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

GENETICS

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. *A. pisum* histone genes are organized in a quintet with the H1 gene followed by H2A and H2B genes that are adjacent and transcribed in same directions, in the opposite strand in respect to the H1 gene. At the 3' end of the histone cluster, genes H3 and H4 constitute an oppositely transcribed pair. The span of the aphid histone genes (more than 7 kb) is greater than the average length of the histone cluster till now reported in insects (about 5 kb). 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.

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Contributions: MM performed the bioinformatic analyses and the chromosomal mapping; GCM analysed the obtained data and performed the cytogenetic analyses; both collaborated to manuscript writing and revision.

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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 fulfil 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 virilis,* where a repeating quartet consisting of the core histones only coexists with the quintet repeat (Schienman *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 cytogenetically mapped in few taxa, with a better detail in Coleoptera and Diptera (Pardue *et al*., 1977; Hankeln *et al*., 1993; Schienman *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 telomere 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 mais* plants. Specimens of *Myzus persicae* were obtained from the laboratory population labelled as *clone 1*, 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 disgregation 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'-ACCACCAAGGCAAACGTATC) and H1-R (5'-CGAGACCTTGG CTGGTTTAG), 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'-AAAATCGAAGGGAGGCAAAT)/H2A-R (5'-AAGAGGACGGCTTGGATGTT) and H2B-F (5'-TGAAGAAATCGTCCGGAAAG)/H2B-R (5'-CGGCCTTAGTTCCTTCACTG), designed on the basis of the *A. pisum* sequences H2A (NM_001163992.1) and H2B (XP_003241030) 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'-ATGGCACGTACCAAGCAAAC) and H3-R (5'-AGGTTGGTGTCCTC GAACAG), 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'-GAATTGTGGCCTCACACACC)/H4-R (5'-CGTACAGTGTTCGGTCTTGG), 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'-CGAAACCGTAAAGGGTACGA) and HIS-CLUST-R (5'-GGCGGCTTTGACTTTATTGA) have been designed on the basis of the *A. pisum* unplaced genomic scaffold 368 (NW_003383857.1, from base 259987 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 for 25 cycles, according to the manufacturer's instructions.

The 5S rDNA repeat unit of *A. pisum* was amplified by PCR using two primers, F (5'-TGCACGTAGTGTTCCCAAGC) and R (5'-ACGACCATACCACGTTGAATAC), deduced from the 5S 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 5S genes as probes were performed as described by Mandrioli *et al.* (2011), whereas staining with chromomycin A_3 (CMA_3) 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.vivo.colostate. 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 ortologues 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_001952 706.2, 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_001 947771.1) have been predicted in *A. pisum* differing each other for a single substitution of an amino acidic 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)

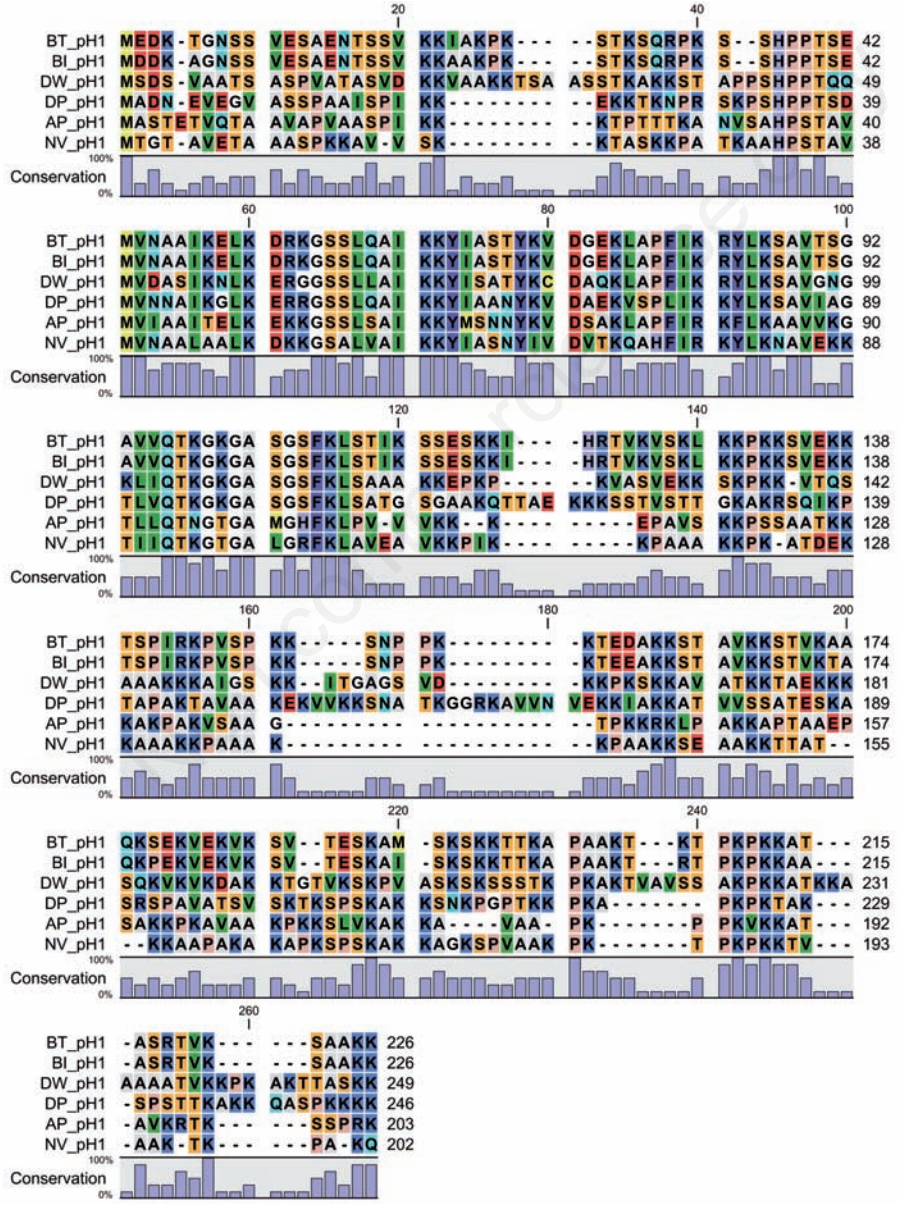

Figure 1. Alignment of the aphid H1 histone amino acidic sequence (AP) with ortologues in *Drosophila willistoni* (DW), *Drosophila ponderosae* (DP), *Nasonia vitripennis* (NV), *Bombus impatiens* (BI) and *Bombus terrestris* (BT). Bars below the alignment show the conservation percentages of each amino 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. melangaster* 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

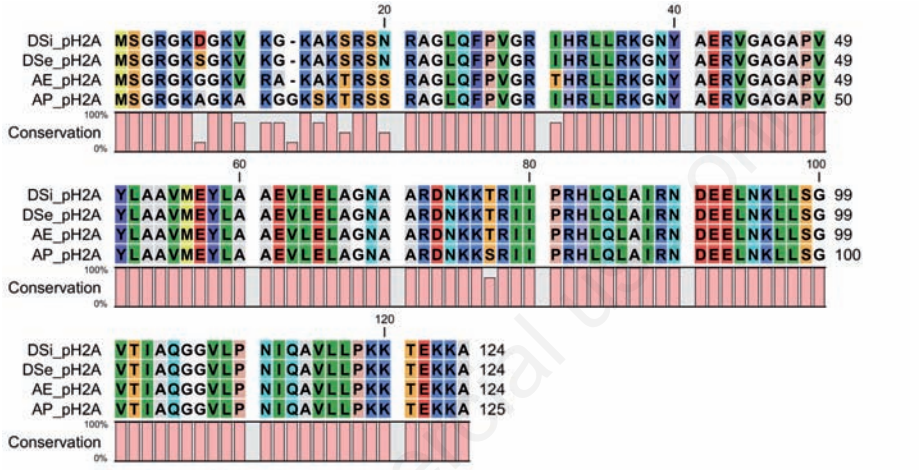

Figure 2. Alignment of the aphid H2A histone protein (AP) with orthologous sequences in *Drosophila sechellia* (DSe), *Drosophila simulans* (DSi) and *Aedes aegypti* (AE). Bars below the alignment show the conservation percentages of each amino acidic residue in the analyzed species.

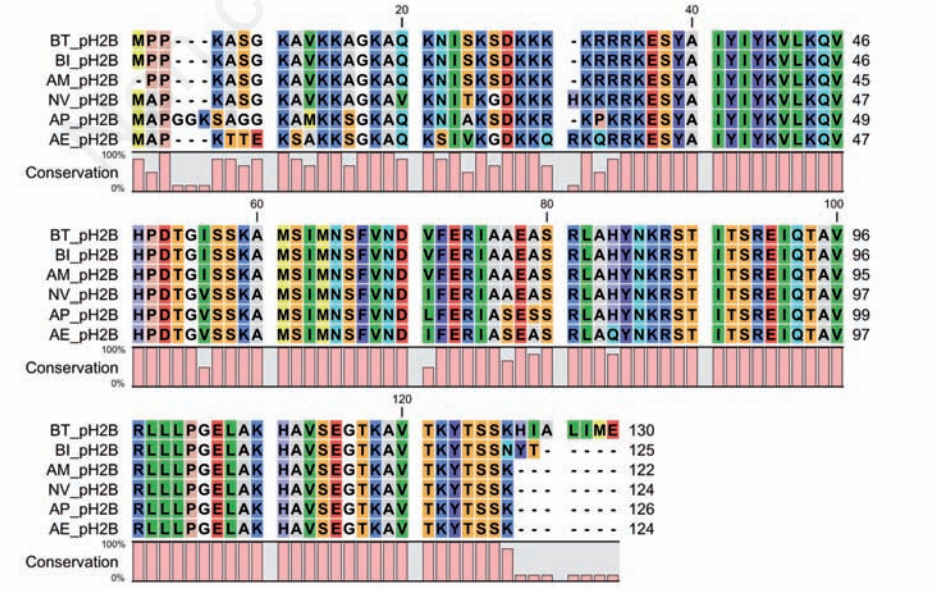

Figure 3. Alignment of the aphid H2B histone amino acidic sequence (AP) with orthologues in *Apis mellifera* (AM), *Bombus impatiens* (BI), *Bombus terrestris* (BT), *Nasonia vitripennis* (NV) and *A. aegypti* (AE). Bars below the alignment show the conservation percentages of each amino acidic residue in the analyzed species.

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,

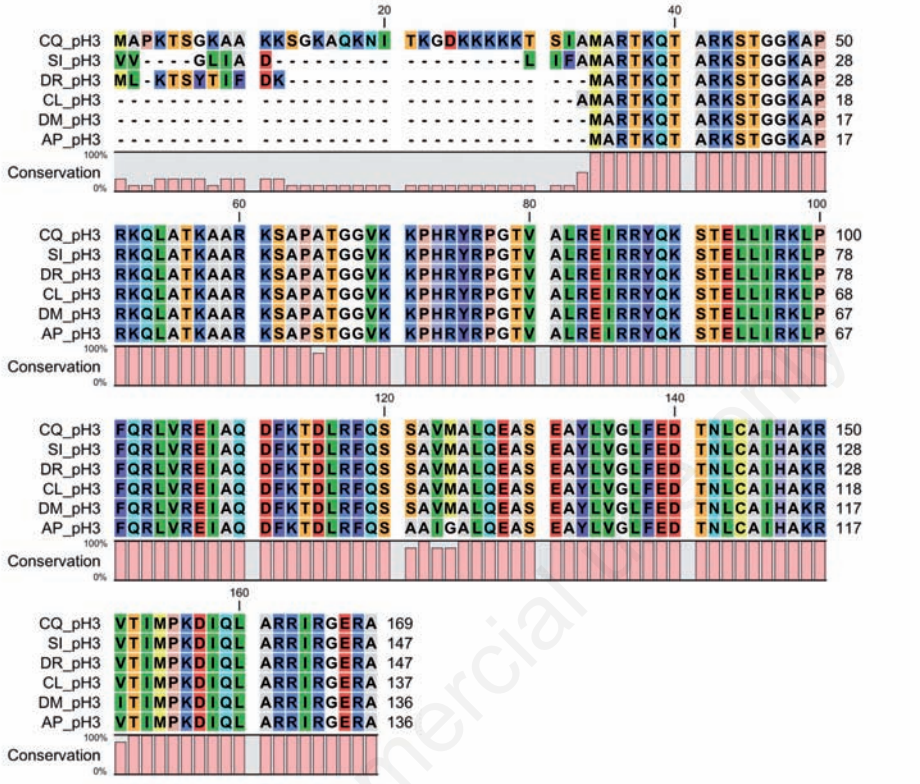

Figure 4. Alignment of the aphid H3 histone protein (AP) with orthologous sequences in *D. melangaster* (DM), *Culex quinquefasciatus* (CQ), *Solenopsis invicta* (SI), *Danio rerio* (DR) and *Columba livia* (CL). Bars below the alignment show the conservation percentages of each amino acidic residue in the analyzed species.

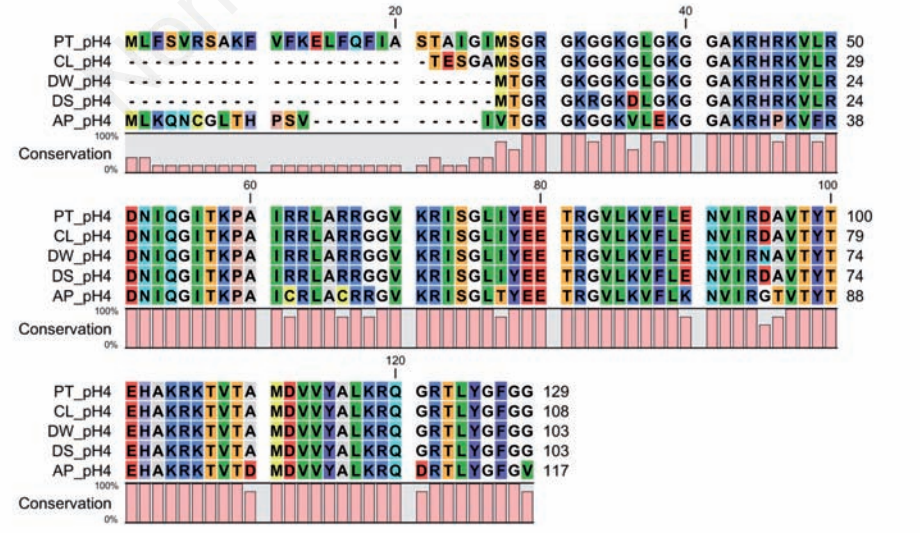

Figure 5. Alignment of the aphid H4 histone amino acidic sequence (AP) with orthologues in *Drosophila willistoni* (DW), *Drosophila sechellia* (DS), *Pan troglodytes* (PT) and *Columba livia* (CL). Bars below the alignment show the conservation percentages of each amino acidic residue in the analyzed species.

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 CMA_3 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 CMA_3 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 CMA_3 (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).

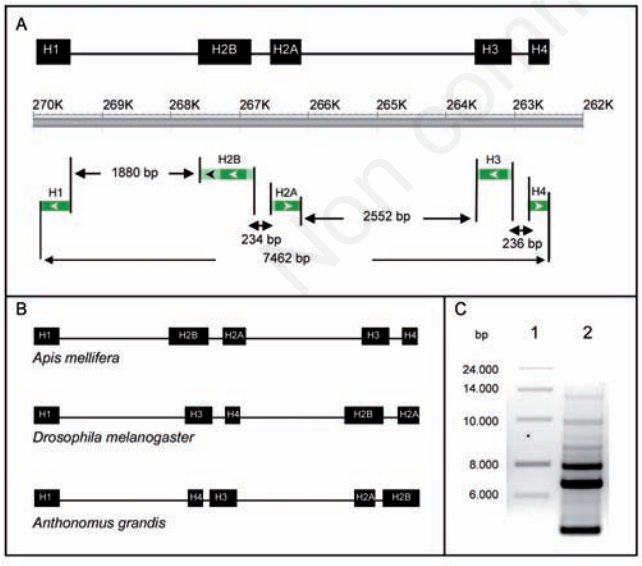

Figure 6. The histone quintet identified in *A. pisum* (A) has the same organization identified in *A. mellifera* and few differences in comparison to the fly *D. melanogaster* and the beetle *A. grandis* (B). Contrarily to what observed in other insect species, the spacers that separate the histone genes within the cluster vary in length as assessed by the presence of different amplified bands after PCR amplification (C, lane 2) (their size has been evaluated using the ultra lane 1Kb DNA ladder, lane 1).

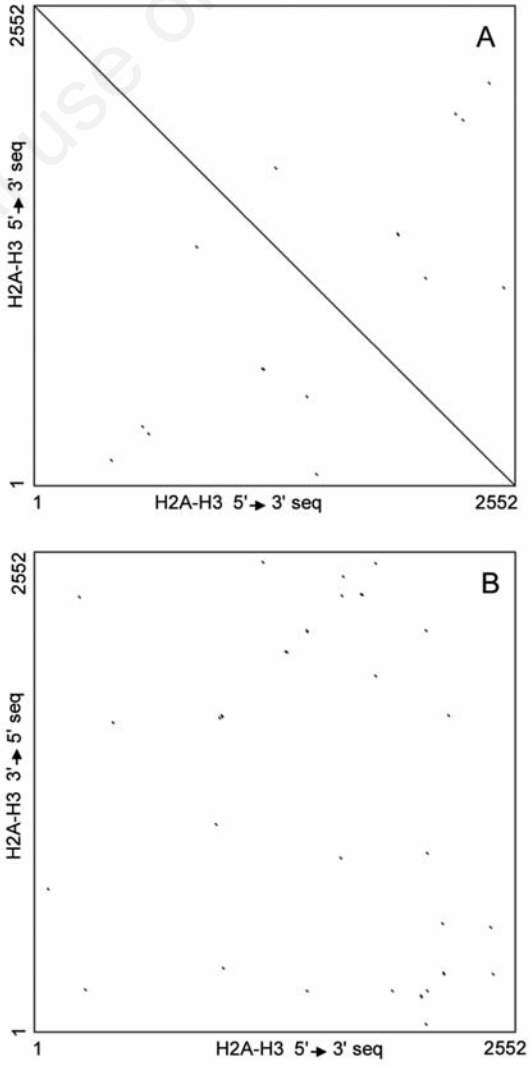

Figure 7. Dot plot analyses of the H2A-H3 spacer indicate that no direct (A) or indirect (B) tandem repeats are present within the spacer sequence. Dot plot windows size = 11.

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 a 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-

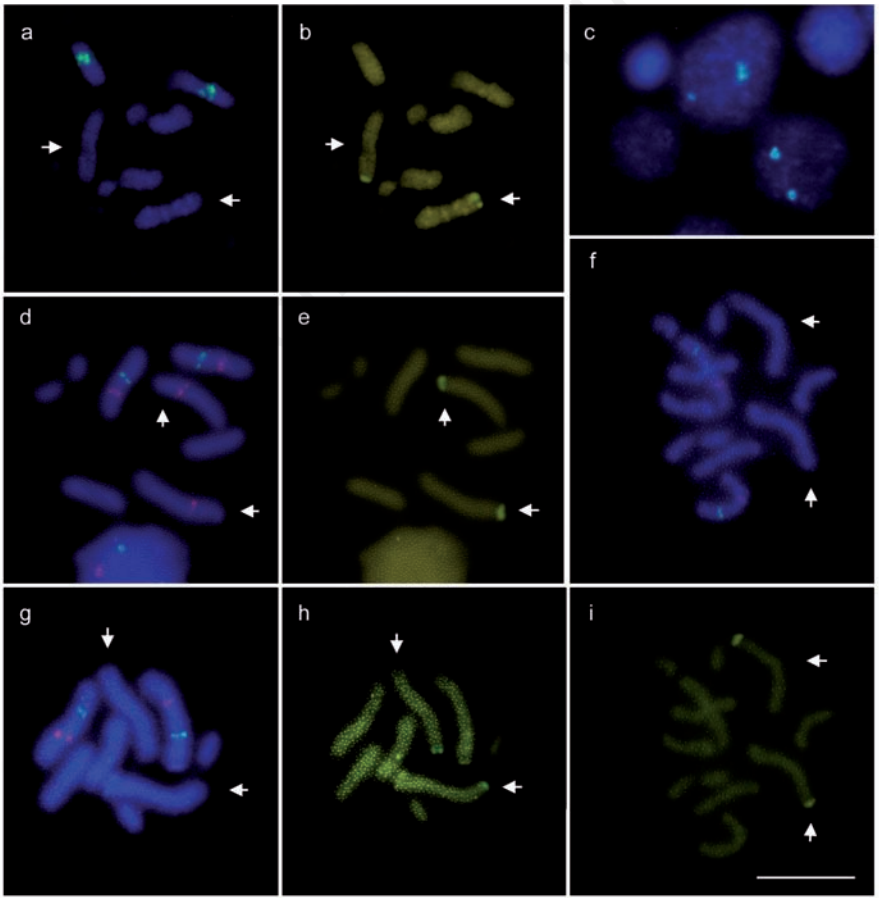

Figure 8. FISH hybridization on DAPI stained chromosomes of the aphids *A. pisum* (a, d), *M. persicae* (f) and *R. padi* (g) with the H3 probe alone (a) or with the simultaneous use of the FITC-labelled H3 (in green) and the Texas red-labelled 5S rDNA probes (in red) (d, f, g), followed by CMA_3 staining 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 (Lifton *et al.*, 1978), but is similar to what reported in other eukaryotes, such as the sea urchin *Strongylocentrotus purpuratus,* where the histone genes were intermingled in a 7 kilobase (kb) repeat unit (Kedes *et al*., 1975). Moreover, the aphis 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. virilise,* where repeating histone clusters consisting of only the core histones coexists 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 (Roehrdanz *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 (Matsuo, 2000; Tsunemoto & Matsuo, 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. mori*, *S. frugiperda* 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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