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Chromosomal Insights: Karyotypic Analysis of Aphid Species in Himachal Pradesh

[1]Kanika Choudhary, [2]Poonam Kumari, [3]Kushal Thakur and [4]Sunil Kumar*

Author's Affiliation:
[1,2,3]Department of Biosciences, Himachal Pradesh University, Shimla 171 005, India
[4]Department of Animal Sciences, School of Life Sciences, Central University of Himachal Pradesh, Academic Block - Shahpur, District – Kangra (H.P.) -176206, India

***Corresponding author:**
Prof. Sunil Kumar
Department of Animal Sciences, School of Life Sciences, Central University of Himachal Pradesh, Academic Block - Shahpur, District – Kangra (H.P.) -176206, India
E-mail: sunilibes@gmail.com

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ABSTRACT:
Aphids are among the most devastating pests impacting agriculture, causing direct harm to plants by ingesting sap and indirectly through the transmission of numerous plant pathogens. This study focuses on the chromosomal characteristics and karyotypic variations of aphid species in the agricultural regions of Himachal Pradesh. By analyzing the chromosomal architecture of key aphid species, the research aims to better understand their reproductive strategies, adaptability, and the genetic mechanisms that enable their effective vectoring of plant diseases. Chromosomes of aphids infesting different host plants from the Kangra district of Himachal Pradesh were studied. These aphid species are, *Aphis craccivora* Koch (2n=8), *Aphis fabae* Scopoli (2n=8), *Aulacorthum solani* Kaltenbach (2n=10), *Hyperomyzus lactuae* (Linnaeus) (2n=12), *Uroleucon ambrosiae* Linneaus (2n = 10). The lengths of individual chromosomes were measured during the metaphase stage of cell division, and their relative lengths were calculated. Additionally, the total complement length of the chromosome set was determined. Based on the relative length data, karyotypes were prepared to visually represent the chromosomal arrangements. Subsequently, idiograms were constructed, providing a schematic representation of the chromosomes, scaled according to their relative lengths and characteristic features. This approach facilitates a comparative analysis of chromosomal structure and organization, aiding in cytogenetic studies and genome characterization. The study reveals significant karyotypic diversity among the aphid species, which may correlate with their varied reproductive strategies and host adaptability.

Keywords:
Chromosomes, Aphids, Total Complement Length, Karyotype, Idiogram.

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1. INTRODUCTION

In agriculture, aphids pose a serious problem despite the fact they are one of the smallest groups of insects (Morales-Hojas 2017). About 5000 species of sap-sucking insects are known around the world. They are classified into about 510 genera and are found in about 300 plant families (Wojciechowski et al. 2015). In temperate regions of the world, aphids are specialized herbivores that feed on the phloem found in vascular plants (Züst and Agrawal 2016). These minute insects are classified under the order *Hemiptera* and exhibit a cosmopolitan distribution. The majority of aphid species are predominantly found in temperate regions, where nearly 25% of plant species are colonized by at least one aphid species (Singh and Singh 2021). An aphid's life cycle is characterized by several peculiar characteristics, including polymorphism, viviparity, telescoping generation, host alternation, and holocentric chromosomes. The phenomenon of cyclic parthenogenesis, as well as polymorphism within the life cycle of aphids, makes them a topic of great interest. Due to their parthenogenetic reproduction, aphids are able to have a very high reproductive potential while recombination of genes takes place during bisexual reproduction, which increases genetic diversity (White 1951). The Tramini tribe of aphids is hypothesized to have an extended evolutionary history of parthenogenesis, as no instances of functional sexual reproduction have been documented among its members (Blackman et al. 2000).

Life cycle and occurrence of several morphs in the life cycle of aphids pose many taxonomical problems. Chromosomal investigations in aphids were initiated in the early twentieth century. The evolutionary changes in aphid chromosomes have been complex, leading to ongoing debate regarding whether a high or low chromosome number represents the ancestral state in this group (Kuznetsova 1968). Gut (1976) conducted a study on the chromosome numbers of parthenogenetic females across 55 aphid species. He pointed out that since the taxonomy of aphids is not stabilized at genetic and species level, one should not draw taxonomic conclusion from the available chromosomal data. Aphids serve as a valuable experimental model in cytogenetics due to their holocentric chromosomes, characterized by centromeric activity distributed along the entire length of the chromosomal axis (Manicardi et al. 2015). From Himachal Pradesh, studies on aphid chromosomes were done by various workers (Gautam 1990, 2003). Gautam (2003) reported the karyotype of green apple aphid, *Aphis pomi* De Geer for the first time from India.

Several aphid species have been documented to exhibit chromosomal variation. The sex chromosomes exhibit multiple unusual characteristics such as a sex-specific inheritance pattern, hemizygosity, and a reduced tendency to recombinate as a result of the sexual selection (Vicoso and Charlesworth 2006). Due to this, the response of these chromosomes to the evolutionary factors is expected to differ from that of the autosomes.

2. MATERIAL AND METHODS

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 aphid's species. The detail of aphid species along with host plants, place & collection time are given in Table 1.

Chromosome Studies

Somatic embryonic tissue was employed for chromosomal analysis. Embryos were obtained by dissecting parthenogenetic females through puncturing the posterior region of the abdomen. The extracted embryos underwent a pretreatment process in 0.7% trisodium citrate solution for 25–30 minutes to facilitate chromosomal preparation. Following pretreatment, the embryos were fixed using a 1:3 acetic acid-ethanol solution for 15–20 minutes at ambient temperature. Subsequently, they were transferred onto a glass slide and treated with 45% acetic acid for 3–5 minutes to enhance chromosome spreading. Finally, a coverslip was

carefully placed over the preparation for further examination.

The prepared slides were stained using a working solution comprising 25 ml Giemsa stain, 1.5 ml methanol, 1.5 ml 0.2 M DHP, and 50 ml distilled water. The slides were immersed in the solution for 20–25 minutes to achieve optimal staining. After staining, the slides and coverslips were gently retrieved, rinsed with distilled water to remove excess stain, and allowed to air-dry at room temperature. Once dried, the slides were mounted using DPX mounting medium. To enhance durability and fixation, the mounted slides were incubated overnight in an oven set at 60°C.

The permanent slides were examined under a research-grade binocular microscope. For photomicrography, well-spread chromosome plates were meticulously selected and analyzed using a LEICA DML-S2 microscope. Chromosomal measurements were conducted by determining the total complement length for each species. Relative lengths of individual chromosomes were derived from their absolute measurements, enabling the construction of idiograms. These idiograms, based on relative length data, provided a visual representation of chromosomal organization and structure.

3. RESULTS

In the present study, chromosomes of five aphid species infesting different host plants of Kangra (Himachal Pradesh) have been studied.

Table 1: **Chromosomes of five species of aphids infesting different host plants of Kangra district of Himachal Pradesh.**

S. No.	Aphid species	Host plant	Chromosome number
1	*Aphis craccivora* Koch	*Amaranthus Spinosus* (Amaranthaceae)	8
2	*Aphis fabae* Scopoli	*Solanum melongena* (Solanaceae)	8
3	*Aulacorthum solani* (Kaltenbach)	*Ocimum gratissimum* (Lamiaceae)	10
4	*Hyperomyzus lactuae* (Linnaeus)	*Sonchus arvensis* (Asteraceae)	12
5	*Uroleucon ambrosiae* (Linneaus)	*Sonchus olerceaus* (Asteraceae)	12

***Aphis craccivora* Koch**

Shiny black-colored aphids of *Aphis craccivora* were found concentrated on the growing tips and leaves of *Amaranthus spinosus.*

The diploid chromosome number was determined to be eight (2n = 8) (Figs. 1a and 1b). Analysis of well-spread metaphase plates revealed that the average actual chromosome length varied from 1.47 µm ± 0.07 S.E. for the shortest chromosome to 2.73 µm ± 0.07 S.E. for the longest. The mean total complement length (TCL) was calculated to be 17.08 µm ± 0.30 S.E. Relative chromosome lengths ranged from 8.62 ± 0.41 S.E. for the shortest chromosome to 15.97 ± 0.26 S.E. for the longest (Table 2). The idiogram representing this species is illustrated in Fig. 1c.

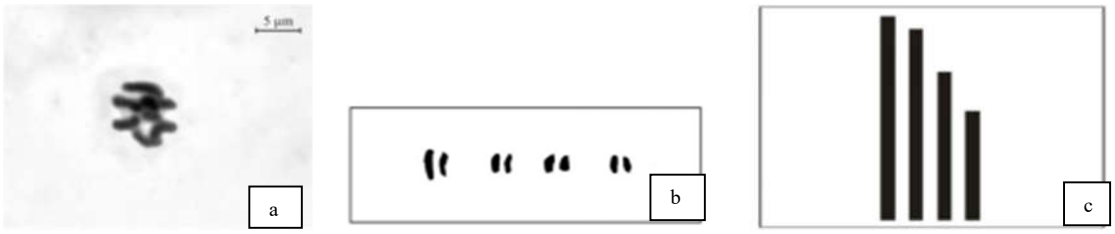

Figure 1: ***Aphis craccivora*** **a. Somatic metaphase chromosomes b. Karyotype c. Idiogram.**

Aphis fabae **Scopoli**

Blackish brown aphids of *Aphis fabae* were dusted with wax. Heavy infestation was found on the flowers and leaves of *Solanum melongena* and these aphids were often ant attended.

The diploid chromosome number in this species was identified as eight (2n = 8) (Figs. 2a and 2b). Measurements from ten metaphase plates indicated that chromosome lengths ranged from 1.75 µm ± 0.13 S.E. for the shortest chromosome to 3.32 µm ± 0.06 S.E. for the longest. The mean total complement length (TCL) was recorded as 21.70 µm ± 0.43 S.E. Relative chromosome lengths varied between 8.00 ± 0.46 S.E. for the shortest and 15.52 ± 0.35 S.E. for the longest (Table 2). The species' idiogram is depicted in Fig. 2c.

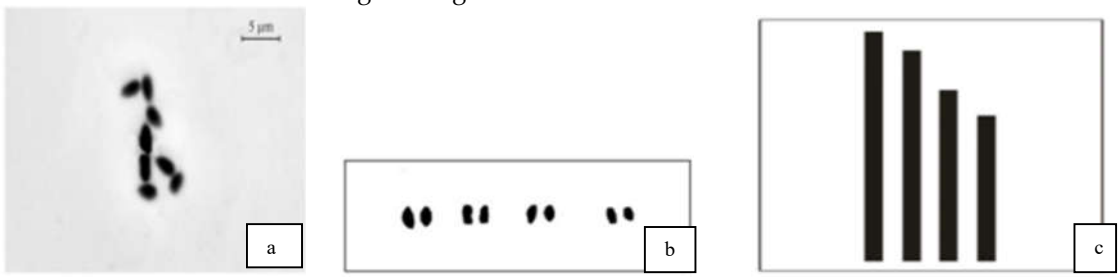

Figure 2: ***Aphis fabae*** **a. Somatic metaphase chromosomes b. Karyotype c. Idiogram.**

Aulacorthum solani **(Kaltenbach)**

Whitish green or yellow aphids of *Aulacorthum solani* were gregarious in habit, showed moderate activity and were ant attended found on *Ocimum gratissimum.*

The diploid chromosome number for this species was determined to be ten (2n = 10) (Figs. 3a and 3b). Measurements of chromosomes from well-spread metaphase plates revealed that the average actual length ranged from 1.54 µm ± 0.05 S.E. for the shortest chromosome to 3.67 µm ± 0.05 S.E. for the longest. The mean total complement length (TCL) was calculated to be 24.50 µm ± 0.13 S.E. The relative lengths of the chromosomes varied from 6.28 ± 0.22 S.E. for the shortest to 14.99 ± 0.23 S.E. for the longest (Table 2). The idiogram for this species is illustrated in Fig. 3c.

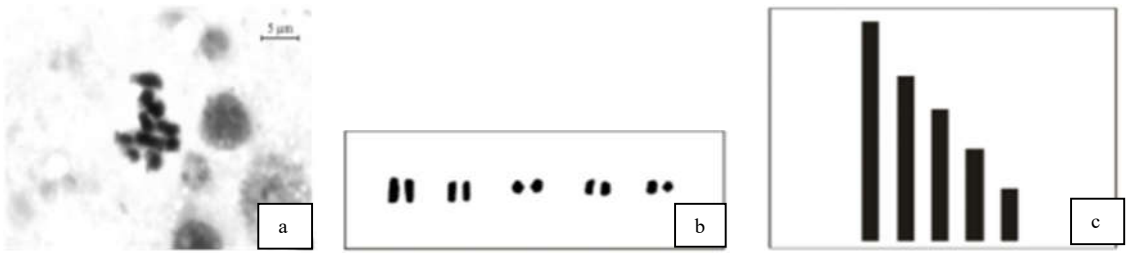

Figure 3: ***Aulacorthum solani*** **a. Somatic metaphase chromosomes b. Karyotype c. Idiogram.**

***Hyperomyzus lactuae* (Linnaeus)**
Yellowish green aphids of *Hyperomyzus lactuae* were collected from stem, leaves and flowers of host plant *Sonchus arvensis.*

The diploid chromosome number for this species was determined to be twelve (2n = 12) (Figs. 4a and 4b). The actual chromosome lengths ranged from 1.21 µm ± 0.06 S.E. for the shortest chromosome to 2.90 µm ± 0.05 S.E. for the longest. The total complement length (TCL) was measured as 24.90 µm ± 0.52 S.E. Relative chromosome lengths varied between 4.87 ± 0.19 S.E. for the shortest and 11.67 ± 0.02 S.E. for the longest (Table 2). The idiogram representing this species is shown in Fig. 4c.

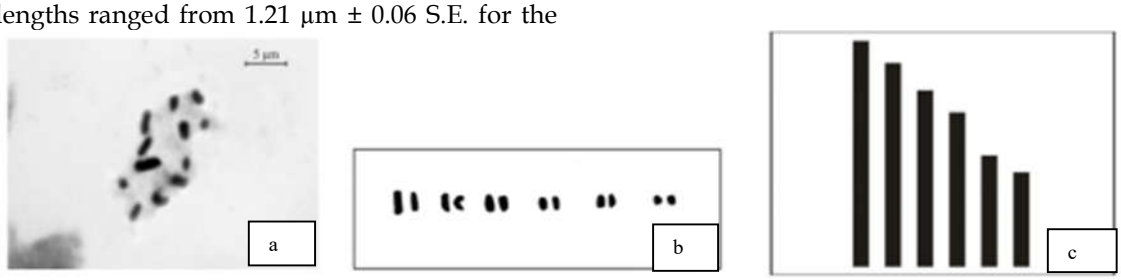

Figure 4: ***Hyperomyzus lactuae*** **a. Somatic metaphase chromosomes b. Karyotype c. Idiogram.**

***Uroleucon ambrosiae* (Linnaeus)**
Uroleucon ambrosiae are dark reddish-brown to nearly black in color. They were collected from the stem leaves and flower buds of the host plant Sonchus oleraceus. The diploid chromosome number was identified as twelve (2n = 12) (Figs. 5a and 5b). Measurements from well-spread metaphase plates revealed that the average actual chromosome lengths ranged from 1.47 µm ± 0.09 S.E. for the shortest chromosome to 3.71 µm ± 0.06 S.E. for the longest. The mean total complement length (TCL) was calculated to be 29.01 µm ± 2.20 S.E. Relative chromosome lengths varied from 4.63 ± 0.09 S.E. for the shortest to 11.99 ± 0.78 S.E. for the longest (Table 2). The idiogram of this species is illustrated in Fig. 5c.

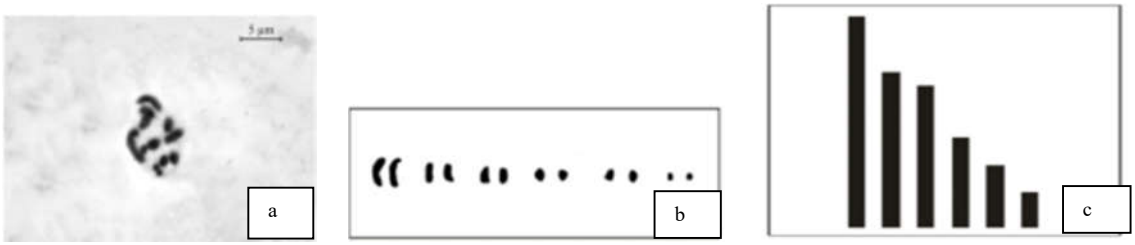

Figure 5: ***Uroleucon ambrosiae.*** **a. Somatic metaphase chromosomes b. Karyotype c. Idiogram.**

Table 2: Actual and relative lengths of somatic metaphase plates of different aphid species

S. No.	Aphid's species	Host plants	Chromosome number													Total Complement Length TCL (µm) ± S.E.
				1	2	3	4	5	6	7	8	9	10	11	12	
1	*Aphis craccivora* Koch	*Amaranthus Spinosus* (Amaranthaceae)	A.L.(µm) ±S.E.	2.73 ± 0.07	2.73 ± 0.07	2.34 ± 0.07	2.34 ± 0.07	1.99 ± 0.05	1.99 ± 0.05	1.47 ± 0.07	1.47 ± 0.07					17.08 ± 0.30
			R.L. ±S.E.	15.97 ± 0.26	15.97 ± 0.26	13.72 ± 0.33	13.72 ± 0.33	11.66 ± 0.17	11.66 ± 0.17	8.62 ± 0.41	8.62 ± 0.41					
2	*Aphis fabae* Scopoli	*Solanum melongena* (Solanaceae)	A.L.(µm) ±S.E.	3.32 ± 0.06	3.32 ± 0.06	3.04 ±0.05	3.04 ±0.05	2.69 ±0.05	2.69 ±0.05	1.75 ±0.13	1.75 ±0.13					21.70 ± 0.43
			R.L. ±S.E.	15.52 ± 0.35	15.52 ± 0.35	14.03 ± 0.09	14.03 ± 0.09	12.42 ±0.08	12.42 ±0.08	8.00 ±0.46	8.00 ±0.46					
3	*Aulacorthum solani* (Kaltenbach)	*Ocimum gratissimum* (Lamiaceae)	A.L.(µm) ±S.E.	3.67 ±0.05	3.67 ±0.05	2.97 ±0.05	2.97 ±0.05	2.20 ±0.05	2.20 ±0.05	1.85 ± 0.05	1.85 ± 0.05	1.54 ±0.05	1.54 ±0.05			24.50 ± 0.13
			R.L. ±S.E.	14.99 ± 0.23	14.99 ± 0.23	12.14 ±0.22	12.14 ±0.22	8.99 ±0.17	8.99 ±0.17	7.56 ±0.17	7.56 ±0.17	6.28 ±0.22	6.28 ±0.22			
4	*Hyperomyzus lactuae* (Linnaeus)	*Sonchus arvensis* (Asteraceae)	A.L.(µm) ±S.E.	2.90 ± 0.05	2.90 ± 0.05	2.62 ± 0.05	2.62 ± 0.05	2.27 ± 0.05	2.27 ± 0.05	1.99 ± 0.08	1.99 ± 0.08	1.43 ± 0.03	1.43 ± 0.03	1.21 ± 0.06	1.21 ± 0.06	24.90 ± 0.52
			R.L. ±S.E.	11.67 ± 0.02	11.67 ± 0.02	10.54 ± 0.10	10.54 ± 0.10	9.13 ± 0.10	9.13 ± 0.10	7.97 ± 0.18	7.97 ± 0.18	5.77 ± 0.12	5.77 ± 0.12	4.87 ± 0.19	4.87 ± 0.19	
5	*Uroleucon ambrosiae* (Linneaus)	*Sonchus olerceaus* (Asteraceae)	A.L.(µm) ±S.E.	3.71 ± 0.06	3.71 ± 0.06	3.29 ± 0.09	3.29 ± 0.09	2.80 ± 0.15	2.80 ± 0.15	2.27 ± 0.09	2.27 ± 0.09	1.96 ± 0.06	1.96 ± 0.06	1.47 ± 0.09	1.47 ± 0.09	29.01 ± 2.20
			R.L. ±S.E.	11.99 ± 0.78	11.99 ± 0.78	10.71 ± 0.73	10.71 ± 0.73	8.98 ± 1.06	8.98 ± 1.06	7.20 ± 0.33	7.20 ± 0.33	6.43 ± 0.18	6.43 ± 0.18	4.63 ± 0.09	4.63 ± 0.09	

TCL-Total Complement Length, AL- Actual lengths

4. DISCUSSION

In the present investigation, karyotypes of 5 species of aphids have been studied. Two aphid species have diploid chromosome number of 2n=8 or 12 while one aphid species has a diploid chromosome number of 2n=10. This study highlights the chromosomal diversity within aphid species and chromosome number may contribute to their adaptability in different environments. The observed diploid numbers (2n=8, 10, or 12) indicate not only genetic variability but also potential evolutionary adaptations within aphid populations to optimize their survival and reproductive success. The tendency of some genera to stabilize their chromosome numbers suggests that certain chromosomal configurations might confer specific advantages, possibly influencing traits like host specificity, fecundity, and resistance to environmental stressors. The finding that aphid species are more prevalent in polluted areas opens up intriguing questions regarding their epigenetic adaptability. Environmental stressors such as pollution might induce epigenetic modifications, which could affect gene expression and phenotypic traits in aphids. This adaptability could be an evolutionary advantage, enabling aphids to thrive under suboptimal conditions, which is critical for pests that inhabit variable agricultural landscapes. Additionally, the role of aphids as model organisms for studying polyphenism where they exhibit distinct morphs in response to environmental conditions, suggests that their epigenetic mechanism could provide insights into phenotypic plasticity. The polyphenism in aphids such as the development of winged and wingless forms, is known to be influenced by factors like crowding and food availability and this may be partly governed by chromosomal and epigenetic regulations.

5. CONCLUSION

The chromosomal architecture and epigenetic adaptability of aphids not only aid in grasping their evolutionary resilience but also have practical implications. Insights gained from this research could inform pest management strategies, allowing for the development of environmentally adaptive approaches that consider the aphid's genetic and epigenetic flexibility. This could be particularly useful in controlling aphid populations in regions like Himachal Pradesh, where agricultural productivity is vulnerable to such pests.

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Author contribution
SK: Conceptualization; KC: Data analysis, curation and validation; PK: Data interpretation and literature review, KC: Original draft preparation; PK: Editing, final draft preparation and critical revision.

Conflicts of interest
The authors declare that they have no conflict of interest.

REFERENCES

Blackman RL (1971) Chromosomal abnormalities in an anholocyclic biotype of *Myzus persicae* (Sulzer). Experientia 27:704–706

Blackman RL, Spence JM, Normark BB (2000) High diversity of structurally heterozygous karyotypes and rDNA arrays in parthenogenetic aphids of the genus *Trama* (Aphididae: Lachninae). Heredity (Edinb) 84:254–260. https://doi.org/10.1046/j.1365-2540.2000.00667.x

Gautam DC (1990) Chromosome numbers and idiograms of seven species of aphids from North-western Himalayas. Chromosome Inf Serv 49:16–18

Gautam DC (2003) Karyotype of Aphis pomi-First report from India. J Cytol Genet 4:95–96

Gut J (1976) Chromosome numbers of parthenogenetic females of fifty-five species of Aphididae (Homoptera) new to cytology. Genetica 46:279–285

Kuznetsova VG (1968) Aphid karyotypes of the subtribe Anuraphidina (Aphididae) and the possible paths of their evolution. Entomol Obozr 47:767–781

Mandrioli M, Manicardi GC (2014) Mapping the aphid genome: the cytogenetic dimension of a pest crop insect.

Manicardi GC, Mandrioli M, Blackman RL (2015) The cytogenetic architecture of the aphid genome. Biol Rev 90:112–125

Morales-Hojas R (2017) Molecular ecology of insect pests of agricultural importance: the case of aphids. Ecol Entomol 42:18–27

Singh R, Singh G (2021) Aphids. Polyphagous pests Crop 105–182

Vicoso B, Charlesworth B (2006) Evolution on the X chromosome: unusual patterns and processes. Nat Rev Genet 7:645–653

White MJD (1951) Animal cytology and evolution

Wojciechowski W, Depa Ł, Kanturski M, et al (2015) An annotated checklist of the Aphids (Hemiptera: Aphidomorpha) of Poland. Polish J Entomol 84:

Züst T, Agrawal AA (2016) to aphids. Nat Publ Gr 2:1–9. https://doi.org/10.1038/nplants.2015.206

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