ELSEVIER

Journal of Thermal Biology 31 (2006) 416–421

Journal of THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Effects of early incubation constancy on embryonic development: An experimental study in the herring gull *Larus argentatus*

Sin-Yeon Kim, Pat Monaghan*

Division of Environmental and Evolutionary Biology, IBLS, University of Glasgow, Glasgow G12 8QQ, UK

Received 23 November 2005; accepted 7 February 2006

Abstract

1. In many birds, parental nest attendance in early incubation is variable, with eggs incubated only intermittently. The effect of this on chick hatching success is unknown.
2. We allowed clutches of a semi-precocial species, the herring gull, to experience different levels of early incubation constancy and examined the effects on embryonic development and hatching.
3. We found evidence that high early incubation constancy enabled some eggs to develop and hatch faster. Chicks that experienced high thermal constancy during early incubation had heavier body mass at hatching.
4. We discuss the factors that might constrain parental attendance early in incubation.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Embryonic development; Hatching; Herring gull; Incubation condition; Incubation constancy; Incubation length

1. Introduction

Most aspects of parental care involve substantial expenditure of energy by the parents (Clutton-Brock, 1991), and there is a need for parents to optimise resource allocation within and among reproductive events (Stearns, 1992). An avian reproductive event involves three readily identifiable phases: egg production, incubation and chick rearing, but the focus of experimental work on reproductive investment has been geared mainly towards the last of these. However, incubation is a very important component of avian parental care, providing a warm and steady temperature and humidity for embryo development (Deeming et al., 1987; Deeming, 2002; Reid et al., 2000, 2002; Turner, 2002). Furthermore, thermal conditions experienced during the embryonic stage may influence the performance of the chicks during the post-hatching period (Gorman and Nager, 2004), and probably beyond (Metcalfe and Monaghan, 2001).

Constancy of incubation, that is the proportion of time that eggs are in contact with an incubating parent, will determine the pattern and tempo of development. However, parental attendance can change during the early incubation period, which may be linked to external and internal factors affecting parental behaviour and resource allocation. For example, Drent (1970) showed that daily constancy of incubation in the immediate post-laying period, in particular, is lower than that of the later incubation period in the semi-precocial herring gull. It is unknown whether the pattern of parental attendance early in incubation has consequences for the subsequent development of the chicks.

In the present study, we investigated the effect of early incubation constancy (IC) in herring gulls by an exchange of clutches between nests at different stages. This experimental manipulation allowed us to examine the effects of different parental constancy early in incubation on embryonic development and hatching success.

---

*Corresponding author. Tel.: +44 141 330 6640; fax: +44 141 330 5971.
E-mail address: P.Monaghan@bio.gla.ac.uk (P. Monaghan).

0306-4565/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2006.02.002

2. Methods

2.1. Study area and species

This study was carried out at a mixed colony of herring gulls *Larus argentatus* and lesser black-backed gulls *L. fuscus* at the South Walney Nature Reserve, Walney Island, Cumbria, UK (54°08′N, 03°16′W) from early April to late July 2003. Approximately 5000 pairs of herring gulls were recorded breeding at this colony in 2003 (count organised by Cumbria Wildlife Trust). In the herring gull, eggs are laid generally every 2 days to complete a three egg clutch, and incubation is shared by both parents (Harris, 1964; Hébert and Barclay, 1986). Herring gulls generally begin incubation on the laying of the second egg (Drent, 1970). Accordingly, incubation length of the second and third laid eggs in the clutch was taken as the number of days from laying to hatching; for first laid eggs, incubation length was counted from the day of laying of the second egg. The daily IC increases over approximately the first 12 days of incubation (i.e. from the laying of the second egg), reaching a 98% IC; it then does not change until hatching of the first egg (Drent, 1970). The semi-precocial young hatch asynchronously, with the third chick hatching a day or so after the other two (Parsons, 1975).

2.2. Field methods for breeding performance

Nests in the study area were visited daily during egg laying from late April to late May until clutch completion. A total of 163 nests with a complete clutch of three eggs were randomly selected for the present study. Eggs were marked and weighed on the day of laying. Based on the normal incubation period of 27 days, we checked each nest daily (beginning 3 days before the estimated hatching date) until all the chicks were hatched to record the exact date of hatching. We weighed chicks with an electronic balance (to nearest g), and measured the wing length with a ruler (to nearest mm) on the day of hatching.

2.3. Clutch exchange experiment

We used 48 pairs of experimental nests that differed from each other in the laying date of the first egg of their clutch by 6 days. We exchanged the completed clutches pairwise in order to allow them to experience different incubation conditions during the early developmental period. Clutch exchange was conducted on day 4 of incubation (i.e. 4 days after laying of the second egg) in one nest (low incubation constancy (low IC) nest) and on day 10 of incubation in the other nest (high incubation constancy (high IC) nest). Therefore, the first and second eggs of the exchanged clutches in high IC and low IC nests had been incubated approximately 4 and 10 days each by the original parents; for the third laid eggs (laid 2 days after the onset of incubation), the incubation duration was 2 days less than this. Thus, parents that started incubation 10 days ago received clutches whose incubation had started only 4 days ago and vice versa. Following the exchange, clutches were then incubated and reared by the foster parents.

Among the remaining 67 control nests, we selected 12 pairs of two nests that had the same laying date and exchanged the clutches pairwise 4 days after laying of the second egg in the clutch to control for the manipulation itself. The proportion of chicks that hatched did not differ between the control nests with and without clutch exchange (Mann–Whitney $U = 433.50$, $n_1 = 43$, $n_2 = 24$, $p = 0.21$). Clutch mass did not differ among the treatment groups (means ± SE: control: 249.93 ± 2.21 g, high IC: 248.25 ± 2.46 g, low IC: 254.15 ± 2.25 g; Kruskal–Wallis test: $H_2 = 2.50$, $p = 0.29$).

2.4. Incubation constancy

Drent (1970) gives detailed figures for the amount of time herring gull parents incubate their eggs from the onset of incubation. We estimated daily IC, using his figures, throughout incubation in control, low IC and high IC nests. Since the incubation stage of birds on the day of clutch exchange differed between low IC and high IC nests, the IC experienced by eggs will also have differed as shown in Fig. 1. While eggs in control nests experienced daily IC of 98% from day 12 of incubation onwards, the first and second eggs in high IC and low IC nests were expected to have experienced such constancy from day 6 and day 18 onwards until hatching (2 days less than this for the third eggs).

As the clutch exchange manipulated not only the incubation conditions of the eggs but also the duration of time that incubating birds had to sit on the nests (means ± SE: control: 28.55 ± 0.10 days, $n = 56$; high IC: 34.07 ± 0.12 days, $n = 42$; low IC: 22.85 ± 0.13 days, $n = 39$), we examined whether this influenced IC in the late incubation stage. In order to estimate daily constancy

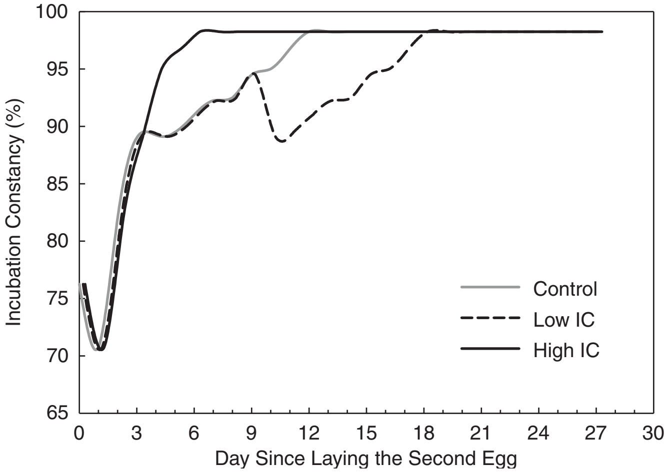

Fig. 1. Estimated daily incubation constancy in control, low IC and high IC nests. Duration of time that eggs were covered by an incubating bird was estimated from Drent (1970).

of incubation, we measured nest temperature in a sample of treatment nests. Nest temperature was measured on day 24 of incubation in control, low IC and high IC nests. We placed thermistors connected to TinyTag data loggers (Gemini Data Loggers, UK Ltd, Chichester, UK) recording temperature at 5-s intervals over 24 h on the side of the nest cup above the eggs and below the edge of the nest, such that they were in contact with incubating birds, and this allowed us to record the presence and absence of the birds. Air temperature in the vicinity of the nest site in lesser black-backed gulls in the same colony rarely rose above 30 °C during the same breeding season (temperature ranged 2.2~41.3 °C; mean of daily average temperature ± SE: 13.61 ± 0.374 °C, $n = 63$; Kim and Monaghan, 2005). Thus, we estimated IC as the daily percentage of time in which the nest temperature was above 30 °C, indicating the presence of an incubating bird. Average daily IC (duration of time with nest temperature $>30\,^{\circ}$C) did not differ among treatment groups on day 24 of incubation (mean percentage of time ± SE: control: 88.67 ± 3.07%, $n = 34$; high IC: 89.03 ± 4.14%, $n = 28$; low IC: 89.63 ± 3.69%, $n = 31$; ANOVA: $F_{2,90} = 0.02$, $p = 0.98$).

2.5. Statistical analysis

Clutch exchange was performed simultaneously for all the eggs in a clutch. Therefore, since the onset of incubation differed for the third eggs, we analysed the outcome for the three eggs in the clutch separately. We used parametric tests unless the data distributions violated the assumptions, when equivalent nonparametric tests were used. All tests were two tailed, and means ± SE are presented in the results. We performed ANCOVA tests using linear models to compare the incubation length of eggs and hatching condition of chicks among the treatment groups. For comparison of hatching success between the treatment groups, generalised linear model (GLM) with a binomial error distribution and a logit link was used (Crawley, 2003). Models initially included all explanatory variables and two-way interactions. Final models were selected by sequentially dropping nonsignificant interactions and then nonsignificant main effects. Laying date of incubating parents was taken into account in all the models since timing of breeding is correlated with breeder quality in herring gulls (Brouwer et al., 1995). Data were analysed using R v1.8.0 (2003) and SPSS v10.0 (1999).

3. Results

3.1. Incubation length

Incubation length was compared separately in each incubated egg in a clutch among control, high IC and low IC nests, controlling for any seasonal effect (laying date of the incubating parents) (Table 1). The first egg hatched earlier in high IC nests (which had experienced greater constancy in earlier incubation), and later in low IC nests (which had experienced a protracted period of relatively low parental attendance) than those in control nests (Tables 1a and 2a). A similar treatment effect appeared in the second egg, but this was only marginally significant (Tables 1b and 2b). Incubation length did not differ in the third egg among control, high IC and low IC nests (Tables 1c and 2c). Laying date affected incubation length in the first and second eggs, but not third eggs, with incubation length being shorter later in the season (Table 1a,b).

3.2. Hatching success and hatchling condition

Overall hatching success (proportion of eggs hatched in the clutch) did not differ among control, high IC and low IC nests (means ± SE: control: 0.82 ± 0.03, $n = 67$; high IC: 0.87 ± 0.03, $n = 48$; low IC: 0.82 ± 0.04, $n = 48$; GLM: $F = 0.40$, df = 2, $p = 0.67$) when the laying date of incubating parents was taken into account (GLM: $F = 5.99$, df = 1, $p<0.05$).

Body mass and wing length of chicks at hatching were compared between control, high IC and low IC nests taking egg mass into account. While hatching mass of chicks was highly correlated with egg mass as would be expected, independent of this effect, the treatment effect was significant in all three hatching positions of chicks (Table 3). Hatchling body masses in high IC nests were heavier than those in control and low IC nests at hatching in the first and second chicks (Fig. 2). However, the third hatched chicks in both high and low IC nests did not differ from each other, but were heavier than those in control nests (Fig. 2). Wing length of chicks at hatching did not differ among the treatment groups, and it was significantly correlated with the egg mass as would be expected (Table 3).

4. Discussion

Daily IC increases during the early incubation phase (approximately until day 12), after which it is constantly

Table 1
Summary of ANCOVA tests examining effects of treatment group and laying date of the incubated clutch on the incubation length in different sequences of eggs

Variable	*F*	df	*p*
(a) First egg			
Group	14.70	2, 133	<0.001
Laying date	12.43	1, 133	<0.001
Group: laying date	1.10	2, 131	0.34
(b) Second egg			
Group	2.81	2, 135	0.06
Laying date	7.05	1, 139	<0.01
Group: laying date	3.61	2, 135	<0.05
(c) Third egg			
Group	1.59	2, 126	0.21
Laying date	0.34	1, 127	0.56
Group: laying date	0.88	2, 123	0.42

Table 2
Number (%) of eggs with each incubation length in control, high IC and low IC nests

	Incubation length						
	23	24	25	26	27	28	29
(a) First egg							
Control	0 (0)	0 (0)	5 (8.9)	26 (46.4)	22 (39.3)	3 (5.4)	0 (0)
High IC	1 (2.4)	1 (2.4)	9 (21.4)	20 (47.6)	11 (26.2)	0 (0)	0 (0)
Low IC	0 (0)	0 (0)	1 (2.6)	14 (35.9)	13 (33.3)	10 (25.6)	1 (2.6)
(b) Second egg							
Control			0 (0)	17 (30.4)	34 (60.7)	5 (8.9)	
High IC			0 (0)	21 (48.8)	20 (46.5)	2 (4.7)	
Low IC			1 (2.4)	12 (28.5)	22 (52.4)	7 (16.7)	
(c) Third egg							
Control			12 (23.0)	37 (71.2)	3 (5.8)		
High IC			13 (32.5)	21 (52.5)	6 (15.0)		
Low IC			5 (13.5)	26 (70.3)	6 (16.2)		

Table 3
Summary of ANCOVA tests examining effects of treatment group and egg mass on mass and wing length at hatching in different sequences of hatchlings

Variable	Mass			Wing length		
	F	df	*p*	*F*	df	*P*
(a) First chick						
Group	4.46	2, 133	<0.05	1.85	2, 133	0.16
Egg mass	273.63	1, 133	<0.001	17.03	1, 135	<0.001
Group: egg mass	0.49	2, 131	0.62	0.80	2, 131	0.45
(b) Second chick						
Group	4.49	2, 137	<0.05	1.15	2, 137	0.32
Egg mass	686.30	1, 137	<0.001	47.97	1, 139	<0.001
Group: egg mass	0.00	2, 135	1.00	0.74	2, 135	0.48
(c) Third chick						
Group	5.79	2, 125	<0.01	0.04	2, 125	0.96
Egg mass	691.62	1, 125	<0.001	23.86	1, 127	<0.001
Group: egg mass	0.98	2, 123	0.38	0.10	2, 123	0.90

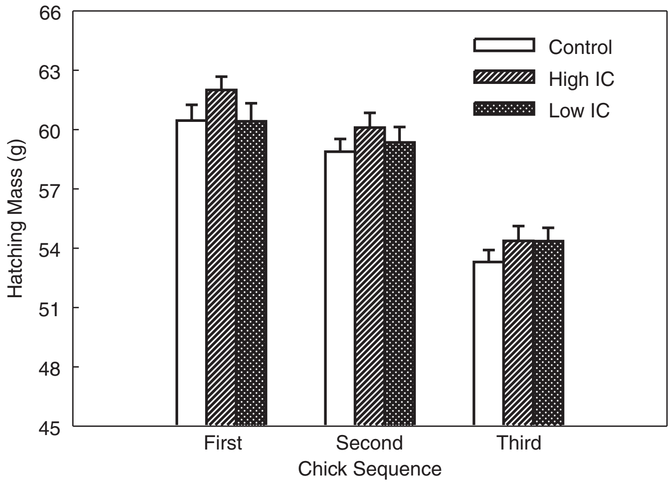

Fig. 2. Body mass at hatching of the first, second and third chicks in control, high IC and low IC nests (means ± SE).

high until hatching in herring gulls, maintaining a steady egg temperature (Drent, 1970). In our study, the IC in the late incubation stage estimated from the measurement of nest temperature (around 89%) was slightly lower than the 98% directly measured by Drent (1970), but this may be due to differences in measurement technique. Our estimates do suggest that clutch exchange did not influence IC during the late incubation phase, since there was no difference among the treatment groups.

In the present study, clutch exchange allowed eggs incubated in high IC nests to experience on average a higher IC, and those incubated in low IC nests experienced a longer period of lower IC, during the early embryonic phase. Estimated period of low IC for the first two eggs in the clutch before the maximum level is reached was 6 days in high IC nests, 12 days in control nests, and 18 days in low IC nests.

The experimental change of incubation conditions during the early embryonic phase influenced the time to hatching of eggs and the condition of hatchlings, but not hatching success. In the first laid eggs, incubation length was longer in low IC nests and shorter in high IC nests than that in control nests, whereas incubation length did not differ among the treatment nests in the second and third eggs. Other studies also showed similar effects of IC on incubation length. Experimentally enlarged clutch size increased incubation length in blue tits (Smith, 1989), and in nests where males assisted females with incubation, incubation length was shorter in starlings (Reid et al., 2002). Improved incubation conditions in high IC nests during the early incubation phase might enable the embryos to develop and hatch faster, and poorer incubation conditions in low IC nests might lead to slower embryonic development. While higher incubation temperature during the late incubation period close to hatching can also shorten the hatching time (Evans, 1990; Evans et al., 1995), this is unlikely to be the case in the present study since the IC did not differ across the treatment nests during this period (day 24). The earlier laid eggs in the clutch seemed particularly affected by the experimental treatments compared to the later laid eggs in the clutch. Since herring gulls start incubation on laying of the second eggs, the development probably had started already in the first and second eggs before exchange of the clutches (Harris, 1964; Drent, 1970; Hébert and Barclay, 1986), and thus they were presumably at a more critical developmental stage and affected by changes in IC.

Incubation levels in high IC nests during the early embryonic periods positively influenced mass at hatching in the first and second chicks. In precocious and semi-precocious avian species, embryos increase their metabolic and heart rates rapidly during the early incubation phase (Vleck and Vleck, 1980; Pearson et al., 2000). This is important for increasing the circulation of nutrients from the yolk to the embryo. It is known that incubation temperature is correlated with heart rate of embryos in gull species, and thus IC is important (Bennett and Dawson, 1979). Since thermal energy provided by the parents during the early incubation phase was more constant in high IC nests than that in control or low IC nests, the embryonic blood circulation may have been faster, and thus enabled them to increase embryonic growth (Deeming and Ferguson, 1991), and thereby to heavier mass at hatching. Alternatively, they might be able to use more nutrients for growth instead of self-maintenance by benefiting from improved incubation conditions (Vleck and Vleck, 1980). It is also possible that variation in hatchling mass correlates with different water loss during incubation. Eggs in high IC nests that had a shorter incubation might have lost less water than those in control and low IC nests, and thus the hatchlings would have been heavier.

Greater IC early in incubation generally appears to have a positive effect on chick development. In general, while the effects we observed were relatively small, the question arises as to why parents not show a higher IC from the outset. This may be a consequence of the competing demands of other activities such as nest defence at that time, or the foraging requirements of females, who still need to obtain resources for albumen production for the last egg and also recover from the demands of producing the clutch. Alternatively, there may be physiological constraints in that parent birds may need time for hormonal adjustment to incubation, for example building up of prolactin, which influences onset of incubation and IC (Meijer et al., 1990; Müller et al., 2004; Criscuolo et al., 2005). It may be that the beneficial effects in terms of chick hatching conditions do not translate into sufficient fitness benefits to outweigh the cost to parents of high IC.

Acknowledgements

We thank Liliana D'Alba for invaluable help in fieldwork. Anonymous reviewers provided very helpful comments on the manuscript. The Cumbria Wildlife Trust kindly allowed S. Y. K to conduct the research in the South Walney Nature Reserve. S. Y. K. was funded by a University of Glasgow postgraduate research scholarship and the Overseas Research Scheme. The experiments we performed did not involve any licensed procedures and complied with the current laws of the UK.

References

Bennett, A.F., Dawson, W.R., 1979. Physiological responses of embryonic Heermann's gulls to temperature. Physiol. Zool. 52, 413–421.

Brouwer, A., Spaans, A.L., De Wit, A.A.N., 1995. Survival of herring gull *Larus argentatus* chicks: an experimental analysis of the need for early breeding. Ibis 137, 272–278.

Clutton-Brock, T.H., 1991. The Evolution of Parental Care. Princeton University Press, Princeton.

Crawley, M.J., 2003. Statistical Computing: An Introduction to Data Analysis Using S-Plus. Wiley, Chichester.

Criscuolo, F., Chastel, O., Bertile, F., Gabrielsen, G.W., Le Maho, Y., Raclot, T., 2005. Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders *Somateria mollissima*, but does modify long term reproductive success. J. Avian Biol. 36, 306–312.

Deeming, D.C., 2002. Behaviour patterns during incubation. In: Deeming, D.C. (Ed.), Avian Incubation: Behaviour, Environment, and Evolution. Oxford University Press, Oxford, pp. 63–87.

Deeming, D.C., Ferguson, M.W.J., 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: Deeming, D.C., Ferguson, M.W.J. (Eds.), Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. Cambridge University Press, Cambridge, pp. 147–171.

Deeming, D.C., Rowlett, K., Simkiss, K., 1987. Physical influences on embryo development. J. Exp. Zool. 1 (Suppl.), 341–345.

Drent, R.H., 1970. Functional aspects of incubation in the herring gull. Behaviour 17 (Suppl.), 1–132.

Evans, R.M., 1990. Effects of low incubation temperatures during the popped egg stage on hatchability and hatching times in domestic chickens and ring-billed gulls. Can. J. Zool. 68, 836–840.

Evans, R.M., Wiebe, M.O., Lee, S.C., Bugden, S.C., 1995. Embryonic and parental preferences for incubation temperature in herring gulls: implications for parent-offspring conflict. Behav. Ecol. Sociobiol. 36, 17–23.

Gorman, H.E., Nager, R.G., 2004. Prenatal developmental conditions have long-term effects on offspring fecundity. Proc. R. Soc. London B 271, 1923–1928.

Harris, M.P., 1964. Aspects of the breeding biology of the gulls *Larus argentatus, L. fuscus* and *L. marinus*. Ibis 106, 432–456.

Hébert, P.N., Barclay, R.M.R., 1986. Asynchronous and synchronous hatching: effect on early growth and survivorship of herring gull, *Larus argentatus*, chicks. Can. J. Zool. 64, 2357–2362.

Kim, S.-Y., Monaghan, P., 2005. Effects of vegetation on nest microclimate and breeding performance of lesser black-backed gulls (*Larus fuscus*). J. Ornithol. 146, 176–183.

Meijer, T., Daan, S., Hall, H., 1990. Family planning in the kestrel (*Falco tinnunculus*): the proximate control of co-variation of laying date and clutch size. Behaviour 114, 117–136.

Metcalfe, N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? Trends Ecol. Evol. 16, 254–260.

Müller, W., Eising, C.M., Dijkstra, C., Groothuis, T.G.G., 2004. Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed gulls. Behav. Ecol. 15, 893–897.

Parsons, J., 1975. Asynchronous hatching and chick mortality in the herring gull *Larus argentatus*. Ibis 117, 517–520.

Pearson, J.T., Moriya, K., Yanone, M., Tazawa, H., 2000. Development and regulation of heart rate in embryos and hatchlings of gulls (*Larus schistisagus* and *Larus crassirostris*) in relation to growth. J. Comp. Physiol. B 170, 429–438.

Reid, J.M., Monaghan, P., Ruxton, G.D., 2000. The consequences of clutch size for incubation conditions and hatching success in starlings. Funct. Ecol. 14, 560–565.

Reid, J.M., Monaghan, P., Ruxton, G.D., 2002. Males matter: the occurrence and consequences of male incubation in starlings (*Sturnus vulgaris*). Behav. Ecol. Sociobiol. 51, 255–261.

Smith, H.G., 1989. Larger clutches take longer to incubate. Ornis Scand. 20, 156–158.

Stearns, S.C., 1992. The Evolution of Life History. Oxford University Press, Oxford.

Turner, J.S., 2002. Maintenance of egg temperature. In: Deeming, D.C. (Ed.), Avian Incubation: Behaviour, Environment, and Evolution. Oxford University Press, Oxford, pp. 119–142.

Vleck, C.M., Vleck, D., 1980. Patterns of metabolism and growth in avian embryos. Am. Zool. 20, 405–416.