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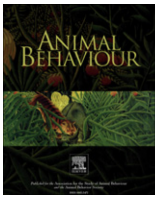

Context-dependent costs of incubation in the pied flycatcher

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Although previously disputed, it is now clear that the demands from avian incubation put parents under considerable energetic stress, sometimes to an extent where the costs of incubation constrain clutch size evolution. However, the behavioural mechanisms involved in manifesting such costs remain largely unknown. We manipulated the demands of incubation by enlarging and reducing clutch size during 2 years in a pied flycatcher, *Ficedula hypoleuca*, population in southern Sweden, and measured the resulting effects on incubation temperature and incubation behaviour. In addition, we assessed possible effects on later reproductive stages by restoring clutch size prior to hatching and subsequently monitoring female nest provisioning and nestling growth rate. The length of both attentive and inattentive bouts, as well as the total time spent incubating, was longer for females incubating enlarged clutches. These females also maintained eggs at lower temperatures, but only at the beginning of incubation. Thus, increased incubation demands were met by investing more time in incubation, but females were still not able to maintain incubation temperature at the same level as control females. Furthermore, females paid costs of increased incubation demands in terms of a longer incubation period in both years, and reduced nestling production in one, but did not appear to transfer any additional costs to their nestlings. We conclude that costs of incubation are context dependent, and suggest that the demands from incubation may be important in brood size determination in this species, at least in deteriorating ambient conditions.

Life history theory predicts that parents should produce the number of offspring that maximizes lifetime fitness. In birds, research in this area has been strongly influenced by the findings of Lack (1947), who argued that brood size in birds is ultimately determined by the number of nestlings that parents can successfully provision. It has since been found that parents frequently produce clutches smaller than predicted by their nestling feeding capacity and, in contrast to Lack's (1947) suggestions, reproductive success is sometimes higher for parents tending experimentally enlarged broods (Tinbergen & Sanz 2004). The difference between clutch size and the most productive brood suggests that demands from earlier reproductive stages may be important in clutch size determination (reviewed in Monaghan & Nager 1997). While this notion has received comparatively little attention, it is now generally accepted that both egg production and incubation often warrant substantial reproductive effort, and thus must be taken into account in the evolution of clutch size.

At least superficially, avian incubation appears to be an undemanding process during which parents remain relatively inactive in more or less well-insulated nests. However, foraging opportunities are often restricted for an incubating bird (Reid et al. 2002) and, because temperature outside the nest rarely matches the optimal requirements of embryos (35–38 °C; Webb 1987; but see De Marchi et al. 2008), parents must actively produce enough heat to provide a high and stable thermal environment regardless of ambient conditions. Thus, outside thermoneutrality most birds spend considerable amounts of energy on incubation (Williams 1987, 1996; Williams & Dwinnel 1990; Thomson et al. 1998a; Tinbergen & Williams 2002). This workload may sometimes even approach that of birds feeding nestlings in some species (Williams 1993; Thomson et al. 1998b).

Although a substantial parental effort is involved, a critical assessment of the role of incubation in clutch size determination requires experimental manipulation of incubation effort. To date, the majority of such studies have focused on adding or removing eggs from the clutch for the duration of incubation and measuring subsequent effects on adults and nestlings. This work has resulted in accumulating evidence that clutch enlargement directly impinges on a wide array of potentially important fitness correlates, such as adult survival (Visser & Lessells 2001; de Heij et al. 2006) and fecundity (Hanssen et al. 2005), as well as nestling production (Moreno et al. 1991; Siikamäki 1995; Reid et al. 2000b; Engstrand & Bryant 2002; Ilmonen et al. 2002; de Heij et al. 2006) and quality (Heaney & Monaghan 1996; Cichon 2000). Experimental clutch enlargement is frequently also associated with a protracted incubation period (Smith 1989; Siikamäki 1995; Dobbs et al. 2006).

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An integrated understanding of the proximate nature by which the incubation of enlarged clutches reduces reproductive success is fundamental in furthering our knowledge on the ecology of avian incubation. However, surprisingly little attention has been paid to explaining how reproductive costs are manifested in parents and subsequently transferred to nestlings. Parents sustain higher workloads when incubating enlarged clutches (Moreno et al. 1991; Moreno & Sanz 1994; but see Engstrand et al. 2002; de Heij et al. 2008), and normally also increase their metabolic rate while on the nest (Biebach 1981, 1984; Vleck 1981; Haftorn & Reinertsen 1985; Weathers 1985; de Heij et al. 2007; Nord et al. 2010), thereby depleting body reserves at a higher rate. Such energetic costs probably affect the thermal environment that parents are able to provide for developing nestlings, potentially at the cost of reduced embryonic growth and development (Webb 1987; Nilsson 2006; Nord & Nilsson 2011). In line with this, zebra finches, *Taeniopygia guttata*, do not maintain clutch temperature when incubating enlarged clutches (Nord et al. 2010) and female tree swallows, *Tachycineta bicolor*, are energetically constrained from maintaining optimum incubation temperature when ambient conditions deteriorate (Ardia & Clotfelter 2007; Ardia et al. 2010) and, conversely, invest more heavily in warming eggs when costs are reduced (Ardia et al. 2009). If parental maintenance of incubation temperature is constrained by the demands from incubation, quantifying temperature will be important in understanding how incubation varies qualitatively between nests with different clutch sizes. In addition, a thorough consideration of incubation temperature, in parallel with behavioural adjustments, is ecologically relevant, because it directly reflects parental investment in nestling development.

In this study, we experimentally manipulated clutch size during the incubation stage in a pied flycatcher, *Ficedula hypoleuca*, population in southern Sweden. By monitoring incubation temperature and incubation behaviour at enlarged and reduced clutches, we aimed to clarify the mechanisms underlying potential fitness costs of incubation, and ultimately to address whether demands from incubation are important in clutch size determination in this species. We predicted that the higher energetic demands associated with the incubation of enlarged clutches would decrease the length of attentive bouts and increase the length of inattentive bouts, and also result in decreased incubation constancy and incubation temperature. By analogy, we expected females on reduced clutches to spend more time incubating, while simultaneously maintaining eggs in more favourable thermal conditions. Provided that enlarged clutches were incubated at lower temperatures, we also expected nestlings from these clutches to be phenotypically smaller throughout the developmental period, because low incubation temperature may directly reduce the size and growth of altricial nestlings (Olson et al. 2006; Nord & Nilsson 2011).

METHODS

Study Area

The experiment was conducted in a nestbox-breeding population of pied flycatchers from May to July 2007 and 2008 in the Vomb area, about 25 km east of the city of Lund in southern Sweden (55°39′N, 13°33′E). The study area is part of a continuously forested pine, *Pinus sylvestris*, plantation with a dense deciduous understory composed mainly of oak, *Quercus robur*, birch, *Betula pendula*, rowan, *Sorbus aucuparia*, and sallow, *Salix* spp. The area contained approximately 200 nestboxes scattered over 219 ha that have been monitored since the early 1980s.

General Procedures and Clutch Size Manipulations

Pied flycatchers in our population commence breeding during mid-May. The female produces a clutch of six to seven eggs, which she incubates alone for some 12 days (Lundberg & Alatalo 1992). We visited nests at least once weekly during nest building and egg laying to determine clutch initiation date, and from the fifth egg onwards (assuming one egg was laid per day) every other day to determine clutch size. Incubation was arbitrarily defined to start on the day of clutch completion (incubation day 0). Two days later (i.e. on incubation day 2) we experimentally reduced or enlarged clutches in a random order by moving two eggs between pairs of nests ($N = 17$ pairs in both years; transport time ≤ 10 min) with similar clutch sizes (±1 egg) and start of breeding (±1 day). We randomly allocated some nests to act as controls ($N_{2007} = 10$, $N_{2008} = 11$). We sham-manipulated half of these nests on day 2 by temporarily removing two eggs for 5 min. Incubating females were temporarily removed from the nest and kept in the hand while the clutch size or control manipulations proceeded. All control females, including those that were not sham-manipulated, were handled in the same way. The whole operation took less than 30 s, after which females were put back on the eggs. Transferred eggs were uniquely marked with a permanent, nontoxic, felt-tipped pen for subsequent identification. The original clutch size was restored on the morning of day 11 (i.e. 1 day before estimated hatching; see below) by returning the original eggs. Original clutch size (reduced: 6.6 ± 0.1; control: 6.2 ± 0.2; enlarged: 6.6 ± 0.1), the start of breeding and the date of manipulation did not differ between treatments ($P = 0.08$, 0.99 and 0.98, respectively). Following the experimental manipulations, the mean increase in clutch size in enlarged broods ± SD was 30 ± 0.5% and the mean decrease in reduced broods was 31 ± 0.7%. This resulted in a significant difference in clutch size between all treatment categories ($P < 0.001$ for all pairwise comparisons).

Incubation Behaviour and Clutch Temperature

To monitor incubation behaviour and clutch temperature we positioned a small temperature data logger (iButton DS1922-L, Maxim Integrated Products Inc., Sunnyvale, CA, U.S.A.; accuracy = 0.5 °C, diameter 17 mm) beneath the clutch in the centre of the nest cup when performing the clutch size or control manipulations on day 2. The data logger, which was set to record temperature in 1 min intervals with a resolution of 0.0625 °C, was replaced with an identical unit on day 5, and was subsequently left in the nest until day 8. Because pied flycatchers construct solid nests composed of root fibres and dry grass with a deep nest cup (Lundberg & Alatalo 1992), the logger stayed in the centre of the nest with the temperature-sensitive side upwards without being covered by nest material, as confirmed by visual inspection on days 5 and 8. Inspection also revealed that eggs were always aggregated around the temperature logger, the latter therefore being in direct contact with the brood patch during incubation. Thus, the iButton recorded female heat allocation during on-bouts, and were as exposed to ambient conditions as was the clutch during off-bouts. This potentially interfered with heat transfer among eggs, but was important because it allowed us to assess incubation dynamics adequately (see below). In support of this, using the same method we have previously found that nocturnally incubating zebra finches adjust incubation temperature predictably to ambient temperature and clutch size (Nord et al. 2010). Because these measurements were performed during steady state incubation at night, this was not a result of undesired experimental artefacts, such as placement or physical properties of the data logger. We are therefore confident that our current measurements adequately reflected female heat transfer to the clutch. The ability of the temperature logger to