

Chromosome studies in southern Brazilian wheat pest aphids *Sitobion avenae*, *Schizaphis graminum*, and *Methopolophium dirhodum* (Homoptera: Aphididae)

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ABSTRACT

We examined the chromosome set of the aphid species *Sitobion avenae*, *Schizaphis graminum* and *Methopolophium dirhodum* by means of conventional staining and C, NOR, *Alu*I and *Hae*III banding methods. These species are considered important pests to several plants of economic interest in Brazil. No variation was observed in the number of chromosomes of *S. avenae*, whereas there was intraspecific variation in the other two species. Interspecific differences in the response to the banding treatments were observed. Whereas these techniques allowed the identification of several *S. graminum* chromosome pairs, only the *Alu*I treatment was capable of inducing differential staining in the *M. dirhodum* chromosomes and no clear patterns emerged when the *S. avenae* preparations were treated.

INTRODUCTION

Cytogenetic studies that consider both the morphology and the chromosomal numbers of aphids can be extremely useful to the taxonomist (Blackman, 1980a,b, 1981). The ubiquitous condition of holocentrism, found in the aphid chromosomes, however, makes the proper recognition of each chromosome pair very difficult (reviews in Blackman, 1981, 1990). In this type of chromosome the centromeric activity is diffused throughout the length of the chromosomes (Blackman, 1987). As stressed later by this author (Blackman, 1990),

due to such characteristics, aphid chromosomes are difficult material for the cytogeneticist.

Great variability in chromosome number seems to be the main characteristic of these insects (Blackman, 1978, 1980, 1990; Khuda-Bukhsh and Kar, 1990; Panigrahi and Patnaik, 1991). As a result of this variability, these authors considered that aphids have not yet achieved complete stability in their chromosomal number, probably due to the holocentric nature of the latter. A considerable body of knowledge on this subject is presently available but it was obtained without using banding techniques, thus limiting their usefulness for cytotaxonomic purposes.

Recent studies such as those of Manicardi *et al.* (1991) applied C, G, *Alu*I and *Hae*III banding techniques to the chromosomes of the aphid *Megoura viciae*, which considerably improved the ability to identify chromosome pairs, especially the X, and autosomes 1 and 2.

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Until now only a single study has been performed with Neotropical pest aphids at the chromosomal level (Rubín de Celis and Ortiz, 1993), which described the chromosome number of *Chaitophorus leucomelas*, an aphid pest to the *Populus* sp. álamo (Salicaceae) crops in Lima, Peru.

In southern Brazil, *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (Linnaeus) and *Sitobion avenae* (Fabricius) are considered important pests of wheat and several other crops (Gassen, 1988). *Methopolophium dirhodum* also causes much damage to wheat strains (Gassen, 1988). These species are currently being studied using different approaches to obtain more information about their biology, and allow for planning of biological control programs.

MATERIAL AND METHODS

Samples of 35 individuals each of *S. graminum*, *S. avenae* and *M. dirhodum* were collected from leaves of the wheat strain BR-35 at the experimental fields of the Centro Nacional de Pesquisa do Trigo of the Empresa Brasileira de Pesquisa Agropecuária (CNPT-EMBRAPA) in the city of Passo Fundo (28°15'S, 52°24'W), southern Brazil in 1994.

The sample preparation for the karyotype study was performed as follows: the aphids were dissected in Ephrussi and Beadle (1936) saline solution and the embryos were kept in a hypotonic solution of colchicine for about 30 min. These embryos were then fixed in acetic acid-ethanol (1:3) for 20 min and squashed in a drop of 45% acetic acid. The cover slips were dislodged using a razor blade, after the immersion of the slides for two seconds in liquid N₂ and air dried. Conventional staining of 10 slides per species was performed with 60% acetic orcein.

For C banding we put the slides in 0.2 M hydrochloric acid for 30 min, and rinsed them in distilled water. Then, the slides were placed in 4% Ba(OH)₂ at 50°C for 1 or 2 min and transferred to 2X SSC at 60°C for 1 h and finally stained with 5% Giemsa for 15 min.

For NOR banding, the slides prepared as previously mentioned remained at 4°C for 24 h. Then, the coverslips were removed after immersion in liquid N₂, and the slides remained in a fixation solution of metanol:acetic acid (1:6) for 15 min, were air dried and submerged in 50% silver nitrate for 3 h at 37°C. The slides were stained with 5% Giemsa and mounted with Entellan Merck.

For *Alu*I and *Hae*III banding, the restriction enzymes were dissolved in the appropriate incubation buffers as supplied by Pharmacia, to provide a final

concentration of 200 units/slide, covered with a siliconized coverslip and incubated in a moist chamber at 37°C overnight as described by Manicardi *et al.* (1991). After the banding treatments the slides were stained with 5% Giemsa in buffer pH 6.8 for 10 min and mounted with Entellan Merck.

RESULTS AND DISCUSSION

The absence of localized centromeres prevents accurate recognition, especially of the small pairs of aphid chromosomes, because they have the appearance of rods or dots, except in those rare cases where it is possible to see consistent constrictions in the X chromosomes such as those of *Aphis idaei* and *Acyrtosiphon rubi* (Blackman, 1980a).

When we analyzed the conventionally stained slides of our samples of *S. avenae*, the regularity in the chromosome number appeared clearly. In fact, after the observation of at least 8 metaphase plates of 35 individuals of this species we did not find any variation. Thus, in Figure 1, we show the female karyotype of *S. avenae* based on our observations. This karyotype consisted of one long pair (probably the sexual pair, by analogy with other aphid species), which presented a slight constriction, indicated by an arrowhead, three medium and five short chromosome pairs. The chromosome number found ($2n = 18$) is in accordance with that reported by Blackman and Eastop (1985), but it is not the same observed by Kapoor and Gautam (1994). In the samples of the latter authors it is mentioned that the *S. avenae* complex has $2n = 12$. The chromosome morphology observed by us and by the latter authors are similar in respect to the single long pair and to the three medium pairs. However, we observed five small pairs against the only two pairs re-

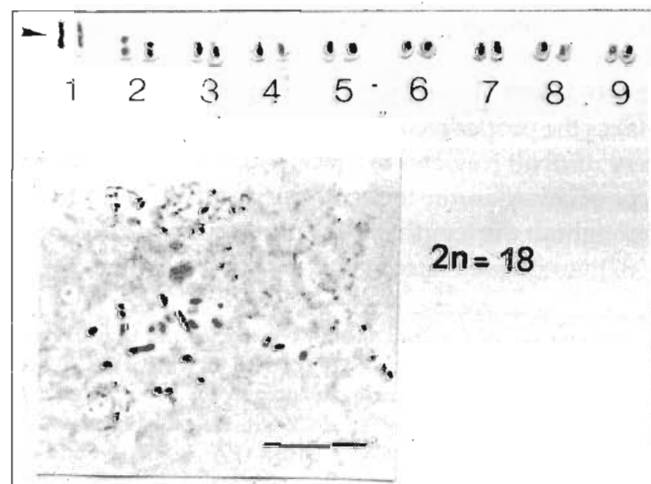


Figure 1 -Karyotype of *Sitobion avenae*. The arrowhead shows a constriction in pair 1. Bar corresponds to 10 μ m.

ported by those authors. Nevertheless, it is possible that these authors observed other members of *Sitobion avenae* complex such as: $2n = 12$ in *Sitobion sp.* and $2n = 18$ in *S. rosaeiformis* and *S. nisconthi*.

In *Sitobion avenae*, the *AluI* and *HaeIII* digestions did not promote distinct bands in any of the 25 slides treated with both restriction enzymes. As a consequence, no improvement could be added to the resolution of its karyotype as done by Manicardi *et al.* (1991) to *Megoura viciae*. Although technical problems that might be responsible for the failure to detect bands cannot be ignored, these findings may otherwise be the result of a lack of AT and GC target sites in the chromosomes of this species. Manicardi *et al.* (1991) observed great variation (from very low to almost complete digestion) in the chromosomes of *M. viciae* by the same enzymes, suggesting that these results may also be the consequence of different kinds of topological chromatin organization in such organisms. Furthermore, no clear responses to C and NOR banding treatments were obtained in the chromosomes of *S. avenae*.

The analysis of the *S. graminum* chromosomes as a rule revealed a chromosome number of $2n = 8$, as previously reported by Blackman and Eastop (1985). However, greater variation in such chromosome numbers was observed in the metaphase plates, after the screening of 25 individuals of this aphid, ranging from $2n = 6$ to $2n = 8$.

Such number variations found in several insects appear to be common, but, as stressed by White

(1973), the most frequently occurring number in a group can be considered the "type number". That probably occurred in our material. Blackman (1980a, 1981) suggests that these differences in aphid chromosome numbers may be due to dissociations or fusions involving elements of the normal diploid set, or to the presence of supernumerary B chromosomes.

Our observations of the metaphase plates of *S. graminum* by using *AluI*, *HaeIII*, C and NOR banding procedures besides conventional staining do not enable us to suggest the cause of the variation encountered. But, despite such number variation, several conspicuous morphological characteristics of the chromosomes of *S. graminum* emerged, when the metaphase plates from slides subjected to the different treatments were analyzed. For instance, clear primary constrictions were observed in the chromosomes of this aphid (Figure 2a,b,e). In Figure 2, we can also see metaphase plates with $2n = 8$ (Figure 2a,c) and $2n = 6$ (Figure 2b). The response to the different treatments allows us to identify certain particularities of the pairs.

When subjected to *HaeIII* digestion the two higher chromosomes showed terminal bands (Figure 2a), similar to those obtained by C banding, in which one additional intercalary band was observed in one of the pairs (Figure 2b). *AluI* digestion produced a conspicuous banding pattern only in one of the higher chromosomes (Figure 2e) that lies in one intercalary band.

Manicardi *et al.* (1991) also observed such patterns in *M. viciae* in a single pair that was indicated as the X chromosome. Those authors found in *M. viciae* results of C, *AluI* and *HaeIII* banding patterns similar to those verifiable in *S. graminum* and by comparison of chromosome morphology, length and response to the treatments, it is possible that the chromosomal pair banded by *AluI* of *S. graminum* also corresponds to the X chromosome.

Finally the response to the NOR banding treatment revealed only a single chromosome pair banded (Figure 2d) in *S. graminum*. A more precise definition of each chromosomal pair of *S. graminum* as a result of all the banding treatments,

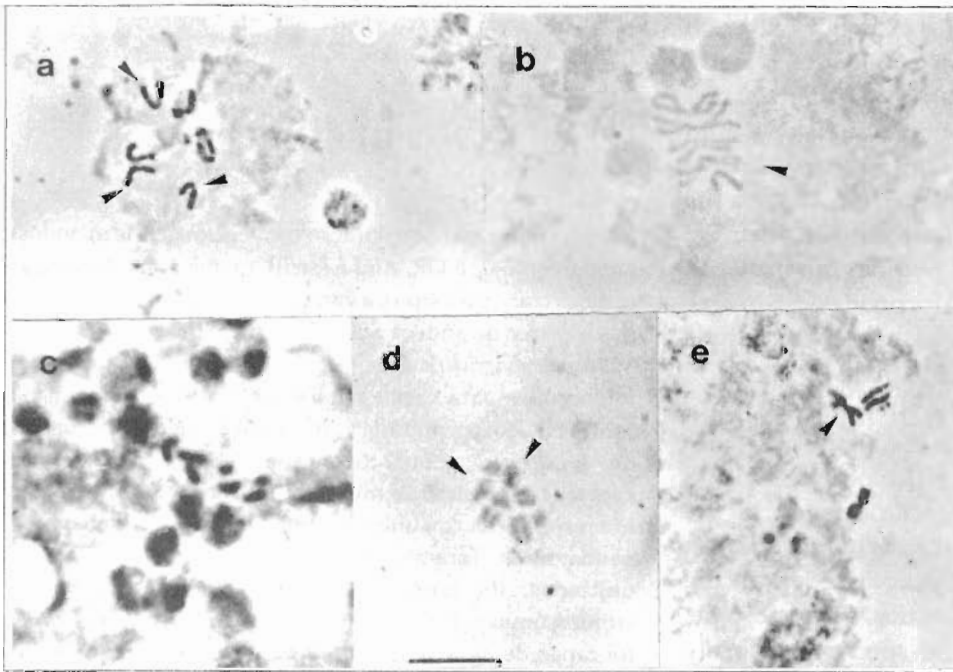


Figure 2 - Metaphase plates of *Schizaphis graminum*: (a) *HaeIII* banded chromosomes ($2n = 8$); (b) C banding ($2n = 6$); (c) acetic orcein staining ($2n = 8$); (d) NOR banding ($2n = 8$); (e) *AluI* banding. Arrowheads show the conspicuous bands obtained by each treatment. Bar corresponds to 10 μm .

however, was limited by the variation in the chromosomal number found.

A certain variation in the chromosomal number was already observed in the metaphase plates of 10 female individuals of *Methopolophium dirhodum*. In Figure 3, we can see that both $2n = 18$ (Figure 3a,c) and $2n = 16$ (Figure 3b,d) chromosomal sets were encountered.

For this species, Blackman and Eastop (1985) reported the chromosomal number of $2n = 18$, but for other species of the same genus, such as *M. festucae* (Theobald), and *M. festucae cerealium* Stroyan and *M. friscum* (Hille Ris Lambers), the $2n = 16$ chromosomal set was recorded by Blackman (1980a). Variation in the chromosomal number within a single aphid species, however, is a very common fact as reviewed by Blackman (1980b). According to this author, a higher variation of the karyotype within aphid species is expected than in other insects, due to their holocentrism. Thus, if the centromeric activity of these chromosomes is dispersed along its full length, broken chromosomal fragments are still capable of segregating at mitosis (Ris, 1942). Blackman (1980a), however, emphasized that this is no strong evidence that such damaged chromosomes remain stable. He already considered that the thelytokous reproduction of the aphids is a factor that allows karyotype variation within populations of the same species. This reproductive strategy, according to Blackman (1980a), allows the rearranged chromosomes to surpass mitosis, attaining high frequencies in apomictic populations prior to being selected in the meiotic process. Similar considerations on chromosomal variation in aphids were also made by Panigrahi and Patnaik (1991).

The *AluI* treatment in this species only produced differential staining in the biggest chromosome pair (arrowhead in Figure 3d). No clear *HaeIII*, *C* and *NOR* banding patterns were obtained in the metaphase plates of female individuals of *M. dirhodum*, but one chromosome pair is distinguished from the others. It probably corresponds to the X chromosome, by analogy with the other aphid species previously mentioned. This assumption, however, needs to be confirmed by future studies.

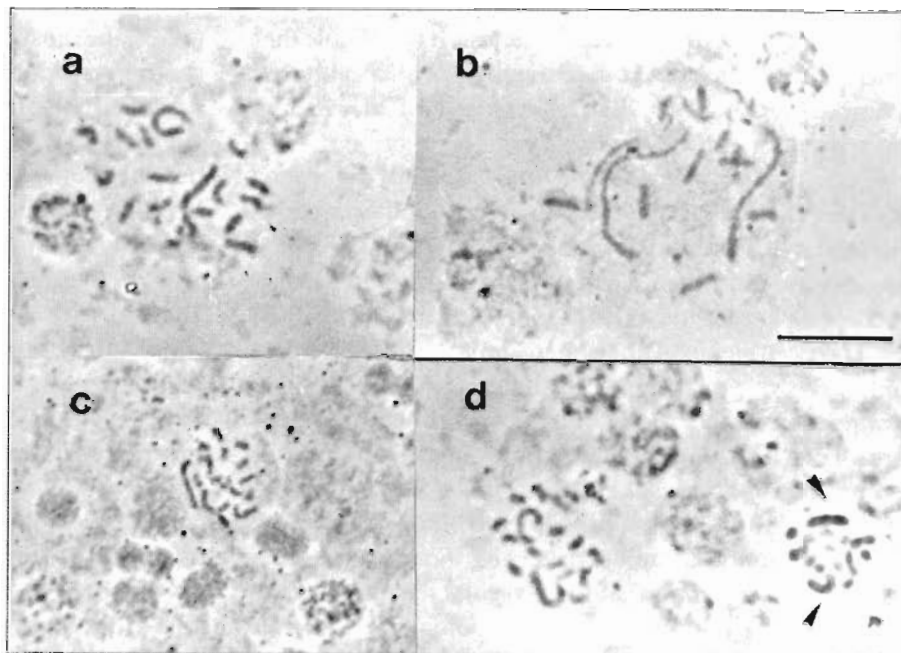


Figure 3 - *Methopolophium dirhodum* metaphase plates: (a) acetic orcein staining ($2n = 8$); (b) acetic orcein staining ($2n = 16$); (c) acetic orcein staining ($2n = 18$); (d) *AluI* digestion and Giemsa staining ($2n = 16$). Arrowheads show the differentially stained chromosome pair. Bar corresponds to 10 μm .

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RESUMO

Por meio de coloração convencional e de métodos de bandamento *C*, *NOR*, *AluI* e *HaeIII*, foi feita uma tentativa de caracterizar o cariótipo e a variação no número cromossômico das espécies de afídeos *Sitobion avenae*, *Schizaphis graminum* e *Methopolophium dirhodum*, que são considerados como pragas importantes para várias plantas de interesse econômico no Brasil. Não foi encontrada variação no número cromossômico de *S. avenae*, enquanto que as outras duas espécies apresentaram variação numérica intra-específica. Diferenças interespecíficas quanto à resposta aos tratamentos de bandamento foram observadas. Através dos métodos utilizados, foi possível a identificação de vários pares cromossômicos de *S. graminum*, mas só o tratamento com *AluI* foi capaz de induzir coloração diferencial nos cromossomos de *M. dirhodum*, enquanto que nenhum padrão claro de bandamento apareceu nos preparados de *S. avenae*.

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