the clutch is still covered following departure of one partner from the nest, contributed to 11.6 ± 3.51 % of total incubation time ($n = 16$ pairs). Shared incubation was included in calculations of individual (male and female) time budgets because any investment of time must be traded-off against other activities (Reid *et al.* 2002a); but it was not included in calculations of total incubation attentiveness, where I was concerned with the amount of time over which heat was transferred to the developing

embryo. Total incubation attentiveness was not normally distributed so I used non-parametric Wilcoxon rank sum tests with continuity correction to see whether it was influenced by the treatment in the first and second breeding attempts separately, and Spearman's rank correlation to see whether male attractiveness affected total attentiveness (using mean total attentiveness over both breeding attempts). I then ran a GLMM on the full sample of sexed embryos ($n = 121$ embryos from 15 nests), expressing the pre-hatching survival of each individual as a binary response variable, to see whether pre-hatching mortality differed between males and females. Sex, treatment and breeding attempt were fixed factors, male attractiveness was a covariate and nest identity was a random factor.

I tested the hypothesis that individuals from TC nests are smaller than those from CT nests by investigating the effects on tarsus length at fledging and at age 100 days of all 73 individuals from 6 CT and 7 TC nests that reached at least 30 days of age. Tarsus length data from the two days were combined in a single model and were normally distributed. Competition with nestmates over food can affect offspring growth and survival (Mock and Parker 1997) and the effects of early developmental conditions may influence tarsus length (Gorman and Nager 2004), so I took brood size at hatching into account. To avoid problems due to collinearity of brood size at hatching with other explanatory variables (see hatching success results), I regressed tarsus length (mm) against brood size minus one (to avoid double-counting the focal individual) in a general linear model with normal errors, and used the raw residuals from this as the response variable in a linear mixed effect models fit by the Restricted Maximum Likelihood (REML) method from the lme4 library. In addition to treatment, breeding attempt and father's attractiveness, I included offspring sex as a fixed factor and age in days as a covariate. Offspring identity was included as a random factor nested within parental nest identity in order to control for repeated measures on days 30 and 100. I give estimates of the model coefficient (β), highest posterior density (HPD) intervals calculated at the 95 % level using Markov Chain Monte Carlo (MCMC) sampling with the chain length fixed at 10,000 (reported in brackets after the coefficient), and P -values based upon the posterior probability distribution. The pvals function from the languageR library (Baayen 2009) was used to calculate these estimates. Significance is denoted by HPD intervals that do not include zero, as well as by P -values < 0.05 .

Next I compared the proportion of hatched offspring that reached the age of 100 days between treatment groups and breeding attempts, including the father's attractiveness as a covariate. This cut-off was chosen because twenty individuals were removed from the

experiment after reaching 100 days of age, and only three of the remaining individuals died after that point (TC $n = 1$, CT $n = 2$ from different nests). I combined offspring that did and did not survive to 100 days using the cbind function and used this single vector as the response variable in a GLMM as described above for hatching success. Finally, I re-ran the model with total incubation attentiveness in place of treatment.

I tested the hypothesis that females that compensated for the reduction in their partners' incubation attentiveness in the first breeding attempt would respond by producing smaller clutches in the second breeding attempt. To take into account potential differences in egg volume between clutches, I regressed mean egg volume against clutch size in a general linear model and used the raw residuals as the response variable in a second general linear model. The previous treatment group, the partner's attractiveness and the number of offspring that the parents fledged in the previous attempt were explanatory variables. I then re-ran the latter model, this time replacing treatment with female incubation attentiveness. Clutch size was not correlated between breeding attempts ($r_s = 0.11$, $P = 0.690$; non-parametric statistics were used here because clutch size in the first breeding attempt was not normally distributed).

Finally, I tested whether TC females allocated more male embryos to their subsequent clutch. I used the cbind function to combine all male and female offspring of known sex, including embryos, into a single vector, and this was used as the response variable in a generalized linear model (GLM). I refer to the proportion of males and females analysed here as the egg sex ratio, and this comprises 88.3 % of all eggs laid in the second breeding attempt ($n = 77$). The egg sex ratio may differ from the primary sex ratio because it does not include embryos that were too small to be detected when they died or those that were not sexed. It is not possible to say whether eggs in which embryos were not detected were infertile or whether early embryonic mortality occurred (Birkhead *et al.* 1995). Treatment group was included as a fixed factor; male attractiveness and the number of offspring that were raised to fledging in the first breeding attempt as covariates. The model was then run a second time with female attentiveness in the first breeding attempt as an explanatory variable in place of treatment group. At the first breeding attempt, there were no differences between treatment groups in egg sex ratio (TC: 51.3 ± 5.91 % male biased, $n = 8$, no embryos were detected in one clutch; CT: 53.3 ± 10.39 male biased, $n = 7$; $t_{13} = 1.30$, $P = 0.217$). I was unable to test whether adult survival was greater in CT than in TC mothers because only one female had died at the time of writing (TC, aged 733 days).

Results

Overall offspring performance

158 eggs were laid by the 16 pairs across two breeding attempts. 116 of these eggs hatched and many individuals were still living 1000 days after the first egg was laid (figure 4.1).

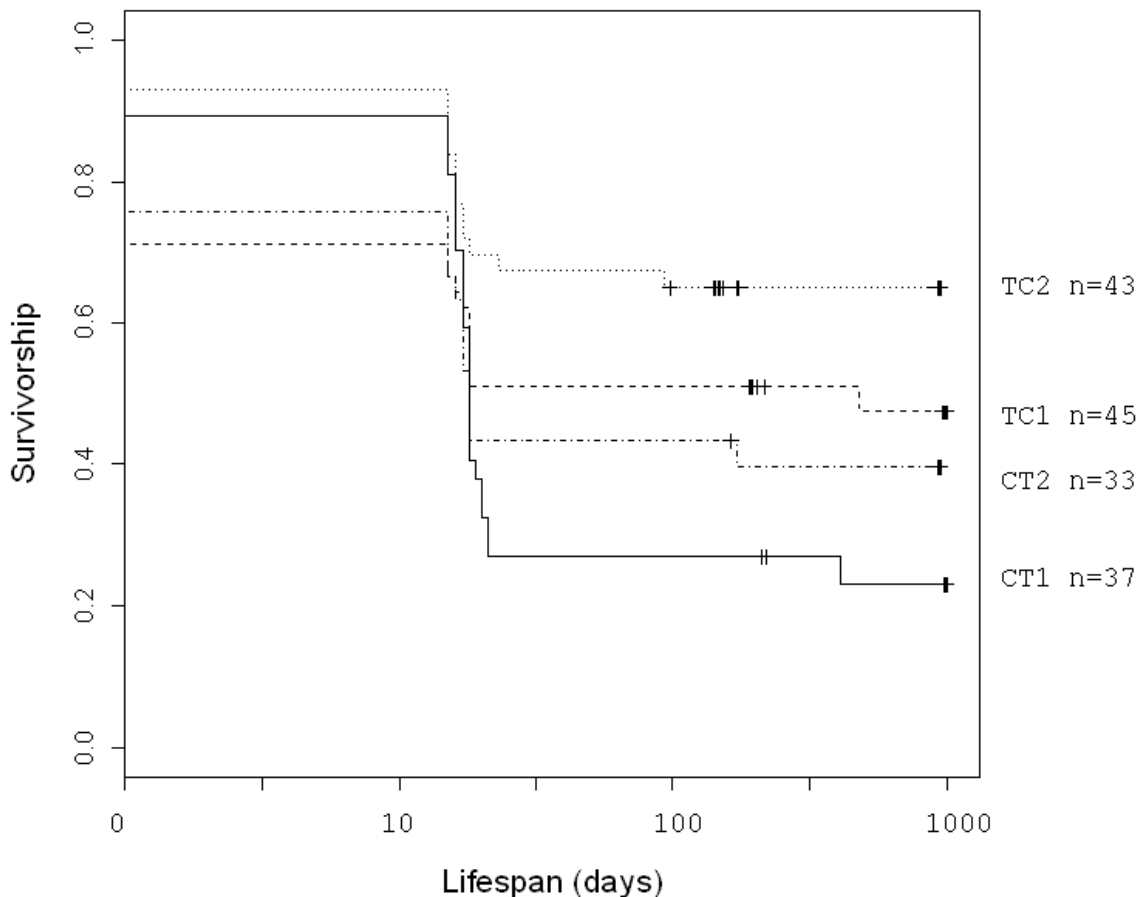


Figure 4.1 Cumulative survival curve to show total offspring mortality from day 0 (laying) to day 1000 per treatment group (TC or CT) per breeding attempt (1 or 2). The survivorship curve is based on all 158 hatched and unhatched eggs from 16 pairs across 2 breeding attempts. Embryonic development was not detected in 31 eggs (including 7 for which data is incomplete), and for simplicity, these are represented as embryos that died on day 0. Thirteen embryos suffered pre-hatching mortality (including one egg that broke) and are represented as dying on day 15 because incubation began 3.9 ± 0.03 days after laying (based on clutch size) and eggs began to hatch 11.1 ± 0.23 days later ($n = 15$ hatched nests). Crosses indicate the point at which individuals that were removed from the experiment or outlived it were last seen alive.

Treatment effects on offspring hatching success

Hatching success was affected by interactive effects between treatment group, breeding attempt and the father's attractiveness (table 4.1). CT pairs hatched a greater proportion of offspring in the first breeding attempt, whereas TC pairs were more successful in the second breeding attempt (figure 4.2a). Hatching success decreased as the father's

attractiveness increased in TC nests, but this was not the case in CT nests (figure 4.2b). There was no correlation between the proportions of chicks hatching in the first and second breeding attempts ($r_s = 0.33$, $P = 0.218$).

Total incubation attentiveness did not differ between treatment groups in the first (TC: 94.3 ± 2.98 %, $n = 9$; CT: 90.6 ± 6.46 %, $n = 7$; $W = 40$, $P = 0.408$, $n = 16$) or second (TC: 95.2 ± 2.19 %, $n = 9$; CT: 94.9 ± 3.78 %, $n = 7$; $W = 40$, $P = 0.408$, $n = 16$) breeding attempt, and was not influenced by male attractiveness ($r_s = 0.10$, $P = 0.725$, $n = 16$). Hatching success was not influenced by total incubation attentiveness (β : -0.01 ± 0.03 , $Z = 0.20$, $P = 0.842$), but by the proportion of time that each sex spent incubating and by the father's attractiveness (table 4.2). Hatching success increased with the father's incubation attentiveness and decreased with the mother's attentiveness in the nests of preferred males, while it decreased with the father's attentiveness and increased with the mother's attentiveness where the father was less preferred (figure 4.3). Pre-hatching survival did not differ between the sexes (females: 98.7 ± 1.33 %, $n = 50$ eggs; males 80.6 ± 6.54 % hatched, $n = 71$ eggs; β : -0.586 ± 0.88 , $Z = 0.67$, $P = 0.504$, $n = 15$ nests, no embryos detected in one TC nest), treatment groups (TC: 93.3 ± 4.71 %, $n = 68$ eggs, 8 nests; CT: 87.1 ± 6.31 %, $n = 53$ eggs, 7 nests; β : 1.051 ± 1.52 , $Z = 0.69$, $P = 0.490$) or breeding attempts (attempt 1: 90.2 ± 5.43 %, $n = 64$ eggs, 15 nests; attempt 2: 90.7 ± 4.96 %, $n = 57$ eggs, 15 nests; β : -0.549 ± 0.82 , $Z = 0.67$, $P = 0.502$). It was not influenced by male attractiveness (β : -0.002 ± 0.03 , $Z = 0.06$, $P = 0.951$, $n = 15$ nests) or by interactions between any of these variables ($P \geq 0.114$).

Table 4.1 GLMM showing the effects of increased male extra-pair copulation (EPC) opportunity (treatment) on hatching success in 7 CT and 9 TC nests over two breeding attempts each. Parameter estimates (β) \pm 1 standard error, Z-values and P-values are presented for all main effects and significant interactions. All other interactions $P \geq 0.595$.

	$\beta \pm 1 SE$	Z	P
Intercept	0.91 ± 1.06	0.86	0.398
Treatment group ^a	3.09 ± 1.59	1.95	0.052
Breeding attempt ^b	-1.17 ± 0.59	2.00	0.045
Father's attractiveness	0.02 ± 0.02	0.78	0.436
Treatment group x breeding attempt	2.40 ± 0.88	2.72	0.006
Treatment group x father's attractiveness	-0.07 ± 0.03	2.64	0.008

^a 'CT' is the reference group

^b '1' is the reference breeding attempt

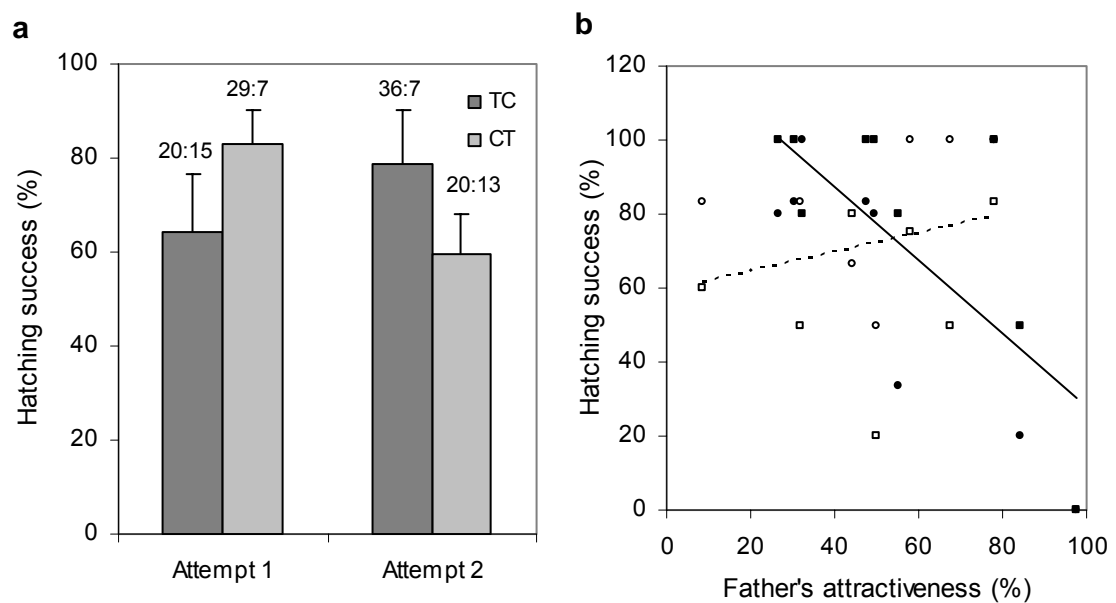


Figure 4.2 Interactions between (a) the treatment group and breeding attempt and (b) the treatment group and the father's attractiveness on the proportion of offspring that hatched as a total of the clutch size in each nest (TC: $n = 9$; CT: $n = 7$) in each of two breeding attempts. **(a)** Bars show the proportion of offspring that hatched (means ± 1 SE) from each group; the total number to hatch from each group compared to the number that did not hatch is indicated above each bar. There was a treatment group \times breeding attempt interaction on hatching success (see table 4.1). **(b)** Each symbol represents the proportion of offspring that hatched per nest per breeding attempt. Circles represent hatching success in the first breeding attempt; squares represent the second. Hatching success decreased with the father's attractiveness in TC nests (closed symbols, solid line, $n = 9$), but not in CT nests (open symbols, dashed line, $n = 7$).

Table 4.2 GLMMs showing the effects of (a) the father's attentiveness and (b) the mother's attentiveness on hatching success in 7 CT and 9 TC nests over two breeding attempts each. Parameter estimates (β) ± 1 standard error, Z-values and P-values are presented for all main effects and significant interactions. All other interactions (a) $P \geq 0.062$ and (b) $P \geq 0.158$.

	a) Father's attentiveness			b) Mother's attentiveness		
	$\beta \pm 1$ SE	Z	P	$\beta \pm 1$ SE	Z	P
Intercept	6.654 ± 2.004	3.32	< 0.001	-7.024 ± 4.713	1.49	0.136
Attentiveness	-0.103 ± 0.043	2.41	0.016	0.146 ± 0.070	2.07	0.038
Father's attractiveness	-0.095 ± 0.035	2.71	0.007	0.202 ± 0.089	2.27	0.024
Attentiveness \times father attractiveness	0.002 ± 0.001	2.18	0.029	-0.003 ± 0.001	2.55	0.011
<i>Main effect not in final models</i>						
Breeding attempt ^b	0.141 ± 0.413	0.34	0.733	0.479 ± 0.459	1.04	0.296

^b '1' is the reference breeding attempt

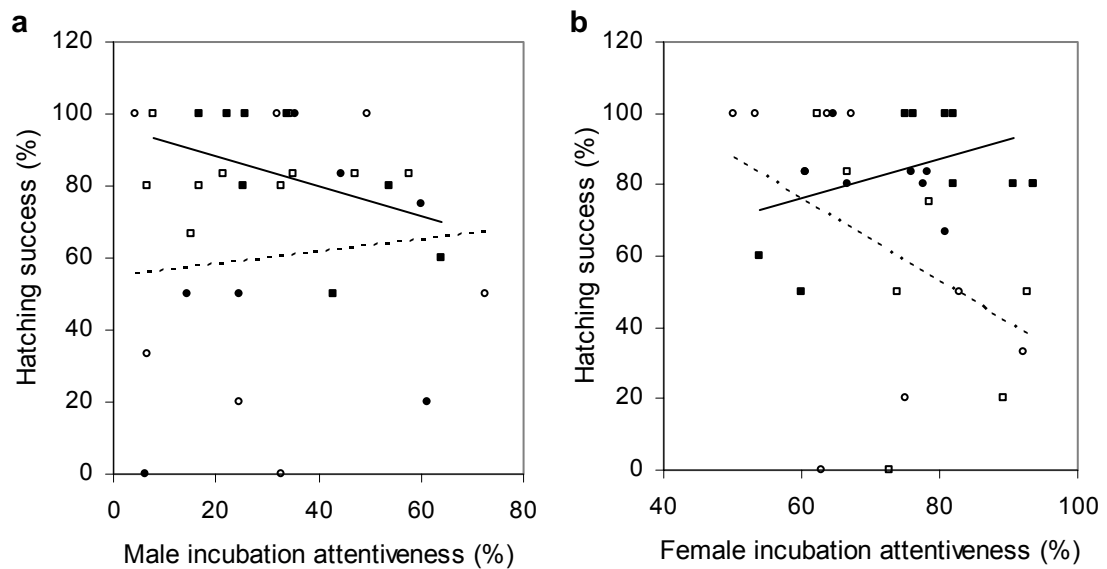


Figure 4.3 Interactions between (a) the father's incubation attentiveness and the father's attractiveness and (b) the mother's incubation attentiveness and the father's attractiveness on hatching success. Hatching success is presented here as a percentage of the total clutch size. For purpose of illustration only, males with attractiveness scores ≥ 50 % or < 50 % are grouped together and termed 'preferred' (open symbols, dashed line, $n = 8$) and 'less preferred' (closed symbols, solid line, $n = 8$) respectively. Circles represent hatching success in the first breeding attempt; squares represent the second, and lines of best fit summarise both attempts. (a) Hatching success increased with the father's incubation attentiveness in more attractive males, and decreased with it in less preferred males. (b) Hatching success decreased with the mother's incubation attentiveness in the nests of preferred males, and increased with it in the nests of less preferred males.

Treatment effects on post-hatching offspring size and survival

Offspring tarsus length, measured at days 30 and 100, was not influenced by the treatment (CT $n = 6$ broods; TC $n = 7$ broods; β : -0.2243 (-0.5298 to 0.0746), $P = 0.146$), the breeding attempt (β : -0.0729 (-0.2505 to 0.0905), $P = 0.399$), the father's attractiveness (β : -0.0016 (-0.0087 to -0.0056), $P = 0.625$), the offspring's sex (β : 0.0611 (-0.1190 to 0.2411), $P = 0.512$) or its age (β : -0.0001 (-0.0023 to 0.0022), $P = 0.927$), nor by any interaction between these variables ($P \geq 0.055$).

A greater proportion of offspring survived to age 100 days from TC (74.7 ± 8.92 %, $n = 8$ nests) than CT nests (41.8 ± 9.95 %, $n = 7$ nests; β : 1.71 ± 0.71 , $Z = 2.41$, $P = 0.016$). The proportion surviving did not differ between breeding attempts (attempt 1: 51.6 ± 10.06 %, $n = 16$; attempt 2: 67.1 ± 9.61 %, $n = 16$; β : 0.71 ± 0.45 , $Z = 1.589$, $P = 0.115$) and was not influenced by the father's attractiveness (β : 0.01 ± 0.02 , $Z = 0.29$, $P = 0.770$). There was no interaction between any of the variables ($P \geq 0.329$). A separate model showed that the proportion of offspring that survived was positively related to total incubation attentiveness (β : 0.06 ± 0.03 , $Z = 1.98$, $P = 0.048$).

Effects of female compensation on subsequent clutch size

The number of eggs a female laid in her second breeding attempt, corrected for egg volume, was not influenced by the treatment (β : -0.2682 ± 0.57 , $t = 0.47$, $P = 0.644$), her partner's attractiveness (β : 0.0003 ± 0.01 , $t = 0.03$, $P = 0.980$) or the number of offspring she had fledged in the first breeding attempt (β : 0.2731 ± 0.14 , $t = 1.91$, $P = 0.076$), and nor was it influenced by interactions between these variables ($P \geq 0.220$). However, when treatment was replaced by female incubation attentiveness in the model, I found an interaction between female incubation attentiveness and the number of offspring previously fledged on the number of eggs a female laid in the second breeding attempt (female attentiveness: β : 0.05 ± 0.03 , $t = 1.900$, $P = 0.082$; offspring fledged: β : 2.42 ± 0.93 , $t = 2.61$, $P = 0.022$; interaction: β : -0.03 ± 0.01 , $t = 2.31$, $P = 0.039$). Females that had previously fledged more offspring showed a decrease in subsequent clutch size with previous incubation attentiveness; subsequent clutch size was positively related to incubation attentiveness in females that had previously fledged few offspring (figure 4.4).

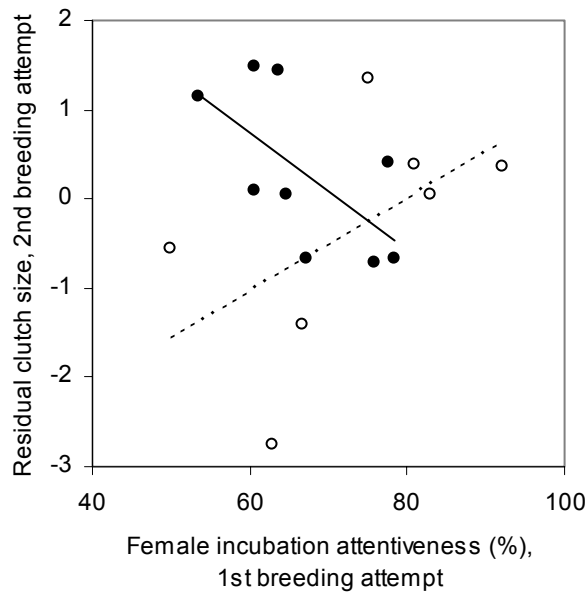


Figure 4.4 The interaction between a female's incubation attentiveness and the number of offspring she fledged on her subsequent clutch size. For purpose of illustration only, females that fledged ≥ 2 offspring ($n = 9$) are represented by closed symbols and the solid line; females that fledged < 2 offspring ($n = 7$) are represented by open symbols and the dashed line. The mean number of offspring to fledge from each nest was 2.1 ± 0.77 . Clutch size decreased with a female's previous incubation attentiveness in females that had previously fledged ≥ 2 offspring, but it increased with attentiveness in females that had previously fledged fewer than two.

Effects of female compensation and male attractiveness on the egg sex ratio

The egg sex ratio of the second breeding attempt was influenced by an interaction between the treatment group and the father's attractiveness (treatment group: β : -2.92 ± 1.56 , $t_{14} = 1.87$, $P = 0.061$; male attractiveness: β : -0.01 ± 0.02 , $t_{14} = 0.50$, $P = 0.617$; interaction: β : 0.06 ± 0.03 , $t_{14} = 2.00$, $P = 0.046$). The proportion of male offspring in a clutch increased with the attractiveness of the father when he had previously received EPC opportunities, but this was not the case in CT nests (figure 4.5). The number of offspring that were raised to fledging in the previous attempt did not influence sex allocation into the subsequent attempt (β : 1.68 ± 0.93 , $t_{14} = 1.81$, $P = 0.101$). In a separate model, I found that the proportion of male offspring in a clutch increased with the father's attractiveness (β : 0.03 ± 0.01 , $t_{14} = 2.05$, $P = 0.041$) and there was a non-significant trend for it to increase with the mother's previous incubation attentiveness, (β : 0.05 ± 0.03 , $t_{14} = 1.88$, $P = 0.060$).

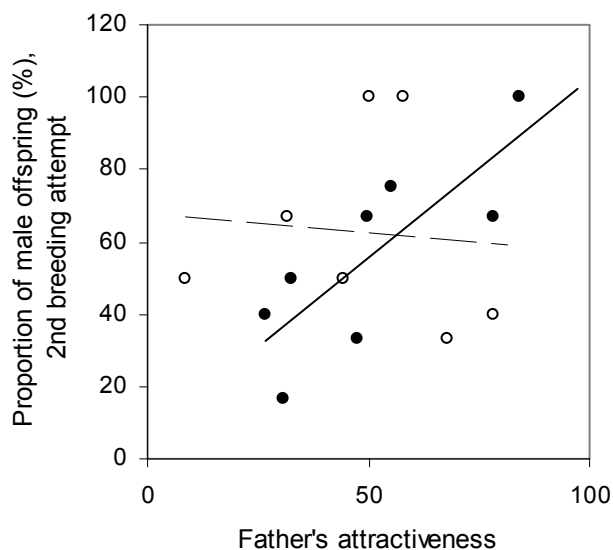


Figure 4.5 The interaction between treatment group and the father's attractiveness on the egg sex ratio of the second clutch. Symbols represent the pre-hatching proportion of male offspring in a clutch. The proportion of male offspring in a clutch increased with their father's attractiveness in TC nests ($n = 8$, closed symbols, solid line), but not in the nests of CT parents ($n = 7$, open symbols, dashed line).

Discussion

I manipulated male EPC opportunity in the Zebra Finch and investigated the extent to which the associated change in the relative contributions of males and females to incubation attentiveness influenced offspring performance and the pair's subsequent reproductive output. Since females compensated for the deficit in their partners' effort, I

expected females, rather than the current offspring, to pay the cost. However, the treatment appeared to influence both parties to some extent. Hatching success was lower in the nests of TC pairs in the first breeding attempt and CT pairs in the second attempt, and was influenced by the father's attractiveness. A greater proportion of hatched offspring survived from TC compared to CT nests in both breeding attempts, but the offspring did not differ in size at adulthood for a given brood size. In the subsequent breeding attempt, females that had raised a larger number of offspring produced clutch sizes that were negatively related to her previous incubation attentiveness, but this was not the case in females that had fledged few offspring. The proportion of male to female offspring in a clutch increased with the father's attractiveness but only when the father had experienced increased EPC opportunities.

Hatching success

In both breeding attempts, hatching success was lower in nests where fathers had experienced increased EPC opportunities, and this suggests that there is a direct fitness cost of increased mating opportunities in the father in terms of decreased hatching success. However, the variation in hatching success was not influenced by the reduction in male incubation attentiveness because males adjusted attentiveness according to the type of treatment that they experienced in their first breeding attempt rather than to the actual variation in EPC opportunity. In fact, hatching success was not related to the total amount of time that the pair incubated the eggs. Gorman *et al.* (2005a) found that hatching success in an aviary population of the same species increased with total incubation effort, but the latter probably did not vary enough in the current study to have had a detectable impact on hatching success.

The outcome of the second breeding attempt is likely to be influenced by the costs to the mother as well as to the offspring, and this may shed light on the different patterns observed in the different breeding attempts. One explanation for the treatment by breeding attempt interaction on hatching success is that pairs that hatched a greater proportion of their eggs in the first breeding attempt (CT pairs) experienced decreased hatching success in the second attempt as a result of increased provisioning effort to care for their larger first brood since there is a trade-off between current and future reproduction (Williams 1966; Trivers 1972). Those that had fewer mouths to feed in the first attempt (TC pairs) were able to invest more resources into the subsequent clutch and achieved a higher hatching success.

While there was little variation between pairs in total incubation attentiveness, there was plenty of variation between the sexes and between individuals. This observation raises the possibility that hatching success may be influenced by differences in the quality of incubation provided, and this could be the case if there are differences in incubation ability between and within the sexes. Sex differences in incubation temperature or the rate at which the eggs are warmed have been documented in a number of passerine species (e.g. Kleindorfer *et al.* 1995; Reid *et al.* 2002b; Voss *et al.* 2008), and male and female Zebra Finches may potentially differ in incubation ability since the female alone possesses a brood patch (Zann and Rossetto 1991). Bartlett *et al.* (2005) found that hatching success was negatively related to male nest attentiveness in the House Sparrow, *Passer domesticus*, but this was no longer the case once the female contribution was taken into account. In support of these findings, they also found that the female House Sparrow has a warmer ventral surface than the male. Hatching success was indeed influenced by the respective amounts of time that males and females spent incubating in the current study, but these relationships depended on the father's attractiveness. In the nests of more attractive males, hatching success increased with the father's incubation attentiveness and decreased with the mother's attentiveness, while the reverse was true in the nests of less attractive males. The Compensatory Investment Hypothesis predicts that females constrained to breed with low quality partners should be more willing to compensate for them (Gowaty *et al.* 2007; Bolund *et al.* 2009; Harris and Uller 2009), and this may be why less attractive males that showed reduced incubation attentiveness had the highest hatching success, but why hatching success decreased with female attentiveness in the nests of more attractive males remains to be seen. The lower hatching success of TC compared to CT pairs in the first breeding attempt suggests that males did not provide a lower quality of care than females during incubation since a greater proportion of the incubation in TC pairs was undertaken by females. Individuals may potentially differ in their willingness to maintain optimum incubation conditions, and it has been suggested that parents can modify their incubation temperature either directly or by adjusting their contact with the eggs (White and Kinney 1974). It is possible that there may have been variation between males in their willingness to care in response to the actual variation in EPC opportunities, irrespective of the amount of time they spent incubating, and that females may have responded by adjusting their own quality of care in the same direction (Johnstone and Hinde 2006).

Pre-hatching mortality did not differ between male and female embryos, and the levels of sex-specific hatching success presented in this study are similar to those found by Rutkowska and Cichoń (2002) in the same species (males: 82 %, females: 97 %). The

majority of studies on the Zebra Finch suggest that the female is more vulnerable to developmental conditions, but these have generally focussed on differences measured at the post-hatching stage (e.g. Kilner 1998; Gorman and Nager 2004; Martins 2004). However Gorman *et al.* (2005b) did not find evidence of sex-specific hatching mortality. Potential differences between studies of sex-specific embryo mortality could arise due to variation in the stage of development at which mortality occurs because deaths that occur early in embryonic development cannot be detected. Differences in the developmental pathways of males and females may mean that the sexes vary in their response to a given developmental stress according to its nature, intensity and the point in time at which it occurs (Badyaev 2002; Gorman *et al.* 2005b).

Post-hatching offspring size and survival

Neither the treatment group nor the father's attractiveness influenced the tarsus length of adult offspring at a given brood size. This is probably due to female compensation for the reduction in male incubation attentiveness since the effects of suboptimal incubation conditions have previously been shown to carry over into subsequent life history stages (Gorman and Nager 2004). Females that compensate for their partners during incubation may be unable to provision the offspring sufficiently due to the trade-off between investment into different reproductive stages (Heaney and Monaghan 1996; Reid *et al.* 2002c), but this does not appear to have been the case here. Although surviving offspring from TC and CT nests did not differ in size, a greater proportion of hatched offspring from TC nests compared to CT nests survived to sexual maturity. In contrast with the results of the hatching success analysis, the proportion of surviving offspring increased with total incubation attentiveness. This suggests that the effects of incubation conditions influence subsequent life history stages, as found in a number of other studies (Gorman and Nager 2004; Gorman *et al.* 2005b; Nilsson *et al.* 2008). However, the survival difference reported in the current study could also arise from variation in the parents' provisioning behaviour due to differences in previous incubation effort, and a cross-fostering experiment would be necessary to separate these effects. Assuming that incubation and provisioning are sufficiently costly in a laboratory environment, I would expect TC females, which compensated for the partners' lower incubation attentiveness, to reduce their reproductive effort into a subsequent breeding attempt.

Subsequent female reproductive output

Females whose partners experienced increased EPC opportunities in the first breeding attempt responded by increasing their own incubation effort, but their subsequent clutches

were not smaller than those of controls (taking into account variation in egg volume). However, subsequent clutch size was negatively related to incubation attentiveness in females that had previously raised a larger number of offspring, presumably due to the trade-off between effort invested into different breeding attempts (Williams 1966), while it was positively related to previous incubation effort in females that had fledged fewer offspring. The failure of treatment group to explain variation in subsequent female reproductive output suggests that females varied within treatment groups in the extent to which they compensated. The positive relationship between incubation attentiveness and subsequent clutch size among females that fledged few offspring may imply that females varied in condition, and female Zebra Finches in good condition have been found to both produce larger clutches (Selman and Houston 1996) and to spend more time incubating than controls (Gorman and Nager 2003). The high incubation attentiveness shown by some of these females (of which four were TC and three CT) in combination with the low fledging rate may also imply a trade-off between incubation and provisioning effort within the same breeding attempt, similar to that described for the Starling, *Sturnus vulgaris*, by Reid *et al.* (2000b). This may explain why the three females that showed the highest incubation attentiveness failed to fledge any young. The effects of incubation effort and fledging success (which can reflect both incubation and provisioning effort) on subsequent clutch size suggest that these measures of parental effort do influence female fecundity, and moreover, that these effects can be detected after (and perhaps even within) a single breeding attempt. Therefore, incubation effort is likely to be a good proxy for parental investment.

The proportion of male offspring in a clutch increased with the father's attractiveness in TC but not CT pairs. Burley (1981) used coloured leg rings to manipulate the attractiveness of Zebra Finches and found that females paired to attractive males fledged a higher proportion of sons, and suggested that this difference may be due to facultative sex ratio manipulation. However, since their results do not include offspring that died between fertilisation and early adulthood, these results may also reflect differences in incubation or provisioning effort, which can also be influenced by the colour of the leg ring (Burley 1988; Gorman *et al.* 2005a), as well as by differences in the sensitivity of the sexes to sub-optimal developmental conditions (Badyaev 2002). By taking detectable embryos into account, the current experiment provides stronger support for the idea that the relationship between the father's attractiveness and the sex ratio may be subject to primary sex ratio adjustment. It is less clear, however, why male attractiveness did not influence the sex ratio in CT pairs.

Conclusions

A number of studies have described a relationship between paternal effort and mating effort, but few of these have explored the fitness consequences of such a trade-off. I found that hatching success was lower in the nests of males that had experienced increased EPC opportunities, which suggests that there is a direct fitness cost of increased mating opportunities in the father, although the mechanism by which this occurred is unclear. A greater proportion of hatched offspring survived to sexual maturity from TC nests, and this was positively related to total incubation attentiveness. The primary sex ratio became more male biased as the father's attractiveness increased, but this was only the case in nests where the father had previously experienced EPC opportunities. The number of eggs a female laid in the second breeding attempt was influenced by her previous incubation effort and the number of offspring she had fledged, and this suggests that, at least in the short-term, or for short-lived socially monogamous species such as the Zebra Finch, females pay the costs of compensating for a deficit in their partner's care. Thus incubation attentiveness is an appropriate proxy for parental investment since it enhanced offspring survival at a cost to the mother's future fitness.

Chapter 5 - Sex differences in parental ability in a biparental incubator

Abstract

Sexual conflict over biparental care occurs when there are differences between males and females in the relative costs and benefits of care. Theory predicts that the sex that experiences the greater costs or lower benefits of parental care will make the lesser contribution to it. A number of avian studies have investigated factors expected to influence variation in incubation attentiveness (time spent incubating), but few have considered whether males and females differ in their abilities to provide care. The Zebra Finch, *Taeniopygia guttata*, is a biparentally incubating passerine in which the female alone has a brood patch and shows greater incubation attentiveness than the male. Based upon these observations, I predicted that incubating females would have a warmer ventral surface, be able to re-warm cool eggs more quickly and incubate eggs to a higher temperature than males. I compared the sexes' incubation abilities under standard conditions and also where the energetic costs of incubation were increased by clutch size enlargement. I used a temperature probe inside a dummy egg to measure egg core temperature and rate of re-warming, and a novel application of infra-red thermography (IRT) to measure ventral and egg surface temperature. IRT data were comparable to those obtained via the traditional method, and it provided quicker, more repeatable measurements that incorporated the whole clutch rather than a single egg. Under standard incubation conditions the ventral surface was warmer in females than males, and ventral temperature was higher than baseline (throat) temperature in females but not males. Incubating females increased their ventral surface temperature following clutch size enlargement, whereas males showed no response. The within-clutch variance in egg temperature increased with clutch size more rapidly in males than in females following the manipulation, but no sex differences were detected in clutch surface temperature or rate of re-warming. In fact, clutch temperatures were positively correlated within pairs, probably due to the nature of biparental incubation, which allows partners to benefit from each other's previous contributions of heat and avoid the costs of re-warming. The difference between males and females in ventral temperature suggests that the sexes differ in their ability or willingness to incubate, presumably as an adaptive consequence of sex differences in parental certainty or mate availability.

Introduction

Parental care is costly and each parent must optimise their allocation of resources (Williams 1966; Maynard Smith 1977) between different stages of reproduction (Heaney and Monaghan 1996; Reid *et al.* 2000b), different breeding attempts (Magrath and Elgar 1997; Reid *et al.* 2000b) and self-maintenance activities such as foraging (Drent *et al.* 1985; Moreno and Hillström 1992). Differences between the sexes in mate availability, the intensity of sexual selection and parental certainty are thought to promote sex role divergence (Trivers 1972; Queller 1997; Kokko and Jennions 2008; Kunz and Hosken 2009), resulting in one sex, usually the female, being selected to invest a greater proportion of time into care or to provide care of a higher quality. Sex role divergence is particularly pronounced in mammals, where the female gestates the offspring and then shoulders the high energetic cost of lactation (Speakman 2008), with males contributing to biparental care in just 10 % of mammal species (male-only care is absent) compared to 90 % of avian species (Clutton-Brock 1991). In species with biparental care, division of labour reflects a co-operative equilibrium between the costs and benefits of care to each partner (Trivers 1972; Houston and Davies 1985; Winkler 1987), and biparental systems, where both parents are required to contribute to care to ensure offspring survival, offers a useful platform for investigating conflict resolution over parental investment.

Many studies have investigated factors expected to influence variation in the temporal aspect of care in biparental birds (Gorman and Nager 2003; Paredes *et al.* 2005; Griggio and Pilastro 2007; Hinde and Kilner 2007), but few have considered whether males and females differ in their abilities to provision the offspring (e.g. Dickens *et al.* 2008; Thaxter *et al.* 2009; Welcker *et al.* 2009) or to incubate the eggs (e.g. Kleindorfer *et al.* 1995; Voss *et al.* 2008). Incubation is costly to the parent (reviewed in Reid *et al.* 2002a; and Tinbergen and Williams 2002), but crucial to the offspring, and the embryo must be maintained within a specific range of temperature and humidity for optimal growth and development (Rahn and Ar 1974; Webb 1987). Variation in incubation behaviour can have a profound impact on the conditions experienced by the embryo (Ricklefs and Smeraski 1983; Webb 1987; Reid *et al.* 2002b; Gorman *et al.* 2005b), and also affect the offspring in adult life (Gorman and Nager 2004).

Moreover, incubation might be a particularly promising stage in which to explore sex role divergence in terms of parental ability because the sexes often differ in their levels of

brood patch development. The brood patch typically consists of a defeathered area of highly vascularised ventral skin that facilitates heat transfer from parent to egg (Bailey 1952; Lea and Klandorf 2002), and it can also play a role in channelling heat away from the clutch (Vleck 1981). Sensory receptors in the brood patch can enable an incubating parent to detect non-optimal egg temperatures, and the parent can then adjust its body position and contact with the eggs accordingly (Drent *et al.* 1970; White and Kinney 1974). In most passerine species the female possesses a brood patch but its occurrence in males is less common (Bailey 1952; Drent 1975), and it has been hypothesised that a greater level of brood patch development should confer greater incubation ability (Jones 1971). The sex with the more developed brood patch may be able to maintain higher egg temperatures, to re-warm cold eggs more rapidly or to sustain longer or more frequent incubation bouts. It may also be better equipped to detect fluctuations in egg temperature. The female appears to warm the eggs more rapidly or to a higher temperature than the male in a number of socially monogamous passerine species (e.g. Kleindorfer *et al.* 1995; Voss *et al.* 2008), but in others the male is able to incubate equally well even without a brood patch (Skutch 1976; Reid *et al.* 2002b; Auer *et al.* 2007). While these studies focussed on egg temperature, few have measured the temperature of the brood patch itself (for exceptions see Criscuolo *et al.* 2001; Deeming and Du Feu 2008), and only Bartlett *et al.* (2005) compared the relative warmth of male and female ventral surfaces in a biparentally incubating species. Bartlett *et al.* (2005) attached thermistors to the abdomens of male and female House Sparrows, *Passer domesticus*, and showed that females, who possess a brood patch, have warmer ventral surfaces than males, who do not.

I studied sex differences in incubation ability in a domesticated population of the Zebra Finch, *Taeniopygia guttata*, a socially monogamous passerine native to Australia and eastern Indonesia (Zann 1996). Division of labour over day-time nest attentiveness is thought to be equal between free-living males and females (Zann and Rossetto 1991), although the male contributes a lesser share than the female in domesticated populations (El-Wailly 1966; Delesalle 1986; Burley 1988; Gorman and Nager 2003; chapter two; chapter three), and night-time incubation is performed almost exclusively by the female in the wild and in captivity (Zann and Rossetto 1991). In the Zebra Finch, the female alone possesses a brood patch (Zann and Rossetto 1991; Zann 1996) and so I predicted that (a) the ventral surface would be warmer in the female than in the male and (b) the female would incubate the eggs at a higher mean temperature. I compared the sexes' incubation abilities under standard incubation conditions and also under more challenging conditions where the energetic costs of incubation were experimentally increased. The energetic cost

of heat transfer is greater when Zebra Finches re-warm cold eggs (Vleck 1981) and so I predicted that (c) females would re-warm the eggs more quickly than males. Larger clutches are more energetically costly to incubate (Biebach, 1981; Biebach, 1984; Haftorn & Reinertsen, 1985; de Heij *et al.*, 2007) and clutch size is under female control, so low quality females may be able to reduce these costs by producing and incubating smaller clutches. Therefore I enlarged the clutches of treatment pairs and predicted that (d) females would be able to would increase their ventral surface temperature to meet the challenge of incubating an enlarged clutch and (e) maintain enlarged clutches at higher temperatures than males. I used a novel application of infra-red thermography (IRT), which uses known properties of an object's surface and simple physical laws to estimate the surface temperature from the infra-red radiation it emits (Speakman and Ward 1998), to quantify egg and ventral surface temperature. I validated the method by comparing egg surface temperatures measured using IRT with those obtained using the conventional method of inserting a temperature probe connected to a data logger into a dummy egg (e.g. El-Wailly 1966; Kleindorfer *et al.* 1995; Reid *et al.* 2000a; Reid *et al.* 2002b).

Methods

Subjects and maintenance

I used 35 virgin pairs of Zebra Finch that had been bred in captivity at the University of Glasgow in 2007 and did not have previous breeding experience. Prior to pairing, the sexes were maintained separately at a density of six individuals per cage (41 x 122 x 45 cm). Each pair cage (60 x 40 x 50 cm) was lined with hemp and supplied with perches, food and water. Birds received a standard diet of *ad libitum* mixed seed, consisting of pearl white, yellow, panicum, red, Japanese and Canadian canary millets (foreign finch mixture, supplied by Galloway MacLeod, U.K.), oyster grit, cuttlefish bone and water. They were also given egg biscuit protein with vitamin (Daily Essentials 2: The Birdcare company, Nailsworth, U.K.) and calcium (Calcivet: The Birdcare company, Nailsworth, U.K.) supplements three times a week and fresh spinach leaves twice a week. Provision of supplements and spinach began eight weeks before pairing and continued throughout the experiment. Coconut fibre nesting material was replenished on the floor of the cage once a week until the onset of incubation.

Although the Zebra Finch is an opportunistic breeder (Zann 1996), reduced maternal reproductive investment has been observed during the winter months in a similar captive population, even though temperature, photoperiod and relative humidity were held constant (Williamson *et al.* 2008). I increased daylight hours one week before pairing began from 10.5 hr L: 13.5 hr D to 12 hr L: 12 hr D. The ‘short day’ light regime lasted eight weeks and the ‘long day’ regime continued until the end of the experiment. Lighting was provided by daylight-spectrum fluorescent tubes (Arcadia Bird Lamp FB36). During both regimes, dawn and dusk were simulated by timing four lamps to activate 15 minutes before the central lighting was switched on and to deactivate with a delay of 15 minutes after the central lighting went out.

Males and females were size matched according to tarsus length and paired at staggered intervals in January and February 2009. Five pairs did not produce a clutch and eight constructed ‘open nests’ on the floor of the cage, although all had access to plywood nestboxes (outer dimensions: 137 x 145 x 145 mm, plywood thickness: 9 mm) filled with hemp. The remaining 22 pairs nested within the nestboxes and were allocated alternately to control or treatment group according to the date that the first egg was laid. I manipulated clutch size in 11 treatment nests by adding two eggs to the natural clutch size during incubation. Individuals allocated to the two groups did not differ in natural clutch size (control: 4.8 ± 0.44 eggs (mean \pm SE), $n = 11$; treatment: 5.3 ± 0.51 eggs, $n = 11$; $F_{1,20} = 0.46$, $P = 0.507$), tarsus length (control males = 13.9 ± 0.14 mm, $n = 11$; treatment males = 14.2 ± 0.14 mm, $n = 11$; $F_{1,20} = 1.87$, $P = 0.187$; control females = 14.2 ± 0.17 mm, $n = 11$; treatment females = 14.5 ± 0.15 mm, $n = 10$ (one measurement missing); $F_{1,19} = 0.84$, $P = 0.372$, measured December 2008) or in age at pairing (control males = 657.5 ± 7.64 days, $n = 11$; treatment males = 663.8 ± 13.83 days, $n = 11$; $F_{1,20} = 0.16$, $P = 0.691$; control females = 592.5 ± 36.15 days, $n = 11$; treatment females = 606.5 ± 28.16 days, $n = 11$; $F_{1,20} = 0.08$, $P = 0.780$).

Experimental design

I measured ventral surface temperature, incubation temperature and rate of re-warming in male and female Zebra Finches to compare the sexes under standard incubation conditions and where incubation effort was experimentally increased through clutch size manipulation. The incubation period of the Zebra Finch spanned 11-15 (median 14) days in a study of 13 wild-caught birds (Zann and Rossetto 1991), and tends to be slightly shorter in captivity (Zann 1996). Incubation appears to begin (‘day 0’) on the fourth day of the laying sequence in clutches of five or more, and on the final day of laying in clutches

of a smaller size (Zann and Rossetto 1991). On day 2 of the incubation period, I replaced all eggs with the same number of dummy eggs made of Fimo modelling clay (Eberhard Faber, Neumarkt, Germany). These have similar thermal properties to fresh Zebra Finch eggs (Gorman 2005) and the replacement of natural eggs for these does not influence the birds' incubation behaviour (Gorman *et al.* 2005a). The use of dummy eggs allowed me to quantify heat transferred by the parents independently of the potentially confounding effects of offspring heat production. The embryo produces heat as it grows (Ar and Sidis 2002), but this may be offset by heat loss from its developing circulatory system towards the end of incubation (Turner 1991; Turner 2002).

In the evening of incubation day 7, I added two extra eggs to the natural clutch sizes of the 11 pairs allocated to the treatment group. These pairs continued to incubate enlarged clutches until the experiment ended on incubation day 12, and are referred to as 'pre-treatment' pairs on days 3-7 and 'treatment' pairs on days 8-12. The remaining 11 pairs incubated their natural clutch sizes throughout, and are always referred to as 'control'. This 'between- and within-groups' design allowed me to test for differences in incubation ability between the sexes and treatments groups, and also for temperature changes in the control group over time, as temperature varies over the incubation period in certain passerine species (Webb 1987).

Infra-red thermography

IRT is a non-invasive technique that provides quick and accurate readings and has been used to measure birds' body temperature (Bakken *et al.* 2005) and energy expenditure (Ward and Slater 2005), and to investigate the cooling rate of artificially heated eggs (Lamprecht and Schmolz 2004). I used the ThermaCamTM E300 (FLIR Systems, Burlington, Canada) to take thermal images of birds and clutches and the software ThermaCAMTM QuickReport 1.1 (FLIR Systems 2007a) to visualise the stored digital false colour images on a P.C. Since the recorded radiation is influenced by the object's emissivity, the ambient temperature and absorption and scattering by atmospheric humidity, I set the surface emissivity value to 0.98 for the bird's plumage (Hammel 1956) or 0.91 for the eggs (fired clay) (Bramson 1968), while temperature estimates were standardised for the mean room temperature (22.2 ± 0.003 °C) and relative humidity (13.6 ± 0.04 %) recorded during the measurement period.

Ventral temperature

Ventral surface temperature measurements comprise the heat generated by the bird and the insulative properties of its plumage and skin, and so I measured the thickness of the plumage overlying the skin to take this variation into account. Ventral plumage surface temperature was recorded as a proxy for ventral temperature, and for simplicity I refer to this hereafter as ventral temperature. Throat plumage surface temperature (hereafter, throat temperature) was also measured to take account of individual variation in baseline body temperature. IRT was carried out on both parents on days 6 and 8 of incubation when nest attentiveness is at its highest (Gorman and Nager 2003). Nineteen pairs participated in the ventral temperature experiment (equipment was unavailable when one treatment and two control pairs of the original 22 were incubating) and complete data is available for all but two treatment pairs and one control female on day 6 of incubation and the partner of the latter female on day 8 who were unwilling to incubate. For each measurement, I gently displaced the incubating bird with a tap to the nestbox and caught it while taking care not to touch its underside. The camera was supported on a tripod, erected at the same site each time to standardize thermal and radiative conditions between readings, and the subject was held at a distance of 0.20 m. I took three replicate images of the ventral surface and recorded the time elapsed between the displacement of the bird from the nest and the image being taken ('image latency'). I ensured that the bird's aluminium leg ring (4.0 mm) was well resolved to provide a scale.

I used ThermaCAMTM Reporter 8.3 (FLIR Systems 2007b) to analyse the best resolved of the three replicates taken on each occasion. With the polygon tool, I traced a straight digital line along the length of the bird's leg ring (figure 5.1: Li1), noting its length in pixels, and then produced three more lines, or thermal transects, of the same length (figure 5.1: Li2, Li3, Li4). I extended Li2 by a factor of three, since the brood patch is at least three times the length of the ring, and placed it along the sagittal plane on the region of the thermal image corresponding to the bird's ventral surface. Li3 was placed at the apex of Li2, along the same plane, in order to standardise the distance between Li2 and Li4; Li4 was placed on the throat area at the apex of Li3. The relative size and positioning of the sampled areas were thus constant across thermal images. I then calculated the mean pixel temperature along the first (ventral surface) and third (throat) thermal transects.

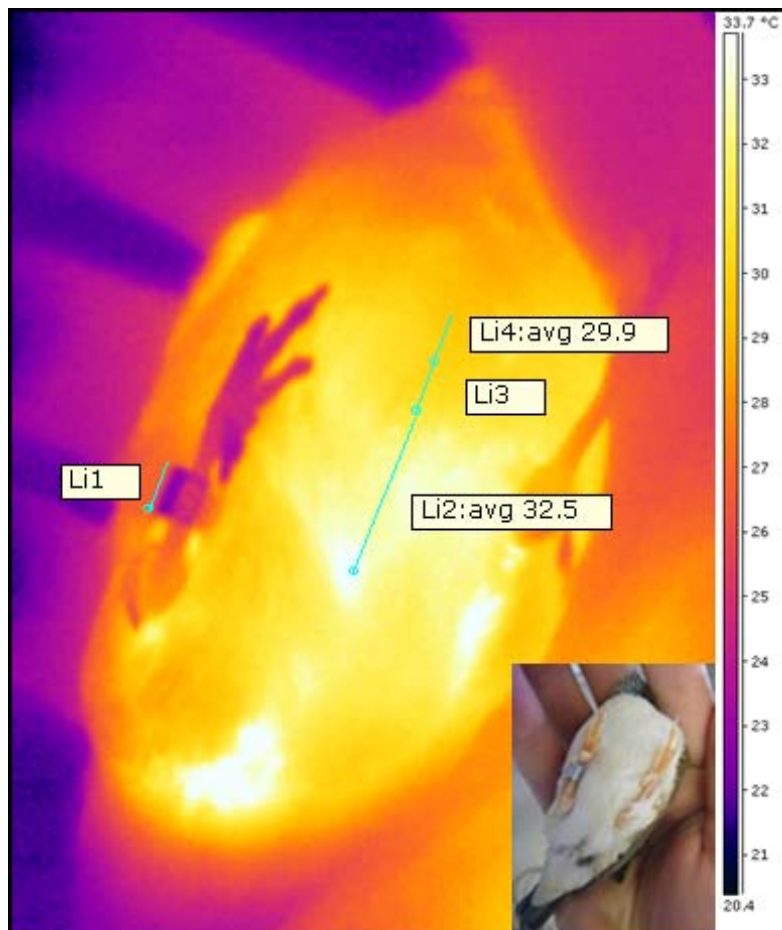


Figure 5.1 Sample thermal image of the ventral surface of a male on day 6 of incubation illustrating the procedure used for image analysis, and (inset) the digital reference image of the same bird.

I measured plumage thickness at the two points where measurements of surface temperature were obtained: one site over the brood patch and another more rostral site over the throat where Li4 was measured. Following IRT measurements on days 6 and 8 of incubation, I measured the birds' plumage thickness to the nearest 0.5 mm by pushing a primary feather, marked along the shaft at 1 mm intervals, through the plumage down to the skin. Ventral temperature was not influenced by image latency in control pairs on days 6 and 8 of incubation (β : 0.35 (-1.21 to 1.95) °C, $P = 0.661$, $n = 9$ pairs; mixed effect model controlling for pair and individual identities, see statistical analysis), and did not differ between pre-treatment (32.1 ± 0.54 °C, $n = 8$ pairs) and control (32.1 ± 0.45 °C, $n = 9$ pairs) groups on day 6, prior to the manipulation (β : -2.91 (-92.70 to 91.46) °C, $P = 0.945$, controlling for pair identity).

Clutch temperature

IRT was carried out on the entire clutches of 21 pairs (IRT data were not collected for one treatment clutch) on incubation days 3-12 after either the male or the female had been incubating. I gently displaced the incubating bird with a tap to the nestbox, noting the

bird's sex, and took three successive thermal images of each clutch on each day from a constant distance of 0.20 m. Using ThermaCAMTM Reporter 8.3 (FLIR Systems 2007b), I analysed the best resolved of the three replicates taken on each occasion, then, using the polygon tool, I traced an outline around the image of each egg (figure 5.2) and calculated the mean pixel temperature within the polygon at the time the image was taken.

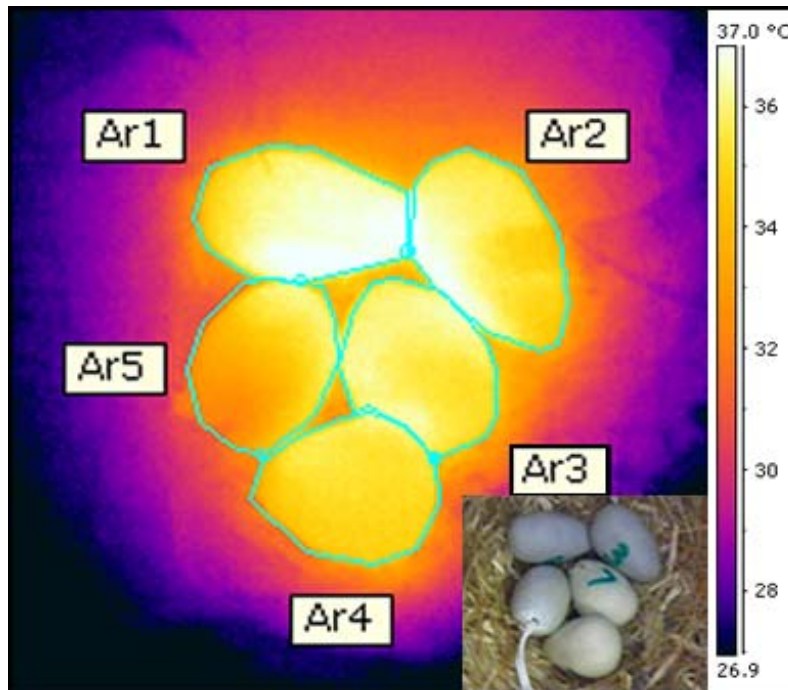


Figure 5.2 Sample thermal image of a clutch of four standard Fimo eggs (Ar1-Ar4) plus a probe egg (Ar5) on day 6 of incubation, illustrating the procedure used for image analysis, and (inset) the digital reference image of the same clutch.

Since exposed eggs cool very quickly and this cooling rate depends on the clutch size, I recorded the time elapsed between the departure of the bird from the nest and the image being taken ('image latency'), and modelled cooling rate by regressing egg surface temperature on \log_{10} -transformed image latency (to obtain a linear relationship) and included clutch size as a covariate (see Appendix table 1). I then used this model to extrapolate the egg surface temperature that an individual egg from a given clutch size and at a given image latency would have had when the incubating bird was displaced. I used these back-calculated egg surface temperatures in all subsequent analyses. I calculated mean egg surface temperature per clutch by averaging the temperatures over all eggs in the clutch and within-clutch variability in egg surface temperature among the eggs of a clutch as a coefficient of variance (CV).

On incubation days 6 and 8, thermal images were taken while a dummy egg with a thermocouple (the probe egg, see below) was inside the nest. This allowed me to compare egg surface temperature measurements from IRT with measurements taken simultaneously of the temperature at the egg's core, and so these values were not corrected for image latency. A digital reference image was also taken to allow the probe egg to be distinguished among the other eggs in the clutch (see inset in figure 5.2).

Probe eggs

I measured egg core temperature using a thermistor (TinytagTM Tinytalk Flying Lead Thermistor PB-5005-0M6, Gemini, West Sussex, U.K.) secured in the centre of two dummy eggs with SuperglueTM mixed with Heat SinkTM thermal compound (hereafter referred to as 'probe eggs'). Probe eggs were made out of Fimo to the same dimensions (diameter at widest points: 17.9, 10.5 mm), individually marked and always connected to the same data logger (TinytagTM Talk 2 TK-4023) where measurements were stored. I used a third data logger with an internal probe (TinytagTM Talk 2 TK-4014) to record ambient temperature in the bird room. Prior to experimental work, I compared readings from the three thermistors by placing them together in a fanned kiln where temperature was raised at 5 °C increments every 30 minutes from 20 to 40 °C. Measurements from the two probe eggs differed only by 0.05 ± 0.002 °C, and the difference between the ambient temperature thermistor and one of the probe eggs was $\leq 0.27 \pm 0.005$ °C. As this difference was less than the minimum accuracy of 0.5 °C, I did not correct for it in later analyses. Data loggers were programmed using TinytagTM Explorer 4.6. (Gemini Data Loggers 2007) to record time and temperature data every two seconds for probe eggs and every 15 seconds for the ambient temperature logger during experimental observations.

Probe eggs were used to collect data on re-warming rate and the temperature at which eggs were maintained (steady-state incubation temperature) for 21 nests (one control pair did not incubate during observations) on days 6 and 8 of incubation. An example of a temperature curve obtained using the probe egg method is shown in figure 5.3.

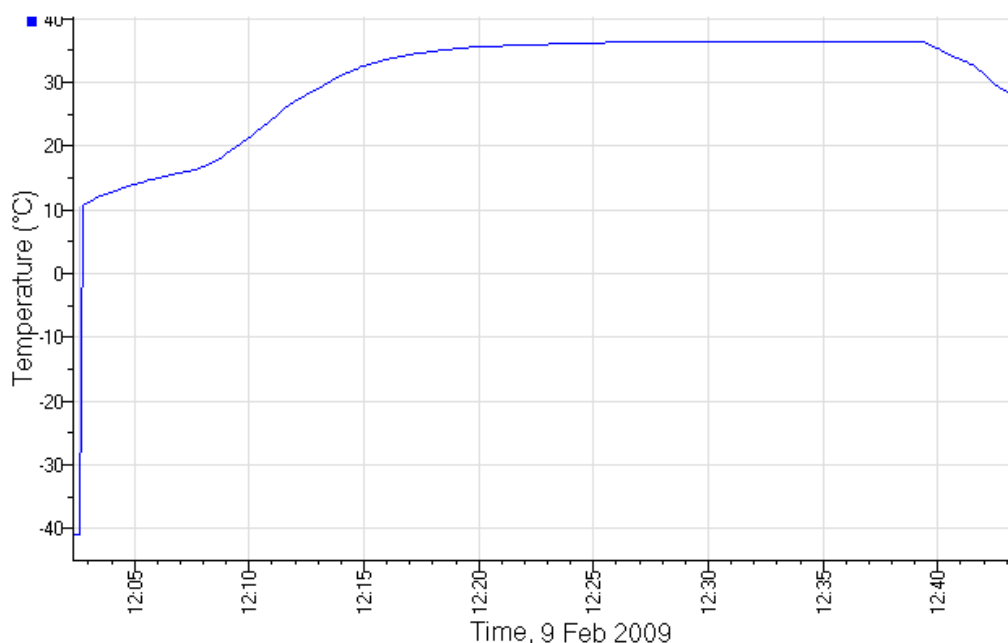


Figure 5.3 Sample temperature curve obtained using the probe egg method to illustrate re-warming of cooled eggs and steady-state incubation. The incubation bout began around 12:08, and the re-warming period continued until approximately 12:18. Steady-state incubation proceeded until the bird was displaced from the nest.

To measure re-warming rates, the incubating bird was displaced from the nest and its dummy clutch was replaced with an equal number of dummy eggs (including the probe egg) that had been cooled in the refrigerator (3-6 °C). Egg temperature at the onset of the re-warming incubation bout did not differ for males and females (males: 17.8 ± 0.56 °C, $n = 21$; females: 17.4 ± 0.66 °C, $n = 19$; β : 0.32 (-1.18 to 1.86) °C, $P = 0.680$, controlling for clutch size). The probe egg was placed in the centre of the clutch with its cable flattened against the nest and connected to a logger outside the cage. A small infra-red video camera was mounted above the nestbox and connected to a JPEG 2000 DVR system and television monitor hidden behind a curtain. I monitored nest activity live in order to establish incubation bout onset time (when one of the partners settled on the clutch by performing a shuffling motion), to identify the bird's sex and to document interruptions to incubation. The observation ended when the focal bird's incubation bout ended or after 30 minutes had passed, whichever occurred first. Data were downloaded and the process was repeated with the bird's partner. Since the same individual often continued to enter the nest or the incubation bout ended before incubation temperature had stabilised, for each partner on each day (6 or 8) I attempted to make at least one observation in which one partner (selected at random) was transferred to an adjacent cage and one in which both partners were present. Excluding one of the partners had no effect on re-warming rate (partner excluded: 0.21 ± 0.01 °C min⁻¹, $n = 31$ individuals; partner present: 0.23 ± 0.02 °C

min^{-1} , $n = 18$ individuals; β : -0.02 (-0.06 to 0.02) $^{\circ}\text{C min}^{-1}$, $P = 0.329$) or steady-state incubation temperature (partner excluded: 35.9 ± 0.19 $^{\circ}\text{C}$, $n = 31$ individuals; partner present: 35.7 ± 0.30 $^{\circ}\text{C}$, $n = 18$ individuals; β : 0.22 (-0.42 to 0.88) $^{\circ}\text{C}$, $P = 0.501$; nested mixed effects models controlling for individual and nest identities).

To estimate steady-state incubation temperature and rate of re-warming (figure 5.3), I fitted a non-linear regression model $C_d = (b-a)(1-\exp((-cd))) + a$, where C_d ($^{\circ}\text{C}$) is the incubation temperature at time d , a is temperature at the onset of incubation ($^{\circ}\text{C}$), b is the steady-state incubation temperature ($^{\circ}\text{C}$) and c is the rate of egg warming ($^{\circ}\text{Cs}^{-1}$) using SYSTAT 10 (SPSS Inc 2000). Unusually short bouts (≤ 6 minutes) and those in which the subject displaced the probe egg were not included in the analysis as steady-state incubation was not reached, and the data were truncated to exclude periods in which the bird stopped incubating. Reid *et al.* (2000a) used a to denote ambient temperature rather than egg temperature at the onset of incubation in the original formulation of this model, but I found that the latter provided a better fit under laboratory conditions. The model used least square estimation and the Gauss-Newton method, and it provided a good fit with the estimates of parameter values b and c in all cases ($R^2 \geq 0.93$). I then calculated repeatability (r) of b and c within individuals within a single day of incubation (6 or 8, whichever gave the greater number of observations) using the formulae given in Lessells & Boag (1987) and in Becker (1984) for standard errors. Re-warming rate (c) was significantly repeatable within individuals ($F_{17,23} = 2.34$, $r = 0.357 \pm 0.18$, $P = 0.030$) so mean values were taken as the unit of analysis. Steady-state incubation temperature (b) was not repeatable ($F_{17,23} = 0.74$, $r = -0.123 \pm 0.18$, $P = 0.737$) and was not subject to further analysis.

Statistical analysis

All data were analysed in R version 2.9.2. (R Development Core Team 2009). IRT measurements of ventral and throat temperature were normalised by square and square-root transformation respectively to allow parametric statistics to be used. Throat plumage thickness measurements were normally distributed, but ventral plumage thickness measurements were not and so non-parametric tests were used in analyses of the latter. All clutch data were normally distributed except within-clutch temperature variance and the re-warming rate data used in the main analysis (the sample used to calculate repeatability was normally distributed); the two non-normal variables were square-root and \log_{10} transformed respectively. I fitted linear mixed effect models by Restricted Maximum Likelihood using the lme4 library (Bates and Maechler 2009). Pair identity was fitted as a

single random factor where data from the same day of incubation were used; individual identity was nested within pair identity in repeated measures analyses. I use n_p to indicate whether the sample size refers to the number of pairs, n_i for the number of individuals and n_m for the number of measurements. Random effects were fitted with random intercepts only, and were controlled for regardless of statistical significance. I present the variance *between* pairs (pair identity) and individuals (individual identity) where appropriate and *within* individuals (residual variation) as a percentage of the total variance. I tested all possible two-way interactions between main effects and used backwards stepwise regression to reach the final model, removing interaction terms $P \geq 0.05$ in order of least significance and then main effects. Significance is denoted by a P -value < 0.05 or a Higher Posterior Density (HPD) interval that does not include zero. For each significant fixed effect and non-significant main effect, I present the model coefficient β and HPD interval (given in parentheses if not tabulated), which is similar to the confidence interval, calculated at the 95 % level using Markov chain Monte Carlo (MCMC) sampling with the chain length fixed at 10,000 and the P -value based upon the posterior probability distribution. These estimates were calculated using the `pvals` function from the `languageR` library (Baayen 2009). Statistical tests are two-tailed and means are presented \pm SE.

Ventral plumage thickness and temperature

First, I tested whether males and females ($n = 17$ pairs) differ in the thickness of plumage overlying their ventral and throat skin on day 6 of incubation (before the manipulation). Since they did not, I combined all males and females for which both days of data were available ($n = 16$) to test whether ventral and throat plumage thickness measurements were correlated between days 6 and 8. Second, I assessed within-pair differences in ventral temperature between days 6 and 8 of incubation using control pairs ($n = 9$ pairs; one female day 6 and one male day 8 measurement missing, as mentioned above). Zann and Rossetto (1991) observed an increase in brood patch development over the incubation period in six wild caught females, and thus ventral temperature might show a similar increase. I then calculated the within-individual repeatability (r) of ventral temperature between days 6 and 8 (Lessells and Boag 1987) and its standard error (Becker 1984). I used control birds, as before, since the treatment was expected to affect brood patch temperature ($n = 8$ pairs), and analysed the sexes separately.

I tested the hypothesis that females have a warmer ventral surface than males using single measures taken on day 6 of incubation ($n = 17$ pairs), with sex as a fixed factor and throat temperature and ventral plumage thickness as covariates in the model. It was not

necessary to correct for the thickness of throat plumage on throat temperature because the two were not related in control pairs (β : 0.02 (-0.04 to 0.01), $P = 0.169$, $n = 9$ pairs). I considered clutch size because it can be an indicator of female quality (Slagsvold and Lifjeld 1990) and influence incubation behaviour (Komdeur *et al.* 2002). I also used day 6 measurements to determine whether ventral temperatures are related between partners using Pearson's product-moment correlation.

If females show a true brood patch but males do not, we should expect a difference between ventral and throat temperature in females only. Examining the sexes separately, I ran a mixed model with surface temperature (ventral and throat data combined, which were normally distributed) as the response variable, body part (ventral or throat surface) as a fixed factor and plumage thickness as a covariate. This analysis used measurements taken on day 6 of incubation ($n = 17$ pairs), controlling for individual identity.

Next, I tested whether males and females differ in ventral temperature when challenged to incubate an enlarged clutch size compared to their natural clutch size. Since ventral temperature did not differ between days 6 and 8 in control pairs, I used a repeated measures design to compare the same individuals ($n = 10$ pairs) before (day 6) and after (day 8) the manipulation. Sex and treatment group were included in the model as fixed factors, and throat temperature and ventral plumage thickness were entered as covariates.

Clutch temperature

All mixed model analyses described below, except when comparing probe and IRT measurements, controlled for natural clutch size. As well as influencing incubation behaviour (Komdeur *et al.* 2002), it can affect the physical properties of the clutch, such as temperature and humidity (Reid *et al.* 2000a), and it can also be an indicator of female quality (Slagsvold and Lifjeld 1990) or condition (Selman and Houston 1996). Analyses of egg surface temperature measured using IRT take the mean egg temperature for the clutch as the unit of analysis. The number of males and females sampled by IRT was not equal for a given day of incubation since a single IRT measurement was taken of each clutch each day, regardless of the sex that was incubating. Since individuals that incubated more often were more likely to be sampled and they may also have been better incubators, I used a binomial mixed model controlling for pair identity to test whether there was a relationship between sampling frequency and incubation temperature in control pairs ($n_p = 11$). Individuals that incubated more frequently did not incubate at a higher mean temperature (β : 0.04 ± 0.16 , $Z = 0.29$, $P = 0.775$).

Sex differences in egg surface temperature, CV and re-warming rate

I calculated the within-individual repeatability (Lessells and Boag 1987) of egg surface temperature over incubation days 3-12 and its standard error (Becker 1984). Control pairs were used since the manipulation was expected to affect egg temperature. Repeated measures were available for 10 females and 7 males, and repeatability was calculated separately for each sex. I then used a mixed model to test for within-pair differences in egg surface temperature, variance in egg surface temperature (CV) and rate of re-warming respectively, with day of incubation (3-12 for egg surface temperature and CV; 6 and 8 for re-warming rate) as a covariate. Next, using separate mixed models, I assessed whether egg surface temperature, CV and re-warming rate were influenced by ventral temperature in control birds. For each bird, I used the mean ventral temperature of days 6 and 8 since this was repeatable between these days. Since the ventral surface was warmer in females than in males, I hypothesised that females might incubate at higher temperatures, that CV might be lower or that eggs might be re-warmed more quickly. I tested this under standard incubation conditions by using egg surface temperature, CV and re-warming rate as response variables in separate mixed models with sex as a fixed factor. This was a between-group analysis of control and pre-manipulation birds (days 3-7 for egg surface temperature and CV; day 6 for re-warming rate). Pairs allocated to the two treatment groups did not differ prior to the manipulation (days 3-7) in clutch surface temperature (control: 39.1 ± 0.27 °C, $n_p = 9$; pre-treatment: 39.2 ± 0.39 °C, $n_p = 8$; β : 0.54 (-0.05 to 1.17) °C, $P = 0.085$), in CV (control: 2.69 ± 0.24 , $n_p = 9$; pre-treatment mean: 2.85 ± 0.39 , $n_p = 8$; β : 0.10 (-0.17 to 0.37), $P = 0.462$) or in the rate at which they re-warmed cool eggs (control: 0.21 ± 0.02 °C min⁻¹, $n_p = 9$; pre-treatment: 0.22 ± 0.03 °C min⁻¹, $n_p = 9$; β : -0.02 (-0.12 to 0.09), $P = 0.767$). These mixed model analyses controlled for pair and individual identities and clutch size.

Next, I tested whether the sexes differed in incubation ability following the clutch enlargement. I used a within-individuals approach to compare pre- (incubation days 3-7 for egg surface temperature and CV; 6 for re-warming rate) and post-manipulation (days 8-12 for egg surface temperature and CV; 8 for re-warming rate) birds, with sex and treatment group as fixed factors. I then used Pearson's Product-Moment Correlation test to see whether male and female egg surface temperatures were correlated within control pairs. For each individual, I took the mean measurement across days 3-12. Since re-warming rate data were not normally distributed, I used Spearman's Correlation test to

examine the same relationship in control pairs, taking the mean of day 6 and 8 measurements for each individual.

Comparison of probe and IRT measurements

I used a paired t-test to see whether temperature measurements taken simultaneously from the surface and core of an egg using IRT and a probe, respectively, differ, and a mixed model to examine the relationship between the two sets of measurements, with egg surface measurements as the response variable and core measurements as the explanatory variable. Individual identity was nested within pair identity to control for the identities of the incubating bird and the nest.

Results

Ventral and throat plumage thickness

Pre-treatment (day 6) males and females did not differ in the thickness of their ventral (males: 4.4 ± 0.52 mm, $n = 17$; females: 3.7 ± 0.31 mm, $n = 16$; $W = 116$, $P = 0.470$) or throat plumage (males: 5.3 ± 0.48 mm, $n = 17$; females: 5.3 ± 0.42 mm, $n = 16$; $t_{31} = 0.08$, $P = 0.941$). An individual's plumage thickness was positively correlated between days 6 and 8 (ventral plumage: $r_s = 0.59$, $P < 0.001$; throat plumage: $r_p = 0.40$, $P = 0.022$; $n = 16$ pairs, sexes pooled).

Repeatability in ventral temperature between days

Among control pairs ($n = 9$), there was no difference in ventral temperature between days 6 (32.1 ± 0.45 °C) and 8 (32.3 ± 0.41 °C) of incubation (β : 5.62 (-32.12 to 43.99), $P = 0.751$). Ventral temperature was repeatable within individual control females ($F_{7,8} = 10.87$, $r = 0.832 \pm 0.11$, $P = 0.002$), but not males ($F_{7,8} = 3.42$, $r = 0.548 \pm 0.26$, $P = 0.053$).

Sex differences in ventral temperature

Prior to the manipulation (day 6), the ventral temperature of control and treatment birds ($n = 17$ pairs) was influenced by ventral plumage thickness and an interaction between the bird's sex and its baseline temperature (as indicated by throat temperature), but not by its natural clutch size (table 5.1). Ventral temperature was positively related to throat temperature, but the slope of the relationship was steeper for males (figure 5.4). Ventral temperature increased with decreasing plumage thickness. Male and female ventral surface temperatures were not correlated within pairs ($r_p = -0.18$, $P = 0.499$, $n = 16$ pairs).

Table 5.1 General linear mixed model showing the effects of sex on ventral temperature (squared) in 17 pairs on day 6 of incubation. Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for main effects and the only significant interaction. All other interactions $P \geq 0.431$.

	β	HPD interval	P
<i>Fixed effects</i>			
Intercept	355.13	-433.12 to 1134.31	0.366
Sex ^a	-1250.20	-2239.12 to -253.56	0.015
Ventral plumage thickness	-20.92	-36.71 to -4.50	0.015
Throat temperature	25.11	0.79 to 50.28	0.051
Sex x throat temperature	39.62	8.05 to 71.88	0.016
<i>Main effect not in final model</i>			
Clutch size	-0.62	-18.20 to 16.58	0.945
<i>Random effects</i>			
			Variance (%)
Individual identity	12.25	0 to 35.15	40.4
Residual	72.03	52.77 to 92.83	59.6

^a 'female' is the reference sex

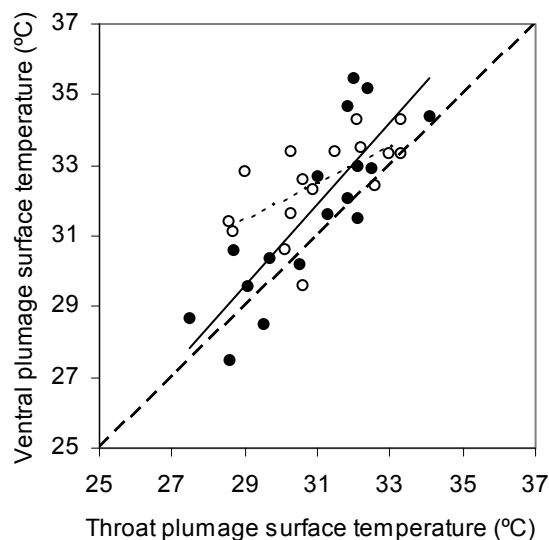


Figure 5.4 The relationship between ventral and throat surface temperature in males and females. Before the manipulation, ventral temperature was positively related to throat temperature, but the slope of the relationship was steeper for males (closed circles, solid line, $n = 17$) than females (open circles, dotted line, $n = 16$). Each point represents one individual on day 6. The dashed line shows where points would lie if ventral and throat temperatures were equal. Throat temperature did not differ between the sexes (β : -0.02 (-0.12 to 0.09), $P = 0.697$, $n = 17$ pairs). Throat plumage was thicker than ventral plumage (β : 0.29 (0.10 to 0.48), $P = 0.004$, $n = 17$ pairs), but this was not influenced by sex (β : 0.06 (-0.15 to 0.25), $P = 0.577$) or an interaction between the two ($P = 0.435$).

The ventral surface (32.5 ± 0.33 °C, $n = 16$) was warmer than the throat (31.1 ± 0.39 °C, $n = 16$; β : -1.42 (-2.43 to -0.48), $P = 0.007$) in females, but there was no difference between the surface temperatures of the ventral area (31.7 ± 0.59 °C, $n = 17$) and the throat (30.9 ± 0.43 °C, $n = 17$; β : -0.42 (-1.62 to 0.74), $P = 0.473$) in males.

Effects of the clutch size manipulation on ventral temperature

Among treatment group birds on days 6 and 8 of incubation, ventral temperature was influenced by throat temperature, ventral plumage thickness and an interaction between sex and treatment group (table 5.2). Female ventral plumage was warmer after the clutch size enlargement, but male ventral temperature did not change (figure 5.5).

Table 5.2 General linear mixed model showing the effects of sex on ventral temperature (squared) in 10 pairs on days 6 and 8 of incubation (repeated measures within individuals). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for main effects and the only significant interaction. All other interactions $P \geq 0.117$.

	β	HPD interval	
<i>Fixed effects</i>			P
Intercept	-771.14	-1127.97 to -400.67	< 0.001
Throat surface temperature	60.70	49.57 to 71.37	< 0.001
Ventral plumage thickness	-24.03	-39.77 to -9.48	0.004
Treatment group ^b	46.89	-0.56 to 93.65	0.051
Sex ^a	11.64	-41.58 to 60.67	0.635
Sex x treatment group	-75.18	-141.57 to -11.19	0.026
<i>Random effects</i>			Variance (%)
Pair identity	13.72	0 to 32.90	27.4
Individual identity	6.04	0 to 20.24	< 0.1
Residual	49.51	36.21 to 64.29	72.6

^a 'female' is the reference sex

^b 'pre-treatment' is the reference group

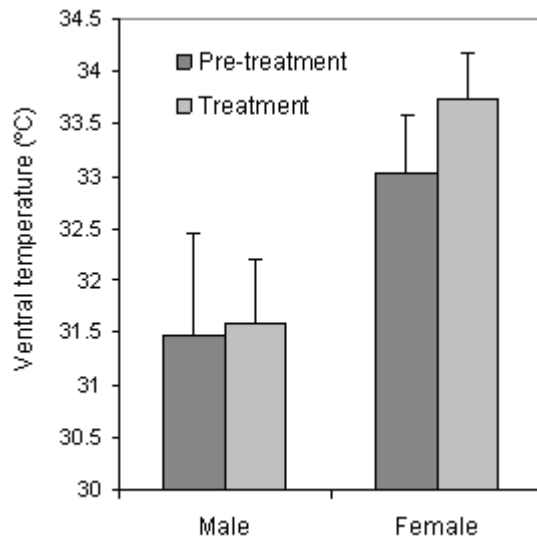


Figure 5.5 Ventral temperature (°C) before and after clutch enlargement (means + 1 SE) in males (pre-treatment $n = 9$, treatment $n = 10$) and females (pre-treatment $n = 8$, treatment $n = 9$). There was a treatment group x sex interaction on ventral temperature (see table 5.2).

Repeatability of egg surface temperature across the incubation period (IRT)

Egg surface temperature was repeatable within control females ($F_{9,30} = 4.80$, $r = 0.491 \pm 0.17$, $P < 0.001$, $n = 10$) and males ($F_{10,19} = 5.93$, $r = 0.399 \pm 0.23$, $P < 0.001$, $n = 7$). Over the incubation period (days 3-12) among control pairs ($n = 11$ pairs) there was no change in egg surface temperature (β : 0.02 (-0.06 to 0.12), $P = 0.622$) or its variance (β : 0.01 (-0.03 to 0.04), $P = 0.728$). Egg surface temperature decreased with clutch size (β : -0.50 (-0.76 to -0.22), $P = 0.001$), while its variance increased with clutch size (β : 0.12 (0.05 to 0.20), $P = 0.006$), so clutch size was statistically controlled for in subsequent models. Within control pairs ($n = 9$) on days 3-12 of incubation, egg surface temperature (β : -0.14 (-0.35 to 0.06), $P = 0.185$) and within-clutch egg temperature variability (β : -0.01 (-0.08 to 0.07), $P = 0.841$) were not related to brood patch temperature

Pre-manipulation sex differences in egg surface temperature (IRT)

Prior to the manipulation (incubation days 3-7), there was no difference in egg surface temperature between females (39.1 ± 0.32 °C, $n = 17$) and males (39.3 ± 0.31 °C, $n = 14$; β : 0.12 (-0.42 to 0.71), $P = 0.659$) nor in within-clutch egg temperature variability (males: 2.92 ± 0.52 %, $n = 13$; females: 2.63 ± 0.21 %, $n = 17$; β : -0.08 (-0.32 to 0.17), $P = 0.539$).

Post-manipulation sex differences in egg surface temperature (IRT)

Among treatment group birds on days 3-12 of incubation, egg surface temperature was influenced by natural clutch size and treatment group, and the variance in clutch surface

temperature was influenced by treatment group and an interaction between sex and natural clutch size (table 5.3). Egg surface temperature decreased with clutch size, while within-clutch variance increased with clutch size. Experimentally enlarged clutches were cooler (pre-treatment: 39.1 ± 0.51 °C, $n_p = 8$; treatment: 37.8 ± 0.38 °C, $n_p = 10$) and showed greater variance in temperature (pre-treatment: 2.75 ± 0.50 %, $n_p = 8$; treatment: 3.75 ± 0.37 %, $n_p = 10$). The slope of the relationship between clutch size and within-clutch variance in temperature was steeper for males than females (figure 5.6). Females did not heat the eggs to warmer temperatures (38.8 ± 0.43 °C, $n = 9$) than males (38.1 ± 0.46 °C, $n = 9$), although there was a trend in the predicted direction.

Table 5.3 General linear mixed models showing the effects on egg surface temperature (°C) and within-clutch egg surface temperature variability (square-root transformed) in pre-treatment (days 3-7: female $n = 8$, male = 6) and post-treatment (days 8-12: female $n = 7$, male $n = 9$; 10 pairs, repeated measures within individuals). Parameter estimates (β), Higher Posterior Density (HPD) intervals and P -values are presented for main effects and the only significant interaction. All other interactions $P \geq 0.226$.

	Egg surface temperature			Within-clutch variability		
	β	HPD interval		β	HPD interval	
			P			P
Intercept	42.16	40.98 to 43.32	< 0.001	1.471	0.88 to 2.05	< 0.001
Clutch size	-0.58	-0.80 to -0.37	< 0.001	0.03	-0.08 to 0.13	0.630
Treatment ^b	-1.17	-1.67 to -0.73	< 0.001	0.23	0.04 to 0.44	0.028
Sex ^a	-0.42	-0.89 to 0.06	0.087	-0.68	-1.37 to 0.01	0.054
Sex x clutch size			NS	0.15	0.03 to 0.28	0.020
Random effects			Variance			Variance
			(%)			(%)
Pair identity	0.35	0 to 0.64	34.0	0.15	0 to 0.28	33.6
Individual identity	0.16	0 to 0.41	7.7	0.07	0 to 0.18	18.4
Residual	0.7841	0.62 to 0.96	58.4	0.34	0.27 to 0.42	48.0

^a 'female' is the reference sex

^b 'control' is the reference group

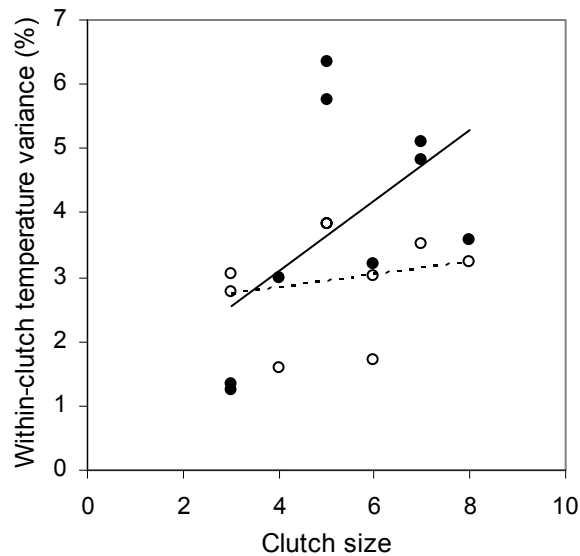


Figure 5.6 Within-clutch variance in surface temperature (%) increased with natural clutch size in treatment pairs, but the slope of the relationship was steeper for males (closed circles, solid line, $n = 9$) than for females (open circles, dashed line, $n = 9$). Each point represents the mean of each individual over days 3-12 of incubation. On days 3-7, individuals incubated their natural clutch size, but on days 8-12, clutches contained two eggs more than is indicated on the axis.

Relationship between partners' egg temperature and rates of re-warming

An individual's egg surface temperature was positively correlated with that of its partner in individuals incubating their natural clutch size on days 3-12 of incubation (figure 5.7), but there was no relationship between an individual's re-warming rate and that of its partner ($r_s = 0.46$, $P = 0.186$, $n = 10$ pairs).

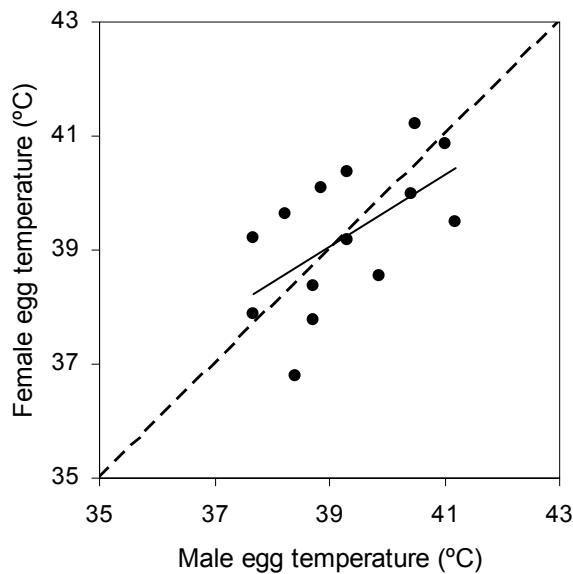


Figure 5.7 Male and female clutch surface temperatures (°C) were positively correlated ($r_p = 0.59$, $P = 0.027$, $n = 14$ pairs). Each point represents the mean egg surface temperature of each individual over incubation days 3-12.

Differences in rate of re-warming between days (probe egg)

Re-warming rate did not differ between days 6 (0.22 ± 0.02 °C min⁻¹, $n = 10$ pairs) and 8 (0.21 ± 0.02 °C min⁻¹, $n = 10$ pairs) of incubation in control pairs (β : -0.02 (-0.07 to 0.03), $P = 0.402$), but it did decrease with clutch size (β : -0.08 (-0.11 to -0.04), $P < 0.001$). Rate of re-warming was not related to the bird's ventral temperature (β : 0.02 (-0.01 to 0.06), $P = 0.196$) in control pairs ($n_p = 8$) on days 6 and 8 of incubation.

Sex differences in re-warming rate (probe egg)

Before the manipulation (day 6), eggs were not re-warmed more quickly by females (0.22 ± 0.02 °C min⁻¹, $n_i = 16$) than by males (0.21 ± 0.02 °C min⁻¹, $n_i = 15$; β : -0.02 (-0.12 to 0.08), $P = 0.646$). In treatment group birds on days 6 and 8 of incubation, experimentally enlarged clutches were not re-warmed more slowly after the manipulation (0.20 ± 0.01 °C min⁻¹, $n_p = 11$) than before (0.22 ± 0.03 °C min⁻¹, $n_p = 9$; β : -0.02 (-0.11 to 0.07), $P = 0.691$), and females did not heat the eggs more quickly (0.19 ± 0.01 °C min⁻¹, $n = 17$) than males (0.21 ± 0.02 °C min⁻¹, $n = 17$; β : 0.03 (-0.06 to 0.12), $P = 0.513$). Re-warming rate was not influenced by natural clutch size (β : 0.08 (-0.05 to 0.01), $P = 0.283$).

Comparison of probe and IRT measurements

IRT estimates of egg surface temperature were lower than the corresponding measurements of the core (IRT: 33.4 ± 0.27 °C; probe egg: 34.4 ± 0.22 °C, $t_{38} = 8.28$, $P < 0.001$), but the two sets of values were positively related and the slope of the relationship

did not differ from 1 (β : 1.13 (0.94 to 1.30), $P < 0.001$, $n_p = 18$, $n_i = 26$, $n_m = 39$, figure 5.8).

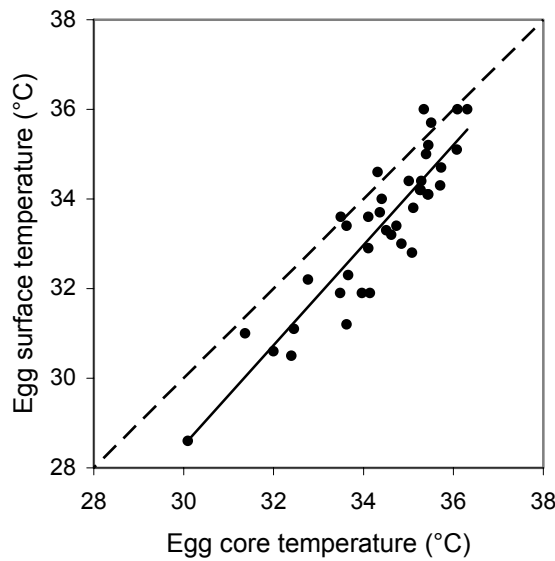


Figure 5.8 Comparing core temperature recorded by the probe egg with the surface temperature of the same egg recorded simultaneously using IRT. Egg temperature was higher at the core than the surface, but the two measures were positively related. The dashed line indicates where points would fall if egg surface and core temperature were equal.

Discussion

I examined sex differences in incubation ability in the Zebra Finch, using IRT to compare the ventral and clutch surface temperatures of incubating males and females and thermistors to compare the rate at which they re-warmed cool eggs. The female alone possesses a brood patch (Zann and Rossetto 1991; Zann 1996), so I predicted that she would have a warmer ventral surface, incubate the eggs to a higher temperature and re-warm them more quickly than the male. The female did have a warmer ventral surface than the male under standard incubation conditions, and the ventral surface was warmer than baseline body temperature in females but not males. There was no difference between the sexes in clutch surface temperature or rate of re-warming, but naturally larger clutches were cooler, took longer to heat up and were more variable in terms of within-clutch egg temperature. I carried out a clutch size enlargement to test whether individuals increase incubation effort under more challenging conditions, and whether the sexes differ in their response. Females responded to the manipulation by increasing ventral temperature, whereas males showed no change. The sexes did not differ in rate of re-warming or egg

surface temperature following the manipulation, but the variance in egg temperature increased with clutch size more steeply in males than in females. Experimentally enlarged clutches were cooler than control clutches, but were not re-warmed more slowly. The internal probe recorded higher temperatures than did IRT, and this is probably because an egg's surface loses heat more quickly than its core due to heat transfer and convection from the surface. However, core and surface measurements, obtained by the probe egg and IRT respectively, were closely and positively correlated. IRT is non-invasive, simple to use and gives precise, detailed measurements.

The ventral temperatures reported here underestimate actual ventral surface temperature because the plumage is likely to insulate heat loss. However, the aim was to compare ventral temperature between the sexes rather than to provide absolute values. The sex difference in ventral temperature was not a result of individual differences in plumage thickness, as this was controlled for statistically and there was no difference between males and females in ventral plumage thickness. The possibility remains that the higher ventral temperatures recorded in females were due to inferior ventral plumage quality or composition (e.g. fewer down feathers), although no such differences were noted. This could be tested by extracting the plumage and skin layer from culled specimens of males and females and placing them upon heat pads of known and constant temperature. Nevertheless, the variation in ventral plumage surface temperature recorded here most likely reflects variation in generated heat.

Sex differences in incubation ability under standard incubation conditions

The warmer ventral surface measured in the female is consistent with the observation that brood patch development is morphologically more advanced in the female Zebra Finch than in the male (Zann and Rossetto 1991; Zann 1996). The female also has the warmer ventral surface in the House Sparrow, *Passer domesticus*, another biparentally incubating species in which the male lacks a brood patch (Bartlett *et al.* 2005). However, under standard incubation conditions, I did not detect any differences between male and female Zebra Finches in incubation temperature or rate of re-warming. Although these findings are consistent with those of Zann and Rossetto (1991), they are surprising given the sex difference in brood patch development and ventral temperature. In addition to this, ventral surface temperature was not related to egg surface temperature or rate of re-warming.

Males have been reported to show similar incubation ability to their partners in a number of species in which the male lacks a brood patch (Skutch 1976). The male Chestnut-vented

Tit-babbler, *Parisoma subcaeruleum*, incubates at a higher temperature than the female, but rates of re-warming and nest attentiveness are equal between the sexes (Auer *et al.* 2007), and the male re-warms the clutch more rapidly but to a slightly lower temperature than his partner in a facultatively polygynous population of the Starling, *Sturnus vulgaris*, (Reid *et al.* 2002b). Maintaining a warmer ventral surface is likely to be costly in terms of increased heat loss (Haftorn and Reinertsen 1985), and so we would expect to find an associated fitness benefit, such as an increased ability to keep the eggs at conditions conducive to embryo development and survival. The brood patch may be less costly to maintain during the day in Australia's hot climate, but this is probably not the case at night when ambient temperatures fall.

However, there may also be costs associated with not having a brood patch. By being more poorly adapted for incubation, males without brood patches may have to work harder to reach the same ends (Auer *et al.* 2007), and Zann and Rossetto (1991) suggest that they could achieve this through increases in basal metabolic rate. Baseline (throat) measurements of males and females did not differ in the current study, which suggests that the sexes' basal metabolic rates are comparable. Nevertheless, future studies should measure basal metabolic rates of incubating males and females since a better understanding of sex differences in the energetic costs of incubation would allow us to make more detailed predictions of how optimum levels of investment might differ between the sexes. In addition to potential energetic costs males may also risk tissue damage without the protective thickening of the epidermal skin that is part of brood patch development (Jones 1971).

The effects of ventral surface temperature on egg temperature may be more difficult to detect in a system of biparental incubation. Measurements of egg surface temperature recorded in the current experiment were probably influenced by the insulative properties of the nest and the previous contribution of the partner, and this may explain the positive within-pair correlation in egg surface temperature. Since this correlation was based upon the mean of each individual's egg temperature over the incubation sequence, with each partner of a pair being measured on a different day, it suggests that, as a pair, they are capable of maintaining the eggs at a stable mean temperature throughout the incubation sequence. Re-warming cool eggs is energetically more expensive than maintaining them at a steady state (Vleck 1981), so biparental incubators can sidestep some of these costs by taking advantage of their partner's previous heat transfer (Voss *et al.* 2008). The mean ventral surface temperature for each pair may be a better predictor of egg temperature than

individual ventral temperatures. Since ventral surface temperatures were not correlated between partners, I can exclude the possibility of assortative mating for incubation ability. We would not expect to find a correlation between the re-warming rates of partners because cooled eggs were used and thus the partner's previous contribution was erased, and indeed no relationship was found. It would be interesting to see whether the correlation between the partners' incubation temperature still stands if cooled eggs are used.

Incubation attentiveness may have a greater effect than ventral temperature on the steady-state incubation temperature. The amount of time that eggs are covered and the number and duration of interruptions to incubation bouts are likely to influence egg temperature considerably, whereas the returns from increased ventral temperature may decline after a certain point. The observations of Zann and Rossetto (1991) offer some support to the suggestion that incubation attentiveness is an important determinant of steady-state temperature. They found that wild Zebra Finches incubated at higher temperatures at night than during the day, in spite of ambient temperatures falling to much lower levels at night. This is probably because night-time incubation consists of a single bout which is subject to fewer disturbances. However, the relationship between incubation attentiveness and egg temperature should be explored in more detail, and incubation temperature may also be modified by tightness of sit (White and Kinney 1974) and the frequency at which eggs are turned (Deeming 2002).

If the female is better adapted to provide care, we would expect her to incubate when the demands of incubation are greatest, such as at night (Bailey 1952; Zann and Rossetto 1991), and for the male to relieve her when she experiences greatest energetic stress, allowing her to forage and recoup her energy (Smith and Montgomerie 1992; Kleindorfer *et al.* 1995; Voss *et al.* 2008). Indeed, the female incubates at night in the Zebra Finch (Zann and Rossetto 1991; pers. obs.), in the Barn Swallow (Ball 1983) and in the Chestnut-vented Tit-babbler (Auer *et al.* 2007), and the brood patch is absent in the male of all these species.

Female response to the clutch size manipulation: ventral temperature

While control females showed high within-individual repeatability in ventral temperature when incubating their natural clutch sizes under constant conditions, treatment females responded to the challenge of incubating an enlarged clutch size by increasing brood patch temperature. This increase could have been a response to the number of eggs present in

the nest since larger clutches represent increased reproductive value or to the decrease in mean egg temperature for a given unit of incubation effort (energy per unit time) since heat is dissipated over a larger area in larger clutches. White and Kinney (1974) found that female Village Weaver-birds, *Ploceus cucullatus*, with anaesthetised brood patches incubated more intensively than control birds. This suggests that the brood patch is involved in sensory perception and that changes in egg temperature should also be detectable through the brood patch. The observation that brood patch temperature was not influenced by natural clutch size in the current experiment suggests that females were adjusting brood patch temperature in response to the change in egg temperature and that brood patch temperature is not influenced by the actual number of eggs present. Natural clutch size can however positively influence incubation attentiveness (Komdeur *et al.* 2002; chapter three), and a pair incubating a naturally larger clutch size may achieve higher incubation temperatures by keeping the eggs covered for longer.

The female brood patch appears to be able to respond fairly quickly to stimuli since the change in brood patch temperature was recorded approximately 14 hours after the clutch size manipulation took place. It would be interesting to see if female brood patch temperatures fall to their previous levels if enlarged clutches are returned to their natural size and also how females respond to clutch reduction.

Male response to the clutch size manipulation: ventral temperature

Treatment males, unlike females, did not respond to the clutch size manipulation by increasing their ventral temperature. The absence of a brood patch may affect their ability to detect changes in clutch size or egg temperature or their ability to adjust their ventral temperature appropriately. Alternatively, they may have responded to the clutch enlargement by increasing a different form of effort, such as incubation attentiveness. However, it may be maladaptive for males to work harder (even if they are capable of doing so) since they tend to be less certain of their relatedness to the offspring (Trivers 1972; Queller 1997) and can potentially obtain greater fitness benefits from investing effort into seeking extra-pair copulations (EPCs) rather than attending to the eggs or offspring, depending on the availability of receptive females and their own competitive ability (Bateman 1948; Magrath and Komdeur 2003; Kokko and Jennions 2008). Perhaps for this reason the more complex and costly morphological adaptations associated with the brood patch have not developed to the same extent (or have not been conserved) in many male passerines. Male incubation may serve to relieve the female in times of energetic stress (Smith and Montgomerie 1992; Kleindorfer *et al.* 1995) because there is a risk that the

embryos will suffer if the eggs are left unattended (Reid *et al.* 2002b; Gorman *et al.* 2005b) and to allow the female to escape the substantial costs of re-warming the clutch (Voss *et al.* 2008). In practice it may not be possible to determine whether males are unable or unwilling to adjust ventral temperature, and sex differences in ability and willingness are likely to have arisen as a consequence of the same evolutionary pressures.

Sex differences in incubation temperature under more challenging conditions

Although I found no differences between males and females in incubation temperature or rate of re-warming under standard incubation conditions, subtle differences were detected when individuals incubated an enlarged clutch size. There was a trend towards females incubating at a higher temperature following clutch enlargement but this was not significant. Nevertheless, the variance in egg surface temperature increased more rapidly as clutch size increased in males than in females. Males managed to keep variance at a low level in smaller clutches, but otherwise varied greatly in their ability to keep the whole clutch at a constant temperature. Females, by contrast, were able to maintain a more similar temperature across all eggs of the clutch, even at the upper limits of the natural range in clutch size. Experimentally-induced fluctuations in incubation temperature have been shown to retard embryonic growth rate (Olson *et al.* 2006) and lead to reduced embryo mass in the Zebra Finch (Olson *et al.* 2008). Since males and females did not differ significantly in mean egg surface temperature in the current study, it is possible that males concentrated their ventral heat on one specific part of the clutch, presumably the centre, whereas females distributed their ventral heat over the entire clutch. Analysis of surface temperatures of individual eggs in relation to their position in the clutch would clarify this issue: if this is the case, the central egg of each clutch should be warmer when incubated by the male than the female. Females may maintain low within-clutch temperature variance if the warm, defeathered area covers a greater area in the female than the male, or if they turn the eggs more frequently.

The effects of clutch size on incubation temperature

Mean egg temperature decreased with natural clutch size and was lower in experimentally enlarged clutches, while the variance in temperature followed the opposite trend. Larger clutch sizes are likely to be cooler since a given input of heat is dissipated over a greater area, and the upper limit on effective incubation is probably constrained by the size of the brood patch (Niizuma *et al.* 2005). Eggs within larger clutches may not receive sufficient contact with the ventral surface, and egg turning distributes that warmth over the entire clutch. Bortolotti and Wiebe (1993) found that smaller females of the American Kestrel,

Falco sparverius, had lower hatching success than larger females. They mention anecdotally that exposed eggs were commonly observed in clutches incubated by smaller females and attribute the difference in hatching success to difficulty in covering the clutch. Such a constraint is consistent with the finding in the present study that the variation in temperature within a clutch was greater in enlarged and naturally larger clutches. Reid *et al.* (2000a) carried out a similar clutch size manipulation in the Starling, *Sturnus vulgaris*. They found that the rate of re-warming was slower, temperature variance larger and hatching success lower in enlarged clutches, and attributed these differences to increased water loss as a result of the manipulation.

Control clutches in the present study were re-warmed more slowly as natural clutch size increased, but no trend was found in enlarged clutches. The probe egg technique measures the warming rate of a single egg in the clutch, and the rate at which that egg was warmed was expected to be influenced by the number of eggs present in the nest. Whether the probe egg can provide an accurate reflection of clutch re-warming rate probably depends how much it is turned relative to the other eggs and where it lies in the clutch. The probe egg was placed in the centre of the clutch before each observation took place, but the extent to which it moved during the observation probably differed between nests. Differences in egg temperature and re-warming rate are likely to be subtle, and it is perhaps only when the whole clutch is taken into account that these can be detected. The effects of clutch size (and possibly even sex) may be easier to detect if re-warming rates were available for the whole clutch. Although IRT cannot be used to measure re-warming rates, it can provide an accurate picture of the relationship between clutch size and temperature because measurements can be obtained for the whole clutch for a given time period. Compared to the probe, IRT may be better at picking up more subtle temperature differences since surfaces are likely to gain and lose heat more rapidly than an object's core.

Conclusions

Under standard incubation conditions, I found that the ventral surface was warmer in the female Zebra Finch than in the male, presumably due to the presence of the brood patch in the female alone. Females responded to clutch size enlargement by increasing ventral surface temperature, whereas males showed no change. It is likely that females increased their incubation effort in response to a decrease in mean egg temperature rather than to the actual number of eggs present since the natural clutch size did not influence ventral temperature. These findings suggest that females are better adapted than males to provide

care during incubation, presumably as a consequence of differences between the sexes in parental certainty and the availability of mates. In spite of females having a warmer ventral surface, no sex differences were detected in clutch surface temperature or rate of re-warming under standard incubation conditions. In experimentally enlarged clutch sizes, by contrast, the variance in egg temperature increased with clutch size more rapidly in males than in females. The temperatures at which individuals maintained the eggs were positively correlated within pairs, and this may reflect the biparental nature of incubation in the Zebra Finch.

Chapter 6 - General Discussion

Overview

How two parties with common yet conflicting interests should act is a question that is central to evolutionary ecology, and has implications that extend far beyond it into fields such as politics, philosophy and economics (e.g. Binmore 1991). In this thesis I explored this question in terms of sexual conflict between male and female parents over division of labour of shared (and also unrelated) young due to potential sex differences in the costs and benefits of parental care relative to other activities.

To investigate sexual conflict over division of parental care, I focussed upon a number of factors that might influence variation in male and female parental effort in a biparentally incubating bird, the Zebra Finch, *Taeniopygia guttata*. The factors I manipulated or considered include male mating opportunities, paternal certainty, male attractiveness, clutch size, experience and the partner's behaviour. Parental effort during incubation was measured in terms of time spent incubating (incubation attentiveness), the temperature of the parents' ventral surfaces, incubation temperature and the rate at which parents re-warm cooled eggs. I examined the fitness consequences of incubation attentiveness (the most widely used of these measures, in order to assess whether it is an appropriate proxy for parental investment, defined as any investment by the parents that enhances offspring survival at a cost to the parents' future fitness (Trivers 1972). Here I discuss my findings in the light of the field of sexual conflict and evaluate how well this work addressed the central aims. I then draw together a number of the themes that ran through the work and speculate about the implications of these findings and discuss the further research questions that these provoke.

How do individuals respond to each other's effort?

Most models predict that partial compensation is the best response to a reduction in a partner's investment under biparental care (e.g. Chase 1980; Maynard Smith 1982; Houston and Davies 1985; Winkler 1987; Kacelnik and Cuthill 1990; McNamara *et al.* 1999), although complete compensation can be predicted under certain circumstances (e.g. Jones *et al.* 2002). Johnstone and Hinde (2006) show that parents may even match the direction of their partners' response when they are less informed about the quality or need of the brood. Compensatory and matching responses can be distinguished by the direction of the relationship: the parental effort of the two partners will be negatively related where compensation occurs, while matching predicts a positive relationship.

In **chapters two** and **three**, I attempted to influence the variation in male parental effort by manipulating paternal certainty and extra-pair copulation behaviour, respectively, and then compared the relationship between male and female incubation effort. In **chapters two** and **three** I found that males whose paternal certainty or EPC opportunity had been manipulated in their first breeding attempt showed a reduction in incubation effort that carried over across to the next breeding attempt. There was a negative relationship between male and female incubation attentiveness in treatment pairs, but control pairs showed a more variable response. The negative, or compensatory, relationship between the parental effort of treatment males and females is consistent with the majority of previous studies that have investigated the response of one partner to the parental effort of another (Harrison *et al.* 2009).

In spite of the abundance of studies that have tested for compensation, there is little consistency between them in the way that compensation is measured and interpreted. This problem was recently highlighted by Harrison *et al.* (2009) and it strikes me as an area for further attention on two levels. First, parents may adjust their parental effort in ways that are not captured by the particular proxy of care measured, and thus it is important to choose a measure of parental effort that is appropriate to the particular stage of parental care and study system. For example, Wright and Cuthill (1990b) found that parents that had been induced to work harder were more likely to switch to a different feeding site and, perhaps as a consequence of this, they brought smaller prey items back to the nest. Most studies of parental effort measure provisioning rate and do not take into account the quality or quantity of food obtained (Harrison *et al.* 2009). For this reason, Harrison *et al.* (2009) suggest that incubation or brooding might be less ambiguous stages in which to test for compensation. I will discuss some of the advantages and disadvantages of testing for compensation or matching at the incubation stage at a later point.

Second, although it is easy to distinguish between compensation and matching based on the direction of the slope, it is less straightforward to determine at what point a response can be termed full or partial and a consensus needs to be reached on how partial and complete compensation (or matching) are distinguished. Most authors have used a ‘between-groups’ approach, comparing treatments and controls (e.g. Wright and Cuthill 1990b; Smith *et al.* 1995), while others have compared the same individuals before and after the manipulation (e.g. Schwagmeyer *et al.* 2002; Lendvai *et al.* 2009). The latter approach has the advantage that it takes into account within-individual variation, but it is necessary to make sure that parental effort does not change over time. Figure 6.1

illustrates the method I recommend for interpreting whether partners compensate for (or match) a *decline* in each others' effort and the extent to which they do this.

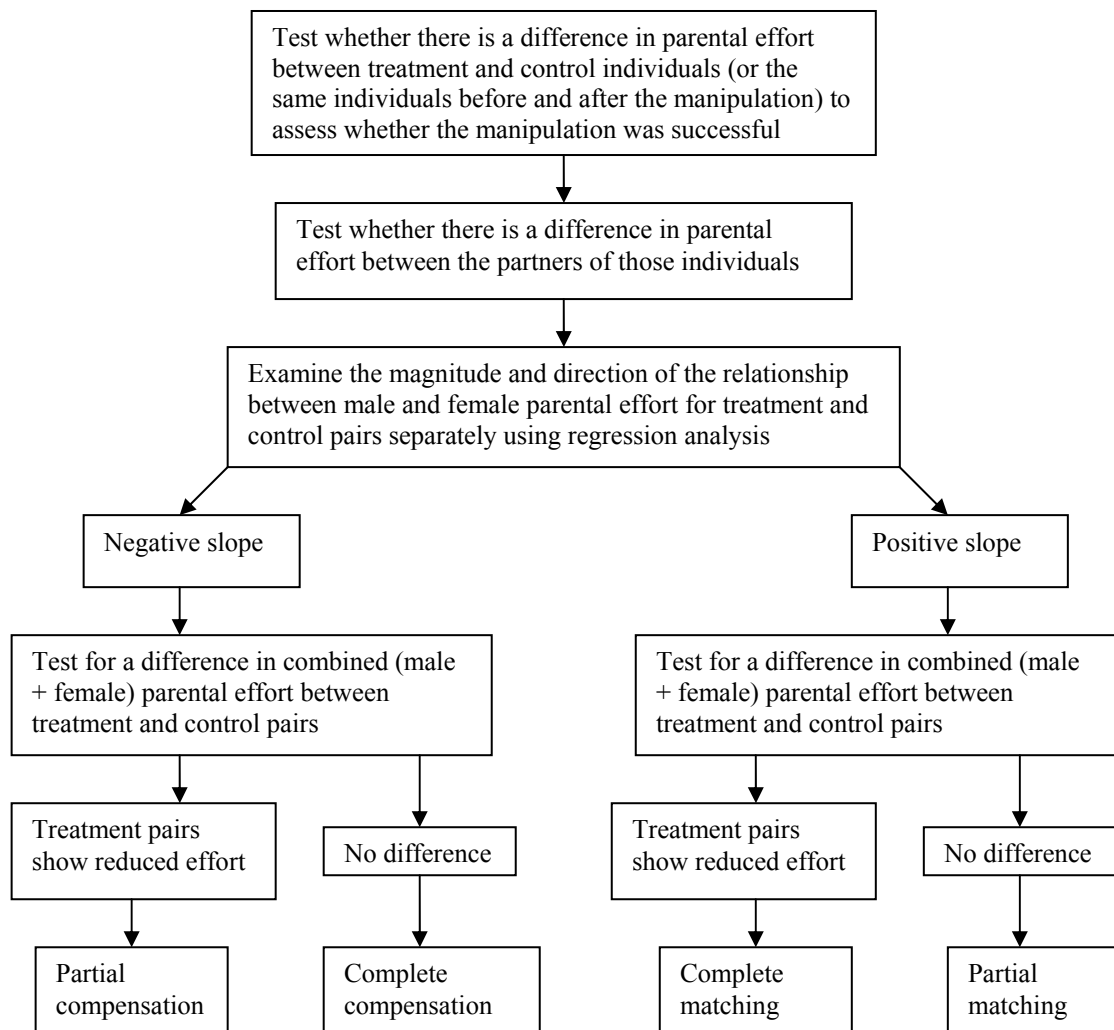


Figure 6.1 Flow chart to illustrate my recommended framework for determining whether partners compensate for (or match) a decline in each others' effort and to what extent. Regression analysis examines the magnitude and direction of the slope of the response of individuals against their partners.

However, full compensation in its strictest sense can only be said to occur when the offspring have not been detrimentally affected (Harrison *et al.* 2009), but most studies do not measure the effects of the manipulation on the offspring, and those that do cannot guarantee that some unmeasured quality has not been affected. Where parents compensate for a reduction in partner effort during incubation, it is feasible that they may be unable to provision the nestlings sufficiently since there can be a trade-off between investment into incubation and subsequent reproductive stages (Heaney and Monaghan 1996; Reid *et al.* 2002c). Therefore, I also recommend that, as in **chapter four**, the effects of the manipulation on offspring performance are measured where possible.

Who drives the negotiation process: the chicken or the egg?

Negotiation models predict that within a biparental system each partner influences the behaviour of the other to some extent (McNamara *et al.* 1999). Central to understanding how compensation and matching might operate over ecological time, but as yet rarely considered, is the question of which partner, if any, drives the negotiation process. Whilst observing incubation behaviour for **chapters two** and **three**, I noted that both sexes often gave the appearance through their interactions with each other of being keen to incubate, contrary to most theoretical predictions (Houston and Davies 1985; McNamara *et al.* 1999). Males in particular made a number of ‘visits’ to the nest during their partner’s bout, sometimes bringing nest material, but often ‘empty-handed’. Similar behaviour has been documented in the House Sparrow, *Passer domesticus*, and it has been postulated that such behaviours may play a role in negotiation (Schwagmeyer *et al.* 2008). In the Zebra Finch, the female often tried to evict the male from the nest by pushing and prodding him with her bill during these visits; this behaviour seemed to occur less frequently in the male. Individuals also attempted to roll the eggs from beneath the incubating partner with the bill, and this sometimes led to changeover. Females made much fewer visits to the nest, but when these occurred, they usually resulted in the male moving aside to let the female incubate. My impression is that a pair’s pattern of effort is directed by the female, but that males vary in their persistence.

Assuming that the negotiation process is driven by the female, it is worth noting that although manipulated males in **chapters two** and **three** appeared willing to incubate, they showed a reduction in incubation attentiveness relative to controls (in the first breeding attempt), as predicted. One possibility is that females gather, perhaps through differences in levels of male persistence, that manipulated males place less value on the current clutch and females are thus less likely to entrust them with incubating. An alternative explanation is that the male gives a display of ‘keenness’ to the female as a signal of his paternal quality. Females may be more likely to increase their own effort when paired with a good quality partner or they might opt to breed with him again. Males may attempt to cheat by feigning willingness, and females may assess the validity of the offer by rejecting the first few bids.

The response of an individual to its partner’s effort is conventionally examined by manipulating factors expected to bring about a decrease (and exceptionally an increase. e.g. Hinde 2006) in effort, and where an effect is found in the predicted direction, it is

assumed that the manipulated partner is the one that has driven the change. While this would seem to be the most parsimonious explanation, we should be aware that animals do not necessarily interpret the world as we do. Handicapping manipulations, for example, have been criticised on the grounds that they could influence the unmanipulated partner's perception of his or her partner's attractiveness (Schwagmeyer *et al.* 2002), and other types of manipulation can present similar difficulties. In **chapter two**, where I aimed to manipulate variation in paternal certainty by allowing females to interact with extra-pair males during their fertile periods, I identified a number of possibilities as to how the treatment (and even the control) could have been construed by experimental subjects. Researchers should try to consider different ways that animals might interpret aspects of their experimental design and judge whether there are any alternative explanations for the behaviours observed, giving particular consideration to how the manipulation might influence the partner.

How do females respond to an attractive partner?

Males vary in quality, and this can influence their contribution to parental care and the levels of primary and secondary effort invested by their partners (Burley 1981; Burley 1988; Sheldon 2000; Bolund *et al.* 2009). However, differences in attractiveness between males remain a source of unexplained variation in many studies of parental effort, and those that have investigated its effects have found two opposing patterns of investment: positive or negative differential allocation. A recent model by Harris and Uller (2009) has helped to bridge the gap in our understanding by exploring the conditions under which these two patterns can be predicted. In **chapters two, three and four**, I considered the effects of male attractiveness on primary and secondary parental effort. The proportion of time that female Zebra Finches spend associating with males during spatial association trials has been shown to reflect sexual preferences (Clayton 1990a; Clayton 1990b; Forstmeier 2007), and so I conducted a series of two-way mate choice trials and used the resulting scores as a measure of male attractiveness. Male attractiveness did not influence male or female incubation effort in **chapter two**, but females paired to more attractive males laid smaller clutches. However, in **chapter three**, male attractiveness did not influence clutch size but it did affect parental care: male incubation attentiveness was negatively related to female attentiveness when the female was paired with her less preferred partner, but there was no relationship when she was paired with her preferred partner. As male attractiveness and male incubation attentiveness decreased, hatching success increased (**chapter four**), and since total incubation attentiveness (or, strictly speaking, the amount of time that the eggs were covered) did not differ between treatment

groups nor was correlated with male attractiveness, the most likely explanation for this interaction is that females compensated for the low work-rate and low quality of their partners.

The Compensatory Investment Hypothesis (CIH) predicts that females should be more likely to compensate when they are constrained to breed with a male that they would not breed with otherwise (Gowaty *et al.* 2007; Gowaty 2008). The negative relationship between male and female incubation effort that was found only when females bred with their less preferred male is consistent with this idea. Harris and Uller (2009) show that compensatory investment is more likely when a female has a low likelihood of achieving future mating opportunities, and it is thus likely to be influenced by female age. The females used in **chapters three** and **four** were 316 ± 11.31 days old when they began to lay, and this is a relatively late age to make a first breeding attempt considering that wild and domesticated Zebra Finches can reach sexual maturity at two or three months of age (Zann 1994; Zann 1996). All relationships between male attractiveness and primary and secondary parental effort discussed so far support the CIH, but in **chapter two** reproductive compensation acted on clutch size, and in **chapter three** it influenced parental care, and this suggests that females may use different mechanisms of compensatory investment depending on the situation. Boland *et al.* (2009) found that females paired to less attractive males produced larger eggs and also deposited more testosterone and carotenoids into the yolks. They concluded that compensatory investment is more likely in a species such as the Zebra Finch that forms longterm pair bonds (Bolund *et al.* 2009).

Incubation attentiveness as a measure of incubation effort

The relative contributions of the partners to incubation have traditionally been measured as nest attentiveness, a general term which has been used to refer to nest occupancy (time spent in the nest. e.g. Smith and Montgomerie 1992; Smith *et al.* 1995; Komdeur *et al.* 2002; Bartlett *et al.* 2005; Kopisch *et al.* 2005; Schwagmeyer *et al.* 2005) or incubation attentiveness (time spent in contact with the eggs. e.g. Gorman and Nager 2003; Bouwman *et al.* 2005; Gorman *et al.* 2005a). The majority of previous studies have favoured nest occupancy as a measure of incubation effort since it is easier to quantify and does not require the use of infra-red nest cameras and video equipment, which may be particularly inconvenient in the field. For **chapters two** and **three**, I measured both nest occupancy and incubation attentiveness, and show that female incubation attentiveness was closely correlated with nest occupancy, but this was not true for males: there was no relationship

between attentiveness and occupancy on two of four days on which it was measured in **chapter two** and on three of four days in **chapter three**.

I chose incubation attentiveness rather than nest occupancy as my proxy for incubation effort since this reflects the component of incubation behaviour that is costly in terms of both time and energy. However, nest occupancy does serve as a good indicator of the temporal input of parents since the range of activities an individual can perform within the nestbox is limited (e.g. a male cannot seek EPCs or forage). Nest maintenance, which can be a component of nest occupancy but not incubation attentiveness, is arguably a form of parental care, and poorly insulated nests are associated with a longer incubation period and retarded nestling growth rates (Winkler 1993; Lombardo *et al.* 1995). However, studies using nest occupancy as a measure of incubation effort may underestimate the energetic component of investment since more energy is used when a bird is incubating compared to sitting still in a nestbox (Vleck 1981). No information is available on the energetics of nest maintenance. A better understanding of the relationship between nest occupancy and incubation attentiveness will allow researchers to make more informed decisions when designing and interpreting experiments, and here I speculate on the reasons for the sex difference in the relationship between incubation attentiveness and nest occupancy with reference to **chapter three**, where the sex difference was more pronounced.

Females spent 92.5 ± 1.37 % of nest occupancy time incubating, while males incubated for just 49.1 ± 4.70 % of the time they were in the nestbox ($n = 17$ pairs). The proportion of time that individuals occupied the nestbox but did not incubate is substantial for both sexes, but this is especially true in males. Males and females participated in a number of activities in the nestbox aside from incubation, and these included clumping with the incubating partner, nest maintenance (adjustments to the fabric of the nest) and nest ‘visits’ (where an individual enters and exits the nestbox without relieving its partner, as defined by Schwagmeyer *et al.* (2008). Male nest maintenance made up 22.3 % of nest occupancy in 15 nests from the first breeding attempt (6.6 ± 1.16 % of observation time). Mate guarding is not likely to contribute to nest occupancy since the fertile period of the female ends around 24 hours before the last egg is laid (Birkhead *et al.* 1989). However, the male may clump with his partner while she is incubating in order to strengthen the pair bond or to preserve body heat. Long nest occupancy bouts can reduce the chances of attracting predators to the nest (Conway and Martin 2000), and predation risk might be greater among males in sexually dimorphic species. However, it could also be argued that incubating females are more vulnerable to predation since the reduction of protein content

in flight muscles associated with recent egg production can negatively affect flight take-off speed (Veasey *et al.* 2001). An alternative explanation is that a proportion of the time that individuals spend co-occupying the nestbox is used in behavioural negotiation. Visit rate was found to be positively associated with the lengths of the partner's incubation bout and the visitor's off-bout in the House Sparrow, *Passer domesticus* (Schwagmeyer *et al.* 2008) and this played a large role in explaining patterns of female compensation. Further work should investigate the relationship between incubation attentiveness and nest occupancy under natural conditions to determine whether the observation reported here is an artefact of the laboratory environment or an aspect of behavioural negotiation.

Shared incubation

One problem I encountered during this project is how 'shared incubation', when both partners incubate a part of the clutch simultaneously, should be interpreted. Shared incubation contributed to approximately 10 % of an individual's incubation attentiveness (see **chapters two** and **three** for actual values). I think that the way that shared incubation should be interpreted depends on the question being asked. When exploring the dynamics of the relationship between male and female, I included shared incubation in calculations of individual (male and female) time budgets because any time that is invested by either party must be traded-off against investment into other activities (Reid *et al.* 2002a). I think the same logic applies when comparing incubation attentiveness between treatment and control groups in order to assess the level of compensation. However, in **chapter four**, where I was concerned with the amount of time over which heat was transferred to the developing embryo rather than sexual conflict, I calculated total incubation attentiveness as $((\text{male incubation attentiveness} + \text{female incubation attentiveness}) / 0.5 \text{ shared incubation})$. This assumes that shared and independent incubation are equally valuable to the offspring, which may not be the case, and this issue needs further attention. It is unclear how common a phenomenon shared incubation is in other studies, as many authors sidestep the issue by expressing individual incubation attentiveness as a ratio of the incubation attentiveness of one sex to that of the other (i.e. $(\text{female attentiveness} / (\text{male} + \text{female attentiveness}))$ (e.g. Gorman *et al.* 2005a) rather than as a proportion of the total time available to each partner, as recommended by Wright (1998). It is possible that shared incubation is a more common occurrence in captivity, where the costs of incubation and of foraging might be lower than in the wild.

Incubation temperature as a measure of parental effort

Egg surface temperature was not influenced by ventral plumage surface temperature, although both measures were repeatable between days. Egg temperature is likely to reflect incubation attentiveness (Zann and Rossetto 1991), the tightness of sit (White and Kinney 1974) and the insulative qualities of the nest (Ar and Sidis 2002). Nest construction and maintenance is arguably a form of parental care and well insulated nests are associated with shorter incubation durations, larger nestlings and greater fledging success in the Tree Swallow, *Tachycineta bicolor* (Lombardo *et al.* 1995). Nest construction has been investigated as a potential indicator of a male's parental quality (Hoi *et al.* 2003; Szentirmai *et al.* 2005) but few studies have investigated sexual conflict over nest construction or maintenance (but see Wiebe 2008). In some species, egg temperature is also influenced by the sex of the incubator (Kleindorfer *et al.* 1995; Reid *et al.* 2002b; Voss *et al.* 2008). In **chapter five**, I found that female Zebra Finches have warmer ventral surfaces than males, and females increased their ventral temperature following clutch enlargement. The variance in egg temperature increased with clutch size more rapidly in males than in females, but no sex differences were detected in clutch surface temperature or rate of re-warming. These subtle differences in incubation ability between the sexes raise the possibility that incubation attentiveness alone may not capture everything that is essential to the offspring in terms of providing the optimal conditions for growth and survival. Olson *et al.* (2008), for example, found that higher egg temperature variability can have negative consequences on bird embryo mass. Indeed, in **chapter four**, I found that hatching success was not influenced by the total amount of time that the eggs were covered by either parent, but by interactions between male or female incubation attentiveness and the father's attractiveness. Further work needs to be done on the differences between males and females in incubation quality. As touched upon earlier, the tightness of sit may influence the quality of incubation, as might the frequency at which the eggs are turned (Deeming 2002) or the number of times the incubating bird re-settles on the eggs or changes its orientation. While collecting egg temperature traces using a temperature probe inside a dummy egg for **chapter five**, I observed that the male tended to incubate more erratically than the female. As I was interested in incubation ability for the purpose of that chapter, I truncated the data to remove such interruptions to incubation. However, this may lead to a difference in incubation temperature as a possible consequence of a lower willingness to incubate the eggs irrespective of any difference in incubation ability.

The sexes may also differ in the amount of information they possess on the temperature of the eggs due to differences in the morphology of the skin that is in contact with the eggs during incubation. The brood patch is involved in sensory perception in many species (White & Kinney, 1974) and it is more developed in the female Zebra Finch (Zann, 1996), so we might expect her to be better equipped to detect changes in the thermal requirements of the eggs. This may mean that she is better informed in terms of the need of the brood or her partner's previous incubation effort. In addition to this the Information Hypothesis predicts that the direction of partners' responses to each other is influenced by the amount of information each parent has at its disposal (Johnstone and Hinde 2006). White and Kinney (1974) found that female Village Weaver-birds, *Ploceus cucullatus*, with anaesthetised brood patches incubated more intensively than control birds, but few other studies have investigated the ability of the birds to vary their incubation temperature. My finding that female Zebra Finches increased their ventral temperature after clutch size enlargement but males did not and that females showed less variability than males in within-clutch incubation temperature suggests that this may be the case. Incubation temperature might be a promising measure of parental effort in which to look for a matching response if individuals are able to vary their incubation temperature, whether directly through changes in ventral surface temperature or indirectly through changes in tightness of sit, and if more information on egg temperature is available to the female than to the male.

So far no studies have tested for compensation or matching in terms of egg temperature. This could potentially be done by manipulating the temperature upon changeover to give one individual the impression that its partner has invested less (or more) than it actually did. I helped to design a piece of equipment called the Egg Thermal Cycler, which can warm up or cool down an artificial 'clutch' of aluminium eggs. However, I discovered during a series of trials that I was unable to manipulate temperature upon changeover rapidly enough and that the birds were reluctant to incubate an artificial clutch that was fixed into place. Another problem with this idea is that it is difficult to know what animals perceive and whether a given response is due to a lack of perception, an inability to respond or an unwillingness to respond. In addition to this, manipulating the temperature upon changeover also changes the costs of re-warming the eggs for the partner who takes over a cooled clutch (Vleck 1981), and this may influence the results observed.

Measuring incubation temperature using IRT and the probe egg method

In **chapter five**, I compare egg surface and core measurements taken using IRT (infra-red thermography) and the conventional method of inserting a temperature probe into a dummy egg connected to a data logger, respectively, and show that measurements taken using these two methods are highly correlated. Here I take the opportunity to discuss the advantages and disadvantages of the two methods.

The IR radiation detected by the camera must be converted to an accurate estimate of surface temperature (McCafferty 2007), which involves taking into account image latency, distance to the subject, surface emissivity, ambient temperature and relative humidity. Image latency can be modelled from rate of cooling, and the other parameters can be easily measured (or taken from the literature, in the case of emissivity). IRT is a less invasive means of recording egg temperature than the internal probe since readings can be taken from the bird's natural clutch. The probe method requires the clutch to either be supplemented with a probe egg, which can increase the costs of incubation and affect the thermal properties of the clutch (Reid *et al.* 2000a; this study) or, since the probe egg is non-viable, at least part of the clutch has to be destroyed, which means that data on offspring survival and performance are lost. The nest has to be disturbed prior to data collection in order to place the probe, and again following data collection to retrieve the probe and loggers, and the trailing wire that connects the probe to the data logger can sometimes restrict movement of the probe egg, particularly when it is small. It is now possible to obtain wireless probes (ibuttons), but these are still too big for small passerines such as the Zebra Finch. The probe method may be more appropriate for longer term studies where it can be placed in the nest at the beginning of the season and retrieved at the end, compared to IRT where the nest has to be disturbed each time a reading is required. However, data loggers can malfunction (e.g. Voss *et al.* 2008) or be removed by the incubating bird. IRT may be more reliable since the user can check that the image has been saved to the camera immediately after it has been taken. A further advantage of IRT is its ability to measure spatial variation within a single image, providing a simultaneous measurement of the entire clutch (and surrounding nesting material), from which the relative positions of the eggs are known. This is not the case with the probe method since the eggs are turned by the incubating parent while measurements are being recorded. IRT is quick and simple to use, but the imaging camera and software are expensive. The probe egg technique has the advantages of providing continuous information on rate of warming as well as asymptotic incubation temperature rather than a snapshot of egg temperature at a particular moment. However, rate of re-warming cannot be modelled for species with

short or erratic incubation bouts since the regression line will not provide a good fit to the data. In comparing the incubation abilities of male and female Zebra Finches in captivity, I found IRT to be a more suitable method since it provided repeatable, precise data that could be collected quickly, and the main disadvantage was that it cannot be used to calculate the rate of warming. Both methods have a number of advantages and disadvantages, and the choice of method should depend on the particular research question.

Conclusions

I showed that variation in male and female incubation effort in a biparentally incubating passerine, the Zebra Finch, is influenced by paternal certainty, a male's EPC opportunities, male attractiveness, clutch size and previous experience. Partners responded to each other over ecological time, as predicted by negotiation models (McNamara *et al.* 1999), but the outcome of negotiation during previous reproductive attempts may influence current reproductive decisions. Variation in parental effort can have fitness consequences on the offspring. I found that the number of offspring that survived to maturity was positively related to parents' incubation attentiveness, and that the number of eggs laid by a female was negatively related to her previous incubation effort in pairs that had fledged a large brood. These findings suggest that incubation attentiveness is an appropriate measure of parental investment, as defined by Trivers (1972). However, further attention is required to establish how differences in incubation quality, as well as attentiveness, influence offspring performance. Differences in incubation quality may arise from differences between the sexes in the morphology and temperature of the surface of the skin that is in contact with the eggs, and these can lead to subtle differences in incubation ability.

This work focussed on sexual conflict over parental care in the Zebra Finch, but the implications of this study are broad. The evolutionary pressures that shape conflict over parental care discussed in this thesis act in a similar way upon sex differences in the costs and benefits of mating behaviour, and this can lead to pre-zygotic sexual conflict over, for example, the timing of oviposition (Barbosa 2009) or duration of copulations (Mazzi *et al.* 2009). Likewise, evolutionary arms races occur between parents and offspring (Trivers 1974), hosts and parasites (e.g. Kilner *et al.* 1999) and predators and prey (e.g. Gallet *et al.* 2009).

Appendix

Modelling clutch temperature at the time of displacement

I modelled cooling rate for each clutch size (the number of eggs present, as opposed to the natural clutch size) using parameter estimates from Appendix table 1 and extrapolated clutch temperature at the time that the bird was displaced from the nest.

Appendix table 1 General linear mixed model showing the effects on the surface temperature of individual eggs from 21 nests. Clutch-, individual- and nest-identity were fitted as nested random factors (see statistical analysis, chapter five).

	β	<i>HPD interval</i>	
<i>Fixed effects</i>			<i>P</i>
Intercept	41.6	38.9 to 44.2	< 0.001
Image latency (\log_{10})	-5.2	-6.9 to -3.4	< 0.001
Clutch size	-0.5	-1.0 to -0.1	0.019
Clutch size x image latency (\log_{10})	0.3	-6.9 to -3.4	0.014
<i>Random effects</i>			<i>Variance (%)</i>
Clutch identity	0.4	0.3 to 0.5	15.0
Individual identity	0.2	0 to 0.5	1.6
Nest identity	0.5	0.3 to 0.8	12.4
Residual	1.4	1.3 to 1.5	71.0

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