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Genetic variability among different Italian populations of the aphid *Myzus persicae*

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Abstract — Aphids life cycle includes cyclical or obligate parthenogenesis, therefore low genetic variation is expected in these insects. Genetic diversity in crop pest aphids such as *Myzus persicae,* is also influenced by the extensive use of insecticides which favoured the selection of few resistant strains. In the present work, 18 *M. persicae* populations collected in different Italian regions, were studied by RAPD-PCR (Random Amplified Polymorphic DNA) to assess genetic variability among populations. Twelve different random primers, selected out of 24 previously investigated, were employed for genomic DNA amplification. A total of 150 fragments from the aphid *M. persicae* populations, and 31 fragments in two outgroups were identified. An UPGMA cluster analysis based on Nei and Li's genetic distance revealed that the *M. persicae* populations could be divided into two groups: aphids from Central and Southern Italy were generally located in the same cluster, while aphids from Northern Italy were more often located in the other. The presence of a positive correlation between genetic and geographical distance suggests that at least a portion of the interpopulation polymorphism evidenced could be caused by restricted gene flow.

Key Words: Aphid, Genetic variability, Interpopulation polymorphism, Molecular markers, *Myzus persicae*, RAPD-PCR.

INTRODUCTION

A peculiar feature of aphids biology is their viviparous reproduction consisting in several generations of apomictic (asexual) parthenogenesis, followed by a single sexual generation at the end of each season (holocicly). Moreover, some species or populations within species have completely or partially abandoned any sexual stage and are obligately parthenogenetic (anholocicly) (for a review see Hales *et al*. 1997). Limited genetic variation is thus expected in aphid species as a consequence of their extensive use of apomictic parthenogenesis, especially in those consisting mainly or exclusively of anholocyclic clones. Since many aphid species are major plant pests, their genetic diversity is also lowered by the extensive use of insecticides. Among crop pest insects, the peach-potato aphid *Myzus persicae* is, without any doubts, one of the most representative species and one of the most serious pest on a wide range of crops. It is presumably of Asian origin like its primary host, the peach (*Prunus persica*), but it is globally distributed today (Blackman & Eastop 2000). This very polyphagous species accepts secondary host plants from over 40 different botanic families, including many economically important crops in which it can cause direct damage and/or it can transmit more than 100 plant pathogenic viruses (Blackman & Eastop 2000). *M. persicae* normally reproduce by cyclical parthenogenesis but this species also shows numerous and irreversible transitions from cyclical to obligate parthenogenesis.

Since the parthenogenetic eggs are produced by mitosis in which there is no recombination (Blackman 1979), parthenogenetic generations are often regarded as clones in which only mutations could produce genetic variability. Moreover, recent anthropogenic selection, such as insecticide treatment, could further explain the low genetic variability observed at a large scale since pesticide resistance has often evolved from the recent appearance and spread of single (or a few) adaptive genes, which are selected for over large geo-

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graphical areas (VORBURGER 2006). Studies addressing genetic diversity in aphid populations started with allozyme analysis which, as expected, revealed much less allozyme variation in aphids than in other invertebrate taxa (TOMIUK & WOHRMANN 1980). A novel range of molecular biology-based techniques are being widely used to study population genetics, which include mitochondrial DNA, RAPD-PCR (Random Amplified Polymorphic DNA) and microsatellites (SUNNUCKS 2000). The use of short arbitrary oligonucleotides as primers for DNA amplification in PCR reactions (i.e., RAPD-PCR), demonstrated the potential of this approach to detect both interspecific (MARTINEZ TORRES *et al.* 1997) and interpopulation (ZITOUDI *et al.* 2001) genetic variations among aphids. Reproducible RAPD banding pattern differences were also observed between winged, wingless and sexual (male or female) morphs of the same species (LUSHAI *et al.* 1997). The disadvantages of RAPDs as a consequence of dominance and low reproducibility are counterbalanced by the essentially infinite number of genomic markers generated together with the possibility to elute and sequence a peculiar RAPD band to produce a codominant marker (LUSHAI *et al.* 1997; BASHASAB *et al.* 2006). In this study, RAPD-PCR was chosen as a convenient molecular method for assessing the genetic variability among 18 *M. persicae* populations collected in different Italian peach-growing regions.

MATERIAL AND METHODS

M. persicae collections were made on peach orchards from 18 sites in different Italian regions (Fig. 1, Table 1). In order to reduce the variables which could influence genetic variability, we focused our attention on populations all collected from the same host (*Prunus persicae*) during spring time. This sampling strategy eliminated the genetic variability caused by different host adap-

Fig. 1 — Geographic distribution of the sampling sites and numbers of collected samples per site. The Region Emilia-Romagna is indicated in lighter grey.

Table 1 — Population codes, geographic origin, number of positive '1' bands per population, and latitude/longitude coordinates of the sampling sites

Population	Region – Town and (province)	Number of positive '1' bands per population	Lat/Long
Mp-1	Toscana – Pisa (PI)	47	10° 24' 53'' / 43° 42' 42''
Mp-3	Emilia Romagna – Imola (BO)	51	11° 43' 0'' / 44° 21' 11''
Mp-12H	Emilia Romagna – Argenta (FE)	45	11° 50' 11'' / 44° 36' 47''
Mp-17	Emilia Romagna – Piacenza (PC)	56	9° 42' 0'' / 45° 2' 51''
Mp-18	Emilia Romagna – Brisighella (RA)	55	11° 46' 33'' / 44° 13' 21''
Mp-19	Emilia Romagna – Piacenza (PC)	44	9° 42' 0'' / 45° 2' 51''
Mp-20	Emilia Romagna – Brisighella (RA)	51	11° 46' 33'' / 44° 13' 21''
Mp-24	Emilia Romagna – Imola (BO)	51	11° 43' 0'' / 44° 21' 11''
Mp-28	Emilia Romagna – Faenza (RA)	48	11° 52' 34'' / 44° 17' 32''
Mp-34	Calabria – Lamezia Terme (CZ)	40	38° 54' 28'' / 16° 14' 30''
Mp-36	Emilia Romagna – Porotto (FE)	46	44° 50' 0'' / 11° 37' 0''
Mp-37	Emilia Romagna – Argenta (FE)	48	11° 50' 11'' / 44° 36' 47''
Mp-38	Emilia Romagna – Ravenna (RA)	48	12° 12' 0'' / 44° 25' 0''
Mp-46	Piemonte – Torino (TO)	52	7° 40' 34'' / 45° 4' 41''
Mp-58	Marche – Ascoli Piceno (AP)	51	13° 37' 52'' / 42° 50' 53''
Mp-64	Abruzzo – Paglieta (CH)	52	14° 29' 53'' / 42° 9' 50''
Mp-70	Calabria – Castrovillari (CS)	51	16° 12' 47'' / 39° 49' 16''
Mp-TO1	Piemonte – Torino (TO)	62	7° 40' 34'' / 45° 4' 41''
Mp-1	Toscana – Pisa (PI)	47	10° 24' 53'' / 43° 42' 42''

tation which, although *M. persicae* is one of the most polyphagous aphids known, has been repeatedly demonstrated (ZITOUDI *et al*. 2001; VORBURGER *et al*. 2003). Moreover, limiting the collection time to spring, we intended to reduce the effect of different reproductive strategies, since we assumed that practically all populations analysed were holociclics.

Samples were taken from one leaf per plant, infested with nymphs, *apterae* and *alatae* and were maintained on pea-seedlings (*Pisum sativum*) under constant conditions: 21±1°C, 16h light: 8h dark. For use as outgroups in RAPD analysis *Aphis pomi* DeGeer and *Achyrthosiphon pisum* Harris were also collected. Our outgroup taxa are all members of the Family Aphididae, subfamily Aphidinae. All the populations were analysed using the RAPD-PCR method. Genomic DNA was extracted from 10-15 living adults per population according to CASSANELLI *et al.* (2005).

Twelve decamer primers (Operon Technologies) were used in the study (OPA-01, OPA-02, OPA-05, OPA-11, OPB-08, OPB-10, OPB-11, OPE-19, OPQ-05, OPQ-15, OPW-19, OPZ-08) selected from 24 previously tested. Polymerase chain reactions were performed by using the same thermalcycler (Hybaid) in 25 μl reaction mixture containing 300 ng of template DNA, 1.5 U of Taq polymerase (Polymed), 0.2 mM dNTPs, 20 ng of each primer, 2.5 mM MgCl2, and 1X reaction buffer supplied by the enzyme manufacturer. Amplification conditions involved one preliminary denaturation at 94°C for 2 min, a total of 35 cycles of a strand denaturation at 94°C for 1 min, annealing at 35°C for 1 min, and primer extension at 72 for 1 min, followed by final extension period of 10 min at 72°C. Then the amplified products were screened in 1.5% agarose gel containing 0,5 μg ml^{-1} Ethidium Bromide in 1X TBE buffer. Each gel was run at 60 V for 2h 30', DNA bands were visualized under UV light (Fig. 2) and were scored for their presence or absence. The molecular weight of each band was estimated by comparison with a co-migrating 100 bp ladder (O'Range Ruler 100 bp ladder, MBI Fermentas). To avoid problems related to variation in the number and intensity of amplification products, band mobility comparisons were made only within gel and based on the same PCR reaction. Each PCR reaction was replicated twice to check for reproducibility of the RAPD patterns produced. For detecting genetic similarity among populations, a data matrix for each primer was produced, each individual being represented as a vector of 1 and 0, for the presence or absence of a fragment.

A binary matrix was obtained and was transformed into a similarity matrix using the similarity coefficient of NEI & LI (1979). From this matrix a dendrogram was obtained by cluster analysis following the unweighted pair-group method of analysis (UPGMA)(SNEATH & SOKAL 1973), using TreeCon Software version 1.3 (VAN DE PEER & DE WATCHER 1994).

The bootstrap support of the nodes (1000 replicates) was calculated for the RAPD tree. Differentiation between clusters was estimated calculating and testing (with 10000 permutations) F_{ST} statistics (WRIGHT 1951) by using the TFPGA software (MILLER 1997).

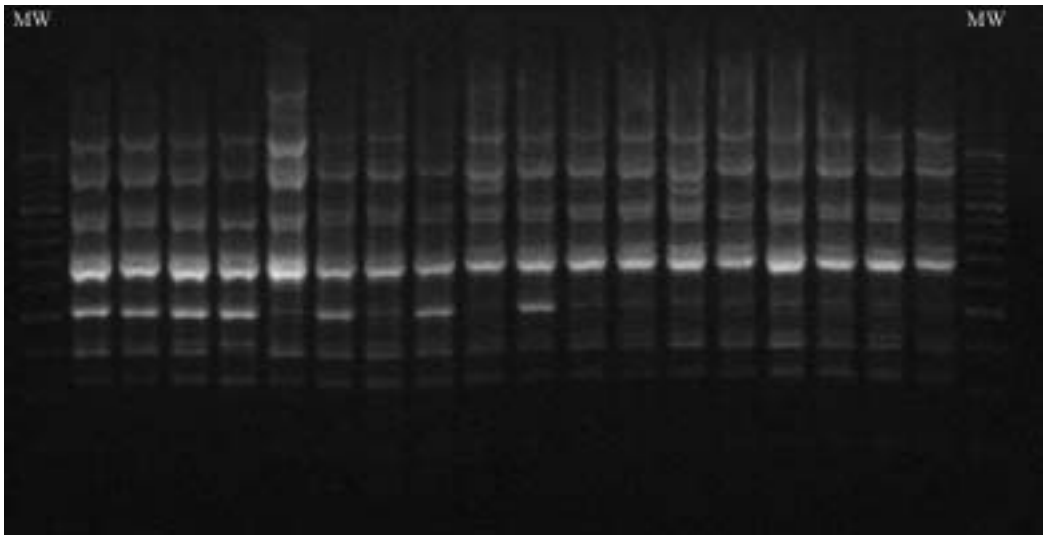

Fig. 2 — Amplified sequences obtained with the primer OPQ5 and separated in 1.5% agarose gel.

To test if differences among populations were influenced by geographic distance, we correlated the genetic distance between pairs of populations against the $\log_{10}$ transformed geographical (linear) distance (Km) between pairs. The significance of the correlation (P) was tested using the MANTEL test (1967) with 10000 random permutations and using the AIS software (MILLER 2005).

Distances among collecting sites were estimated from the latitude and the longitude. Moreover, for this analysis when dominant markers are available, AIS uses a simple genetic distance that is the proportion of mismatched loci between any pairs of individuals *i* and *j* in a data set.

RESULTS

Random amplified polymorphic DNA (RAPD) analyses applied to 18 population of *M. persicae* collected in different Italian regions using 12 decamer primers produced a number of fragments varying from 7 to 14 per primer, ranging in size from 250 to 1500 bp. All primers used allowed the detection of a total of 150 reproducible and well-resolved bands for the 18 *M. persicae* populations (Table 1) and 31 exclusive markers in the two outgroups analysed, for a total of 181 markers analysed. Similarity indices calculated following NEI & LI (1979) obtained by a data matrix considering all the RAPD markers (data not shown), showed that the highest genetic similarity was registered between the Mp-46 and Mp-58 populations (0.775), whereas the lowest between Mp-34 and Mp-TO1 (0.133). Distance matrix used to construct a phenogram with 1000 bootstrap replicates produced a dendrogram showing that the separation of the *M. persicae* complex as a whole from the two outgroups, was strongly supported with 96% bootstrap value (Fig. 3). Moreover, within the *M. persicae* populations, aphids from Northern Italy were generally differentiated from those collected in Central and Southern Italy since they were preferentially located in different clusters The two clusters were supported with 61% and 66% bootstrap values, which suggests they were not somewhat arbitrary. The upper cluster, including most Northern populations, showed in general a lower genetic diversity within the strains. Among them, the MpTO1 population collected near Torino, in the North-West of Italy (see Fig. 1), resulted differentiated from the others with 100% bootstrap confidence. On the contrary, the other cluster, which included all Central and Southern Italy populations analysed (with the only exception of Mp-58, from Ascoli Piceno, which grouped with those from Northern Italy) generally showed a higher divergence among the

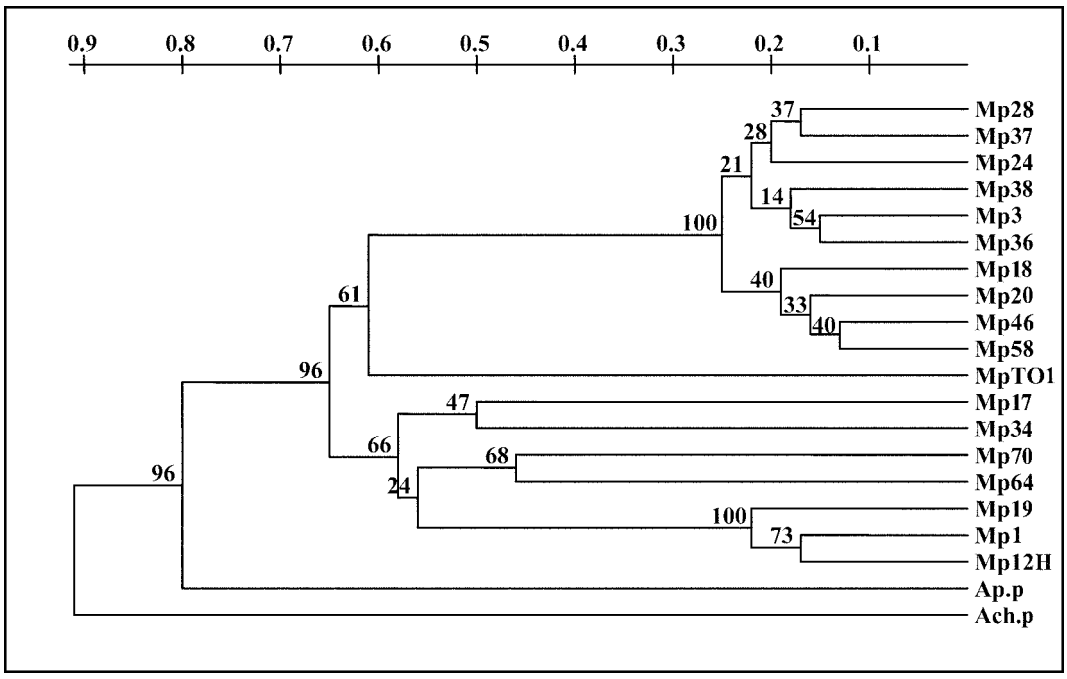

Fig. 3 — UPGMA dendrogram generated from the similarity distance matrix. Bootstrap confidence levels for 1000 pseudo-replicated data matrices are indicated. The two outgroups are indicated as following: Ap.p = *Aphis pomi*, Ach.p = *Acirthosiphon pisum*

included populations. Genetic differentiation between the two clusters was strong and significant (F_{ST} = 0.399 P<0.01).

When the entire dataset was considered, the correlation between genetic and geographic distances among population-bulks resulted highly significant and positive ($r = 0.429$, $P = 0.0046$) (Figure 4A). Moreover, the correlation was also significant when only the Emilia Romagna region was considered ($r = 0.568$, $P = 0.0228$) (Figure 4B).

DISCUSSION

Reproductive strategies, together with genetic drift and natural selection are generally invoked to account for low genetic variability among aphid species. In the peach-potato aphid, *Myzus persicae* genetic diversity is also lowered by the large use of the insecticides which, starting from 1950s favoured the selection of resistant strains (ZAMOUM *et al.* 2005). However, both cytogenetics (SPENCE & BLACKMAN 2000) and molecular studies (for a review see WILSON *et al.* 2003) show that an appreciable level of genetic variation in *M. persicae* does exist. For example, biochemical studies carried out in *Myzus persicae* populations, show that allozyme loci were found to be excessively monomorphic thus suggesting that they could be under selection pressure (DELMOTTE *et al.* 2002). By using hypervariable microsatellite markers or ITS fingerprints, several studies have shown that asexually reproducing populations of *M. persicae* sampled on herbaceous plants displayed a low genotyping variability, both within and among populations at a regional scale (FENTON *et al.*1998; GUILLEMAUD *et al.* 2003; VORBURGER *et al.* 2003). On the contrary, molecular characterization of the Tunisian *M. persicae* clones using microsatellite markers made possible the detection of an unexpected high number of genotypes (DJILANI KHOUADJA *et al.* 2003).

In order to increase the knowledge above the extent of genetic variation for this important crop pest aphid, we have applied the RAPD-PCR technique to 18 *M. persicae* populations collected in different Italian areas, with a particular emphasis regarding the Emilia and Romagna Region, which represents one of the largest peach growing areas in Europe.

The RAPD-PCR technique, based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence, has provided an advantageous molecular technique able to detect genetic markers both in plants and animals.

Some RAPD analyses had been previously carried out also within *M. persicae* populations, but the conclusion differed. For example, AL-ABOODI and FFRENCH-CONSTANT (1995) detected no variation when comparing 32 clones from Canada and two from England besides CLEMENTS *et al.* (2000) reported that RAPD polymorphisms didn't separate populations on the basis either of host-plant association or of geographic origin. However, CENIS *et al.* (1993) were able to distinguish a German clone from five identical Spanish clones and MARTINEZ-TORRES *et al.* (1997) discovered that almost every clone of *M. persicae* from a Spanish region differed. Moreover, genetic variation associated with host-plant and life cycle but not with

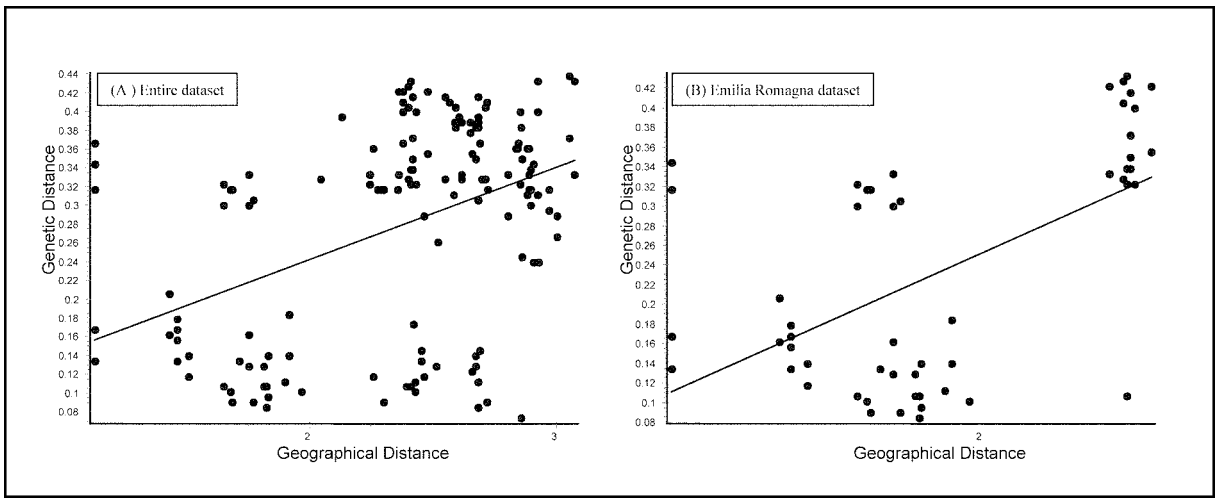

Fig. 4 — Relationships between genetic divergence and geographic distance among the *M. persicae* Italian populations when the entire dataset (A) or only the Emilia Romagna dataset (B) are considered.

geographic distance was observed in Greek populations (ZITOUDI *et al*. 2001)

In the present study, the 150 reproducible and well-resolved RAPD-PCR bands obtained by us put in evidence a substantial amount of variation in RAPD profiles among the 18 populations of *M. persicae* examined.

The level of interpopulation genetic similarity was found to vary from 0.133 to 0.775 and, owing to the cluster analysis, it was associated with geographic origins. In fact, *M. persicae* populations collected from Central and Southern Italy were generally located in the same cluster while those from Northern Italy, with few exception, were located in a different one. Moreover, within the two clusters there was a different level of variability since populations collected from Central and Southern Italy presented an higher genetic divergence than those collected in Northern Italy. A possible reason for this last result could be that *M. persicae* from Northern Italy were mostly collected in Emilia-Romagna, which is the main region in Italy in the peach production.

The observed significant correlation between genetic and geographic distance is consistent with other studies on this species (LOXDALE 1990; MARTINEZ-TORRES *et al*. 1997; WILSON *et al.* 2002; GUILLEMAUD *et al*. 2003) but ZITOUDI *et al*. (2001) failed to show an association between genetic divergence and geographic distance when examining 96 asexual lineages of *M. persicae* collected in different Greek regions. The presence of a significant population differentiation with increasing geographical distance could be explained by suggesting that, contrary to what previously supposed (VAN EMDEM *et al.* 1969; HALES *et al*. 1997) long distance migration is not so common in *M. persicae*. The presence of a restricted gene flow among sites is also supported by the observation that the correlation remains significant also when the analysis is restricted only to the Emilia Romagna region (i.e. not only at the country scale but also at a smaller geographical scale). Alternatively it could be hypothesized that, even in presence of long distance migration, the success rate of migration may be low (LOXDALE and LUSHAI 1999; GUILLEMAUD *et al*. 2003). Moreover, the relationship between genetic differentiation and geographical distance found in *M. persicae* by us and other Authors (LOXDALE *et al*. 1993; SUNNUCKS *et al*. 1997; GUILLEMAUD *et al.* 2003) resulted higher than that observed in other aphid species (DELMOTTE *et al*. 2002; DOLATTI *et al*. 2005) thus suggesting that, as already discussed (LOXDALE *et al.* 1993), migration capacity results largely variable among different aphid species.

Since our collection strategy was aimed to avoid effects on genetic variability due to host adaptation and reproductive strategy (see Material and methods) among other possible reasons able to explain the observed genetic difference between *M. persicae* populations we could suggest climatic (temperature) variations in the Italian environments, quali/quantitative differences regarding natural enemies (parasitoids and predators) but also differences in pest management strategies.

Temperature has long been identified as one of the most important environmental variables in aphid ecology (HALES *et al.* 1997) and genetic variation among clones associated with their different temperature tolerance has been detected in *M. persicae* (VORBURGER *et al.* 2003).

On the other hand, it is possible that populations collected in different regions are under different selection factors depending from the species and the natural enemies present in the collecting field.

Last but not least also the different phytosanitary history can account for the observed results as, in many cases, the populations were collected in insecticides sprayed orchards. Further analyses, carried out utilizing increasing number of populations and different molecular markers could give additional important contributions to the stimulating study of genetic variability in *M. persicae*.

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