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Chromosome studies on some aphid species from two tehsils in Himachal Pradesh's Kangra district

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Abstract
Chromosomes of aphids infesting different host plants from two tehsils namely; Jawali and Kangra in Kangra district of Himachal Pradesh were studied. These aphids species are, z*Acyrthosiphon gossypii* Mordvilko (2n=6), *Aphis punicae* Passerini (2n=8), *Brachycaudushelichrysi* (Kaltenbach) (2n=12), *Hyperomyzuslactuae* (Linnaeus) (2n=12), *Macrosiphoniella sanborni* (Gillette) (2n=12), *Macrosiphum euphorbiae* (Thomas) (2n=10), *Myzusascalonicus* Doncaster (2n=12), *M. ornatus* Liang (2n=12), *Toxopteraaurantii* (Boyer de Fonscolombe) (2n=8), *Toxopteraodinae* (van der Goot) (2n=8), *Uroleuconambrosiae* (Linnaeus) (2n=12). The actual lengths, relative lengths and total complement length (TCL) of chromosomes were measured at metaphase. Karyotypes were prepared and idiograms were constructed based on relative lengths data.

Keywords: chromosomes, aphids, total complement length, karyotype, idiogram

Introduction

Aphids inflict significant harm to a variety of agricultural and horticultural crops by feeding on plant sap and spreading viral infections to various parts of plant (Kennedy *et al*., 1962) [13]. This group of plant sap sucking insects contains approximately 5000 species described worldwide in about 510 genera infesting about 300 plant families (R L Blackman & Eastop, 2006; Roger L Blackman & Eastop, 2000; Gavrilov-Zimin *et al*., 2015) [1, 2, 9]. Aphids have a number of peculiar phenomenon in their life cycle such as polymorphism, viviparity, telescoping of generation, host alternation and holocentric chromosomes. Most of the aphids are heteroecious, exploiting both primary and secondary host plants. Holocyclic aphids have a sequence of parthenogenesis in few generations followed by a single sexual generation, whereas in anholocyclic aphids sexual portion of life cycle lost (Moran, 1992) [20].

In aphids, the XX-XO type of sex determination is used. Aphid X chromosomes have received a lot of interest since they contain a lot of structural limitations (Manicardi *et al*., 2002) [19]. All fertilized eggs of sexual females produce parthenogenetic females only. To generate a male, an egg must lose half of the sex chromatin of the parent female during a single maturation division. To produce a male, an egg must shed 50% of the parent female's sex chromatin during a single maturation division. During development of the male oocyte, the X chromosomes are coupled end-to-end to create a condensed C-shaped bivalent, according to (Orlando, 1965). When the pairing products split, one X chromosome proceeds to the spindle's end while the other divides equationally with autosomes, leaving just one chromatid of the original X bivalent in the developing male egg. The X chromosomes in presumptive male oocytes form a looping structure and then in middle, a nucleolus like spherical body develops which maintains cohesion between two X chromosomes Blackman (1986). In female developing oocytes, there occurs no such association of X chromosomes. According to scientist (Jaquiéry *et al*., 2012), evolutionary pressures have a significant influence in evolution of X chromosome in aphids, which have an XO system in which males are hemizygous (XO) and females are homozygous (XX).

Aphid cytogenetic investigation is beneficial because it aids in the discovery of species-specific chromosomal markers, which makes a work that is now deemed tough much easier (Rakauskas, 1998) [23]. Classical and molecular cytogenetics work together to investigate the structure and evolutionary history of aphid holocentric chromosomes (Mandrioli & Carlo Manicardi, 2012) [18].

Kangra in Himachal Pradesh is rich in diverse type of vegetation. Keeping this in view the frequent karyotype variations in many species of aphids, it was considered worth to investigate the chromosomes of some species take over different host plants in Jawali and Kangra tehsils of Kangra district of Himachal Pradesh.

Research Materials and Techniques

Identification

Female aphids infesting twigs, leaves, and influorescence of several host plants were gathered from various locations as apterous, viviparous, parthenogenetic female aphids. Key developed by Blackman & Eastop (1984) was used for identification of different aphids species. The detail of aphid species along with host plants, place & collection time are given in Table I.

Table 1: Aphid species along with host plant, place and period of collection. Fig.1.

Species of Aphids	Host plants	Collection site	Collection time
Acyrthosiphon gossypii	*Abelmoschus esculentus*	Nagrota Surian, Kangra	October, 2017
Aphis punicae	*Punica granatum*	Nagrota Surian, Kangra	October, 2017
Brachycaudus helichrysi	*Ageratum houstonianu*	Nagrota Surian, Kangra	October, 2017
Hyperomyzus lactuae	*Sonchus arvensis*	Nurpur, Kangra	April, 2017
Macrosiphoniella sanborni	*Chrysanthemum indicum*	Ansoli, Kangra	October, 2017
Macrosiphum euphorbiae	*Malva parviflora*	Ichhi, Kangra	September, 2017
Myzus ascalonicus	*Dahlia pinnata*	Ansoli, Kangra	September, 2017
M. ornatus	*Ajuga bracteosa*	Ansoli, Kangra	September, 2017
Toxoptera aurantii	*Citrus medica*	Matour, Kangra	October, 2017
T. odinae	*Duranta erecta*	Ichhi, Kangra	October, 2017
Uroleucon ambrosiae	*Sonchus olerceaus*	Jawali , Kangra	April, 2017

For the chromosomal study, somatic embryonic tissue of parthenogenetic females was used. The aphids dissected by puncturing the posterior part (end) of the abdomen and then processed for 25–30 minutes with a 0.7 percent trisodium citrate solution. For 15 to 20 minutes, these embryos were preserved in a 1:3 acetic-ethanol solution. After stabilising the embryos, they were put for 3 to 5 minutes on a glass slide in a drop of 45 percent acetic acid. A coverslip was placed on the slide. The slide and coverslip then lightly tapped with the blunt end of the forcep between two layers of blotting sheets. With a sharp jerk, the coverslip was thrown off the slide. After that, both the coverslips and the slides were dried in a dust-free environment. 20–30 minutes were spent staining chromosome coverslips and slides with 2% Giemsa. Dipped slides in xylene and mounting them in DPX made them permanent.

Photomicrographs were obtained, while the slides were being inspected using a binocular microscop. Using an ocular micrometre, the lengths of the chromosomes were determined. Actual chromosomal lengths, the total complement lengths, and relative chromosome lengths were determined. Data about relative lengths was used to create the idiograms.

Results

In the current study, the most common diploid chromosomal numbers reported are 8 and 12.

Acyrthosiphon gossypii Mordvilko

Green aphids with bands were discovered infesting the lower side of leaves and flowers, with six diploid chromosomes (2n=6) (Figs. 1a, 1b). The mean actual length of chromosomes measured on well dispersed metaphase plates ranged from 2.34 m 0.09 to 3.60 m 0.05. The total complement length (TCL) was 16.90 µm ±0.30 on average. The chromosomes' relative lengths ranged from 11.32µm ±0.30 to 0000. This species' idiogram revealed two pairs of lengthy and two pairs of increasingly shrinking chromosomes (Fig. 1c).

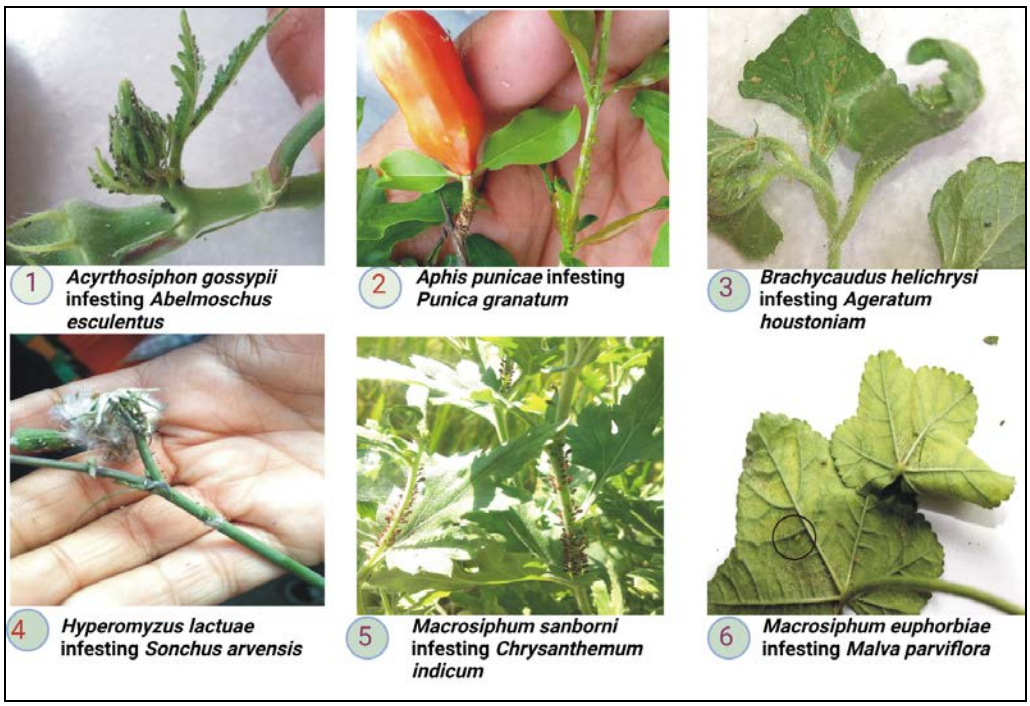

Fig 1: Aphids infestind different plants

***Aphis punicae* Passerini**

Aphis punicae, a green-colored aphid, was collected from the immature leaves and stem of Punica granatum. The number of chromosomes was discovered to be eight (2n=8) (Figs. 2a, 2b). The mean actual length of chromosomes measured on metaphase plates ranged from 1.29 µm ±0.74 to 2.97 µm ±0.05. Total complement length (TCL) was 17.78 µm ±0.55 on average.

The chromosomes' relative lengths ranged from 6.87µm ±0.40 to 16.77µm ±0.35. This species' idiogram revealed a pair of lengthy chromosomes and three pairs of increasingly decreasing chromosomes (Fig. 2c).

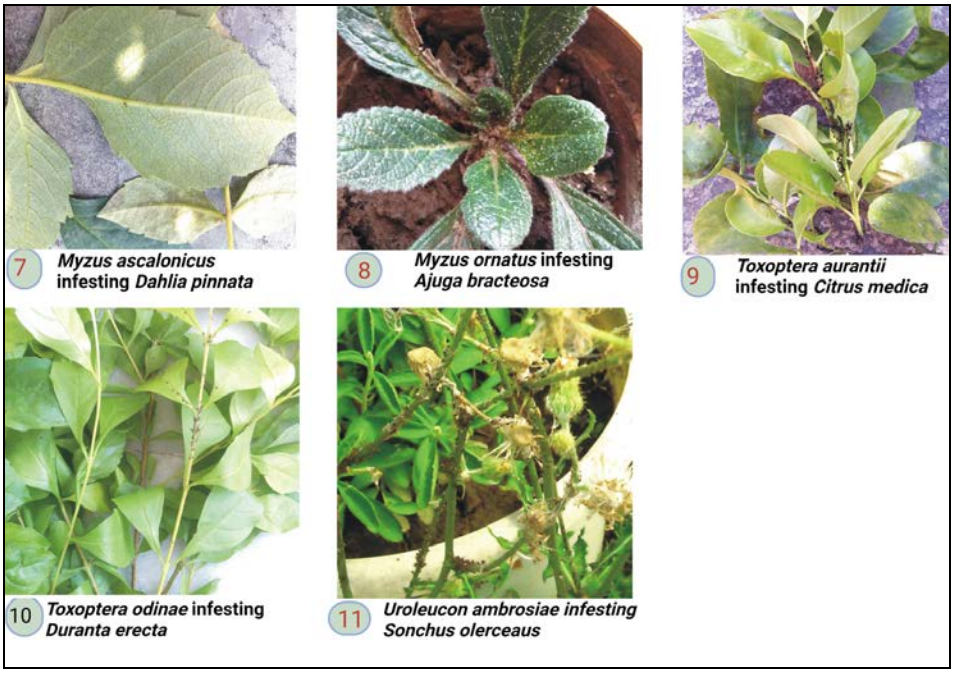

Fig 2: Figures of Karyotype

***Brachycaudus helichrysi* (Kaltenbach)**

Brachycaudus helichrysi aphids were wax-coated green aphids. The infoldings of juvenile Ageratum houstonianum leaves were colonised by these. 2n=12 was discovered to be the chromosomal number (Figs. 3a, 3b). Chromosome lengths varied between 1.45µm ±0.05 and 3.08 µm ±0.05. TCL measured 26.39 µm ±0.70. Chromosome relative lengths ranged from 5.58µm ±0.16 and 11.62µm ±0.42. This species' idiogram revealed a progressive reduction in the length of chromosomes (Fig. 3c).

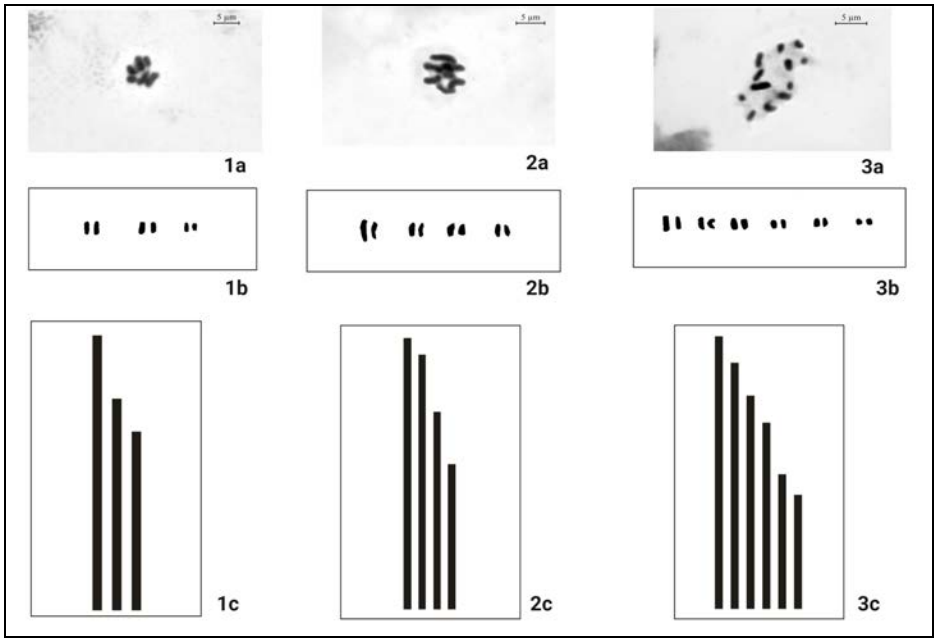

Fig 3

***Hyperomyzus lactuae* (Linnaeus)**

Hyperomyzus lactuae aphids were collected from the stems, leaves, and flowers of the host plant Sonchus arvensis. The number of diploid chromosomes (2n=12) was discovered to be twelve (Figs. 4a, 4b). Chromosome lengths ranged from 1.21 µm ±0.06 to 2.90 µm ±0.05. The length of the total complement was 24.90 µm ±0.52. Chromosome relative lengths varied from 4.87µm ±0.19 to 11.67µm ±0.02. The idiogram revealed a progressive decrease in the length of chromosomes (Fig. 4c).

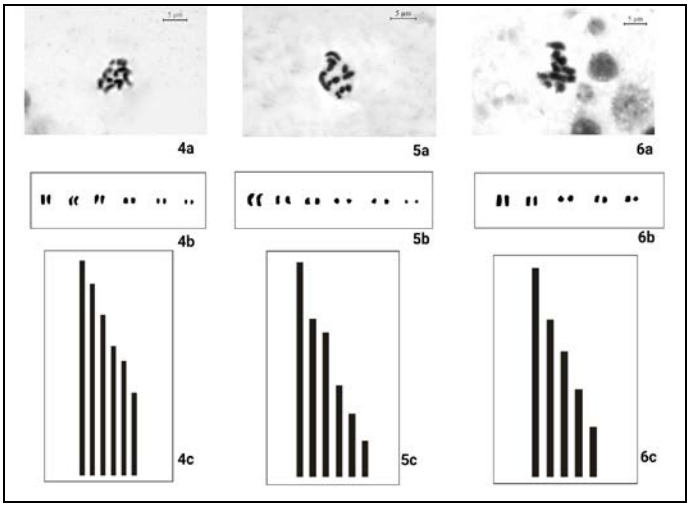

Fig 4

***Macrosiphoniella sanborni* (Gillete)**

This species of aphid was found on immature apical shoots and stems of plants and was dark reddish brown in colour. Twelve (2n=12) diploid chromosomes were discovered (Figs. 5a, 5b). The actual length of chromosomes ranged from 0.45 µm ±0.05 to 2.69 µm ±0.05, according to measurements of well scattered metaphase plates. TCL was 17.60 µm ±0.42. Chromosome relative lengths varied between 2.55 µm ±0.23 to 15.36 µm ±0.49. A pair of long, two pairs of medium, and three pairs of short chromosomes were displayed in the idiogram (Fig. 5c).

***Macrosiphum euphorbiae* (Thomas)**

This species' aphids had a light green colour to them. This species had a total of ten (2n=10) diploid chromosomes (Figs. 6a, 6b). Chromosome lengths varied between 0.94 µm ±0.14 and 3.79 µm ±0.29. 23.05 µm ±0.82 was the total complement length. Chromosomes ranged in relative length from 3.97 µm ±0.36 to 16.57µm ±0.52. This species idiogram revealed a pair of chromosomes that is long, a pair of medium-sized chromosomes, and three pairs of short chromosomes that steadily decreased in size (Fig. 6c).

***Myzus ascalonicus* Doncaster**

This species' aphids ranged in colour from pale yellow to dark green. The number of diploid chromosomes in these aphids is twelve (2n=12) (Figs. 7a, 7b). The actual length of chromosomes in these aphids ranged from 1.47 µm ±0.07 to 3.46 µm ±0.08. The length of the total complement was 29.54 µm ±0.66. The chromosomes' relative lengths ranged from 4.88 µm ±0.21 to 11.76 µm ±0.14. This species' idiogram revealed a progressive decrease in chromosomal length (Fig. 7c).

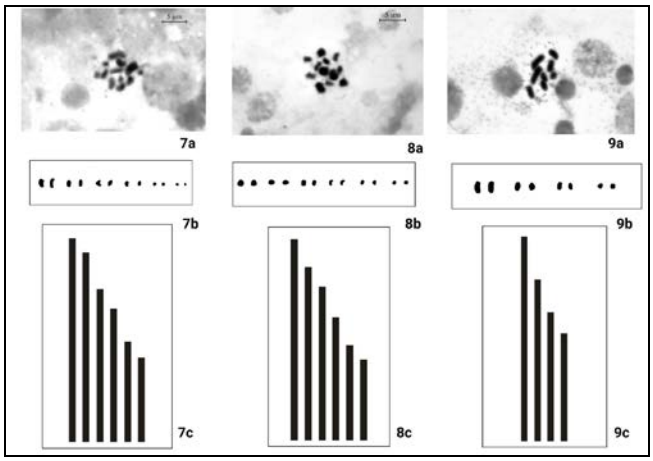

Fig 5

***M. ornatus* Laing**
This kind of aphid is tiny and light green to pale yellow in colour. The number of diploid chromosomes in these aphids is twelve (2n=12) (Figs. 8a, 8b). Actual chromosome lengths ranged from 1.57µm ±0.06 in the shortest chromosome to 3.89 µm ±0.04 in the longest chromosome. The length of the total complement was 32.18 µm ±0.70. The chromosomes' relative lengths ranged from 4.89 µm ±0.09 to 12.14 µm ±0.24. This species' idiogram revealed a progressive decrease in chromosomal length (Fig. 8c).

***Toxoptera aurantii* (Boyer de Fonscolombe)**
Aphids had black and white banded antennae, as well as black siphunculi and cauda, and were tiny, round, shiny, brownish red. The number of diploid chromosomes in this group is eight (2n = 8). (Figs. 9a, 9b). Actual chromosomal lengths ranged from 1.82 µm ±0.04 to 3.46 µm ±0.07. The length of the total complement was 20.37 µm ±0.22. The chromosomes' relative lengths ranged from 8.92 µm ±0.12 to 16.98 µm ±0.18. This species' idiogram revealed a pair of long, a pair of medium, and two pairs of short chromosomes (Fig. 9c).

***T. odinae* (van der Goot)**
These were little to medium in size, ant-attended and greenish dull or grey brown in colour. The abdomen of alatae is reddish-brown to dark brown in colour. The number of diploid chromosomes in these aphids is eight (2n = 8). (Figs. 10a, 10b). Actual chromosomal lengths ranged from 1.82 µm ±0.04 to 2.87 µm ±0.08. The length of the total complement was 18.90 µm ±0.30. The chromosomes' relative lengths varied from 9.64µm ±0.24 to 15.16µm ±0.25. This species' idiogram revealed a progressive reduction in chromosomal length (Fig. 10c).

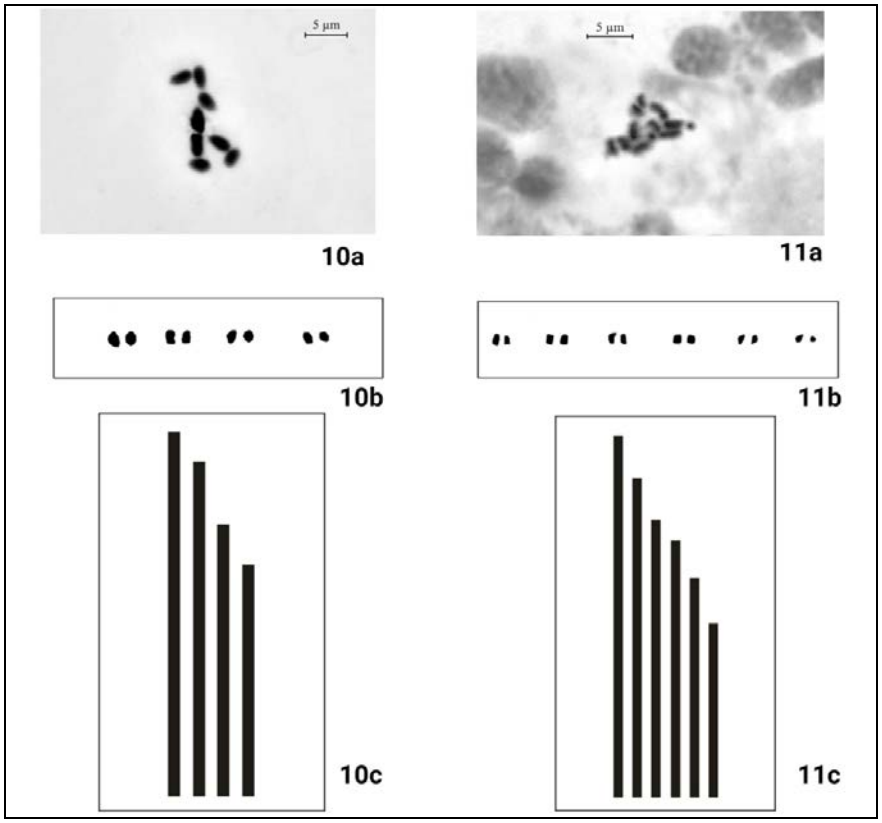

Fig 6

***Uroleucon ambrosiae* (Linnaeus)**
Uroleucon ambrosiae ranges in colour from dark reddish brown to nearly black. These were taken from the stem leaves and near the flower bud of Sonchus olerceaus, the host plant. The number of diploid chromosomes was discovered to be twelve (2n=12) (Figs. 11a, 11b). The mean actual length of chromosomes measured on well dispersed metaphase plates ranged from 1.47µ m ±0.09 to 3.71 µm ±0.06. Total complement length (TCL) was 29.01µm ±2.20 on average. The chromosomes' relative lengths ranged from 4.63µm ±0.09 to 11.99µm ±0.78. This species' idiogram revealed a progressive decrease in chromosomal length (Fig. 11c).

Discussion
The chromosome number 2n=8 was found in three species in this investigation, while 2n=12 was found in six species. In addition, 2n=10 and 2n=6 were identified in one species each.
In the current study, *Acrythosiphon gossypii* with the lowest chromosome number 2n=6 was found in *Abelmoschus esculentus*. However, earlier variations have been recorded in this species with 2n=6, 8, 10, 12, 14 and 16. Many workers reported 2n=8 in *A. gossypii* but from different host plants (Kulkarni & Kacker, 1979; Kurl, 1978) [14, 15]. Gautam reported karyotypes of 21 species of aphids and various chromosomes were studied (Gautam, 2002, 2003) [7]. Gautam

and Kumar reported karyotypes of aphids infesting *Quercus* and *Rhododendron* (Gautam, 2006) [8].

The chromosome number (2n=8) was reported in 3 species of present study, *Aphispunicae* (Passerini) collected from *Punica granatum*, *Toxoptera aurantii* and *T. odinae* collected from *Citrus medica* and *Duranta erecta*. The diploid chromosome number 2n=8 in *A. punicae* was in confirmity with earlier chromosomal accounts given by (Panigrahy & Patnaik, 1987) [22]. (Samkaria *et al*., 2010) [25] Same diploid chromosome number in *T. aurantii* also reported. *T. odinae* had the same diploid chromosomal number (2n = 8) as Kurl (1980) [15]. *Macrosiphum euphorbiae* has the diploid chromosomal number 2n=10. *Macrosiphum* is a polyphagous North American species that eats up to 200 different plant species from 20 different families (Le Roux *et al*., 2010) [17]. In the present investigation, *M. euphorbiae* was collected from *Malva parviflora* revealed the diploid chromosome number as 2n=10.

In present investigation chromosome number as 2n=12 was observed in six species namely; *Brachycaudus helichrysi* (Kaltenbach) collected from *Ageratum* sp., *Hyperomyzus lactuae* collected from host plant *Sonchus*, *Macrosiphoniella sanborni was* collected from *Chrysanthemum*, *Myzus ascalonicus* collected from *Dahlia pinnata*, *M. ornatus* collected from *Ajuga bracteosa* and *Uroleucon ambrosiae* collected from host plant *Sonchus olerceaus*. 2n=12 in *B. helichrysi* confirmed by earlier reports of (Dutta, 1993) [5] from *Artemisia vestita*, (Kuznetsova, 1968) reported 2n + 10 in aphids from *Prunus* sp. While (Raychaudhuri & Das, 1987) [24] reported 2n=10 in *B. helichrysi* collected from *Brassica napus*. In *M. sanborni*, the same diploid chromosomal number (2n=12) was reported by (R L Blackman & Eastop, 2006; Boschetti, 1963; Dutta, 1993) [1, 3, 5]. 2n=10 has also been found in *M. sanborni* by (Chen, 1985) [4]. The number of diploid chromosomes in the genus Myzus ranges from 8 to 20 in different species. Here, two species of *Myzus* has been investigated. *M. ascalonicus* collected from *Dahlia pinnata* and *M. ornates* collected from *Ajuga bracteosa*. *Uroleucon ambrosiae* collected from host plant *Sonchus olerceaus*. Diverse researchers have counted the number of diploid chromosomes in this genus's diverse member (Gut, 1976; Kar *et al*., 1990) [10].

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Author Contribution

Conceptualisation: Kanika Choudhary, Sarita Kumari, Preparation of manuscript: Kanika Choudhary, Sarita Kumari Literature review: Kanika Choudhary, Sarita Kumari, Editing and Drafting of Manuscript, Rajni. Both corresponding authors equally participated in the preparation of manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest

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