MAINTENANCE OF NORMAL STRUCTURE IN HETEROPLOID SALAMANDER LARVAE, THROUGH COMPENSATION OF CHANGES IN CELL SIZE BY ADJUSTMENT OF CELL NUMBER AND CELL SHAPE

G. FANKHAUSER
Department of Biology, Princeton University, New Jersey

SIX FIGURES

INTRODUCTION

Our knowledge of the effects produced by deviations from the normal, diploid chromosome number ("heteroploidy")[1] is based largely on studies on plants in which polyploidy is widespread under natural conditions and easily induced by experimental means. Investigations on polyploidy in animals were limited almost entirely to a few species of arthropods until it was recognized, a few years ago, that abundant material for such studies is furnished by amphibians where aberrations in chromosome number occur spontaneously with surprising frequency and may also be induced experimentally (for a general review and bibliography see Fankhauser, '45). The analysis of the consequences of such large-scale quantitative changes in the genetic system is of particular interest in animals and, especially, in vertebrates in which the closer integration of the individual produces special conditions that may well modify the usual effects of heteroploidy. Moreover, the strong tendency shown by amphibian embryos to return into normal channels of development following the most severe operations performed by the experimental embryologist, a tendency usually referred to as "regulation," may express itself here also, although the interference is of a much more subtle nature than usual since it affects the intimate constitution of the nucleus.

The most outstanding feature of polyploid salamander larvae is the absence of gigantism that is so generally associated with an increase in chromosome number in plants. Young larvae whose chromosome numbers range from the diploid to the pentaploid are all of approximately the same size. Even among haploid larvae that are smaller as a rule,

[1] Heteroploidy = any deviation from the normal diploid chromosome number; polyploidy = presence of three, four, or more complete sets of chromosomes (Winkler, '16).

the most vigorous may approach the size typical for diploids (cf. Sharp, '43, fig. 157; Fankhauser, '45, fig. 7). In later stages of development triploids may or may not grow slightly larger than the controls; tetraploid and pentaploid larvae grow more slowly but usually reach normal size at metamorphosis the onset of which is delayed correspondingly ('45, figs. 5 and 6).

Examination of whole-mounts of amputated tailtips, from which the chromosome number of living larvae may be determined, and of sections through preserved animals shows at once that the change in the number of chromosome sets produces the usual primary effect: the size of the nuclei and cells increases approximately in proportion to the chromosome number ('45, figs. 1 and 4). However, the change in cell size is compensated by a reciprocal change in cell number in the various organs. In triploid individuals of the newt, Triturus viridescens, preserved during metamorphosis, which have been studied most closely so far, this compensation is visible in all organs examined, with the exception of the gonads and, possibly, of the notochord (Fankhauser, '41). Preliminary observations on sections of tetraploid and pentaploid animals at metamorphosis show that in these the still larger cell size is again balanced by a correspondingly greater reduction in cell number. The question how various organs and tissues of higher polyploids with their larger but less numerous cells compare with the diploid in functional efficiency will be an important one to investigate.

In haploid larvae the compensation of the small cell size by an increase in cell number is only partial as a rule. In the single haploid newt that has been raised to metamorphosis so far (Baltzer, '22; Fankhauser, '38) most organs were of considerably more than half normal size, while some glands (Harder's glands, thyroid) had reached approximately diploid dimensions. The cartilages of the larynx alone showed about the same cell number as in the diploid and, therefore, about one-half normal size.

While the normal size of organs may thus be preserved in heteroploid individuals by changes in cell number, this adjustment alone will not always suffice to assure maintenance of the normal structure of an organ, particularly when it contains cells arranged in a single layer. Such layers cannot retain the normal thickness unless the shape of the individual cells is also modified. Such a far-reaching secondary adjustment actually takes place in heteroploid salamander larvae as has been mentioned briefly before ('45, pp. 47 and 66) and will be described in more detail in the present paper.

The haploid, triploid, and pentaploid larvae of Triturus viridescens from which the drawings were made all originated spontaneously and were found among larvae that developed at room temperature from untreated eggs laid in the laboratory. The larvae were fixed in Michaelis' or Bouin's fluid, sectioned at 15 micra, and stained with Harris' acid hemalum and Orange G. Obviously, animal tissues are less favorable for studies on cell shape than those of plants because the boundaries between adjacent cells of a layer usually are not distinct. However, the shape and the spacing of the nuclei indicate clearly the approximate position of the cell limits.

OBSERVATIONS

1. Epidermis of tail fin

The epidermis of the fin of young larvae actually consists of two layers of greatly flattened cells that are closely associated. The nuclei of the lower layer occupy spaces between those of the upper and are almost at the same level even under high magnifications. They are very flat discs that take up the greater part of the cell body. The area of twenty nuclei drawn from whole-mounts of the fin and measured with a planimeter increases in almost direct proportion to the chromosome number from the haploid to the pentaploid levels. If the area of haploid nuclei of larvae of Triturus viridescens is taken as 1.0, that of the diploid is 2.32, of the triploid 3.5, and of the pentaploid 5.0 ('45, table 2). In axolotl larvae, the addition of successive chromosome sets changes the area from 1.0, in the haploid, to 1.96 (diploid), 2.99 (triploid), 3.86 (tetraploid), and 4.19 (pentaploid) (Fankhauser and Humphrey, '43, table 1).

The area of the nuclei thus increases almost as rapidly as the volume which is explained by the fact that the thickness of the nuclei, as seen in optical section at the crest of the tailfin of axolotl larvae, remains almost constant from the haploid to the tetraploid levels but shows a slight increase in the pentaploid ('45, fig. 3). The thickness of the epidermis thus remains nearly constant in spite of the differences in cell size.

2. Pronephros

Figure 1 shows that the over-all dimensions of the cross sections of the tubules are approximately the same in young haploid, diploid, and pentaploid larvae of Triturus viridescens because of the progressive reduction in cell number with increasing cell size. What is even more striking, the diameter of the wall of the tubules also remains constant as a result of pronounced changes in the shape of the component cells.

In the haploid the cells are nearly cuboidal; in the pentaploid they are greatly flattened and reach about the same thinness as in the diploid, except at the site of the nuclei which seem to offer more resistance to the forces that bring about the change in shape.

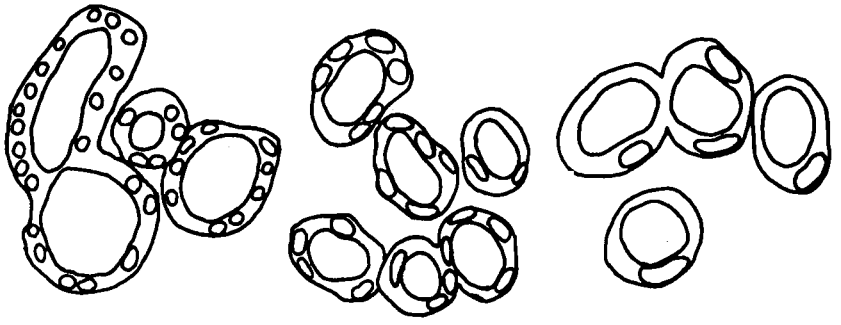

Fig. 1 Cross sections of pronephric tubules from a haploid larva (35 days old), from a diploid (40 days, first indication of hindlimb buds), and from a pentaploid (40 days, slightly less advanced in development than diploid). Size of tubules and diameter of wall remain approximately the same in spite of differences in cell size, through changes in cell shape. × 245.

3. Pronephric duct

The dimensions of the cross sections of the pronephric ducts vary widely in one individual, between different levels and between right and left, presumably because of variations in the amount of fluid present. However, the range of variation of the diameter of the duct and of the thickness of its wall are approximately the same in haploid, diploid, and pentaploid larvae as a result of a combination of changes in cell number and cell shape similar to those in the pronephric tubules (fig. 2).

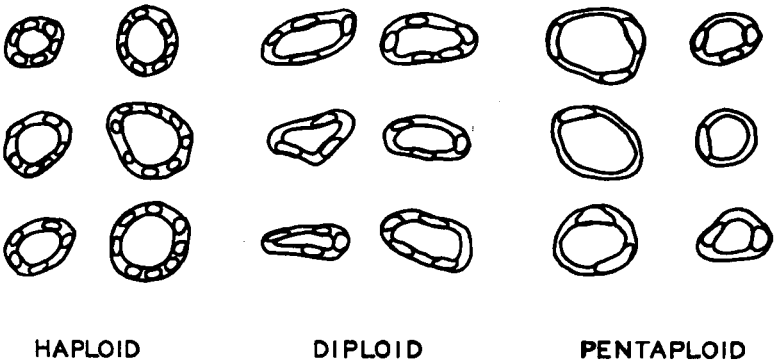

Fig. 2 Cross sections of pronephric ducts of the same larvae, taken at different levels. Similar adjustment of cell number and cell shape as in pronephric tubules. × 245.

4. *Epithelium of lens*

In diploid larvae the epithelium covering the outer half of the lens consists of a single layer of flattened cells. In a corresponding portion of the epithelium of a slightly younger haploid larva the same thickness of the cell layer is maintained by an increase in cell number and a modification in cell shape (fig. 3). In a pentaploid of exactly the same age and developmental stage as the diploid the thickness of the lens epithelium is again approximately normal because of the extreme flattening of the cells. In contrast to the pronephros and pronephric duct, the nuclei also are reduced to the thickness of the diploid. The same adjustment of the cell shape is visible in the lens epithelium of an older pentaploid larva (fig. 3, right).

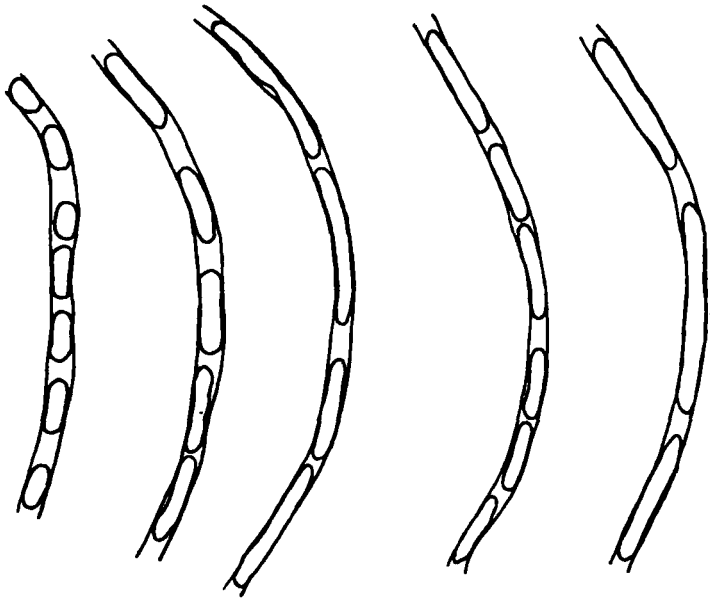

Fig. 3 Left: small portions of epithelium of lens of the same haploid, diploid, and pentaploid larvae. Thickness of epithelium remains nearly constant. Right: corresponding portions of lens epithelium of an older diploid larva and of a pentaploid of the same age and stage (50 days, long hindlimb buds). Cells and nuclei of pentaploid again flattened to about same diameter as in diploid. × 575.

5. *Nuclear pattern of the retina*

A comparison of the nuclear patterns of the retina in three triploid animals at metamorphosis and in their controls showed that the diameter of the retina as a whole and of its various layers was slightly greater in the triploids although the triploid eyes as a whole were of normal size

(Fankhauser, '41, p. 164, figs. 15 and 16). At that time it was assumed that the pattern of differentiation of the retina, including the number of rows of cells in the different layers, was more rigidly fixed than that of other organs so that a complete adjustment in cell number in a radial direction was not possible. A partial compensation of cell size through reduction in cell number was evident in both the ganglionic and inner nuclear layers. In the outer nuclear layer, consisting of a single row of elongate nuclei of visual cells, no adjustment could be expected.

However, examination of sections through the eyes of two young pentaploid larvae fixed at the ages of 40 and 50 days, respectively, demonstrated that the pattern of differentiation of the retina is readily modified, to such an extent that the whole retina and its various layers are actually thinner than in the diploid.

The eyes of the younger pentaploid are slightly smaller than those of the diploid of the same age. In the diploid retina from three to four rows of nuclei are visible in the ganglionic layer; in the pentaploid there are two (fig. 4). In the inner nuclear layer, the diploid retina

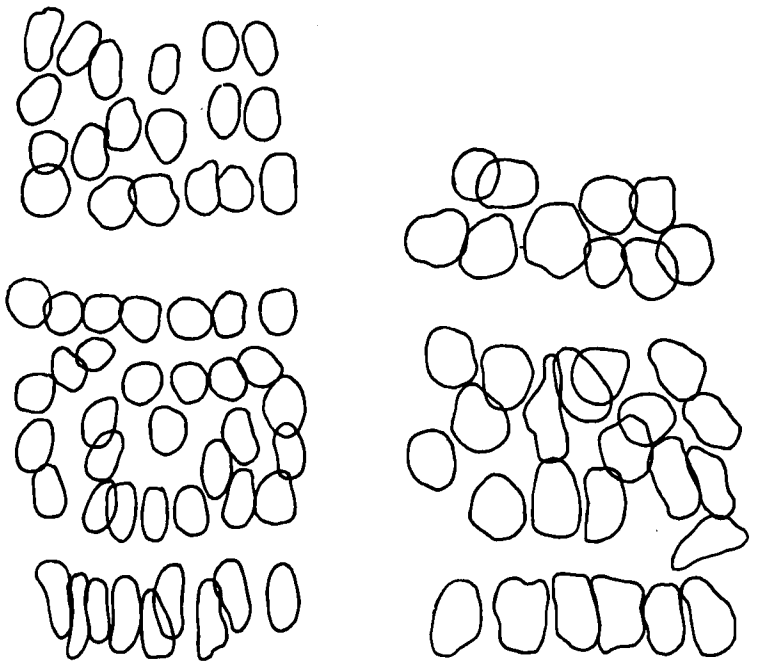

Fig. 4 Nuclear pattern of retina of the same diploid and pentaploid larvae as in figure 1 (40 days). Smaller number of rows of cells in ganglionic and inner nuclear layers reduces diameter of retina of pentaploid. Nuclei of visual cells shown at bottom of figure are much wider than diploid but of about same height. × 575.

shows from four to five rows of nuclei, the pentaploid from two to three. Furthermore, there is a pronounced change in the appearance of the outer nuclear layer. In the pentaploid retina the nuclei of the visual cells are much wider than those in the diploid but have about the same height so that the diameter of this layer is the same in both. Unfortunately, the cytoplasmic portions of the visual cells are too poorly preserved by the fixatives employed to allow a comparison.

In the older pentaploid larva the eyes are slightly larger than in the diploid control; the retina, however, is again thinner (fig. 5). Progressive differentiation of the retina has resulted in a reduction in its width

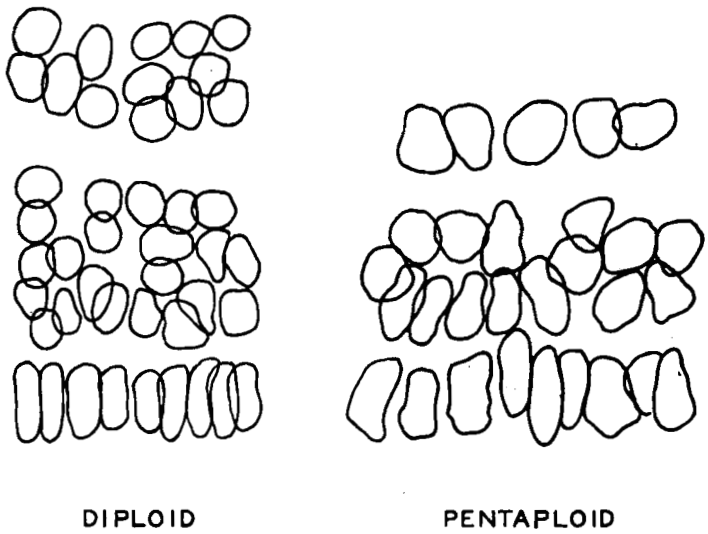

Fig. 5 Nuclear pattern of retina of older diploid and pentaploid larvae (50 days). Reduction of thickness of retina connected with further differentiation in both diploid and pentaploid. × 575.

in both animals. The ganglionic layer of the diploid is reduced to two or three rows of nuclei, that of the pentaploid to a single row. In the inner nuclear layer there are from three to four and two rows, respectively. The nuclei of the visual cells are again considerably wider in the pentaploid but of approximately the same height as in the diploid.

A tendency toward an increase in width of these nuclei is also noticeable in a more advanced triploid larva (fig. 6). The retina has the same diameter as in the diploid because of a decrease in the number of cell layers which neutralizes completely the increased size of the individual elements. At this stage of development the nuclei of some visual cells show a further differentiation into a longer, basal portion and an en-

larged, lightly stained distal part. The nuclei of the triploid show the same degree of differentiation as those of the diploid but are slightly wider in proportion to their height.

It is possible that the thinness of the pentaploid retina, an "over-compensation" of the greater cell size, is a transitory phenomenon, perhaps an early expression of the slower growth rate typical for pentaploid larvae in later stages of development, and that the retina reaches the normal diameter at metamorphosis. Sections through fully

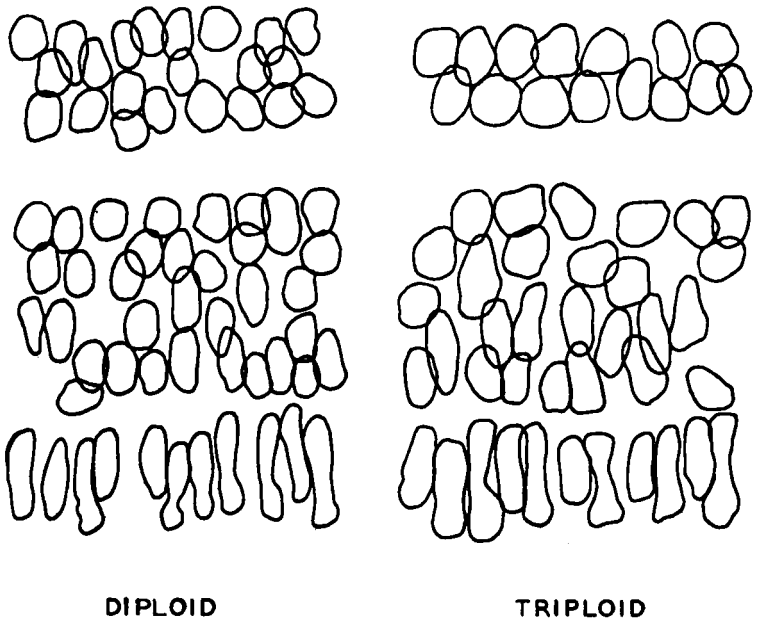

Fig. 6 Nuclear pattern of retina of a more advanced diploid larva and of a triploid of the same age and stage (formation of third toe-bud). The larger size of the triploid cells (nuclei) is almost exactly compensated by a decrease in the number of rows of cells. Nuclei of visual cells show further differentiation and are slightly wider in the triploid. × 575.

differentiated pentaploid eyes will also show whether or not the modification of the shape of the nuclei of the visual cells remains as extreme at this stage as it is in young larvae.

DISCUSSION

Changes in the number of chromosome sets present in the cells of an organism are of importance chiefly because they produce individuals that differ from the normal in the size of the building units. The change in cell size leads to a change in body size if it is not compensated by an inverse change in cell number. Such a neutralization of the primary

effect of heteroploidy is evident not only in salamander larvae but also in triploid and tetraploid silkworms (Kawaguchi, '36; Astaurov, '40). It is remarkable that the same animal body, of normal or nearly normal size and structure, can be built from cells ranging from the haploid to the pentaploid size, although the latter are approximately five times as large as the former.

While tetraploid plants of normally diploid species usually show pronounced gigantism, it should not be forgotten that this reaction may be greatly influenced by environmental and hereditary factors. Tetraploid cucumber plants do not show gigas characters when grown in the field, in contrast to their behavior in the greenhouse (Shifriss, '42). Tetraploid maize plants are much shorter and less vigorous than diploids if they are from inbred strains (Randolph, '42). In such cases, however, a reduction in both cell number and cell size may be involved in the prevention of gigantism.

In this connection the fact should also be stressed that in plants the effects of the initial increase in cell size may be cancelled in the later development of certain organs, such as the fruit. In tetraploid squashes the secondary expansion of the individual cells by increased vacuolization, which is responsible for the later phases of growth of the fruit, is much reduced so that the mature tetraploid fruit is of normal size, although the early fruit primordium was about twice as large as the diploid (Sinnott and Franklin, '43).

A partial loss of the original gigas characteristics during later development has been described in leaves of tetraploid maize plants (Randolph, Abbe, and Einset, '44). The cells of the shoot apex of tetraploid seedlings are almost exactly twice as large as those of the diploid. In the mature tetraploid leaf the average size of the cells is only 1.6 times the diploid size while the cell number is about the same as in the diploid. The total volume of the mature leaf is thus not more than 1.6 times the normal. Similar changes in the size of polyploid cells during later phases of growth will undoubtedly be discovered in other species of plants as well.

In contrast with the quantitative changes in cell size shown by most heteroploid organisms, changes in cell shape either occur but rarely, or have not received much attention so far. Peculiar changes of unknown significance have been reported for various races of the moss, Physcomitrium, where doubling of the normal, haploid chromosome number leads to a broadening of the cells in races that have narrow cells in the haploid condition, to a lengthening in races with short cells (Barthelmess, '41).

A modification of cell shape has also been found in the apical meristem of tetraploid plants of the periwinkle, Vinca rosea (Cross and Johnson, '41). In the tetraploid cells the lateral dimensions are increased by one-half to two-thirds, while the vertical dimensions may be slightly reduced. As a result of the greater width of the meristem both the stem and the leaf primordia are wider than in the diploid, i.e., the change in cell shape is responsible for important distinguishing features of the tetraploid plant.

In heteroploid amphibian larvae the changes in cell shape described in this paper do not produce changes in the appearance of the individuals but, on the contrary, help to maintain the normal proportions and structure of certain organs. The individual building blocks are molded until they fit into the normal pattern. It is probable that similar changes in cell shape take place in the wall of the capillaries, in various membranes, and, in older larvae, in the corpuscles and tubules of the mesonephros, perhaps in all structures where normal permeability is of the utmost importance.

From these observations one may conclude that both cell number and cell shape are subject to some controlling mechanism that operates during development and tends to produce a normal end result, even to the very details of organization, in spite of the apparently irreversible changes in cell size.

SUMMARY

Young triploid and pentaploid larvae of Triturus viridescens and other species of salamanders are of approximately normal dimensions because the size of the nuclei and cells, which increases in proportion to the chromosome number, is neutralized by a corresponding decrease in the number of cells. In haploid larvae, an increase in cell number brings about at least a partial compensation of the smaller cell size.

In organs with single cell layers normal dimensions and structure are maintained in heteroploid larvae by changes in cell number combined with changes in the shape of the individual cells which show a progressive flattening with increase in chromosome number. The diameter of the wall of the pronephric tubules and pronephric ducts, and the thickness of the epithelium of the lens of the eye thus remain about the same from the haploid to the pentaploid levels.

In the retina of young pentaploid larvae the number of rows of cells in the ganglionic and inner nuclear layers is reduced to about one-half of that in the diploid so that the total diameter of the retina is actually smaller in the pentaploid. In the single layer of visual cells, on the

other hand, the nuclei are wider in the pentaploid, but of about the same height as in the diploid so that the diameter of this nuclear layer remains about the same.

These observations show that in the amphibian embryo both cell number and cell shape may be modified to allow the formation of organs of normal size and structure. This indicates that both are subject to some control by the developing organism.

LITERATURE CITED

ASTAUROV, B. L. 1940 Artificial parthenogenesis in the silkworm (Bombyx mori L.). Moscow.

BALTZER, F. 1922 Ueber die experimentelle Erzeugung und Aufzucht eines haploiden Triton taeniatus. Verh. Schweiz. Naturf. Ges., Bern, S. 248–249.

BARTHELMESS, A. 1941 Mutationsversuche mit einem Laubmoos, Physcomitrium piriforme. I. Morphologische und physiologische Analyse der univalenten und bivalenten Protonemen einiger Mutanten. Z. ind. Abst. Vererb. Lehre, Bd. 79, S. 153–170.

CROSS, G. L., AND T. J. JOHNSON 1941 Structural features of the shoot apices of diploid and colchicine induced tetraploid strains of Vinca rosea. Bull. Torrey Bot. Club, vol. 68, pp. 618–635.

FANKHAUSER, G. 1938 The microscopical anatomy of metamorphosis in a haploid salamander, Triton taeniatus Laur. J. Morph., vol. 62, pp. 393–413.

———— 1941 Cell size, organ and body size in triploid newts (Triturus viridescens). J. Morph., vol. 68, pp. 161–177.

———— 1945 The effects of changes in chromosome number on amphibian development. Quart. Rev. Biol., vol. 20, pp. 20–78.

FANKHAUSER, G., AND R. R. HUMPHREY 1943 The relation between number of nucleoli and number of chromosome sets in animal cells. Proc. Nat. Acad. Sci. Wash., vol. 29, pp. 344–350.

KAWAGUCHI, E. 1936 Der Einfluss der Eierbehandlung mit Zentrifugierung auf die Vererbung bei dem Seidenspinner. I. Ueber die Auslösung der polyploiden Mutation. J. Fac. Agric., Hokkaido Imp. Univ., vol. 38, pp. 111–133.

RANDOLPH L. F. 1942 The influence of heterozygosis on fertility and vigor in autotetraploid maize. Genetics, vol. 27, p. 163.

RANDOLPH, L. F., E. C. ABBE AND J. EINSET 1944 Comparison of shoot apex and leaf development in diploid and tetraploid maize. J. Agric. Res., vol. 69, pp. 47–76.

SHARP, L. W. 1943 Fundamentals of Cytology. New York.

SHIFRISS, O. 1942 Polyploids in the genus Cucumis. J. Hered., vol. 33, pp. 144–152.

SINNOTT, E. W., AND A. H. FRANKLIN 1943 A developmental analysis of the fruit in tetraploid as compared with diploid races of cucurbits. Am. J. Bot., vol. 30, pp. 87–94.