Cytogenetic analysis on the gall generation of two aphid species: *Tetraneura nigriabdominalis* and *T. ulmi*

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SUMMARY - We carried out a comparative cytogenetic study on gall-forming generations of *Tetraneura nigriabdominalis* and *Tetraneura ulmi* using NOR and C-banding followed by Giemsa, Chromomycin A3 (CMA3) and DAPI staining reactions in order to improve our knowledge of these species and better define their identification. In both species, C-banding revealed that heterochromatin was not equilocated on each chromosome. Moreover, in *T. nigriabdominalis* the C-banding technique identified B chromosomes. The different response to the CMA3 and DAPI staining reactions after C-banding points out heterogeneity of the heterochromatic DNA composition in both species. Moreover, both CMA3 and silver staining revealed heteromorphism of NORs.

INTRODUCTION

The bulk of the entomological literature refers to studies carried out on leaf-dwelling aphids (BLACKMAN 1987). Instead, data on gall-forming and root-dwelling aphids is scanty, even though these are among the most important parasites of crops (such as *Anuraphid 'maidisradicis* for wheat, *Eriosoma lanigerum* for apple trees, *Pemphigus bursarius* on lettuce). Many gall-forming and root-dwelling species belong to the genera *Pemphigus* and *Tetraneura*. The knowledge of these species remains rather limited. For example, among the *Pemphigus* species, we only have a partial knowledge of their life cycle, and the secondary hosts of most of them remain an enigma (BLACKMAN and EASTOP 1994). Thus, the systematics of this genus is a best uncertain.

The genus *Tetraneura* Hartig includes about thirty species, of which at least seven alternate hosts between galls on *Ulmus* and roots of Gramineae (BLACKMAN and EASTOP 1994). Cohabitation of different species on the same secondary host plant makes identification even more problematic. Within this genus, the species *T. nigriabdominalis* is known for Southern Europe, Japan, Korea and the eastern United States. It is widely represented in Italy. In the past *T. akinire* and *T. nigriabdominalis* were frequently con-
founded. After Hille Ris Lambers (1970) the sub genus *Tetraneuretta* was erected, and the two species were separated. On the contrary, *Tetraneura akinire* Sasaki is now thought to be a synonym of *Tetraneura nigriabdominatis* (Blackman and Eastop 1994). This reflects a persisting uncertainty in the identification of these species and the incomplete information available in the literature.

Lacking significantly different morphological characters, cytogenetic studies may prove particularly efficacious for distinguishing neighbouring species. Gautam et al. (1993a) and Manicardi and Gautam (1994) performed cytogenetic studies on a root population of *T. nigriabdominatis*.

This paper aims to broaden the cytogenetic knowledge on specimens of *T. nigriabdominatis* from gall-forming generation. Moreover, the study was also extended to gall-forming animals of *T. ulmi*; with the aim of underscoring the role of the cytogenetic approach as a tool for helping distinguish species that cohabitate on the same host and often present similar morphological features.

MATERIALS AND METHODS

Specimens of *Tetraneura nigriabdominatis* (Sasaki) and *Tetraneura ulmi* (L.) were collected from the galls on *Ulmus campestris* trees in Modena (Italy), near the University Campus. Fundatrices were dissected in Ringer's saline solution, and the embryos were immersed in a 1% hypotonic solution of sodium citrate for about 10 min. The embryos were then fixed in methanol-acetic acid (3:1) for a few seconds and then immediately squashed in a drop of 45%, acetic acid. The coverslip was dislodged from the preparation, and the slides were air dried at room temperature.

C-banding was performed according to Sumner (1972). After treatment, some slides were stained with 5% Giemsa solution in Soerensen buffer, pH 6.8 for 10 min. Chromomycin A, (CMA₃) staining was performed according to Schweizer (1976). DAPI treatment was carried out according to Donlon and Magenis (1983). Silver staining of NORs was performed following the technique of Howell and Black (1980).

RESULTS

Analysis of a large number of mitotic plates of *T. nigriabdominatis* showed a variable chromosome number ranging from $2n=13$ to $2n=19$, with a prevalence of plates having $2n=18$, which must be considered the modal complement number for parthenogenetic females of this population (Fig. 1A, B). Male metaphase showed a constant lack of one of the second long pair of chromosomes, which we identified as an X chromosome (Fig. 1C).

C-banding technique applied to *T. nigriabdominatis* mitotic chromosomes revealed the presence of C-positive regions located on the two longer chromosome pairs (Fig. 1D).
An intercalary band was observed on the longest autosomal pair (A1). The second longest pair of chromosomes, the sex chromosomes, presented a heterochromatic band located at one of the two telomeric ends. Moreover, a pair of dot-shaped chromosomes was strongly heterochromatic (Fig. 1D). The
other autosomes did not show signs of C-banding. Identification of the sex chromosomes and homologues of some pairs of autosomes allowed us to reconstruct the karyotype (Fig. 1E). The intercalary band which appeared on the A1 chromosome pair after C-banding treatment and Giemsa staining also appeared after C-banding and DAPI staining (Fig. 1F), whereas C-banding and CMA3 staining of chromosomes identified only one X telomere, which appeared as a brightly fluorescent zone (Fig. 1G). Moreover, whereas CMA3-positive heterochromatin was clearly appreciable in C-banded untreated control preparations (Fig. 1H), DAP heterochromatin did not appear in untreated chromosomes which were uniformly fluorescent (Fig. 1H). CMA3 staining also revealed heteromorphism between sex chromosomes, with one of the GC-positive zones consistently more fluorescent than the other. The results of silver staining were similar to those obtained with Chromomycin A3 (Fig. 1J).

The chromosome number of *T. ulmi* ranged from $2n=14$ to $2n=16$ (Fig. 2A, B). C-banding and Giemsa staining revealed a clear intercalary band on the X chromosomes, which could be divided into two sub-bands (Fig. 2C). The A1 chromosome pair presented two bands, each located at the opposing subtelomeric regions. One of these bands generally stained more densely than the other, and the lightly stained band was sometimes nearly indistinguishable. None of the other autosomes showed C-banding. In *T. ulmi* all the C-positive bands that stained with DAPI corresponded to those stained with Giemsa (Fig. 2E), except the heterochromatic band located on one X telomere, which was brightly fluorescent after CMA3 treatment (Fig. 2F). The same X telomere resulted also strongly positive after silver staining (Fig. 2I). As previously observed in *T. nigriabdominalis* a certain degree of NORs heteromorphism was observed between homologous X telomeres also in *T. ulmi* (see Fig. 2F and 2I).

**DISCUSSION**

The variability in chromosome number together with the holocentric nature of aphid chromosomes make this taxon a subject of great cytogenetic interest. Currently, more than 4,000 species of aphids have been described, but the chromosome number has only been reported for about 20% of these (GAUTAM et al. 1993).

The main objective of our study was to perform a cytotaxonomic and cytogenetic analysis of the chromosomes of *T. nigriabdominalis* and *T. ulmi*, which could contribute to improve the knowledge of these species and better their identification. Within the genus *Tetraneura* (as in other genera of the
family Pemphigidae), species identification remains a problem, and, as retained by BLACKMAN and EASTOP (1994), cytotoxic data can be useful for revising this genus.
In *T. nigriabdominalis*, we found a variable diploid chromosome number ranging from 2n=13 to 2n=19, with a net prevalence of 2n=18 mitotic plates, which represent the typical diploid chromosome number of the species reported in the literature (BLACKMAN and EASTOP 1994). Instead, in *T. ulmi* we found a chromosome number ranging from 2n=14 to 2n=16, whereas for this species the literature reports a value of 2n=14 (BLACKMAN and EASTOP 1994). C-banding treatment allows definitive identification of at least a part of the homologous chromosomes and this improves the construction of nonambiguous karyotype. Indeed, h010centric chromosomes lack primary conscription, whereas attempts to identify homologous chromosomes based only on their length can be misleading due to the phenomenon of allocyclia.

In both species, C-banding revealed that heterochromatin was not equilocated on each chromosome. This pattern, observed in all the aphid species cytogenetically studied to date, (MANICARDI et al. 1991a, b, 1992, 1996, 1998a; BIZZARO et al. 1998) could be considered typical of h010centric chromosomes and differs from that observed on monocentric chromosomes, where the heterochromatic regions typically occupy specific zones of all chromosomes, corresponding to centromeres or sometimes telomeres (SCHWEIZER and LOIDL 1987).

An interesting element emerging from C-banding studies in the population of *T. nigriabdominalis* was a pair of strongly heterochromatic dot-shaped chromosomes. Based on that feature, these chromosomes must be considered B chromosomes. To date, reports of B chromosomes in aphids are rare (BLACKMAN 1976, 1988, 1990). In particular, BLACKMAN (1976, 1988) describes an X chromosome / B chromosome system in the genus *Euceraphis*, where 'B' chromosomes are maintained at a constant number and transmitted through the male germinal line. The author suggests that these B chromosomes may represent a non-functional part of the X chromosome system. In light of this hypothesis, the finding of heterochromatic B chromosomes in the complement of *T. nigriabdominalis* could give rise to the small accumulation of heterochromatin on its sex chromosomes, contrasting with that in other aphid species studied to date (MANICARDI et al. 1991b, 1996, 1998a; BIZZARO et al. 1998).

The C-banding pattern of *T. nigriabdominalis* reported by MANICARDI and GAUTAM (1994) differs from that described in this paper for the same taxon, whereas it overlaps that described here for *T. ulmi*. These findings may be explained by considering that the specimens utilized in the previous report (MANICARDI and GAUTAM 1994) were obtained from root generations, for which exact species identification is complicated by a frequent mix with individuals from other species, in particular with *T. ulmi* with which it shares the secondary host plant.

In light of the present results, unequivocal since obtained on animals collected from the specific and unmistakable galls of the two species, we can...
affirm that the previous data published in MANICARDI and GAUTAM (1994) should be attributed to *T. ulmi*. The appreciable difference in C-banding between the two species reported here underscores the importance of C-banding as a diagnostic tool, particularly useful when traditionally distinctive characters do not allow precise species identification.

The different response to CMA3 and DAPI staining after C-banding observed in both species, points out the heterogeneity of heterochromatic DNA Composition. In fact, GC-rich NOR-associated heterochromatin differs from other heterochromatic bands that are characterized by AT-rich DNAs. This pattern of heterochromatin heterogeneity seems to be a general characteristic of aphid chromatin since it has been described in all species investigated at cytogenetic level (MANICARDI *et al*. 1996, 1998a; BIZZARRO *et al*. 1998).

Silver staining of mitotic chromosomes of both *T. nigriabdominalis* and *T. ulmi* reveals two dots located on one telomere of each sex chromosomes. This seems to be a highly conservative characteristic of aphid chromosomes in that the same NOR location has been described in almost all species of aphids studied to date (KUZNETSOVA and GANDRABUR 1991; MANICARDI *et al*. 1992, 1996, 1998b; BLACKMAN and SPENCE 1996).

Chromomycin A3 is a fluorochrome that preferentially forms stable complexes with double helix DNA rich in G-C sequences (BEHR *et al*. 1969). The convergence between CMA3 and AgNO3 positive regions in *T. nigriabdominalis* and *T. ulmi* can probably be attributed to a high G-C content in the regions coding for the 18S and 28S subunit or perhaps in the intergenic spacers (RITossa and SPIEGELMAN 1965; WALLACE and BIRNSTIEL 1969). NOR- and CMA3-positive regions are also known to converge in other plants (SCHWEIZER 1976; LOZANO *et al*. 1990), invertebrates (SCHWEIZER *et al*. 1983; GOSALVEZ *et al*. 1987; LOPEZ FERNANDEZ *et al*. 1989) and vertebrates (SCHMID 1980; MAYR *et al*. 1978). However, we should point out that, given the specificity of the silver nitrate stain for acid proteins associated with newly transcribed rRNA and not for rDNA, only the NOR transcriptionally active in the preceding interphase are stained with AgNO3 (HOWELL 1977; SCHWARZACHER *et al*. 1978). Instead, CMA3 determines a positive fluorescence that is independent of the activity of the nucleolar organizing region (SCHMID and GUTTENBACH 1988). Since all the mitoses observed in the two species being studied were positive to both CMA3 and silver staining, we can conclude that all the NORs had undergone synthetic activity in the preceding interphase. Indeed, these treatments were carried out in rapidly proliferation embryonal cells. Moreover, since an appreciable level of heteromorphisms between homologous NORs has been observed after both CMA3 and AgNO3 staining it is possible to hypothesize that the observed heteromorphism may be due to different distributions of rDNA genes between homologues and not to different levels of transcriptional activity. The NORs heteromorphism occasionally reported for several organism (SCHMID 1982; SANCHEZ *et al*. 1989; SUZUKI *et al*. 1990; ZURITA *et al*.).
1997), has been repeatedly described in different aphid species (BLACKMAN and SPENCE 1996; MANICARDI et al. 1998).

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