

Original article (Orijinal araştırma)

The karyotype studies of some aphid species (Hemiptera: Aphidoidea) from Niğde province in Türkiye¹

Türkiye'nin Niğde ilinden bazı yaprakbiti türlerinin (Hemiptera: Aphidoidea) karyotip değerlendirilmesi

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Abstract

The biological features of aphids as holocentricity, parthenogenetic reproduction, and telescopic generation have fascinated researchers to conduct chromosomal studies. Because of their chromosomes' holocentricity, the fragmentation fusion leads to karyotypic variations in aphid species. In phytophagous insects like aphids, holocentrism can be related to production of compounds that induce chromosomal variations. In the current study, the evaluation of karyotypes of six aphid species belonging to six genera that infest different host plants at the Niğde Ömer Halisdemir University campus area was conducted between September and November 2022. Evaluated species were *Acyrtosiphon (Acyrtosiphon) ilka* Mordvilko, 1914, *Aphis (Aphis) spiraecola* Patch, 1914, *Brachycaudus (Brachycaudus) helichrysi* (Kaltenbach, 1843), *Cinara (Cinara) curvipes* (Patch, 1912), *Macrosiphum (Macrosiphum) rosae* (L., 1758), and *Pterochloroides persicae* (Cholodkovsky, 1898). The *C. curvipes* and *A. ilka* karyotypes were determined for the first time in this study.

Keywords: Aphid, chromosome, Hemiptera, karyotype, Türkiye

Öz

Kromozomlarının holosentrik doğası, partenogenetik üreme, teleskopik jenerasyon gibi biyolojik karakteristik özellikleri, yaprakbitlerini kromozomal çalışmalar için çekici hale getirmektedir. Farklı konak bitkiler için önemli zararlı türler olan yaprakbitleri holosentrik kromozomlara sahiptir. Füzyon veya parçalanma, kromozomlarının holosentrik doğası nedeniyle karyotipik varyasyonlara yol açabilmektedir. Yaprakbitleri gibi fitofag böceklerde holosentrizm, kromozomal varyasyonlara neden olan bileşiklerin üretimi ile ilişkili olabilir. Bu çalışmada, Niğde Ömer Halisdemir Üniversitesi kampüs alanında farklı konak bitkileri istila eden altı cins'e ait altı yaprakbiti türünün karyotiplerinin değerlendirilmesi Eylül ve Ekim 2022'de yapılmıştır. Bu türler *Acyrtosiphon (Acyrtosiphon) ilka* Mordvilko, 1914, *Aphis (Aphis) spiraecola* Patch, 1914, *Brachycaudus (Brachycaudus) helichrysi* (Kaltenbach, 1843), *Cinara (Cinara) curvipes* (Patch, 1912), *Macrosiphum (Macrosiphum) rosae* (L., 1758) ve *Pterochloroides persicae* (Cholodkovsky, 1898)'dir. *Cinara curvipes* ve *A. ilka'nın* karyotip verileri ilk kez bu çalışmada belirtilmiştir.

Anahtar sözcükler: Yaprakbiti, kromozom, Hemiptera, karyotip, Türkiye

¹ This study is a part of the Master Thesis of first author.

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Received (Alınış): 12.12.2023 Accepted (Kabul ediliş): 19.06.2024 Published Online (Çevrimiçi Yayın Tarihi): 20.06.2024

Introduction

Nearly 6000 aphid species worldwide and 675 aphid species from Türkiye have been described up to date (Görür et al., 2012, 2023; Kök & Özdemir, 2021; Favret, 2024). The aphids have been recorded on almost 25% (nearly 94.000 plant species) of the known species of host plants, but nearly 100 aphid species were evaluated as economically significant. Currently, the chromosome numbers of 1.039 aphid species belonging to 14 families have been reported, comprising nearly 22% of all the described aphid species (Potan & Gautam, 2019; Sharma & Gautam, 2019; Kuznetsova et al., 2021).

The aphid cytological studies started at the beginning of the 20th century (Morgan, 1909). Blackman (1980) presented chromosome numbers of 180 aphid species, and pointed out that diploid chromosome numbers of them range from 6 [*Sarucallis kahawaluokalani* (Kirkaldy, 1907)] to 72 [*Amphorophora* (*Amphorophora*) *sensoriata* Mason, 1923]]. Gautam & Dutta (1994) provided information about the chromosomal compositions of 52 aphid species belonging to 34 genera and 21 of them were reported for the first time. The sex diagnosis and karyotype of *Cavariella aegopodii* (Scopoli, 1763) and *Tuberolachnus salignus* (Gmelin, 1790) were detected in different localities of India in a study by Dhatwalia & Gautam (2009). Although they determined the diploid chromosome number of *C. aegopodii* as $2n=8, 9$ and 10 , the male diploid chromosome number comprised eight autosomal and a single X chromosome. The common diploid chromosome number of *T. salignus* was $2n=20$, but in the Solan region, the population showed variations in diploid chromosome number as $2n=18-20$. In a study related to three clones of *Myzus persicae* (Sulzer, 1776) detailed karyotype analyses were conducted by using Hind200 satellite and subtelomeric repeat chromosomal markers. The results of the study showed that clone 1 diploid chromosome number was ten autosomal and double X ($2n=12$), clone 50 was $2n=13$, and clone 70 was $2n=14$ (Monti et al., 2012). Rivi et al. (2012) reported cytogenetic data of 66 *M. persicae* populations, infected aubergine, peaches, potato tobacco, and tomato host plants, distributed in different localities of Italy. The researchers indicated that the diploid chromosome number of *M. persicae* generally was $2n=12$, but the diploid chromosome number of populations that were collected from tobacco host plants was $2n=11-14$. In a study conducted in different regions of India, 27 aphid species belonging to 14 genera were evaluated karyomorphologically. It was determined that the chromosome number varied between $2n=6-18$ in the aphid species evaluated (Sharma & Gautam, 2019). Kumari et al. (2022) aimed to give information about the karyotypes of four aphid species that damage to medically significant and common in host plants in India. It was shown that the chromosome number of *Macrosiphum euphorbiae* (Thomas, 1878) infecting *Malva parviflora* L. (Malvales: Malvaceae) host plant was $2n=10$, the chromosome number of *Myzus ornatus* Laing, 1932 infecting *Ajuga integrifolia* Buch.-Ham. ex D. Don (Lamiales: Lamiaceae) host plant was $2n=12$, and the chromosome number of *Aphis odinae* (van der Goot, 1917) infecting *Duranta erecta* L. (Lamiales: Verbenaceae) host plant was $2n=8$ respectively.

The holocentric structure of the aphid chromosomes results in centromeric activity that diffuses the full length of chromosomes. Thus, the holocentricity in their chromosomes has deep implications for chromosomal development (Normark, 1999; Blackman et al., 2000; Wilson et al., 2003). Holocentric chromosomes have several kinetochores along the length of the chromosome instead of the single centromere that is characteristic of other chromosomes. In 1935, the term holocentric was defined for the first time and currently stands for some features as follows;

- i. The monocentric chromosomes show a lack of primary tightness, which corresponds to that of the centromere.
- ii. There are several kinetochores at the chromosome axis.
- iii. Microtubules move from the metaphase plate towards the poles and are attached to the chromosomes along their entire length. The term holokinetic chromosome stands for the chromatids that do not form the standard V-shaped during the cell division, characteristic of monocentric chromosomes; instead, they separate each other in parallel. Holocentric chromosomes have undergone many changes during the evolution of both animals and plants.

iv. Holocentric chromosomes can stabilize chromosomal fragments through extensive kinetochores, promoting karyotype rearrangements (Mandrioli & Manicardi, 2012; Manicardi et al., 2015).

However, holocentricity can also lead to restrictions for crossing over in homologous chromosomes that are adjacent to each other during meiosis due to the limitation of the number of chiasmata (Mandrioli & Manicardi, 2003, 2012; Melters et al., 2012; Manicardi et al., 2015; Lukhtanov et al., 2018). Both host plants and geographical conditions play important roles in chromosomal variation. Therefore, it is necessary to study the chromosomes of aphids from different host plants and geographical regions. Under these general approaches, this study aimed to determine the chromosome numbers of certain aphid species distributed in the campus area of Niğde Ömer Halisdemir University and to contribute to the karyological characteristics of various aphids.

Materials and Methods

This study was conducted in the campus area of Niğde Ömer Halisdemir University in 2022. The parthenogenetic, viviparous female individuals were collected from different host plants (Table 1), and aphid species were identified according to the key provided by Blackman & Eastop (2024).

Table 1. The information about studied samples and ant attendance of aphid populations (+: presence of ant attendance; -: absence of ant attendance)

Sample no.	Host plant	Species	Collection date
S1	<i>Rosa</i> sp. L. (Rosaceae)	<i>Macrosiphum rosae</i> L., 1758	27. IX. 2022
S2	<i>Sonchus</i> sp. L. (Asteraceae)	<i>Acyrtosiphon ilka</i> Mordvilko, 1914	29. IX. 2022
S3	<i>Prunus domestica</i> L. (Rosaceae)	<i>Pterochloroides persicae</i> (Cholodkovsky, 1898)	30. IX. 2022
S4	<i>Cedrus</i> sp. Rich (Pinaceae)	<i>Cinara curvipes</i> (Patch, 1912)	3. X. 2022
S5	<i>Hibiscus</i> sp. L. (Malvaceae)	<i>Aphis spiraeicola</i> (Patch, 1914)	6. X. 2022
S6	<i>Lepidium latifolium</i> L. (Brassicaceae)	<i>Brachycaudus helichrysi</i> (Kaltenbach, 1843)	7. X. 2022
S7	<i>Sonchus</i> sp. Britton & Brown (Asteraceae)	<i>Acyrtosiphon ilka</i> Mordvilko, 1914	11. X. 2022
S8	<i>Acacia</i> sp. Miller (Fabaceae)	<i>Aphis spiraeicola</i> Patch, 1914	18. X. 2022

The slide preparation for karyological studies was conducted as follows (amended from Manicardi et al., 1996);

1. Adult female individuals from each population were dissected primarily in Ringer's saline solution.
2. The embryos were taken into the mini tubes that included a 1% hypotonic solution of potassium chloride and kept for 10 minutes.
3. Embryos were transferred into new sterile mini tubes and centrifuged at 3000 Rpm for 15 minutes.
4. The fixative was added to the mini tubes that included pellets (3: 1 methanol: acetic acid) and then kept in deep freeze at -20°C for 15 minutes.
5. Then each mini tube was centrifuged at 3000 Rpm for 15 minutes.
6. The 4th step was repeated with fresh fixative.
7. The samples were kept in deep freeze at -20°C for 60 minutes.
8. 20 µL of the cell suspension was dropped onto clean slides by pipette at a distance of 30cm and air-dried.
9. Dried slides were kept in a chalet that includes 10% of Giemsa stain for 15 minutes.
10. After the samples were removed from the stain, they were washed and left to dry for 24 hours.

Detection of chromosomes was conducted under the bright field microscope using immersion oil at 100x ocular.

Results and Discussions

In this study, six aphid samples collected from different host plants from the Niğde Ömer Halisdemir University campus area between September and October 2022 were used and chromosomal data was obtained from viviparous adult females of different species.

Acyrthosiphon (Acyrthosiphon) ilka Mordvilko, 1914

The diploid chromosomal number of *A.ilka* that was collected from the host plant *Sonchus* sp. was $2n=8$ and a single X chromosome (Figure 1 a-b). The idiogram of this species revealed a single X chromosome and two partners long, a partner medium-sized, and a partner of short chromosomes (Figure 1 c).

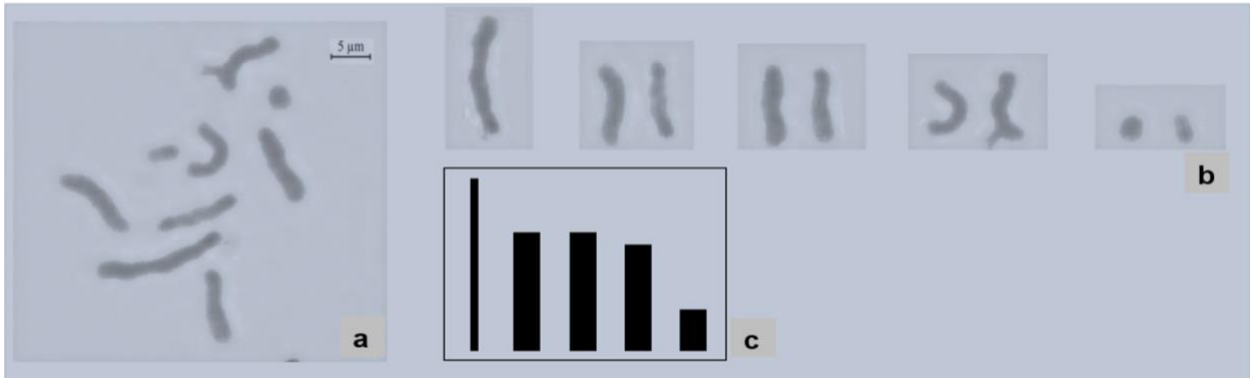


Figure 1. *Acyrthosiphon ilka*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

Aphis (Aphis) spiraecola Patch, 1914

The diploid chromosomal number of *A.spiraecola* that was collected from the host plant *Acacia* sp. and *Hibiscus* sp. was $2n=8$ (Figure 2 a-b). The idiogram of this species revealed a partner of long, a partner of medium-sized, and two partners of gradually decreasing short chromosomes (Figure 2 c).

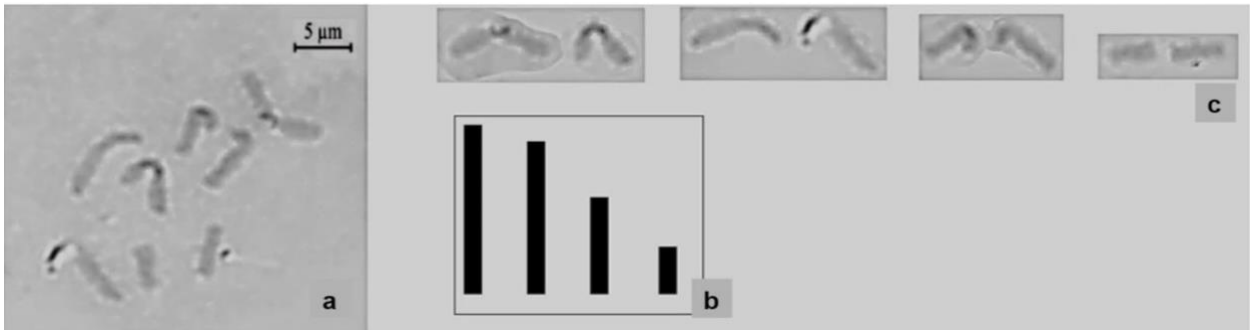


Figure 2. *Aphis spiraecola*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

Brachycaudus (Brachycaudus) helichrysi (Kaltenbach, 1843)

The diploid chromosomal number of *B.helichrysi* that was collected from the host plant *Lepidium latifolium* was $2n=12$ (Figure 3 a-b). The idiogram of this species revealed two partners of long, two partners of medium size, and two partners of short chromosomes (Figure 3 c).

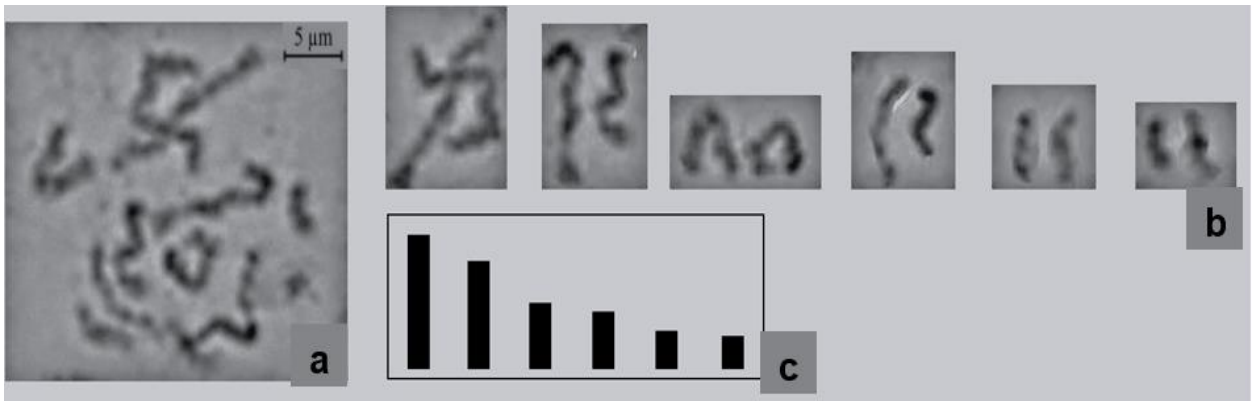


Figure 3. *Brachycaudus helichrysi*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Cinara (Cinara) curvipes* (Patch, 1912)**

The diploid chromosomal number of *C. curvipes* that was collected from the host plant *Cedrus* sp. was $2n=10$ (Figure 4 a-b). The idiogram of this species revealed a partner of long, two partners of medium size, and two partners of short chromosomes (Figure 4 c).

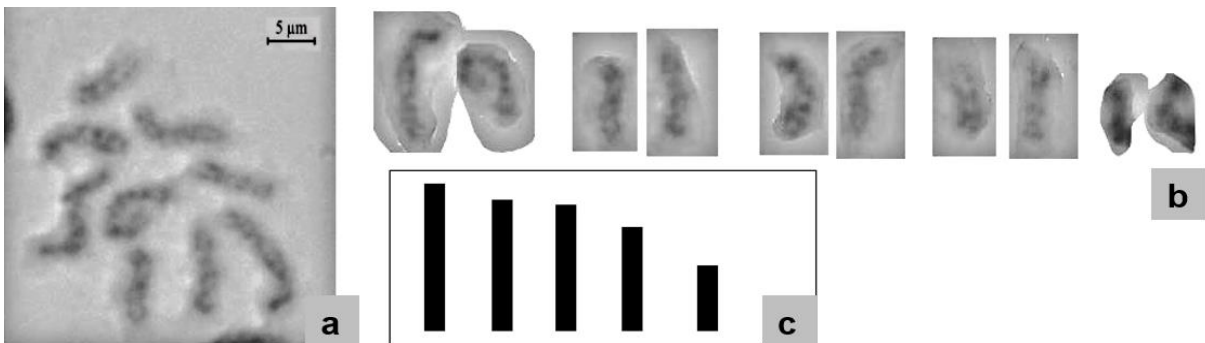


Figure 4. *Cinara curvipes*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Macrosiphum (Macrosiphum) rosae* (L., 1758)**

The diploid chromosomal number of *M. rosae* that was collected from the host plant *Agropyron* sp. was $2n=10$ (Figure 5 a-b). The idiogram of this species revealed a partner of long, a partner of medium-sized, and three partners of gradually decreasing short chromosomes (Figure 5 c).

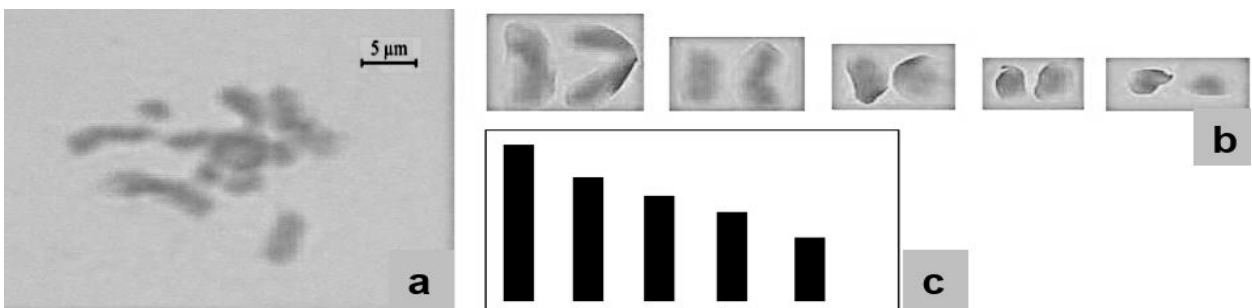


Figure 5. *Macrosiphum rosae*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

Pterochloroides persicae (Cholodkovsky, 1898)

The diploid chromosomal number of *P. persicae* that was collected from the host plant *Prunus cerasifera* was $2n=12$ (Figure 6 a-b). The idiogram of this species revealed a partner of long, two partners of medium-sized, and three partners of short chromosomes (Figure 6 c).

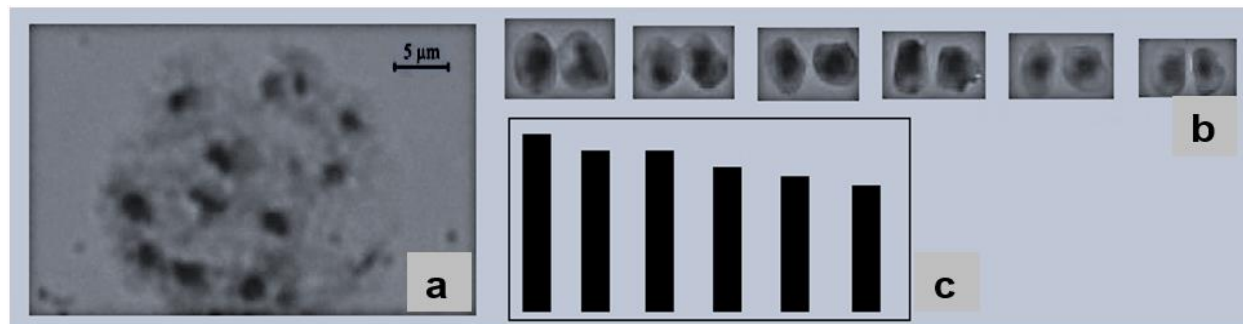


Figure 6. *Pterochloroides persicae*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

Most of the data on holocentric chromosomes obtained so far have been derived from studies conducted on aphids and members of the Lepidopteran order. The phytophagous lifestyle of aphids can lead to the conservation of their chromosomal fragments. The tendency to favor the inheritance of chromosomal fragments causes repetitive substitutes in the karyotypes of some aphids like *Myzus persicae*. Furthermore, aphids have a repetitive expression of the gene encoding telomerase, therefore they can also start the resynthesis of telomeres at the inner cut-off points, leading to the stabilization of chromosomal fragments (Wilson et al., 2003; Dhatwalia & Gautam, 2009).

Currently, the standard chromosome number for all Aphidinea members can be considered as $2n=8$, 10 and 12. Cytogenetically, 601 species in 119 genera belonging to Aphididae, the largest family with 3035 species in approximately 273 genera, were studied and the findings support this data. These chromosome numbers, or at least some of them, are also common in other relatively well-studied families such as Drepanosiphidae ($2n=8$, 4, and 18), Eriosomatidae ($2n=6$, 8, 10, 12 and 20) and Lachnidae (usually $2n=10$, 12 and 14). In Hormaphididae, $2n=12$ is the common chromosome number. However, all other families are too poorly studied to allow the determination of standard values (Manicardi et al., 2015; Kuznetsova et al., 2021). In previous studies, diploid chromosome numbers of *A. spiraecola* (Kapoor, 1994; Blackman & Eastop, 2024), *B. helichrysi* (Raychaudri & Das, 1987; Blackman & Eastop, 2024), *Macrosiphum rosae* (Samkaria et al., 2010; Blackman & Eastop, 2024) and *Pterochloroides persicae* (Blackman & Eastop, 2024) were determined as 8, 12, 10 and 20, respectively. The current study evaluated the karyotypes of six species that preferred different host plants and the chromosome numbers of them varied from 9 to 12. The karyotype data of *A. spiraecola* ($2n=10$), *B. helichrysi* ($2n=12$), *M. rosae* ($2n=10$) showed similarity with previous studies (Dutta, 1993; Kapoor & Gautam, 1994; Samkari et al., 2010; Sharma & Gautam, 2019; Potan & Gautam, 2019; Blackman & Eastop, 2024). Although the chromosome number of *P. persicae* was indicated as $2n=20$ by Blackman & Eastop (2024), as a result of this study it was $2n=12$. This difference in the number of chromosomes in *Pterochloroides persicae* may be due to differences in the environmental conditions (geographical conditions, climate, host plant, etc.).

A range of unique cytogenetical processes are involved in the changeover between parthenogenetic and bisexual reproduction in the complex life time of the aphid. For example, in the case of cyclic parthenogenesis to happen, every descendants that develop from fertilised eggs must be XX females, while all of the sperm must have only one X chromosome. This occurs when one of the two X chromosomes is eliminated throughout the annual meiosis of the egg. However, the formation of parthenogenetic progeny consisting exclusively of females from bisexuals including the exclusion of male reproductive cells. Aphid

sex is controlled by endocrine factors responding to environmental cues, rather than to be reached by the random combination of male and female chromosomes during fertilisation. Such a complex and unique system emphasises a special "Aphidoid-type" sex determination system in parallel with such rare systems. The fact that some aphid species have multiple sex chromosomes most likely arose through X chromosome divisions, but other mechanisms can also be envisaged. The fact that some aphid species have multiple sex chromosomes probably results from X chromosome divisions, but other mechanisms are possible. Some species in the Adelgid and Greenid families have up to four pairs of X chromosomes, and some species in the Phylloxerid, Eriosomatid, Lachnid and Drepanosiphid families have two pairs of sex chromosomes. In some species, despite having multiple sex chromosomes, their sex determination system remains $X_nX_n/X_n(0)$ (male/female) (Manicardi et al., 2015; Kuznetsova et al., 2021). The karyotype data of *C. curvipes* and *A. ilka* were given for the first time in the current study as $2n=10$ and $2n=8+X$, respectively. Identification of chromosomal landmarks is crucial in organisms with holocentric chromosomes, as the absence of a primary constriction and the difficulty in obtaining a clear banding pattern make cytogenetic studies in species with this unique chromatin organization challenging. The relevance of a cytogenetic approach to aphid chromosomes have shown that information on aphid genomes is not only scientifically important but also economically relevant. Manicardi et al. (2015) assessed *M. persicae* populations and suggested that, when their impact on economically important crops is considered, there is a need for chemical and/or biological control. Without a full understanding of its heredity, it may be hard to accurately assess the existence of infectious and adaptive variability that makes biological and chemically based controls less effective. The concept that populations of aphids are resistant over time and across geographical areas continues to be controversial since aphid colonies do not seem genetically uniform, as was previously thought. Aphid colonies can be aggregations of individuals of distinct karyotypes and thus respond differently to selective external factors. Therefore, a more detailed cytogenetic effort, expected to be supported by the identification of more chromosomal regions, would supply valuable data to assess the adaptive potential of aphids at short temporal and regional scales. Thereby, it could make a significant difference to our understanding of traits such as reproductive rate, host selection, resistance to pesticides, and the mechanisms of speciation (Wilson et al., 2003; Monti et al., 2011; Manicardi et al., 2016). Chromosomal variation occurs in aphids depending on the host plant and different geographical conditions (Sharma & Gautam, 2019). Chromosomal variation occurs in aphids depending on the host plant and different geographical conditions (Sharma & Gautam, 2019). Considering Turkey's geographical location and different climatic zones, it is assumed that chromosomal variations of aphids distributed in our country are quite diverse. In this regard, it is necessary to study the chromosomes of aphids from different host plants and geographical regions.

Acknowledgment

The authors would like to thank Dr. Teoman Kankılıç and Dr. Zübeyde Kumbıçak for their valuable assistance.

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