Letters to the Editor

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could be due to the fact that Aedes albopictus culture was more sensitive to JE virus than Aedes w-albus culture as evidenced from the comparative yield of infectious virus from the respective cultures (unpublished data). In general, the intracellular material showed slightly higher concentration of CF antigen than the extracellular medium.

With CHP virus the CF antigens remained mostly intracellular both in Aedes albopictus and Aedes w-albus cultures (on the 6th PI day). None of the extracellular culture media of CHP infected Aedes albopictus cultures reacted in CF test whereas extracellular media of CHP infected Aedes w-albus cultures showed presence of CF antigens only in the undiluted samples. However, the intracellular material (CHP infected Aedes w-albus cultures) contained higher concentration of CHP complement fixing antigens than that present in the extracellular medium.

It was also found that a higher dose (7.75, 6.75 and 5.75 log mouse LD$_{50}$) of CHP virus both in Aedes albopictus and Aedes w-albus cultures resulted in lower yield of CF antigens as compared to the inoculum containing lower concentrations of virus (1.75 to 4.75 log mouse LD$_{50}$). This type of prozone phenomenon has been also observed with regards to infectivity of CHP virus in vivo, Aedes albopictus and Aedes w-albus cultures (VRC unpublished data 1971 and 1972). However, this was not observed in case of JE infected cultures.

The results thus indicate that in JE infected Aedes albopictus and Aedes w-albus cultures, CF antigens were distributed both extra- and intracellularly whereas with CHP virus the CF antigens were located mainly intracellularly.

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S. N. Ghosh

S. S. Tongaonkar

C. N. Dandawate

5. —, Goverdhan, M. K. and Bhat, U. K. M., Ibid. (Submitted for publication).

CHROMOSOME STUDIES IN THE LIVER FLUKE, FASCIOLA GIGANTICA COBBOLD, 1856, FROM ANDHRA PRADESH

The genus Fasciola claimed to consist of three to six species enjoys a wide range of mammalian hosts including the humans. The variation in the elongated shape of F. hepatica from ox in contrast to the oval of that from sheep is considered to be due to adaptive factors acting in the final host. Experimental infection of F. gigantica in rabbits indicates that apart from genetic factors, physiology and the environment of the host play a major role on the parasite morphology. Apparently a thin line of distinction exists between F. hepatica smaller than F. gigantica with the consequential changes in the orientation of the reproductive organs and slight variations in life-histories. Comparative cytology may throw some light in this direction. However, the trematode cytology has been considered to be difficult in view of the application of elaborate mammalian chromosome schedules and heavy condensation of mitotic chromosomes precluding clear morphology. The cytology of digenetic trematodes is still a matter of speculation and divergence of opinion in spite of the chromosome numbers being reported in several of them by Britten and Walton. The diploid chromosome number of F. hepatica has been variously claimed as eight, twelve or as eighteen or twenty. The haploid number has been reported to vary from six to nine and ten bivalents. There is also a lack of knowledge on the karyotype of this form. While Srivastava and Jha have described the chromosome number of F. gigantica from the liver of buffalo as sixteen with eight bivalents, based on observations from only spermatogenesis, our studies, however, indicate the same to be twenty with a haploid count of ten bivalents. The chromosome number and the karyotyping from mitotic as well as mitotic cells of F. gigantica have been reported here.

The adult parasites from the bile ducts of sheep and the larval forms from the infected snails, Lymnaea luteola, were collected from Banswada in the Nizamshagar Irrigation area in Andhra Pradesh, where the incidence of Fascioliasis is known to be high. The technique of handling the adult parasite material has been described elsewhere. The first report of obtaining mitotic chromosome complements from larval stages of trematodes was by Cary and later followed by Short and Menzel and Ching Tsong Lo. As the older rediae lack the germinal material the
posterior ends of small to medium sized rediae containing the germ balls were dissected and squashed for mitotic chromosomes by the haematoxylin squash procedure which was essentially the same as reported earlier.

The twenty chromosomes constituting the diploid number in *F. gigantica* from the rediae are seen in Figs. 1 and 2. The karyotypes, prepared by the method of Levan *et al.* seen in Figs. 3 A and 3 B, show that they consist of two pairs of large, four pairs of medium and four pairs of small submetacentric chromosomes. The first two pairs have their short arms more than half the lengths of their counterparts. Table I indicating the lengths


Table 1

Arm and total lengths of chromosomes

(in microns)

<table>
<thead>
<tr>
<th>Chromosome Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Arm</td>
<td>2.50</td>
<td>2.27</td>
<td>2.03</td>
<td>1.98</td>
<td>0.62</td>
<td>0.93</td>
<td>1.28</td>
<td>1.36</td>
<td>0.55</td>
<td>0.74</td>
</tr>
<tr>
<td>Long Arm</td>
<td>4.22</td>
<td>4.15</td>
<td>3.24</td>
<td>3.09</td>
<td>3.93</td>
<td>3.45</td>
<td>3.05</td>
<td>2.88</td>
<td>3.38</td>
<td>3.19</td>
</tr>
<tr>
<td>Total chromosome length</td>
<td>6.72</td>
<td>6.42</td>
<td>5.27</td>
<td>5.07</td>
<td>4.55</td>
<td>4.38</td>
<td>4.33</td>
<td>4.24</td>
<td>3.93</td>
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</table>

<table>
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<tr>
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<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Arm</td>
<td>1.00</td>
<td>1.00</td>
<td>0.69</td>
<td>0.69</td>
<td>0.72</td>
<td>0.93</td>
<td>0.69</td>
<td>0.52</td>
<td>0.88</td>
<td>0.55</td>
</tr>
<tr>
<td>Long Arm</td>
<td>2.58</td>
<td>2.45</td>
<td>2.22</td>
<td>2.21</td>
<td>1.93</td>
<td>1.71</td>
<td>1.65</td>
<td>1.80</td>
<td>1.45</td>
<td>1.76</td>
</tr>
<tr>
<td>Total chromosome length</td>
<td>3.58</td>
<td>3.45</td>
<td>2.91</td>
<td>2.90</td>
<td>2.65</td>
<td>2.64</td>
<td>2.34</td>
<td>2.32</td>
<td>2.33</td>
<td>2.31</td>
</tr>
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</table>

of the chromosomes and those of shorter and longer arms measured from enlarged photomicrographs and the idiogram (Fig. 4) also emphasize this feature. Srivastava and Jha, on the other hand, claim the first two out of the eight pairs described by them to be the biggest and metacentric and the rest presumed as acrocentrics. While the morphology of chromosomes is clearly demonstrable in somatic cells of larvae, in gametogenesis although the number can be counted the centromeres and arms are rarely detectable in view of heavy condensation. The ten bivalents counted from a large number of meiotic cells do not help in identifying the individual chromosomes (Figs. 5 and 6) which is analogous to the situation existing in F. hepatica. This is not surprising since Rees had also reported in another digenetic trematode, Parorchis acanthus, that the chromosomes do not present typical shapes during gametogenesis and revert to normal form at fertilization.

Variations in the chromosome numbers have been recorded by several investigators in F. hepatica. With the exception of Sanderson all have based their observations on sectioned material and such analysis may not be so reliable. Ching Tsong Lo, who established the diploid chromosome number in Fasciolopsis buski, another genus of the same family Fasciolidae as fourteen, has provided its karyotype. Even in F. hepatica where the number has been variously determined the karyotyping and chromosome classification have not been shown. They have been described here for the first time in F. gigantica to consist of 20 from the mitotic cells of larvae (see Karyotype, Figs. 3 A and 3 B; Idiogram, Fig. 4 and Table) and as 10 bivalents from the spermatocyte divisions (Figs. 5 and 6). On the basis of chromosome numbers alone it is possible to establish generic boundaries in the family, Fasciolidae, with F. hepatica investigated by Sanderson and F. gigantica described here falling into one and Fasciolopsis buski reported by Chin Tsong Lo into another category. There is a lacuna in our knowledge on the comparative cytology and karyotype analyses of many digenetic trematodes. These would probably help in drawing conclusions on chromosomal evolution, determine the actual taxonomic status and examine the phylogenetic relationship between them.

We are thankful to Prof. O. S. Reddi, Department of Genetics, Osmania University, Hyderabad, for his interest in the studies and to Dr. S. S. Shukla, Fascioliasis Scheme, Zone No. 1, Banswada, Government of Andhra Pradesh, for the supply and identification of the material. One of us (P. V. R.) is grateful to Sri. G. S. Chary, Principal, Government College, Jagtial, for encouragement and the University Grants Commission, New Delhi, for financial assistance.

MEIOSIS IN SPIRONEMA FRAGRANS LINDLE

A clone of Spiromena fragrans which apparently gathered from South India never flowered at the University Gardens, Calcutta. A part of this clone when transplanted to Banaras flowered in April when subjected to sunlight for about 4-6 hours daily. Under shade, unlike other plants of the tribe Tradescantiae, it never produced any spike. The spikes, however, did not yield any seed and to know the causes of this sterility the meiosis of the clone was studied.

Meiotic count unfailingly revealed $n=6$ without any aberration (Figs. 1, 2). In pachytene there was complete synopsis in bivalents, but it was virtually impossible to note detailed karyomorphological observations due to their tangled nature.

The intermittent light and deeply stained regions which were clearly visible in the chromosomes at early prophase and continued right up to the pachytene and diplonete stages can best be attributed to the differential coiling of the chromosomes.

Figs. 1-3. Microphotographs of meiotic stages in Spiromena fragrans, $\times$1,000. Fig. 1. Prometaphase I. Fig. 2. Metaphase I. Fig. 3. Meiosis II.

In diakinesis the smallest bivalent was found to be associated with the nucleolus with the short satellite arms and remains visible till the prometaphase. At prometaphase (Fig. 1) there are 1-3 chiasmata per bivalent. More than three chiasmata have never been observed. The values of average number of chiasmata per nucleus and terminalization coefficient were worked out to be 9.8 and 0.62 respectively; these values being slightly different than those of the earlier observation. There was normal segregation of chromosomes to each pole at anaphase of meiosis I and II (Fig. 3). Pollen fertility though very high (95%) seed setting was absent.

In the diploid Tradescantia crassifolia and T. bracteata meiotic abnormalities reduce seed production. But in Khova a kind of balanced lethal system that maintains hybridity, permits seed production. Spiromena though apparently diploid and possesses a perfect meiotic system is completely sterile, and reproduces vegetatively. The