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## Natural occurrence of monoploids and polyploids in the Indian catfish, *Heteropneustes fossilis*

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**Natural occurrence of male and female haploid, triploid and tetraploid *Heteropneustes fossilis* is reported for the first time. Karyotypic and nuclear volumetric evidences are described to confirm the haploid, triploid and tetraploid nature of the identified individuals. Studies on spermatogenesis also confirm the observed unusual ploidies in these individuals.**

OWING to the absence of well-defined sex chromosomes in fishes, polyploidy has spontaneously originated, perhaps repeatedly, and has been sustained in populations of diverse orders<sup>1</sup>. In fact much has been written on this subject by Ohno<sup>2</sup>. Expectedly, natural triploid populations have evolved in 8 genera representing 3 orders of fish; the viviparous Poeciliids (*Poecilia*, *Poecilopsis*)<sup>2</sup>, oviparous Cyprinids (*Carassius*<sup>4</sup>; *Misgurnus*<sup>5</sup>; *Phoxim*<sup>6</sup>; *Rutilus*<sup>7</sup>) and Athernids (*Menidia*)<sup>8</sup>. Viable hybrid unisexual triploids have also been recorded in *Poecilia*<sup>9</sup>: (i) *P. latipinna-2 mexicana*, and (ii) *P. latipinna-mexicana*; *Poecilopsis*<sup>10</sup>: (i) *P. 2 monacha-lucida* (ii) *P. monacha-2 lucida*, and (iii) *P. monacha-viriosa-lucida*; and *Phoximus*<sup>11</sup>: (i) *P. 2 eos-neogaeus*, and (ii) *P. eos-2 neogaeus*<sup>1</sup>. Likewise, naturally occurring tetraploids have been reported in *Carassius auratus*<sup>4</sup>, the European *Barbus* sp.<sup>12</sup>, *Misgurnus anguillicaudatus*<sup>5</sup> and *Cobitis biwae*<sup>13</sup>. The frequency with which unusual base replacements, inactivating mutations can occur in the duplicated genes, and the workload for replication has perhaps minimized the abundance of tetraploid fish species<sup>14</sup>.

In India, Pandey and Lakra<sup>15</sup> recorded tetraploidy in a single individual of *Clarias batrachus*, using karyotype ( $2n = 50$ ;  $4n = 100$ ) as the evidence. While listing chromosome number of several fishes, Manna<sup>16</sup> doubted the possible occurrence of polyploidy in *Heteropneustes fossilis*, but provided no supporting evidence for it. Tiwary *et al.*<sup>17</sup> claimed successful induction of triploidy in *H. fossilis*, but have not provided acceptable evidence for their claim. To the best of our knowledge, no report is as yet available for natural occurrence of haploids (male and female) in any fish species, although the publication of Varadaraj<sup>18</sup> stands out as a single publication on the induction of viable haploid gynogenetic *Oreochromis mossambicus*.

This present communication reports on the natural occurrence of monoploids and polyploids (triploids and tetraploids), both in males and females in the South Indian populations of the catfish, *H. fossilis*, on the basis of the evidences of erythrocyte nuclear volume and karyotype. We have also shown the unconventional mechanism of spermatogenesis in these naturally occurring polyploids.

Collections of *H. fossilis* were made from different sources in Tamilnadu and Kerala during April–October 1998. As many as 120 individuals were randomly selected from these populations, for confirmation of their ploidy groups. Initially, a number of individuals were sacrificed to acquire blood for erythrocyte nuclear measurement<sup>19</sup>, and tissues for karyotyping<sup>20</sup>. However, all subsequent analyses were made following noninvasive procedures, as live monoploids and polyploids were required for further studies: Hence, blood was collected by caudal puncturing. Smears were fixed in methanol for 1 min and stained in 4% Giemsa in phosphate buffer (pH 6.4) for 10 min, and were subjected to nuclear measurements using stage and ocular micrometer (Erica, Japan) under a phase contrast microscope (Nikon, Japan).

Table 1 shows the occurrence of monoploids, diploids, triploids, and tetraploids at the frequency of 1.7, 91.7, 4.2 and 2.5% respectively. In all these unusual ploidy groups, both males and females were recorded, though the frequency of female triploid and tetraploid was only one each, against 4 and 2 males, respectively.

Ploidy was identified on the basis of both erythrocyte (RBC) volume, and chromosome number. The nuclear volume of RBC increased from  $4.1 \mu\text{m}^3$  in a haploid to 8.7, 13.7 and  $19.5 \mu\text{m}^3$  in diploid, triploid and tetraploid, respectively (Figure 1). In tetraploids, the nuclear volume widely varied, compared to other ploidy groups.

The diploid chromosome number varied between 56 and 58. Of the 110 individuals analysed, as many as 86 individuals had 58 chromosomes, 15 individuals had 56, and the rest 57. Of the 120 individuals analysed, 2 proved to be haploids, of which the female bore 30

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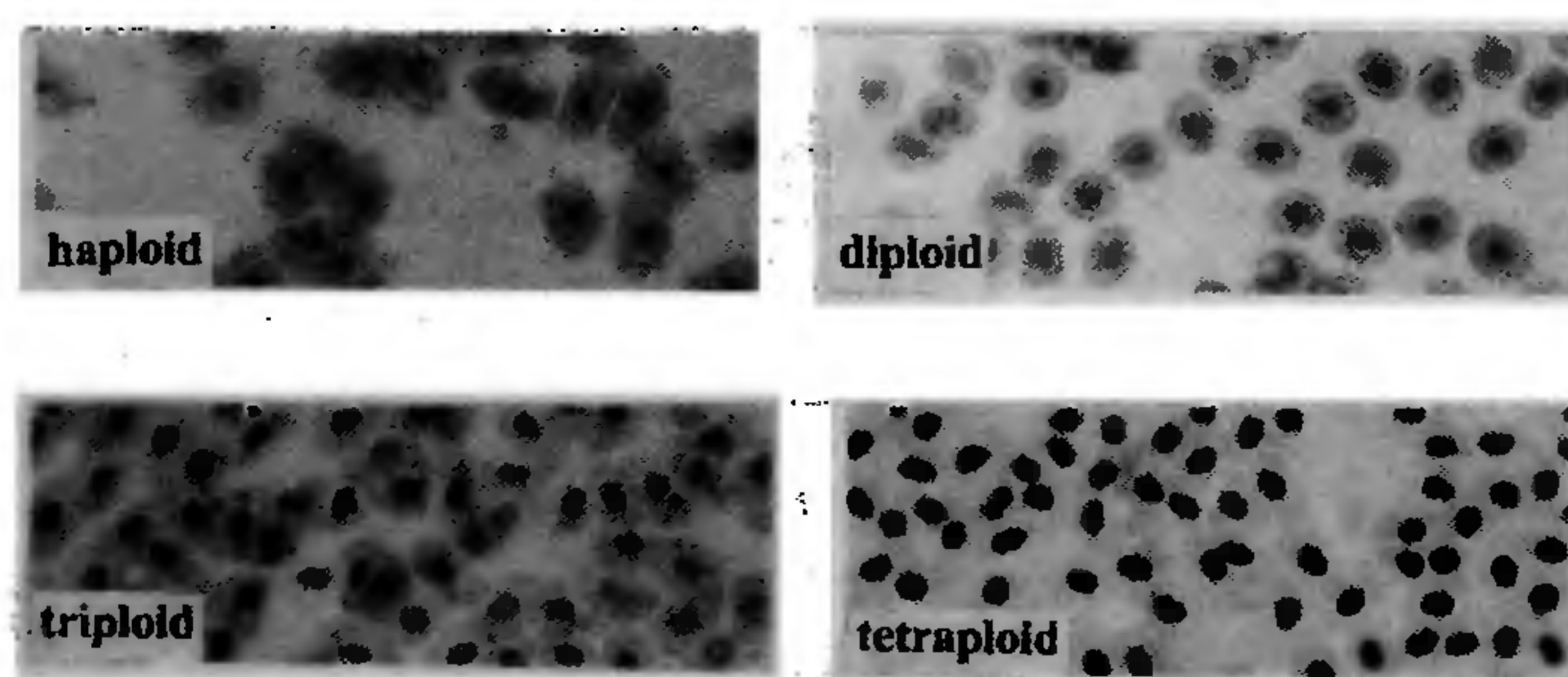
**Table 1.** Natural occurrence of monoploids and polyploids in the South Indian population of the catfish *H. fossilis*

Ploidy type	Chromosome number	RBC nuclear volume ( $\mu\text{m}^3$ )	Number of males	Number of females	Occurrence frequency (%)
Haploid	29*/30	$4.1 \pm 1.17$	1	1	1.7
Diploid	56–58	$8.7 \pm 1.35$	47	63	91.7
Triploid	87	$13.7 \pm 1.70$	4	1	4.2
Tetraploid	116	$19.5 \pm 3.04$	2	1	2.5

Total number of individuals subjected to karyotype and RBC analyses is 120; \* = male.

**Table 2.** Estimation of sperm head diameter to determine the percentage of euploid and aneuploid sperms in the haploid, diploid, triploid and tetraploid males of *H. fossilis*

Ploidy (n)	Sperm head diameter ( $\mu\text{m}$ )	Haploid (%)	Diploid (%)	Triploid (%)	Tetraploid (%)
<i>Euploid sperm</i>					
Haploid (n)	1	30	94	19	2
Diploid (2n)	2	—	3	21	90
Triploid (3n)	3	—	—	12	—
Tetraploid (4n)	4	—	—	5	—
<i>Aneuploid sperm</i>					
Hypohaploid	< 1	70	2	29	—
Hypodiploid	< 2	—	1	8	4
Hypotriploid	< 3	—	—	4	4
Hypertriploid	> 3	—	—	2	—

**Figure 1.** Erythrocytes of haploids, diploids, triploids and tetraploid Indian catfish, *H. fossilis*. All photomicrographs were made at the same magnification of 40 $\times$ . Note the increase in diameter of the darkly stained nucleus in the haploids, diploid and polyploids.

chromosomes and the male 29 only. All the 5 identified as triploids consistently had 87 chromosomes. No difference was noted in the chromosome number between male and female triploids. The tetraploid carried 116 chromosomes. The presence of microchromosomes were observed in all these ploidy groups (Figure 2).

Table 2 shows the head diameter of euploid and aneuploid sperms generated by haploids, diploids, and polyploids of *H. fossilis*. In diploids, spermatogenesis is typical and conventional, resulting in the production of 94% euploid (n) sperms; likewise, spermatogenesis was also typical and conventional in tetraploids, resulting in the production of 90% euploid (2n) sperms. But, the

spermatogenesis in triploids was unconventional and hazardous resulting the production of 56% euploid sperms and 43% aneuploid sperms. Among the euploid sperms, some were haploids while others were deploids, triploids, and some even tetraploids (Figure 3). The hazardous nature of spermatogenesis is indicated by the presence of hypohaploids, hypodiploids, hypotriploids and hypertriploids. The ability of triploid and tetraploid sperms to activate/fertilize diploid *H. fossilis* eggs (n) has been reported elsewhere<sup>21,22</sup>.

To the best of our knowledge this is the first report on the natural occurrence of male and female haploids in fish; triploids and tetraploids have already been reported, mostly from China, Japan, Europe, and North America<sup>1</sup>. Based on a single individual of *Clarias batrachus*, natural occurrence of tetraploidy in India was claimed<sup>15</sup>.

In India, the presence of the genus *Heteropneustes* is predominantly represented by a single species *H. fossilis*. In recent years the occurrence of *H. microps*, which hails from Sri Lanka, has been reported (S. Sridhar and M. A. Haniffa, pers. commun.). The morphological character by which these two species are distinguished is by the anal fin, which remains confluent with caudal fin in *H. microps*, but separate in *H. fossilis*. That all the 120 individuals analysed in the present work had anal and caudal fins, typical of *H. fossilis* clearly indicates that the individuals were not hybrids of these

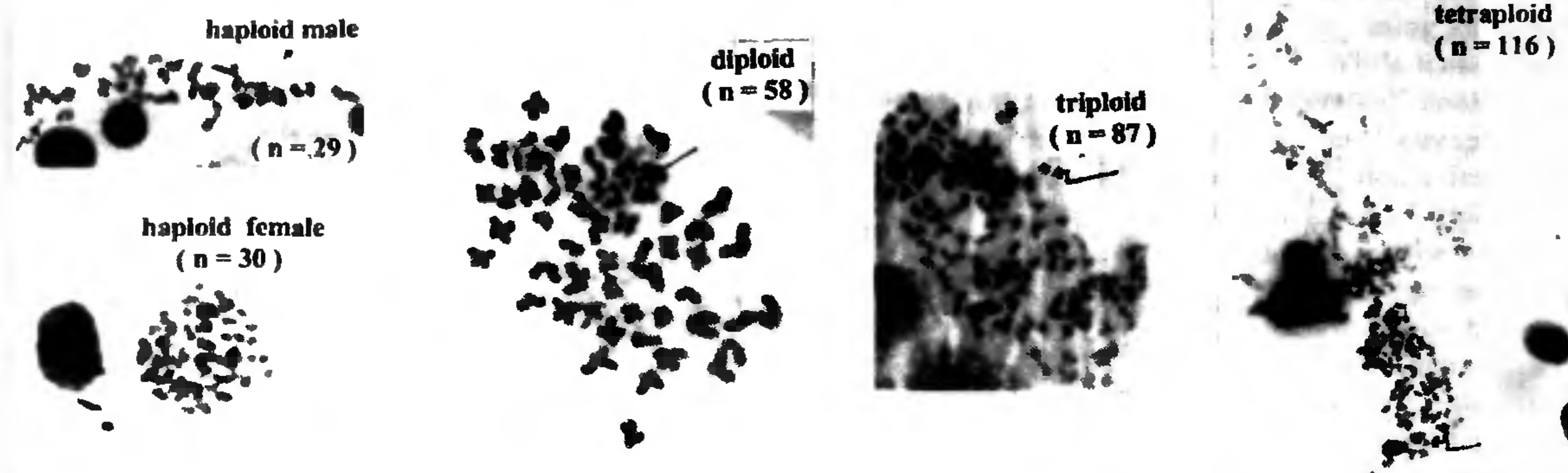


Figure 2. Karyotypes of the male and female haploids, diploid, triploid, and tetraploid *H. fossilis*. Photomicrographs for haploids, diploid and triploid were made at the magnification of 100 $\times$  and for tetraploid at 40 $\times$ . Arrows indicate the microchromosome(s).

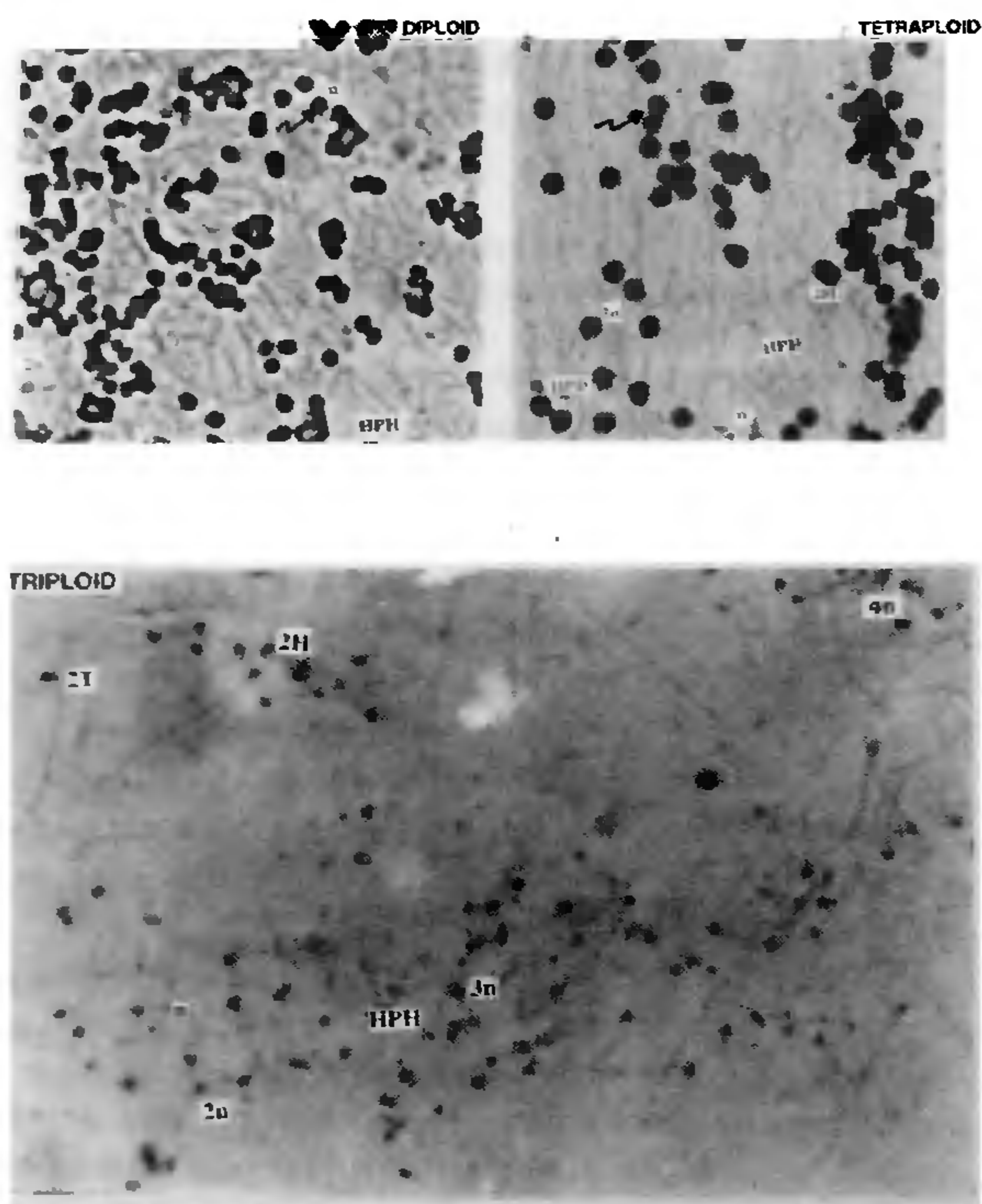


Figure 3. Sperms of diploid and tetraploid (upper panel) and triploid (lower panel) *H. fossilis*. Based on nuclear diameter (lightly visible in the diploid and tetraploid, as indicated by arrows), genomic levels of sperms can be identified; note the presence of hyperdiploid (HPD) and hypohaploid (HPH) sperms in tetraploids. Note also the presence of haploid ( $n$ ), diploid ( $2n$ ), triploid ( $3n$ ) and tetraploid ( $4n$ ) sperms in diploid and polyploids. Sperms with two tails (2T) and two heads (2H) (also indicated by an arrow in the right upper panel) are indicated. All photomicrographs were made at the magnification of 40 $\times$ .

monoploids and polyploids were neither a new species nor a hybrid.

It is an established fact that whereas triploid females are sterile, males are partially fertile and produce viable sperms<sup>1</sup>. However, fertile female triploids have been reported in a couple of carp species<sup>23-25</sup>; in these, the pathways through which fertile oocytes are produced differ in general from the conventional one<sup>1</sup>. The hazardous pathways through which sperms are generated in these partially fertile triploid males have been broadly indicated by a couple of previous authors from their flow cytometric and cytological studies<sup>26,27</sup>. In triploid *Pagrus major* Kawamura *et al.*<sup>28</sup> reported the production of sperm carrying haploid to heptaploid genome. In the present study too, sperms with haploid to tetraploid genome were recorded. It is not clear how from a triploid, sperms carrying  $4n-7n$  genome can be generated.

Secondly, some sperms generated by triploids were aneuploids sometimes with 2 heads and 2 tails (e.g. *Pagrus major*)<sup>29</sup>. These two kinds of abnormalities appear to be common for triploid *H. fossilis* too. Therefore, an intensive study on cytological pathways, through which sperms carrying polyploid genomes are generated in these unusual ploidy groups, is presently being studied.

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two species. Moreover, the fact that almost conventional but partially hazardous spermatogenesis occurs in tetraploid individuals also confirms that these analysed

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## The fungistatic action of oleic acid

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**Oleic acid (C 18:1) has been found to be fungistatic against a wide spectrum of saprophytic moulds and yeasts. The fatty acid causes a delay of 6-8 h in the germination of fungal spores and is very effective at a low concentration of 0.7% (v/v). The application of this property of oleic acid finds great use in preserving foodstuffs including bakery products like cakes and pastries with sweet soft cream as a topping which are prone to quick spoilage under conditions of non-refrigeration.**

FATTY acids of varying chain lengths are known for their antimicrobial action primarily against Gram-positive bacteria and yeasts at low pH<sup>1-6</sup>. The observed inhibition is explained as a consequence of the uptake of undissociated fatty acids which dissipate the transmembrane proton gradient and thereby affect ATPase activity<sup>1,7</sup>. The undissociated form of fatty acids is highly soluble in membrane phospholipids and has been shown to enter the cell by passive diffusion<sup>8,9</sup>.

Toxicity studies using fatty acids have been well documented during bacterial and yeast alcoholic fermentations of grape must<sup>2,4</sup>.

In the present communication, we report the wider ability of oleic acid to inhibit a variety of moulds and yeasts. The fungistasis is effected at the germination stage of the spores. This observation can find use in the preservation of foodstuffs, as oleic acid can be metabolized easily *in vivo* in the same manner as fatty acids normally found in food.

A number of saprophytic yeasts and moulds (as listed in Table 1) were grown and maintained on slants of potato dextrose agar (PDA) medium<sup>10</sup> at 30 ± 1°C or 37 ± 1°C. Following incubation for five days, the cultures were then stored at 4 ± 1°C till use. The organisms were subcultured once in every fifteen days and the purity of the cultures was checked regularly under microscope.

The inhibition tests were carried out in two stages. (i) The plate method; where oleic acid (0.2 ml) was loaded in wells (8 mm diameter) on PDA plates containing a luxuriant lawn of the different yeasts and moulds; (ii) *Candida albicans* and *Aspergillus* sp. (showing largest zones of inhibition) were grown in potato dextrose broth containing 0.1-2% (v/v) oleic acid at 30 ± 1°C and 37 ± 1°C, respectively in a controlled environment, using new Brunswick shaker (G25-KC) at 250 rpm. Biomass in each case was measured using predried (80°C for 48 h) and preweighed discs of Whatman filter paper no.1 or aluminium foil cups.

For the study of inhibition of aerial microflora, PDA plates containing 1%, 2%, and 3% (w/v) of dextrose were exposed to air for 1 h. A high concentration of 3% dextrose was tried, as bakery products often contain a

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