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Karyotype studies on grape phylloxera (*Daktulosphaira vitifoliae* Fitch)

by

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Summary: A cytogenetic technique was developed to produce suitable chromosome spreads for phylloxera karyotype analysis. The karyotype for pathogenetic phylloxera was 2 n = 10. Karyotypes from haploid sex cells were found to vary between n = 5 and n = 6, the latter possibly indicating an aneuploidic aberration. Tetra- and polyploid cells were detected in somatic trophocytes. Preparation of phylloxera sex and somatic cells for chromosomal analysis reported here enables the study of genetic variation on a chromosomal scale.

Key words: chromosome, Daktulosphaira vitifoliae, karyotype, phylloxera.

Introduction

Phylloxera, *Daktulosphaira vitifoliae* Fitch, is one of the most important grape pests. Their importation and subsequent spread on the susceptible roots of European vineyards in the last half of the 19th century led to the development of hundreds of resistant rootstocks upon which most of the world's vineyards are now planted. Phylloxera reproduce through cyclical parthenogenesis on grape roots and leaves. Populations with a complete life cycle (holocyclic) bridge these parthenogenetic stages with dimorphic forms that produce sexual offspring (*sexuparae*). These offspring mate and lay an overwintering egg that initiates another parthenogenetic cycle (the *fundatrix*). Populations that only reproduce parthenogenetically are termed anholocyclic.

Phylloxera resistant rootstocks have successfully controlled this pest for over 100 years. However, strains with more aggressive feeding habits have been reported in Germany (Boubals 1994) and California (Granett *et al.* 1987). Studies on the population dynamics and biology of these and other phylloxera revealed the occurrence of new phylloxera biotypes that are host plant-adapted. These discoveries led to descriptions of genetic variation within and among phylloxera populations in North America and Europe (Fong *et al.* 1995; Forneck *et al.* 1998; Lin *et al.* 1999). Efforts to associate phylloxera feeding behavior with a distinct genotype, or genetic marker have not yet been successful. Genetic markers linked to aggressive feeding behavior would be useful in pest management and rootstock breeding. It might also be possible to associate karyotypes or levels of aneuploidy with changes in phylloxera behavior. Such chromosomal changes have been found in aphids and have been associated with the evolution of insecticide resistance (Blackman and Takada 1975; Blackman 1987). The objective of the study presented here was to define the karyotypes of 6 phylloxera populations from Germany, France, Italy and California (USA). This process required the development and modification of cytogenetic techniques, and will aid future investigations into the genetic basis of phylloxera behavior and biotype development.

Material and Methods

Phylloxera material: Phylloxera originating from different European sources (Table) were raised on grape host plants using isolation chambers under controlled greenhouse conditions (16 h photoperiod, 24-26 °C temperature). Under theses conditions parthenogenetic and sexual phylloxera morphs were produced.

Chromosome preparations: Ovarioles, preoviposited eggs and sexual larvae were dissected from anholocyclic adults or *sexuparae* in Ringer's saline solution for later examination of mitotic and meiotic cell divisions. These tissues were kept in a 1 % hypotonic solution of sodium citrate for about 15 min. Ten to 15 reproductive systems were pooled, transferred into a 500 µl Eppendorf tube, and fixed with three parts of acetic glacial acid, one part of methanol (abs.) for 10 min. After centrifuging (5 min at 500-800 *g*) the supernatant was poured off and the pellet resuspended in fresh fixative. The ovary tissue was macerated passing the suspension through a syringe 3 to 5 times. Cells were spread by dropping one or two drops of the suspension on a clean microscope slide, followed by air-drying. Chromosomes were stained with DAPI (4',6-diamino-2-phenylindole: 2 $\mu g \cdot ml^{-1}$ in McIlvaine's buffer, pH 7) after a protocol published by Manicardi *et al.* (1997) with minor modification, air-dried and examined through episfluorescent filter sets (Zeiss 01). For whole body preparations

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Table

Populations and specimens used to examine phylloxera chromosomes. All populations were maintained under greenhouse culture on their original hosts

Geographical origin	Original host plant	Specimen analyzed
Bingen/Germany	Teleki 5C	Parthenogenetic ovaries
Gundelsheim/Germany	Teleki 5C	Parthenogenetic ovaries
Caprino di Veronese/Italy	Teleki 5C	Parthenogenetic ovaries and eggs, ovaries from *sexuparae*, sexual larvae
Montpellier/France	C3309	Parthenogenetic ovaries and eggs
California/USA	Cabernet Sauvignon	Parthenogenetic ovaries and eggs, ovaries from *sexuparae*,sexual larvae

adult parthenogenetic phylloxera were placed on a microscope slide, mounted in a drop of dd H_2O, squashed with a coverslip, and stained with a 0.01 % 3,6-bis (dimethylamino) acridine solution and examined as described above.

Results and Discussion

Phylloxera female reproductive system: Phylloxera ovarioles are telotrophic like those commonly found in Hemiptera (Fig. 1 A, B). The number of ovarioles per adult female ranges from 9 to over 50 in fundatrices (females that initiate the first parthenogenetic generation of the season), the number being influenced by host and seasonal time (MAILLET 1957 b).

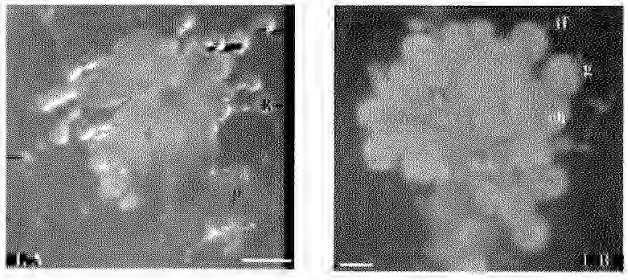

Fig. 1: Dissection of reproductive system of an adult parthenogenetic phylloxera in dorsal view. **A** Vital squash preparation prior to staining; **B** Stained with acridine orange. g: germarium; p: phylloxera; tf: terminal filament; ch: chorion. Bar represents 100 µm.

Karyotypes: Karyotypes of parthenogenetic females from all 1998 collections were 2n = 10 (Fig. 2). Phylloxera chromosomes are similar to those of other Hemiptera; they are holocentric with no localized centromer. This makes condensed chromosomes in late prophase and metaphase appear as simple rods. Four pairs of autosomes and one pair of sex chromosomes could be observed, as reported in previous studies (MAILLET 1957 a). These 10 chromosomes could be divided into two classes: one pair of large chromosomes comprising an appendix similar to a chromosomal satellite, and 4 pairs of smaller chromosomes.

Endomeiotic recombination (COGNETTI 1961) was not observed during the mitotic division cycle in parthenogenetic phylloxera (Fig. 3 A, B). One karyotype containing 2n = 9 chromosomes was found in somatic cells of what appeared to be male sexual phylloxera. MAILLET (1957 a) described phylloxera sex cells and found ova with n = 5 chromosomes and spermatids with n = 4. Spermatids were not observed but ova with n = 5 and n = 6 chromosomes were frequently found (Fig. 3 C, D). These cells had one additional chromosome that paired with one of the smaller autosomes. Supernumerary chromosomes have been described in aphids where they are termed B-chromosomes and do not affect the phenotype (BLACKMAN 1976). These B-chromosomes are usually shorter than A-chromosomes and do not pair with the standard chromosomes. The extra chromosome that we detected in ova cells did not meet the requirements in regard to morphology for B-chromosomes and thus the role of these aneuploidic cells remains unclear and requires further work.

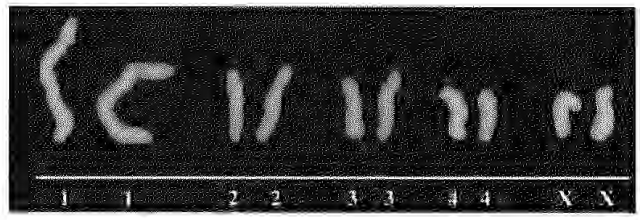

Fig. 2: Karyotype for parthenogenetic phylloxera (2n = 8 + XX).

Tetra- and polyploid cells derived from endomitosis were found in ovary cells of parthenogenetic females and are likely trophocytes that nurture developing oocytes (Fig. 3 E). Endopolyploidy is a common occurrence in aphids and widespread in somatic tissues like salivary glands, fat body tissue or trophycytes of oviparous females (BLACKMAN 1987). Multiple crossing over events were observed during diakenisis of meiosis II (Fig. 3 F).

Conclusion

The cytogenetic techniques utilized here produced suitable chromosome spreads for karyotype analysis. The karyotype for parthenogenetic phylloxera was 2n = 10. Karyotypes from haploid sex cells were found to vary between n = 5 and n = 6. Endomeiosis was not observed. Polyploid cells were

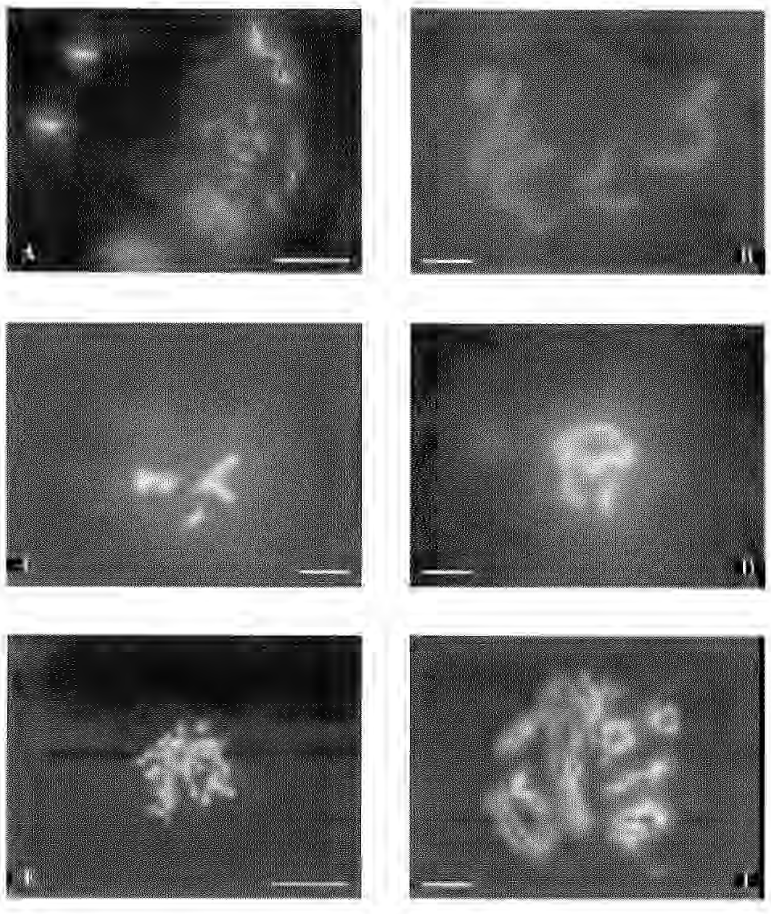

Fig. 3: Phylloxera chromosomes displaying various karyotypes (A-F). **A** Partial mitotic cell division cycle of phylloxera: prometaphase, metaphase, early and late telophase; **B** Karyotype of a parthenogenetic female (2n = 10); **C** Karyotype of a sexual phylloxera's ovum (n = 4 + X); **D** Karyotype of a sexual phylloxera's ovum with additional chromosome (n = 4 + A + X); **E** Tetraploid cell derived from trophocytes; **F** Multiple chiasmata in diakenisis during meiosis in a sexual phylloxera ovum. Bar represents 100 µm.

detected in somatic trophocytes. The successful preparation of phylloxera sex and somatic cells for chromosomal analysis reported here is now being used to study genetic variation and its association with feeding behavior on a chromosomal scale.

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