Chromosomal mapping reveals a dynamic organization of the histone genes in aphids (Hemiptera: Aphididae)

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Abstract

Despite their involvement in different processes, histone genes have been analysed in few insects. In order to improve the knowledge about this important gene family, genes coding for histones have been analysed in the aphid Acyrthosiphon pisum showing that at the amino acid level, aphid histones are highly conserved. In particular, data from A. pisum confirm that H1 is the most variable of the five histones, whereas histones H3 and H4 are highly conserved with the H3 almost identical from insects to vertebrates. Furthermore, spacers that separate the aphid histone genes vary in length. The histone genes have been mapped in A. pisum and successively in the aphids Myzus persicae and Rhopalosiphum padi showing that they are present in a single large cluster located in an interstitial position of autosomes 1, differently from what reported in the Russian wheat aphid Diuraphis noxia, where histone genes have been localized in a telomere of the two X chromosomes suggesting a dynamic organization of this multigene family in aphids.

Introduction

Histone proteins are the major constituents of chromatin and they are involved in the packaging of DNA into nucleosomes and in the regulation of gene expression through their post-translational modifications (Schaffner et al., 1978; Rea et al., 2000; Jenuwein & Allis, 2001). The histone family can be divided into two groups, four core histones (H2A, H2B, H3 and H4) and the linker histone (H1), that are coded by a multigene family, where each histone gene is reiterated in a hundred or more copies in order to fulfill the cell’s constant requirement for histones (Schaffner et al., 1978). The multiple copies of the histone genes may be clustered into distinct chromosomal regions and in invertebrates they are typically clustered as quartets (H2A, H2B, H3 and H4) or quintets (H2A, H2B, H3, H4 and H1), although scattered solitary histone genes have also been reported (Lifton et al., 1977; Engel & Dogson, 1981; Maxon et al., 1983).

Among insects, the typical arrangement of the histone genes is a repeating quintet comprising the four core histones (H2A, H2B, H3, and H4) along with histone H1. Partial exceptions to this rule have been described in the flies Drosophila americana and Drosophila viridis, where a repeating quartet consisting of the core histones only coexists with the quintet repeat (Schieman et al. 1998; Nagel & Grossbach, 2000; Nagoda et al., 2005). Since the quartet arrangement is viable in many other invertebrates (Baldo et al., 1999; Barzotti et al., 2000; Eirín-López et al., 2004), it has been suggested that the quartet is derived from the quintet via deletion of H1 (Nagoda et al., 2005).

In insects, histone genes have been cyrogenetically mapped in few taxa, with a better detail in Coleoptera and Diptera (Pardue et al., 1977; Hankeln et al., 1993; Schieman et al., 1998; Cabrero et al., 2009; Cabral-de-Mello et al., 2011a,b,c). In particular, in many Drosophila species the histone quintet is restricted to a single cytological locus (Fitch et al., 1990), whereas FISH mapping revealed that histone genes are generally co-located with the genes coding for the 5S rRNA in Coleoptera (Cabral-de-Mello et al., 2011a,b,c). Besides the association of 5S rDNA and histone genes, co-localization or linked organization of histone genes with other gene families is not unusual in insects, since they have been found, for instance, associated to the major rDNA in the beetles Anthonomus grandis and Anthonomus texanus, where the five histone genes were inserted into the intergenic spacer regions (IGS) that separate the single transcriptional units in the rDNA array (Roehrdanz et al., 2010).
At present, the study of histone genes in aphids has been limited to the chromosomal mapping of the H3 and H4 genes in the Russian wheat aphid *Diuraphis noxia*, where they have been localized in a single telome of the two X chromosomes within the nucleolar organizing regions (NORs) (Novotná et al., 2011). As previously reported, the co-localization of major rDNA genes within the histone cluster is not new in insects, but it could be particularly interesting in aphids in view of the role that rDNA genes play in the sex determination (Mandrioli et al., 1999). Indeed, it has been repeatedly suggested that rDNA genes have a crucial role in the X chromosome association occurring during the maturation division of the parthenogenetic oocytes, which is at the basis of the sex determination of the X0 males (Orlando, 1974; Hales & Mitler, 1983; Blackman & Hales, 1986). In particular, Mandrioli et al. (1999) reported that the *A. pisum* rDNA IGS contains numerous 247 bp long repeats that show homologies with the 240 bp repeats located in the *Drosophila melanogaster* rDNA intergenic spacers that are responsible for fly sex chromosomes pairing (Mckee & Karpen, 1990) suggesting that these repeats could be at the basis of the rDNA-mediated association of the aphid X chromosomes (Mandrioli et al., 1999). Considering that histones genes were clustered within IGS in the beetles *A. grandis* and *A. texanus*, the co-localization of histone genes in this critical region of the aphid genome deserve further research in order to better understand a possible role of the histone genes in the X chromosome association. At this regards, in the present paper we localized the genes coding for the histones H1, H2A, H2B, H3 and H4 in the pea aphid *Acyrthosiphon pisum* (Harris, 1776), also studying their organization in the histone gene cluster. Lastly, we analysed the localization of the histone genes in the peach potato aphid *Myzus persicae* (Sulzer, 1776) and in the wheat/ oat aphid *Rhopalosiphum padi* (Koch, 1854) in order to better evaluate if the histone gene localization at NORs observed in *D. noxia* is a common feature in aphids.

**Materials and Methods**

The specimens of *A. pisum* used for this research were obtained from the LSR1 laboratory lineage, kindly furnished by Manuel Plantagenest (INRA, France), and maintained on broad bean *Vicia faba* plants. *R. padi* specimens were collected in Modena and maintained on *Zea mays* plants. Specimens of *Myzus persicae* were obtained from the laboratory population labelled as *clone I*, kindly supplied by Emanuele Mazzoni, Università Cattolica di Piacenza (Italy) and maintained on pea *Pisum sativum* plants. The three aphid species were bred at 20°C with 16 hours of light and 8 of darkness.

DNA extraction from aphids was performed as described in Mandrioli et al. (1999). For chromosome spreads, adult females were dissected in a 0.8% hypotonic solution of sodium citrate saline solution and then embryos were kept in the same solution for 30 minutes (min). Embryos were then transferred to minitubes and centrifuged at 3000 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 disaggregation of the material followed by a further centrifugation at 3000 g for 3 min. This step was repeated with fresh fixative. Finally the pellet was resuspended in new fixative and 20 ml of cellular suspension was dropped onto clean slides.

Primers H1-F (5’-ACCACCAAGGAACTGATATC) and H1-R (5’-CGAGAAGCAATTGCATTAG), designed on the basis of the *A. pisum* H1 sequence XM_001944253.2, have been used in order to amplify a 330 bp fragment by PCR. The amplification was performed with a thermal-cycler Hybaid at an annealing temperature of 55°C for 1 min and making extension at 72°C for 30 sec.

Genes coding for histone H2A and H2B have been amplified with the primers couples H2A-F (5’-AAAATCGAAAGGGAGGCAAT)/H2A-R (5’-AAGAGGACCGTGGAGTGT) and H2B-F (5’-TGAAGAATCGTCCGGAAAG)/H2B-R (5’-CGGCTTAGTTCTTCTCAGT), designed on the basis of the *A. pisum* sequences H2A (NM_001163992.1) and H2B (XP_003240130.3) respectively. Both primer couples have been used in order to amplify a 320 bp fragment by PCR with a thermal-cycler Hybaid at an annealing temperature of 54°C for 1 min and making extension at 72°C for 30 sec.

Primers H3-F (5’-ATGCGACGTACCACAAGAAAC) and H3-R (5’-AGTGGTTGTCCTCCAGAACAG), designed on the basis of the *A. pisum* H3 sequence XM_003240984.1, have been used in order to amplify a 327 bp fragment by PCR at an annealing temperature of 55°C for 1 min and making extension at 72°C for 30 sec.

Genes coding for histone H4 have been amplified with the primers couple H4-F (5’-GAATTGCTCCCTCACCACCC)/H4-R (5’-CGTAGCAGTGTTCGCTTTGG), designed on the basis of the *A. pisum* sequence H4 (XM_001950998.1). Primers have been used in order to amplify a 329 bp fragment using an annealing temperature of 50°C for 1 min and making extension at 72°C for 30 sec.

In order to entirely amplify a segment from the H1 to the H4, the primers HIS-CLUST-F (5’-CGAAACCGATTAAGGTAGCAG) and HIS-CLUST-R (5’-GGCCGCTTTGACCATTGTA) have been designed on the basis of the *A. pisum* unplaced genomic scaffold 368 (NW_00338357.1, from base 259867 to 272662). Primers have been used in order to amplify a 7379 bp fragment by an Hybaid thermal-cycler using the Fermentas Long PCR Enzyme Mix making annealing and extension at 68°C for 8 minutes and 25 cycles, according to the manufacturer’s instructions.

The SS rDNA repeat unit of *A. pisum* was amplified by PCR using two primers, F (5’-TGCACTGATTGTTCCAGAACAG) and R (5’-ACGCCATACCCGGTTGATATC), deduced from the SS coding sequences of insects available in GenBank. The two primers employed here, were designed in such a way that primer cross-hybridization with other pol III-controlled genes was prevented (Geiduschek & Tocchini-Valentini, 1988). The amplification mix contained 100 ng genomic DNA, 1 mM of each primer, 200 mM dNTPs and 2 U of DyNAzyme II polymerase (Finnzymes Oy). The amplification was performed with a thermocycler Hybaid at an annealing temperature of 59°C for 30 seconds and making extension at 72°C for 45 seconds.

Random priming probe biotin-labelling was performed with the Biotin High Prime (Roche), whereas the PCR digoxigenin labelling were performed using the Dig High Prime (Roche). Both labelling were done according to the Roche protocols.

Fluorescent *in situ* hybridization (FISH) experiments with the H3 and SS genes as probes were performed as described by Mandrioli et al. (2011), whereas staining with chromomycin A3 (CMA3) fluorochrome was performed as described by Mandrioli et al. (1999).

Photographs of the fluorescent images were taken using a Zeiss Axioplan epifluorescence microscope equipped with a CCD camera (Spot, Digital Instrument, Madison, USA) and with the Spot software supplied with the camera.

Bioinformatic analyses have been performed using the software CLC sequence viewer (Aarhus, Denmark) and using BLAST tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi) available at NCBI. Search for direct and indirect repeats within the histone spacers has been performed using the Tandem Repeat Finder tool (freely available at the address http://tandem.bu.edu/trf/trf.html) and the Nucleic Acid Dot Plot online tool (http://www.colestate.edu/molkit/dnadot/).

**Results**

During the analysis of the *A. pisum* genome, several genes have been annotated as histone-like, but no further investigations have been performed.
The alignments of the aphid H1 histones with orthologues in GenBank revealed that the peptide XP-001944288 is a complete H1 histone. This protein, that is 203 amino acid (aa) long, had a 60% similarity/45% identity to H1 histones isolated in the fly *Drosophila willistoni* and the beetle *Dendroctonus ponderosae* and a 70% similarity/60% identity to the hymenopteran *Nasonia vitripennis* and the lepidopterans *Bombus impatiens* and *Bombus terrestris* (Figure 1).

Five proteins (NP_001157464.1, XP_001952706.2, XP_003244391.1, XP_003241036.1, XP_003241023.1) have been annotated as histone H2A-like. They are almost identical with the exception of a single amino acid change in the protein NP_001157464.1 and a deletion of an amino acidic residue in the protein XP_003241023.1. Among them, the *A. pisum* protein XP_001952706.2 (125 aa) represents a complete and highly conserved histone H2A with a 98% similarity/94% identity to H2A histones isolated in the flies *Drosophila sechellia* and *Drosophila simulans* and a 96% similarity/94% identity to the mosquito *Aedes aegypti* H2A (Figure 2).

Three 126 aa long H2B histone-like proteins (XP_003241030.1, XP_001950269.1, XP_001947771.1) have been predicted in *A. pisum* differing each other for a single substitution of an amino acid residue, with the protein XP_001947771.1 showing the strongest similarity/identity to H2B histones isolated in other insects. Similarly to what previously reported for the H2A, the aphid H2B histone showed a 98% similarity/91% identity to H2B histones of *Apis mellifera*, *B. impatiens* and *B. terrestris*, whereas a lower similarity/identity was present in respect to *N. vitripennis* (91/86%) and *A. aegypti* (89/86%) (Figure 3).

Four H3 histone proteins have been predicted in the aphid genome, but three of them (XP_001949472.1, XP_001950199.1 and XP_003246588.1)

![Figure 1](image1.png)

Figure 1. Alignment of the aphid H1 histone amino acidic sequence (AP) with orthologues in *Drosophila willistoni* (DW), *Drosophila ponderosae* (DP), *Nasonia vitripennis* (NV), *Bombus impatiens* (BI) and *Bombus terrestris* (BT). Bars below the alignment show the conservation percentages of each amino acidic residue in the analyzed species.
were partial sequences, whereas the protein XP_003241032.1 represented a full length 157 aa long H3 histone. The aphid H3 protein had a 98% similarity/97% identity to H3 histones isolated in the dipterans D. melanogaster and Culex quinquefasciatus and in the ant Solenopsis invicta. Interestingly, the percentages of both similarity and identity are high also in comparison to vertebrate H3 proteins, such as Danio rerio (96/95%) and Columbia livia (99% identity to aphid H3) suggesting a particularly high conservation of the aphid H3 in respect to orthologous histones (Figure 4).

The protein XP_001951033.1 was the unique one annotated as H4 like in A. pisum and indeed it had a high similarity/identity to other insect H4 proteins ranging from the 88/87% in D. willistoni to the 87/85% in D. sechellia. Despite its lower conservation in respect to H3, H4 still have high value of similarity to vertebrate H4 histone as assessed in comparison to Pan troglodytes (87%) and C. livia (88%) (Figure 5).

The bioinformatic analysis of the A. pisum genome evidenced that the contig NW_003383857.1 contained a complete histone cluster consisting of the histone quintet (H1, H2B, H2A, H3 and H4) (Figure 6). The span of the histone genes is 7462 bp, making the aphid cluster larger than the range reported for other insects that is about 5000 bp.

In order to confirm the size of histone cluster, the primers HIS-CLUST-F and HIS-CLUST-R have been used to entirely amplify a segment from H1 to H4 evidencing several bands suggesting that histone genes were spaced by differently long spacers that separate the histone genes within the histone unit (Figure 6C). This result has been confirmed also by the analysis of three contigs (NW_003384165.1, NW_003384165 and NW_003386216.1) that, despite the presence of a...
partial histone cluster, evidenced different distances among the histone genes. In particular, the H2A-H3 spacer was the most variable in length and sequence ranging from 1.514 to 2.552 bp, but no direct or inverted repeat has been identified using tandem repeat finder or DNA dot plot analyses (Figure 7).

According to the previous bioinformatic analyses, it emerged that the aphid histone quintet consisted in the H1 gene followed by the H2A and H2B genes that were adjacent and transcribed in opposite directions. At the 3’ end of the histone cluster, H3 and H4 genes constituted an oppositely transcribed pair (Figure 6A).

Interestingly, some contigs contained a partial histone cluster flanked by non-histone genes, such as the contig NW_003383520,
where only the histone genes H2A, H3 and H4 were present and the contigs NW_003383520.1 and NW_003383520.1 containing an histone cluster consisting of the genes H3-H4 and H1-H2A only respectively.

FISH experiments with the H3 gene as a probe revealed that in *A. pisum* the histone genes were clustered on a single pair of long chromosomes (Figure 8A) that have been identified as the autosome 1 couple on the basis of the absence of CMA3 staining of the NOR region, used as a marker of the X chromosomes, the only other long chromosomes of *A. pisum* complement (Figure 8B). The restricted presence of the histone genes to a single region has been confirmed by the results of the H3 FISH in the *A. pisum* interphase nuclei (Figure 8C).

Considering that 5S rDNA genes have been previously located in multiple clusters on the X chromosomes and on autosomes 1 (Bizzaro et al., 2000) and that a co-localization between histones and the 5S genes was reported in literature (Cabral-de-Mello et al., 2011b), a double FISH has been performed in *A. pisum* showing that the 5S and the histone coding genes are both located on autosome 1, but at different regions (Figure 8D).

The same set of probe has been also used in the aphids *M. persicae* (Figure 8F) and *R. padi* (Figure 8G) revealing the localization of the 5S and histone genes on different portions of the autosomes 1, as assessed by the CMA3 staining (Figure 8H, I).

### Discussion

Multigene families constitute a functionally important portion of the eukaryotic genomes and some of them have been very useful cytogenetic markers for studying chromosomal diversification and genome organization (e.g. Cabral-de-Mello, 2011a, b, c). Among them, both the major ribosomal DNA cluster (encoding for the 28S, 18S and 5.8S rRNAs) and the 5S rDNA array (made of tandemly repeated 5S rDNA units) have been frequently mapped on insect chromosomes, whereas the localization of histone genes is till now restricted to few species of insects, mainly consisting of Diptera and Coleoptera (Pardue et al., 1977; Hankeln et al., 1993; Schienman et al., 1998; Cabrero et al., 2009; Teruel et al., 2010; Cabral-de-Mello et al., 2011a, b, c; Novotná et al., 2011).

At present the three most relevant gene families (major and minor rDNA units and histone gene cluster) have been studied in few aphid species making very difficult to understand aphid chromosome evolution through a comparative approach.

In aphids major rDNA genes are arranged as tandemly repeated clusters that have been localized at one telomere of each X chromosome by silver staining (Blackman & Hales, 1986; Kuznetsova et al., 1993; Mandrioli et al., 2011), staining with the GC-specific fluorochrome CMA3 (Manicardi et al. 2002; Mandrioli et al. 2011) and in situ hybridization with rDNA probes (Blackman et al., 2000; Manicardi et al., 2002; Mandrioli et al., 2011). Exceptions include the interstitial position of rDNA genes in *Amphorophora idaei* (Fenton et al., 1994) and the autosomal localization of NORs in *Schoutedenia lutea* (Hales, 1989).
Differently from the conserved position of the major rDNA genes, the 5S rDNA localization on aphid chromosomes vary between species, since FISH experiments evidenced a single cluster located on autosome 1 in *Aphis nerii* (Mandrioli et al., 2011) and three interstitial clusters on X chromosomes, together with a 5S rDNA cluster in an intercalary region of autosome 1, in *A. pisum* (Bizzaro et al., 2000).

The histone coding genes have been studied at present in a single aphid species, the wheat aphid *D. noxia*, where the histone H3 and H4 genes are co-localized at the NOR-bearing ends of the two X chromosomes (Novotná et al., 2011). As previously reported, this co-localization is not a unique feature of aphids and it suggests that histone genes could be inserted into the intergenic spacer region, as reported in Coleoptera (Roehrdanz et al., 2010). This hypothesis is further supported by FISH experiments with the H3 probe showing that the histone gene cluster is, like the rDNA genes, present in the silver nitrate positive bridge connecting the two X chromosomes at prometaphase, during the male determination (Novotná et al., 2011).

In the three aphid species analysed in the present paper, the histone genes have been mapped in a single cluster located in an interstitial position of autosome 1. This result, while confirming that aphid histone genes are present in a single chromosome in the aphid karyotype, highlighted that they occur in different positions of the chromosome complement. The conservation in the number of histone gene clusters strongly resembles the results observed in several coleopteran species, were the histone genes have been mainly located on one autosomal bivalent only (Cabral-de-Mello et al., 2011a, b, c) indicating that a strong purifying selection acts on the histone clusters, preventing the spread of these genes through the aphid genome, as previously proposed in grasshoppers (Cabrero et al., 2009). On the contrary, the different localization of the histone genes in *D. noxia* in comparison to *A. pisum, M. persicae* and *R. padi* reveals a dynamic repositioning of the histone cluster during aphid evolution due chromosomal rearrangements, as previously reported for species belonging to the *Drosophila* genus (Steinemann et al., 1984).

The four aphid species studied for the histone gene mapping belong to the family Aphididae, but are part of different tribes since *R. padi* belongs to Aphidini, whereas *D. noxia, A. pisum* and *M. persicae* to Macrosiphini. Considering that Aphidini have been suggested as primitive to Macrosiphini (Kim et al., 2011), the interstitial position of histone genes observed in *R. padi* could be considered more ancestral in respect to the localization of the histone genes within the rDNA of *D. noxia*. However, further analyses on other aphid species are necessary to support phylogenetic considerations about the ancestrality of histone localization.

According to previous data on *A. pisum*, 5S rDNA genes cluster in an intercalary position of the autosome 1 (Bizzaro et al., 2000). Due to the overlapping of the histone and 5S rDNA genes in Coleoptera, simultaneous FISH experiments with the histone and 5S probes have been performed showing that they map on the same chromosome, but in differ-

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**Figure 8.** FISH hybridization on DAPI stained chromosomes of the aphids *A. pisum* (a, d), *M. persicae* (f) and *R. padi* (g) with the H3 probe alone (a) or with the simultaneous use of the FITC-labelled H3 (in green) and the Texas red-labelled 5S rDNA probes (in red) (d, f, g), followed by CMA3 staining of the same chromosomal plates (b, e, h, i), evidenced the presence of a single histone gene clusters in aphids, as confirmed also in *A. pisum* interphase nuclei. Arrows indicate X chromosomes. Bar corresponds to 10 μm.
ent positions. In addition, the relative localizations of these clusters on autosome 1 are similar in the three studied aphid species, supporting the previously stated suggestion that a substantial synteny of gene order and orientation is present in Aphidinae (The International Aphid Genomics Consortium, 2008).

At the amino acid level, aphid histones are highly conserved and they confirm that H1 is the most variable of the five histones, whereas histones H3 and H4 are highly conserved with the H3 almost identical from insects to vertebrates. Histones H2B and H2A differs from orthologous proteins only for few amino acids. As a whole, the amino acid sequences of core histones in aphids overlap literature data stating that H3 and H4 evolve most slowly, with H2A and H2B evolving ten times as fast and H1 evolving even more rapidly (Thatcher & Gorovsky, 1994).

The span of the histone genes observed in the pea aphid (more than 7 kb) is over the range reported for other insects, such as different Drosophila species (Litton et al., 1978), but is similar to what reported in other eukaryotes, such as sea urchin Strongylocentrotus purpuratus, where the histone genes were intermingled in a 7 kilobase (kb) repeat unit (Kedes et al., 1975). Moreover, the aphid histone genes were spaced by differently long spacers that separate the histone genes within the histone unit. This is an unusual feature in insects since, as reported for instance in flies, histone genes are organized into repeats of 4.8 and 5.0 kb that did not varied in size comparing more than 20 Drosophila strains (Strausbaugh & Weinberg, 1982), with some exceptions to this rule noted in D. americana and D. virilis, where repeating histone clusters consisting of only the core histones coexist with the quintet repeat (Nagoda et al., 2005). Interestingly, histone gene repeats have been shown to be of variable length in S. purpuratus, where variations have been found to occur at many regions of the repeat and in particular at the spacer regions (Overton & Weinberg, 1978). However, contrarily to what observed in the ribosomal spacer separating the major rDNA genes, the heterogeneity observed in aphids and in the sea urchin is not due to the presence of repeats or other specific class of repeated DNAs interspersed within the histone spacers.

Up till now in the typical insect histone gene cluster, H2A and H2B are generally adjacent and transcribed in opposite directions and similarly genes H3 and H4 constitute a similar oppositely transcribed pair (Roebrdanz et al., 2010). However, there are several exceptions to this general rule. In Drosophila, for instance, H1, H2A and H3 genes are transcribed from the same strand (Matsuou, 2000; Tsunemoto & Matsuou, 2001; Kakita et al., 2003; Nagoda et al., 2005). Interestingly, in the aphid histone cluster H1, H2B and H3 are transcribed in the same strand so that they have the same polarity previously observed in A. mellifera and, more recently, in some coleopteran species belonging to the genus Anthonomus, even if these beetles have the H3/H4 and H2A/H2B pairs in switched positions in respect to the H1 gene and they possess a histone order (H4H3)(H2AH2B)(H1), in place of the more common (H2BH2A)(H4H3)(H1) (Figure 6B).

As a whole, the presence of highly conserved coding regions within the histone genes could furnish new opportunities to develop probes for labelling chromosomal markers for the study of chromosome changes during aphid evolution. Interestingly, despite the holocentric nature of the aphid chromosomes that could facilitate chromosome rearrangements (including translocations) (Monti et al., 2012), the similarity in the chromosomal distribution of major rDNA, 5S rDNA and core histone gene clusters suggests that a substantial synteny of gene order and orientation could be present in Aphidinae, as previously suggested by the International Aphid Genomics Consortium (2008). However, further studies will be necessary to properly evaluate the size of the conserved syntenic blocks of genes that could be very small, as previously reported in the holocentric chromosomes of the moths B. morti, S. rugipiera and H. armigera, where it has been estimated a chromosomal evolution rate (approximately two chromosome breaks per Mb DNA per My) much higher than among Drosophila species and that have been related to the holocentric nature of the lepidopteran genomes (d’Alençon et al., 2010).

A definitive response about the evolution rate of the aphid chromosomes therefore deserve for further analyses that could be greatly favoured by the inclusion of the chromosomal mapping of the identified contigs/scaffolds in the main goals of the ongoing genome projects of aphids.

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