

²⁰ Krakow, J. S., and S. Ochoa, these PROCEEDINGS, 49, 88 (1963).

²¹ Inman, R. B., and R. L. Baldwin, *J. Mol. Biol.*, 5, 172 (1962).

²² The melting point of poly (A + U) complexed in the presence of Mg^{++} is considerably higher than that of the complex prepared in 0.02 M NaCl alone. The latter melts at 44°. Product and primer were precipitated with alcohol in the reaction represented in Figure 5. The Mg^{++} bound to the precipitate was therefore present in the mixture during the melting experiment. This accounts for the melting point between 60 and 64°. The melting point of pure poly (A + U) in 0.02 M NaCl and $10^{-4}M$ $MgCl_2$ was 64°. Exhaustive dialysis against 0.02 M NaCl reduced the value to 50°.

POLYPLOIDY IN THE MEXICAN AXOLOTL (*AMBYSTOMA MEXICANUM*) RESULTING FROM MULTINUCLEATE OVA*

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Communicated by Robert W. Briggs, October 7, 1963

Spontaneous polyploidy in the Amphibia has been reported by several investigators.¹⁻⁴ Such polyploids among the progeny of diploid parents generally have been interpreted as resulting from one or more of the theoretically possible accidents which may occur in meiosis, fertilization, or the first cleavage division. This interpretation is much in accord with the fact that polyploidy may be induced by experimental procedures which suppress the second maturation division or disturb the normal progress of the first cleavage division, and it doubtless accounts for the great majority of spontaneous polyploids. Investigators rarely have offered the interpretation that polyploidy may result from a multinucleate condition in the egg. Humphries,⁵ for example, hesitated to assume that diploidy in the egg of *Triturus* might be the result of a binucleate condition, in view of "the direct pathways actually seen to exist." More recently, however, Parmenter *et al.*⁶ have reported the occurrence of binucleate and trinucleate young oöcytes in *Rana pipiens*. Their discovery of this condition suggested, as they point out, an important possible source of diploid parthenogenetic individuals. They point out, too, the possibility that the failure of one of the nuclei in a binucleate egg to undergo one or both meiotic divisions "could produce various chromosome numbers in mature eggs and in embryos resulting from their parthenogenetic stimulation or from fertilization."

Whether the multinucleate young oöcytes of *R. pipiens* observed by Parmenter *et al.* would have given rise to mature ova of unusually large size is uncertain. Briggs⁷ states that he has observed eggs from females of this species with two or even three first polar bodies, but that they were of essentially the same size as mononucleate eggs, and could not be distinguished without the aid of a microscope. Multinucleate eggs of the axolotl, however, are readily distinguished by their larger size when eggs of a spawning including them are examined with the naked eye (Fig. 1). Their markedly large size, when they were first observed by the writer, caused them to be recorded as "giant" eggs.

The first oversized, or "giant," egg was found in 1953 in a spawning from a homozygous dark (D/D) female. No others were observed until 1956 when several appeared in two successive spawnings of a second female which was closely related to

the first (the daughter of a sister). All of these eggs happened to be fertile and were in cleavage stages when first observed. Later, when similar large eggs began to appear with considerable frequency in the spawnings of descendants of this second female, some were observed before cleavage began. Such eggs were usually found to have first polar bodies formed at two sites, commonly at some little distance apart (Fig. 1). If the egg was one which had been fertilized, second polar bodies later appeared at these sites. In a few spawnings, however, some of the oversized eggs were distinctly larger than the others, and in these, polar bodies were observed at three or even four sites instead of two. In one instance a giant egg was found with a first polar body formed at only one site instead of the two or more characteristic of such eggs. Unfortunately, this egg was not sectioned to determine whether a second nucleus was present in a submerged position, such as was described by Humphries⁵ for the nucleus of occasional mononucleate eggs of *Triturus*.

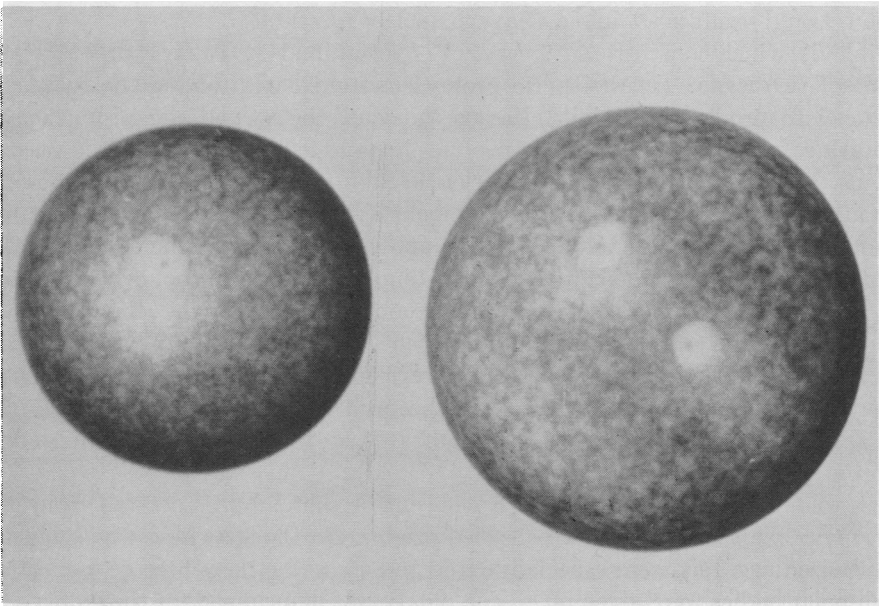


FIG. 1.—Mononucleate and binucleate eggs of the axolotl, fixed after formation of the first polar body. $\times 24$.

Females which produced giant eggs in one spawning tended to do so in others. Percentages of such eggs, in the spawnings including them, ranged from 0.13 per cent (1 in 768) to 6.24 per cent (29 in 465). In a group of 51 spawnings for which complete records were available, 209 giant eggs were included in the total of 24,588, an incidence of 0.85 per cent. This rate is probably slightly below the actual one, since large eggs which were at all doubtful because of damage or cytolytic changes were disregarded.

Determinations of the ploidy of embryos or larvae developing from giant eggs were made whenever possible. This was generally done from tailtip preparations stained with azure B, in which the maximum number of nucleoli per cell can easily be ascertained. This number serves to indicate the number of sets of chromosomes present.⁸ In a few embryos the number of nucleoli was found by examination of un-

TABLE 1
 PLOIDY OF 145 INDIVIDUALS WHICH DEVELOPED FROM OVERSIZED OR GIANT EGGS, PRESUMABLY ALL MULTINUCLEATE

| Type | Number found | Percentage of total |
|-------------------------------------|--------------|---------------------|
| Diploid ($2N$) | 26 | 17.9 |
| Triploid ($3N$) | 113 | 77.9 |
| Tetraploid ($4N$) | 4 | 2.8 |
| Mosaics ($2N/4N$ and $2N/3N + N$) | 2 | 1.4 |

stained cells with a phase microscope. In all, the ploidy of 145 individuals was determined. These are included in Table 1.

The great majority of the embryos from giant eggs (77.9%) were found to be triploids. Since most such eggs showed two sites at which first and second polar bodies were formed, they would appear to be binucleate, and after completion of meiosis would contain two pronuclei, each with the haploid number of chromosomes. These, combining with the set of chromosomes introduced by the spermatozoon, would result in a triploid cleavage nucleus.

Diploids, which constitute 17.9 per cent of the animals in Table 1, probably arose in one of two ways: (1) the sperm pronucleus united with one of the two female pronuclei to produce a diploid cleavage nucleus, the second egg pronucleus degenerating; or (2) the two female pronuclei combined to form a diploid cleavage nucleus, the spermatozoon inducing cleavage but contributing no chromosomes. Although this second mode of development (gynogenesis) sometimes occurs in mononucleate eggs of the axolotl,³ it is impossible to say whether it actually accounts for any of the diploid embryos from binucleate eggs. Proof of the participation of the chromosomes of the spermatozoon in producing the diploid cleavage nucleus was obtained in one instance in which a dark female (D/D) had been mated with a white male (d/d). One of her giant eggs produced a diploid larva which was reared and mated with a white animal; the resulting progeny numbered 67 darks and 59 whites, showing that this one diploid, at least, had received a chromosome set from the spermatozoon.

Tetraploids, which constituted 2.8 per cent of the embryos derived from giant eggs, may, like the diploids, be of varied origin. In the spawning which included 29 oversized eggs, six were especially large and showed either three or four sites at which polar bodies were given off. The ploidy was determined for the embryos developing from four of these eggs. Three were triploids and one was a tetraploid. The latter could have come from a trinucleate egg in which all three nuclei completed meiosis, the three pronuclei resulting then combining with the sperm pronucleus to produce a tetraploid cleavage nucleus. Another possibility is that this tetraploid was derived by gynogenesis from one of the eggs with four nuclei, all of which had completed meiosis. The three triploids derived from eggs with three or four nuclei could have arisen by various combinations of three haploid nuclei from the four or five present (sperm pronucleus, plus three or four egg pronuclei).

Whether the mosaics listed in Table 1 came from binucleate eggs or ones with three or four nuclei is uncertain. One possible origin for the $2N/4N$ mosaic is that it came from an egg with three nuclei, one of which, after meiosis, combined with the sperm pronucleus to form a diploid cleavage nucleus; the other two egg pronuclei, if shifted into one of the first two blastomeres, could then unite with the diploid nucleus of that blastomere to produce the tetraploid condition found in one lateral

half of the mosaic. The reader may find other possible origins for this mosaic, and perhaps also an explanation for the one which was $2N/3N + N$.

Possible Genetic Basis for Tendency to Produce Multinucleate Ova.—The occurrence of binucleate eggs in any amphibian species must depend upon the occasional failure of completion of a final oögonial division. Nuclear division without formation of separate daughter cells, followed shortly by onset of meiosis in the two nuclei, would give rise to the binucleate young oöcytes described by Parmenter *et al.*⁶ for *Rana pipiens*. Mitotic division of one of the two daughter nuclei before meiosis begins, again without cell division, would result in a trinucleate oöcyte, and if such mitosis occurred in both daughter nuclei, an oöcyte with four nuclei. Whether the tendency toward incomplete division in the final mitoses of the oögonia in frog and axolotl is inherent in the character of these cells themselves or results from some peculiarity of their environment (some unusual feature of ovarian structure, possibly) remains problematic. In either case, the phenomenon may very well have a genetic basis.

With minor exceptions which will be mentioned subsequently, all of the axolotl females known to have produced giant (multinucleate) eggs are descended through both parents from the second female which spawned such eggs. The dark strain to which these animals belong was derived from a few young animals obtained in 1951 from Dr. J. Holtfreter of the University of Rochester. These animals were descendants of axolotls imported by Dr. E. Caspari, then at Cold Spring Harbor; most of them had been procured for him by Dr. H. Gloor from various European dealers and were, as Gloor states,⁹ "of an entirely obscure origin."

Figure 2 shows the earlier ancestry of the animals in the writer's colony which have produced giant eggs. The animals numbered 2, 3, and 4 were among those obtained from Holtfreter. From spawning no. 542, four females were reared, of which one (542-1) produced a single giant egg. For the 24 spawnings from four females of spawning no. 607, not a single unusual egg was recorded. The third-generation female no. 755-4, on the other hand, produced 16 giant eggs in three of her four spawnings. Only two of her daughters were reared (872-3, 872-4); these laid no oversized eggs in nine and eight spawnings, respectively. From matings with their brother, no. 872-2, however, they both produced daughters which spawned a few such eggs. The descendants of these three animals of spawning 872 now include 179 homozygous dark (D/D) females which have spawned one or more times, of which 69 (38.6%) have produced one or more giant eggs. The high percentage of females of this particular dark strain showing a tendency to lay multinucleate eggs is in striking contrast with the absence of such females in the white

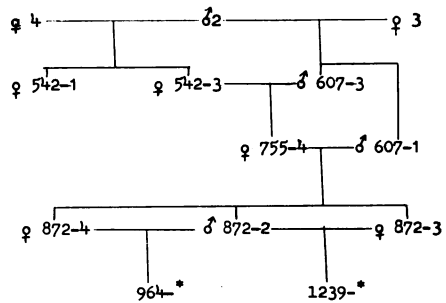


FIG. 2.—Chart showing matings of dark axolotls from which all females producing oversized (multinucleate) eggs are descended. The animals numbered 2, 3, and 4 were obtained from the Holtfreter colony at the University of Rochester. Female 542-1 was the first known to lay an unusually large egg. Female 755-4 laid several such eggs; all other females known to have spawned such eggs are her descendants by way of the three animals of spawning no. 872.

*Some of the many females from these spawnings produced multinucleate eggs.

strain and four other dark strains from which, all told, well over a thousand spawnings have been obtained.

The data on record would appear to support the interpretation that the tendency to produce multinucleate eggs depends upon homozygosity for a recessive gene. If we assume that this gene was carried by female no. 4 and male no. 2, it could have been transmitted through both parents to every homozygous dark (D/D) female of this strain which has since spawned multinucleate eggs. Although a few heterozygous dark (D/d) females have also produced such eggs, their white fathers could have carried the recessive gene for this peculiarity as well as their dark mothers, since two of these white males were actually descended from male no. 2, and the third had as one ancestor a dark male obtained from the Holtfreter colony at the same time as male no. 2 and assumed to be his sib. Ten heterozygous dark (D/d) females whose white (d/d) father was of an unrelated strain (inbred since 1937) produced not a single oversized egg in 20 spawnings (9,921 eggs) although their mother had laid such eggs and two of her five daughters by a dark male had likewise done so.

One rather exceptional finding may be mentioned. A dark female whose right ovary had been partially replaced by a graft from a white donor spawned a single giant egg in each of two spawnings. She was always mated with white males, and since the triploid larvae from her two giant eggs were white rather than dark (the dominant color), these eggs must have come from her ovarian graft rather than her own ovary. Both parents of the white embryo furnishing this ovarian graft were descended from the dark male already mentioned as a probable sib of male no. 2 of Figure 2, and it is possible, therefore, that this white donor was homozygous for the postulated recessive gene. Among the few siblings of this white embryo which were reared were four females, none of which ever spawned giant eggs. We are left with the question whether the giant eggs derived from the ovarian graft were the result of the genotype of the donor embryo or were induced by influences depending upon the constitution of the dark host, which, however, never laid giant (multinucleate) eggs coming from her own ovary.

Comment.—The discovery of multinucleate eggs in the spawnings of Mexican axolotls of this dark strain, and the fact that a high percentage of these eggs give rise to heteroploids, does not affect the validity of the interpretations offered by Fankhauser and Humphrey³ for the heteroploids they reported in this species. Those heteroploids, with the exception of nine at most, were the progeny of white axolotls or of animals of dark strains for which multinucleate eggs were never observed. Their heteroploidy, therefore, must have resulted from the various accidents of meiosis, fertilization, or the first cleavage division which were postulated by the authors. Substantial evidence for the stated origins of many of those heteroploids was afforded by their color or sex, or their genotypes as determined by test matings.

That multinucleate eggs do occur in the Mexican axolotl, however, must be borne in mind by investigators undertaking studies involving spontaneous or experimental heteroploidy in this species. Descendants of the dark axolotls "of obscure origin" sent by Gloor to Cold Spring Harbor are now a part of many other laboratory colonies. The dark stock in the writer's own colony at Indiana University for the past several years has consisted exclusively of the descendants of the animals of this

strain included in Figure 2, and eggs or larvae of this stock have been furnished to several investigators desiring to establish colonies of their own. Fortunately, the multinucleate eggs of these axolotls are easily recognizable because of their large size and hence can be excluded from investigations in which their character might lead to erroneous conclusions. Their occurrence and easy recognition are, of course, advantageous to an investigator who has use for the polyploids to which they commonly give origin.

Summary.—Multinucleate eggs, much larger than the ordinary mononucleate ones, have been found in the spawnings of dark Mexican axolotls of a strain derived from European stock of obscure origin. The majority of these oversized eggs have two nuclei, but a few have three or four. In 51 spawnings including them, such large eggs constituted 0.85 per cent of the total (209 out of 24,588). Of 145 embryos or larvae which developed from these eggs, 113 (77.9%) were triploids, 26 (17.9%) were diploids, and the remaining 6 were tetraploids and mosaics.

The females spawning multinucleate eggs, with one exception, have been descended through both parents from the two animals whose mating produced the first female known to have spawned such eggs. This suggests that the tendency to produce multinucleate eggs may depend upon homozygosity for a recessive gene or genes. Such eggs have not been found in the spawnings of white axolotls or of dark females of four other strains, but have been included in spawnings of over 38 per cent of the females of the dark strain showing this peculiarity. Their occurrence in this strain must be taken into account in any study involving consideration of chromosome number.

* This investigation was supported in part by research grants from the U.S. Public Health Service (RG 5850), and from the National Science Foundation (G 4856).

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⁷ Briggs, R. W., personal communication (1963).

⁸ Fankhauser, G., and R. R. Humphrey, these PROCEEDINGS, **29**, 344 (1943).

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