of chromosome 10 exhibited a unique behavior in that centric regions were formed by portions of the chromosome other than the centromere.


HEAT-INDUCED TRIPLOIDY IN THE NEWT, TRITURUS VIRIDESCENS

By Gerhard Fankhauser and Rita Crotta Watson
Department of Biology, Princeton University
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Heat and cold have both been widely used to induce polyploidy in plants.1, 2, 3, 4 Depending on whether the treatment is applied during meiosis or during the early divisions of the zygote, triploid or tetraploid plants are produced. In general, the abnormal temperature seems to disturb the formation of the spindle and the normal separation of the daughter chromosomes at anaphase, probably through changes in the viscosity of the cytoplasm. Exposure during early prophase of meiosis may also affect the behavior of the chromosomes directly and prevent pachytene pairing,3, 4 or completely suppress both meiotic divisions so that a diplotene nucleus changes directly into a pollen-grain nucleus.5

Similar effects of temperature have been described in some invertebrate animals. Refrigeration of normally parthenogenetic eggs of the brine shrimp, Artemia salina, may double the chromosome number from diploid to tetraploid through inhibition of the single, equational maturation division.6, 7 Heat treatment of unfertilized eggs of the silkmoth, Bombyx mori, induces parthenogenesis and also causes retention of the diploid chromosome number, sometimes with subsequent fusion of diploid cleavage nuclei to form tetraploid or partially tetraploid animals.8, 9, 10

Among vertebrates, spontaneous and experimentally induced polyploidy have been studied extensively in several species of salamanders, because of the ease with which the chromosome number of living young larvae may be determined in whole-mounts of the amputated tailtip.11 Spontaneous deviations from the normal, diploid chromosome number occur rather frequently.12 Among 1878 larvae of the newt, Triturus viridescens, which were examined from November, 1937, to August, 1942, 38 were found to possess various deviating chromosome numbers; the majority of these, 25 (1.33% of the total), were triploid.

Experimental triploidy was first induced in salamanders by refrigeration
of freshly fertilized eggs of *Triturus viridescens*. Before insemination, which occurs during egg-laying, the amphibian egg has reached the metaphase of the second maturation division; it remains in this stage until fertilization has taken place. In the newt, late anaphase is reached about 30 minutes after fertilization. The low temperature presumably suppresses the second maturation division and produces a diploid egg nucleus which fuses with a normal, haploid sperm nucleus to form a triploid cleavage nucleus. A cytological investigation of the events taking place in refrigerated eggs is in progress.

The range of low temperatures which will induce triploidy is considerable. Temperatures from $0^\circ$ to $+4^\circ$C. seem to be most effective; however, lower temperatures also give good results. The figures include all experiments performed so far, except those involving temperatures above $4.35^\circ$C. that are not as effective in inducing triploidy.

### TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature</th>
<th>Number of eggs treated</th>
<th>Number of larvae obtained</th>
<th>Chromosome</th>
<th>% 3N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triturus viridescens</em></td>
<td>$0^\circ$ to $+4.35^\circ$C.</td>
<td>466</td>
<td>228 (48.9%)</td>
<td>167</td>
<td>50</td>
</tr>
<tr>
<td><em>Triturus pyrrhogaster</em></td>
<td>$+1.5^\circ$ to $+2.5^\circ$C.</td>
<td>117</td>
<td>29 (24.8%)</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td><em>Amblystoma mexicanum, Azolotl</em></td>
<td>$+1^\circ$ to $+3^\circ$C.</td>
<td>154</td>
<td>31 (20.1%)</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

These figures include all experiments performed so far, except those involving temperatures above $4.35^\circ$C. that are not as effective in inducing triploidy.

1 Plus one mixed hyperdiploid-triploid larva.
2 Plus one hyperdiploid larva.

Triploid larvae have also been obtained in considerable numbers following exposure of eggs to $+5.5^\circ$ and $+6.38^\circ$C. The upper and lower limits of the effective range have not yet been determined accurately. The duration of the treatment varied from 5 to over 24 hours; a few experiments of shorter duration produced diploid larvae only. The percentage of triploid larvae varied in different series of experiments, largely because the eggs of individual females seem to react differently to the same treatment.

The refrigeration method has also been successfully applied to eggs of *Triturus pyrrhogaster* and, more recently, in collaboration with Dr. Humphrey of the University of Buffalo, to axolotl eggs (table 1). Although the total number of eggs treated in these two species was rather
small, the results indicate that specific differences exist, both in the rate of mortality during early stages of development and in the percentage of triploid individuals among the surviving larvae. It is also of interest that this treatment occasionally produces haploid larvae in all three species. The mechanism which leads to the formation of a haploid cleavage mitosis under cold treatment is not known at present.

In view of the effectiveness of heat treatment as a polyploidy-inducing agent in plants, experiments to test the influence of high temperatures on salamander eggs had been considered for some time. Following preliminary tests of the heat resistance of eggs of *Triturus viridescens* by several undergraduate students, the first extensive series of heat treatments were carried out by Rita Crotta Watson in the spring of 1942. The eggs were transferred immediately after laying to dishes kept in an incubator running at 34.2° to 37.2°C. Following the treatment which lasted for from 5 to over

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULTS OF HEAT TREATMENT OF FRESHLY FERTILIZED EGGS OF <em>Triturus viridescens</em></td>
</tr>
<tr>
<td>Beginning of treatment: immediately after laying.</td>
</tr>
<tr>
<td>Duration of treatment: 5 to over 50 minutes (see table 3).</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>No. of eggs treated</td>
</tr>
<tr>
<td>No. of larvae obtained</td>
</tr>
<tr>
<td>Triploid</td>
</tr>
</tbody>
</table>

50 minutes (tables 2 and 3) the eggs were returned to water at room temperature and allowed to develop. The chromosome number of each surviving larva was determined from counts in the amputated tailtip.

The rate of mortality during early stages of development did not differ significantly at the various temperatures used (table 2). On the other hand, at any one of these temperatures, treatments lasting for over 19 minutes seemed to be increasingly harmful (table 3). The mortality among heat-treated eggs was highest during the blastula stage; in the refrigeration experiments, on the other hand, it reached its peak during early cleavage.

The four larvae which developed from eggs exposed to 34.2° were all diploid. Of the larvae obtained from treatments at 35 to 37.2°C, 84.4% were triploid; within this range, different temperatures seemed to be about equally effective in inducing triploidy (table 2). The duration of the treatment also had no clear-cut influence on the resulting percentage of
triploid larvae (table 3). The shortest treatments that produced triploid larvae were 5 minutes at 37.0°C., and 8 minutes at 36.0°C.

More experiments will be needed to determine the range of effective temperatures and lengths of treatments. Already it is evident that short heat treatments are at least as effective in inducing triploidy as long cold treatments, if not more so (compare tables 1 and 2). Furthermore, the rate of mortality among the heat-treated eggs may be slightly lower.

It is interesting to compare these results with those obtained by heat treatments of various species of plants during the early divisions of the zygote (cf. ref. 15, table 1). The temperatures used ranged from 38°C to 45°C., and the duration of the treatment varied from 30 minutes to 48 hours. The yield of polyploids (predominantly tetraploids) varied from 0.25 to 8%. The greater effectiveness of heat treatments of amphibian eggs is probably determined largely by three factors: (1) the treatment can be applied directly to the isolated egg cell; (2) it reaches the egg always in exactly the same stage of the mitotic cycle, the metaphase of the second maturation division; (3) the movements of the chromosomes are normally suspended until about 10 to 15 minutes after fertilization, with the chromatids still closely associated in pairs (dyads); the treatment thus merely prevents these movements from resuming their course instead of stopping an actively progressing mitosis.

Attempts to induce tetraploidy by refrigeration of newt eggs during the first cleavage mitosis have been a complete failure so far, probably because of the difficulties encountered in timing the treatment. The living egg is opaque and does not indicate in any way when it reaches the metaphase of the first division; moreover, the study of sections through preserved eggs has shown that the interval between fertilization and metaphase varies widely between individual eggs kept at the same temperature. It is possible that heat treatments, which are more easily controlled because of the short exposure that is needed, will be more successful.

<table>
<thead>
<tr>
<th>Duration, Minutes</th>
<th>5-9</th>
<th>10-19</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>Over 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eggs treated</td>
<td>4</td>
<td>47</td>
<td>24</td>
<td>13</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Number of larvae obtained</td>
<td>5 (71.4%)</td>
<td>34 (72.3%)</td>
<td>12 (50%)</td>
<td>5 (38.5%)</td>
<td>2 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Triploid</td>
<td>5 (100%)</td>
<td>29 (85.3%)</td>
<td>8 (66.7%)</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>..</td>
</tr>
</tbody>
</table>
Summary.—One hundred and thirteen freshly fertilized eggs of the newt, Triturus viridescens, were treated at temperatures ranging from 34.2° to 37.2°C., for from 5 to over 50 minutes, and raised at room temperature. Sixty-two (54.9%) of the treated eggs developed into larvae; 49 of these (79%) were found to be triploid, the rest diploid. The shortest heat treatments which produced triploid larvae were 5 minutes at 37.0°C., and 8 minutes at 36.0°C.

A comparison of the results with those obtained by refrigeration of eggs shows that short heat treatments are at least as effective in inducing triploidy as cold treatment lasting for 5 or more hours. Both heat and cold presumably suppress the second maturation division of the egg which is not completed normally until about one hour after fertilization.

3 Sax, K., Jour. Arnold Arboretum, 17, 153-159 (1936).
9 Astaurov, B. L., "Artificial Parthenogenesis in the Silk Worm (Bombyx mori L.)," Moscow (1940).
14 Griffiths, R. B., Genetics, 26, 69-88 (1941).

THE INCREASE OF B VITAMINS IN GERMINATING SEEDS*

BY PAUL R. BURKHOLDER AND ILDA McVEIGH

OSBORN BOTANICAL LABORATORY, YALE UNIVERSITY

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The rapid synthesis of provitamins A and vitamin C in germinating seeds has been clearly demonstrated by many investigators in recent years.1 2 Much less is known, however, concerning the possible changes in the amounts of B vitamins during germination. Mung bean sprouts are known to contain vitamins of the B complex.3 It has been reported that the B₁ of pea embryos increases during germination while the content