Sex differences in embryo development periods and effects on avian hatching patterns

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Competitive interactions among siblings can be an important determinant of parental fitness. These are strongly influenced by relative offspring size and therefore also by the extent to which parents can influence offspring size hierarchies. The temporal pattern of hatching in an avian clutch has a large effect on size and developmental disparities among chicks. Hatching spread is generally assumed to be mainly determined by the onset of incubation in relation to egg laying. However, the extent to which factors other than incubation onset, such as development rate, also influence timing of hatching has received little empirical investigation. We compared incubation periods of male and female black guillemot (Cepphus grylle) embryos to ascertain whether the time taken for an egg to hatch varies with embryo sex. Laying date and egg mass had no significant effect on incubation time, but male embryos hatched on average a day sooner than did females. The onset of incubation and hatching spread vary in black guillemots. However, in mixed-sexed clutches in which the first-laid embryo is male, a faster development time of males should mean asynchronous hatching regardless of parental incubation regime. This was supported by empirical investigation. These results demonstrate that factors other than incubation behavior can be important in establishing avian hatching patterns. Whether these sex differences in development rate are a result of constraints on the degree of parental control, or an adaptive strategy to manipulate hatching patterns, remains to be established. [Behav Ecol 15:205–209 (2004)]

The competitive dynamics among siblings can have a profound effect on their growth and survival and, thereby, also on parental fitness. Elucidating the proximate factors that determine these dynamics is therefore an important component of behavioral and evolutionary ecology. In birds, the competitive ability of chicks within a brood is strongly influenced by their relative size and developmental stage (Mock and Parker, 1997). Female parents can potentially alter brood dynamics through manipulation of various egg production parameters such as the number, size, and contents of the eggs in a clutch (see Lipar and Kettersson, 2000; Parsons, 1970; Quinn and Morris, 1986; Schwabl, 1993; Slagsvold et al., 1984; Sockman and Schwabl, 2000; Williams, 1994) and the sex of the embryos (Bradbury and Griffiths, 1999). However, it is generally considered that the temporal pattern of hatching is of particular importance in determining competitive dynamics among chicks within a brood. This is by virtue of the initial size disparity and the ensuing self-perpetuating growth and competitive advantages that even a small amount of hatching asynchrony can establish (Bancroft, 1984; Bryant, 1978; Howe, 1978; Lamoy, 1990; Magrath, 1992; Ricklefs, 1984; Stokland and Amundsen, 1988; Vinuela, 1997). Although many hypotheses have been put forward to account for the evolution of hatching asynchrony in birds (for reviews, see Amundsen and Stokland, 1988; Clark and Wilson, 1981; Magrath, 1990; Stoleson and Beissinger, 1995), two fundamental aspects have largely been ignored: (1) the underlying proximate mechanisms that determine hatching spread (Stoleson and Beissinger, 1995), and (2) the extent to which asynchrony is in fact under parental control (Magrath, 1992). Most studies of hatching asynchrony assume that eggs within a clutch develop at more or less identical rates for a given effort of incubation and that, as a consequence, the temporal pattern of hatching is mediated via the timing of effective incubation in relation to the laying sequence (see Clark and Wilson, 1981; Drent, 1975). Although some support for this assumption is derived from studies in which eggs hatch according to laying order (Cargill and Cooke, 1981; Inoue, 1985; Magrath, 1992), the role of parental incubation behavior as the primary determinant of hatching spread has received only modest empirical scrutiny (Stoleson and Beissinger, 1995; Viega and Vinuela, 1993). Indeed, the general consensus has been that the ability to influence hatching patterns independently of incubation regime is restricted to some precocial species, in which synchrony is achieved either through accelerated hatching of eggs laid toward the end of the laying sequence (Davies and Cooke, 1983; Vince, 1964) or through retardation of development in more advanced embryos; such effects are mediated largely through calls emitted by the embryos themselves (Persson and Andersson, 1999; Vince, 1968). However, recent studies have shown that a number of nonprecocial species also have the ability to influence hatching patterns by means other than parental incubation regime. In these studies, factors such as egg-size, laying order and laying date affected embryo development rates (St. Clair, 1996; Vinuela, 1997). Moreover, circumstantial evidence suggests that disparate development rates may be more prevalent among birds than previously considered.

One potentially important factor that could influence the development time of an embryo is its sex. Sex differences in development rate occur in many taxa and, although generally linked to sexual size dimorphism, are also likely to be influenced by hormonal and other factors that differ between the sexes during development; such differences may be mediated maternally or by the phenotype of the developing embryo itself (Anderson et al., 1997; Badyaev, 2002; Burke, 1992; Cordero et al., 2001; Kochhar et al., 2001; Mead et al., 1987; Petrie et al., 2001; Sinervo, 1990). Maternal manipulation of individual embryo development rate...
through control of egg contents is conceivable in birds and other egg-laying vertebrates because eggs are self-contained developmental units. This contrasts with the eutherian mammal system in which developmental resources are supplied to all siblings from a single source, the uterine blood supply, which limits maternal control. Although gender-mediated effects on hatching patterns have been reported in captivity in domesticated avian species, (Burke, 1992; Davies and Payne, 1982), no such effects in a wild bird population have yet been reported.

The black guillemot (Cepphus grylle) is a sexually monomorphic seabird that exhibits marked interpair variation in a number of breeding parameters. Although the majority of breeding pairs lay a two-egg clutch, variation among pairs has been reported in the interval between laying the two eggs, the overall incubation period, and in whether or not the two eggs hatch on the same day (Asbirk, 1979; Ewins, 1989). In the present study, we examined the extent to which male and female eggs differed in their development rates, and whether this is linked in the predicted manner to the temporal pattern of hatching.

METHODS

Study species and site
Black guillemots are burrow or cavity nesting seabirds. Our data were collected on the Holm of Papa Westray, Orkney, Scotland (59°22'N, 2°53'W), from May–August in the years 1996 and 1997. The Holm is approximately 0.8 × 0.3 km, is uninhabited by humans, is free from mammalian predators, and has a colony of approximately 65 breeding pairs of black guillemots.

Egg laying and embryo development period

Nests were checked daily for the presence of an egg so that the laying date of each egg and laying interval for each clutch were obtained. On the day of laying, eggs were weighed to the nearest 0.1 g by using a 200-g electronic balance, and each egg was marked according to its laying sequence by using a permanent marker pen (a- and b-eggs refer to first- and second-laid eggs, respectively). On completion of the clutch, nests were undisturbed until a few days before their estimated hatching date (eggs are incubated for approximately 28 days; Ewins, 1986), whereupon daily nest checking resumed to determine hatching date, and thereby development period, and the hatching interval between the eggs. Because the first signs of hatching (i.e., pipping) can be seen at least 2 days before the chick emerges from the shell (Harris and Birkhead, 1985), we could identify the approximate level of hatching spread before hatching. In those nests in which it appeared that the clutch would hatch synchronously, we undertook more frequent checks to ensure that the egg origin of each chick was ascertained. This also provided an accurate measure of whether a clutch hatched synchronously (within 12 h) or not. All eggs hatched according to laying order, and in all clutches that we predicted to hatch synchronously, eggs hatched within 12 h of each other. Within each brood, siblings are referred to as either a- (first-hatched) or b- (second-hatched) chicks. To facilitate sibling identification, the a-chick in each brood was regularly marked on the head with a small streak of correcting fluid (Tipp-Ex®).

Initiation of incubation relative to clutch completion in the black guillemot is variable. Full incubation of the first egg begins before the second egg is laid in some broods, whereas in others full incubation starts only on clutch completion (Asbirk, 1979; Cook MI, Monaghan P, personal observation). Thus, the onset of incubation is variable with respect to a-eggs, making it difficult to examine development period accurately, but b-eggs are fully incubated from the day of laying. Accordingly, we used the incubation period of b-eggs to compare male and female embryo development times. Because all eggs were second-laid eggs, this had the added advantage of removing any potentially confounding effects of laying order.

Identification of embryo sex
Chick sex was ascertained by using a molecular DNA technique based on the presence or absence of the highly conserved W-linked CHD1 gene (chromodomain-helicase-DNA-binding protein W-linked) using genomic DNA isolated from blood (live chicks) or muscle samples (for chicks that died before blood samples could be obtained) using the procedure of Griffiths et al. (1996). Blood samples from live chicks were obtained under a UK Home Office license from 5–20-day-old nestlings by means of tarsal venepuncture using a sterile hypodermic needle. Approximately 50 μl of blood was transferred via capillary tube to an equal volume of BLB buffer (2% sodium dodecyl sulphate [SDS], 50 mM EDTA, 50 mM Tris at pH8). Blood samples were kept cool in the field, placed in a refrigerator within 5 h of collection, and returned to the laboratory for sexing. Chicks appeared unaffected by this procedure.

After digestion of a small portion of the sample by proteinase K digestion for 12 h, DNA was extracted by using phenol/chloroform and recovered by ethanol precipitation. The protocol for the extraction of muscle DNA followed that of the blood samples, but muscle tissue required at least 48 h in proteinase K for complete digestion. Polymerase chain reaction (PCR) amplification of DNA followed that of Griffiths et al. (1996). The PCR products were subsequently electrophoresed in agarose gel and photographed under ultraviolet light.

Data analysis
Because data met the parametric assumptions of normality and homoscedasticity, appropriate parametric statistics were applied. To examine the influence of embryo sex on development period, we used an ANCOVA (general linear model procedure) with egg mass and laying date as covariates. Laying dates for each of the 2 years over which data were collected were standardized by subtracting the date of clutch initiation from the median laying date of that year. Normality was tested by using the Kolmogorov-Smirnov goodness-of-fit test, and all probabilities given are two-tailed. Analyses were undertaken by using SPSS for Windows 7, and all tests were as described by Zar (1996).

RESULTS

Development period
During the breeding seasons of 1996 and 1997, we examined 59 b-eggs in 42 nests. For 17 of these nests, data were collected in both years. To avoid possible pseudoreplication, we randomly assigned each of these nests to one of the 2 years, and used only data from the allocated year in the analysis. Mass of b-eggs did not differ between years (1996: mean mass = 47.7g, n = 40, SD = 4.2; 1997: mean mass = 47.6g, n = 23, SD = 3.1; t40 = 0.11, p = ns).

Figure 1 shows the mean development period in days for the male and female b-eggs. Development period was not
influenced by egg mass or laying date; however, there was a highly significant difference between the development period of male and female embryos, with the mean male development time being shorter (ANCOVA effect of sex: $F_{1,40} = 11.6$ and $p < .002$; effect of egg mass: $F_{1,39} = 0.38$, $p = ns$; effect of laying date: $F_{1,38} = 0.75$, $p = ns$; all interactions, $p = ns$).

**Hatching pattern**

This sex difference in development period could therefore influence the temporal pattern of hatching. In mixed sex clutches where a male is laid first (M-F clutches), the onset of incubation before the first egg is laid will increase the degree of asynchrony; even if incubation begins simultaneously on the two eggs, the male should still hatch about a day before the female. Hatching synchrony should not occur. All such clutches should be asynchronous, with the male hatching first. We examined the incidence of synchronous (same day) and asynchronous hatching in mixed sex clutches in which a male was laid first. The predicted pattern of hatching was observed; of 17 such M-F clutches in which hatching pattern was known, none hatched synchronously. In contrast, in 18 same sex clutches (M-M and F-F clutches), we found that nine hatched on the same day, and the other nine hatched asynchronously, a highly significant difference from the pattern found in the M-F clutches ($X^2 = 15.05$ and $p < .001$). In mixed sex clutches in which a female is laid first (F-M clutches), both synchrony and asynchrony in hatching is possible. However, we did not have a sufficient sample of such F-M broods to compare with the other combinations, because this combination is rare in our study population (Cook, 1999).

**DISCUSSION**

In the present study, we found a difference between male and female embryos in the time from the onset of incubation to hatching, with males hatching on average about 1 day sooner than did female embryos. Other factors that might potentially influence embryo development times in birds include egg size and laying date. Rahn and Ar (1974) demonstrated a clear positive relationship between egg mass and incubation period among avian species, but the results of studies investigating such effects within species are equivocal (Viñuela, 1997). For example, although some studies are in accord with the results of Rahn and Ar (1974), others have failed to show a significant effect of egg mass (see Drent, 1975; Kattan, 1995; Martin and Arnold, 1991; Parsons, 1972; Ricklefs and Smeraski, 1985; Runde and Barrett, 1981). Back guillemots are sexually monomorphic, and we found no effect of egg mass on development period in this species. Variation in the degree of egg mass variation within a species, and the possibility of interacting egg compositional changes, may account for the differing results between studies. A reduction in incubation period as the season progresses has been demonstrated for a number of species (see Parsons, 1972; Runde and Barrett, 1981; St. Clair, 1996), although interpreting such effects is often statistically problematical owing to the confounding effects of other breeding parameters and environmental factors that also vary with date (St. Clair, 1996). Again however, we found no effect of laying date on development rate in the black guillemot. Given that the black guillemot is unusual among temperate and arctic seabird species in that relatively few breeding parameters are affected by season (Cook, 1999), it is perhaps not surprising that date of clutch initiation had no effect on development in this species.

For the present study, we used b-egg incubation period to ascertain development rates because we could not accurately determine the onset of incubation of a-eggs. Egg size generally decreases with laying order in the black guillemot (Ewins, 1986, 1989; Cook MI, Monaghan P, unpublished data), but because development rate was not influenced by egg size, it is likely that the observed differences in development rate will be similar in a-eggs. This is supported by the pattern of hatching in M-F clutches, which hatched only asynchronously, as expected. For such clutches, parents would only be able to alter the degree of hatching asynchrony but could not achieve a synchronously hatching brood. On the other hand, the sex difference in development period could give parents an additional means of influencing hatching patterns independently of incubation regime if they could control the sex of their brood. In the black guillemot, for example, an asynchronous brood could be achieved by laying a M-F clutch, without the need for an early onset of incubation. Starting incubation before the clutch is complete may be incompatible with the demands of egg formation for some females. The capacity to facultatively adjust the production of male and female offspring has been demonstrated in a number of bird species (for a review, see Sheldon, 1998), and maternal condition has been shown to be an important factor (Albrecht, 2000; Korpimaki et al., 2000; Nager et al., 1999; Wittingham and Dunn, 2000). Alternatively, if females cannot control the sex of the eggs that they lay, sex differences in embryo development rates will constrain the extent to which optimum hatching patterns can be attained.

A number of potential mechanisms may underpin the observed sex differences in development period. Given that the black guillemot is sexually monomorphic, differences in development rate are not linked to sexual size dimorphism, as is the case in many other species (Badley, 2002). A likely candidate for effects on development rate is the hormonal environment during development, which is influenced by both maternally derived hormones present in the yolk and, later in development, by the embryo itself. Both positive and negative effects of maternally derived androgens on offspring growth and development have been reported in birds (Eising et al., 2001; Schwabl, 1997; Sockman and Schwabl, 2000). Eising et al. (2001) recently reported that black-headed gull eggs injected with androgens hatched sooner, although the opposite was found to be the case in American kestrels (Sockman and Schwabl, 2000) suggesting possible interspecific differences in the sensitivity of embryos to maternal androgens. Of particular relevance here is the finding that maternally derived yolk hormones can influence the
development of the hatching muscle (Lipar and Ketterson, 2000), which suggests a possible route whereby females could selectively influence the time taken to hatch. Alternatively, the shorter development time of male black guillemots may, as with mammals, be a feature of the development of the male phenotype and not something that females can alter independently of offspring sex.

However the reduction in the development time of males is achieved, the present study demonstrates that factors other than incubation regime can be important in determiningavian hatching patterns and hence the competitive dynamics of the brood. Not only may the determinants of embryo development period provide clues as to the potential constraints operating during incubation but also, until these determinants are established, designating an adaptive function to a species’ hatching pattern should be treated with caution. More studies are required to determine the general applicability of sex-specific development rates in wild birds and their role in avian breeding strategies.

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REFERENCES


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