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ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 5, Issue, 7, pp.1338-1341, July, 2014

International Journal of Recent Scientific Research

RESEARCH ARTICLE

CHROMOSOMAL STUDIES ON TWO SPECIES OF APHIDS FROM JAMMU (J&K), INDIA

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ARTICLE INFO

Article History:

Received 12th,June, 2014
Received in revised form 22th,June, 2014
Accepted 11th, July, 2014
Published online 28th, July, 2014

Key words:

Aphids, chromosomes, *Aphis fabae*, *Myzus persicae*, TCL %, RL %

ABSTRACT

In the present study, the chromosomes of two species of aphids i.e. *Aphis fabae* and *Myzus persicae*, were studied from Jammu (J&K). The observed diploid chromosome number for both the species was 2n=8 and 2n=12 respectively. The mean total lengths, RL % and TCL % were measured at metaphase stage for both species. The TCL% and RL% of *Aphis fabae* was 29.97 to 20.88 and 100 to 69.66 respectively and of *Myzus persicae* was 25.66 to 9.15 and 100 to 35.63 respectively.

INTRODUCTION

Aphids are small plant sucking insects and are very important as agricultural pests not only in view of their parasitic action against crops, but also because they represent active vectors of crop viruses. In this respect, a wide knowledge of aphid genome organization could be crucial in the layout of protocols aimed at the manipulation of the aphid genome in order to diminish their parasitic impact on plants of agricultural interest. The chromosomes of aphids, like those of other hemipteran insects, have diffuse centromere so that kinetic activity is dispersed along the entire length of each chromatid at least in mitotic divisions, thus influencing chromosome behaviour (White, 1973). The interest of a cytogenetic approach towards this taxon is also emphasized by the lymph-sucking feeding of these insects which represent a serious problem for agriculture, not only in view of their parasitic action against crops, but also because they represent active vectors of crop viruses. But also, the holocentric nature of aphid chromosomes represented a great drawback for cytogenetic studies as homologues, in view of the absence of primary or secondary constrictions can be paired on the basis of their size only (Blackman, 1980a).The mitotic chromosomes can be easily observed in aphid embryonic tissues. For this reason, aphids represent a suitable insect model for determining the differences and similarities in the structure and activity of holocentric and monocentric chromosomes.

The earliest studies on aphid karyology, many are suspecting due to uncertainties in proper identification of the respective aphid species (Kuznetsova and Shaposhnikov, 1973) and lack of voucher material. Importance of the aphid karyotype and chromosomal numbers has been addressed in detailed reviews by several authors (Blackman, 1980a; Kuznetsova and Shaposhnikov, 1973; Blackman, 1980b; Blackman and Eastop, 1984; Hales *et al*, 1997).

In order to clear up the rearrangements in holocentric chromosome complements, the general procedure up till now has been to measure the length of the chromosomes on prints of cells in mitotic metaphase and estimate the mean relative lengths of the chromosomes in a number of cells. Chromosomal rearrangements in species with holocentric chromosomes are, however, difficult to interpret, as there is no localized centromere that can serve as a landmark on the rearranged chromosomes. The subject of this study is the normal karyotype of metaphase complement of two important agricultural pests i.e. *Myzus persicae* and *Aphis fabae.*

MATERIAL AND METHODS

The aphids, *Aphis fabae* and *Myzus persicae* were collected from host plants viz. *Vicia fabae* and *Raphanus sativus* respectively from Jammu district (Jammu and Kashmir, India). Chromosome preparations were obtained from young embryos in which eye pigment were absent or not visible under dissection microscope. The parthenogenetic females were dissected and their embryos were given pretreatment of 0.8% sodium citrate as hypotonic solution for about 30 min. After pretreatment, the embryos were fixed in freshly prepared fixative i.e. 3:1 methanol-aceticacid for about 20 min and 3 changes were given to the material. Then embryos were placed on a glass slide in a drop of 2% lacto-

aceto orcein stain for 5 min. The coverslip was gently placed on material put on glass slide. Then slides were air dried for observation.

Then after, the prepared chromosomal slides were scanned and photographed by using YS100 binocular research microscope and Samsung SDC-313 camera respectively. Well spread mitotic metaphase stages were photomicrographed at a magnification of 1000X. Histogram was prepared by taking chromosome pair number on X-axis and corresponding relative length percentage (RL %) on Y-axis. The chromosomes were paired on the basis of their size only as they lack primary or secondary constriction (Blackman, 1980a).

RESULT

During the present investigation, chromosomes of two species of aphids infesting different plants have been studied and

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observed that both species are of holocentric nature.

Aphis fabae

The aphids of this species were blackish grey in color and infested ventral and dorsal side of leaves of Beans *(Vicia fabae)*. The observed diploid chromosome number was eight (2n=8) (Fig. 1). About 50 metaphase stages were observed and in majority of the diploid chromosome number of 8 was found confirming 2n=8. The karyotype (Fig. 2) of parthenogenetic female was prepared from well spread metaphase complement that revealed the presence of eight elements and showed four pairs of chromosomes gradually decreasing in length. Histogram (Fig. 3) was prepared on the basis of decreasing value of RL% from chromosome pair one to four. The maximum of TCL % i.e. 29.97% was in case of first chromosome pair and the minimum of it i.e. 20.88% was recorded in case of last chromosome pair. The RL% varied from 69.66% (for chromosome pair number four) to 100% (for the first chromosome pair). The total complement length of haploid set was calculated to be 29.7µm (Table 1).

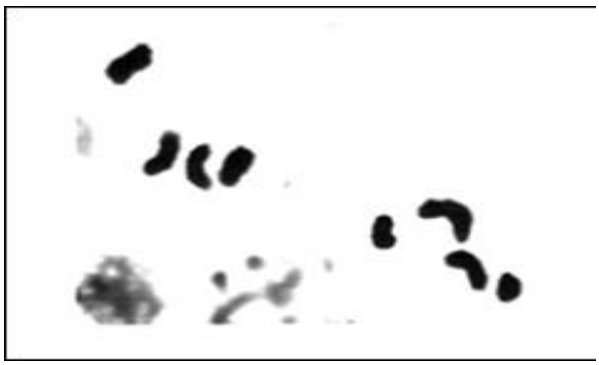

Fig. 1: Somatic metaphase of parthenogenetic female *Aphis fabae* showing

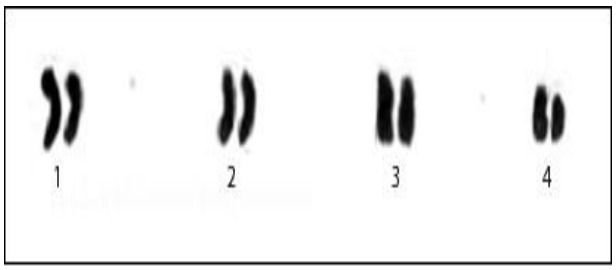

Fig. 2: Karyotype of somatic metaphase of *A. fabae* (2n=8)

Table 1: Morphometric data of karyotype of *Aphis fabae* (embryo) 2n=8.

Chromosome Pair Number	Mean Total Length (µm)	Total Complement Length Percentage (TCL %)	Relative Length Percentage (RL %)
1	8.9	29.97	100
2	7.4	24.92	83.15
3	7.2	24.24	80.89
4	6.2	20.88	69.66

Morphometric Data Of Karyotype

- Actual mean length of the largest chromosome (autosome) = 8.9µm
- Actual mean length of the smallest chromosome (autosome) = 6.2µm
- RL% of the largest chromosome = 100%
- RL% of the smallest chromosome = 69.66%
- Ratio of the largest chromosome to the smallest chromosome=1.435
- Total complement length of haploid set = 29.7µm

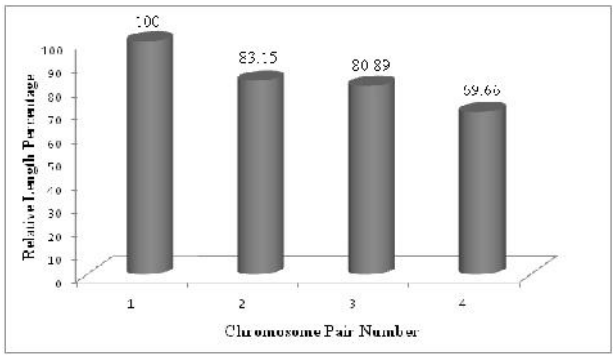

Fig. 3: Histogram of parthenogenetic female *Aphis fabae*

Myzus persicae

The aphid species were yellowish green, grey green or olive green infested the stem of *Raphanus sativus.* A metaphase preparation revealed that *M. Persicae* has diploid chromosome number i.e. 2n=12 (Fig. 4). About 50 metaphase stages were observed and in majority of the diploid chromosome number of 12 was found confirming 2n=12. The karyotype (Fig. 5) revealed the presence of six pairs of medium to small-sized autosomes, showing gradation in size. Histogram (Fig. 6) was prepared on the basis of decreasing value of RL% from chromosome pair one to six. The karyo-morphometrical analysis showed that actual mean length of chromosomes vary from 3.1µm (smallest) to 8.7µm (longest).The total complement length of haploid set was calculated to be 33.9µm. The first autosomal pair is largest in the complement with actual mean length of 8.7µm, TCL% of 25.66 and RL% of 100. The sixth autosomal pair is smallest in the complement with actual mean length of 3.1µm, TCL% of 9.15 and RL% of 35.63 was observed (Table 2).

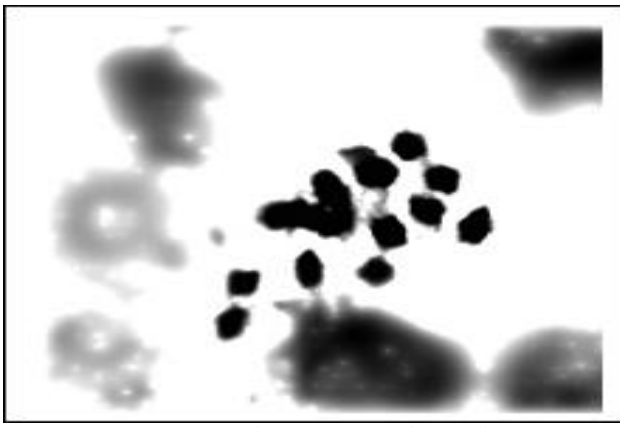

Fig. 4: Somatic metaphase of parthenogenetic female of *Myzus persicae* showing 12chromosomes

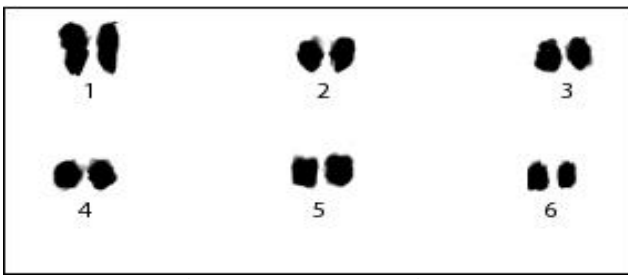

Fig. 5: Karyotype of metaphase complement of *Myzus persicae* (2n=12)

Table 2 Morphometric data of karyotype of *Myzus persicae* (embryo) 2n=12.

Chromosome Pair Number	Mean Total Length (µm)	Total Complement Length Percentage (TCL %)	Relative Length Percentage (RL %)
1	8.7	25.66	100
2	6.5	19.17	74.71
3	6.0	17.70	68.97
4	5.2	15.33	59.77
5	4.5	13.27	51.72
6	3.1	9.15	35.63

Morphometric data of karyotype

- Actual mean length of the largest chromosome (autosome) = 8.7µm
- Actual mean length of the smallest chromosome (autosome) = 3.1µm
- RL% of the largest chromosome =100%
- RL% of the smallest chromosome = 35.63%
- Ratio of the largest chromosome to the smallest chromosome= 2.80
- Total complement length of haploid set = 33.9µ

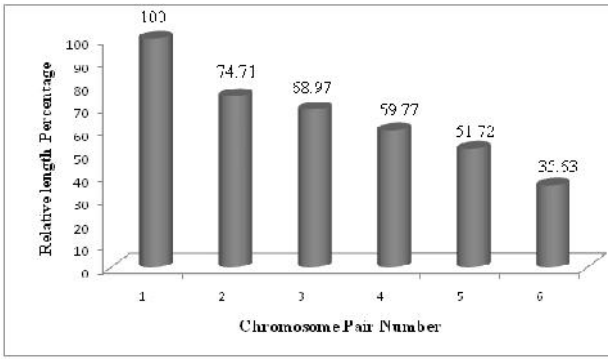

Fig. 6 Histogram of parthenogenetic female *Myzus persicae.*

DISCUSSION

In organisms possessing holocentric chromosomal organization, chromosome fusions and fissions can occur without any duplication or loss of centromeres. This has consequences for the survival of the *de novo* chromosomal changes through mitosis and meiosis, and hence for karyotype evolution. Autosomal fusions and fissions, particularly the latter, seemed to play a pivotal role in aphid karyotype evolution (Blackman, 1980a).

The genus *Myzus* comprises of 55 species (Blackman and Eastop, 1984) out of which the chromosome number of 22 species are reported in literature (Kuznetsova and Shaposhnikov, 1973; Blackman, 1971,1986) The diploid chromosome number varies from 8 to 20 in different species of this genus. Seventeen species of this genus have diploid chromosome number 12 i.e., 2n=12. *Myzus persicae* (Sulzer), commonly known as green peach aphid was collected from *Raphanus sativus*. In the present study, the diploid chromosome number in this species was found to be twelve which in conformity with the earlier workers (Criniti *et al*, 2006; Spence and Blackman; 1998; Lauritzen, 1982; Sun and Robinson, 1966; Gupta and Kurl, 2011). However some chromosomal variation in *M. persicae* was also reported (Blackman, 1980a; Blackman, 1971; Gautam *et al*, 1993; Xiao-Wen and Xiao-Xi, 2000; Sethi and Nagaich, 1972). These variations may be due to fusion or dissociation in the chromosomes. However, no such variation has been observed in this aphid species in the present study. In populations of *Myzus persicae*, from many parts of the world also structural heterozygosity in chromosomes has been reported and this is because of a translocation between the first and third pair of autosomes.

Aphis is the largest aphid genus containing nearly 500 species (Blackman and Eastop, 1984). Cytology of as many as 59 species from the genus *Aphis* is already known (Kurl, 1986. Panigrahi and Patnaik, 1987). It seems to be characterized by the chromosome number of 2n=8, although some exception have been reported e.g. in *Aphis farinosa*, 2n= 6 (Stevens, 1906) and in *A. solenella* and *A. umbrella*, 2n=7. In present study, *A. fabae* the diploid chromosome number was observed to be 2n=8, which is same as reported by some earlier workers (Dutta and Gautam, 1993; Kapoor and Gautam, 1994; Marco *et al*, 2009). No variation was observed during present investigation. So it is evident that *A. fabae has* diploid chromosome number 2n=8, as in majority of representatives of the genus *Aphis*. Sex chromosomes could be the longest (Blackman, 1986; Kuznetsova and Gandrabur, 1991)

In organisms possessing holocentric chromosomal organization, chromosome fusions and fissions can occur without any duplication or loss of centromere. This has consequences for the survival of the *de novo* chromosomal changes through mitosis and meiosis, and hence for karyotype evolution. Autosomal fusions and fissions, particularly the latter, seemed to play a pivotal role in aphid karyotype evolution (Blackman, 1980a). The identification of chromosomal markers in organisms possessing holocentric chromosomes is extremely important since the lack of a primary constriction, together with the difficult in obtaining a clear-cut banding pattern, have greatly hampered cytogenetic studies in species possessing such a peculiar chromatin organization (Hales *et al*, 1997). The interest of a cytogenetic approach towards aphid chromosomes is emphasized by the consideration that information regarding aphid genomes could be important not only from a scientific, but also from an economic point of view. Also, the chromosomal rearrangements in species with holocentric chromosomes are, however, difficult to interpret, as there is no localized centromere that can serve as a landmark on the rearranged chromosomes. Thus, there is much scope for refinement of banding techniques like Q-, NOR- and G- banding.

Acknowledgements

Authors are very thankful to Prof. K.K. Sharma, Head, Department of Zoology for providing the necessary lab facilities.

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