

vided helpful information, and Larry L. Wolf and John J. Morony assisted me in the field.

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APPARENT DOUBLE BLASTODERMS IN ADÉLIE PENGUIN EGGS

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As part of a study of yolk formation in the Adélie Penguin (*Pygoscelis adeliae*), we removed 148 freshly-laid eggs from 94 marked pairs nesting at the Cape Crozier rookery, Ross Island, Antarctica, in November 1981. While analyzing yolk structure, we discovered that 17 (11%) of these eggs had yolks supporting two apparent blastoderms (fertilized ova). Multiple blastoderms and blastodics (unfertilized oocytes) have been noted in domestic chicken (*Gallus gallus* var. *domesticus*), quail, and turkey (*Meleagris gallopavo*) eggs at low frequencies (Abbott, pers. comm.; Romanoff and Romanoff 1949). Olsen (1962) found this multiple condition most frequently in unfertilized eggs laid by turkeys vaccinated with fowl pox virus. The occurrence of dizygotic embryos developing on a single yolk has been reported as rare (Dareste 1874, Riddle 1924, Newman 1940, Levi 1957). Out of over 15,000 yolks examined from more than 80 non-domestic species, these Adélie Penguin eggs are the first eggs we have seen to clearly exhibit this anomaly.

Of the 17 eggs considered here, 8 were from four two-egg clutches and 9 were single eggs from complete clutches. Twelve eggs were laid by 9 females all nesting within an area of approximately one ha. Our study plot inscribed an area of over 100 ha with penguin nests occurring on all available snow-free patches of open ground. Since egg collection within the plot was well distributed, we were surprised at the clustered occurrence of these unusual eggs. All females from which eggs had been taken (total = 148) had been given an oral dose of non-toxic lipophilic dye (Sudan black B) before laying as part of another experiment. This dye was transferred to the layer of yolk deposited on the dose date and was used to define the timing of yolk formation. For the eggs containing double blastoderms, the dye served to tint the yolk blue and enhanced the visibility of the blastoderms, which appeared as 2-5

mm undyed discs on the surface of the yolk. The presence of a second blastoderm may have gone unnoticed without the dye since the blastoderm is hard to see on the pale yellow yolk surface of penguin eggs. Blastoderms are easily visible on egg yolks of most species where the female is feeding while the yolk is formed; female Adélie Penguins are fasting at this time.

Yolk is deposited in a regular and predictable manner with lipoprotein material laid down in layers, enlarging the growing yolk over a period of days. The number of days of yolk deposition is species-specific. No yolk is deposited at the position of the blastodisc, either because yolk material cannot pass between the follicle's granulosa cells or because the oolemma outside the blastodisc is unable to incorporate the yolk (Perry et al. 1978). The result is visible as the neck of the latebra—a tubelike extension of the pale yellow yolk seen in a yolk cross-section—which connects the central latebra, or primordial yolk, with the nucleus of Pander under the blastodisc (Romanoff and Romanoff 1949). Cross-sections of yolk from penguin eggs with double blastoderms show two distinct latebral necks (Fig. 1), although one neck often is less noticeable. This duplication of yolk structure indicates that both apparent blastodiscs were present many days before ovulation, usually days before the females arrived at the rookery. Thus, the possibility is eliminated that these anomalies resulted from any postovulatory events, such as polyspermy or structural disturbances owing to rough handling or shaking—treatments known to induce monozygotic "double-monsters" in single blastodermal eggs handled later in development (Ulshafer and Clavert 1979). Similarly, a high incidence of monozygotic twins can result from inducing hypothermia in egg-laying chickens (Sturkie 1946) or from exposing waterfowl eggs to low temperatures before incubation (Batt et al. 1975). These two conditions are effective only after fertilization, however, and thus are unlikely causes of the present problem. Because all our eggs were subjected to the same procedures of transport, storage, and fixation, artifacts of preparation are effectively precluded. The angle formed between the two latebrae averaged 85° (10°-170°) but did not prove to be a clue as to their origin.

Preparation of yolk for analysis of its formation requires freezing, fixation in a 4% formalin, and slicing (Grau 1976). These procedures do not preserve the vitelline membrane and the adherent blastodermal tissue for histology. As a result, hematoxylin-eosin staining of sections of the apparent blastoderms failed to reveal conclusive evidence that both locales supported cellular material. We were

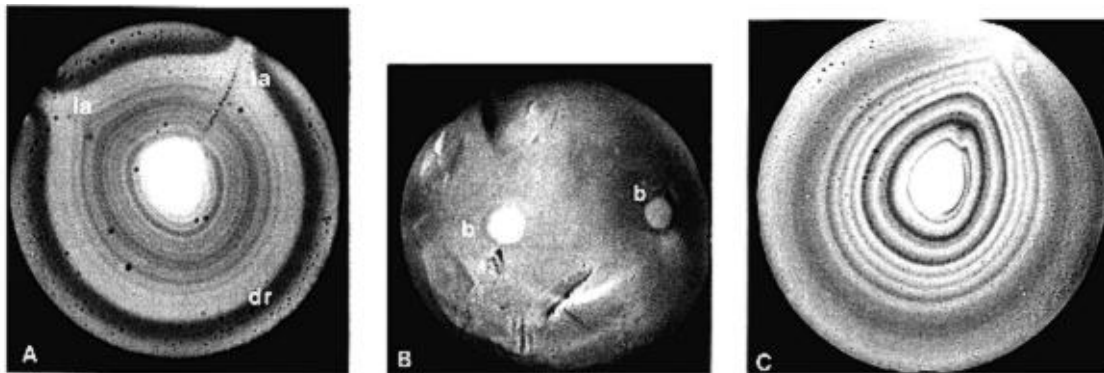


FIGURE 1. A—central slice through a yolk showing apparent double blastoderms and two lateral necks (la); B—yolk A prior to slicing with two apparent double blastoderms (b) on the yolk surface; C—example of a normal single blastoderm yolk with one lateral neck in this central slice. The “blastoderms” in A and B are unintentionally enhanced by the presence of a Sudan black dye ring (dr) incorporated in the yolk.

unable to determine whether the anomalies we saw on the yolk surface were, in fact, embryonic or gametic tissue, or were possibly the result of an abnormality of the follicle wall which affected deposition. Had the yolks not been frozen before examination, we would have had more chance of removing and identifying embryonic tissue.

The cause of the apparent double blastoderms in penguins cannot be determined with the information available. We suggest the following possibilities: (1) fission of the blastodiscs or migration of nonviable gametic tissue, perhaps polar bodies, early in yolk formation, causing differential yolk deposition across the follicle wall; (2) formation of oogonia each with two or more nuclei, resulting in multiple blastodiscs; (3) envelopment of two primary oocytes within a single follicle, resulting in two viable blastodiscs and the possibility of double fertilization (double blastoderm); and (4) aberrations in the follicle wall such that yolk deposition was restricted over a small area, thus creating a second latebra neck and a surface anomaly superficially similar to a blastodisc.

That a genetic factor may be contributing to the occurrence of double blastoderms is suggested by the high incidence (11%) of these unusual eggs in the population, coupled with the nonrandom occurrence in individual females. In addition, young Adélie Penguins are known to return to their natal site in selecting their first nest site and mate (LeResche and Sladen 1970). This may have contributed to a high degree of relatedness among female birds showing this reproductive aberration. Our study birds had not been previously banded and, therefore, their lineage and breeding histories are unknown. Because the eggs were collected fresh and were prepared for other analyses, the methods of which precluded satisfactory examination of the yolk membrane and the material underlying it, we were unable to tell whether there was more than a single true blastoderm on any single yolk. The developmental consequences of such a condition are also unknown. It is not surprising that twinning in penguins has not been reported; twins developing from nutrients provided by a single yolk would probably never hatch.

We report our incomplete observations here to alert other researchers who may have the opportunity to observe yolk structure. Our observations point to a curiosity which may have genetic implications within a penguin population. We draw attention to the potential information which may be gained by examining both eggs which fail to hatch and fresh eggs in future studies of the reproductive biology of wild birds.

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