An Experimental Analysis of the Development of the Haploid Syndrome in Embryos of
Xenopus laevis

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INTRODUCTION

HAPLOID vertebrates may occur spontaneously but are very rare (Fankhauser, 1941; Humphrey & Fankhauser, 1957); however, haploids may be experimentally produced in fish (Swarup, 1959) and in mammals (Beatty, 1953), while amphibian eggs may be so treated that all developing embryos are haploid (Porter, 1939; Gurdon, 1960).

The full descriptions of the development of haploid Rana pipiens (Porter, 1939) and R. nigromaculata (Miyada, 1960) apply so well to Xenopus laevis that only the most important points will be touched on here.

Haploid amphibians may be identified at the beginning of gastrulation since their animal pole cells are smaller at a given stage than are those of diploids. In all haploid Anura the onset of gastrulation is delayed, and thereafter haploids become progressively more retarded in their development. Their neural plates are shorter, and when the neural folds have closed it can be seen that the embryos are microcephalic and suffer from lordosis and a bulging abdomen. These anomalies are common to all haploids; but after hatching it can be seen that the majority of haploids have a feeble heart and sluggish blood circulation, which may be the reason for the oedema to which the animals are subject. They are much less active than diploids and will lie at the bottom of the dish unless provoked into violent but short-lived activity. Their muscle fibres remain spindle-shaped and do not develop into compact blocks of tissue as do those of diploids.

Whereas 90–95 per cent of haploid Xenopus develop the haploid syndrome as described, the remainder become normal looking tadpoles despite having shown the early signs of haploidy before hatching. Haploids of other Anura

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also occasionally appear normal, but until Miyada's report (1960) none were known to have metamorphosed. However, he managed to rear 8 (0.05 per cent) of his haploid *Rana nigromaculata* through metamorphosis, but at all times they were physically weaker than diploids.

In this paper a distinction will be made between the primary signs of haploidy (e.g. short myotomes) which are a direct consequence of small cell size, and the secondary signs of haploidy (e.g. oedema, feeble heart and poorly coiled gut), which together define the term haploid syndrome.

Indeed, one of the earliest theories to explain the haploid syndrome (Tchou-Su, 1931) implied that small cell and nuclear size caused the syndrome by upsetting the balance of surface area to volume. Since the nuclear surface was then considered to be the site of oxidative reactions Tchou-Su suggested that haploid cells produced more energy than the cell could use. However, we know from the work of Hertwig (1927) that haploid cells will survive in a diploid environment as well as diploid cells will, and hence any theory demanding that haploid cells are inevitably short-lived must be wrong.

Other theories to explain the syndrome are that proposed by Darlington (1937) to explain the early death of homozygous diploids, which involves the unmasking of recessive lethal genes; and that of Briggs (1949) which suggests that early nuclear cytoplasmic imbalance is of primary importance.

By means of grafting experiments Hadorn (1936, 1937) was able to show that hybrid andromerogons, developing from enucleate *Triturus palma* eggs fertilized with *T. cristatus* sperm, died apparently from causes acting specifically on the cells of the head mesenchyme, Epidermal cells from these haploid hybrids survived well on diploids. Since both heterospermic (Hadorn, 1937) and homospermic (Hertwig, 1927) haploid epidermis can survive to adult stages when grafted embryonically on to diploid hosts, one might expect to find that the mesenchyme of these two types would also behave similarly. However, Beetschen (1960) has shown that there is no tissue in haploid *Pleurodeles waltlii* that behaves like the mesenchyme of the previously mentioned hybrid andromerogons, and is a centre of degeneration. He parabiosed haploid and diploid *P. waltlii* and reported that many pairs metamorphosed. In all cases the haploid partner was malformed and more or less a parasite, so that it was impossible to determine the degree of haploid organ function, since the diploid partner may have been carrying the functional load.

The present work was an attempt to elucidate some of the causes of the haploid syndrome in *Xenopus*, particularly by efforts to relieve haploids, or their tissues, of the syndrome. It was thought that lower temperature might reduce the mortality of haploids if a vital process which they performed subnormally was not slowed so much by cold as other functions which they performed more normally. Haploid/diploid anterior/posterior chimaerae were made in order to assess the capacity of diploid tissues to improve the development of haploid, while ensuring that the haploid organs had to function.
MATERIALS AND METHODS

Adult *Xenopus laevis* were induced to spawn by injection of commercial gonadotrophin. Ten to twenty minutes after laying, fertilized eggs were irradiated with 2000 ergs/sq. mm. u.v. light to produce androgenetic haploids (Gurdon, 1960).

Haploid and diploid embryos used in the temperature experiment were reared in groups of about forty, in tap water that had been allowed to stand and become dechlorinated. The tadpoles hatched naturally and were fed on a suspension of nettle powder in water.

Operations were performed on decapsulated embryos at Stage 22/23 (Nieuwkoop & Faber, 1956) in full strength Holtfreter solution on agar in 2 in. Petri dishes. A haploid and a diploid embryo were brought together and both were transected at the required level. The corresponding portions were squeezed into a trough in the agar which was slightly longer and narrower than the embryo. Occasionally it was necessary to hold the embryo in position with slivers of cover glass.

Healed chimaerae were transferred to 50 per cent Holtfreter solution in agar-lined dishes where they remained overnight (15 hours). Next morning they were moved to 10 per cent Holtfreter solution. 2 g. sodium sulphadiazine was added to every litre of culture medium for embryos up to Stage 40. After Stage 40 they were treated without special precautions.

Whole mounts of tail tips, cut from living tadpoles, were stained and inspected to determine ploidy. Chromosome counts were not made since nuclear size and maximum number of nucleoli seen in any one nucleus are excellent indicators of ploidy.

RESULTS

Survival of haploid Xenopus

It was recognized early in this work that 90 per cent or more of *Xenopus* haploids die before hatching or develop the syndrome, while the remaining 5–10 per cent may feed and grow for some time but do not reach metamorphosis. In order to gain more precise information on the pattern of haploid mortality, survival curves were constructed for haploid and control diploid populations. The experiment was conducted at 22°C (which is near the optimum for diploids) and 16°C to discover whether the overall metabolic rate, as controlled by temperature, affected the pattern or survival.

During the experiment, moribund tadpoles were removed from culture when it was considered they would not live another day. This means that the number of survivors at any time may have been underestimated but it was possible to preserve the animals for further inspection. Removal of haploids just before their death and decay may also have had a beneficial effect on the remaining...
animals since as many as 15 per cent of all the haploids in this experiment fed and were free of the syndrome.

Temperature did not appear to affect anything but the rate of development, in the sense that there were no obvious morphological differences between animals of the same stage that had been reared at different temperatures. Nevertheless development was nearly twice as rapid at 22°C as it was at 16°C.

Text-fig. 1 illustrates these points. The mortality of diploids at these stages is normally negligible at either temperature. However, the curve for diploids at 22°C flatters the culture conditions of these animals since one bowl became foul and its occupants died. The data from these animals have not been included in the curve. The days on which 'cool' and 'warm' haploids, that appeared normal, reached a named stage are linked by broken lines so that it can be seen that by reducing the temperature from 22°C to 16°C the life- but not the stage-expectancy of these haploids was increased.
The mean expectations of life of all haploids were 14.4 days at 22°C and 22 days at 16°C, which is very close to the age at which the majority of 'syndromic' haploids, raised at these temperatures, died. Since these haploids became arrested at Stage 43+, which they reached on the fifth and ninth days respectively, they spent more than half their lives in this state.

The most advanced haploid in this experiment lived for 4.4 months and died at Stage 57, but after 2 months both 'warm' and 'cool' tadpoles were cultured together at room temperature, so its early history is unknown. It was one animal out of many hundreds.

Development of haploid/diploid chimaerae

These experiments were performed to see which path of development combinations of haploid and diploid tissue would follow, and how much the tissues would influence each other. The results can only be evaluated with knowledge of the survival curves and development of haploid and diploid *Xenopus laevis*. Since the abnormalities found in haploid *Xenopus* are similar to those described in detail by Porter (1939) for *Rana pipiens* they will not be repeated here.

When chimaerae were made the junction was at one of the three levels indicated at the left of Text-fig. 2. The convention used to describe such chimaerae is to put the letter for the level of the cut first, followed by the hyphenated symbols for haploid and diploid with the anterior one first. Thus an animal consisting of anterior diploid and posterior haploid tissue joined at the most posterior level was designated C D–H.

In a group of six control diploid operations (C D–D) one showed signs of the 'haploid' syndrome (oedema and not feeding) and died within 9 days; two otherwise normal animals swam in spirals and died within 13 days of the operation and the other three were indistinguishable from unoperated diploid controls and were discarded at 1 month. Hence a high proportion of all chimaerae may be adversely affected by the operation itself. All haploid chimaerae were more seriously affected since all eight C H–H died within 5 days of the operation and their unoperated controls lived another 3 days.

The separate parts of mixed chimaerae developed as one would expect from their ploidy, up to Stage 35/36, so that all haploid eyes were retarded in pigmentation compared with diploid ones. Later, some diploid optic cups failed to enlarge so that the cavity was often obliterated and the retina much folded and disorganized, and they looked like the eyes of 'syndromic' haploids. Many eyes of haploid origin became perfect. This phenomenon applies to other organ systems too (regardless of how far the organ in question is from the site of the cut); in fact, it appears that the development of a chimaera is so integrated that the whole animal tends to be either haploid-like or diploid-like.

The chimaerae in experiment 28.xi.60. (Text-fig. 2) were each given scores
for various organ systems which depended upon the extent to which they were diploid- or haploid-like or intermediate. The groups with the most diploid scores were A H–D, C H–D and C D–H.

<table>
<thead>
<tr>
<th>Control diploid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A H–D</td>
<td>6</td>
</tr>
<tr>
<td>B H–D</td>
<td>12</td>
</tr>
<tr>
<td>C H–D</td>
<td>6</td>
</tr>
<tr>
<td>C D–H</td>
<td>6</td>
</tr>
<tr>
<td>B D–H</td>
<td>12</td>
</tr>
<tr>
<td>A D–H</td>
<td>6</td>
</tr>
<tr>
<td>Control haploids</td>
<td></td>
</tr>
<tr>
<td>No survivors</td>
<td></td>
</tr>
</tbody>
</table>

**Text-fig. 2.** Camera lucida drawings of living embryos in experiment 28.xi.60., made 6 days after the operation (right). On the left are schematic representations of the embryos at the time of operation, with the original number of each type of chimaera indicated.
None of the B H–D embryos had well developed hearts and yet many swam vigorously. At this stage the length of the tail, which is a direct consequence of ploidy and cell size, determines how much a tadpole swims. That diploid tails are longer than haploid can be seen in Text-fig. 2.

That the degree of gut coiling was only intermediate in group C H–D can be explained by postulating a coiling centre in the anterior portion which exerts its influence on the rest of the gut. Since wholly haploid animals have even more poorly coiled intestines than H–D animals, diploid endoderm may respond better to the weak stimulus from the haploid coiling centre. C D–H intestines are well coiled, which suggests that the diploid centre is highly efficient.

Ten days after the operation the survivors were fixed and sectioned. Inspection of the sections confirmed the earlier scores of the same animals. It was observed that five out of six A H–D animals had a defective branchial skeleton, as did both B H–D survivors but none of the three remaining groups of animals. This malformation occurs in the region of transection and was probably caused by a combination of operative damage and the inadequacy of the haploid cells in this region, since it differed from the defects seen in the syndrome. Those animals (B D–H) with the operation at this level, but in which diploid cells made up the skeleton, showed no such damage; neither did those with haploid skeletal elements and a more posterior site of operation. In fact these animals (C H–D) had better formed jaws than most control haploids.

The development of haploid eyes and hearts also appeared to be improved in chimaerae, provided the site of operation did not lie close to the primordia of these organs, which shows that the mere presence of diploid tissue anywhere in a chimaera may be enough to prevent haploid tissues from developing in a way characteristic of the haploid syndrome. It is difficult, however, to say that those diploid parts of a chimaera failing to become normal were specifically affected by the adjoining haploid tissue, since one-sixth of D–D controls developed signs of the haploid syndrome.

Another aim of these experiments was to rear partial haploids through metamorphosis, and because there were strong indications that C chimaerae were the most healthy, more of them were made.

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>No. of animals on day of op.</th>
<th>Coiled gut no oedema</th>
<th>Poorly coiled gut. Oedema</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 21/22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haploid control</td>
<td>20</td>
<td>1</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>C H–D</td>
<td>24</td>
<td>5</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>C D–H</td>
<td>24</td>
<td>14</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Diploid control</td>
<td>20</td>
<td>19</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Another aim of these experiments was to rear partial haploids through metamorphosis, and because there were strong indications that C chimaerae were the most healthy, more of them were made.
In experiment 13.vi.61. there was similar evidence that it is better to have diploid tissue in front than an equal volume behind. Twenty-nine haploid and twenty-nine diploid embryos were used but only twenty-four each of C D–H and C H–D healed satisfactorily. From the time of the operation (Stage 22) until Stage 36 the two portions of the chimaeras seemed to develop according to their ploidy. After this stage it became more difficult to distinguish between the portions on morphological grounds, since the whole chimaeras were becoming either diploid-like or haploid-like. Seven days after the operation C H–D and C D–H chimaeras and their haploid and diploid controls were divided into two groups on the basis of their state of oedema and gut coiling together (Table 1). In no cases did a tadpole have a well coiled gut and oedema or vice versa. Also associated with a well coiled gut and lack of oedema was diploid-like development of the eyes, heart and branchial skeleton.

![Text-fig. 3. Survival curves for chimaeras and their unoperated controls in days after the operation (experiment 13.vi.61.). The original numbers were controls twenty each and chimaeras twenty-four each.](image_url)
The viability of diploid-like chimaerae was higher than that of haploids and the curves in Text-fig. 3 demonstrate that the best survival was of the groups with the most diploid-like development. The maximum age reached by two C D-H was 5½ months, which represented Stage 57 for one and 62 for the other.

One may conclude that there are slight differences in viability between haploid and diploid tissues and that when the total reduction in viability falls to a certain level the haploid syndrome may develop. The presence of diploid tissues in a chimaera may allow increased development and efficiency of haploid organs; however, the fact that no chimaerae metamorphosed suggests that the haploid tissues were deleterious. It is possible that diploids may contain a 'vitalizing' substance, as Fankhauser suggested in 1952; or that they can remove haploid 'devitalizing' substances from the environment.

DISCUSSION

The theories of the haploid syndrome may now be reconsidered. Tchou-Su (1931) expected the nuclei of haploid cells to be the same shape, and the haploid nuclear volume and surface area to be 0.5 and 0.63 respectively of those of a diploid. However, haploid and diploid nuclei are not the same shape, for haploid nuclei are more nearly spherical than diploid ones, as Böök (1941) and Miyada (1960) emphasized. This means that since the ratio between volumes is 0.5, that between surface areas must be other than 0.63. If one postulates that the ratio of surface areas is less than 0.63 and approaches 0.5 then the nuclei (in any tissues in which the diploid nuclei are not themselves nearly spherical) should be more nearly spherical in haploids than diploids. This is indeed the case, so that it appears that the area of nuclear membrane may be related to the number of chromosomes rather than their volume.

The electron microscopic studies of Barer, Joseph & Meek (1960) on the fate and reformation of the nuclear membrane suggest that, in fact, each chromosome plays an important part in the membrane's formation. They state that after nuclear division, elements of the endoplasmic reticulum assemble on the chromosomes before fusing with each other to form an intact double membrane. With only half the number of 'crystallization' centres one might expect half-size haploid nuclear membranes, and the nuclei to be more nearly spherical than diploid. As this is so, there is no reason to suppose that the surface activity of haploid nuclei is disproportionally increased.

The prolonged life of haploid tissues, and even half-animals, when grafted to diploid does not help to confirm the theories that the syndrome is caused by either the presence of unmasked recessive lethal genes, or nucleo-cytoplasmic imbalance. But it does suggest that diploid tissues act as a prophylactic. The diploid cells may be removing harmful substances from, or providing beneficial substances for the haploid cells.
Not all diploids, however, are free from the signs of the haploid syndrome as Subtelny (1958) showed with homozygous diploid *Rana pipiens*. Relatively fewer of these tadpoles were affected than were their haploid sibs, in spite of the fact that the diploids were as homozygous as the haploids. Because they had the nucleo-cytoplasmic ratio of a diploid he concluded that the embryos not developing the syndrome were those that would have been affected by an underdose of nucleus; and the ones that did develop the syndrome did so by an undetermined condition of the nucleus. The fact that homozygous diploids develop the syndrome late does not support any idea that specific genes are acting, for these animals should develop the syndrome earlier, since in all other respects they are more advanced than haploids.

Although the development of haploids is uniform enough to lead to the haploid syndrome, yet variation among haploids is greater than among normal diploids; and in this respect a parallel can be drawn with other ‘homozygous’ animals.

McLaren & Michie (1954) and Grüneberg (1954) showed that inbred mice that were nearly homozygous were more variable than hybrids in response to ‘Nembutal’, or in development, respectively.

It therefore seems possible that the facts can be explained by the hypothesis that ‘homozygous’ haploids and diploids are unable to counteract stresses in development owing to their lack of developmental stability; and that such stresses may be caused by the presence of recessive lethal genes or nucleo-cytoplasmic imbalance. As a result their development diverges from normal and proceeds towards the signs of the haploid syndrome. The added ‘stability’ of diploid tissue in chimaerae is enough to turn the development of the haploid tissues towards normality and reduce the effects of unfavourable conditions.

**SUMMARY**

1. The only observed effect of rearing haploid and diploid embryos of *Xenopus laevis* at 16°C instead of 22°C was retarded development. The same proportion of haploids (90–95 per cent) develop the syndrome (oedema, feeble heart, poorly coiled gut) and the stage expectancy is similar.

2. Haploid tissues may live longer and develop more normally when telobiosed to diploid tissues, but anterior diploid tissue promotes better development than the same quantity of posterior tissue.

3. It is suggested that the haploid syndrome develops because haploids lack hybrid vigour completely, and cannot regulate the abnormalities caused by smaller cell size and/or unmasked recessive lethal genes.

4. An hypothesis is put forward to explain the fact that haploid nuclei are more nearly spherical than diploid nuclei.
HAPLOID SYNDROME IN XENOPUS

RÉSUMÉ

1. Le seul effet que l'on peut observer chez des embryons haploïdes et diploïdes de *Xenopus laevis*, élevés à 16°C au lieu de 22°C est un retard de développement. La même proportion des haploïdes (90-95%) montre le syndrôme (œdème, coeur faible, intestin malformé) et ils meurent aux mêmes stades.

2. Les tissus haploïdes peuvent vivre plus longtemps et se développer plus normalement quand il est pratiqué une télobiose avec des tissus diploïdes; mais ils se développent meilleur si les tissus diploïdes sont antérieurs.

3. Il est suggéré que le syndrôme haploïde se développe parce que les haploïdes manquent complètement de la 'vigueur hybride', et ne peuvent pas compenser les anomalies occasionnées soit par le plus petit volume des cellules soit par des gènes recessifs létaux (de ce fait non-masqués).

4. Une hypothèse est proposée pour expliquer pourquoi les noyaux haploïdes sont plus proches de la forme sphérique que les noyaux diploïdes.

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REFERENCES


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