NOTE ON THE PIGMENTATION OF FROGS' EGGS.

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On page 4 of the second volume of the new Text-book of Embryology (Vol. ii., Vertebrata, Macmillan, London, 1919) Professor Graham Kerr makes some observations upon the pigmentation of the egg in Ganoids and Amphibians. These remarks would appear to be based upon the experience of a limited group of workers with a small number of frog species. While nothing is stated dogmatically by Professor Kerr, his remarks have the appearance of a general statement, yet they are not in accord with my own observations upon the eggs of Australian Anura. It has appeared to me, therefore, worth while to place on record the discrepancies I notice, particularly as the Text-book will be used largely by students whose powers of discrimination have not been developed, and who are apt to consider as conclusive any statement that they read in such a work.

Professor Kerr writes:—"Many eggs on the other hand especially amongst the Ganoid fishes and the Amphibians are given a dark colour by the presence within them of brownish-black pigments belonging to the melanin group. Such pigment appears to be of definite biological significance, providing as it does an opaque coat which protects the living protoplasm from the harmful influence of light. Eggs in which it occurs develop, as a rule, under conditions where they are exposed to intense daylight. The eggs of ordinary Frogs and Toads for example which are surrounded by clear transparent jelly have a well developed pigment coat. On the other hand in the case of Frogs and Toads whose eggs are surrounded by light-proof foam or are deposited in burrows underground they are commonly without pigment.

"In all probability this deposition of melanin pigment in the superficial protoplasm of the egg (normally in its *upper* portion) is to be interpreted as having been originally a direct reaction to the influence of light, the metabolism being so affected as to bring about the formation of this particular iron-containing excretory pigment.

"It may be objected that the pigment is produced before the egg is laid (*e.g.* the Common Frog) and therefore before it is exposed to the action of light, but as a matter of fact the body-wall of the adult is by no means opaque to light rays and even while still in the ovary the eggs are exposed to the influence of faint light. If we may take it, as seems probable, that the

influence of natural selection has gradually developed in such cases the particular type of sensitiveness to light which leads to the formation of melanin, on account of its protective value, then there is nothing surprising in the developing of this sensitiveness at earlier and earlier periods until at last it has resulted in the pigmentation of the still intra-ovarian egg in response to the feeble light rays which penetrate the body wall."

There can be no disagreement with a statement that the eggs of most Anura have a pigmented upper pole. This may vary in size from a small polar cap (*Pseudophryne*) to considerably more than a hemisphere (*Rana temporaria*); and in colour from light brown (*Hyla ewingii*) to intense black (*Crinia signifera*). But several instances have been given of Anuran eggs which are without pigment.

Thus Agar (1909, p. 895) writes of *Phyllomedusa sauvagii*—"The eggs are quite unpigmented, and any that are exposed to the surface, as happens often in less perfectly formed nests, turn yellow and die." Budgett (1899, p. 315) mentions the spawn of *Phyllomedusa hypochondrialis* as—"batches of white eggs in masses of firm jelly." Agar's Figure 2 shows quite plainly that the intra-ovarian eggs are without pigment. In the case of *Phyllomedusa,* then, we have undoubted white eggs, presumably as the eggs are enclosed in light-proof foam. But *Phyllomedusa* is a small, comparatively translucent frog, which lies by day on the upper surfaces of leaves, fully exposed to light (Budgett, 1899, p. 314); so that if pigment be a direct reaction to light, one would expect to find in this case the eggs definitely pigmented.

In three species of *Limnodynastes* with the breeding habits of which I am familiar, the eggs are also laid in a mass of foam, which from its nature must reflect and refract so much incident light as to be practically light-proof. Yet the eggs are densely pigmented, as are the developing larvae. *L. dorsalis* is a burrowing frog, never seen by daylight at ordinary times, and resorting to water only for a day or two in the breeding season. *L. peronii* is a cryptozoic form, found under stones, etc., by day, and resorting to water at night. This species may, however, be found in the water during the daytime within the limits of its spawning season. *L. tasmaniensis* is also cryptozoic, and I have only once found it in water by daylight. None of these species would appear to be susceptible to the influence of daylight acting through their robust body-wall, yet the intra-ovarian eggs are densely pigmented. It may be noted in passing that eggs entangled in the upper portion of the foam die, not from exposure to light, but because of insufficient moisture in this region.

There is thus a very marked difference between *Phyllomedusa* and *Limnodynastes,* and it would seem that the explanation of pigmentation is further to seek than Graham Kerr's general statement would have us believe.

Besides *Phyllomedusa,* certain other frogs have been reported as laying eggs without pigment. Of *Paludicola fusomaculata* Budgett writes (1899, p. 309)—"The eggs . . . are found embedded in a frothy mass floating upon the surface of the water, ... and are without pigment." Bles (1907, p. 445) dealing with Budgett's material of this species writes—"The ovum is quite free from pigment. In the earliest stage examined, the blastopore is very small." In view of what I have to say below, the latter sentence may bear some significance. Otherwise *Paludicola* spawn would appear to resemble that of *Limnodynastes,* except for the absence of pigment.

Budgett (1899, p. 311) records the nests of *Engystoma ovale* as—"holes in the ground beneath fallen tree trunks, of the size of a cricket ball and lined with a froth containing white eggs, and also tailed larvae."

Bles (1907, p. 451), describing Budgett's Gambia material of *Hemisus marmoratum,* writes:—"The earliest stages which were preserved are late segmentation stages of which there is nothing special to note except that the eggs are quite without pigment."

Finally Dakin (1920, p. 242) describes the eggs of *Heleioporus albopunctatus* as "somewhat large and without pigment." These eggs were laid at the bottom of burrows 18 inches deep, in friable sandy soil, and were enclosed in a mass of frothy mucus.

It would appear then on the face of it that unpigmented frog's eggs are of comparatively common occurrence. But the condition that I find in the eggs of *Pseudophryne australis* and *P. bibronii* causes me to suspect that many eggs described as being without pigment may not really be so. In these species the unsegmented egg has a black upper pole, occupying in section about one-sixth of the circumference of the egg. During the early stages of segmentation the micromeres are confined to this cap, which retains its black colour. As overgrowth proceeds, however, the pigmented area extends, but loses its intensity of colour, so that by the time the blastopore has become circular the whole pigmented area is of so pale a grey as to be distinguishable with difficulty from the white yolk. In eggs which have been preserved the distinction is even less marked. Pigment appears again during the formation and closure of the neural groove, but the embryo, when folded off, is practically without pigment.

In this case unless the eggs were observed in the early segmentation stages they might easily be described as without pigment. As most of the eggs described as unpigmented are of a similar heavily yolked type, I believe the same thing may have happened in some at least of these cases. Eggs of *Heleioporus* kindly sent me by Professor Dakin have all passed beyond the stage of early segmentation, and have an appearance precisely similar to that of *Pseudophryne* eggs. In Budgett's material mentioned above, of *Paludicola* and *Hemisus* there are no early segmentation stages. The material of *Engystoma* would appear to have been lost, as no mention of it occurs in Bles' report (1907).

From appearances observed in two species of *Pseudophryne,* and in several species of *Hyla,* the amount of pigment present seems to bear a definite relation to the protoplasmic mass of the egg. The addition of further yolk to the bulk of the egg in the course of evolution is not accompanied by a compensating addition of pigment. As overgrowth proceeds, the amount of pigment present appears to remain practically constant. In small yolked eggs which are densely pigmented there is little or no diminution of intensity of colour during overgrowth. In large-yolked eggs, however, owing to the much greater area which has to be covered by the pigment, there is a very definite lightening in colour, from the black to palest grey in *Pseudophryne,* from dark brown to light yellowish brown in several species of *Hyla,* as overgrowth proceeds.

This view is opposed to that of T. H. Morgan (1891, p. 758) who maintains that black cells do not overgrow light, but that new pigmented cells are cut off from the upper corners of the yolk cells, and—"there is a continuous formation of new pigment taking place at the periphery of the black area within the new cells that are being formed." Pigment production is admittedly a product of active metabolism, and one would expect to find pigment being formed in the region of the germ ring, but, in the eggs I mention, this would not appear to be sufficient to greatly affect the colour of the egg surface.

Finally, both species of *Pseudophryne* are cryptozoic in habit hiding by day under logs and heaps of refuse, and laying their eggs in similar situations.

Here again it seems impossible that the pigment of the egg should have been produced in response to the direct stimulus of light, and a supposition that *Pseudophryne* has only recently taken to a cryptozoic habit, and has not yet succeeded in eliminating pigment, hardly helps matters.

Regarding the pigmentation of tadpoles, I can confirm the observations of Wenig (1913) that those which develop in water with clay in suspension do not develop normal pigment. I recently captured some tadpoles of *Limnodynastes* at a depth of five feet in a muddy creek, in a net sunk for crayfish. The normal tadpole has a uniform dense pigmentation, but these individuals were almost transparent, except for two lines of chromatophores dorso-laterally along the main muscle mass, and the whole brain showed white through the dorsal surface. Here there has been an obvious relation between light and pigment development.

For the eggs, however, the matter is apparently not so simple, and an enquiry into the nature and mechanism of production of these pigments, and into the purpose they subserve, seems called for.

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