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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 (Wrench *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 influencing chromosome 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				
Forli 1	13 frm 3	G	Peach				
Forli 2	12	G	Peach				

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

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

61 The standard female karyotype of this species is $2n = 12$, but
62 specimens with a chromosome complement of either $2n = 13$ or
63 14 have also been reported (Blackman, 1980; Lauritzen, 1982).
64 On the basis of relative chromosome lengths, Blackman (1971)
65 concluded that the $2n = 13$ karyotype raised from a break in

one autosome of the pair A3, whereas a break in one 66
67 chromosome of either the A2 and A3 pairs led to a $2n = 14$
68 karyotype. Rare cases of strain possessing $2n = 11$ and $3n = 18$
69 have also been reported (Blackman, 1980; Yang & Zhang,
70 2000). Very recently, the analysis of mitotic metaphase
71 chromosomes of a *M. persicae* laboratory strain revealed
72 different chromosome numbers, ranging from 12 to 17, within
73 each embryo (intraclonal genetic variation *sensu* Loxdale &
74 Lushai (2003)). Chromosome length measurements revealed
75 that the observed chromosomal mosaicism is due to recurrent
76 fragmentations of chromosomes X, 1 and 3 (Monti *et al.*, 2012).

The present study shows cytogenetic data regarding 66
77 *M. persicae* strains collected in different Italian regions
78 showing several chromosomal rearrangements, the most 79



Fig. 1. Geographic distribution of the sampling sites.

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

84 **Material and methods**

85 *Myzus persicae* populations were collected mainly from
 86 peach (*Prunus persicae* L.) orchards (48), but also from

herbaceous hosts like tobacco (10), tomato (5), potato (1) and
 87 aubergine (2) at various locations in different areas of Italy (see
 88 table 1, fig. 1) and maintained as parthenogenetic female
 89 colonies on pea-seedlings (*Pisum sativum* cv 'Meraviglia
 90 d'Italia') under constant environmental conditions: 21°C,
 91 16 h light:8 h dark photoperiod.

For chromosome spreads, adult females were dissected in
 92 Ringer saline solution and embryos were kept in a 1%
 93 hypotonic solution of sodium citrate for 30 min. The embryos
 94
 95

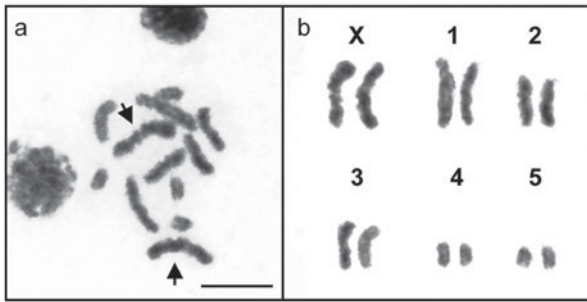


Fig. 2. Metaphase plate of the *M. persicae* strain Ferrara 03 stained with (a) Giemsa and (b) relative karyotype. Arrows indicate X chromosomes. Bar corresponds to 10 μ m.

96 were then transferred to minitubes and centrifuged at 350 g
 97 3 min. Methanol-acetic acid 3:1 was added to the pellet, which
 98 was made to flow up and down for 1 min through a needle of a
 99 1 ml hypodermic syringe to obtain disaggregation of the material
 100 followed by a further centrifugation at 1000 \times g for 3 min. This
 101 step was repeated with fresh fixative. Finally, the pellet was
 102 resuspended in new fixative, and 20 μ l of cellular suspension
 103 was dropped onto clean slides and stained with 5% Giemsa
 104 solution in Soerensen buffer, pH 6.8 for 10 min. Silver staining
 105 of nucleolar organizing regions (NORs) was achieved follow-
 106 ing Howell & Black (1980). Slides were examined using a
 107 Nikon Eclipse 80i fluorescence microscope with UV filters, and
 108 photographs were taken using Nikon digital sight DS-U1.
 109 Morphometric analyses of mitotic plates were carried out on
 110 30 metaphases using the software MicroMeasure, freely
 111 available at the Biology Department at Colorado State
 112 University website ([http://rydberg.biology.colostate.edu/](http://rydberg.biology.colostate.edu/MicroMeasure)
 113 [MicroMeasure](http://rydberg.biology.colostate.edu/MicroMeasure)). Male induction for Salerno 03, Pescara 02,
 114 Cosenza 02 and Pisa 01 strains was evaluated by exposing
 115 parthenogenetic female aphids to short photoperiods (8 h
 116 light:16 h dark) according to Crema (1979).

117 Results

118 The analysis of mitotic cells of embryos, obtained from
 119 parthenogenetic females, confirmed that $2n=12$ is the stan-
 120 dard chromosome number in *M. persicae* (fig. 2), but 14 out of
 121 66 strains analysed showed intraspecific karyotype variants
 122 due to both structural and numerical variations in chromo-
 123 some complements (table 1, figs 3–6).

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

135 NOR staining (figs 3a, c, g, h and 6c) revealed the presence
 136 of heteromorphism in the size of rDNA genes in strains
 137 Salerno 3 (fig. 4c) and Cosenza 2 (fig. 6c) and evidenced that
 138 the fission of the X chromosomes observed in Cosenza 2
 139 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).

140
 141
 142 Considering the geographical distribution, it is evident that
 143 almost all karyotype variations (11 out of 14) were present in
 144 central and southern Italian regions, whereas only three were
 145 found in northern locations. Furthermore, all but one of the
 146 strains collected on tobacco showed chromosomal rearrange-
 147 ments; and, in particular, all the strains possessing the A1–3
 148 reciprocal translocation were found on this plant and were red
 149 in colour.

150 Male induction revealed that the *M. persicae* strains Salerno
 151 03, Pescara 02 and Cosenza 02, all possessing different kinds of
 152 karyotype variations, are anholocyclic since it was not possible
 153 to induce the sexual generation differently from that obtained
 154 under the same experimental conditions with the *M. persicae*
 155 strain Pisa 1, which showed a normal karyotype.

156 Discussion

157 The typical aphid karyotype consists of pairs of rod-like
 158 chromosomes, whose number is typically stable within a
 159 genus, as shown in the large genus *Aphis*, where the typical
 160 chromosome number is eight with the exception of *A. farinosa*
 161 with $2n=6$ (Blackman, 1980; Hales *et al.*, 1997). Nevertheless,
 162 exceptions have been published as revealed in the genus
 163 *Amphorophora*, where the chromosome number varies from
 164 $2n=4$ to $2n=72$ (Blackman, 1980).

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

175 The most common chromosomal variant described in
 176 *M. persicae* complement is a reciprocal translocation between
 177 the first and the third autosome pairs, leading to females with
 178 $2n=12$ karyotype showing a marked structural heterozygosity
 179 (Blackman, 1980).

180 The empirical data, as presented in this paper, reveal for
 181 the first time that this chromosomal aberration also occurs in
 182 Italy since seven out of the 14 strains showed karyotype
 183 variations due to the A1–3 reciprocal translocation. In view of
 184 the absence of any primary constriction, which is typical of the
 185 holocentric chromosomes, together with the lack of specific
 186 banding patterns after conventional banding procedures, we
 187 combined procedures of standard chromosome staining (such
 188 as Giemsa and silver staining) with chromosome length
 189 evaluation. In particular, we used silver staining to confirm
 190 the exclusive localization of NORs regions on X chromosome
 191 telomeres in *M. persicae* and analyzed the involvement of sex
 192 chromosomes in the translocation event (Manicardi *et al.*,
 193 2002). Afterwards, in the absence of any other cytogenetic
 194 markers, the morphometric analysis was employed to identify
 195 autosomes A1 and A3 as the chromosomes engaged in the
 196 rearrangement.

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

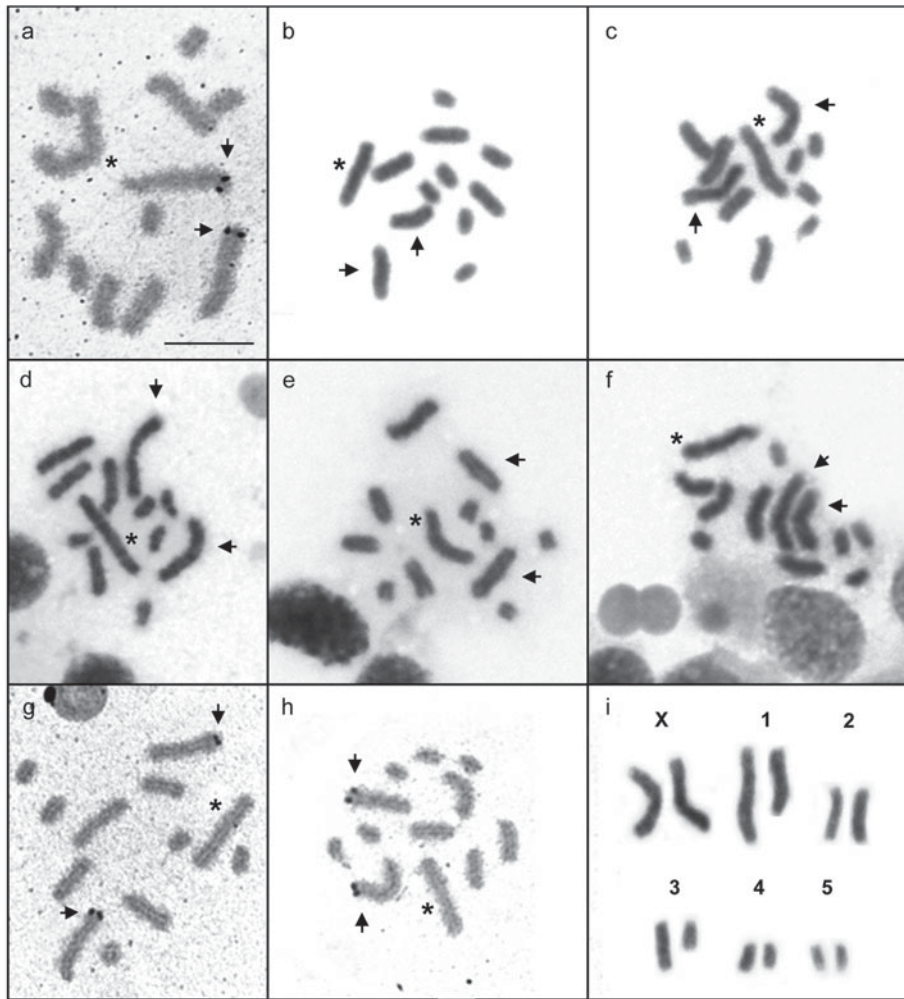


Fig. 3. *M. persicae* chromosome complements showing A1–3 reciprocal translocation. (a) Benevento 01 is silver stained, (b) Salerno 01, (c) Chieti 02 and (e) Chieti 03 are stained with Giemsa, whereas (d, g) Chieti 1 and (f, h) Chieti 4 are both Giemsa and silver stained. The (i) karyotype is derived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

202 controls (Blackman *et al.*, 1978). Preliminary data involving
 203 PCR and southern blot analysis revealed that, in one of
 204 the Italian populations with this chromosomal aberration
 205 (Chieti 1), the FE4 gene (electrophoretically fast variant (allele)
 206 of the normal expressed carboxylesterase 4 (E4) enzyme) only
 207 was present (Rivi *et al.*, 2009). This strain showed a moderate
 208 increase in esterase activity and was considered an S/R1
 209 (susceptible/first resistance level) strain *sensu* Devonshire *et al.*
 210 (1992). The aforementioned data allows us to suggest that this
 211 is the first *M. persicae* strain possessing the A1–3 chromosomal
 212 reciprocal translocation linked to an FE4 and not directly
 213 related to a high level of esterase-based insecticide resistance.
 214 Experiments currently in progress are aimed to extend this
 215 experimental procedure to all Italian strains possessing A1–3
 216 reciprocal translocations, in order to better clarify the
 217 relationships between this chromosomal rearrangement and
 218 the insecticide resistance in *M. persicae* populations.

219 Other fissions relatively frequent in the studied Italian
 220 *M. persicae* populations occurred at autosomes 3 and 4,

221 whereas in one case only the fission involved the X 221
 222 chromosome. Different autosome fragmentations have been 222
 223 repeatedly described in *M. persicae* populations collected 223
 224 worldwide, whereas the X fragmentation has been observed 224
 225 only in a *M. persicae* laboratory strain characterised by an 225
 226 extensive chromosomal mosaicism (Monti *et al.*, 2012). In this 226
 227 connection, it must be emphasized that in both such cases, the 227
 228 X fission occurs in X chromosomes possessing a low number of 228
 229 rDNA genes and in the telomeric region opposite to the NORs- 229
 230 bearing one. The recurrent fission of the same chromosomes in 230
 231 the same region argues that the *M. persicae* genome possesses 231
 232 some fragile/labile sites that could be the basis for the 232
 233 observed changes in the chromosome number.

234 For many years, chromosome evolution has been generally 234
 235 explained by considering the random-breakage model (Becker 235
 236 & Lenhard, 2007). On the contrary, a number of comparative 236
 237 cytogenetic studies evidences a relationship between chromo- 237
 238 somal rearrangements and specific chromosomal architecture 238
 239 and suggests a role of the repetitive DNAs in chromosome 239

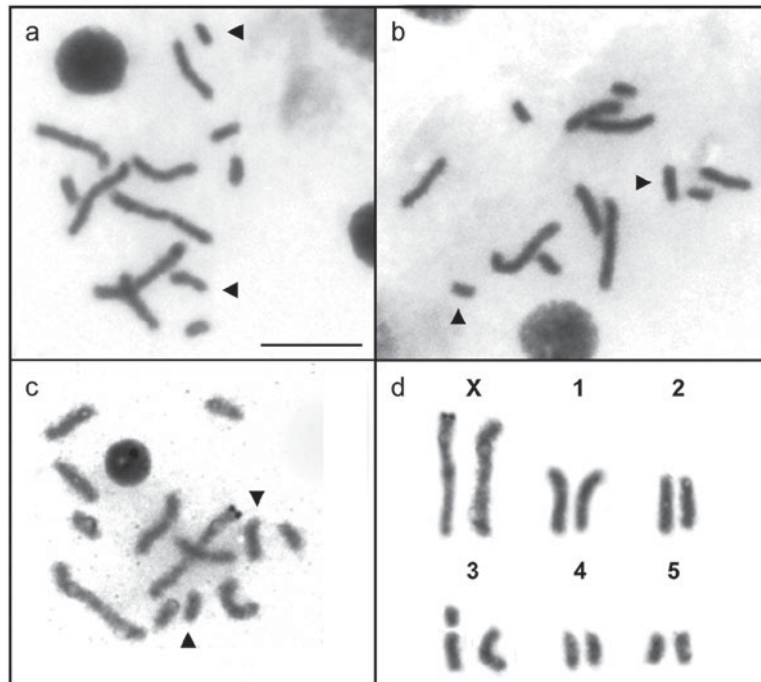


Fig. 4. *M. persicae* chromosome complements showing A3 fission. (a) Forli 01 and (b) Salerno 02 are stained with Giemsa, whereas (c) Salerno 03 and (d) its relative karyotype are silver stained. Arrow heads indicate chromosomes involved in the fission. Bar corresponds to 10 μ m.

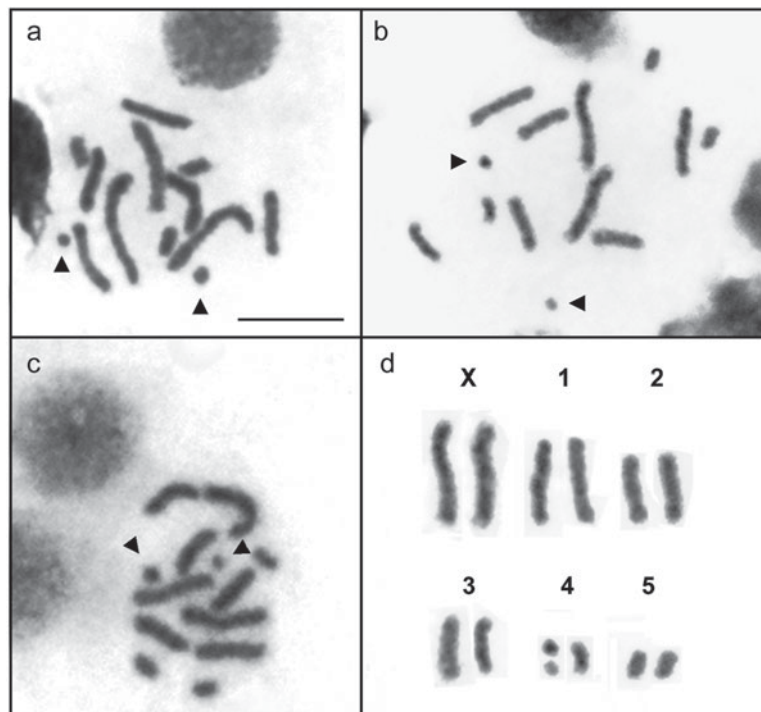


Fig. 5. Giemsa staining of *M. persicae* chromosome complements showing A4 fission: (a) Cosenza 01, (b) Ravenna 06 and (c) Piacenza 10. The (d) karyotype is derived from (b) Ravenna 06. Arrow heads indicate chromosomes involved in the fission. Bar corresponds to 10 μ m.

240 rearrangements. The nature of the repetitive DNA within
241 chromosomal breakpoint regions varies significantly, from
242 clusters of rRNA and tRNA genes to simple di- and

tri-nucleotide expansions (Caceres *et al.*, 1999; Carlton *et al.*, 243
2002; Coghlan & Wolfe, 2002; Kellis *et al.*, 2003; Renciuk *et al.*, 244
2011). The data reported in this paper confirmed recent 245

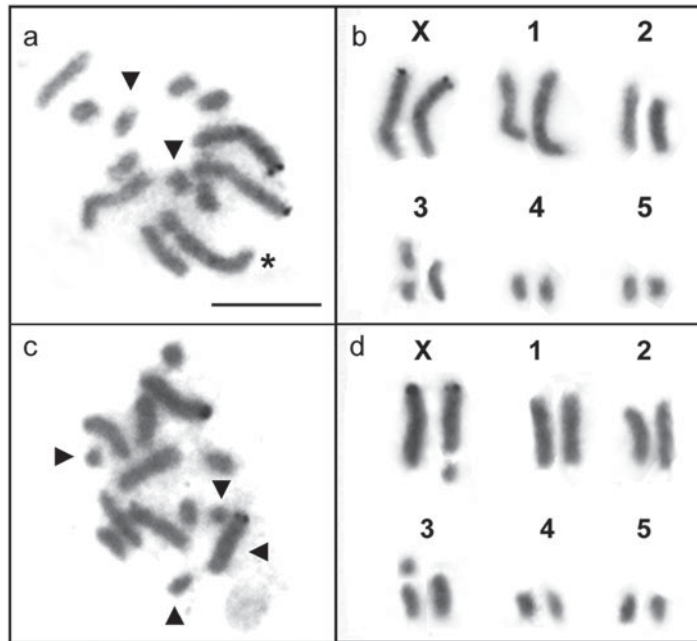


Fig. 6. (a) Pescara 02 complement stained with AgNO_3 and (b) relative karyotype. (c) Cosenza 02 complement silver stained with (d) relative karyotype. Arrow heads indicate chromosomes involved in the fissions. Asterisk indicates A1–3 translocated chromosomes. Bar corresponds to 10 μm .

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

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

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

274 Our data put in evidence that all but one of the strains
275 collected on tobacco plants showed karyotype variations,
276 whereas only four of the 56 population collected on other hosts
277 (corresponding to about 7% of the total) displayed chromo-
278 somal rearrangements. A suggestive explanation for the
279 observed relationships between chromosomal rearrangements

and tobacco plants could rely in the clastogenic effect of
nicotine.

280
281
282 Nicotine is a naturally occurring alkaloid found primarily
283 in members of the solanaceous plant family, including
284 *Nicotiana tabacum*. Several reports showed that nicotine, as a
285 consequence of DNA replication fork stress (Richards, 2001;
286 Freudenreich, 2005), produces genotoxic effects on Chinese
287 hamster ovarian (CHO) cells (Trivedi *et al.*, 1990, 1993) and
288 sister chromatid exchanges and chromosome aberrations in
289 bone marrow cells of mice (Sen *et al.*, 1991). Extensive
290 chromosomal rearrangements have also been described in a
291 mice population known as 'tobacco mice' since they live close
292 to kiln for drying tobacco (Fraguedakis-Tsolis *et al.*, 1997). In
293 addition, DNA fragmentation by nicotine has been demon-
294 strated both in peripheral lymphocytes (Sassen *et al.*, 2005) and
295 in human spermatozoa (Arabi, 2004). Nicotine, together with
296 ultraviolet exposure, has also been considered an exogenous
297 factor which can contribute to the generation of mutations
298 which could be at the basis of chromosomal mosaicism (De,
299 2011), a very rare phenomenon we have observed in Salerno
300 02, one of the strains collected on tobacco plants.

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

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

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