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Karyotype variations in Italian populations of the peach-potato aphid *Myzus persicae* (Hemiptera: Aphididae)

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Abstract

In this study, we present cytogenetic data regarding 66 *Myzus persicae* strains collected in different regions of Italy. Together with the most common $2n=12$ karyotype, the results showed different chromosomal rearrangements: $2n=12$ with A1–3 reciprocal translocation, $2n=13$ with A1–3 reciprocal translocation and A3 fission, $2n=13$ with A3 fission, $2n=13$ with A4 fission, $2n=14$ with X and A3 fissions. A $2n=12$–13 chromosomal mosaicism has also been observed. Chromosomal aberrations (and in particular all strains showing A1–3 reciprocal translocation) are especially frequent in strains collected on tobacco plants, and we suggest that a clastogenic effect of nicotine. Further benefited by the holocentric nature of aphid chromosomes, could be at the basis of the observed phenomenon.

Keywords: karyotype variations, chromosomal rearrangements, holocentric chromosomes, nicotine, clastogenic effect, *Myzus persicae*, Aphididae

(Accepted 27 March 2012)

Introduction

Classical and molecular cytogenetics provide an integrated approach for structural, functional and evolutionary analyses of chromosomes. This ranges from karyotype analyses to molecular mapping of chromosomes.

To date, studies concerning chromatin structure and organization have been mainly focused on eukaryotes having monocentric chromosomes, whereas species possessing holocentric/holokinetic chromosomes have been rather neglected. Chromosomes with diffused centromeric activity have been found in Protista, as well as in plant and animal species (Wrensch *et al.*, 1994). The chromosomes of aphids, like those of other hemipteran insects, have diffuse centromeres so that kinetic activity is dispersed along the entire length of each chromatid at least in mitotic divisions, thus influencingchromosome behaviour (White, 1973). In organisms possessing this kind of chromatin organization, chromosome fusions and fissions can occur without any duplication or loss of centromeres. This has consequences for the survival of the *de novo* chromosomal changes through mitosis and meiosis, and hence for karyotype evolution. Autosomal fusions and fissions, particularly the latter, seemed to play a pivotal role in aphid karyotype evolution (Blackman, 1980), although this view is at present somewhat speculative due

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Table 1. List of the Italian populations of *M. persicae* analyzed.

Northern Italy				Central-southern Italy			
Population	Chromosome number	Color	Host plant	Population	Chromosome number	Color	Host plant
Torino 1	12	G	Peach	Pisa 1	12	G	Peach
Torino 2	12	G	Peach	Pisa 2	12	G	Peach
Torino 3	12	G	Peach	Pisa 3	12	G	Peach
Torino 4	12	G	Peach	Ascoli 1	12	G	Peach
Cuneo 1	12	G	Peach	Pescara 1	12	G	Tobacco
Como 1	12	G	Peach	Pescara 2	12t + frm3	R	Tobacco
Lodi 1	12	G	Peach	Chieti 1	12t	R	Tobacco
Padova 1	12	G	Peach	Chieti 2	12t	R	Tobacco
Padova 2	12	G	Peach	Chieti 3	12t	R	Tobacco
Piacenza 1	12	G	Tomato	Chieti 4	12t	R	Tobacco
Piacenza 2	12	G	Tomato	Salerno 1	12t	R	Tobacco
Piacenza 3	12	G	Tomato	Salerno 2	12/13 frm3	R	Tobacco
Piacenza 4	12	G	Tomato	Salerno 3	13 frm3	R	Tobacco
Piacenza 5	12	G	Tomato	Benevento 1	12t	R	Tobacco
Piacenza 6	12	G	Peach	Cosenza 1	13 frm 4	G	Peach
Piacenza 7	12	G	Peach	Cosenza 2	14 frm X + 3	G	Peach
Piacenza 8	12	G	Peach	Catanzaro 1	12	G	Potato
Piacenza 9	12	G	Aubergine	Catanzaro 2	12	G	Peach
Piacenza 10	13 frm 4	G	Aubergine	Cagliari	12	G	Peach
Piacenza 11	12	G	Peach				
Bologna 1	12	G	Peach				
Bologna 2	12	G	Peach				
Bologna 3	12	G	Peach				
Bologna 4	12	G	Peach				
Bologna 5	12	G	Peach				
Bologna 6	12	G	Peach				
Ferrara 1	12	G	Peach				
Ferrara 2	12	G	Peach				
Ferrara 3	12	G	Peach				
Ferrara 4	12	G	Peach				
Ferrara 5	12	G	Peach				
Ferrara 6	12	R	Peach				
Ferrara 7	12	G	Peach				
Ravenna 1	12	G	Peach				
Ravenna 2	12	G	Peach				
Ravenna 3	12	G	Peach				
Ravenna 4	12	G	Peach				
Ravenna 5	12	G	Peach				
Ravenna 6	13 frm 4	G	Peach				
Ravenna 7	12	G	Peach				
Ravenna 8	12	G	Peach				
Ravenna 9	12	G	Peach				
Ravenna 10	12	R	Peach				
Ravenna 11	12	G	Peach				
Ravenna 12	12	G	Peach				
Forlì 1	13 frm 3	G	Peach				
Forlì 2	12	G	Peach				

to a lack of knowledge concerning the mechanisms involved in rearrangements of the holocentric chromosomes (Spence & Blackman, 2000).

A recurrent chromosomal rearrangement found in the peach-potato aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) populations collected worldwide involves a A1–3 reciprocal translocation associated with increased levels of resistance to organophosphate and carbamate insecticides (Blackman *et al.*, 1978; Spence & Blackman, 1998).

The standard female karyotype of this species is $2n = 12$, but specimens with a chromosome complement of either $2n = 13$ or 14 have also been reported (Blackman, 1980; Lauritzen, 1982). On the basis of relative chromosome lengths, Blackman (1971) concluded that the $2n = 13$ karyotype raised from a break in one autosome of the pair A3, whereas a break in one chromosome of either the A2 and A3 pairs led to a $2n = 14$ karyotype. Rare cases of strain possessing $2n = 11$ and $3n = 18$ have also been reported (Blackman, 1980; Yang & Zhang, 2000). Very recently, the analysis of mitotic metaphase chromosomes of a *M. persicae* laboratory strain revealed different chromosome numbers, ranging from 12 to 17, within each embryo (intraclonal genetic variation *sensu* Loxdale & Lushai (2003)). Chromosome length measurements revealed that the observed chromosomal mosaicism is due to recurrent fragmentations of chromosomes X, 1 and 3 (Monti *et al.*, 2012).

The present study shows cytogenetic data regarding 66 *M. persicae* strains collected in different Italian regions showing several chromosomal rearrangements, the most

Fig. 1. Geographic distribution of the sampling sites.

common being the A1–3 reciprocal translocation, which we here reported for the first time in Italy. We have also looked for the presence of a relationship between karyotype variations and the host plants.

Material and methods

Myzus persicae populations were collected mainly from peach (*Prunus persicae* L.) orchards (48), but also from herbaceous hosts like tobacco (10), tomato (5), potato (1) and aubergine (2) at various locations in different areas of Italy (see table 1, fig. 1) and maintained as parthenogenetic female colonies on pea-seedlings (*Pisum sativum* cv 'Meraviglia d'Italia') under constant environmental conditions: 21°C, 16 h light:8 h dark photoperiod.

For chromosome spreads, adult females were dissected in Ringer saline solution and embryos were kept in a 1% hypotonic solution of sodium citrate for 30 min. The embryos

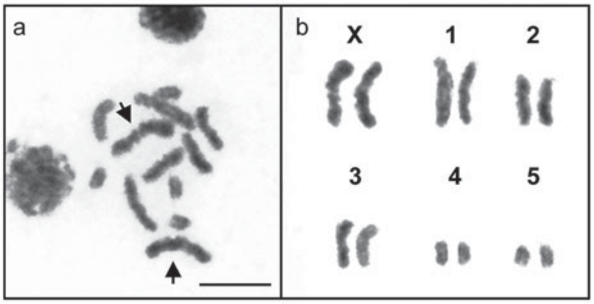

Fig. 2. Metaphase plate of the *M. persicae* strain Ferrara 03 stained with (a) Giemsa and (b) relative karyotype. Arrows indicate X chromosomes. Bar corresponds to 10 µm.

were then transferred to minitubes and centrifuged at 350 g for 3 min. Methanol-acetic acid 3:1 was added to the pellet, which was made to flow up and down for 1 min through a needle of a 1 ml hypodermic syringe to obtain disgregation of the material followed by a further centrifugation at 1000 × g for 3 min. This step was repeated with fresh fixative. Finally, the pellet was resuspended in new fixative, and 20 µl of cellular suspension was dropped onto clean slides and stained with 5% Giemsa solution in Soerensen buffer, pH 6.8 for 10 min. Silver staining of nucleolar organizing regions (NORs) was achieved following Howell & Black (1980). Slides were examined using a Nikon Eclipse 80i fluorescence microscope with UV filters, and photographs were taken using Nikon digital sight DS-U1. Morphometric analyses of mitotic plates were carried out on 30 metaphases using the software MicroMeasure, freely available at the Biology Department at Colorado State University website (http://rydberg.biology.colostate.edu/MicroMeasure). Male induction for Salerno 03, Pescara 02, Cosenza 02 and Pisa 01 strains was evaluated by exposing parthenogenetic female aphids to short photoperiods (8 h light:16 h dark) according to Crema (1979).

Results

The analysis of mitotic cells of embryos, obtained from parthenogenetic females, confirmed that $2n=12$ is the standard chromosome number in *M. persicae* (fig. 2), but 14 out of 66 strains analysed showed intraspecific karyotype variants due to both structural and numerical variations in chromosome complements (table 1, figs 3–6).

The most frequent chromosomal rearrangement found in Italian populations is related to the A1–3 reciprocal translocation, which was found either alone (fig. 3) or together with an A3 fission (in one strain; fig. 6a, b). Other chromosome fissions involved A3 (found in two cases; fig. 4) and A4 (found in three cases; fig. 5), whereas a strain possessing 14 chromosomes as a consequence of both X and A3 fissions was also found (fig. 6c, d). Lastly, we identified a strain showing an intra-individual chromosome mosaicism due to the presence of mitotic plates with 12 (24% of the observed plates) and 13 (76%) chromosomes as a consequence of an A3 fission (fig. 4b).

NOR staining (figs 3a, c, g, h and 6c) revealed the presence of heteromorphism in the size of rDNA genes in strains Salerno 3 (fig. 4c) and Cosenza 2 (fig. 6c) and evidenced that the fission of the X chromosomes observed in Cosenza 2 always occurred in the X chromosome bearing the smallest NOR-positive telomere and involved the X telomere opposite to the rDNA-bearing one (fig. 6c).

Considering the geographical distribution, it is evident that almost all karyotype variations (11 out of 14) were present in central and southern Italian regions, whereas only three were found in northern locations. Furthermore, all but one of the strains collected on tobacco showed chromosomal rearrangements; and, in particular, all the strains possessing the A1–3 reciprocal translocation were found on this plant and were red in colour.

Male induction revealed that the *M. persicae* strains Salerno 03, Pescara 02 and Cosenza 02, all possessing different kinds of karyotype variations, are anholocyclic since it was not possible to induce the sexual generation differently from that obtained under the same experimental conditions with the *M. persicae* strain Pisa 1, which showed a normal karyotype.

Discussion

The typical aphid karyotype consists of pairs of rod-like chromosomes, whose number is typically stable within a genus, as shown in the large genus *Aphis*, where the typical chromosome number is eight with the exception of *A. farinosa* with $2n=6$ (Blackman, 1980; Hales *et al.*, 1997). Nevertheless, exceptions have been published as revealed in the genus *Amphorophora*, where the chromosome number varies from $2n=4$ to $2n=72$ (Blackman, 1980).

Rearrangements most commonly involved autosomes, as shown in *M. persicae*, where, despite a standard chromosome number of $2n=12$, several strains possessing karyotypes consisting of 11–14 chromosomes have previously been reported (Blackman, 1980). On the contrary, Hales (1989) and Monti *et al.* (2012) demonstrated a complex pattern of associations and fissions occurring on both autosomes and X chromosomes in *Schoutedenia lutea* (van der Goot) (Hemiptera: Aphididae) and *M. persicae*, respectively, suggesting different scenarios for understanding aphid karyotype evolution.

The most common chromosomal variant described in *M. persicae* complement is a reciprocal translocation between the first and the third autosome pairs, leading to females with $2n=12$ karyotype showing a marked structural heterozygosity (Blackman, 1980).

The empirical data, as presented in this paper, reveal for the first time that this chromosomal aberration also occurs in Italy since seven out of the 14 strains showed karyotype variations due to the A1–3 reciprocal translocation. In view of the absence of any primary constriction, which is typical of the holocentric chromosomes, together with the lack of specific banding patterns after conventional banding procedures, we combined procedures of standard chromosome staining (such as Giemsa and silver staining) with chromosome length evaluation. In particular, we used silver staining to confirm the exclusive localization of NORs regions on X chromosome telomeres in *M. persicae* and analyzed the involvement of sex chromosomes in the translocation event (Manicardi *et al.*, 2002). Afterwards, in the absence of any other cytogenetic markers, the morphometric analysis was employed to identify autosomes A1 and A3 as the chromosomes engaged in the rearrangement.

According to the literature, a link exists between the A1–3 chromosomal reciprocal translocation and resistance to organophosphate and carbamate insecticides due to E4 gene amplification (Blackman *et al.*, 1995), perhaps involving the removal of a repressor gene away from the structural genes in

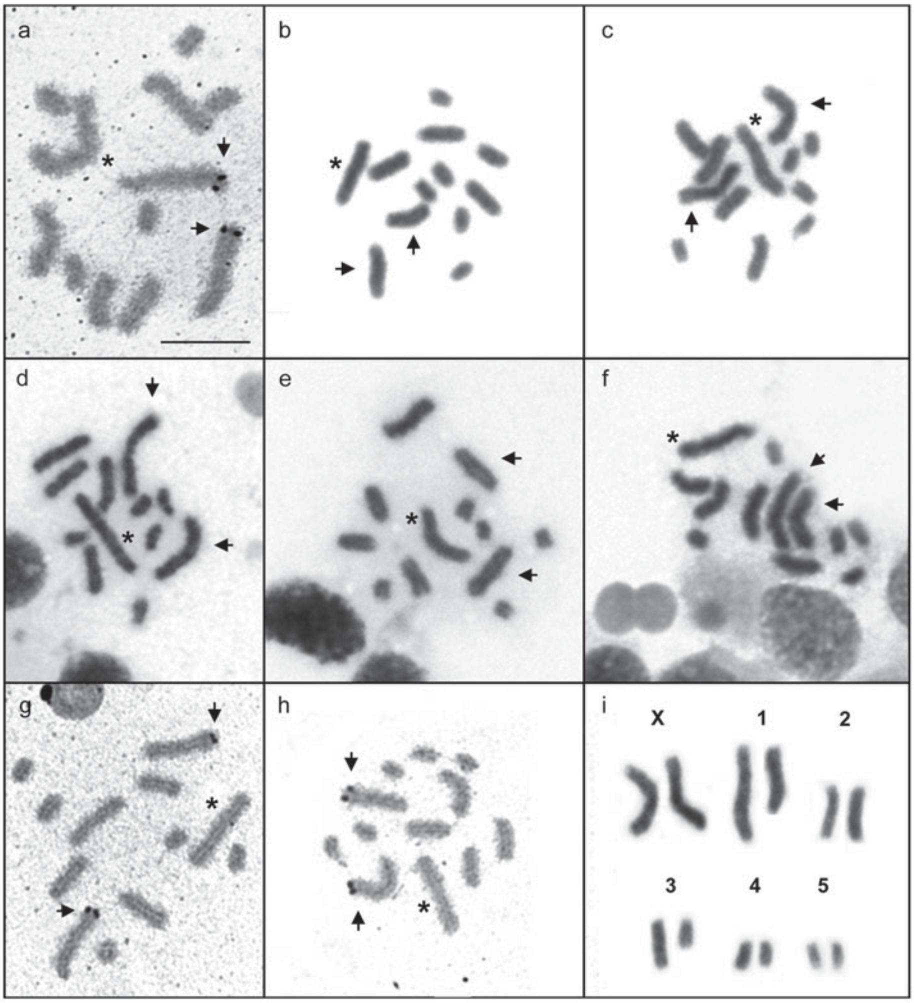

Fig. 3. *M. persicae* chromosome complements showing A1–3 reciprocal translocation. (a) Benevento 01 is silver stained, (b) Salerno 01, (c) Chieti 02 and (e) Chieti 03 are stained with Giemsa, whereas (d, g) Chieti 1 and (f, h) Chieti 4 are both Giemsa and silver stained. The (i) karyotype is derived from (c) Chieti 02. Arrows indicate X chromosomes. Asterisks indicate A1–3 translocated chromosomes. Bar corresponds to 10 μm.

controls (Blackman *et al.*, 1978). Preliminary data involving PCR and southern blot analysis revealed that, in one of the Italian populations with this chromosomal aberration (Chieti 1), the FE4 gene (electrophoretically fast variant (allele) of the normal expressed carboxylesterase 4 (E4) enzyme) only was present (Rivi *et al.*, 2009). This strain showed a moderate increase in esterase activity and was considered an S/R1 (susceptible/first resistance level) strain *sensu* Devonshire *et al.* (1992). The aforementioned data allows us to suggest that this is the first *M. persicae* strain possessing the A1–3 chromosomal reciprocal translocation linked to an FE4 and not directly related to a high level of esterase-based insecticide resistance. Experiments currently in progress are aimed to extend this experimental procedure to all Italian strains possessing A1–3 reciprocal translocations, in order to better clarify the relationships between this chromosomal rearrangement and the insecticide resistance in *M. persicae* populations.

Other fissions relatively frequent in the studied Italian *M. persicae* populations occurred at autosomes 3 and 4, whereas in one case only the fission involved the X chromosome. Different autosome fragmentations have been repeatedly described in *M. persicae* populations collected worldwide, whereas the X fragmentation has been observed only in a *M. persicae* laboratory strain characterised by an extensive chromosomal mosaicism (Monti *et al.*, 2012). In this connection, it must be emphasized that in both such cases, the X fission occurs in X chromosomes possessing a low number of rDNA genes and in the telomeric region opposite to the NORs-bearing one. The recurrent fission of the same chromosomes in the same region argues that the *M. persicae* genome possesses some fragile/labile sites that could be the basis for the observed changes in the chromosome number.

For many years, chromosome evolution has been generally explained by considering the random-breakage model (Becker & Lenhard, 2007). On the contrary, a number of comparative cytogenetic studies evidences a relationship between chromosomal rearrangements and specific chromosomal architecture and suggests a role of the repetitive DNAs in chromosome

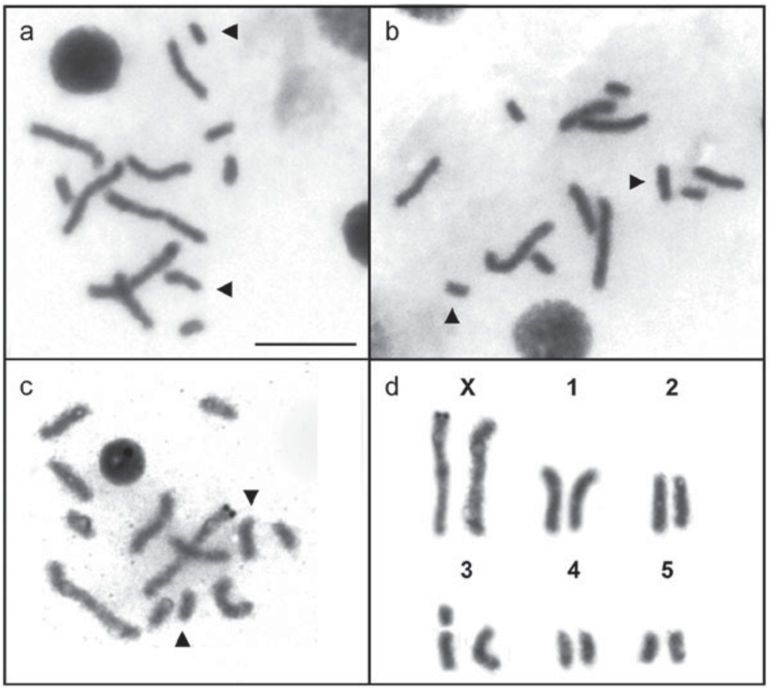

Fig. 4. *M. persicae* chromosome complements showing A3 fission. (a) Forlì 01 and (b) Salerno 02 are stained with Giemsa, whereas (c) Salerno 03 and (d) its relative karyotype are silver stained. Arrow heads indicate chromosomes involved in the fission. Bar corresponds to 10 μm.

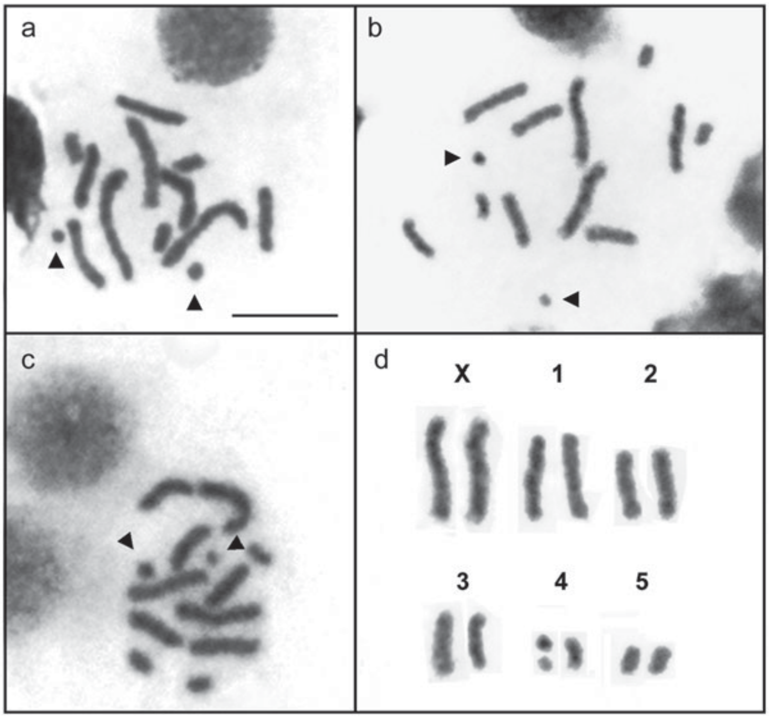

Fig. 5. Giemsa staining of *M. persicae* chromosome complements showing A4 fission: (a) Cosenza 01, (b) Ravenna 06 and (c) Piacenza 10. The (d) karyotype is derived from (b) Ravenna 06. Arrow heads indicate chromosomes involved in the fission. Bar corresponds to 10 μm.

rearrangements. The nature of the repetitive DNA within chromosomal breakpoint regions varies significantly, from clusters of rRNA and tRNA genes to simple di- and tri-nucleotide expansions (Caceres *et al.*, 1999; Carlton *et al.*, 2002; Coghlan & Wolfe, 2002; Kellis *et al.*, 2003; Renciuk *et al.*, 2011). The data reported in this paper confirmed recent

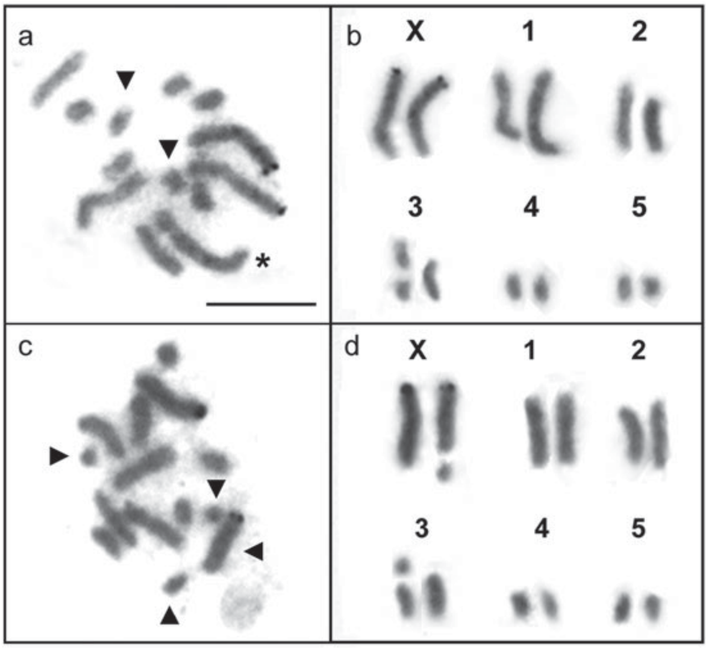

Fig. 6. (a) Pescara 02 complement stained with $AgNO_3$ and (b) relative karyotype. (c) Cosenza 02 complement silver stained with (d) relative karyotype. Arrow heads indicate chromosomes involved in the fissions. Asterisk indicates A1–3 translocated chromosomes. Bar corresponds to 10 μm.

observations regarding the recurrent fission of the same chromosomes in the same region (Monti *et al.*, 2012), allowing us to further support the hypothesis concerning the presence of fragile/labile sites in the *M. persicae* holocentric chromosomes.

Chromosomal rearrangements in aphids have been hypothesized to affect some complex phenotypic traits, such as the host plant choice (Blackman, 1987; ffrench-Constant *et al.*, 1988). For example, karyotypic variants observed in the corn leaf aphid *Rhopalosiphum maidis* (Fitch) have been associated with changes in the host choice. Similarly, an association of chromosome number with host plant has been described within the *Sitobion* genus, which shows $2n = 12$ on ferns and $2n = 18$ on grasses (Brown & Blackman, 1988; Hales *et al.*, 1997).

A peculiar example of host adaptation concerns *M. persicae* strains feeding on tobacco. Morphometric analyses of specific taxonomic markers revealed that they are distinguishable from those living on other host plant so that the tobacco-feeding form was elevated to the status of a separate species by Blackman (1987). Further molecular evidences failed to confirm the genetic isolation of the population living on tobacco (Field *et al.*, 1994; Clements *et al.*, 2000), although other data, as well as behavioural/pheromonal evidence, suggests that the two forms undergone some significant degree of ecological-evolutionary divergence (Kephalogianni *et al.*, 2002; Margaritopolous *et al.*, 2003; Blackman *et al.*, 2007).

Our data put in evidence that all but one of the strains collected on tobacco plants showed karyotype variations, whereas only four of the 56 population collected on other hosts (corresponding to about 7% of the total) displayed chromosomal rearrangements. A suggestive explanation for the observed relationships between chromosomal rearrangements and tobacco plants could rely in the clastogenic effect of nicotine.

Nicotine is a naturally occurring alkaloid found primarily in members of the solanaceous plant family, including *Nicotiana tabacum*. Several reports showed that nicotine, as a consequence of DNA replication fork stress (Richards, 2001; Freudenreich, 2005), produces genotoxic effects on Chinese hamster ovarian (CHO) cells (Trivedi *et al.*, 1990, 1993) and sister chromatid exchanges and chromosome aberrations in bone marrow cells of mice (Sen *et al.*, 1991). Extensive chromosomal rearrangements have also been described in a mice population known as 'tobacco mice' since they live close to kiln for drying tobacco (Fraguedakis-Tsolis *et al.*, 1997). In addition, DNA fragmentation by nicotine has been demonstrated both in peripheral lymphocytes (Sassen *et al.*, 2005) and in human spermatozoa (Arabi, 2004). Nicotine, together with ultraviolet exposure, has also been considered an exogenous factor which can contribute to the generation of mutations which could be at the basis of chromosomal mosaicism (De, 2011), a very rare phenomenon we have observed in Salerno 02, one of the strains collected on tobacco plants.

Even if there are no literature data analyzing nicotine effects on organisms possessing holocentric chromosomes, the previously reported data allows us to propose at least that chromosome architecture, rather than random breakages, has a pivotal role in aphid chromosome evolution and rearrangements.

The high telomerase expression, previously reported in *M. persicae* (Monti *et al.*, 2011), that stabilized chromosomes involved in fragmentations, coupled to reproduction by obligate apomictic parthenogenesis, could be at the basis of the stabilization of the observed chromosome instability on *M. persicae* strains collected on tobacco plants favouring the inheritance of the variant karyotypes.

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