SHORT COMMUNICATIONS

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YOLK FORMATION AND OVIPOSITION IN CAPTIVE EMUS

KATHERINE V. HIRSCH

AND

C. R. GRAU

In Western Australia, Emus (*Dromaius novaehollandiae*) mature at two years, females lay 5–10 eggs from April to June, and the male incubates them for 56 days, during which time he does not eat or drink (Davies 1976). Serventy and Whittell (1976) reported the mean egg weight as 580 g, with considerable variation among eggs. The structure of the thick, dark shell (Tyler and Simkiss 1959), and the proteins of the albumen (Osuga and Feeney 1968) and yolk (Burley 1973) have been described.

Emu eggs included in a general survey of yolk formation were found to contain many rings of yolk that were apparent after dichromate staining. Some rings were so thin that we could not be sure whether they should be counted as being laid down during a 24-h period, as in domestic fowl (*Gallus gallus*, var. *domesticus*), in which lightly staining yolk is deposited at night, and darkly staining yolk is deposited during the active feeding period of the day (Grau 1976). Because yolk deposition had previously been studied in captive domestic birds by feeding dyes that are deposited in the yolk (Riddle 1908, 1910, Gilbert 1972), we were able to use the combined techniques of staining and dye feeding to investigate yolk formation and subsequent oviposition by examining eggs from Emus that had been fed dyes periodically.

Two pairs of three-year old Emus were maintained in an outdoor, unpaved pen (65 × 35 m). Water and a commercial turkey breeder ration were freely available. In addition, lettuce was provided occasionally. In the third breeding year, females began to lay 24 October 1977, and continued until 26 April 1978. During one 40-day period, the two females laid 23 eggs; six of the eggs were made available for our study. Every 10 days for 80 days, beginning 1 December 1977, we gave each of the four birds a capsule containing a dye (either 300 mg Sudan IV, a red dye, or 300 mg Sudan black B, a blue dye). The capsules were wrapped in lettuce leaves, tied with string, and placed on the ground. Males were given dyes in order to provide controls for any possible adverse effects of the experiment. Eggs, which were laid on the ground, were collected daily. The eggs used in this study were degassed in a vacuum overnight to prevent bubbles, frozen at −20°C, and fixed in 4% formalin for 16 h at 65°C. Each yolk was cut in half, and one half was placed in 6% potassium dichromate for 16 h at 65°C. Slices were made from both stained and unstained halves, and rings were counted in the stained slices, utilizing a magnifying lamp. Profiles were drawn representing dark and light rings of stained yolk (Fig. 1), a peak for a dark ring and a valley for a light ring (Roudybush et al. 1979). The dye rings were not easily visible in the dichromate-stained slices, so their locations were determined by comparison with the unstained half of the same egg.

In addition to the count of rings in the six eggs containing dye, we counted dichromate-stained rings of two other eggs to help estimate total time of yolk formation.

Two of the eggs, laid at least 24 days after dye feeding at 10-day intervals had begun, showed three widely separated dye rings, thus indicating that yolk formation takes more than 20 days but less than 30 days. Four eggs had two dye rings. After we stained halves of the eggs with dichromate, we counted the dark rings from one dye ring to the next and found 9 rings in three dye-to-dye periods, 10 rings in four periods, and 11 rings in one period. Because the interval between dye feedings was actually 10 days, we concluded that each ring, however thin, should be counted.

An example of one of the eggs studied is presented in Figure 1, which is a photograph of the surface exposed when the yolk was cut through the center. Red dye was deposited in rings 3 and 24, and blue dye in ring 12. In this example, the egg contained three dye rings with counts of 9 and 11 dichromate-stained rings from one dye ring to another, as shown in Table 1. The expected numbers were 10 and 10. The inner rings were irregular, with considerable folding, a condition that was not unusual among the eggs studied.

By counting the rings in the two other yolks as well as the six yolks that contained dye we found that the time for deposition of yolk varied from 25 to 28 days, with a mean of 26.1 days (Table 1). Counting from the dates of dosing with dyes to the dates of oviposition, we determined that the number of days elapsing between the onset of rapid yolk deposition and oviposition varied from 31 to 39 days, with a mean of 36 days. Thus the difference between the date of completion of yolk deposition and oviposition varied from 6 to 13 days, with a mean of 10.3 days.

DISCUSSION

In contrast to other eggs that have been examined after dichromate staining (Grau 1976, Roudybush et al. 1979), Emu yolks were observed to have a series of

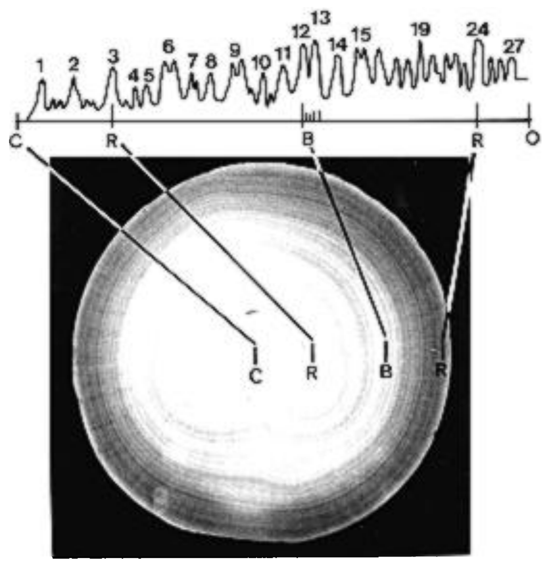

FIGURE 1. Slice through a dichromate-stained half of the Emu yolk marked (*) in Table 1. The female had been dosed with Sudan IV (R) and Sudan black B (B) at alternating 10-day intervals. The hand-drawn profile above shows the location of the dye rings in relation to the center (C) and outer margin (O) of the yolk. The B line was broad. From the positions of the dyes, it appears that peak 12 should have been marked peak 13. The diameter of the yolk was 69 mm.

TABLE 1. Ring patterns in Emu egg yolks. Six eggs contained two or three dye rings. Yolk completion and laying days are Julian dates for the years 1977–1978. The egg marked (*) is shown in Figure 1.

Number of dye rings	Ring pairs during the period from: Initiation to first dye ring	Black dye ring to red ring	Red dye ring to black ring	Black dye ring to red ring	Last dye ring to outer margin		Total days of yolk formation	Day yolk formation complete	Day laid	Difference in days
2	8	9			8		25	353	359	6
2	13	10			4		27	349	360	11
3	4	9	10		2		25	357	5	13
2	5	10			11		26	356	4	13
3*	3		9	11	4		25	4	12	8
2	10			10	6		26	6	17	11
0							27			
0							28			
						Means	26.1			10.3

wide and narrow rings that made interpretation difficult. The feeding of dyes at 10-day intervals gave time markers to particular yolk rings, and permitted counting of rings laid down over a 10-day period. The results indicate that in Emus both wide and narrow rings should be counted in order to determine days required to form the yolk.

When the data on yolk formation times and dates of feeding dyes were related to laying dates, we discovered an unexpected delay in the total time of egg formation. It was unexpected because the time sequences in laying hens, as reported first by Warren and Scott (1935), and later by others, indicated that ovulation quickly followed yolk completion, and oviposition occurred some 24 h later. Similar findings have been made on turkeys (*Meleagris gallopavo*; Bacon and Cherms 1968) and Japanese quail (*Coturnix* sp.; Bacon and Koontz 1971). In each of these gallinaceous birds, yolk formation times were determined by either feeding or injecting dyes daily. Formation times for albumen, membranes, and shell were determined by noting the position and state of completion of eggs at specific times of autopsy in relation to time of laying of the previous egg, or, in anesthetized birds, by direct observation (Warren and Scott 1935). In these birds, which were bred to lay many eggs, the interval between oviposition and ovulation of the next egg in the sequence was approximately 30 min. The ovulated ovum was surrounded by the folds of the infundibulum, and the egg was laid 24–26 h later. Gilbert (1972) found that in 20% of cases of first eggs of a sequence of eggs laid daily, the egg that was expected to be the last one of a sequence actually was delayed in formation by a day, and became the first in the next sequence. Thus even a delay of one day is rare in chickens, probably fewer than 1% of the eggs being delayed.

The various time interrelationships that have been so well documented in domestic birds have not been investigated in wild birds until recently. In Cassin's Auklets (*Ptychoramphus aleuticus*), for example, four days usually elapse between yolk deposition and laying (Grau et al. 1978, Astheimer et al. 1980).

In the two Emus we studied, yolk was deposited for 26 days, but the eggs were not laid until 10 days later. We cannot, however, determine whether this delay in egg formation, as compared with chickens, is due to holding the mature ovum within the follicle, thus delaying ovulation, or to slow passage through the oviduct.

LITERATURE CITED

ASTHEIMER, L., T. E. ROUDYBUSH, AND C. R. GRAU. 1980. Timing and energy requirements of egg synthesis in Cassin's Auklet. Pac. Seabird Group Bull. 6(2):29 (Abstract).

BACON, W. L., AND F. L. CHERMS. 1968. Ovarian follicular growth and maturation in the domestic turkey. Poult. Sci. 47:1303–1314.

BACON, W. L., AND M. KOONTZ. 1971. Ovarian follicular growth and maturation in coturnix quail. Poult. Sci. 50:233–236.

BURLEY, R. W. 1973. Isolation and properties of a low molecular weight protein (Apovitellenin I) from the high-lipid lipoprotein of Emu egg yolk. Biochemistry 12:1464–1470.

DAVIES, S. J. J. F. 1976. The natural history of the Emu in comparison with that of other ratites. Proc. XVI Int. Ornithol. Congr. (1974):109–120.

GILBERT, A. B. 1972. The activity of the ovary in relation to egg production, p. 3–21. *In* B. M. Freeman and P. E. Lake [eds.], Egg formation and production. British Poultry Science, Ltd., Edinburgh.

GRAU, C. R. 1976. Ring structure of avian egg yolk. Poult. Sci. 55:1418–1422.

GRAU, C. R., T. E. ROUDYBUSH, S. H. MORRELL, AND D. G. AINLEY. 1978. Relation of yolk deposition to oviposition in Cassin's Auklets (*Ptychoramphus aleuticus*). Pac. Seabird Group Bull. 5(2):71 (Abstract).

OSUGA, D. T., AND R. E. FEENEY. 1968. Biochemistry of the egg-white proteins of the ratite group. Arch. Biochem. Biophys. 124:560–574.

RIDDLE, O. 1908. The rate of growth of the egg-yolk in the chick, and the significance of white and yellow yolk in the ova of vertebrates. Science 27:945.

RIDDLE, O. 1910. Studies with Sudan III in metabolism and inheritance. J. Exp. Zool. 8:163–184.

ROUDYBUSH, T. E., C. R. GRAU, M. R. PETERSEN, D. G. AINLEY, K. V. HIRSCH, A. D. GILMAN, AND S. M. PATTEN. 1979. Yolk formation in some charadriiform birds. Condor 81:293–298.

SERVENTY, D. L., AND H. M. WHITTELL. 1976. Birds of Western Australia, p. 65–67. Univ. Western Australia, Perth.

TYLER, C., AND K. SIMKISS. 1959. A study of the egg shells of ratite birds. Proc. Zool. Soc. Lond. 133:201–243.

WARREN, D. C., AND H. M. SCOTT. 1935. The time factor in egg formation. Poult. Sci. 14:195–207.

Department of Avian Sciences, University of California, Davis, California 95616. Present address of first author: Section of Wildlife, Minnesota Department of Natural Resources, Box 7, Centennial Office Building, St. Paul, Minnesota 55155. Accepted for publication 17 June 1981.