

schooled under the weeds in the aquarium and came out only to feed. The post larvae were fed on chopped chironomid larvae from 8 days post hatching. The post larvae were 8–12 mm TL at 12 day post hatching. The post larval stage continued till 15 day post hatching after which they underwent a transformation to resemble the adult. At this stage, they were termed juveniles. The juveniles were transferred to a cement tank (capacity 1500 l) and fed with finely chopped beef liver. 30 days post hatching juveniles were 18–22 mm TL and resembled the adult with respect to all external characteristics indicating the end of the juvenile period (Figure 5).

Sixty days post hatching (length =  $3.2 \pm 0.2$  cm; weight =  $1.75 \pm 0.25$  g) fingerlings of *O. bimaculatus* were released into the earthen pond ( $8 \times 12 \times 1.5$  m) and fed with finely-chopped chicken intestine at the rate of 15% of their body weight. After three months of culture, they attained a length of  $7 \pm 0.5$  cm and weight of  $30 \pm 2.5$  g. After six months of culture, the fish showed an average length of  $14.5 \pm 1.5$  cm and weight of  $100 \pm 10$  g (Figure 6).

Ovaprim is effective in inducing ovulation in *O. bimaculatus*. The time taken for response (5–6 h) is the lowest recorded for any catfish. The fertilization rate in the present experiment (75%; Table 1) is comparable to earlier reports in *Heteropneustes fossilis* (50% and above) using D-Lys6:sGnRH-A<sup>5</sup>, *Clarias macrocephalus* (60–80%) using LHRHa + PIM<sup>6</sup>, *Silurus asotus* (81.5–98.0%)<sup>7</sup>, *Ictalurus punctatus* (78.6%)<sup>8</sup> and *Mystus punctatus* (85%)<sup>9</sup> using dried carp pituitary extract. The present dosage of 0.5

ml/kg body weight of ovaprim may be used as a standard in future breeding of *O. bimaculatus*.

The Conservation Assessment and Management Plan (CAMP)<sup>2</sup> recommended a captive breeding programme for this fish and also stated that at present there is no existing captive population of *O. bimaculatus*. Keeping their recommendations in mind, induced breeding of *O. bimaculatus* was successfully accomplished and a captive population of 548 individuals are being maintained at CARE.

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## Female heterogamety in Indian populations of *Polypedium nubifer* (Diptera: Chironomidae)

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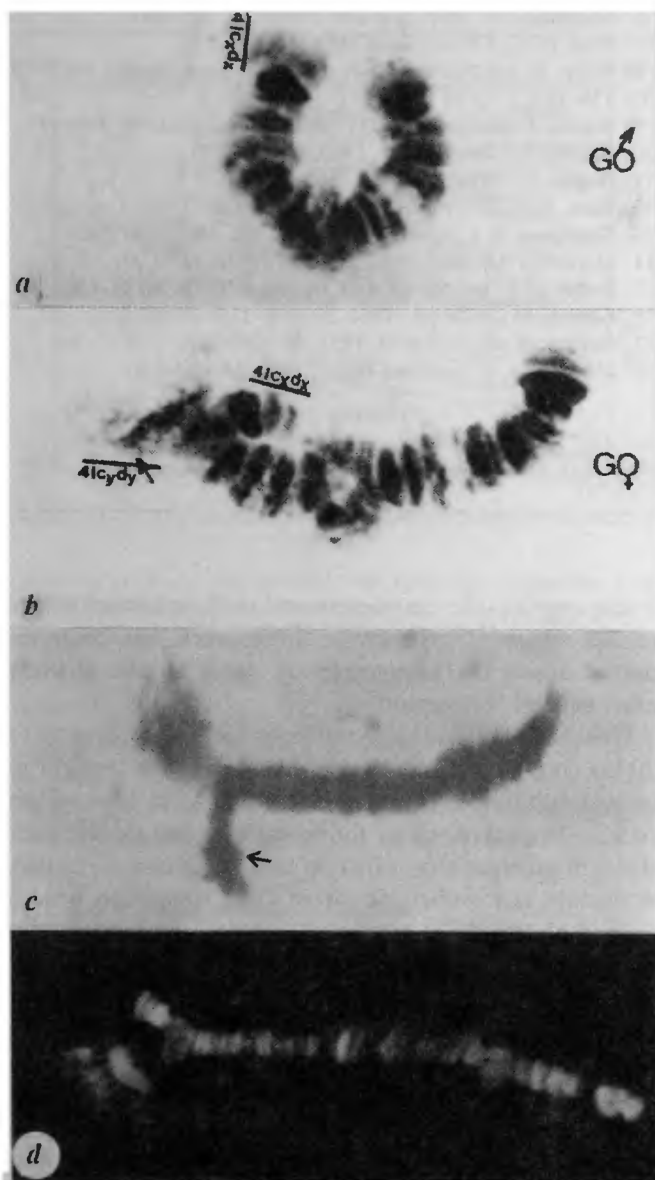
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A considerable number of larvae of *Polypedium nubifer* from two different geographic localities in India were analysed for the first time for male and female heterogametic system of sex determination. The results revealed a consistent occurrence of female heterogamety in Indian populations, the female being heterozygous for 41c-d region at the tip of chromosome IV, while male was always homozygous for this region. A comparative study of female specific region between Indian and Australian populations of this species suggests that Indian populations of *P. nubifer* have distinctly diverged, genetically.

In most groups of bisexual animals it is the male sex which is heterogametic. However, in some major groups

such as Lepidoptera and birds and in a few Diptera, Crustacea, fishes, amphibians and reptiles the situation is reversed, the female being heterogametic. Chironomids (Diptera) are well known for not having morphologically differentiated sex chromosomes. There are, however, considerable evidences that in this group of nematoceran insect sex is determined by one or more translocatable genes as also found in simuliids<sup>1</sup>, house flies<sup>2</sup>, frogs<sup>3,4</sup> and in certain lizards<sup>5</sup> which may or may not be associated with any transposable element. In some species of *Chironomus* the linkage of these sex-associated genes has been demonstrated to an inversion or some other chromosomal aberrations<sup>6–11</sup>, but more rarely by a small differentiated heterochromatinized segment on the chromosome. Further, the results of many studies have demonstrated that in Chironomidae the males are the heterogametic sex and the genetic basis of this lies in the presence of a dominant male specific factor in association with a cytologically demonstrable inversion and heterochromatic band.

There are also instances of divergent evolution of male and female heterogamety. Bush<sup>12</sup> described a case of similar evolution of female heterogamety among



**Figure 1.** Polytene chromosome IV (arm G) of *Polypedilum nubifer*: **a**, Male with homozygous 41cX-dX region; **b**, Female with heterozygous condition for the same region comprising 41cX-dX and 41cY-dY regions; **c**, Female specific region detected as C-band negative, arrow showing a dark centromeric band in male specific region after C-banding; **d**, Female specific region showing an enhanced band and the terminal puff after Hoechst<sub>33258</sub> staining.

Australian Tephritidae, where male heterogamety was predominant. In family Chironomidae, the occurrence of female heterogamety in Wisconsin population of *Chironomus tentans* was reported by Thompson<sup>13</sup>, where other American populations were known to have male heterogametic expression. *P. nubifer* belonging to this family is an unusual species in which the occurrence of both male and female heterogamety has been reported in Australian populations<sup>14,15</sup>. The chromosomes defined by Porter and Martin<sup>15</sup> as X and Y in this species differ markedly, where the Y chromosome in female consti-

tutes a differential heterochromatinized segment at the tip of chromosome IV. Similarly, Gordon<sup>16,17</sup> reported this in Mexican and Honduran populations of platyfish, *Xiphophorus maculatus*. Porter and Martin<sup>15</sup> commented that in the Australian populations at least, a situation has reached where evolution has already proceeded towards the final stage of morphologically and hence genetically differentiated sex chromosomes. With regards to the rare occurrence of male heterogamety, it was argued that the Australian populations are still in the process of evolving from male heterogamety to female heterogamety, so that the ancestral form of the differential end can still occur in males. If this contains weak female determining genes, male development still proceeds normally. They<sup>15</sup> also reported the occurrence of such distinguishing female heterogamety in Israel population of *P. nubifer* and in the morphologically similar New Zealand species, *P. pavidus*. Michailova<sup>18</sup> observed a similar situation of female heterogamety in another closely-related species *P. aberrans* from Hungary and Bulgaria.

With this background, we report results of our studies undertaken for the first time in Indian populations of *P. nubifer*. Over 550 larvae of *P. nubifer* from two different localities were examined. The results from both the populations revealed a consistent occurrence of female heterogamety, characterized by a differential heterochromatinized segment designated as 41cY-dY. On the contrary, all the male larvae were homozygous for 41cX-dX region (Figure 1 *a, b*). A comparison of the heterochromatinized region between Australian and Indian populations revealed some striking differences. In the Australian it was reported as a heterochromatic segment, whereas it was found as a markedly differentiated structure in the Indian material, comprising deeply stained enhanced band (41c) and a puff-like structure at the tip (41d). The male specific region designated as 41cX-dX resembled closely in both populations. The fluorescence staining with Hoechst<sub>33258</sub> showed clear differentiation of the enhanced band and the puff in the female specific region (41cY-dY), whereas the C-banding analysis showed that the whole female specific region including the enhanced band is C-band negative, indicating the absence of constitutive heterochromatin in this region. However, a positive C-band was found in the male specific region, representing the centromeric position of chromosome IV. These observations (Figure 1 *c, d*) clearly indicate that the female specific segment is well differentiated in the Indian material.

Our results on Indian populations of *P. nubifer* not only revealed the consistent occurrence of female heterogamety, but also showed marked differentiation of this large heterochromatinized segment. The enhanced band was found C-band negative, indicating the absence of constitutive heterochromatin in this region. Based on these observations it is inferred that Indian populations

of *P. nubifer* have distinctly diverged, genetically, and probably female heterogamety has been established in them. Following the results of some earlier studies and our present data, it seems clear that the replacement of one sex-determining mechanism (XX-XY) by another (ZZ-ZW) has rather frequently preceded speciation in Chironomidae as it has occurred in Simuliidae.

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## Geothermal and seismic evidence for the fluids in the crust beneath Koyna, India

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The presence of fluids in the crust beneath Koyna region has been examined, by computing the isochoric thermal pressure, pore fluid pressure and pore fluid factor at the pressure-temperature beneath the area. A reduction of 0.2 km/s in compressional wave velocity in the depth range from 6 to 11.5 km beneath Koyna region as revealed by DSS studies is not an unequivocal constraint about the existence of fluids in the shallow crystalline crust. Therefore, these data along with the geothermal seismicity of the region and other relevant data have been considered and isochoric thermal pressure (19 to 21 bar/°C) and pore fluid factor (0.78 to 0.83) were analysed to evaluate the possible presence of fluids. These results indicate the existence of sialic low velocity layer enriched in fluids beneath Koyna area.

THE understanding of the role of the fluids in the earth's crust on various physical properties, attracted much attention in recent years<sup>1-6</sup>. The basic question, 'whether the fluids present in the crust or in what form' has been addressed by many workers in geosciences. A minor quantity of fluids influences all types of physical properties measurable by geophysical means, like elasticity, electrical conductivity, thermal, mechanical and rheological properties of rocks<sup>7-12</sup>. Koyna (73°45'E, 17°33'N) which lies in the western margin of the Deccan Trap (Figure 1) is one such region where a variety of studies have been carried out since the 1967 major earthquake of magnitude 6.8, to understand the seis-

micity, earthquake parameters and tectonothermal nature of the region<sup>13-19</sup>. However, little work has been reported about the occurrence of fluids in the shallow crust beneath this region.

Presence of fluids has been reported in the crust up to 20 km (refs 3, 20, 21). Fluid-filled layers are thought to be responsible for low seismic velocities in low porous rocks<sup>7</sup>. The existence of fluids tends to reduce the rock strength substantially, which in turn decreases the ability to sustain any deviatoric stress. The rocks can be deformed plastically at moderate pressure and temperature conditions<sup>22</sup>. The roles of fluids and their pressure are also significant in understanding the earthquake failure process<sup>6,23</sup>. The presence of fluids beneath Koyna may be of particular interest to explain the seismicity of the region which is hypothesized to be reservoir induced<sup>13</sup> and also due to tectonic strain<sup>24</sup>.

In this context, we have carried out analyses of deep seismic sounding (DSS) velocity structure, heat flow, geochemical data of thermal waters of nearby areas, seismicity of the region. The isochoric thermal pressure, pore fluid pressure and pore fluid factors were computed. These results are discussed in the light of possible existence of fluids in shallow crust beneath Koyna region and their tectonic implications.

Deep Seismic Sounding (DSS) studies were carried out along two profiles, each about 200 km long in and around Koyna region (Figure 1)<sup>16,17</sup>. Krishna *et al.*<sup>25</sup> further refined the velocity model after digitizing the analog records and delineated a low velocity zone (LVZ) between 6 and 11.5 km depth. The amplitude modeling using these digitized records indicated the reduction of *P* wave velocity (*V<sub>p</sub>*) by 0.2 km/s at this depth range which was further confirmed in their later studies<sup>26,27</sup>.

Koyna earthquake of December 1967 has been studied and the focal depth estimated to be 4.5 km (refs 13, 24). An analysis of the earthquakes of magnitudes up to