Cytogenetic Studies on Willow Aphids

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Summary  Cytogenetic study was carried out on willow aphids viz. *Cavariella aegopodii* and *Tuberolachnus salignus* in order to ascertain the karyotype and sex determination in these aphids from different localities of Himachal Pradesh, India. In *Cavariella aegopodii*, collected from Shimla, Mashobra and Shoghi localities, \( 2n = 10 \). There is 1 pair of long, 2 pairs of medium sized and 2 pairs of short chromosomes. However, karyotypic variation was observed in aphid populations collected from the Solan locality, where the variable diploid chromosome numbers such as \( 2n = 8, 9 \) and 10 were also recorded. The male sexuals of *Cavariella aegopodii* are diploid having \( 2n = 9 \) with 4 pairs of autosomes and a single X chromosome. The various stages of meiosis in the testes of the male mainly occur during the early developmental stages and can also be found in the first and second instar nymphs. The *Cavariella aegopodii* has XX-XO type of sex determination system.

In *Tuberolachnus salignus* collected from the Shimla, Mashobra and Shoghi localities, \( 2n = 20 \). The ididiogram of *Tuberolachnus salignus* reveals the gradual decrease in chromosome length. However, karyotypic variation was observed in aphids collected from the Solan locality where the diploid chromosome number (2n) ranged from 18 to 20.

**Key words**  *Cavariella aegopodii*, Karyotypes, Male meiosis, Sex determination, *Tuberolachnus salignus*.

Aphids belong to the insect superfamily Aphidoidea which evolved 280 million years ago. During the course of their evolution, these insect pests underwent genetic or chromosomal changes that had far-reaching consequences in their life cycles. The occurrence of polymorphism, viviparity, telescoping of generations and the small size of holocentric chromosomes in aphids make cytogenetical investigations in these insect pests very difficult but interesting (Blackman 1980a, b, Gautam et al. 1993).

Willow plants are severely affected by aphids that cause considerable damage to these plants. In India, *Cavariella aegopodii* has been reported from Northwestern and Northeastern Himalayas (Raychaudhuri 1980) and *Tuberolachnus salignus* from the Spiti, Kullu and Solan areas of Himachal Pradesh (Hameed et al. 1975, Bhalla and Pawar 1977). However, there is no information available on the cytogenetics of these willow aphids from India.

There are about 4000 aphid species recorded all over the world (Blackman and Eastop 1994). Of these, chromosomes of 750 aphid species have been recorded (Robinson and Chen 1969, Kuznetsova and Shaposhnikov 1973, Gut 1976, Blackman 1980a, 1986, Chen and Zhang 1985a, b). Chromosomes of aphids fauna of Himachal Pradesh have been investigated by several workers (Dutta and Gautam 1993, Kapoor and Gautam 1994, Gautam and Dutta 1994, Gautam and Kapoor 2002, Gautam and Dhatwalia 2003, Gautam and Kumari 2003, Gautam and Kumar 2006) and in many cases karyotypic variations were reported. Keeping in view the frequent occurrence of karyotypic variations in aphids, it was considered appropriate to investigate the chromosomes of willow aphids in different populations from Himachal Pradesh, India.

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Figs. 1–14. 1, 2n=10 in the parthenogenetic wingless female of *Cavariella aegopodii*. 2, 3, 4, 2n=8, 9 and 10 of the parthenogenetic wingless female of *Cavariella aegopodii*. 5, Spermatogonia. 6, Primary spermatocyte. 7, Prophase stage. 8, Metaphase I stages (2n=9). 9, Spermatids. 10, Spermatzoa of male sexuals of *Cavariella aegopodii*. 11, 2n=20 in the parthenogenetic wingless female of *Tuberolachnus salignus*. 12, 13, 14, 2n=18, 19 and 20 in the parthenogenetic wingless female of *Tuberolachnus salignus*. Bar represents 10 μm.
Materials and methods

The chromosomes of different populations of *Cavariella aegopodii* and *Tuberolachnus salignus* collected from different localities *i.e.* Shimla (31°1' N Latitude, 77°1' E Longitude), Mashobra (31°1' N Latitude, 77°2' E Longitude), Shoghi (31°1' N Latitude, 77°5' E Longitude) and Solan (30°9' N Latitude, 77°15' E Longitude) were investigated and their karyotypes analyzed. The fresh embryos of second and third instar nymphs were used for the chromosome studies because the cells of these embryos are mostly in dividing stages. For chromosome studies, a nymph was taken on a slide and the embryos were obtained by puncturing the posterior end of the abdomen with the help of entomological pins. Only young embryos in which eye pigment was not visible were taken for chromosomal preparation.

The embryos were pretreated in 0.9% sodium citrate solution for half an hour. Pretreatment in sodium citrate solution brings about swelling of the tissue which results in well spread chromosomal plates. The pretreated embryos were fixed in 1 : 3 acetic ethanol solution for about 15 to 20 min at room temperature. After fixation, embryos were placed on a glass slide in a drop of 50% acetic acid for 3 to 5 min. A coverslip was placed on the material with 1 edge extended outside the slide. The coverslip was tapped gently with the blunt end of forceps. The slide was then placed between 2 layers of blotting paper and pressed with the thumb gently to spread the material in a uniform layer. The coverslip was dislodged off the slide with a sudden jerk with the help of the sharp edge of a razor blade. It was noticed that most of the material adhered to the coverslip and only some remained adhered to the slide. The slides and coverslips were then dried in a dust free chamber.

Staining of the chromosome slides was done with 2% Giemsa. 1.5 ml of 0.2 M disodium hydrogen phosphate, 1.5 ml of methanol and 2.5 ml of stock solution (25 ml glycerol, 25 ml methanol and 1 gm Giemsa powder) were mixed to prepare working solution. To this working solution, 50 ml of distilled water was added. The solution was shaken and pH was maintained at 6.8. The slide and coverslip were stained with working solution. Excess stain was washed off by rinsing the slide and coverslip with distilled water. Slides and coverslips were then dried in an oven. Slides were made permanent by dipping the slides and coverslips in xylene and mounting in DPX separately. The slides were then dried in an oven at 60°C for about 3 d.

For chromosomal measurements, well spread metaphase plates were selected. Actual lengths of chromosomes were measured using an ocular micrometer and from these, the total complement length (TCL) was calculated for each species. From the data of actual chromosome lengths and total complement length (TCL), the relative lengths (percentages of total complement length) were calculated. Idiograms were constructed based on relative length data. Slides of testes to study male meiosis were also prepared according to the technique used for somatic chromosomes. Photomicrographs of chromosomes plates were taken with LEICA DM LS2 microscope equipped with LEICA DFC 320 camera.

Results

*Chromosome number and karyotype of Cavariella aegopodii*

For all the populations collected from the Shimla, Mashobra and Shoghi localities, the chromosome number (2n) is 10 in this species (Fig. 1). However, karyotypic variation was observed in aphid populations collected from the Solan locality. The variable diploid chromosome number ranged from 8 to 10 (Figs. 2–4). Idiograms revealed 1 pair of long, 2 pairs of medium sized and 2 pairs of short chromosomes (Fig. 15).

The details of the karyotypes of the parthenogenetic wingless and parthenogenetic winged female are summarized in Table 1, 2 respectively.
Table 1. Mean total complement lengths, and chromosome actual and relative lengths in parthenogenetic wingless female of *Cavariella aegopodii*

<table>
<thead>
<tr>
<th>Locality</th>
<th>2n</th>
<th>Total length of complement (μm)</th>
<th>Chromosome Length (μm)</th>
<th>Relative Length (μm)</th>
<th>Figure No.</th>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Shimla</td>
<td>10</td>
<td>33.36±4.7</td>
<td>5.2±0.50</td>
<td>2.00±0.70</td>
<td>15.86±1.65</td>
</tr>
<tr>
<td>Mashobra</td>
<td>10</td>
<td>36.6±6</td>
<td>5.1±1.3</td>
<td>2.7±2.2</td>
<td>13.74±1.17</td>
</tr>
<tr>
<td>Shoghi</td>
<td>10</td>
<td>40±6.1</td>
<td>5.5±1.4</td>
<td>3±2.1</td>
<td>13.81±0.56</td>
</tr>
<tr>
<td>Solan</td>
<td>8</td>
<td>28.2±2.3</td>
<td>4.64±0.54</td>
<td>2.5±0</td>
<td>16.34±0.95</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>34.16±4.08</td>
<td>5.24±0.48</td>
<td>2.74±0.48</td>
<td>15.38±0.54</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>36.6±5.24</td>
<td>5.24±0.48</td>
<td>2.11±0.59</td>
<td>14.54±2.03</td>
</tr>
</tbody>
</table>

Table 2. Mean total complement lengths, and chromosome actual and relative lengths in parthenogenetic winged female of *Cavariella aegopodii*

<table>
<thead>
<tr>
<th>Locality</th>
<th>2n</th>
<th>Total length of complement (μm)</th>
<th>Chromosome Length (μm)</th>
<th>Relative Length (μm)</th>
<th>Figure No.</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Shimla</td>
<td>10</td>
<td>36.6±6</td>
<td>4.99±0.64</td>
<td>2.73±0.74</td>
<td>13.74±1.17</td>
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</tbody>
</table>

Table 3. Mean total complement lengths, and chromosome actual and relative lengths in parthenogenetic wingless female of *Tuberolachnus salignus*

<table>
<thead>
<tr>
<th>Locality</th>
<th>2n</th>
<th>Total length of complement (μm)</th>
<th>Chromosome Length (μm)</th>
<th>Relative Length (μm)</th>
<th>Figure No.</th>
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<tr>
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</tr>
<tr>
<td>Shimla</td>
<td>20</td>
<td>52.9±12.61</td>
<td>3.24±1.05</td>
<td>1.98±0.67</td>
<td>6.08±0.90</td>
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<tr>
<td>Mashobra</td>
<td>20</td>
<td>68.5±7.76</td>
<td>3.63±0.38</td>
<td>3.13±0.67</td>
<td>5.30±0.37</td>
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<tr>
<td>Shoghi</td>
<td>20</td>
<td>51±13.3</td>
<td>3.11±0.83</td>
<td>1.98±0.64</td>
<td>6.11±0.71</td>
</tr>
<tr>
<td>Solan</td>
<td>18</td>
<td>46.2±9.65</td>
<td>2.86±0.55</td>
<td>2.11±0.60</td>
<td>6.25±1.65</td>
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<tr>
<td></td>
<td>19</td>
<td>46.7±10.43</td>
<td>2.86±0.55</td>
<td>1.59±0.60</td>
<td>6.18±0.53</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>50.5±12.26</td>
<td>3.0±0.62</td>
<td>2.0±0.6</td>
<td>6.11±0.7</td>
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</tbody>
</table>

Table 4. Mean total complement lengths, and chromosome actual and relative lengths in parthenogenetic winged female of *Tuberolachnus salignus*

<table>
<thead>
<tr>
<th>Locality</th>
<th>2n</th>
<th>Total length of complement (μm)</th>
<th>Chromosome Length (μm)</th>
<th>Relative Length (μm)</th>
<th>Figure No.</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Shimla</td>
<td>20</td>
<td>50.1±12.28</td>
<td>3.06±1.09</td>
<td>1.88±0.59</td>
<td>6.04±0.93</td>
</tr>
</tbody>
</table>
Meiosis in the male sexuals

The male sexuals of *Cavariella aegopodii* are diploid having $2n=9$ both in the somatic as well as in the germlinal tissues (testes). There are 4 pairs of autosomes and a single X chromosome. The various stages of meiosis in the testes of the male occur mainly during the late embryonic development but can also be found in the second and third instar nymphs. The various stages of meiosis are as follows.

The spermatagonia (Fig. 5) have compact nuclei having diploid chromosome number. They divide to produce other spermatogonia that enter the growth phase. The primary spermatocytes are larger in size and their nuclei are larger and show the granular chromatin masses (Fig. 6) that appear in the form of coiled thread like structures. The chiasma formation and crossing over are not distinctly visible in the prophase I stages (Fig. 7). The metaphase I plates show the male karyotype clearly (Fig. 8). The homologous chromosomes separate during anaphase I and the univalent X chromosome remains on the axis of the spindle. In metaphase II, only secondary spermatocytes with 5 chromosomes were observed. The spermatids are rounded in shape when newly formed. Later, they are transformed into elongated structures and produce various transition stages (Fig. 9). Spermiogenesis leads to the formation of mature spermatozoa (Fig. 10). Ultimately, two spermatozoa are produced from 1 primary spermatocyte.

The *Cavariella aegopodii*, has XX-XO type of sex determination system. The parthenogenetic wingless female, parthenogenetic winged female, winged female sexuparae and ovipara sexual have $2n=10$. The male aphid has $2n=9$. Thus, in all the females, there are 4 pairs of autosomes and a pair of (XX) sex chromosomes, whereas the male has 4 pairs of autosomes and a univalent X chromosome.

Chromosome number and karyotype of *Tuberolachnus salignus*

In parthenogenetic wingless females of *Tuberolachnus salignus* (Gmelin), collected from the Shimla, Mashobra and Shoghi localities, the $2n$ is 20 in these species (Fig. 11). However, karyotypic variations were observed in aphid populations collected from the Solan locality. The variable diploid chromosome number ranged from 18 to 20 (Figs. 12–14). The idiogram reveals the gradual decrease in chromosome length (Fig. 16).
The details of the karyotypes of the parthenogenetic wingless and parthenogenetic winged females of *Tuberolachnus salignus* are summarized in Tables 3, 4 respectively.

The diploid chromosome number \( (2n) \) of 10 \( (2n=10) \) in the parthenogenetic wingless female of *Cavariella aegopodii*, collected from different localities *i.e.* Shimla, Mashobra and Shoghi, confirms the earlier report of Blackman (1980a). However, variable diploid chromosome numbers such as \( 2n=8, 9 \) and \( 10 \) were recorded in populations of the Solan locality. 11 species of aphids belonging to genus *Cavariella* have been studied cytogenetically by different workers (Shinji 1931, Gut 1976, Chen and Zhang 1985b, Blackman 1980a, 1986) and in all of these, \( 2n \) ranges from \( 2n=6 \) to \( 14 \).

Blackman (1980a) reported that aphids tend to show more karyotypic variations because of the presence of holocentric chromosomes and their thelytokous mode of reproduction. The observable karyotype differences between related holocyclic species of aphids are possibly due to fusions and dissociations (Kuznetsova 1974, Blackman 1980a). Karyotypic variation provides information about the pathways along which karyotype evolution has taken place (Blackman 1980a). In the present investigations, the aphid populations of *Cavariella aegopodii* collected from the Solan locality showed karyotypic variation within the species as \( 2n \) ranges from 8 to 10 in these aphid populations.

Prophase I stages of meiosis were not clear and chiasma formation and crossing-over could not be observed as is the case in other aphid species (Blackman 1976). The males of *Cavariella aegopodii* are homogametic and produce only one kind of spermatozoa \( (N=5) \). Earlier, similar findings were reported by Schwartz (1932) in males of *Tetranura ulmi* and by Blackman (1976) in males of the *Euceraphis* species.

The *Cavariella aegopodii* has an XX-XO type of sex determination system as has also been reported in other aphid species by many workers (de Baehr 1920, Morgan 1909a, b, 1912, 1915, Schwartz 1932, Lawson 1936, Ris 1942, Blackman 1976, 1980b).

*Tuberolachnus salignus* is placed in subfamily Lachninae and tribe Lachnini. Ghosh (1982) reported that world fauna of Lachninae includes about 539 species. Only 36 species belonging to 14 genera have been recorded from India (Ghosh 1989). Das *et al.* (1985) reported that this group is cytotaxonomically important because of its high degree of endemism, host plant preference and large body size of the aphids. Karyotypes of more than 60 species of Lachninae are known at present (Kuznetsova and Shaposhnikov 1973, Rukavishnikov 1979, Blackman 1980a, 1986, 1990, Pal and Khuda-Bukhsh 1982, Das *et al.* 1985). In all these populations collected from the Shimla, Mashobra and Shoghi localities, chromosome studies revealed that \( 2n=20 \) in this species, confirming the earlier report of Blackman and Spence (1996). However, karyotypic variation was observed in aphid populations collected from the Solan locality, as in these aphids the diploid chromosome number ranges from 18 to 20 in this species. No sexual morphs of this aphid species were recorded during observations for several years in different localities of Himachal Pradesh.

**References**


— and Spence, J. M. 1996. Ribosomal DNA is frequently concentrated on only one X chromosome in permanently apomorphic aphids, but this does not inhibit male determination. Chromosome Res. 4: 314–320.


