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Histone H1 Genes and Histone Gene Clusters in the Genus *Drosophila*

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Abstract. Whereas the genomes of many organisms contain several nonallelic types of linker histone genes, one single histone H1 type is known in *Drosophila melanogaster* that occurs in about 100 copies per genome. Amplification of H1 gene sequences from genomic DNA of wild type strains of *D. melanogaster* from Oregon, Australia, and central Africa yielded numerous clones that all exhibited restriction patterns identical to each other and to those of the known H1 gene sequence. Nucleotide sequences encoding the evolutionarily variable domains of H1 were determined in two gene copies of strain Niamey from central Africa and were found to be identical to the known H1 sequence. Most likely therefore, the translated sequences of *D. melanogaster* H1 genes do not exhibit intragenomic or intergenomic variations.

In contrast, three different histone H1 genes were isolated from *D. virilis* and found to encode proteins that differ remarkably from each other and from the H1 of *D. melanogaster* and *D. hydei*. About 40 copies of H1 genes are organized in the *D. virilis* genome with copies of core histone genes in gene quintets that were found to be located in band 25F of chromosome 2. Another type of histone gene cluster is present in about 15 copies per genome and contains a variable intergenic sequence instead of an H1 gene. The H1 heterogeneity in *D. virilis* may have arisen from higher recombination rates than occur near the H1 locus in *D. melanogaster* and might provide a basis for formation of different chromatin subtypes.

Key words: *Drosophila melanogaster* — *Drosophila virilis* — Histone H1 — Histone genes — Linker histones — Mobile elements — Penelope

Introduction

Many organisms and cell types contain several different subtypes of histone H1 (Cole 1987; Wells and McBride 1989; Schulze and Schulze 1995). Indirect immunofluorescence with specific antibodies has revealed that different H1 subtypes are distinctly distributed in polytene chromosomes and may establish chromatin domains with different structures (Mohr et al. 1989; Schulze et al. 1993, 1994). In *Drosophila melanogaster,* on the other hand, H1 heterogeneity does not appear to play a role. Wild type strains contain about 100 copies per genome of a single H1 gene type (Lifton et al. 1977). These genes are arranged in clusters of quintets that comprise one copy of H1 and of each of the four core histone genes (Matsuo and Yamazaki 1989).

Two fractions of H1 proteins have earlier been identified in *D. virilis,* each of which appears in a phosphorylated and a dephosphorylated form (Blumenfeld et al. 1978). They were found to interact in vitro in a differential way with the three different satellite DNA sequences that make up 41% of the *D. virilis* genome and that are located in the "heterochromatic" sections of the chromosomes (Blumenfeld et al. 1978). Possibly, therefore, H1 proteins and/or their phosphorylated modifications could contribute to the formation of different types of chromatin in vivo in this organism.

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We have studied the extent of intra- and intergenomic variation of the H1 gene sequence in *D. melanogaster* strains of different origin, including North America and Australia, as well as central Africa, from where *D. melanogaster* is supposed to have originated (David and Capy 1988). The open reading frames of all H1 clones obtained from genomic DNA were found to be identical to the *D. melanogaster* H1 sequence reported earlier (Murphy and Blumenfeld 1986; Matsuo and Yamazaki 1989). In contrast, we have identified and sequenced three different H1 genes in *D. virilis.* They were found to be arranged in one chromosome locus in about 40 quintets with core histone genes. The same locus also comprises about 15 quartets of core histone genes without H1 gene copies.

Materials and Methods

Drosophila *Strains and Library*

D. melanogaster strain Oregon-R (North America) was obtained from G. Reuter (Halle), strain Cardwell (Australia) from the European Drosophila stock center (Umeå, Sweden), and strain Niamey (central Africa) from J.R. David (CNRS, Gif-sur Yvette). The *D. virilis* strains Bochum and Okenoshima were a gift of H. Kress (Berlin). The library of genomic *D. virilis* DNA was constructed by H. Kress by cloning partially Sau3 AI-restricted DNA into the XhoI-site of phage lambda FixTMII and was kindly provided by him.

Isolation and Sequencing of Histone Genes, PCR

Genomic DNA was isolated from flies according to McGinnis et al. (1983). Complete H1 genes were amplified by the PCR with primer oligonucleotides that were synthesized according to a conserved 5′-region and to the 3′-hairpin element (Birnstiel et al. 1985), respectively. For *D. melanogaster* these oligonucleotides were GCYYTCCTCCTCGATTCTCA and CCTGAAAAGGAYTTGTTTWARMAAATTTC; for *D. hydei* TCCTCCTCGATTCTCA and ATTTKGTAGTCCTGAAAAGGACT; and for *D. virilis* TCCTCCTCGATTCTCACTSAAAC (SNPCR6) and CCTKAAAAGGSMTTTGTWKRAWARMAAATTTC (SNPCR5).

The organization of histone genes in phage clone 122 was determined by means of the products of the PCR with the T7 primer oligonucleotide, GTAATACGACTCACTATAGGGC; the *D. virilis* H1 primer oligonucleotide SNPCR6 (see above); an oligonucleotide, AARGCNATGAGAYATHATGAA, constructed according to a sequence within the protein encoding region of the H2B gene (ESPCR12); an oligonucleotide, ATGCCNAARGAYATHCARYTNGC, constructed according to a sequence within the protein encoding region of the H3 gene; and an oligonucleotide constructed according to a sequence within the protein encoding region of the H4 gene (GAAGACAGTCACAGCCATGG). The lengths of the PCR products and their restriction by StuI and XbaI yielded the map of the gene cluster. The intergenic sequence between the histone H2B gene and the histone H3 gene was amplified with primer oligonucleotide ESPCR12 (see above) and the oligonucleotide that corresponded to a sequence within the histone H3 gene (see above). These and other primers described above were also used for amplification of other intergenic regions.

The PCR (Saiki et al. 1988) was performed in 25- or 50-μl samples containing 0.04 m*M* dNTP, 1× buffer (Eurogentec), 1–2 m*M* $MgCl_2$, 50 pg DNA, 1 pmol of each oligonucleotide, and 1 U taq polymerase for analytical PCR or pfu polymerase for cloning, for 35 cycles at 92°C for 2 min; then at 40°C, 45°C, or 50°C for 2 min; and finally at 72°C for 3, 4, or 10 min, depending on the primers used. The PCR products were separated on 0.7% agarose gels in TAE buffer (Sambrook et al. 1989) and cloned into the Ecl136II-site of pUC18 (Yanish-Perron et al. 1985), according to standard protocols (Sambrook et al. 1989). DNA was sequenced according to Sanger et al. (1977) with the Amersham USB sequencing kit. Sequence analyses were performed by the program package PC Gene (release 6.7, Intelligenetics) and alignments and similarity analyses by the program of Myers and Miller (1989). For comparisons with sequences from gene libraries the BLAST server was used (Altschul et al. 1990).

Southern Blots and Estimation of Gene Copy Numbers

Gene probes were hybridized on Southern blots (Southern 1975) to 5 μg of genomic DNA for qualitative analysis or to equivalent molar amounts of genomic DNA and plasmid DNA for quantitative analysis. A series of plasmid DNA concentrations equivalent to increasing numbers of gene copies per genome was used for calibration, based on a genome size of *D. virilis* of 0.77 pg (Kavenoff and Zimm 1973). DNA of plasmid clones containing an H1 gene, an H4 gene, or the complete H2B–H3 intergenic sequence, respectively, were digested with restriction enzymes to yield the genomic insert and were separated on agarose gel together with genomic DNA equivalent to 6.5×10^6 genomes. Southern blotting onto Hybond-N was performed according to standard procedures. The gene probes were labeled with [^{32}P]dATP (Feinberg and Vogelstein 1984), and hybridization was visualized by autoradiography. Labeled bands corresponding to fragments of genomic DNA and plasmid inserts were excised from the membrane, and the radioactivity was counted in a liquid scintillation counter. The results were used to draw a calibration curve to determine the number of gene copies per genome. The gene probes used were as follows: (1) A HincII–StuI fragment encoding part of the N-terminal domain and the entire central globular domain of the H1.1 gene (279 bp), (2) a HincII–Ecl136II fragment encoding a complete H4 gene (about 1000 bp), and (3) a SpeI–BspI fragment encoding 406 bp of the H2B-H3 intergenic sequence.

Results and Discussion

Numerous H1 Sequences from Genomic D. melanogaster *DNA Exhibited Identical Restriction Patterns*

In a search for variations of the H1 gene sequence in the *D. melanogaster* genome, primer oligonucleotides were constructed according to flanking sequence stretches that are identical in all known H1 genes of the genus *Drosophila* (see Materials and Methods). These were used as primers in PCR to amplify H1 genes from genomic DNA of *D. melanogaster* strain Oregon-R. The amplified DNA comprises the complete coding region and about 100 bp flanking regions, both upstream and downstream. The PCR product obtained (about 900 bp) was cloned into pUC18, and 18 of the clones were selected for restriction analyses of the inserts. The restriction patterns obtained by EcoRI (no cut), HindIII (one cut), BamHI (one cut), PstI (two cuts), and StuI (no cut), respectively, were found to be identical in all inserts (not shown) and to resemble the patterns derived from the known H1 gene

```
A D.mel. H1           -147  T.AA...AACTT.G...TAGT..ATGACTG..GTC.GT.G....  -104
  D.hyd. H1           -167  T.------..T.....G----........-...G..........  -135
  D.vir. H1.1 (Boc)   -148  AC--GCATTGAGTAACA----AACGTTCA-ATACCAAAAACATT  -112


  D.mel. H1           G.TAAAGT..T.................TCAG.G.-AAAGGA..T..G--  -56
  D.hyd. H1           ....CG....T...................G....-------........  -91
  D.vir. H1.1 (Boc)   CTCGTTTAGCCCTCCTCCTCGATTCTCACTCAAAC-------GGGTGACC  -68
  D.vir. H1.2 (Boc)           -84                ...G....-------........  -68
  D.vir. H1.3 (Boc)          -101  .................G....C------........  -70
  D.vir. H1.1 (Oke)           -86              ..........-------........  -68
  D.vir. H1.2 (Oke)           -84                ...G....-------........  -68
  D.vir. H1.3 (Oke)           -98  ...............T......-------........  -68


  D.mel. H1           ---TAGGCAGCGC-GCGAGC----------CATTTTTAACAGAAA----A  -24
  D.hyd. H1           AAAATGCCAT-GCCGC-AGCTGGCAAATTTC-GATTAAACAAAAACGTTG  -44
  D.vir. H1.1 (Boc)   --------------------TGGTATATTTCTGTTTTCAC----ACCAAA  -42
  D.vir. H1.2 (Boc)   --------------------....................----......  -42
  D.vir. H1.3 (Boc)   --------------------....................----......  -44
  D.vir. H1.1 (Oke)   --------------------....................----......  -42
  D.vir. H1.2 (Oke)   --------------------AAAG.A..GGA.T....TCG----.A....  -42
  D.vir. H1.3 (Oke)   --------------------....................----......  -42


  D.mel. H1           .A......C..--...GA.A..-----...--------------------  -1
  D.hyd. H1           CATT......TAT...GA.G..--......A.GT..-----T...TAAAA  -1
  D.vir. H1.1 (Boc)   ATAGTGTTTTC--AGTTCACAAAAGTAAAGTAAAGC-GC-ACGAA-----  -1
  D.vir. H1.2 (Boc)   ...........--.......................-..-.....-----  -1
  D.vir. H1.3 (Boc)   ...........--.......................C..C.....-----  -1
  D.vir. H1.1 (Oke)   ...........--.......................-..-.....-----  -1
  D.vir. H1.2 (Oke)   TACAA..GAA.--...GA.T..-..G..-......T-.A-.-.TT--AAG  -1
  D.vir. H1.3 (Oke)   ...........--.......................-..-.....-----  -1
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Fig. 1. The nucleotide sequences of the three histone H1 genes H1.1, H1.2, and H1.3 of *D. virilis* (strain Bochum), aligned to the H1 gene sequences of *D. melanogaster* (Murphy and Blumenfeld 1986; Matsuo and Yamazaki 1989) and *D. hydei* (Kremer and Hennig 1990). Dots indicate sequence identities. The numbering is relative to the start codon. The start and stop codons are underlined. The *D. virilis* Bochum sequence data are available from EMBL/GenBank under the accession numbers L76558, U67772, and U67936. Also included are the sequenced regions of the H1.1, H1.2, and H1.3 genes of another strain of *D. virilis,* Okenoshima. Boc: strain Bochum, Oke: strain Okenoshima. *D. mel.: D. melanogaster; D. hyd.: D. hydei; D. vir.: D. virilis.*

sequence of *D. melanogaster* (Murphy and Blumenfeld 1986; Matsuo and Yamazaki 1989).

H1 Gene Sequences from D. melanogaster *Strains of Different Geographic Origin*

The histone H1 gene sequences in a North American strain (Murphy and Blumenfeld 1986) and a Japanese strain (Matsuo and Yamazaki 1989) of *D. melanogaster* are identical. To investigate interstrain variations between strains collected at geographically distant locations, we have amplified H1 sequences from genomic DNA of the *D. melanogaster* strains Cardwell from Australia and Niamey from central Africa. The PCR products were cloned into pUC18. The inserts of 11 clones obtained from strain Cardwell and of 3 clones from strain Niamey were restricted by EcoRI, HindIII, BamHI, PstI, and StuI, respectively, and were found to yield restriction patterns identical to those obtained from all the H1 clones of strain Oregon-R and to the patterns expected for the originally (Murphy and Blumenfeld 1986, Matsuo and Yamazaki 1989) sequenced H1 genes (not shown).

As *D. melanogaster* is thought to have originated from central Africa (David and Capy 1988), the amplified H1 sequences from strain Niamey are of special interest. We have therefore sequenced 340 bp in the 5′-region and 250 bp in the 3′-region of H1 genes from two

```
B D.mel. H1            .............................T..C..A...GC.....C..C   50
  D.hyd. H1            ...........C.TT......GTA..T..G.....C....C.C.....A.   50
  D.vir. H1.1 (Boc)    ATGTCTGATTCTGCAGTTGCAACGTCCGCATCTCCTGTGATTGCCCAAGC   50
  D.vir. H1.2 (Boc)    ..................................................   50
  D.vir. H1.3 (Boc)    ...................................C.......T......   50
  D.vir. H1.1 (Oke)    ..................................................   50
  D.vir. H1.2 (Oke)    ...........C......................................   50
  D.vir. H1.3 (Oke)    ..................................................   50


  D.mel. H1            ...GA...TT.....G..A...GTC-.A..........TC..GAT.TG.T   99
  D.hyd. H1            ........CT.....G.....AG.T........-.C.....T.CG..T.C   99
  D.vir. H1.1 (Boc)    AGCCTCAGGCGAGAAAAAGGTGTCTACTAAAAAAGGCAGCAGC-ACACCA   99
  D.vir. H1.2 (Boc)    .................................-.........C......   99
  D.vir. H1.3 (Boc)    .................................-.........C......   99
  D.vir. H1.1 (Oke)    ...........................................-......   99
  D.vir. H1.2 (Oke)    ......................G....................GC.....  100
  D.vir. H1.3 (Oke)    .................................-.........C....--   99


  D.mel. H1            .GCA.....GCA...AA.G.CT.T..GA....G..A..T..G........  149
  D.hyd. H1            .C...G...GCA...AA....A.G.....T...A.A..............  149
  D.vir. H1.1 (Boc)    GAATCAAAG---AAGTCAACAGCAGCTCCGCCATCGCACCCACCAACTCA  146
  D.vir. H1.2 (Boc)    A........---......................................  146
  D.vir. H1.3 (Boc)    A........---......................................  146
  D.vir. H1.1 (Oke)    .........---......................................  146
  D.vir. H1.2 (Oke)    ACGAGTCAAAG-...........---...C....................  146
  D.vir. H1.3 (Oke)    .........---......................................  146
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Fig. 1. Continued.

of the Niamey strain PCR clones. The first region includes a 5′-flanking stretch of about 100 bp (not shown) and adjacent 240 bp that encode the N-terminal domain and part of the central globular domain. The latter region includes 155 bp that encode part of the C-terminal domain and the stop codon and a 3′-flanking stretch of about 100 bp. Heterogeneities of protein structure would have been expected to occur primarily within these two regions, because the N-terminal and the C-terminal domain comprise the main regions of intragenomic, intraspecific, and interspecific variability in H1 (Wells and McBride 1989). The nucleotide sequences were found to be identical in both clones and identical to that of the originally sequenced H1 genes in North American and Japanese strains (Murphy and Blumenfeld 1986; Matsuo and Yamazaki 1989), except for a short stretch of three nucleotides immediately preceding the start codon (not shown).

These results do not indicate any intragenomic or intergenomic variation of the H1 histone primary structure in *D. melanogaster,* in spite of the fact that the genomic DNA was obtained from strains of very different origin. The analyzed H1 genes appear to be monomorphic, and their coding region has probably not been mutated since the *D. melanogaster* populations in North America, Japan, Australia, and central Africa became separated.

D. virilis *Contains Three Different Types of H1 Genes*

The strategy that did not yield any indication of H1 sequence variation in *D. melanogaster* allowed the identification and characterization of three different H1 sequence variants in *D. virilis.* Initial attempts to amplify H1 sequences from genomic DNA of *D. virilis,* by oligonucleotide primers constructed according to conserved sequences of histone H1 genes from other species, were not successful. We therefore chose to first amplify and clone a sequence from the related species *D. hydei* that contains the flanking and coding regions of an H1 gene of that organism. A genomic library of *D. virilis* strain Bochum screened with the *D. hydei* sequence then yielded a clone (122), the insert of which contained a complete H1 gene within a 3.4 kb Ecl136II restriction fragment. The coding sequence and the 5′-flanking 250 bp and 3′-flanking 200 bp were sequenced (Fig. 1), and the gene was denominated *D. virilis* H1.1 (GenBank accession no. L76558).

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C D.mel. H1             .........G..C..T..C..T........A...........C..T....  199
  D.hyd. H1             ...G.....G...........AC.......A...................  199
  D.vir. H1.1 (Boc)     GCAAATGGTAGATGCATCGATTAAAAATTTGAAGGAACGTGGTGGCTCAT  196
  D.vir. H1.2 (Boc)     ..................................................  196
  D.vir. H1.3 (Boc)     ..................................................  196
  D.vir. H1.1 (Oke)     .............                                       159
  D.vir. H1.2 (Oke)     ..............................A...................  196
  D.vir. H1.3 (Oke)     ..................................................  196


  D.mel. H1             .A...........C............AC...C..T..T..A.....C...  249
  D.hyd. H1             .....T....T..A..G.........A....C..T...............  249
  D.vir. H1.1 (Boc)     CCCTTCTGGCAATTAAAAAATATATCGGTGCTACATACAAGTGCGATGCC  246
  D.vir. H1.2 (Boc)     ..................................................  246
  D.vir. H1.3 (Boc)     ..................................................  246
  D.vir. H1.2 (Oke)     ..................................................  246
  D.vir. H1.3 (Oke)     ............................                        224


  D.mel. H1             ..A...T....G..A.....C..G......T.A..ATCG..C..G.TC..  299
  D.hyd. H1             ..A..A........A........G........G......T..........  299
  D.vir. H1.1 (Boc)     CAGAAGCTAGCCCCGTTCATTAAAAAGTACCTTAAGAATGCAGTTGCGAA  296
  D.vir. H1.2 (Boc)     .......................G........A........G........  296
  D.vir. H1.3 (Boc)     ..................................................  296


  D.mel. H1             ......GC.T..T.....T.....A........A.....A..T..C....  349
  D.hyd. H1             ......GT.A..T....................A..............GT  349
  D.vir. H1.1 (Boc)     TGGAAACGTGATCCAAACAAAGGGCAAGGGTGCCTCTGGTTCGTTTAAAC  346
  D.vir. H1.2 (Boc)     ......G.............................C.............  346
  D.vir. H1.3 (Boc)     ......G...........................................  346
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Fig. 1. Continued.

Two additional H1 genes of *D. virilis* Bochum were isolated from genomic DNA by a PCR procedure with primer oligonucleotides constructed according to 5′-flanking (oligonucleotide SNPCR6) and 3′-flanking (oligonucleotide SNPCR5) sequences of the *D. virilis* H1.1 gene and of the H1 genes of *D. melanogaster* (Matsuo and Yamazaki 1989) and *D. hydei* (Kremer and Hennig 1990). The PCR product (about 900 bp) was cloned into pUC18, and 15 clones obtained were restricted with EcoRI, HindIII, BamHI, PstI, or StuI, respectively. Whereas most restriction sites were identical in all clones, four of the inserts contained a BamHI site and three others contained a HindIII site not present in the H1.1 gene. Sequencing of the inserts of one of the clones of each group yielded two new H1 genes, which were designated H1.2 and H1.3 (GenBank accession nos. U67772 and U67936).

Figure 1 shows the nucleotide sequences of the three H1 genes of *D. virilis* strain Bochum, and Figure 2 the derived amino acid sequences aligned with those of *D. melanogaster* and *D. hydei* H1. The three *D. virilis* H1 genes exhibit in the coding regions nucleotide sequence identities of 94.9% (H1.1 versus H1.2), 96.0% (H1.1 versus H1.3), and 95.2% (H1.2 versus H1.3). The derived amino acid sequences exhibit a number of substitutions in the N-terminal and C-terminal domains, which include several nonconservative replacements and result in charge differences. Substitutions also occur within the conserved globular domain. In total, the sequence identities on the amino acid level were 93.6% (H1.1 versus H1.2), 94.8% (H1.1 versus H1.3), and 95.2% (H1.2 versus H1.3). Interestingly, the DNA sequence is more conserved than the protein sequence. All three H1 proteins of *D. virilis* differ considerably from the H1 sequence of *D. melanogaster* (sequence identity 63.6% versus H1.1) and also from the H1 of *D. hydei* (77.8% versus H1.1).

As one uniform H1 sequence is found in *D. melanogaster* of diverse geographic origin, we were interested to learn whether the presence of three different types of H1 genes is restricted to strain Bochum or is also found in other strains of *D. virilis*. We therefore amplified H1 genes from genomic DNA of the *D. virilis* wild type

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D D.mel. H1           .G..G..C..T..C..G.....AAAGG.T..G...----.AAA.....A  395
  D.hyd. H1           .G.......TT.C..A.....GC.T.....T....TG...T....A...  399
  D.vir. H1.1 (Boc)   TATCTGCATCCGCTAACAAGGATGCCAAGCCAAAGGCCTCCGCTGTCGAG 396
  D.vir. H1.2 (Boc)   .....CG.........A......C.......................... 396
  D.vir. H1.3 (Boc)   .....CG.........A................................. 396


  D.mel. H1           GGTTTTGT.TGCTGA.A...AAG...AAA.CA.GA..GT.G.CTCT.AG. 445
  D.hyd. H1           ......T...........---.....T.....GC..-..G......AG.  445
  D.vir. H1.1 (Boc)   AAGAAAACCAAGAAGGTAAATGCTTCGGCGGCACGAG-CAACTAAAAGCA 445
  D.vir. H1.2 (Boc)   ........T.......G..............CG..-.........AG.  445
  D.vir. H1.3 (Boc)   ...............................GC..-..........GT  445


  D.mel. H1           ...T.G..GTC..CT...AA....CT..C.TT..G..T.....C...... 494
  D.hyd. H1           .A.C..---TA...G.T..C..G...C..AA.....T.........G... 491
  D.vir. H1.1 (Boc)   AGAGTAGTACGTCTACCACGAAAAAAGCAGCGGGTGCCGCTGATAAAAAG 494
  D.vir. H1.2 (Boc)   ....................G............................. 494
  D.vir. H1.3 (Boc)   CA..................G............................. 494


  D.mel. H1           .CCAAAGCT..GA.GG.T.TG..TA.......G.C..CC..A..T..... 544
  D.hyd. H1           T.----G..G.--.GG.T.TG.TGA.......G.G......C..G..... 535
  D.vir. H1.1 (Boc)   CT----ATCAA--AATCCGCAGCAGCCAAAAAAAATGTTGAGAAAAAGAA 538
  D.vir. H1.2 (Boc)   ..----.....--........C..A....G..G.G..............G 538
  D.vir. H1.3 (Boc)   ..----.....--..................................... 538


  D.mel. H1           AA....G..G.C...A..C........C........T....TC......T 594
  D.hyd. H1           A.....A....CG.....C..A.....A......GTT..T..T..T..A. 585
  D.vir. H1.1 (Boc)   GGCTGACAAAGAAAAGGCTAAGGATGCGAAGAAAACGGGAACCATAAAGG 588
  D.vir. H1.2 (Boc)   ...G......C.......................A............... 588
  D.vir. H1.3 (Boc)   .................................................. 588
  D.vir. H1.1 (Oke)                               ...................... 22
  D.vir. H1.3 (Oke)                               ...................... 22
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Fig. 1. Continued.

strain Okenoshima (Japan), with the 5′- and 3′-flanking sequences (SNPCR6 and SNPCR5, see Materials and Methods) as primers. Cloning of the PCR product (about 900 bp) again yielded clones with three types of inserts: one type with a BamHI restriction site (2 clones), one type with a HindIII site (2 clones), and a third type without these sites (15 clones). Subcloning and sequencing of the 5′-regions (about 330 bp, including a stretch of about 90 noncoding bp) and the 3′-regions (about 250 bp, including about 60 noncoding bp) of the inserts of one clone of each type showed that strain Okenoshima also contains three types of H1 genes (Figure 1). Two of them, H1.1 and H1.3, are almost identical in the two strains of *D. virilis* as far as the sequenced stretches are concerned, whereas the third one (H1.2) in strain Okenoshima is more divergent from H1.2 in strain Bochum and from the other H1 genes, with the highest extent of divergency occurring in the 5′ noncoding region.

The H1 gene polymorphism in *D. virilis* has no counterpart in *D. melanogaster.* The three different H1 genes in *D. virilis* were easily identified by their individual restriction patterns, whereas in *D. melanogaster* the same restriction analysis yielded one uniform type of H1 sequence. Two of the three types of H1 genes may encode the two fractions of H1 proteins of *D. virilis* that were earlier identified by Blumenfeld et al. (1978) and found to interact differentially with different satellite DNA sequences in vitro.

Since these H1 gene sequences of *D. virilis* have been communicated (Nagel 1996) and made available in GenBank (1996), Schienman et al. (1998) have used this information to construct H1 variant-specific primers to study the distribution of the different H1 sequence vari-

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E D.mel. H1              .G.....CG.CG..A.......G..AGTG.CT...G.G.........G.T  644
  D.hyd. H1              .......C..............A............C..........A..T  635
  D.vir. H1.1 (Boc)      CCAAGCCTACAACAGCAAAGGCTAAGTCAAGCGCAACAAAGCCAAAGACC  638
  D.vir. H1.2 (Boc)      .......C..........................................  638
  D.vir. H1.3 (Boc)      ......T...........................................  638
  D.vir. H1.1 (Oke)      ..................................................   72
  D.vir. H1.2 (Oke)                     ...................................   35
  D.vir. H1.3 (Oke)      ......T...........................................   72


  D.mel. H1              GTAGT.G.G..AG.GT...AG..A..G..AGCGGTGTCT.CAAAAC.C.A  694
  D.hyd. H1              .................C........G..T..G..G..T..CG.....G.  685
  D.vir. H1.1 (Boc)      CCTAAACCTAAGACCACAAGTGCTAAACCGAAAAAAGTCGTGTCGGCTAC  688
  D.vir. H1.2 (Boc)      ..A..G..........A.................................  688
  D.vir. H1.3 (Boc)      ..................................................  688
  D.vir. H1.1 (Oke)      ...............C..................................  122
  D.vir. H1.2 (Oke)      ..A..G............................................   85
  D.vir. H1.3 (Oke)      ..................................................  122


  D.mel. H1              AAA...GGTG......G.ATCGGTTTCTGC.A.C.C.........G....  744
  D.hyd. H1              ---.T.G..A..A...G.------------.....C............G.  720
  D.vir. H1.1 (Boc)      ---GACCCCCAAGAAAAC------------TGCTGTCAAGAAGCCAAAAG  723
  D.vir. H1.2 (Boc)      ---...............------------..................G.  723
  D.vir. H1.3 (Boc)      ---...............------------....................  723
  D.vir. H1.1 (Oke)      ---...............------------....................  157
  D.vir. H1.2 (Oke)      ---...............------------....................  120
  D.vir. H1.3 (Oke)      ---...............------------....................  157
```

Fig. 1. Continued.

ants among clones of a *D. virilis* P1 library. Whereas the strain used probably did not contain H1.3 genes, Schienman et al. (1998) found that 7 clones out of 10 P1 clones contained only H1.1 gene sequences and 2 clones contained only H1.2 sequences, whereas one clone contained both types of genes. The authors conclude that H1 genes of a single variant type are often contiguous and speculate that the majority of H1 genes at a chromosome locus may be of a single variant type.

Genomic Organization of the H1 Genes of D. virilis

A cluster of histone genes has earlier been characterized from the genome of *D. virilis* (Domier et al. 1986). It contains the four core histone genes arranged in the same order as in the histone gene quintets of *D. melanogaster* (Lifton et al. 1977; Matsuo and Yamazaki 1989) but lacks the H1 gene (Domier et al. 1986). We have therefore investigated the genomic organization of the H1 genes in *D. virilis* by analyzing the total phage insert containing the H1.1 gene (genomic library clone 122, see above). Cleavage of the insert by the restriction enzyme Ecl136II, which also cleaves in the phage cloning site, yielded four fragments of 3.4 kb, 2.3 kb, 1.4 kb, and 1.2 kb size, respectively. The fragments were subclones and sequenced in part and were found to contain all core histone genes in addition to the H1.1 gene (Nagel 1996; Fig. 3). Their relative order and orientation was unraveled by means of PCR products that were generated by oligonucleotide primers constructed according to sequences within the H1, H3, H4, and H2B genes, respectively (not shown, see Materials and Methods). The H2A sequence was incomplete and comprised the C-terminal domain only. Alignment with the histone gene clusters of *D. melanogaster* and *D. hydei* (Fig. 4) shows a corresponding order and orientation of the *D. virilis* histone genes within the sequenced gene cluster. Schienman et al. (1998), in a systematic study of the heterogeneity of histone gene clusters in *D. virilis,* obtained corresponding results. The *D. virilis* H3 sequence differs from H3 in *D. melanogaster* and *D. hydei* by an insertion of two amino acid residues (Fig. 5). Within the histone gene cluster, two sequenced stretches of 150 bp and 232 bp, respectively, that exhibit a sequence identity of more than 90% with part of the mobile element Penelope (Evgen'ev et al. 1997) were identified in an intergenic region (Fig. 3).

A second type of histone gene cluster of *D. virilis* that

```
D.mel. H1             .............A.PP.TV....VQ..A.GSAGT.A.KAS.T.......   50
D.hyd. H1             ....V..V.....T.Q..SA....AA..PA.ASA..A.K.T...T.....   50
D.vir. H1.1 (Boc)     MSDSAVATSASPVIAQAASGEKKVSTKKGSSTPESK-KSTAAPPSHPPTQ   49
D.vir. H1.2 (Boc)     ............................AAA..K..-.............   49
D.vir. H1.3 (Boc)     ..............V.............AAA..K..-.............   49
D.vir. H1.1 (Oke)     .................................K..-.............   49
D.vir. H1.2 (Oke)     ........................A......ATPKSK....-........   49
D.vir. H1.3 (Oke)     ............................AAA.....-.............   49

D.mel. H1             .........................T...................S..V.  100
D.hyd. H1             .......Q.................S.....................S...  100
D.vir. H1.1 (Boc)     QMVDASIKNLKERGGSSLLAIKKYIGATYKCDAQKLAPFIKKYLKNAVAN   99
D.vir. H1.2 (Boc)     ..................................................   99
D.vir. H1.3 (Boc)     ..................................................   99
D.vir. H1.1 (Oke)     ....                                                 53
D.vir. H1.2 (Oke)     .................................                    82
D.vir. H1.1 (Oke)     ..........................                           75

D.mel. H1             .KL..............|....K.EKD...KSK.LSAE...QSKKVASK.I  150
D.hyd. H1             .KL..............|...SK.EP...V.S-....S...TS...A.K.K  149
D.vir. H1.1 (Boc)     GNVIQTKGKGASGSFKL|SASANKDAKPKASA-VEKKTKKVNASAARATKS  148
D.vir. H1.2 (Boc)     .KL..............|.R..K..P......-.................K  148
D.vir. H1.3 (Boc)     .KL..............|.R..K.........-.............A...R  148

D.mel. H1             GV.SKK.AVG.ADKKP.AK.AV.T..TA.N..TE.A.........I..S.  200
D.hyd. H1             --TI.A...PK.V.......AVVT..S.D....E.A.......V......  197
D.vir. H1.1 (Boc)     KSSTSTTKKAAGAADKKLSKSAAAKKNVEKKKADKEKAKDAKKTGTIKAK  198
D.vir. H1.2 (Boc)     ......................PT..S....R...A..............  198
D.vir. H1.3 (Boc)     S.................................................  198
D.vir. H1.1 (Oke)     ..................................................   50
D.vir. H1.3 (Oke)                                       ................   16

D.mel. H1             .AAT...VT.A...AVVA.ASK...AVSAKPKKTV.K.SVSAT..KPKAK  250
D.hyd. H1             ..........A..................A.AS...A.A........---  244
D.vir. H1.1 (Boc)     PTTAKAKSSATKPKTPKPKTTSAKPKKVVSATTPKKTAVKKPKAKTA---  245
D.vir. H1.2 (Boc)     ....................K..........................---  245
D.vir. H1.3 (Boc)     L..............................................---  245
D.vir. H1.1 (Oke)     ....................P..........................---  100
D.vir. H1.2 (Oke)     ...............................................---   50
D.vir. H1.3 (Oke)     ...............................................---   66

D.mel. H1             TT.A..   256
D.hyd. H1             -.....   249
D.vir. H1.1 (Boc)     -SATKK   250
D.vir. H1.2 (Boc)     -.....   250
D.vir. H1.3 (Boc)     -.....   250
D.vir. H1.1 (Oke)     -.....   106
D.vir. H1.2 (Oke)     -.....    56
D.vir. H1.3 (Oke)     -.....    72
```

Fig. 2. The deduced amino acid sequences of the H1 histones of *D. virilis* (strain Bochum), aligned to the sequence of H1 of *D. melanogaster* (Murphy and Blumenfeld 1986; Matsuo and Yamazaki 1989) and *D. hydei* (Kremer and Hennig 1990). Dots indicate amino acid residues identical with those in H1.1 of *D. virilis.* Also included are the deduced sequences of strain Okenoshima. The central domains are boxed. Boc: strain Bochum, Oke: strain Okenoshima. *D. mel.: D. melanogaster; D. hyd.: D. hydei; D. vir.: D. virilis.*

differs from the earlier described histone gene cluster (Domier et al. 1986) and that in contrast to it contains a complete set of genes for each of the five histone types has thus been identified (Nagel 1996; Schienman et al. 1998; present results). Quantitative Southern blots (see Materials and Methods) revealed about 40 copies of H1 genes and about 50 copies of H4 genes per genome (Fig. 6). A probe for a sequence in the spacer region between the H2B and the H3 gene in clusters without an H1 gene (see below) also allowed us to estimate a number of about 15 copies of this region per genome (Fig. 6). Probably therefore, the histone genes of *D. virilis* are arranged in about 50 gene clusters (see also Domier et al. 1986), about 40 of which contain an H1 gene, whereas the others contain an intergenic spacer linking the H2B and the H3 gene.

We also analyzed genomic DNA of *D. virilis* to check whether additional types of histone gene clusters could be detected. Probes for H1 and H4 genes, respectively, were hybridized to Southern blots of genomic DNA cleaved with Ecl136II or Bgl II. When probed for H1, the Ecl136II fragments revealed one strong hybridization signal at 3.4 kb (Fig. 7C, lane 2), whereas the Bgl II fragments exhibited a strong signal at about 20 kb and

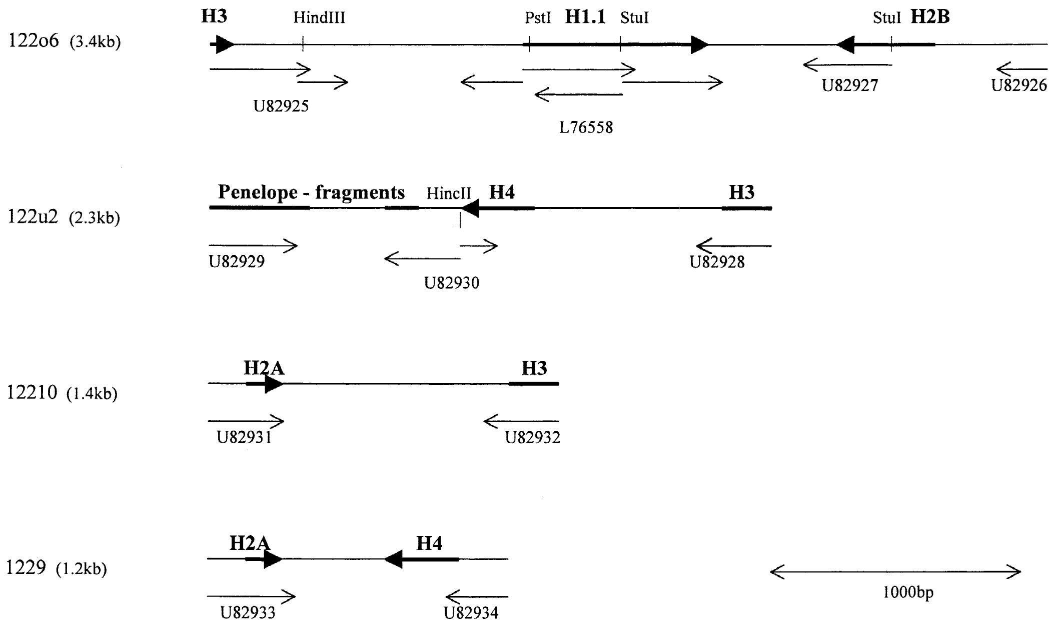

Fig. 3. Restriction fragments of a phage insert that together contain a quintet of histone genes with an incomplete H2A gene. Arrows indicate sequenced regions, and the numbers given are the corresponding GenBank accession numbers. Genes and the fragments of the mobile element Penelope are indicated overneath.

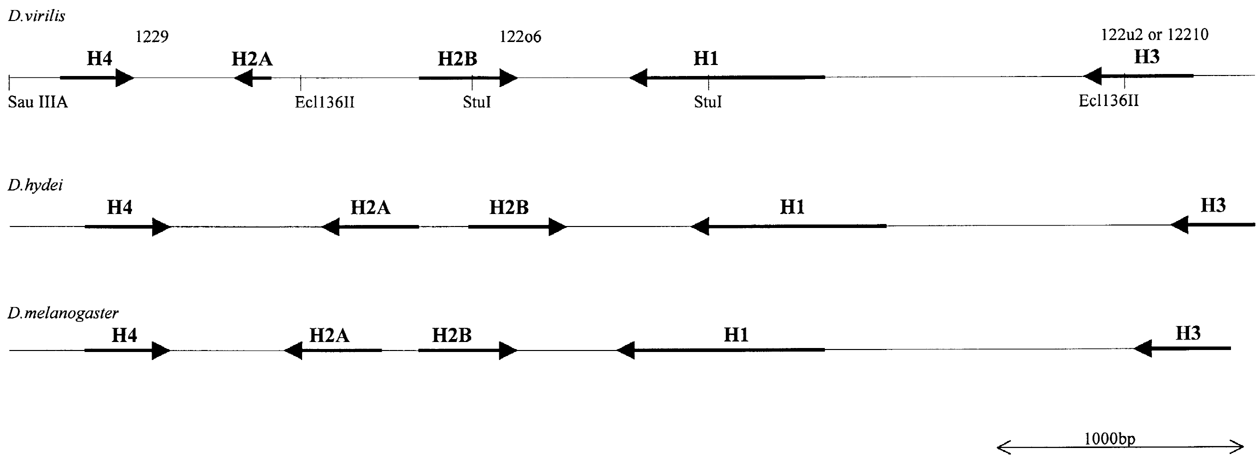

Fig. 4. Organization of a histone gene cluster from *D. virilis* (from the restriction fragments shown in Fig. 3), as compared to the histone gene clusters of *D. melanogaster* (Matsuo and Yamazaki 1989) and *D. hydei* (Kremer and Hennig 1990).

```
D.virilis         MARTKQTARKSTGGKAPRKQLATKAAADRKSAPATGGVKKPHRYRPGTVA   50
D.hydei           ..........................--......................   48
D.melanogaster    ..........................--......................   48

D.virilis         LREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEASE  100
D.hydei           ..................................................   98
D.melanogaster    ..................................................   98

D.virilis         RYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA              138
D.hydei           A.....................................              136
D.melanogaster    A..................I..................              136
```

Fig. 5. The deduced amino acid sequence of the H3 histone of *D. virilis* (clone 122) aligned to the sequence of H3 of *D. melanogaster* (Matsuo and Yamazaki 1989) and *D. hydei* (Kremer and Hennig 1900). Dots indicate amino acid residues identical with those in H3 of *D. virilis.*

two minor fragments (Fig. 7C, lane 3). This indicates the presence in the genome of several different gene clusters comprising an H1 gene. One of them is represented by the insert of the genomic library clone 122 that contains a 3.4-kb Ecl136II fragment with a complete H1 gene (Fig. 3). Schienman et al. (1998), in a systematic study of histone gene clusters in *D. virilis,* have characterized several subtypes of H1-containing clusters that differ in

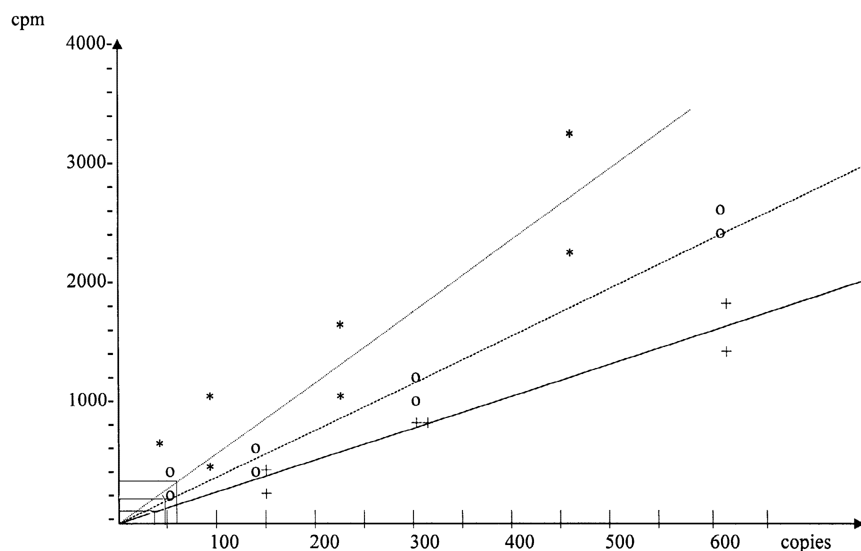

Fig. 6. Calibration curves for the estimation of copy numbers of histone gene clusters in the genome of *D. virilis.* Genomic DNA equivalent to 6.5×10^6 genomes and plasmid DNA were restricted and on Southern blots hybridized to gene probes labeled with [^{32}P] dATP (see Materials and Methods). The curves represent hybridized radioactivity from probes for histone H4 (asterisks), histone H1 (circles), and for an intergenic region specific for a histone quartet that lacks the H1 gene (crosses).

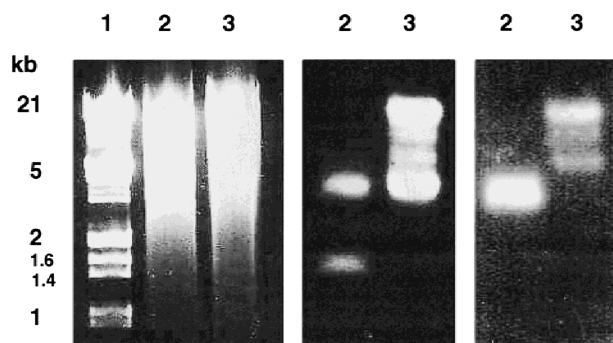

Fig. 7. Southern blots of genomic DNA of *D. virilis.* Genomic DNA restricted with Ecl136II (lane 2) and Bgl II (lane 3) was separated together with marker lambda DNA (lane 1) on agarose gel **(left)**, blotted onto nylon membranes, hybridized with radiolabeled probes for histone H4 **(middle)** and histone H1 **(right)**, and exposed to X-ray films.

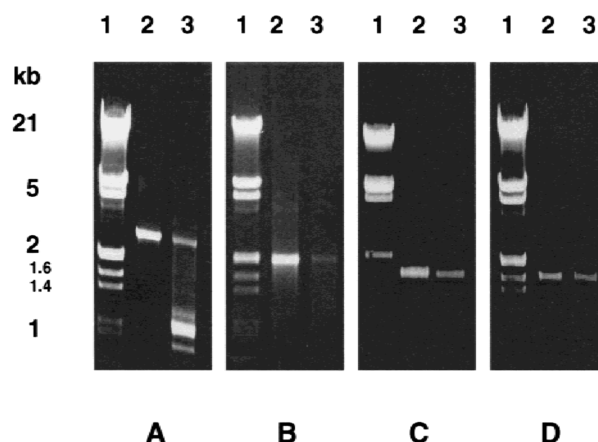

Fig. 8. Isolation of intergenic sequences of histone gene clusters from a cloned histone gene quintet (lane 2) and genomic DNA of *D. virilis* (lane 3) revealed genomic size heterogeneities of the H2B–H3 intergenic region. PCR products amplified by means of primer sequences constructed according to sequences within two adjacent genes (see Materials and Methods) were separated on agarose gels. The sequences chosen were from H2B and H3 **(A)**, H3 and H1 **(B)**, H2B and H1 **(C)**, and H4 and H2B **(D).**

size. The probing of genomic DNA for H4 (Fig. 7B) revealed two Ecl136II fragments (1.4 and 3.4 kb, lane 2) two prominent Bgl II fragments (3.4 and 20 kb), and two less prominent Bgl II fragments with sizes between these values (lane 3). The 1.4-kb Ecl136II fragment obviously originated from histone gene clusters of the type represented by clone 122 and corresponds to its subclone 12210 (Fig. 3), whereas the 3.4-kb Ecl136II fragment is not represented in that gene cluster. Two major types of histone gene clusters containing an H4 gene were also revealed by the hybridization pattern exhibited by the Bgl II fragments. The 3.4-kb fragment obviously originated from histone gene clusters of the type represented by the quartet that has been analyzed earlier and has been shown to contain corresponding Bgl II cleavage sites and to lack an H1 gene (Domier et al. 1986). The larger Bgl II fragments probably originate from histone gene clusters that contain an H1 gene. The genome of *D. virilis* thus contains two types of histone gene clusters comprising H4 genes that differ in that one type contains an H1 gene whereas the other does not. The clusters containing an H1 gene fall under different subtypes that differ in size (Schienman et al. 1998).

On polytene chromosomes, the probes for the H1 gene, the H4 gene, and the histone gene cluster lacking an H1 gene (see below), all hybridized to a single site (not shown), band 25F (see the chromosome map; Kress 1993). No additional hybridization signals could be detected. On the other hand, Anderson and Lengyel (1984),

A

```
122o6   TAAAATTCAGCTAGATGTGCATTGTACGGGAAGCAAACAAAAGATCCAAA   50
        |||||||||||||||||||||||||||||||||||||||||||||| |||
1238    TAAAATTCAGCTAGATGTGCATTGTACGGGAAGCAAACAAAAGATC-AAA   49

122o6   AGGCCCTTTTCATCGGGCACAAATCTCTTTTTCAAGGAATTAAAAATATT  100
        ||||||||||||  || |||||||||||||||||||||||||||||||||
1238    AGGCCCTTTTCAG-GGCCACAAATCTCTTTTTCAAGGAATTAAAAATATT   98

122o6   TTTAAATATCAGAATAATATCTCATATGAATGTA-AGCGTTCTTAAAACT  149
        |||||||||||||||| | || |||||  |  || |   |||  |||| |
1238    TTTAAATATCAGAATATTGTC-CATATATAGATACATATTTCAGAAAATT  147

122o6   TTTCTATTATTTAAATATTAGACCCGAAATTTACACATTACACATTCGTC  199
            || || | ||| |   | |         | | |  | | ||   |
1238    AACATAGTAATCAAACAGCCGTCATCCTTATGAAATAAAAAGATAACTA  197

122o6   TATGCGTACTTTTGAGGG                                 217
            |       |   |
1238    ATATCCGTTGGCTACAGC                                 215
```

B

```
122o6   AAGCTTTTTAATAAGATGA-TTTATTTGGCGACCTATCAATTTCATAATT   49
            |    ||   |   | ||| ||| | |    ||        | |
1238    CTCATAGGCAAGGCGTACAGTTTTTTTCGAGCATGATACTGCAAGTGAAA   50

122o6   GTTTCTTTGACTGGCTCGATGTCTGAGGTTTTTTTATTCCAGACAATATG   99
         ||   |    | |              | |||||           |||
1238    ATTCGGTGAGATAGAAAATATGTAATTTTGTTTTTTAGATTATTCCTATC  100

122o6   AAAATCCAGTTTTGTTTTAAATTTTTACTT-AGTATATTG-ATATTTAAT  147
        | | |   ||    ||||   | |       ||  | ||| | ||| |||
1238    ACAGTGTTGTGACTTTTTTGGTATGATGGACAGATTTTTGTACATTAAAT  150

122o6   TTAATATATTGATACGAAAATAAGAATCTCCTTGTTTAAAATATTTTGTG  197
        | || || |  ||| |||||||  |||||| |||||||||||||||||||
1238    TCAAGATGTATATAGGAAAATATCAATCTCTTTGTTTAAAATATTTTGTG  200

122o6   GTCCT-GAAAAGGACCGATTTTTTTTTATATTTATGTATTAGAGACGAG  246
        ||||| | ||||||||||||||| ||||||||||||||||||||||||||
1238    GTCCTTGGAAAGGACCGATTTTTGTTTATATTTATGTATTAGAGACGAG  250

122o6   GTAGACTTA                                          255
        |||||||||
1238    GTAGACTTA                                          259
```

Fig. 9. The 3′-flanking regions of the H2B genes (**A**) and the H3 genes (**B**) in the two types of histone gene clusters of *D. virilis* that comprise (clone 12206) and lack (clone 1238) an H1 gene. Within the first about 100 bp, these regions exhibit high degrees of sequence identity. The stop codons are underlined, and the hairpin structure palindromes are double-underlined.

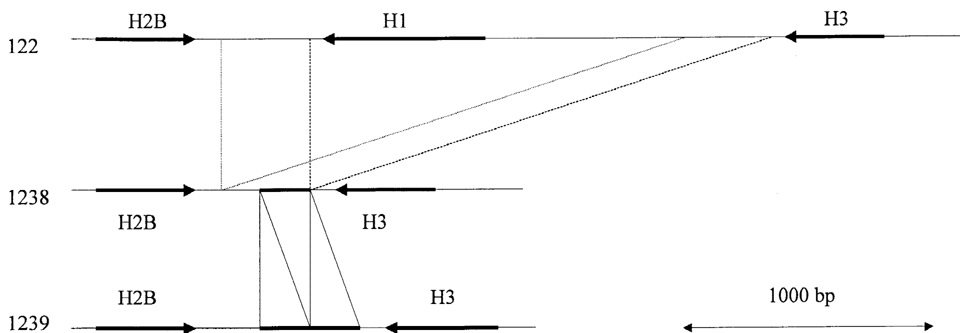

Fig. 10. Scheme of the organization of the histone gene clusters of *D. virilis* between the H2B and the H3 gene. Clone 122 contains an H1 gene, whereas this gene is lacking in the histone gene clusters contained in clones 1238 and 1239. The dashed and dotted lines indicate the borders of the regions with high sequence similarities. Clones 1238 and 1239 differ by about 200 bp that are a repeat of a sequence indicated by bold lines. Arrows indicate genes.

using an entire histone gene cluster as a probe, found two hybridization signals, one in the locus that we now have identified as 25F and an additional weak signal in 43C on chromosome IV. Schienman et al. (1998) also detected hybridization with an H1-specific probe to both loci which thus contain H1-comprising histone gene clusters.

Two Types of Histone Gene Clusters in D. virilis

We have compared sequences in between the H2B and the H3 gene in both types of histone gene clusters with the aim to understand the evolutionary origin of the cluster type without an H1 gene. Two oligonucleotides were

constructed according to sequences within the open reading frames of the H2B and the H3 gene, respectively (see Materials and Methods), and were used as primers for the amplification from genomic DNA of sequences between these two genes. In parallel, the same region was amplified from the insert of the phage clone (122) that contains a complete quintet of histone genes. A PCR product of 2.8 kb size was obtained in both cases which contained the H1 gene and the adjacent intergenic regions (Fig. 8A). Genomic DNA yielded additional PCR products with sizes of about 1100 bp, 1000 bp, 950 bp, and 800 bp (Fig. 8A). In contrast, no genomic size heterogeneities of the regions between the H3 and H1, the H2B and H1, and the H4 and H2B genes were found (Fig. 8B–D). The 1000-bp and the 800-bp fragments (Fig. 8A, lane 3) were cloned, sequenced, and found to represent the intergenic sequence between the H2B and the H3 gene from quartets lacking the H1 gene. Both sequences are very similar to each other (97.3% sequence identity) but differ by a duplication of about 200 bp length (Nagel 1996; sequences available under GenBank accession numbers U88331 and U88330). Schienman et al. (1998) have confirmed the occurrence of two different histone gene quartets that differ by about 200 bp.

For a comparison of the histone gene quartets with a quintet containing an H1 gene, Fig. 9 shows alignments of part of the sequences of the 2.8-kb fragment (which contains the H1 gene and intergenic regions) and the 800-bp segment, beginning from the stop codon of the H2B gene (Fig. 9A), and beginning from the stop codon of the H3 gene (Fig. 9B). In both sites, the alignment of the sequence of the histone gene quintet (clone 12206) with the sequence of the quartet lacking the H1 gene (clone 1238) exhibits a high degree of sequence identity for about 100 bp. From there on, the similarity decreases rapidly.

Based on these results, Fig. 10 shows a schematic representation of the region between the H2B and the H3 gene within the two histone gene clusters and indicates the borders of high sequence identity. As both *D. melanogaster* and *D. hydei* contain only histone gene quintets with a complete set of histone genes, the quintet arrangement in the *D. virilis* genome must be the ancestral organization in this species. Comparison of the intergenic sequences did not provide indications on where the deletion of the H1 gene occurred, but breakpoints in recombination events should lie beyond the borders of high sequence similarity (Figs. 9, 10). The duplication of part of the sequence, which lies out of the area of high sequence similarity, should have taken place after the deletion event.

As to the differences between *D. virilis* and *D. melanogaster* in histone gene organization and the heterogeneity of H1 genes, it may be worthwhile to consider the chromosomal positions of the histone gene loci in both species. In *D. melanogaster,* the histone gene quintets are located close to the centromere heterochromatin in locus 39 D/E in chromosome II (Pardue et al. 1977). In contrast, the histone gene cluster of *D. virilis* is located far from the centromere and can therefore be expected to undergo recombination at a higher rate. DNA polymorphisms have been shown to be correlated to recombination rates in *Drosophila* (Begun and Aquadro 1992). As to the position of the histone gene cluster of *D. melanogaster,* it is located within a region near the centromere that has been shown to exhibit a reduced extent of gene polymorphism (Palopoli and Wu 1996). Whereas the extents of regions of reduced polymorphism vary between different *Drosophila* species (True et al. 1996), it is tempting to speculate that the heterogeneity of histone gene organization and of the H1 gene structure in *D. virilis* arose as a consequence of a higher recombination rate. This might then have provided a basis for the differentiated affinities of H1 subtypes to satellite DNAs observed by Blumenfeld et al. (1978).

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