Causes and consequences of individual variation in the plasticity of incubation and embryonic heart rate in the cooperatively breeding chestnut-crowned babbler (*Pomatostomus ruficeps*)

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

UNIVERSITY OF EXETER

THESIS ABSTRACT

Plasticity is a mechanism by which organisms can alter their phenotype to match their current environment. Selection for behavioural (i.e. reversible) plasticity is expected when organisms experience costly environmental variation that can be mitigated by a phenotypic response in behavioural time. However, plasticity is costly and thus, should generally be selected for when the environment is predictably variable in time or space. It is well known that species differ in the presence and strength of plasticity in different traits, as different species (and their traits) are often under different selection pressures. However, not as much is known about how individuals within populations vary in their plasticity, which has important implications for understanding population-level evolutionary responses. Specifically, behavioural plasticity may be a key adaption for traits related to parental care and resulting offspring developmental trajectories. In this thesis, I investigate the causes and consequences of variation in plasticity in one component of parental care, incubation, and one component of offspring development, heart rate, in the chestnut-crowned babbler, a cooperatively breeding bird native to inland south-eastern Australia. In chapter 2, I analyzed the incubation behaviour of wild babbler mothers in order to understand whether they are variably plastic in their incubation schedules, and if so, what may drive that variation. I used temperature data from gauges placed in wild nests to determine incubation schedules. I found that incubation was indeed plastic in response to abiotic factors, namely temperature and wind speed, and increased with increased helper number. I found significant individual variation in the plasticity of incubation within our population and found that helper number may be a determinant of female responses to temperature variation. In chapter 3, I investigated whether individual embryos differ in the plasticity of their heart rate in response to temperature. As helper number influences developmental environment by affecting incubation, I predicted that helper number would correlate with embryonic plasticity in heart rate. I found that embryos from groups with more helpers were more plastic in their heart rates in response to temperature than those from groups with fewer helpers. This reduced plasticity in embryos from groups with fewer helpers is likely to be adaptive: embryos from groups with fewer helpers are prone to experiencing lower temperatures more frequently, which,

all else being equal, will prolong the pre-hatching period. Together, my studies show that plasticity of multiple traits varies amongst individuals in our population and that such variation could potentially be adaptive. These results help to explain significant variation in both incubation schedules and offspring developmental rates, with important evolutionary and ecological implications. For example, assuming a heritable genetic component to plasticity, this population of babblers appear capable of responding in real time to environmental changes with positive repercussions for population resilience in their arid habitat.

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Chapter 1: THESIS INTRODUCTION

The importance of plasticity

Plasticity is the ability of a single genotype to produce multiple phenotypes in response to a change in the environment (Waddington 1942; West-Eberhard 2003; Pigliucci, Murren & Schlichting 2006). Organisms experience environmental variation during their lifetime, which can cause mismatches between their phenotype and their environment. Since this variation happens within their lifetime, they cannot rely solely on genetic adaptation to match their phenotype to their environment and thus, plasticity can be a useful adaption (Chevin et al 2010). For example, neotropical tadpoles (*Rana palmipes)* adaptively darken their pigmentation, increase their tail muscle and size, and increase their developmental speed when they sense predators (McIntyre, Baldwin & Flecker 2004). Additionally, genetic adaptation may not be beneficial for a generation's offspring if the offspring's environment does not match their parents' environment. Plasticity of trait values can evolve for the same reason that mean trait values evolve: genes underpin plasticity and there is genetic variation in a given population (Mather 1953; Nussey et al. 2005). As a consequence, species vary in the traits showing plasticity and the magnitude of any plastic capacity, suggesting species-specific differences in the costs and benefits of plasticity. For example, species that experience little environmental variation are not expected to pay the costs of plasticity (neurological machinery to sample and respond to the environment) (Chevin et al 2010; Gomez-Mestre & Jovani 2013). Further, plasticity may not always be adaptive, rather, it may be maladaptive. For example, Langerhans and DeWitt found that freshwater snails *(Physella virgata)* plastically reduced growth and changed their shell shape in the presence of non-molluscivorous sunfish, which reduces their fecundity and ability to avoid their predators (Langerhans & DeWitt 2002).

As organisms gain fitness by surviving and successfully reproducing, plasticity is expected to evolve in those traits that increase each of these fundamental fitness parameters in a given environment (Pryzbylo, Sheldon & Merilä 2000). For example, male burying beetles (*Nicrophorus vespilloides*) benefit more than females from decreasing

their parental care in the presence of a partner and thus, are more plastic in their parental care in response to their social environment (Rauter & Moore 2004). However, individual variation in plasticity within populations is not well understood, despite it being hypothesized to have major evolutionary and ecological implications (West-Eberhard 2003; Sultan 2007; Nussey, Wilson & Brommer 2007; Pigliucci, Murren & Schlichting 2006). In order to understand the evolution of plasticity, we need to quantify: (1) individual variation in plasticity; (2) the environments associated with plastic responses; and (3) whether such responses are likely to be adaptive (Sultan 2007; Alonzo 2015).

Plasticity of parental care

A key suite of behaviours likely to be under strong selection for plasticity are those pertaining to parental care (Royle, Russell & Wilson 2014). Parents increase their reproductive success by ensuring that their offspring survive to adulthood and the offspring's developmental environment in turn plays an important role in shaping their survival, quality and phenotype (Arendt 1997; Case 1978; Billerbeck et al. 2001; Brommer 2003; Shine & Olsson 2003). Parental care serves to increase the mean level of support offspring gain during development, as well as to reduce the environmental variation that they experience during development by increasing the mean favorable environment experienced. For example, chimpanzee mothers (*Pan troglodytes*) gestate, suckle and then share food with their offspring to both increase overall levels of nourishment and maintain consistent levels before nutritional independence (McGrew 1975; Nishida & Turner 1996).

Since parental care can reduce the harmful environmental variation otherwise experienced by offspring, even in uni-parental care systems, parental contributions are likely under strong selection for plasticity. For example, a 32-year study on wild great tits (*Parus major*) found evidence for strong positive selection on the plasticity of lay date, which correlates with climate change, i.e. environmental variability (Nussey et al. 2005). Avian parents are also known to be plastic in other expressions of parental care: for example, common blackbirds (*Turdus merula*) decreased nest insulation (by decreasing dry grass mass) with increased ambient temperature (Mainwaring et al. 2014). Further,

species in which care is provided by more than one individual, e.g. bi-parental and cooperative care systems, variation in the social environment is likely to provide an additional selection pressure on plasticity, because the contributions of other helpers will itself be variable (Royle, Russell & Wilson 2014). Thus, helper number may drive among-individual variation in optimal plastic responses, affecting phenotypic variation in plastic capacities within populations (Nussey et al. 2005; Snell-Rood 2013). Despite that the ecological and evolutionary implications of understanding among-individual variation in plasticity and mediating drivers of that variation are poorly documented, there is evidence that variation in plasticity could be a key adaptation for a wide variety of species; particularly in the field of parental care (Royle, Russell & Wilson 2014).

Developmental consequences of parental care

Patterns of parental care have profound impacts on offspring, which are reliant on parental contributions following their production (Lindstrom 1999; Metcalfe & Monagahan 2001; Lummaa & Clutton-Brock 2002). Although parents will be under selection to buffer offspring against abiotic and biotic variation, antagonistic selection on parental survival will lead to variation in the degree to which parents are able to buffer offspring from such variation. As a consequence, offspring will experience variation in abiotic and biotic parameters. Offspring that develop outside of their mothers will be particularly vulnerable to this variation early in development (Huggins 1941; Shilov 1968; Drent 1975; Webb 1988). In many egg-laying species, the thermal environment is the most important constraint on embryonic development as embryos need to maintain a specific range of temperatures in order to survive and develop (White & Kinney 1974; Webb 1988).

Avian parents can control their offspring's developmental environment behaviourally by laying their eggs in a thermally protected location and/or by incubating the eggs until they hatch (White & Kinney 1974; Walsberg & King 1978). However, intermittently incubating species, which routinely leave the nest during embryonic development, will, by definition, experience frequent thermal variation during the embryonic stage (Boersma 1982; Martin et al. 2008). This variation can be detrimental to offspring, for example, it has been shown that periodic cooling of zebra finch (*Taeniopygia guttata*) eggs increased the metabolic

and growth costs of embryos (Olson, Vleck & Vleck 2006). Thus, parents (particularly in uni-parental incubation systems) must balance these cooling costs to offspring with their own foraging needs and predation risk avoidance. Whilst parents are expected to mitigate these costs by leaving the nest for longer when conditions are more favourable (McClintock, Hepp & Kennamer 2014), embryos could themselves react plastically to their parents' incubation schedules by adjusting their developmental rate in response to temperature (Du et al. 2010a). It is known that offspring growth trajectories vary as a function of food acquisition during development (Phillips et al. 1994; Metcalfe & Monaghan 2001). Additionally, it is also known that development is sensitive to temperature in oviparous species. But what is not quantified is whether or not offspring respond differently to the same variation in the environment - in other words whether there is among-individual variation in plasticity. Again, quantifying this variation and understanding its drivers is central to projecting ecological and evolutionary responses to changing environments.

The model system and specific aims

In this thesis, I investigate variation in maternal and embryonic responses to changing abiotic factors (primarily temperature) during incubation and their mediating biotic factors (primarily helper number) using the 50 g, cooperatively breeding, chestnut-crowned babbler (*Pomatostomus ruficeps*) of inland south-eastern Australia (Higgins and Peter 2002). Being an arid zone cooperative breeder, mothers and offspring experience considerable variation in both abiotic and biotic conditions. The breeding female is the sole incubator of the eggs, a period that on average lasts 20 days (Russell et al. 2010; Young et al. 2013). All breeding females experience considerable diurnal and seasonal variation in temperature and wind speed (Russell 2016; Capp et al. 2017). During the day, temperature can vary from a low of 0-10 °C to a high of 20-40 °C, while wind speed, which has exacerbating effects on egg cooling rates can vary from 0-50 km/h (Russell 2016; Capp et al. 2017). Further, in this system, females vary in the support gained from other group members during the breeding season, with the number of helpers varying from one (their partner) to 14 (including non-breeding helpers). Additional helpers have two important consequences of relevance for breeding females: (1) they feed the breeding

female and so potentially reduce the extent to which she needs to leave the eggs to satisfy her own nutritional needs; and (2) for each additional helper, females reduce their subsequent offspring provisioning rate by ca. 10%, allowing females to recoup any additional costs of increased investment during incubation (Browning et al. 2012). Both should lead incubating females to be more attentive in large groups. As such, females and their offspring not only experience predictable abiotic variation, but the impact of such variation is likely to be modified by the current workforce (i.e. helper number).

This thesis aims to investigate the causes and consequences of individual variation in the plasticity of a single component of parental care, incubation, and a consequential metric of embryonic development, heart rate. First, I investigated variation in the plasticity of incubation behaviour in response to the ambient environment by measuring incubation behaviour of wild chestnut-crowned babblers. I tested which factors affected incubation behaviour, whether there was individual variation in incubation plasticity amongst mothers and if so, whether I could explain this plasticity. Due to helper number effects on the ability of mothers to invest in offspring, namely by feeding females during incubation and lightening her provisioning load upon her clutch hatching, I hypothesized that helper number would predict variation in incubation plasticity. Secondly, I investigated the effect of temperature on a key indicator of embryonic development, heart rate. I previously predicted that helper number would be a key influence on incubation behaviour. Thus, helper number is expected to be a predictor of embryonic thermal environment. In light of these predictions, I hypothesized that variation in embryonic heart rate plasticity in response to temperature would be present in the population and relate to helper number.

CHAPTER 2

Positive helper effects on incubation and plasticity

2.1 ABSTRACT

Intermittent incubators, which sporadically leave the nest during the incubation period to forage, must balance their own energetic needs with those of their developing offspring. The energetic constraints of incubation and offspring development are modified by ambient thermal conditions. Thus, it likely benefits incubators to be plastic in their incubation: to adjust their incubation behaviour in response to environmental conditions. Furthermore, the cost-benefit trade-off between incubator and offspring energetic needs may vary within a population due to the social environment (e.g. partner quality or helper number), which may modify the scope for plastic responses in incubation schedules as a function of climatic conditions. In order to investigate whether there is predictable variation in incubation plasticity within a population, I studied the incubation behaviour of the cooperative chestnut-crowned babbler using temperature gauges placed within wild clutches throughout the incubation period. I found that females increased both their on- and off- bout durations with increased ambient temperature and wind and that individual females varied in the breadth of these responses. Further, I found that females with more helpers increased both their mean incubation levels and to some extent, their temperature-dependent incubation response and that this was not confounded by helper effects on clutch warming and cooling rates. These results suggest that females capitalize on increasing numbers of helpers to increase their contribution to incubation, rather than saving resources for themselves.

2.2 INTRODUCTION

Parental care has evolved as a way for parents to increase their fitness by increasing the survivability and quality of their offspring (Clutton-Brock 1991). An offspring's developmental environment can have a range of fitness consequences on their survivability, quality and eventually, fitness (Lindstrom 1999). Thus, controlling the environment of one's offspring may be a strong selective force for populations that experience high amounts of environmental variation during development. However, parental care can be costly to parents by reducing parental survivability and future reproductive success: for example, reproductive success in smallmouth bass (*Micropterus dolomieui*) is positively tied to parental mass loss during the breeding period, which can increase parental mortality (Wiegman & Baylis 1995; Gillooly & Baylis 1999). Since parental care can be a costly investment and these costs vary with environmental conditions, I expect that capitalizing on favourable conditions is under strong selection.

One way in which parents can capitalize on favourable conditions is to respond plastically to variation in environmental conditions. Plasticity is a mechanism by which organisms can respond adaptively to environmental change within the course of a day, breeding event or lifetime, and can be visualized using a reaction norm approach (Schlichting & Pigliucci 1998). The reaction norm is a simple graphical visualization of the phenotypes expressed (on the y axis) as a function of the environmental gradient (on the x axis). The absolute slope value of the resulting line can be thought of as a quantification of the level of plasticity (i.e. the steeper the line(s), the more plastic the genotype/individual/population). For example, the seed beetle (*Stator limbatus*) adaptively reduces the size of its eggs when laid on host plants that are easily consumable by both small and large offspring (Fox, Thakar & Mousseau 1996). Further, orange-crowned warbler (*Vermivore celata*) parents adaptively adjust their nest placement and provisioning rate (y axis) in response to perceived predation risk (x axis) (Peluc et al. 2008). However, because behavioural plasticity is expected to be costly due to neurological and sampling costs (DeWitt, Sih & Wilson 1998; Snell-Rood 2013), populations are not expected to be plastic in all traits and in response to all environmental

variation experienced. Further, different individuals within a population may experience different environmental conditions that affect the cost-benefit ratio of expressing plasticity in a given trait. To understand the ecology and evolution of plasticity, we need an improved understanding of both the degree of plasticity in fitness defining traits, as well as the relative impact of candidate ecological drivers of such plasticity (Royle, Russell & Wilson 2014).

Selection on parents to influence the environmental variation experienced by offspring during the embryonic stage is strong in many oviparous species (Weisrock and Janzen 1999). Birds have taken this to an extreme through the evolution of incubation, with the majority transferring heat directly to their offspring via contact incubation. However, in some such species, particularly some small passerines, incubation is performed by females only, which must routinely leave the nest during incubation to feed (parental departures from the nest are deemed “off-bouts” and times that parents are on the nest are deemed “on-bouts”). In such cases, there exists an obvious trade-off between parental investment into offspring development and self-maintenance (Visser & Lessells 2001). Further, since ambient conditions are expected to influence the optimal resolution of this trade-off, female-only incubators might, in particular, show plasticity in incubation behaviour in response to ambient thermal conditions (e.g. McClintock, Hepp & Kennamer 2014). Population-level plasticity of incubation in response to, for example, ambient temperatures (Conway & Martin 2000), food availability (Zanette, Doyle & Tremont 2000) and partner provisioning rates (Chalfoun & Martin 2007) are well documented. For example, horned larks (*Eremophila alpestris*) plastically increase attentiveness with increasing temperatures at low temperatures and decrease attentiveness with increasing temperature at high temperatures (Camfield & Martin 2009). However, we know almost nothing about individual variation in plastic responses to environmental variation, or candidate drivers of that variation. This is a significant short-coming, as this phenotypic variation provides the raw ingredients for evolution of plasticity through natural selection (Royle, Russell & Wilson 2014).

Here I use the cooperatively breeding chestnut-crowned babbler (*Pomatostomus ruficeps*) as a model to investigate population- and individual-level plasticity during incubation in response to ambient climatic conditions (temperature and wind speed) and the social environment (helper number). This 50g bird, endemic to inland south-eastern Australia, lays clutches of 2 - 6 eggs (mean = ca. 4) in dome-shaped nests. It is an appropriate model for three main reasons. First, the breeding female within a given group is the sole incubator (Capp et al. 2017). Second, they typically breed between late winter and early summer, encapsulating considerable variation in temperature and wind speed, both within and across days (Capp et al. 2017). As females roost alone during the breeding event, often at temperatures progressively below thermal neutrality, they incur progressively large energetic costs (Chappell et al. 2016) and thus, it is likely that ambient temperature has significant repercussions for the costs of incubation. Third, being a cooperative breeder, females in this species experience a variable social environment due to variation in the number of helpers she has (ranging from 0-14, mean = 5), who provide food for the incubating female and for the offspring post-hatching. Importantly, the number of helpers is predictable within a breeding event, but not between them (Russell 2016), meaning that there may be significant selection on capitalizing on favourable social conditions through plasticity in incubation schedules (Russell & Lummaa 2009).

To address the overall aim of this study, I gathered data on the temperatures within nests during incubation using probes inserted into model eggs placed within wild clutches. This method allowed me to gain significant insights into patterns of maternal investment into incubation versus foraging, as well as their underlying predictors. First, I investigated the population-level response of incubation behaviour to environmental variation (both climatic and social). Second, I assessed whether this population-level response is driven by plasticity within females or differences amongst females, which could be driven by a correlation between mean incubation level and the mean environment experienced. Further, I accounted for the possibility of differences in incubation amongst females due to nest quality and/or her ability to warm her clutch. Third, I performed an investigation into variation in individual-level incubation responses to climatic variation (i.e. individual

variation in the plasticity of incubation). I attempt to explain this variation in individual-level incubation plasticity through consideration of female characteristics (age), social environment (helper number) and the thermal environment experienced (mean temperature), as the mean incubation level and plasticity of incubation are likely to be correlated.

2.3 MATERIALS AND METHODS

STUDY POPULATION

This study was conducted August-November 2014 and 2015 at the University of New South Wales Arid Zone Research Station, Fowler's Gap (141°43'E, 51°05'S), New South Wales, Australia. Weather data was collected on-site at the research station through the Australian bureau of meteorology. Between August and November 2014 and 2015, the average air temperature during daylight hours, 6AM-6PM, was 21°C (SD = 8.4 °C) and the average wind speed was 17.5 km/h (SD = 8.9km/h). Our wild chestnut-crowned babbler population has been monitored since 2004, and the majority of birds are identifiable through unique colour-combinations and transponder (PIT) tags inserted subcutaneously in the flank of birds (see Browning et al. 2012, Young et al. 2013 & Nomano et al. 2015 for further details). Briefly, in association with a PIT tag reader (LID650, Trovan Ltd, UK) positioned at the bottom of nest trees, and a camouflaged copper coil antenna fitted in the mouth of the nest, the identity (along with date and time) of each nest visitor can be determined. The readings of unique entrances into the breeding nest, in association with counts during observations and capture sessions, were used to determine the number of unique helpers in each breeding group.

MEASURING INCUBATION

Incubation behaviour was obtained over a total of 1,915 hours of daylight, over 167 days at 27 nests (range = 11 - 160 hours and 1-13 day(s); mean ± SD =67 ± 45 hours and mean ± SD = 6 ± 4 days). This large quantity of data was achieved using temperature probes placed in a single model egg of Fimo clay in each nest (Reid et al. 2000). Model eggs were made to resemble natural eggs in size, shape and colour; with a rejection rate

of model eggs averaging ~1.5% (Berg & Russell unpublished). The temperature logger (Tinytag Plus 2, Gemini Data Loggers, UK) was placed under the nest, while the leading wire was threaded through the nest and the 2 x 12 mm probe fitted into a pre-drilled hole in the model egg within the nest cup above. Each temperature gauge took a temperature measurement every two minutes from the time it was attached to the nest (mean ± SD = 12 ± 4 days into incubation) until it was retrieved at the end of the incubation period (Russell et al. 2010). Days into incubation was estimated by subtracting the current date from the hatch date, assuming an incubation duration of 20 days (Russell et al. 2010). I estimate that doing so would lead to an error in days into incubation of up to 2 days on average, based on known variation in the incubation duration of this population. In 15 nests, clutches contained the natural eggs in addition to a single model egg containing the temperature probe, while in the other 12 nests, all natural eggs were removed, placed into incubators and replaced with models, one containing the temperature probe; so such nests only contained model eggs. Because model eggs cool more quickly than natural eggs, our motivation here was to elucidate whether or not egg temperature at the onset of each bout of incubation might mediate maternal incubation schedules. If the female uses the temperature of the clutch to inform her incubation decisions, we expect that females would differ in their incubation of real and model egg clutches.

QUANTIFYING INCUBATION

The temperature data was used to determine incubation behaviour by using significant drops in temperature to signify the female leaving the nest and a steady or increasing temperature to signify the female remaining on the nest. Any window during which the female was on versus off the nest were considered as on- versus off-bouts, respectively, both of which occurred multiple times throughout each day (mean ± SD = 15 ± 5 on-bouts per day; mean ± SD = 14 ± 5 off-bouts per day). Off-bouts were first identified using the Rhythm software (Cooper & Mills 2005). The Rhythm program turns temperature data into a waveform and is able to identify off-bouts based on pre-specified criteria. In my case, off-bouts were defined by a significant drop in temperature (minimum initial slope of 0.15 °C per minute and overall drop of at least 2 °C over the bout duration) within the nest for at least 2 minutes. These selection settings were chosen through trial and error

to obtain the best match of known off-bouts (n=20). Rhythm selections were then uploaded into the Raven acoustic analysis software to check for errors in off-bout selections (e.g. see Figure 1, Cornell Lab of Ornithology 2013). The Raven software displays a waveform of the temperature variations within the nest, which shows a clear representation of consistent temperature drops within the nest (off-bouts). The Rhythm selections can be overlaid on the waveform as highlighted selection borders, which can then be shortened or lengthened by hand, where necessary to encapsulate each off-bout (Figure 1). These selections were then exported from the program as start and end times for each off-bout (to the nearest minute), with the time between successive off-bouts defining the on-bout durations. From this data, on- and off-bout durations were quantified into hours (e.g. 90 minutes = 1.5h). The start of each on- and off-bout were then matched precisely to nest temperature data and to the nearest hourly measure of ambient temperature (in °C) and wind speed (in km/h) (both are recorded each hour, on the hour on-site at Fowler's Gap research station via the Bureau of Meteorology). Incubation behaviour was measured between twilight sunrise and sunset on each day, defined as civil twilight, which are the hours over which babblers are able to forage. However, I excluded the first and last on-bout of each day. This was simply because females incubate overnight, meaning that the duration of the first and last on-bout of the day cannot be meaningfully quantified.

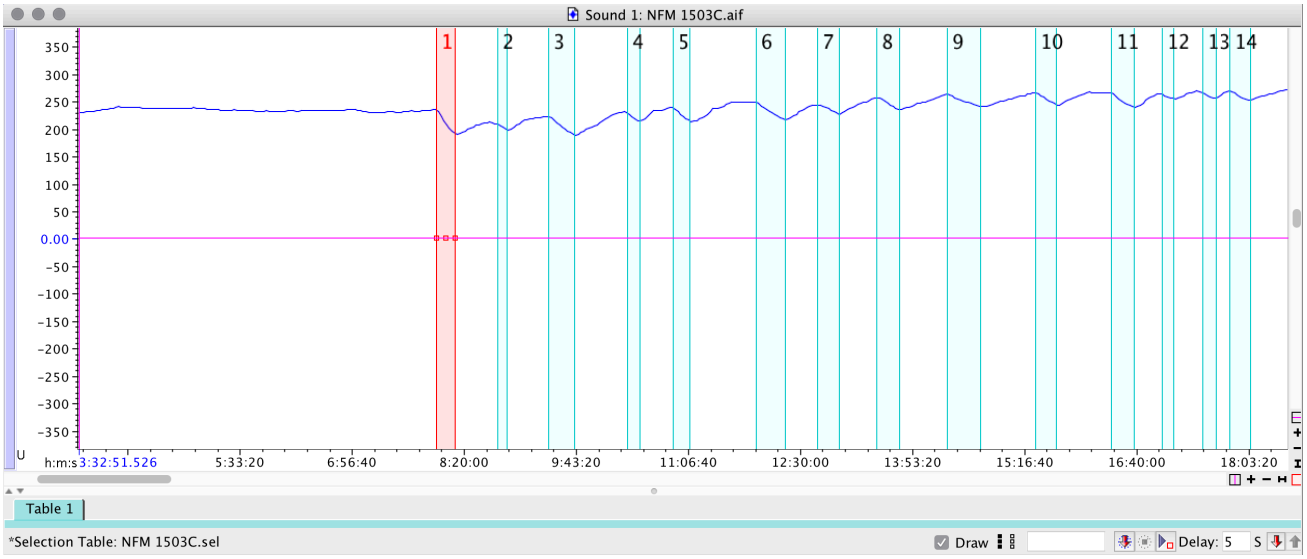

Figure 1. Off-bout selections in Raven. The dark blue waveform represents the temperature within the nest over time (in seconds). The light blue shaded columns represent the off-bout selections that were imported from Rhythm. The red column is an off-bout that is selected and thus, can be edited.

Incubation and nest thermal properties

Measuring investment into incubation using off- and on-bout durations might be confounded if either nests differ in their thermal properties or if females differ in their ability to warm eggs. To test whether either is likely, I selected a subset of nests encompassing the full range of helper numbers (2-8 in this study) and for which model egg temperature was equivalent at either on- or off-bout onset because females from larger groups are expected to have higher quality nests and possibly an increased ability to warm eggs. To test whether females vary in their capacity to warm eggs upon returning from an off-bout, I selected temperature data from the nest probes that were within 2 °C of each other at the end of off-bouts (mean temp = 21.21 °C; n = 9 nests). Similarly, to test whether the thermal properties of nests might impact egg cooling rates, and by extension incubation schedules, I simply did the reverse: selected nests with equivalent temperatures at the end of on-bouts (mean temp = 29.56 °C), and tested cooling rates during ensuing off-bouts (n = 12 nests).

ANALYSIS

All statistical analyses were performed in R (Development Core Team, 2008, version 3.3.1). Three types of analyses were conducted. Linear mixed models (LMMs) were used to investigate the factors affecting population-level incubation behaviour. Random regressions were used to assess individual-level plasticity: in such analyses, one can test whether individuals vary in the steepness of their reaction norms by nesting the key independent variable within individual ID as random effects (Schaeffer 2004). Linear models were used to parse out amongst- and within-individual effects on mean incubation, to test the effect of helper number on nest properties and egg warming rate and to investigate the correlates of individual plasticity level (i.e. slope). AIC scores were used as criteria for model selection; variables that decreased the model AIC score by at least 2 were kept in the model. Subsequently, p-values were calculated by comparing successive models using the native “ANOVA” function and the conditional r-squared for

each term was obtained using the piecewiseSEM package (Lefcheck 2016). Plots were made using the ggplot2 package (Wickham 2009).

Factors affecting mean incubation levels

In order to investigate whether the mean patterns of incubation vary in response to abiotic and biotic factors, I ran two linear mixed models, one each for on- and off-bout durations using the lme4 package (Bates et al. 2015). Further, as on and off-bout duration data were right-skewed, I ensured normality of both by using natural-log transformations prior to each analysis. Next, I tested the effects of ambient temperature, wind speed, helper number, clutch age, size (number of eggs) and type (majority model or real) and previous bout duration (Equations 1 and 2). As on- and off-bout durations were amassed multiple times per day over multiple days per nest, nest identity (i.e. female identity) and date were included in both models as random intercepts.

On-bout ~ temp + wind + helper no. + age + size + type + prev. bout + (1|date) + (1|nestID)
Off-bout ~ temp + wind + helper no. + age + size + type + prev. bout + (1|date) + (1|nestID)

Equations 1 and 2. Linear mixed models used to assess the factors that affect mean on- and off-bout durations.

Amongst- versus within-individual effects

Population-level responses of incubation to environmental variation can be driven by plasticity within individuals or by differences in the mean environment experienced, and subsequently, mean incubation expressed amongst individuals. As such, I ran a separate linear mixed model in order to parse out these two different effects. In order to do so, I analysed the effects of mean ambient environment experienced (among-individual effects) and variance in ambient environment (within-individual effects) on on- and off-bout durations (Van de pol & Wright 2009) (Equations 3 and 4). As previously, nest ID and date were fitted as random intercepts.

On-bout duration ~ mean temp + var. temp + mean wind + var. wind + (1|date) + (1|nestID)

Off-bout duration ~ mean temp + var. temp + mean wind + var. wind + (1|date) + (1|nestID)

Equations 3 and 4. Linear models to assess the effects of variance in temperature and wind speed experienced and mean temperature and wind speed experienced on incubation behaviour.

Additionally, differences amongst individuals in the ability to warm eggs and their nest heat retention may drive amongst-individual differences in incubation responses. As helper number is predicted to drive variation in incubation efficiency, I tested whether it affects clutch warming (by the female upon returning to the nest) and cooling rate (upon the female leaving the nest) in two linear models (Equations 5 and 6).

Warming rate ~ helper no. + (1|date) + (1|nestID)
Cooling rate ~ helper no. + (1|date) + (1|nestID)

Equations 5 and 6. Linear mixed models used to assess helper effects on warming and cooling rate of clutches within wild nests.

Individual variation in plasticity

I investigated whether there was significant among-individual variation in incubation behaviours (on- and off-bout duration) in response to temperature and wind using random regression models with the lme4 package (Equations 7 - 10). In order to investigate individual variation in the plasticity of incubation behaviour in response to temperature, temperature was nested within nest ID as the random regression term (correlation term). The same process was run to test for a plastic response to variation in wind speed separately. Again, date was fitted as a random intercept for the bout duration models and all bout durations were natural-log-transformed for normality as data were skewed. Temperature and wind were both mean-centered. Each random regression model was then compared to a LMM without the random regression term using an ANOVA F-test to obtain a statistical test for the difference in explanatory power with and without the inclusion of individual-level responses to variation in abiotic parameters.

On-bout duration ~ temp + (temp|nestID) + (1|date)
Off-bout duration ~ temp + (temp|nestID) + (1|date)
On-bout duration ~ wind + (wind|nestID) + (1|date)
Off-bout duration ~ wind + (wind|nestID) + (1|date)

Equations 7– 10
Random regressions used to assess individual-variation in the plasticity of bout durations in response to ambient temperature and wind speed.

Analysing candidate drivers of individual-level plasticity is challenging because any analysis that fits predicted values (in this case slope gradients) will fail to account for the accumulation of error inherent in using predicted values in subsequent analyses (Houslay & Wilson 2017). Although the suggested bivariate approach was attempted, I was unable to get the model to run correctly with our data. Here I therefore proceeded with the simpler approach of fitting slope estimates from the random regression models as the response term in linear models, but acknowledge that doing so renders the results explorative. Specifically, I tested in linear models the potential ability of helper number, maternal age and mean temperature experienced during the incubation duration (as we could not control for ambient conditions experienced) to explain any significant among-individual variation in plastic responses to ambient conditions and did so for both on- and off-bouts (Equations 11 and 12).

On-bout plasticity slope ~ helper no. + female age + mean temp
Off-bout plasticity slope ~ helper no. + female age + mean temp

Equations 11 and 12. Linear models to assess the effects of helper number, female age and mean temperature experienced on on- and off-bout plasticity.

2.4 RESULTS

Factors affecting mean incubation responses

On-bout duration ranged between 2 - 238 minutes and averaged at 27 minutes (SD = 22 mins). Ambient temperature and wind speed both had positive effects on on-bout duration, although the effect of the former was more significant than the latter (Table 1). For example, while a 10 °C increase in ambient temperature led to a two minute and 20 second increase in average on-bout duration (Figure 2a), a 10 km/h increase in wind speed led to a one minute and 10 second increase in on-bout durations on average (Figure 2b). Further, helper number also had a positive effect on on-bout duration: average on-bout durations increased by one and a half minutes with the addition of each helper, leading the females in the largest groups to have on-bouts ~9 min longer on average (Figure 2c). By contrast, clutch age, size or type did not significantly affect on-bout duration. Finally, the duration of the previous off-bout had a strong positive effect on proceeding on-bout duration (Figure 2d).

The average off-bout duration ranged between 2 - 90 minutes and averaged at 18 minutes (SD = 11 mins). Ambient temperature also had a positive effect on off-bout duration, with a 10 °C increase in ambient temperature resulting in a two-minute increase in average off-bout duration (Figure 3a). By contrast, neither wind speed nor helper number impacted off-bout duration, and although I found a significant positive effect of clutch age, the magnitude was of dubious biological significance: a five day increase in clutch age led to a 40 second increase in average off-bout duration (Figure 3b). As with on-bout durations, clutch age, size or type did not affect off-bout durations. Finally, duration of the previous on-bout also had a positive effect on the proceeding off-bout (Figure 3c).

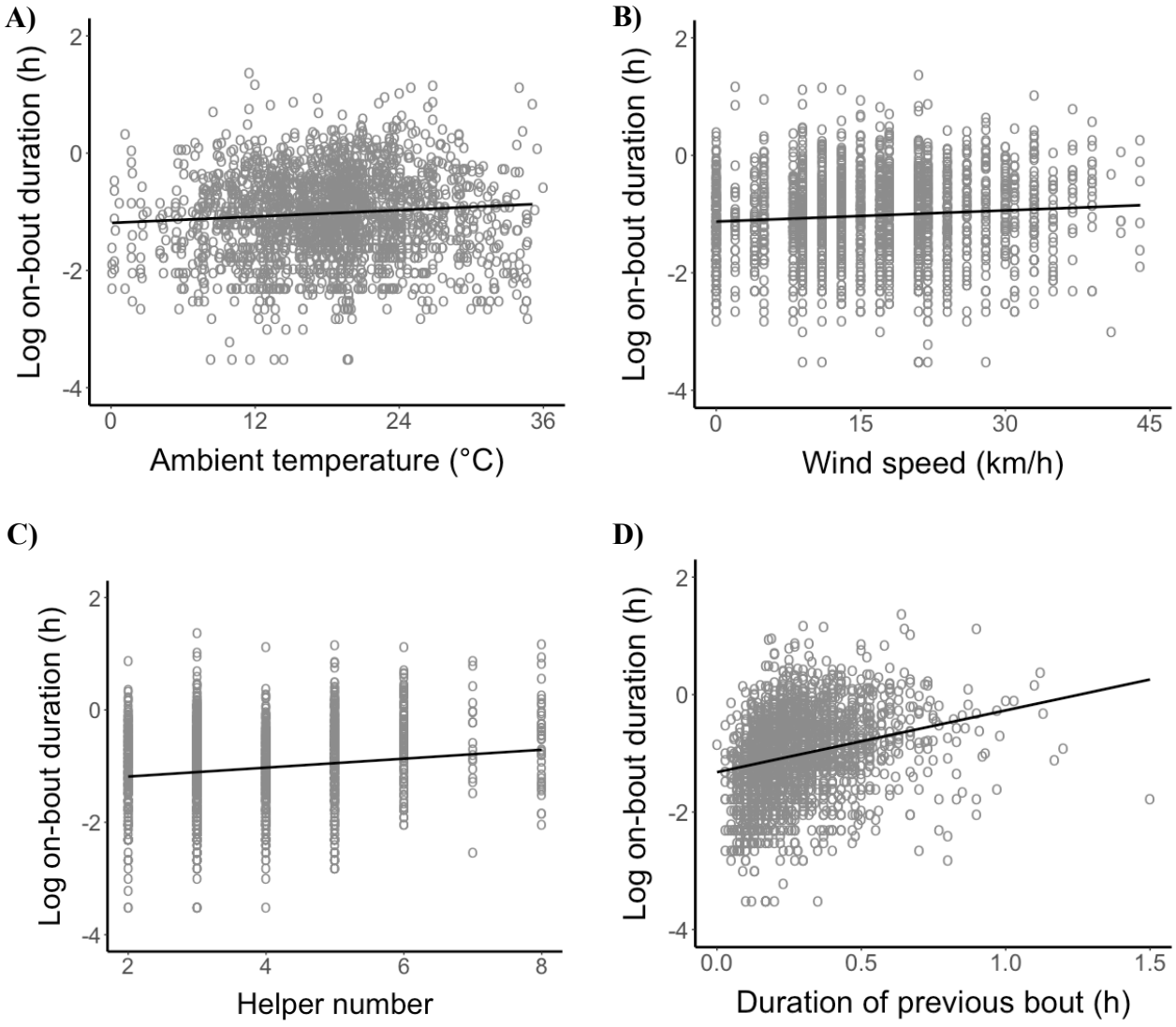

Figure 2. **Factors affecting mean on-bout responses.** (A) Increased on-bout duration with higher ambient temperature, (B) increased on-bout duration with higher wind speed, (C) increased on-bout duration with increased helper number and (D) increased on-bout duration proceeding a longer off-bout. Raw data represented as dots and fitted lines were calculated from the LMM, controlling for all other significant effects.

	β	SE	df	*F statistic*	*P value*	Variance (%)	Δ AIC
(Intercept)	-1.89	0.15					
Temperature	0.0093	0.0029	1, 1189.17	10.050	**0.0016**	2.19	**-7.11**
Wind	0.0056	0.0020	1, 282.73	8.21	**0.0045**	0.77	**-5.94**
Helper No.	0.080	0.0034	1, 26.48	5.23	**0.026**	0.28	**-3.36**
Clutch Age	0.041	0.0062	1, 142.46	0.92	0.34	0.0044	1.04
Clutch Size	0.049	0.054	1, 25.23	0.81	0.38	0.0031	1.09
Clutch Type	-0.020	0.11	1, 23.21	0.039	0.85	0.00037	1.96
Prev. Bout	1.04	0.086	1, 2116.86	147.59	**<0.0001**	2.92	**-140.96**

Table 1. Factors affecting mean on-bout responses. Analysis was conducted using a LMM. Significance testing was performed using F statistics. Percent variance explained refers to the change in the marginal pseudo-R^2 explained by the model by fitting the given term. Δ AIC is the change in AIC from dropping that term from the model.

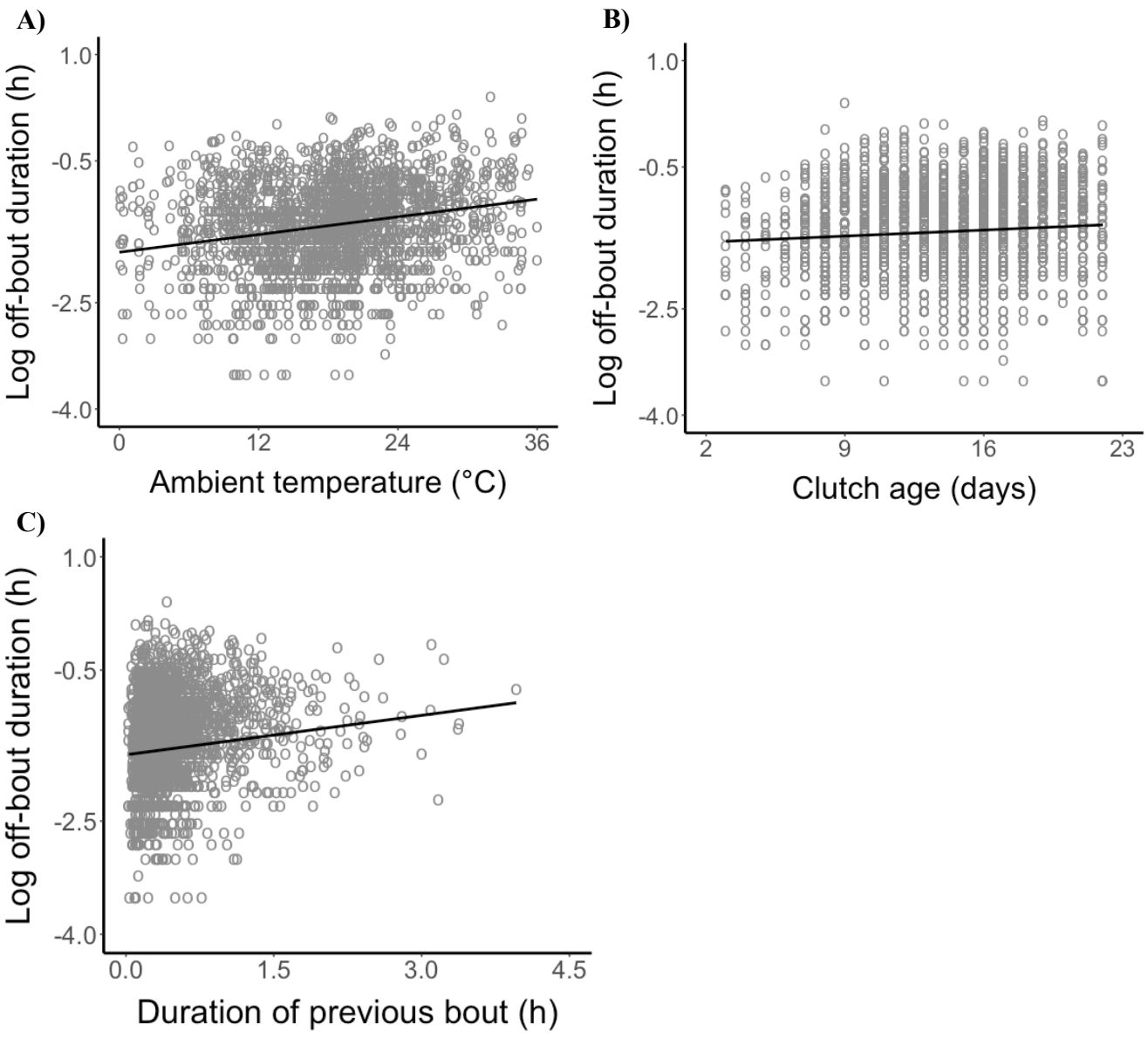

Figure 3. Factors affecting mean off-bout responses. (A) Increased off-bout duration with higher ambient temperature, (B) slightly increased off-bout duration with greater clutch age and (C) increased off-bout duration proceeding a longer on-bout. Raw data represented as dots and fitted lines were calculated from the LMM, controlling for all other significant effects.

	β	SE	df	*F statistic*	*P value*	Variance (%)	Δ AIC
(Intercept)	-2.045	0.092					
Temperature	0.021	0.0022	1, 1272.49	85.89	**<0.0001**	3.31	**-80.57**
Wind	0.00038	0.0016	1, 187.40	0.056	0.81	0.030	1.95
Helper No.	0.041	0.025	1, 25.24	2.74	0.11	0.65	-0.85
Clutch Age	0.012	0.0049	1, 123.54	6.14	**0.015**	0.033	**-4.14**
Clutch Size	0.048	0.043	1, 26.38	1.29	0.27	0.39	0.64
Clutch Type	-0.056	0.083	1, 23.77	23.77	0.51	0.33	1.49
Prev. Bout	0.17	0.029	1, 2157.55	36.62	**<0.0001**	0.079	**-34.11**

Table 2. Factors affecting mean off-bout responses. Analysis was conducted using a LMM. Significance testing was performed using F statistics. Percent variance explained refers to the change in the marginal pseudo-R^2 explained by the model by fitting the given term. Δ AIC is the change in AIC from dropping that term from the model.

Amongst- versus within-individual effects

Population-level incubation responses were driven by plasticity within females: the variance experienced by females in temperature and wind drove their incubation responses. In contrast, incubation responses were not driven by differences amongst females: the mean temperature and wind speed experienced by a female did not impact her incubation response (Table 3 and 4). Furthermore, there were no significant differences amongst females in incubation response due to helper number: both nest warming and cooling rate were unaffected by helper number (LM: *Warming Rate*: estimate $\pm$ se = -0.059 $\pm$ 0.04, $F_{1,7}$ = 2.12, p = 0.19; *Cooling Rate*: estimate $\pm$ se = -0.019 $\pm$ 0.031, $F_{1,10}$ = 0.38, p = 0.55).

	β	**SE**	**df**	***F statistic***	***P value***	**Variance (%)**	**Δ AIC**
(Intercept)	-1.27	0.083					
Temp. Var.	0.014	0.003	1, 1288	20.80	**<0.001**	1.25	**-17.64**
Wind Var.	0.052	0.018	1, 407	8.15	**<0.01**	0.61	**-5.91**
Mean Temp.	-0.02	0.015	1, 30	1.84	0.19	0.41	0.11
Mean Wind.	0.013	0.013	1, 35	1.04	0.31	0.49	0.91

Table 3. On-Bout Van De Pol. Analysis was conducted using a LMM using the van de Pol and Wright method. Significance testing was performed using F statistics. Percent variance explained refers to the change in the marginal pseudo-R^2 explained by the model by fitting the given term. Δ AIC is the change in AIC from dropping that term from the model.

	β	**SE**	**df**	***F statistic***	***P value***	**Variance (%)**	**Δ AIC**
(Intercept)	-1.74	0.058					
Temp. Var.	0.019	0.0023	1, 1432	70.063	**<0.001**	4.31	**-65.77**
Wind Var.	0.0018	0.0087	1, 37	0.041	0.84	0.042	1.51
Mean Temp.	0.0094	0.015	1, 284	0.41	0.52	0.37	1.43
Mean Wind.	-0.0092	0.01	1, 30	0.81	0.37	0.12	1.11

Table 4. Off-Bout Van De Pol. Analysis was conducted using a LMM using the van de Pol and Wright method. Significance testing was performed using F statistics. Percent variance explained refers to the change in the marginal pseudo-R^2 explained by the model by fitting the given term. Δ AIC is the change in AIC from dropping that term from the model.

Individual variation in plasticity

There was significant among-individual variation in responses to variation in ambient temperature for both on- and off-bout durations (Random Regression: *On-bout*: χ^2_2 = 9.62, p<0.01; *Off-bout*: χ^2_2 = 7.39, p = 0.025, Figure 4a and b), but not to variation in wind speed (Random Regression: *On-bout*: χ^2_2 = 3.12, p=0.21; *Off-bout*: χ^2_2 = 3.19, p = 0.2). The significant among-individual differences in response to variation in ambient temperature showed positive associations between intercepts and slopes (on-bouts: r = 0.66; off-bouts: r = 0.28). In other words, those with relatively long on- and off-bouts at low temperatures increased their durations of each to a greater extent under increasing temperatures.

The average on-bout slope ranged from 0 to an increase of 2.5 min per 1 °C increase in ambient temperature (mean = 1 min increase per 1°C, SD = 30 second increase). There was a non-significant, positive tendency for mothers in large groups to respond more positively to increasing ambient temperatures than those in small groups (LM: estimate ± SD = 0.002 ± 0.00099, $F_{1,26}$ = 3.91, p=0.059, Figure 5). Neither female age (LM: estimate ± SD = 0.0006 ± 0.00056, $F_{1,22}$ = 1.16, p=0.29) nor the mean temperature the female experienced during the incubation duration (LM: estimate ± SD = -0.00042 ± 0.00027, $F_{1,25}$ = 2.37, p=0.14) appeared to influence on-bout plasticity.

The average off-bout slope ranged from 0 to an increase of 1.7 mins/°C and averaged at an increase of 1 min/°C (SD = 30 seconds). In contrast, helper number did not affect off-bout slope (LM: estimate ± sd = 0.0014 ± 0.0011, $F_{1,26}$ = 1.79, p = 0.19). As with on-bout plasticity, neither female age (LM: estimate ± SD = -0.000098 ± 0.00058, $F_{1,23}$ = 0.028, p=0.87) nor mean temperature experienced during the incubation duration significantly affected off-bout plasticity (LM: estimate ± SD = 0.00046 ± 0.0003, $F_{1,26}$ = 2.38, p = 0.14).

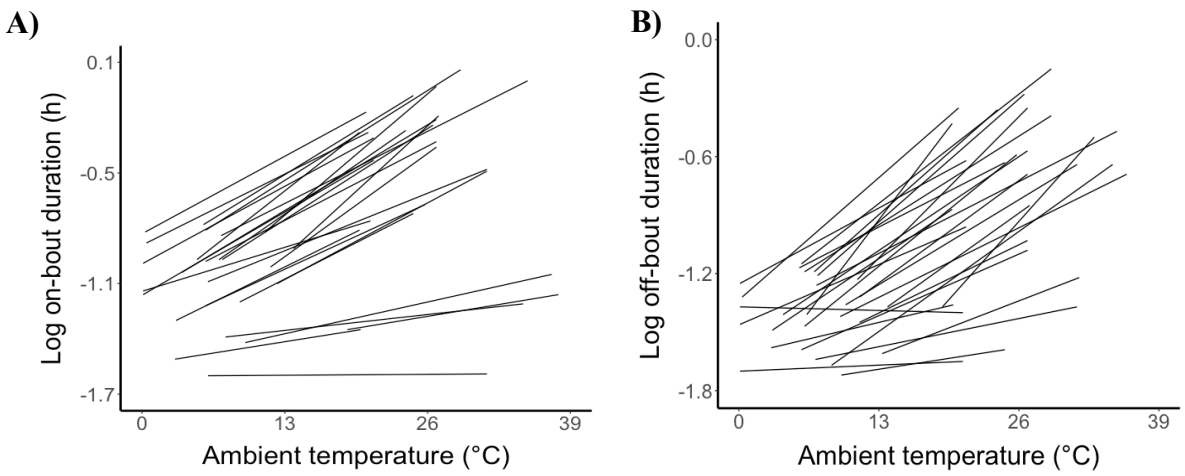

Figure 4. Individual Variation in Plasticity. (A) Individual variation in the slope of on-bout duration in response to ambient temperature and (B) individual variation in the slope of off-bout duration in response to ambient temperature. Fitted lines represent average female plasticity for each nest and were calculated from the Random Regression.

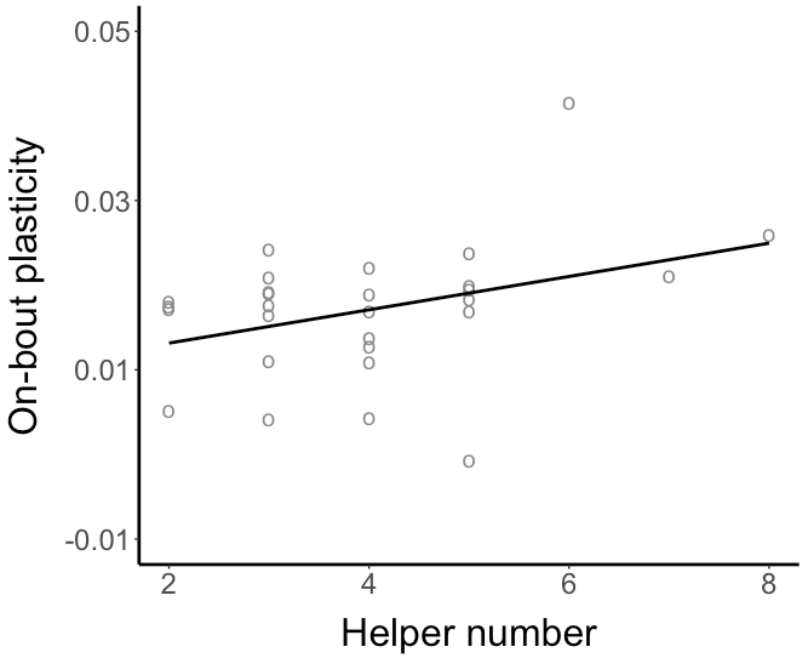

Figure 5. **Individual plasticity slopes analysis.** Positive effect of helper number on the increase in on-bout duration with increased temperature. On-bout plasticity was quantified as the slope of the on-bout duration (in hours) ~ temperature (in °C). Individual slope values are shown as dots. Fitted Line showing positive trend of helper number on on-bout slope in response to ambient temperature.

2.5 DISCUSSION

On average, individuals had longer on- and off-bout durations at higher temperatures and also had longer on-bout durations when wind speeds were high. Further, those living in larger groups also had longer on-bout durations and no change in off-bout durations, suggesting that females in large groups show greater investment into incubation than those in small groups. Moreover, individuals varied in their plastic responses to temperature variation, although not wind speed. Most notably, those with relatively long on- and off-bout bout durations at low temperatures increased their durations of each to a greater extent under increasing temperatures. Finally, I found some evidence to suggest that the magnitude of plastic responses to increasing temperatures was influenced by the number of helpers: those with more helpers tended to perform longer on-bouts with increasing temperatures than those with fewer helpers. These results provide the first demonstration of among-individual variation in plastic responses to environmental variation in patterns of incubation, and a rare example in parental care more generally (Royle et al. 2014). Further, these results suggest that temperature variation and potentially variation in social environments are important pressures selecting for plasticity of incubation patterns in this cooperatively breeding passerine.

Although significant abiotic and biotic predictors of incubation schedules were detected in this study, the amount of variance explained was relatively modest, with ~5% and 1% of the variance explained by each, respectively. While low levels of variance explained can stem from small sample sizes, this is unlikely to be the case here, for I obtained data for 1,915 h encompassing 2,330 on-bouts and 2,166 off-bouts for 27 females. Notwithstanding, at least three difficult-to-measure parameters might be expected to have more significant explanatory power. For example, heritable, additive genetic differences among females should explain significant variance. Although testing this possibility is not currently possible in this babbler population, and remains to be tested more generally, the explanatory power of the random terms provide some support for this possibility. For example, in the LMM analyses of the factors affecting on- and off-bout durations, the random term female identity explained 1-3% of the variance, while in the random regressions, among-female variation in responses explained 1-4% of the variance.

Another possibility is that incubation schedules are affected strongly by fine-scale variation in previous or anticipated foraging success, and/or the amount of food females receive during incubation; which itself might be relatively stochastic owing to variation in the foraging success of potential providers and their current proximity to the nest. Although I was not able to test these possibilities here, it is known from other studies that supplemental feeding experiments often increase attentiveness, as does levels of sustenance provided by partners or other group members (Chalfoun & Martin 2007). Finally, nests might vary in their insulatory properties, with consequences for incubation schedules, as has been found in other species (Mainwaring 2015). While babbler nests are known to vary in their structure (IRK Stewart & AF Russell unpublished), I found no evidence to suggest that the cooling rates of model eggs were influenced by any differences in nest heat retention due to the number of helpers available. These caveats notwithstanding, these results have implications for our understanding of selection on incubation investment in this species and more generally.

I found that incubation schedules are sensitive to ambient temperature and wind speed, with both on- and off-bout durations being positively influenced by the duration of the previous off- or on-bout respectively. Such temperature effects are well-documented: a meta-study on 34 avian intermittently-incubating species found the same patterns (Conway & Martin 2000). Furthermore, an experimental study on tree swallows (*Tachycineta bicolor*), showed that on-bout durations were increased when nest temperatures were artificially increased (Ardia et al. 2009). This could be driven by the fact that favourable ambient temperatures slow egg cooling rates, females can spend longer off the nest foraging, with positive knock-on effects for following on-bout durations. For example, black-capped chickadee (*Poecile atricapillus*) females increased on-bout durations and decreased off-bout frequency with slower egg cooling rates (Cooper & Voss 2013). This hypothesis assumes that incubators have a greater relative fitness interest in their own condition than the developmental rates (or success) of their offspring, which is likely occurring in this system as female babblers produce multiple clutches both within and across breeding seasons (Russell 2016). We found partial support for this hypothesis. For example, on- and off-bout durations were positively associated, although

whether long off-bouts cause long on-bouts, or vice versa is not clear. On the other hand, high winds were associated with increased on-bout, but not off-bout durations despite the potential amplifying effects of high winds on egg cooling rates (Capp et al. 2017). In support of the hypothesis, clutches of model eggs were not associated with reduced off-bout durations compared to real clutches. Reduced off-bout durations would be expected if females are more concerned with their offspring than themselves, since such eggs cool at a faster rate than natural eggs and are presumably routinely cooler at each on-bout onset. In contrast, females increased their off-bout durations with increasing clutch age, likely due to the increasing ability of embryos to create their own metabolic heat: for example, mean egg temperature increases with age, suggesting that females do respond to increasing clutch temperature over the incubation duration (Cooper & Voss 2013). These conflicting results hint that the interplay between investments in offspring care versus self-maintenance might be complex; which would also explain why environmental variation explains a relatively modest amount of variance in incubation schedules.

One way in which increased complexity could arise is if females inherently vary in their level of investment into incubation. In support, not only did we find significant among-female variation in overall contributions to incubation, but females also varied in their responses to changing ambient temperature. Surprisingly, females that contributed more to incubation at low temperatures, were also the ones that increased their contributions most as temperatures increased. One explanation for these results is that female contributions to incubation are condition-dependent. Under a condition-dependent hypothesis, females incubating relatively early in the season (i.e. late winter when ambient temperatures are low) would be expected to suffer greater costs of incubation than those incubating later when ambient temperatures are more favourable. This is because during incubation, female babblers roost alone in the nest overnight, and we have previously shown that lone roosting at 5 °C (the mean night-time temperature during early breeding) incurs a 34% increased metabolic cost compared with lone roosting at 15 °C (the mean night-time temperature later in the season) (Chappell et al. 2015). On the contrary, I found no evidence in the linear model and random regression analyses to suggest that mean ambient temperature experienced during incubation influenced either

the intercept incubation levels or the response slopes. Further, in a previous study, we found no evidence to suggest that nest attentiveness was influenced by breeding phenology in this population (Capp et al. 2017). Finally, we found no evidence that female age, which could correlate with overall condition, influenced female responses to variation in temperature. Together, although further study is required, these results suggest there is little evidence currently to suggest that among-female variation in incubation schedules is driven by their body condition. This is the first study to investigate variation in responses to variation in ambient conditions at the individual level, and so whether or not this result is common-place is not known.

On the other hand, I found that females breeding in large groups showed longer on-bout durations than those breeding in small groups, and some suggestion that those breeding in large groups increase their contribution relatively more under increasingly favourable ambient temperatures. Combined, females in groups of 8 spent about 40 minutes extra on the nest incubating per day than pairs, with these effects neither being confounded by nest properties nor among-female variation in the ability to warm eggs. These results might be expected to arise through a condition-dependent mechanism (i.e. if females in large groups have improved foraging efficiency during off-bouts and/or if larger groups provide more food to incubating females). For example, in cooperatively breeding meerkats (Suricata suricatta), sentinels allow group members, including mothers, to forage more efficiently (Clutton-Brock et al. 1999). However, in chestnut-crowned babblers, incubating females often forage alone during off-bouts, and we have previously shown that large groups are associated with resource depletion near the nest (Sorato et al. 2015). Further, although females are known to be fed by group members on and off the nest, current evidence suggests it to be highly stochastic and not obviously related to group size (E Capp & AF Russell unpublished). This is presumably because of the large foraging range of this species (~ 1 km^2, Portelli et al. 2009; Sorato et al. 2012) and the suspected high costs of travelling to and from the nest when the female may or may not be present (Browning et al. 2012). Indeed, because small groups deplete their resources around the nest less rapidly and benefit more from foraging with an extra group member, we might expect small groups to feed the incubating female more frequently than large

groups, simply because they are more likely to be in the vicinity of the nest. Thus, while again further work is required, I suspect that the group size effects reported are not manifest through condition-dependent effects on the incubating female.

An alternative possibility is that females vary in the degree to which they are selected to invest in their offspring versus themselves, with this effect influenced, at least in part, by their current social environment. There are two reasons to suggest this hypothesis. First, larger groups have substantial positive effects on nestling survival owing to their impact on brood provisioning rates (Browning et al. 2012; Liebl et al. 2016 a, b). In addition, group sizes and breeding conditions are highly variable between years (Russell 2016), meaning that there should be significant selection on females to increase investment in activities for which she alone is responsible (such as incubation) as a function of increasing current group size (Russell & Lummaa 2009). Second, because brood provisioning rates increase as a function of helper number, but consequential effects on nestling survival are diminishing (Browning et al. 2012), mothers are able to reduce their contribution to nestling provisioning by up to 80% in large groups (Browning et al. 2012). As a result, mothers currently breeding in large groups can afford to increase their contribution to incubation, without incurring proportional long-term costs, because they will be able to recoup condition lost during the subsequent nestling phase, relative to those breeding in smaller groups. Given that such helper effects are not uncommon in cooperative breeders (Hatchwell 1999; Russell & Lummaa 2009), I expect this hypothesis to gain widespread support in cooperative breeders. Furthermore, I expect this hypothesis to also be relevant to any system where the expected fitness returns from a current attempt vary as a predicable function of current environment, including nest predation pressure (Fontaine & Martin 2006), partner quality (Sheldon 2000), food availability (Zanette, Doyle & Tremont 2000) and ambient climate (Conway & Martin 2000). However, I caution that such future tests will need to be careful to tease this differential investment/allocation hypothesis from the more commonly invoked role of condition-dependence (Radford 2004; Chalfoun & Martin 2007).

In conclusion, this study presents the first demonstration of among-individual variation in plastic responses to a variable environment, in this case temperature, of incubation schedules, and a rare demonstration of variation in individual-level plasticity in a form of parental care more generally (Westneat et al. 2013; Royle et al. 2014). Although ecological drivers of this variation have yet to be elucidated, one potential candidate is variation in the social environment (see above). Assuming that the variation detected has a heritable genetic basis (Brommer 2013) and fitness advantages (see above), these results have a number of important implications. Not least, it suggests that plasticity in patterns of incubation might be under selection and so potentially evolve. In this chestnut-crowned babbler system, this might be expected since the timing of breeding is dependent upon stochastic rain events, primarily during winter (Russell 2016), leading to breeding females incubating during a potentially wide range of temperatures (Capp et al. 2017). More generally, the results suggest that populations might be more equipped to deal with climate change than might be expected through selection on mean trait values (Charmantier et al. 2008; Przybylo, Sheldon & Merila 2000). I hope that this study lays a foundation for future work aimed at investigating variation in individual-level plasticity, its ecological drivers, heritability and additive genetic variance.

CHAPTER 3

Positive helper effect on embryonic heart rate plasticity

3.1 ABSTRACT

Avian embryos of intermittent incubators experience significant variation in their temperature throughout development. However, much of this thermal variation exceeds the small range of optimal embryonic developmental temperatures. Embryos are known to plastically adjust their heart rate in response to temperature: lowering heart rate and further, developmental rate when temperature drops. Further, if this plastic response can be selected upon, we would find individual variation in a population, but this has not been thoroughly explored. This variation could be driven by differences in how individuals navigate a trade-off between optimal developmental rate and energy use. I investigated possible individual variation in heart rate plasticity within a wild chestnut-crowned babbler (*Pomatostomus ruficeps*) population. In the lab, I experimentally lowered the temperature of wild babbler embryos and recorded their corresponding change in heart rate. I found that heart rate does indeed drop with lowered embryonic temperature and that a variety of other factors such as embryonic age, egg volume and time of day affect mean heart rate. Further, I found that the magnitude of the plastic response of heart rate varied amongst individuals and finally, that the number of helpers that the mother of the egg has positively effects the magnitude of heart rate change with temperature. The mechanism driving this correlation has not been determined but is possibly driven by a positive correlation between maternal incubation effort and helper number. These results suggest that embryos could be adaptively varying in their response to incubation conditions, which could counteract detrimental variation in incubation.

3.2 INTRODUCTION

Temperature plays a key role in embryonic development, and this effect is likely of particular importance for embryos that develop outside of the mother, which are less likely to be thermally buffered (White and Kinney 1974). Externally developing species have evolved different strategies to control their embryo's thermal environment. For example, many reptiles bury their eggs underground to buffer thermal variation. Other species have evolved to regulate the temperature of their embryos behaviourally throughout development, such as birds, many of which directly transfer heat to their embryos via incubation. As expected, the temperature of offspring during development is closely related to development time in a wide range of taxa: embryos that are maintained at higher temperatures during a greater portion of time develop faster (Gillooly & Dodson 2000). However, the sensitivity of developmental time to temperature varies amongst avian species, even when controlling for egg mass (Martin et al. 2007). How this variation in sensitivity arises is not clear, but one possibility is variation in embryonic sensitivity to temperature. For example, fence lizards show adaptive variation in embryonic heart rate in response to temperature, which corresponds with their developmental rate (Du et al. 2010a). However, whether such effects arise through consistent individual differences in phenotypic plasticity, the ability of heart rate to be adjusted in real time in response to temperature exposure, is not known. The plasticity of heart rate can be visualized using a reaction norm approach (Schlichting & Pigliucci 1998). The reaction norm is a simple graphical visualization of the heart rate expressed as a function of the temperature gradient, in which the slope of the line is a quantification of the plasticity of that organism (or population). Whether developing embryos vary in heart rate plasticity as a function of temperature not only has important implications for understanding developmental rates and developmental effects more generally, but also population responses to our changing climate.

Our understanding of all of the early determinants of heart rates is not complete. However, we know from work on ectotherms that heart rates can acclimate to prevailing temperatures. For example, Du et al. found that the embryonic heart rates of three reptile species acclimate to preceding average incubation temperature (Du et al.

2010b). This leads to the intriguing possibility that developing embryos can adjust their heart rate responses during development. A relatively simple way of testing this hypothesis is through the use of inexpensive, easy-to-use heart rate monitors to monitor the change in heart rate during egg cooling experiments. Further, evidence suggests that embryonic heart rate could have important knock-on implications for energy use and developmental rates. For example, a study on 30 avian species suggested a strong link between embryonic heart rate and development, and developmental rate, as higher heart rates were correlated with shorter incubation durations (Ar & Tazawa 1999). The hypothesis that embryos vary in their heart rate sensitivity to temperature will be supported if, during egg cooling, the slope of heart rate decline varies amongst embryos.

The emergence of adaptive phenotypic plasticity of heart rate sensitivity to temperature is most likely found in species for which embryos routinely experience variable temperatures during development. One such group of animals are birds, which can display intermittent, contact incubation by a single parent, as is the case in a portion of passerine species. This is because in such species, the single parent (usually the female) must routinely leave the eggs to forage (Drent et al. 1985). This generates two inevitable sources of among-clutch variation in temperatures experienced during development. First, because females from the same population vary in phenology, eggs which are left unattended early in the season when ambient temperatures are low will, all else being equal, experience longer periods at lower temperatures than females breeding later in the season when ambient temperatures are more favourable (Meijer et al. 1999). Second, because incubation is costly (Price 1998; Visser & Lessells 2001; Hanssen et al. 2005; de Heij, van den Hout & Tinbergen 2006) and females differ in their ability to forage successfully during bouts off the nest, e.g. due to differences in local foraging quality (Zanette, Doyle & Tremont 2000), clutches will inevitably vary in the amount of time they experience temperatures below the expected thermal optimum (Webb 1988). Finally, there is a general selection pressure in such species to develop quickly because early hatching reduces the time in the nest and so the high risk of predation (Martin et al. 2015).

Here, using the cooperatively breeding chestnut-crowned babbler, I provide the first test of the hypothesis that avian embryos within a population show variable plasticity in their heart rates. This 50g endemic passerine bird of inland south-eastern Australia, which lays clutches of 2 - 6 eggs (mean = ca. 4) in dome-shaped nests and has a ~20 d incubation period, represents an ideal model to test this hypothesis. First, it shows intermittent, contact incubation by the breeding female only at each nest. Second, I showed in Chapter 2 that although females spend average bouts on and off the nest of 27 and 18 min, respectively, that there was consistent among-female variation in average bout durations. For example, the average standard deviation in time spent on the nest during bouts of incubation was 22 mins, while the average standard deviation in the time spent off the nest was 11 mins. Third, a significant source of this variation was helper number, with those females breeding with 8 helpers spending an average of 42 min extra on the nest per day. This means that, *a priori*, one can generate predictions regarding which embryos should be expected to show reduced heart rate responses to cooling temperatures, i.e. those from nests with few helpers. Finally, there is likely to be significant selection on rapid development because early hatching reduces the probability of being predated, influences competition with offspring produced by other females breeding in other nests within the social group, and allows parents to have more reproductive attempts within the year, increasing their indirect component of inclusive fitness (Russell 2016).

This study aims to test whether embryos within our population vary in their heart rate plasticity in response to temperature variation, and whether any such variation might be adaptive. In order to do so, I measured the heart rate of developing embryos during artificial incubation in standardized conditions, and then every minute thereafter during egg cooling experiments that mirrored natural cooling patterns in the field. It is important to note that eggs were collected relatively late in development (mean = 5 d before hatching) to ensure that embryos had sufficient time to acclimate to natural, field conditions prior to testing under the standardized conditions of the lab environment, and I found no evidence to suggest that time in the lab influenced heart rate responses to the experiment (see Results). Using these methods I outline specific aims. First, I aim to understand the general factors, both environmental and intrinsic, that affect mean heart

rate. Second, I aim to understand whether the change in heart rate with dropping temperature varies amongst individuals and if so, what factors could be explain this variation.

3.3 MATERIALS AND METHODS

STUDY POPULATION

This study was conducted during the breeding season of 2015 (August-November) at the University of New South Wales Arid Zone Research Station, Fowler's Gap (141°43'E, 51°05'S), New South Wales, Australia. The study population of chestnut-crowned babblers was established in 2004, and details of their socio-ecology have been detailed elsewhere (Russell 2016). I collected 36 eggs from 9 clutches for the purposes of this study. All eggs were collected in a padded container and transported immediately, by vehicle (<5km), to onsite Brinsea® Octagon 20 incubators set at 38.4 °C with 43-46% humidity (Webb 1988). However, egg temperatures sometimes exceeded 38.4 °C upon removal from the incubator. This is possibly due to the ability of embryos to produce their own metabolic heat late in development (Cooper & Voss 2013). Eggs were collected as late as possible during development, to ensure they had maximal time to acclimate to their natural incubation temperatures and that the subsequent standardized conditions provided in the incubators would have minimal impacts on their heart rates during the experiments. On average, eggs were in the incubators for 4.2 d before hatching (SD = 2.5 d) with a range of 1-9 days, meaning that most eggs spent 15.8 d in their nests prior to being moved to the incubator (i.e. 79% of their developmental time in the egg, assuming that artificial incubation does not affect incubation duration). The length and breadth of all eggs was measured using Vernier calipers (± 0.1mm) and labelled, before being entered to the incubators. Volume was subsequently calculated by multiplying egg length by egg width^2 (Hoyt 1979).

During their time in the incubators, eggs were exposed to natural light-dark cycles, and checked every 2-4 h for hatching, as well as once per day for pipping (the beginning of the hatching process). Further, signs of pipping were always checked before the

experiments (see below), since heart rate can fluctuate significantly during this stage (Ar & Tazawa 1999). Pipping can be heard by putting eggs up to the ear, well before cracks appear in the egg shell. Hatching success in the incubator was comparable to that in the field, ca. 88% success, and following hatching, all offspring were returned to their natal nest. All nestlings were accepted without exception and have no evidence that have impaired development or survival compared with naturally-incubated control nests.

Measuring heart rate during egg cooling

In order to assess changes in the heart rate of embryos when cooled, eggs were removed from the incubator and assayed one at a time, before being replaced and the next one assayed. On removal from the incubator, each egg was placed pointed-side up onto the shallow egg cup within the digital heart rate monitor (Buddy® Mark 1, Avitronics, UK, see Lierz, Gooss & Hafez 2006 for further details). Before closing the lid to the monitor, I determined the surface temperature of the pointed end of the egg using a FLIR E8 thermal imaging camera which provides an instantaneous measure on the screen of the temperature (± 0.1 °C) (Kastberger & Stachl 2003). I then simply closed the lid and obtained a measure of the heart rate (in bpm), with stable heart rates usually taking just a few seconds to register. After the heart rate was recorded, I then opened the lid of the monitor briefly to determine the egg temperature. The egg surface temperature at assay onset was approximately 38 °C. From this starting temperature, the eggs were allowed to cool naturally towards, but not beyond, the suggested lowest safe temperature of 25°C (Tazawa & Nakagawa 1985). The time taken to cool and the lowest temperature attained, depended on the ambient lab temperature. Using the thermal imaging camera, in conjunction with knowledge of the lab temperature, I was able to assess the optimal interval between each heart rate measure such that I could obtain 10 heart rate measures per egg at regular temperature intervals. On average, heart rates were obtained following a ~1°C drop in egg surface temperature, which took between 0.5 and 3 min, with the precise temperature always recorded immediately prior to the each record of heart rate obtained. The experiments were conducted between 7:45 and 17:15. Overall, I obtained 941 heart rate measures. On average, the 36 eggs were assayed on 2.6 separate occasions (SD = 1.3, range = 1 – 5), and with an average number of trial per assay of 9.9

per egg (SD = 1.4, range = 5 - 10). This slight reduction from the attempted 10 trials per assay arose either because the lab temperature was not low enough to allow further cooling or the egg temperature dropped to the lowest safe temperature. The system has been assessed for reliability of heart rate and movement detection and were shown to be reliable when movement is accounted for (Pollard, Pitsillides & Portugal 2016). As such, I did not take heart rates during egg movement (which is assessed by the device and displayed on the egg monitor screen in real time).

ANALYSIS

All analyses were conducted in R (R Development Core Team, 2008, version 3.3.1.). Three types of analyses were conducted: mixed effect models (LMMs using lme4, version 1.12, 2016); random regression models; and, bivariate models (the latter conducted by Dr. Thomas Houslay using the package ASReml-R). AIC scores were used as criteria for model selection; variables that decrease the model AIC score by at least 2 were kept in the model. F-statistics were obtained using the native R "ANOVA" function to calculate p-values (Bates et al. 2015). Variance explained by each term was obtained by calculating the change in conditional R^2 between the full model and the model with the term of interest (using the piecewiseSEM package, Lefcheck 2016). All plots were made using the ggplot2 package in R (Wickham 2009).

Factors affecting heart rate

First I used an LMM to investigate the factors affecting heart rate (Equation 13). The response variable, heart rate (beats per minute), was natural log-transformed to obtain a normal data distribution as the data were right-skewed. Egg surface temperature, age, helper number, egg volume, hatch order, time of day and days in the incubator and were fitted as explanatory variables. A non-linear relationship between embryonic heart rate and egg temperature was observed in a similar experiment in chicken embryos (*Gallus gallus domesticus*) and thus, temperature was included as a squared term in the model (Tazawa & Nakagawa 1985). Days to hatching was fitted because embryonic heart rates are known to increase with embryonic age (Pearson & Tazawa 1999). Volume was fitted as squared term because embryonic heart rate in altricial and semi-altricial birds has been

shown to not scale linearly with mass (Tazawa et al. 2001). Hatch order was included as a proxy for lay order. Time of day was included as a squared term in the model to account for any 'circadian' effects (i.e. sunrise and sunset elicit similar heart rate responses). In order to control for acclimation effects, days that the embryo spent in the incubator was also included in the model. Interactions between temperature and helper number and age and hatch order were also fitted into the model to assess whether helper number affects heart rate responses to temperature and whether hatch order affects the developmental speed of heart rate. Individual ID nested within clutch and date were fitted as random intercepts.

$$\textit{Heart rate} \sim \textit{temp} + \textit{temp}^2 + \textit{age} + \textit{helper no.} + \textit{volume} + \textit{volume}^2 + \textit{hatch order} + \textit{time} + \textit{time}^2 + \textit{daysinincubator} + \textit{temp: helper no.} + \textit{age:hatchorder} + (1|\textit{date}) + (\textit{ID:clutch})$$

Equation 13. Linear mixed model to assess the effects of biotic and abiotic factors on mean heart rate and their interactions.

Among-individual variation in responses to temperature

I investigated among-embryo variation in heart rate plasticity in response to temperature using a random regression model with the lme4 package in R (Equation 14, Bates et al. 2015). Random regressions were used to investigate whether individuals significantly vary in the steepness of their reaction norms by nesting the key independent variable (temperature) within individual ID as random effects in a mixed model (Schaeffer 2004). Egg surface temperature (ºC) was mean centered. Although heart rate is obviously expected to decline with declining temperature, the key question is whether the level of this decline varies amongst embryos, measured as variation in the change in heart rate per 1 °C drop in temperature (i.e. the slope of heart rate ~ temperature). The response variable, heart rate, was natural log-transformed to obtain a normal data distribution as it was right-skewed. Embryonic age was included to control for changes of heart rate within individuals due to age effects. Other factors were not included as they did not vary within individuals. In order to investigate individual variation in the plasticity of heart rate in

response to temperature, temperature nested within egg ID was included as the random regression term. Additionally, date and egg ID nested within clutch were fitted as random intercepts to account for repeated measures of eggs across separate assays and eggs from the same clutch. The model outputs a correlation for the random regression term that reflects the relationship between the intercepts and slopes (i.e. plasticity) on average. The model with the random regression term was then compared to a model without this using an ANOVA F-test, which indicates the relative fits of the models with and without the random regression term.

Heart rate ~ temp + age + (temp|ID) + (1|date) + (ID:clutch)

Equation 14. Random regression model to assess individual variation in heart rate as a function of temperature.

Explaining among-embryo variation in plasticity

In order to investigate whether the number of helpers, and so early exposure to incubation patterns, could account for among-embryo variation in plastic responses to temperature, a bivariate model was conducted in ASREML (Houslay & Wilson 2017). In this model, the correlation between helper number and variation in plastic responses to temperature was tested. The bivariate model controlled for embryonic age as a fixed effect in addition to date and ID nested within clutch as random intercepts to reflect the effects included in the random regression described above.

3.4 RESULTS

Factors affecting heart rate

Embryonic heart rate ranged from 89 bpm to 361 bpm, with a mean of 189 bpm (SD = 57.3) across all temperatures (25°C - 40°C). Unsurprisingly, temperature explained the largest amount of variance in heart rate (70%), with an average decrease in heart rate of 4 bpm for every degree decrease in temperature down to 25 °C (Table 5; Figure 6a). As expected, embryonic heart rate increased with age (Figure 6b). On the other hand, helper

number did not significantly affect embryonic heart rate. Embryos in large eggs had higher heart rates than those in smaller eggs (Figure 6c). However, this effect was not significant as a squared term. Hatch order did not affect heart rate. Heart rate showed significant circadian rhythm, peaking in the middle of the day (Figure 6d). The days that the egg spent in the incubator did not affect mean heart rate. After controlling for these effects, I found that the number of helpers in the female's group significantly modified the temperature effect on heart rate: the heart rate of embryos from groups that contained more helpers were more sensitive to reductions in temperature than those in groups containing fewer helpers (Figure 6a). More specifically, at high temperatures, heart rates averaged 374 bpm across the range of group sizes, at low temperatures (i.e. 25 °C), heart rates were 16% higher in the smallest versus the largest groups. Additionally, hatch order modified the effect of age on heart rate: those hatched later in the clutch increased their heart rate more significantly with age than those hatched earlier (Figure 6c).

Among-embryo variation in plasticity

There was significant among-embryo variation in the plasticity of embryonic heart rates (i.e. variation in the slope of heart rate ~ temperature) in response to temperature (ANOVA: χ^2_2=114.85, p<0.001). There was a negative correlation (0.29) between an individual's heart rate y-intercept and their heart rate plasticity: those with lower mean heart rates had greater plasticity.

Embryos from groups with more helpers had higher heart rate plasticity in response to temperature than those in smaller groups (Bivariate Model: *Helper:* estimate $\pm$ se = 0.575 $\pm$ 0.137, χ^2_2 = 10.217, p<0.001, Figure 7). Because of the positive correlation between slope and intercept, this effect could be indirectly driven by a positive effect of helper number on intercept. However, this effect is not driven by a direct helper number effect on heart rate intercept, but caused by a correlation between helper number and heart rate slope (Bivariate Model: *Helper:* estimate $\pm$ se = -0.298 $\pm$ 0.204, χ^2_2= 1.641, p = 0.1).

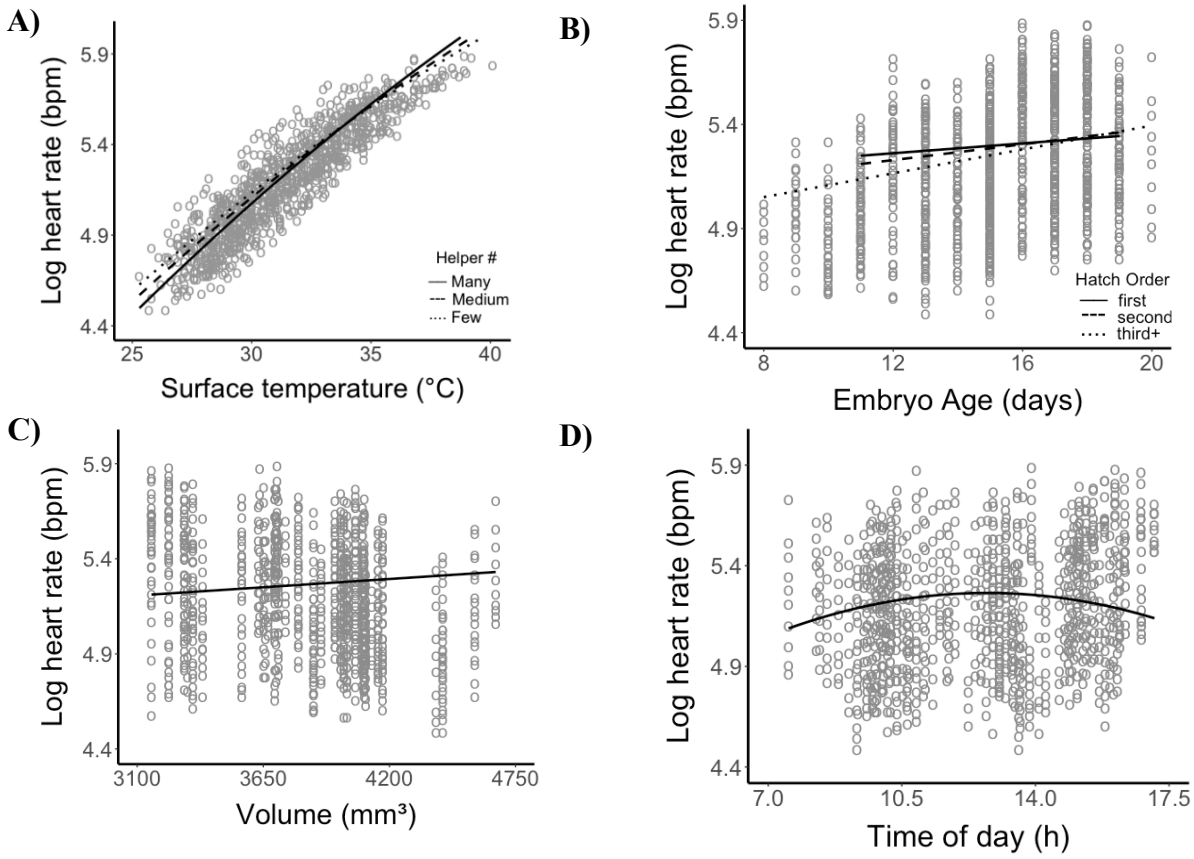

Figure 6. **Factors Affecting Heart Rate**. (A) Interaction between egg surface temperature and helper number on heart rate, (B) interaction between age and hatch order on heart rate, (C) positive effect of egg volume on heart rate and (D) non-linear effect of time of day on egg heart rate. Raw data represented as dots and fitted lines were calculated from the LMM, controlling for all other significant effects.

Table 5

	β	SE	df	*F statistic*	*P value*	Variance (%)	Δ AIC
(Intercept)	-1.36	0.35					
Temperature	0.11	0.0010	1, 897.87	11454.1	**<0.0001**	69.92	**-2236.53**
Temperature^2	-0.0016	0.017	1, 897.03	31.59	**<0.0001**	0.26	**-29.27**
Embryo Age	0.026	0.0033	1, 54.69	62.56	**<0.0001**	12.65	**-38.38**
Helper Number	-0.12	0.0083	1, 34.68	61.11	0.15	0.013	-0.26
Egg Volume	0.000080	0.000039	1, 38.58	4.21	**0.047**	0.27	**-2.34**
Egg Volume^2	0.000000013	0.000000090	1, 40.10	0.16	0.88	0.22	1.97
Hatch Order	-	-	2, 29.37	0.72	0.49	0.019	2.42
Time of day	-0.00067	0.0029	1, 116.86	0.054	0.82	0.10	-1.90
Time of day^2	-0.0065	0.0010	1, 437.98	38.62	**<0.0001**	1.78	**-34.015**
Days in Incubator	-0.011	0.0065	1, 56.85	2.84	0.10	0.13	-0.68
Temp x Helper No.	0.0031	0.00054	2, 201.53	32.62	**<0.0001**	0.33	**-31.09**
Age x Hatch Order	-	-	2, 914.70	20.67	**<0.0001**	1.53	**-33.52**

Table 8. Factors Affecting Heart Rate. Analysis was conducted using a LMM. Significance testing was performed using F statistics. Percent variance explained refers to the change in the marginal pseudo-R^2 explained by the model by fitting the given term. Δ AIC is the change in AIC from dropping that term from the model.

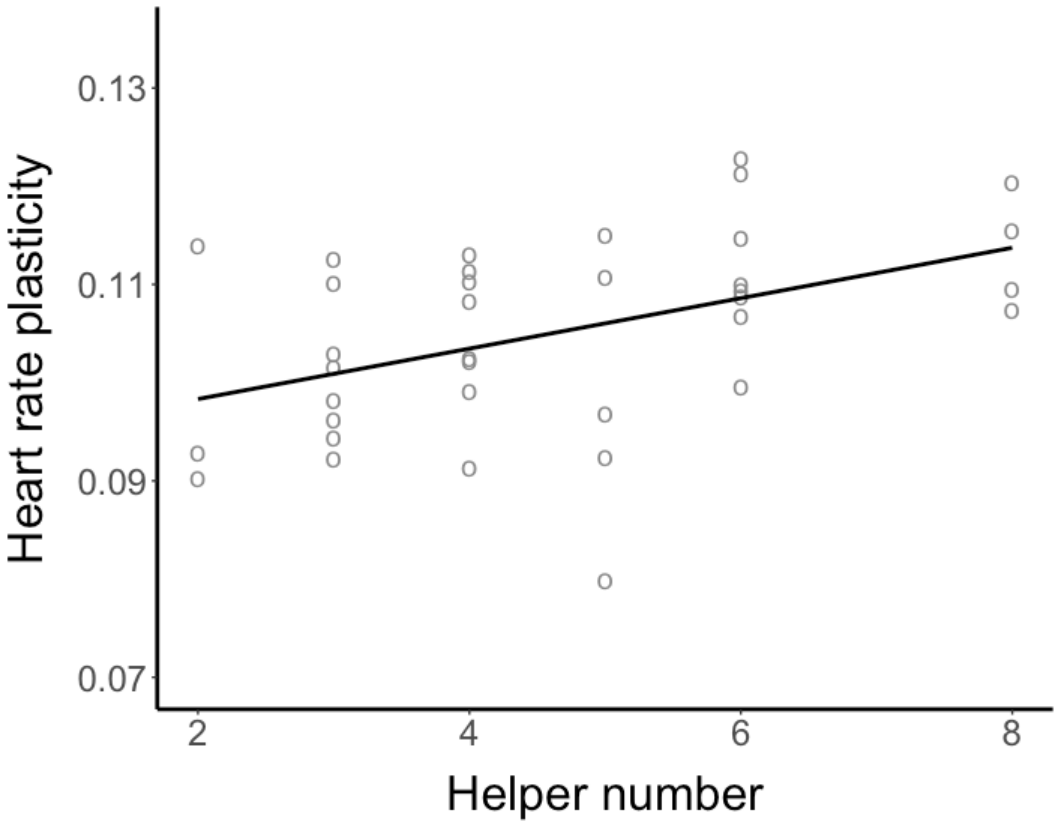

Figure 7. **Helper Effect on Heart Rate Plasticity.** Strong positive effect of helper number on heart rate plasticity (i.e. the value of the slope of heart rate ~ temperature) as predicted from the random regression model (using BLUPs) controlling for embryonic age.

3.5 DISCUSSION

Embryonic heart rate was influenced significantly by egg age, volume, time of day and the interaction between embryo age and hatch order primarily. However, by far the greatest predictor was egg surface temperature, although the influence of temperature was modified by helper number, with those embryos from small groups on average maintaining a higher heart rate at low temperatures than those from large groups. Further the heart rate response of embryos to declining temperature was variable, indicating among-embryo variation in their plastic responses. Finally, the individual level of heart rate plasticity significantly correlated with the number of helpers in their natal group: those from small groups showed lower plasticity than those from large groups.

Heart rates and metabolic rates

Heart rates are expected to be linked to metabolic rates, and by extension developmental rates, because faster growth requires higher metabolic rates and so more oxygen (Ar & Tazawa 1999; Green 2011). Although I was not able to measure metabolic rate directly, given that metabolic rate is known to be temperature-dependent, and I found that egg surface temperature explained 70% of the variance in heart rate, which suggests that heart rate responses correlate with metabolic responses (Gillooly et al. 2001). In further support, heart rates increased significantly with embryonic age, which is expected because during development, older individuals have higher metabolic demands owing to their greater number of cells (Tazawa, Watanabe & Burggren 1994). Similarly, heart rate was elevated in larger eggs, which is again expected with increased metabolic demand, since larger eggs contain larger embryos in birds (Krist 2011). Taken together, these results are consistent with the possibility that heart rate is strongly associated with metabolic rate.

Positive helper effects

This study provides some of the first evidence that avian embryonic heart rates show among-individual variation in their plastic response to temperature within the same population. In addition, this individual variation in plasticity was correlated with the number

of helpers in the group. Embryos laid and incubated by mothers in groups with few helpers retained significantly higher heart rates at low temperatures compared with those laid and incubated by mothers in large groups. This effect is unlikely to be generated by genetic differences, since groups sizes are highly variable within and between years for a given female (Russell 2016), meaning there is little scope for a link between genotype and group size in this species. By contrast, variation in plastic responses are expected because I previously showed that clutches incubated by females in small groups are heated for shorter bouts of time and so experience more time at lower temperatures than those incubated by females in larger groups. These differences were non-trivial: females in larger groups spend an extra hour on the nest per day incubating, meaning that embryos from those groups likely spend more time at developmentally favourable temperatures. In contrast, I did not find and effect of the days that embryos spent in the incubator (versus in the nest) on mean heart rate. However, we were not able to know exactly how long each embryo or clutch had been incubated in the wild. Thus, a change in developmental rate driven by artificial incubation may cloud our estimates of time spent in the nest. Finally, there may have been confounding factors that affected our ability to find nests early in the incubation duration.

The increased plasticity of heart rate in response to temperature may benefit embryos from groups with more helpers by helping them to conserve energy in sub-optimal developmental temperatures (i.e. not wasting energy by maintaining a high heart rate in lower temperatures). In contrast, the reduced plasticity of heart rate in response to temperature may benefit embryos from groups with fewer helpers by increasing overall developmental rate. This highlights the fact that higher levels of plasticity may not always be adaptive for all individuals and that this could drive and maintain variation in plasticity within populations. Embryonic developmental rate is positively tied to temperature (Gillooly & Dodson 2000). Thus, embryos need a certain amount of time at developmentally favourable temperatures in order to develop and hatch. Embryos that experience delayed hatching waste energy as they need to spend extra energy on maintenance during growth (Martin & Schwabl 2008). Further, females that have lower attentiveness must spend more days on the nest, wasting their energy on incubation,

which is known to be costly for future survival and reproduction (Visser & Lessells 2001). Additionally, delayed hatching increases the risk of the embryo dropping below a viable temperature and being predated (Conway & Martin 2000). Thus, it might benefit embryos that experience less favourable developmental temperatures to maintain growth during colder temperatures by maintaining increased heart rate (Du et al. 2010a). This strategy may not pay off for embryos that experience favourable developmental temperatures as expending energy maintaining high heart rates in colder temperatures is costly (Webb 1988). This would support our finding of variation in plasticity in our population, as helper number correlates with that variation.

Plasticity and mechanism

Currently, the mechanism driving this variation in embryonic heart rate is unknown. As helper number is not heritable, this variation is likely due to a maternal effect. One possibility is that physical properties of the egg are driving this variation. For example, egg porosity can affect the ability of embryos to metabolize efficiently (Tullett & Deeming 1982). The porosity of the eggshell can affect the oxygen consumption efficiency of the embryo and heart rate is correlated to oxygen consumption (Ar & Tazawa 1999). Therefore, the strength of the effect of temperature on heart rate may vary due egg porosity which could affect mean heart rate, which I have shown correlates with heart rate plasticity. Additionally, the physical constituents of the egg and/or the egg size or shape may play a role in heart rate and variation amongst embryos from groups with different helper numbers. I did find that egg volume correlated with mean heart rate, but that this effect only explained a small amount of the variance in heart rate. Another possibility is that embryos that consistently experience lowered temperatures during incubation acclimate to maintaining high heart rates in colder conditions. A similar study that investigated the heart rates of fence lizard (*Sceloporus undulates*) embryos found that embryos from colder climates maintained higher heart rates and consequently, developmental speeds in colder conditions than those from warmer climates (Du et al. 2010a). They suggest that this effect may be caused by a thermal acclimation effect, which could be the mechanism acting in this population. Further study is need to elucidate

the mechanism driving the correlation between helper number and heart rate plasticity in this population.

Conclusion

In this study, I found that the plasticity of embryonic heart rate is positively correlated with helper number and that this may be adaptive. I found that embryos from groups with fewer helpers spend less time at developmentally favourable temperatures due to decreased incubation attentiveness. Further, they are able to maintain higher heart rates in cold temperatures. Thus, they could mitigate the costly developmental delays caused by lowered incubation by maintaining growth at cold temperatures. Overall, this study highlights the importance of plasticity as a possible embryonic adaptation and how it may interplay with variation in parental care.

THESIS DISCUSSION

In this thesis, I investigated variation in the plasticity of incubation behaviour and embryonic heart rate within a wild population of cooperatively breeding chestnut-crowned babblers. I found that incubation effort (the amount of time the female spent on the nest) was higher in groups with more helpers. Further, females from groups with more helpers may be slightly more plastic in their incubation in response to temperature: adjusting their incubation effort more drastically in response to ambient temperature. Overall, females in groups with more helpers incubated better, they spent more time on the nest and this effect was not confounded by other factors that affect incubation efficiency such as female warming ability or nest heat retention. Thus, embryos in clutches that belong to groups with more helpers spent more time in developmentally favorable temperatures. Since embryos reduce their heart rate in lower temperatures, embryos from groups with fewer helpers are more prone to developmental delays. However, I found that embryos from groups with fewer helpers were less plastic in their heart rate in response to temperature: they maintained higher heart rates in cold temperatures. This lowered plasticity can possibly counteract the developmental delay caused by less frequent incubation.

Incubation plasticity

The efficiency of incubation, the act of transferring heat directly to developing offspring, is closely tied to ambient thermal conditions (Conway & Martin 2000). Plasticity may be a key adaption for incubation behaviour for multiple reasons. Firstly, variation in the thermal environment during incubation is costly (Nord and Nilsson 2011). Secondly, the thermal environment during incubation is likely to be stochastic and not only change within breeding attempts, but between them. Thirdly, sampling the thermal environment is straightforward as organisms do not need proxies. Moreover, signals of the thermal environment are likely to be reliable. Finally, the benefits of increased incubation efficiency via plasticity are beneficial to both parent and offspring. Increasing incubation efficiency benefits the parent by reducing unnecessary energy expenditure (Conway & Martin 2000). Incubation efficiency also benefits offspring by decreasing detrimental

thermal variation during development (Nord & Nilsson 2011). Taken together, this evidence supports our finding of incubation plasticity within our population and suggests that the plasticity is likely adaptive for mothers.

I predicted that due to incubation feeding by helpers that females with more helpers would be able to be more plastic in their incubation behaviour. Incubation is a costly investment for parents: For example, incubating pectoral sandpipers (*Calidris melanotos*) lose body mass consistently throughout the incubation duration (~24 days) (Cresswell et al. 2004). Incubating parents can counter this by plastically adjusting their nest construction, as nest thermal properties has been shown to directly affect fledgling success (Deeming & Pike 2015). However, I did not find helper effects on nest warming and cooling rate. Instead, I predicted that incubators could reduce their incubation energetic costs by maximizing thermal efficiency via adjusting incubation behaviour in response to ambient temperature. I predict that females are limited by food and leave the nest in order to forage and since helpers feed the female during incubation, that females with more helpers can increase maternal investment into incubation (Russell 2016). There is evidence for this presumption in other intermittent incubators. For example, experimental food supplementation significantly increased time that northern mockingbirds spent incubating (*Mimus polyglottos*) (Londono, Levey and Robinson 2008). Indeed, I did find that females with more helpers spent more time on the nest between off-bouts (Chapter 2: Figure 2c).

I also found that females significantly varied in their plasticity of incubation in response to ambient temperature: they varied in their increase in on-bout duration as a function of higher ambient temperatures (Chapter 2: Figure 4a). However, I did not find strong evidence that helper number was positively correlated with this plasticity, although I did see a positive trend (Chapter 2: Figure 5). I predicted that helper number would positively correlate with increased plasticity as females can be choosier about when they leave the nest to forage in order to maximize thermal incubation efficiency as they are less likely to be food-limited (Russell 2016). This could be driving the trend, but further evidence is needed to confirm that this effect is of biological relevance. I also checked whether female age affected plasticity but did not find any statistical support. Thus, there is significant

individual variation in incubation plasticity that remains unexplained. Further studies in multiple species are needed to uncover the source and possible maintenance of variation in incubation plasticity. I have shown that variation in incubation plasticity exists in a wild population and further studies are needed to investigate possible variation in incubation plasticity in other species and assess the effects of incubation plasticity on individual fitness.

Heart rate plasticity

I predicted that embryos are plastic in their heart rate in response to temperature, that this plasticity may vary amongst individuals and that helper number may correlate with that variation. Indeed, I found that heart rate was plastic in response to temperature (Chapter 3: Figure 6a) and that temperature explained a large portion of the variance in heart rate. This finding was predicted in light of evidence that heart rate decreases with temperature in avian embryos as they are essentially ectothermic (Andrewartha, Tazawa & Burggren 2011). I also found that heart rate increased with embryonic age and volume, as expected as metabolic costs scale with embryonic age and mass, necessitating increased gas exchange (Chapter 3: Figure 6b and 6c). Interestingly, I found that heart rate is plastic in response to time of day when controlling for temperature: heart rate peaked at midday (Chapter 3: Figure 6d). This could be due to a circadian response of the embryo, which has been demonstrated in chicken embryos (Moriya et al. 2004). These results demonstrate that embryos are plastic in response to a multitude of factors (e.g. age and time of day), which could also be explored in future studies.

Plasticity is often seen as an adaptive trait; however, I posit that decreased plasticity may also be adaptive. In chapter 3, I found that embryos from groups with fewer helpers adaptively decreased the plasticity of their heart rate in response to temperature. By decreasing the plasticity of their heart rate, they are predicted to decrease the time needed until hatching (Ar & Tazawa 1999; Du et al. 2010a). Delayed hatching is costly for embryos and parents due to increased energy expenditure by both parties and increased risk of predation (Martin et al. 2015). The decreased plasticity I found in embryos from groups with fewer helpers may be adaptive as they experience more

cooling time as females spend less time on the nest, as I showed in chapter 2. As such, they are already prone to experiencing delayed hatching and increased predation risk due to their lower helper number. Thus, lack of heart rate plasticity may be a unique adaptation for them. However, I found that those with more helpers are more plastic, which can be adaptive as well, but in a different way. Embryos from groups with more helpers are less likely to experience developmental delays and predation due to high helper number and thus, high incubation and sentinelling effort. In this way, the benefits of plasticity could outweigh the costs. Plasticity of heart rate allows embryos to conserve energy when experiencing detrimental developmental conditions (Durant, Hopkins and Hepp 2011). Maintaining a high heart rate in cold conditions is more energetically costly and since embryos are only imbued with a finite set of resources, maximizing heart rate efficiency is beneficial (Olson, Vleck & Vleck 2006; Du et al. 2010a). These results suggest that variance in plasticity correlated with an interplay of intrinsic and extrinsic factors that are unique to each individual. This could induce variation in the plasticity of individuals within populations, altering the predicted evolution of that population as a whole. Thus, studies are needed to assess the population scale impacts of variation in plasticity over time. Further, I found that there is significant variation in plasticity during development and studies are need to understand the effects of this plasticity on individual survival and fitness in later life stages.

The interplay of development and parental care

In this thesis, I have shown that there is an interesting interaction between parental adaptation and embryonic adaptation. I showed that helper number correlated with differences in incubation effort and possibly, plasticity. This increased incubation effort in groups with more helpers in turn affects the environment of offspring. Females from groups with more helpers spent more time on the nest, which in turn can decrease costly thermal variation in their offspring's developmental environment. Further, the positive trend of helper number on incubation plasticity in response to temperature may further reduce embryonic thermal variation. I showed that helper number positively correlates with embryonic plasticity in response to their environment. Plasticity of embryonic heart rate could be more beneficial for those that develop in favorable environments: in this

case, those that belong to groups with more helpers. Thus, the social environment of the incubating female (i.e. helper number) likely influenced her incubation behaviour, which could have influenced the developmental environment of embryonic offspring. This thesis highlights the importance of understanding the interplay of development and parental care in order to understand them each individually. Without understanding the influence of variation in parental care on embryonic environment, my result of variation in the plasticity of embryonic heart rate would be harder to interpret. Many studies investigate embryonic development completely in a lab setting, which neglects the influence of the complex factors that affect selection upon embryonic development, such as incubation behaviour and nest quality. Furthermore, these differences in parental adaptations created variation in selection for plasticity in our population. This variation can be not only adaptive, but can influence the direction of evolution of the population as a whole.

Conclusions

Overall, this thesis aimed to investigate the causes and consequences of variation in the plasticity of parental care and development. I found that chestnut-crowned babblers are plastic in both incubation behaviour and embryonic heart rate in response to the thermal environment. I also found significant individual variation in the plasticity of both traits within the population. I have shown that variation in parental care, in this case incubation, could be affecting selection on offspring developmental plasticity by influencing embryonic environment. The results from this thesis demonstrate that there is individual variation in plasticity within our wild population and that this variation may be adaptive.

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