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Drosophila virilis Histone Gene Clusters Lacking H1 Coding Segments

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Summary. Approximately 30–40% of *Drosophila virilis* DNA complementary to cloned *Drosophila* histone genes is reduced to 3.4-kilobase-pair (kbp) segments by Bgl I or Bgl II digestion. The core histone genes of a 3.4-kbp Bgl II segment cloned in the plasmid pDv3/3.4 have the same order as the *D. melanogaster* core histone genes in the plasmid cDm500: $\overrightarrow{\text{H2B}}$ $\overleftarrow{\text{H3}}$ $\overrightarrow{\text{H4}}$ $\overleftarrow{\text{H2A}}$. Nonetheless, pDv3/3.4 and cDm500 have different histone gene configurations: In pDv3/3.4, the region between the H2B and H3 genes contains 0.35 kbp and cannot encode histone H1; in cDm500, the region contains 2.0 kbp and encodes histone H1. The lack of an H1 gene between the H2B and H3 genes in 30–40% of *D. virilis* histone gene clusters suggests that changes in histone gene arrays have occurred during the evolution of *Drosophila.* The ancestors of modern *Drosophila* may have possessed multiple varieties of histone gene clusters, which were subsequently lost differentially in the *virilis* and *melanogaster* lineages. Alternatively, they may have possessed a single variety, which was rearranged during evolution. The H1 genes of *D. virilis* and *D. melanogaster* did not cross-hybridize in vitro under conditions that maintain stable duplexes between DNAs that are 75% homologous. Consequently, *D. virilis* H1 genes could not be visualized by hybridization to an H1-specific probe and thus remain unidentified. Our observations suggest that the coding segments in the H1 genes of *D. virilis* and *D. melanogaster* are >25% divergent. Our estimate of sequence divergence in the H1 genes of *D. virilis* and *D. melanogaster* seems high until one considers that the coding sequences of cloned H1 genes from the closely related species *D. melanogaster* and *D. simulans* are 5% divergent.

Key words: Histone genes — Histone H1 — Gene organization — Nucleotide sequence — *Drosophila virilis*

Introduction

In many higher eukaryotes, the H1, H3, H4, H2A, and H2B histone genes occur in clustered repeating units containing one copy of each gene. The nucleotide sequences of clusters vary between species and even within the genome of an individual. The variability is detected as differences in the primary sequences, the organization or the intergenic spacers of the histone genes within clusters (Lifton et al. 1978; Maxson et al. 1983; Woodland et al. 1984). In *Drosophila melanogaster,* the H1, H2A, H2B, H3, and H4 histone genes occur in 100–110 serially repeated 4.8- to 5.0-kb clusters, which map to polytene chromosome region 39D-E (Pardue et al. 1977). In this article, we demonstrate that some histone gene clusters of *Drosophila virilis,* a related species, have the same core histone gene organization as *D. melanogaster,* but lack H1 histone coding segments at the expected position within the histone gene cluster. This observation adds another variable to the

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Table 1. Histone gene recombinants

Name[a] (source)	Vector	Contents[b]	Construction	Insert size (kbp)
cDm500 (Dm)	ColE1	H1, H2A, H2B, H3, H4	Shear and AT tailing	9.6
cDm500/1.1 (cDm500)	pUC4	H2A, H4	Ava I digestion, ligation into Sal I site	1.1
pDv3 (Dv)	pUC4	H2A, H2B, H3, H4	Sal I–Mbo I digestion, ligation into Bam HI site	8.8
pDv3/3.4 (pDv3)	pUC12	H2A, H2B, H3, H4	Bgl II digestion, ligation into Bam HI site	3.4
pDv3/2.9 (pDv3)	pUC12	–	Eco RI–Bgl II digestion, ligation into Bam HI–Eco RI site	2.9
pDv3/2.5 (pDv3)	pUC12	H2B, H4, H2A and/or H3	Eco RI–Bgl II digestion, ligation into Bam HI–Eco RI site	2.5
Dv9A (pDv3/3.4)	M13mp9	H2A, H2B, H3, H4	Bgl II digestion, ligation into Bam HI site	3.4
Dv9B (pDv3/3.4)	M13mp9	H2A, H2B, H3, H4	Bgl II digestion, ligation into Bam HI site	3.4
Dv9C (pDv3/3.4)	M13mp9	H2A	Sau 3A digestion, ligation into Bam HI site	1.0
Dv9D (pDv3/3.4)	M13mp9	H2A	Cla I digestion, fill in, ligation into Sma I site	0.6
Dv9E (pDv3/3.4)	M13mp9	H2B	Alu I digestion, ligation into Sma I site	0.2
Dv9F (pDv3/3.4)	M13mp9	H2B$^-$	Alu I digestion, ligation into Sma I site	0.2
Dv9G (pDv3/3.4)	M13mp9	H3	Alu I digestion, ligation into Sma I site	0.2
Dv9H (pDv3/3.4)	M13mp9	H3$^-$	Alu I digestion, ligation into Sma I site	0.2
Dv9I (pDv3/3.4)	M13mp9	H4	Alu I digestion, ligation into Sma I site	0.2

[a] Dm indicates *D. melanogaster*; Dv indicates *D. virilis*
[b] Superscript minus indicates complement of coding strand

complex picture of histone gene organization. It remains to be established whether this difference is reflected in the control of histone gene expression.

Materials and Methods

Materials. Restriction enzymes were purchased from Bethesda Research Laboratories, New England Bio-Labs, and Boehringer Mannheim; large-fragment DNA polymerase I and synthetic 15-base primer, from Bethesda Research Laboratories; deoxynucleotides and dideoxynucleotides, from P-L Biochemicals; and [^{32}P]dATP and [^{35}S]dATP, from Amersham. Enzymes were used according to the respective supplier's suggestions. The *D. melanogaster* histone gene clone cDm500 (Lifton et al. 1978) (Table 1) was a gift from David Hogness.

Preparation and Analysis of Nucleic Acids. D. virilis embryo DNA and polysomal RNA were purified as described elsewhere (Blumenfeld et al. 1973; Chooi et al. 1980). M13 DNAs were purified by alkaline lysis (Birnboim and Doly 1979).

DNAs were separated electrophoretically in 0.15 cm × 13 cm × 23 cm agarose gels containing 0.8–1.4% agarose in 15 mM Tris-HCl (pH 7.4), 20 mM sodium acetate, 2 mM disodium ethylenediaminetetraacetate (Na_2EDTA), for 90 min at 4 V/cm. Hind III-digested lambda phage and Hinf I-digested pBR322 (Sutcliffe 1979) DNAs were included as molecular-weight standards. Gels were stained with 2 μM ethidium bromide, transilluminated with UV light, and photographed with Polaroid type 665 film. The molecular weights of restriction fragments were estimated from 1:1 photographs of agarose gels using a Houston Instruments HiPad digitizer, a molecular-weight-estimation program, and an Apple II+ computer.

^{32}P- and ^{35}S-labeled probes were prepared by nick translation of plasmids (Maniatis et al. 1975) or replication of M13 subclones (Hu and Messing 1982) containing histone genes. The *D. melanogaster* histone gene subclone cDm500/1.1 contains the histone H4 gene, the spacer between the H4 and H2A genes, and the 3′ region of the H2A gene. It was prepared by digesting cDm500 with Ava I, electrophoretically purifying (McDonnell et al. 1977) the 1.1-kb Ava I fragment, and ligating that fragment into pUC4 (Table 1).

D. virilis DNA was singly digested with various restriction enzymes, electrophoresed on 0.8% agarose gels at 10 V/cm for 1 h, and transferred to nitrocellulose filters (Southern 1975). Filters were dried; prehybridized in 1 M NaCl, 0.1 M PIPES (pH 7.0), 0.1% n-lauroyl sarcosine, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone (Denhardt 1966), and 200 μg/ml yeast tRNA, 50% formamide (Casey and Davidson 1977) for 4–18 h at 42°C; and hybridized with labeled probes for 2–18 h

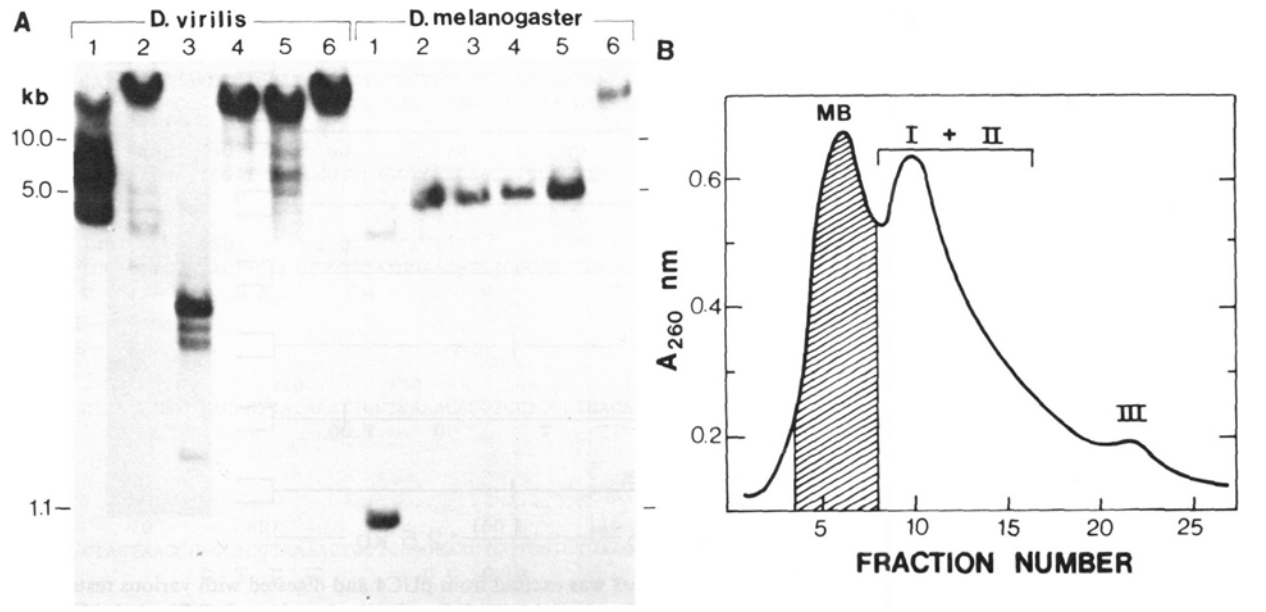

Fig. 1A, B. **A** Restriction enzyme digestion of *D. virilis* and *D. melanogaster* histone genes. *D. virilis* DNA (4 μg) and *D. melanogaster* DNA (0.08 μg) were singly digested with Ava I (lane 1), Bam HI (lane 2), Cla I (lane 3), Eco RI (lane 4), Hind III (lane 5), and Sal I (lane 6); electrophoresed; blotted onto nitrocellulose and hybridized with nick-translated cDm500/1.1. **B** Enrichment of *D. virilis* histone genes by CsCl gradient centrifugation. The DNA from gradient fractions 3–7, indicated by the cross-hatching, was pooled, precipitated, resuspended, digested with Sal I, and fractionated by sucrose gradient centrifugation. The three absorbance peaks contain main band (MB) DNA, satellites I and II, and satellite III

at 42°C. Filters were washed twice with 250 ml 0.30 M NaCl, 0.030 M Na citrate, 0.1% n-lauroyl sarcosine, 0.1% Na pyrophosphate (50°C for 15 min); dried; and exposed at −70°C to Kodak XAR film sandwiched between two Dupont Hi-plus intensifying screens.

RNAs were electrophoresed on methylmercury hydroxide–1.8% agarose gels at 10 V/cm for 2 h (Baily and Davidson 1976) and transferred to nitrocellulose filters (Thomas 1980). Histone band identities were established by hybridizations with subclones Dv9C, Dv9E, Dv9F, and Dv9G, which contain single *D. virilis* histone gene coding segments (Table 1). The expected position of hybrids containing H1 mRNA was estimated by hybridization between *D. melanogaster* mRNA and cDm500.

D. virilis DNA (20 μg/ml in 100 mM NaCl, 10 mM Tris (pH 8.0), 1 mM Na_2EDTA) was adjusted to n = 1.3990 with solid CsCl and centrifuged in a Beckman VTi 65 rotor at 30,000 rpm for 48 h at 20°C. Gradient fractions (0.5 ml) were collected from the bottom with a peristaltic pump and monitored at 260 nm (Mullins and Blumenfeld 1978). Fractions containing main-band DNA were pooled, diluted 1:4 with distilled water, ethanol precipitated, resuspended, digested completely with Sal I, and centrifuged on 10%–40% sucrose gradients in 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM Na_2EDTA, in a Beckman VTi 50 rotor at 50,000 rpm for 90 min at 25°C. Fractions (0.5 ml) were collected from the bottom and analyzed electrophoretically. Fractions containing high-molecular-weight (>20 kb) DNA were pooled, ethanol precipitated, resuspended, partially digested with Mbo I, digested with alkaline phosphatase (Ullrich et al. 1977), ligated into the Bam HI site of pUC4 (Vieira and Messing 1982), and used to transform *E. coli* JM83 from ampicillin sensitive (amp^s) to ampicillin resistant (amp^r) (Kushner 1978). Recombinant colonies were replica plated onto nitrocellulose filters (Hanahan and Meselson 1980) and screened with nick-translated cDm500/1.1.

Analyses of Cloned D. virilis Histone Genes. The *D. virilis* component of the histone gene clone pDv3 (Table 1) was excised with Eco RI, singly digested with various restriction enzymes, and analyzed electrophoretically. The *D. virilis* component of the subclone pDv3/3.4 (Table 1) was excised, end labeled with [^{32}P]dATP (Smith and Birnstiel 1976), digested with Bgl I, and partially digested with Hha I, Sau3A I, or Cla I.

D. virilis DNA (40 μl; 100 μg/ml) and pDv3/3.4 DNA (40 μl; 100 ng/ml) were serially diluted 1:1 with distilled water, denatured with 60 μl 0.7 M NaOH, 1.7 M NaCl, at 22°C for 20 min, dotted onto a prewashed nitrocellulose filter, and neutralized with 2 ml 3 M NaCl, 1.5 M Tris-HCl (pH 7.0) (Kafatos et al. 1979). Filters were washed in 0.60 M NaCl, 0.060 M sodium citrate; baked; hybridized to nick-translated pDv3/3.4; washed; and dried. Dots were excised and counted by liquid scintillation spectroscopy.

M13 cloning and dideoxy sequencing procedures (Sanger et al. 1977; Heidecker et al. 1980; Messing et al. 1981) were used with three modifications: (1) sequencing reactions were performed in 10 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$; (2) [γ-^{35}S]dATP was substituted for [γ-^{32}P]dATP (Biggin et al. 1983); and (3) the Kodak XAR film was preflashed (Bonner and Laskey 1974). DNA sequences were compiled and compared with the histone gene sequences of cDm500 using a sequence-analysis program and an IBM PC.

The M13 subclones Dv9A and Dv9B each contain a copy of the *D. virilis* 3.4-kb histone gene cluster, but in opposite orientations. They were hybridized to the gene-specific subclones Dv9C, Dv9D, Dv9E, Dv9F, Dv9G, Dv9H, and Dv9I (Table 1) and analyzed electrophoretically (Messing 1983).

Results

Cloning and Characterization of pDv3

Southern blot analyses with nick-translated cDm500/1.1 (Fig. 1A) indicated that *D. virilis* histone genes were reduced to 6.5-, 8.4-, and 9.6-kb segments by Ava I (lane 1); *D. melanogaster* histone

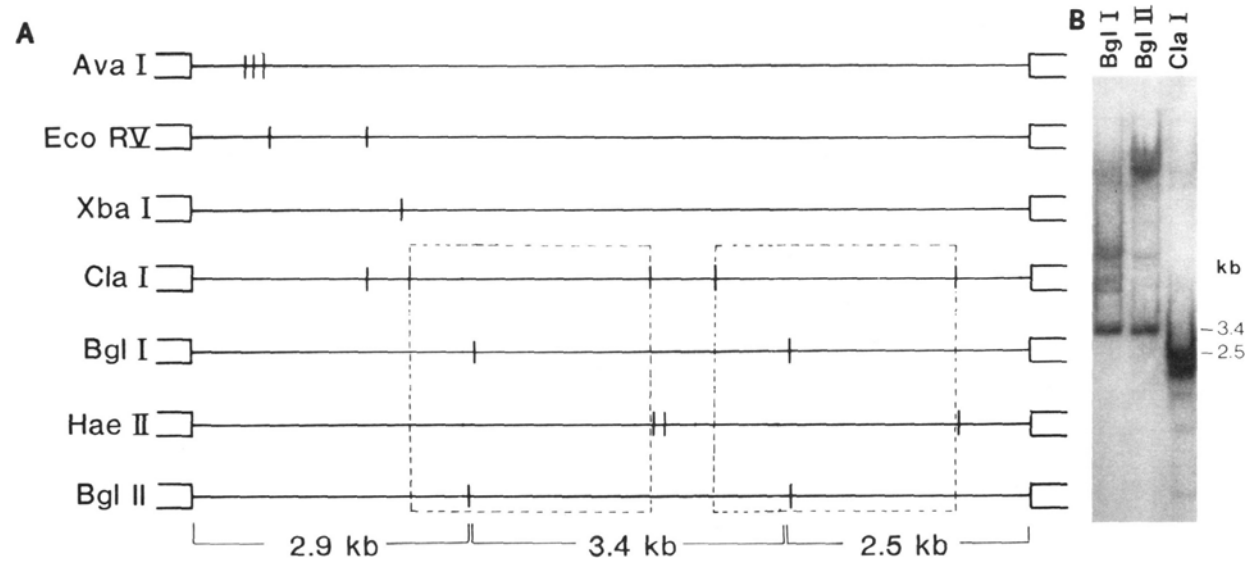

Fig. 2A, B. **A** Restriction map of pDv3. The 8.8-kbp insert of pDv3 was excised from pUC4 and digested with various restriction enzymes. It was not cut by Bam HI, Eco RI, Hind III, Kpn I, Pst I, Pvu II, Sal I, Sst I, Sma I, Xho I, or Xma I. **B** Blot hybridization of *D. virilis* histone genes to pDv3/3.4. *D. virilis* DNA was digested with Bgl II, Bgl I, or Cla I; electrophoresed; blotted; and hybridized with nick-translated pDv3/3.4

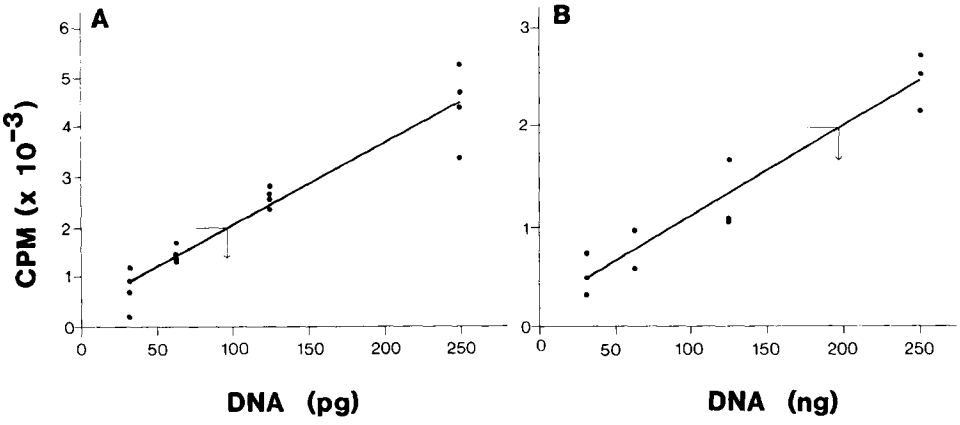

Fig. 3A, B. *D. virilis* histone gene abundance. pDv3/3.4 (**A**) and total *D. virilis* (**B**) DNAs were dotted onto nitrocellulose and hybridized with nick-translated pDv3/3.4. The individual dots were excised and counted. These results indicate that pDv3/3.4 is complementary to approximately 0.05% of the *D. virilis* genome and present in approximately 50 copies per genome

genes were reduced to 1.1-kb segments by the same enzyme (lane 1). *D. virilis* histone genes were essentially undigested by Bam HI, Eco RI, and Hind III (lanes 2, 4, and 5), and were reduced to a major 2.5-kb and five minor (0.7- to 2.3-kb) bands by Cla I (lane 3); the majority of *D. melanogaster* histone genes were digested into 4.8- to 5.0-kb repeating units by each of these enzymes (lanes 2–5) (Lifton et al. 1978). *D. virilis* histone genes were not digested by Sal I (lane 6), a feature that was exploited in the cloning of *D. virilis* histone gene segments in pDv3.

After preparative centrifugation in neutral CsCl, *D. virilis* DNA was resolved into three absorbance peaks. These contained, respectively, main-band DNA, satellites I + II, and satellite III (Blumenfeld et al. 1973; Gall and Atherton 1974). When the main band (fractions 3–7; Fig. 1B) was digested with Sal I and fractionated by sucrose gradient centrifugation, high-molecular-weight (>20 kb) DNA represented <2% of the starting DNA, yet was ca. 90% satellite DNA and contained >90% of *D. virilis* DNA complementary to cDm500/1.1 (data not shown). When this DNA was partially digested with Mbo I, ligated into the Bam HI site in pUC4, and used to transform JM83, seven amp^r colonies that hybridized to cDm500/1.1 were recovered (data not shown). One colony contained pDv3, which had an 8.8-kb *D. virilis* DNA insert. The insert was cleaved by Cla I into two 2.5-kb fragments separated by 0.65 kb. The 2.5-kb Cla I fragments, highlighted in Fig. 2A, each contain one Bgl I and one Bgl II site. Hae II sites are aligned at similar positions to the right

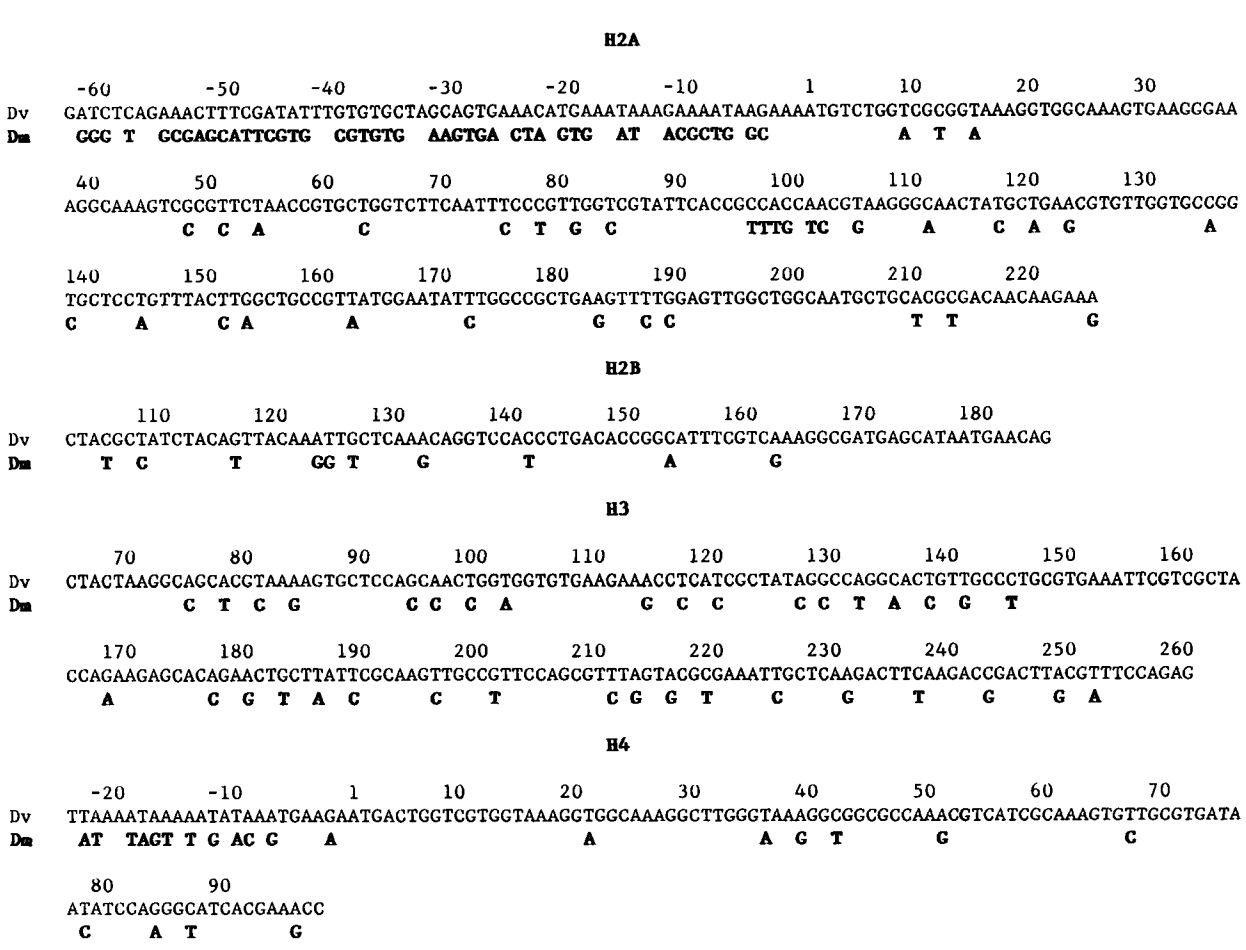

Fig. 4. Homologies between *D. virilis* (Dv) and *D. melanogaster* (Dm) core histone gene segments. Dv9C, Dv9E, Dv9G, and Dv9I were homologous to the *D. melanogaster* H2A, H2B, H3, and H4 histone gene segments, respectively. The *D. melanogaster* nucleotides that differ from their *D. virilis* homologues are indicated in boldface. *D. melanogaster* sequences were taken from Goldberg (1978)

of each 2.5-kb Cla I fragment. Since 1% of the base pairs within the two 2.5-kb Cla I fragments were restriction mapped to homologous positions, we conclude that the 2.5-kb Cla I fragments are tandemly arrayed and separated by 0.65 kb of DNA.

The insert was cleaved into 2.5-, 2.9-, and 3.4-kb segments by Bgl II. Subclones derived by Bgl II digestion of pDv3 were named according to their insert sizes as pDv3/2.5, pDv/2.9, or pDv/3.4. The 3.4- and 2.5-kb Bgl II fragments both contained a Bgl I site and an Hae II site aligned at similar positions relative to the Bgl II site; thus, the Bgl I sites were 3.4 kb apart. The 2.9-kb Bgl II fragment lacked Bgl I and Hae II sites, but contained Ava I, Eco RV, and Xba I sites (Fig. 2A).

pDv3/3.4 Contains H2A, H2B, H3, and H4 Histone Genes

Dot blot experiments indicated that pDv3/3.4 is homologous to 0.05% of the *D. virilis* genome (Fig. 3). The 2×10^{11} dalton *D. virilis* genome (Gall and Atherton 1974) thus contains sequences complementary to 50 copies of the 3.4-kb Bgl II fragment of pDv3. Blot hybridization with completely digested *D. virilis* DNA and nick-translated pDv3/3.4 revealed that *D. virilis* DNA contains 3.4-kb Bgl I, 3.4-kb Bgl II, and 2.5-kb Cla I segments complementary to pDv3/3.4 (Fig. 2B). The 3.4-kb Bgl I and Bgl II fragments together account for 30–40% of the hybridization between pDv3/3.4 and *D. virilis* DNA; the 2.5-kb Cla I fragment accounts for 60–70% of the hybridization. Thus, the histone gene organization of the adjacent 3.4-kb and 2.5-kb segments of pDv3 accounts for 15–20 histone gene complements per genome. Approximately 50% of the *D. virilis* DNA complementary to pDv3/3.4 is not digested by Bgl II (Fig. 2B), and could contain an array of histone genes different from the array contained in pDv3/3.4.

DNA sequencing indicated that subclones of pDv3/3.4 contained DNA segments homologous to the H2A, H2B, H3, and H4 genes of cDm500. The insert of Dv9C was 84% homologous to the region of the *D. melanogaster* histone H2A gene extending from position −63 through the AUG start codon to position +225; the insert of Dv9E was 87% homologous to the region of the *D. melanogaster* H2B

histone gene extending from position +102 to position +185; the insert of Dv9G was 82% homologous to the region of the *D. melanogaster* H3 histone gene extending from position +65 to position +260; and the insert of Dv9I was 90% homologous to the region of the *D. melanogaster* H4 histone gene extending from position −24 through the AUG start codon to position +98 (Fig. 4). The sequences of the H2B, H3, and H4 segments were confirmed by sequencing the complementary strands of each clone; the sequence of the H2A segment was confirmed by sequencing two independently derived subclones of pDv3/3.4 (data not shown).

The 3.4-kb insert of Dv9A was oriented with the Bgl I site proximal to the priming site for dideoxy sequencing, and the 3.4-kb insert of Dv9B was oriented with the Bgl I site distal to the priming site (Domier 1984). Hybridization analyses revealed that (1) the H2A-specific subclone Dv9C and the H3-specific subclone Dv9G both hybridized to Dv9A, and (2) the H2B-specific subclone Dv9E and the H4-specific subclone Dv9I both hybridized to Dv9B (Fig. 5). Thus, the *D. virilis* H2A and H3 genes are transcribed from one of the DNA strands; the H2B and H4 genes, from the complementary strand.

pDv3/3.4 contains five Sau3A I sites and five Hha I sites. The H2B-specific subclone Dv9E hybridized to the 745-bp Sau3A I fragment (a in Fig. 6A) and the 820-bp Hha I fragment (b); the H3-specific subclone Dv9G hybridized to the 870-bp Sau3A I fragment (c) and the 630-bp Hha I fragment (d); the H4-specific subclone Dv9I hybridized to the 570-bp Sau3A I fragment (e), the 110-bp Hha I fragment (f), and the 230-bp Hha I fragment (g); and the H2A-specific subclone Dv9C hybridized to the 1.27-kb Hha I fragment (h) and the 670-bp Sau3A I fragment (i).

The H2A segment of Dv9C contains a Sau3A I site at position −64. The hybridization between Dv9C and Dv9A established that the H2A coding strand extends from right to left. If the H2A gene encodes a protein with 130 amino acids (Palmer et al. 1980), the H2A coding segment occupies bases 64–454 in the 670-bp Sau3A I fragment. The H4 segment of Dv9I contains an Hha I site at position +44, corresponding to position 230 in the 560-bp Sau3A I fragment. Since H4 contains 106 amino acids (Isenberg 1979), the H4 coding region occupies bases 186–504 in the 560-bp Sau 3A fragment. From the placement of the H2A and H3 genes on one strand, restriction mapping with gene-specific probes (Fig. 6B), and the accurate placement of the H2A and H4 genes within known restriction fragments, we mapped the histone genes of pDv3/3.4. Their order is $\overrightarrow{\text{H2B}}\ \overleftarrow{\text{H3}}\ \overrightarrow{\text{H4}}\ \overleftarrow{\text{H2A}}$ (Fig. 6C). Therefore, the polarities and relative positions of the four core histone genes are identical in pDv3/3.4 and cDm500. However, an important distinction can be made between them. The region between the H2B and H3 genes of pDv3/3.4 is ca. 0.35 kb and cannot encode an H1 histone; the corresponding region in cDm500 is ca. 2.0 kb and encodes an H1 histone.

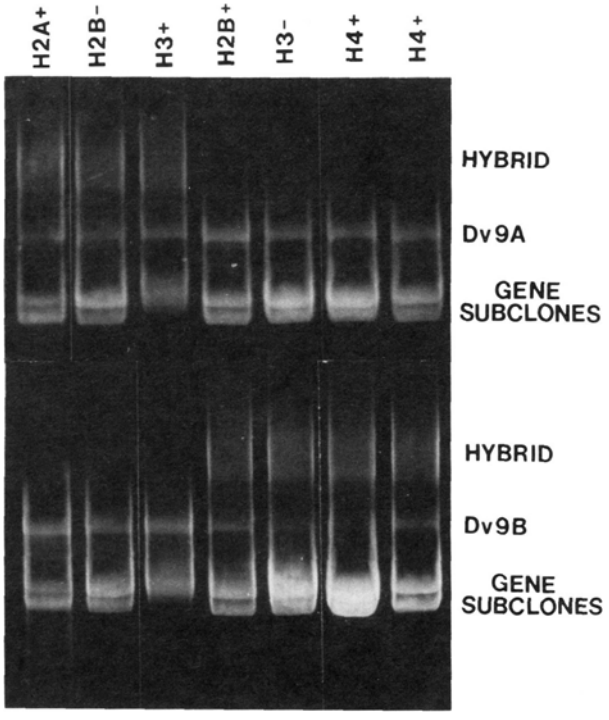

Fig. 5. The polarity of *D. virilis* histone genes. M13 subclones of pDv3/3.4 containing sequences homologous to individual core histone genes were hybridized to the subclones Dv9A and Dv9B. Dv9A hybridized to the sense (+) strands of H2A- and H3-specific subclones and to the antisense (−) strand of an H2B-specific subclone. Dv9B hybridized to the sense (+) strands of H2B- and H4-specific subclones and to the antisense (−) strand of an H3-specific subclone. Therefore, H3 and H2A are transcribed from one strand, and H2B and H4 are transcribed from the complementary strand

pDv3/3.4 and pDv3/2.5 Lack H1 Histone Genes

When electrophoretically separated *D. virilis* polysomal RNA was blotted onto nitrocellulose and hybridized to nick-translated pDv3/3.4 or pDv3/2.5, three bands were detected (Fig. 7). The correspondence between bands and histone mRNAs were established by hybridization to the subclones Dv9C, Dv9E, Dv9G, and Dv9I, which contain single *D. virilis* coding segments. The expected position of hybrids containing H1 mRNA was estimated by hybridization between *D. melanogaster* mRNA and cDm500 (data not shown). In order of decreasing electrophoretic mobility, the bands represented hybridization between the probes and mRNAs for his-

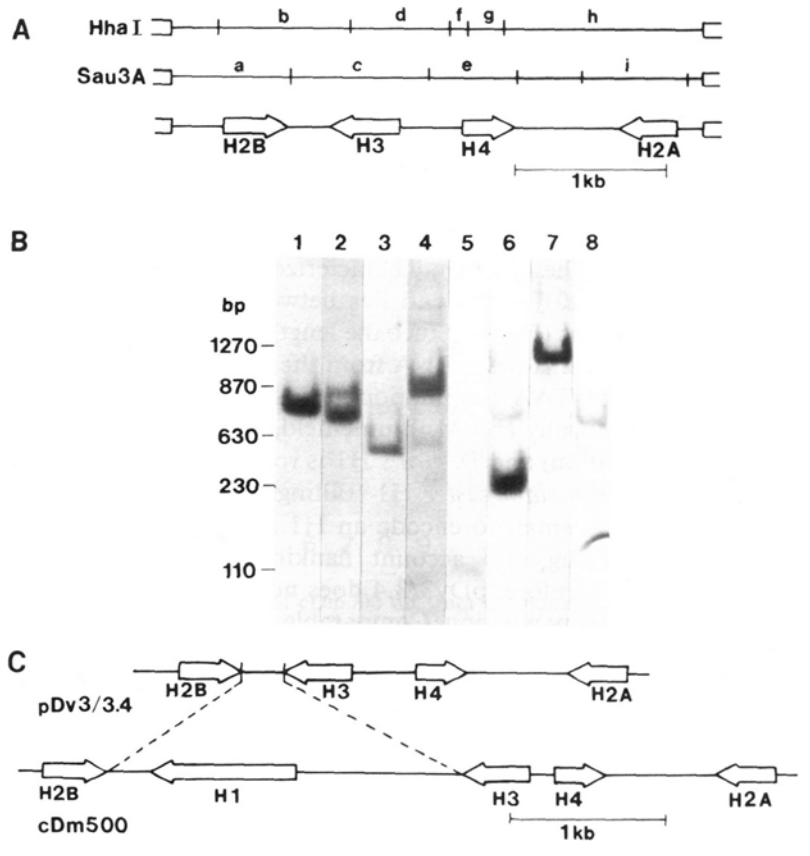

Fig. 6A-C. Histone gene organization in pDv3/3.4. **A** The end-labeled 3.4-kbp insert of pDv3/3.4 was mapped with Hha I and Sau3A I. **B** The insert was digested with Hha I (lanes 1, 3, 5, 7) or Sau3A I (lanes 2, 4, 6, 8), electrophoresed, blotted onto nitrocellulose, and probed with histone-gene-specific probes Dv9E (lanes 1, 2), Dv9G (lanes 3, 4), Dv9I (lanes 5, 6), and Dv9C (lanes 7, 8). **C** Histone gene organization in pDv3/3.4 and cDm500. The map of cDm500 was circularly permuted to accentuate its similarity to the map of pDv3/3.4

tones H4, H2B, and H2A + H3. H1 mRNAs of *D. melanogaster* migrated to the position indicated by the arrow in Fig. 7. Neither pDv3/3.4 nor pDv3/2.5 hybridized to RNA that migrated to this position. pDv3/2.9 hybridized to an mRNA band that migrated between the H2B and H2A + H3 mRNAs and presumably is not an H1 histone mRNA. Therefore, pDv3 contains a 2.9-kb DNA segment that is adjacent to 3.4-kb and 2.9-kb histone gene arrays and complementary to an uncharacterized RNA. The lack of hybridization between pDv3 subclones and H1 mRNA suggested that this cloned segment might lack an H1 gene.

The largest undefined region in pDv3/3.4 is ca. 670 bp and located between the H4 and H2A genes. It includes the 400-bp Sau3A I fragment as well as the flanking termini of the 670-bp and 560-bp Sau3A I fragments, and cannot encode histone H1. Size considerations also indicate that pDv3/2.5, which hybridizes to mRNAs for histones H2B, H2A + H3, and H4, likewise cannot encode histone H1. Therefore, the histone gene clusters in the adjacent 3.4-kb and 2.5-kb Bgl II fragments of pDv3 lack an H1 gene. Because the two Bgl II sites are contained within tandemly arrayed 2.5-kb Cla I fragments, the 3.4- and 2.5-kb fragments are tandemly arrayed. If the repeating units of the *D. virilis* core histone genes contain 3.4 kb of DNA, then pDv3 contains 1.7 tandemly arrayed histone gene clusters that lack an H1 gene.

The H1 Genes of D. melanogaster and D. virilis Do Not Hybridize In Vitro

The 615-bp Pst I fragment of cDm500 (Goldberg 1978) represents 82% of the H1 histone coding segment (T.J. Murphy and M. Blumenfeld, manuscript in preparation). Southern blot analyses established that it hybridized intensely to the 4.8- to 5.0-kb histone gene repeating unit liberated by Hind III or Bam HI digestion of *D. melanogaster* DNA, but did not hybridize to *D. virilis* DNA (Fig. 8A). Conversely, nick-translated pDv3 hybridized to every segment of Pst I-digested cDm500 except the 615-bp fragment. We could not detect hybridization between pDv3 and the Pst I fragment—even when the radioautograph was deliberately overexposed to maximize the likelihood of detecting a weak signal (Fig. 8B) or the hybridization stringency was lowered (data not shown). Since a *D. melanogaster* histone H1 probe did not hybridize to cloned or genomic *D. virilis* DNAs, we could not use blot hybridization to visualize *D. virilis* H1 genes, and thus could not completely exclude the possibility

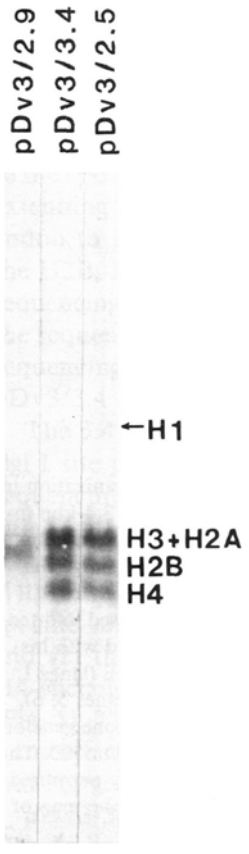

Fig. 7. Hybridization of *D. virilis* mRNAs to subclones of pDv3. *D. virilis* polysomal RNA was separated on CH_3HgOH–agarose gels, blotted to nitrocellulose, and hybridized to nick-translated pDv3/2.9, pDv3/3.4, or pDv3/2.5. pDv3/3.4 and pDv3/2.5 hybridized to mRNAs for histones H4, H2B, and H2A + H3. They did not hybridize to the mRNA for histone H1, which migrates to the position indicated by the arrow. pDv3/2.9 hybridized to an mRNA band that migrated between H2B and H2A + H3

that pDv3/2.9 contains an H1 gene. However, this possibility seems remote, since pDv3/2.9 did not hybridize to an mRNA large enough to encode H1.

Discussion

In *D. virilis,* H1 histone phosphorylation is developmentally regulated and involves approximately 5% of the chromosomal protein. The phosphorylation occurs within a single tryptic fragment located in the N-terminal third of the molecule (Blumenfeld et al. 1978; Billings et al. 1979). Since the *D. melanogaster* H1, H2A, H2B, H3, and H4 genes are present in serially repeated 4.8- to 5.0-kb clusters (Lifton et al. 1978), we expected to subclone H1 genes from *D. virilis* histone gene clusters, sequence the H1 coding segment, deduce the amino acid sequence of the H1 histone, and, by referring to the known composition of the phosphopeptide (Billings 1979), map the phosphorylation site within the H1 molecule. This approach proved untenable because the H1 coding segment of *D. melanogaster* did not hybridize to cloned *D. virilis* histone gene clusters or to *D. virilis* DNA. The lack of hybridization between the H1 histone genes of *D. melanogaster* and *D. virilis* has a precedent: Early and late sea urchin histone genes do not form stable hybrids (Maxson et al. 1983).

The largest uncharacterized region in pDv3/3.4 is ca. 740 bp and lies between the H4 and H2A genes (Fig. 8). Since the length of a *D. melanogaster* H1 histone mRNA from the initiation site through the TAA termination site is 805 ± 2 bases (T.J. Murphy and M. Blumenfeld, manuscript in preparation) and *D. virilis* H1 is roughly the same size as *D. melanogaster* H1 (Billings 1979), this region is too small to encode an H1 histone, even without taking into account flanking control sequences. Therefore, pDv3/3.4 does not contain an H1 gene at any position. Comparable size considerations indicate that the 2.5-kb Bgl II fragment, which is complementary to mRNAs for histones H2B, H2A + H3, and H4, does not encode an H1 histone. Thus, the two adjacent *D. virilis* histone gene clusters in pDv3 lack an H1 coding segment.

The core histone genes within pDv3/3.4 are 82–90% homologous to their counterparts in cDm500, and are arranged in the same order. These homologies underscore the intriguing difference between the histone gene complements of these two clones. The region between the H2B and H3 genes of pDv3/3.4 is too small to encode an H1 histone; the corresponding region of cDm500 encodes histone H1. Since the histone gene insert in pDv3/3.4 is present 15–20 times per *D. virilis* genome, the lack of an H1 gene at the expected site is significant, and suggests that changes in histone gene arrays have occurred during the evolution of *Drosophila.*

The ancestors of modern *Drosophila* may have possessed multiple varieties of histone gene clusters, which have been lost differentially in the *virilis* and *melanogaster* lineages. Alternatively, they may have possessed a single variety, which has been rearranged one or more times during evolution. Virtually all *D. melanogaster* histone gene clusters contain an H1 gene between the H2B and H3 genes (Goldberg 1978; Lifton et al. 1978). By analogy to the 3.4-kbp histone gene cluster of *D. virilis,* if any *D. melanogaster* histone gene clusters lacked H1 genes, they would be reduced in size and lack the restriction enzyme cleavage sites that occur in and around the H1 gene. Both of these features should be detectable by restriction mapping. Although individual histone genes, in the form of orphons, are sprinkled throughout the *D. melanogaster* genome (reviewed by Maxson et al. 1983), we are aware of no results suggesting that any *D. melanogaster* his-

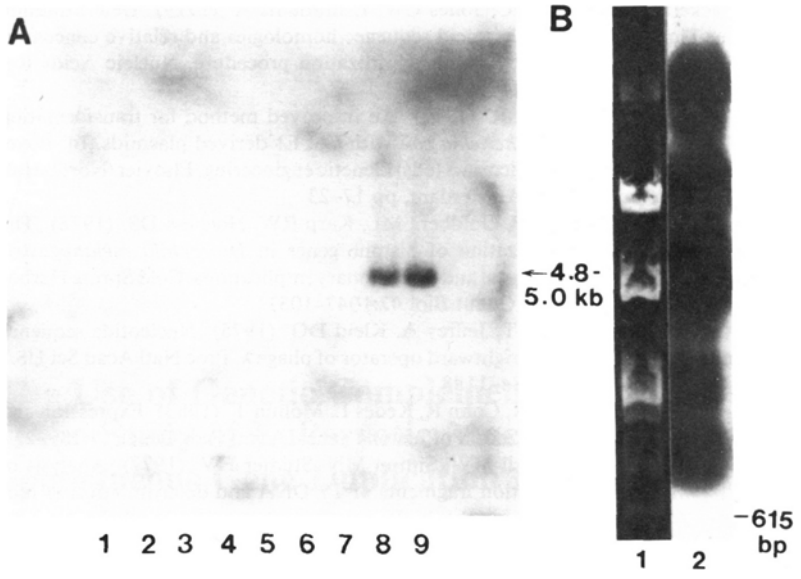

Fig. 8A, B. The H1 gene of cDm500 does not hybridize to *D. virilis* DNA. **A** *D. virilis* DNA (4 μg/lane; lanes 1–7) was singly digested with Ava I, Bam HI, Bgl II, Cla I, Hind III, Sal I, or Xba I; *D. melanogaster* DNA (0.05 μg/lane) was digested with Bam HI (lane 8) or Hind III (lane 9). DNAs were electrophoresed, blotted to nitrocellulose, and hybridized to the nick-translated 615-bp Pst I fragment of cDm500. **B** cDm500 was digested with Pst I, electrophoresed (lane 1), blotted onto nitrocellulose, and hybridized to nick-translated pDv3 (lane 2). pDv3 hybridized to every Pst I fragment of cDm500 except the 615-bp fragment containing 82% of the H1 coding region. The radioautograph of lane 2 was deliberately overexposed to illustrate the absence of even a weak signal of hybridization between the 615-bp Pst I fragment and pDv3

tone gene cluster lacks the H1 gene. While this negative inference is suggestive, it cannot be construed as evidence that histone genes have become rearranged during the evolution of *Drosophila.*

pDv3/3.4 and pDv3/2.5 hybridized to the core histone mRNAs, but did not hybridize to H1 histone mRNA; pDv2.9 hybridized to an uncharacterized mRNA that migrated ahead of *D. melanogaster* histone H1 mRNA, but did not hybridize to core histone mRNAs. The relative electrophoretic mobility of the mRNA complementary to pDv3/2.9 makes it unlikely that pDv3/2.9 contains an H1 gene. However, the question remains open, because we have been unable to identify stable hybrids between the 615-bp Pst I fragment of cDm500 (which contains 82% of the H1 coding region) and *D. virilis* DNAs. Restriction map heterogeneity (Figs. 1A and 2B) (Domier 1984) suggests that the histone genes of *D. virilis* might be arrayed in multiple configurations, only one of which is carried in pDv3. For instance, approximately 50% of the *D. virilis* DNA complementary to pDv3/3.4 is not digested by Bgl II (Fig. 2B) and conceivably might contain genes for histone H1 and one or more core histones. Examination of this possibility has been hindered by the lack of sequence homology between the H1 histone genes of *D. virilis* and *D. melanogaster.*

Our hybridization conditions (1 M NaCl, 50% formamide, 42°C) maintain stable duplexes between DNAs that are 75% homologous. Our inability to form stable hybrids between the H1 genes of the two species suggests that the coding segments in the H1 genes of *D. virilis* and *D. melanogaster* are at least 25% divergent. The estimated sequence divergence of the H1 genes is higher than the observed divergence of coding regions within the core histone genes (Fig. 4). This relationship is consistent with the fact that H1 histones are less conserved than the core histones. At first glance, our minimum estimate of sequence divergence may seem high. However, it becomes plausible when one considers that the coding sequences of cloned H1 genes from the more closely related species *D. melanogaster* and *D. simulans* are 5% divergent (T.J. Murphy and M. Blumenfeld, manuscript in preparation).

In situ hybridization of a *D. melanogaster* histone gene probe to *D. virilis* polytene chromosomes suggests that the *D. virilis* histone genes are located at two different sites: 20g and 4K (Anderson and Lengyel 1983). It will be interesting to learn whether the *D. virilis* H1 genes are located at one, both, or neither of these sites. The problem, however, will be made difficult by the lack of sequence homology between the H1 genes of *D. virilis* and *D. melanogaster.*

The clustered organization of the five types of histone genes in many higher eukaryotes may reflect their coordinate expression during early development (Lifton et al. 1978). The demonstration that at least 30% of *D. virilis* histone gene clusters lack an H1 gene is not compatible with this notion, unless these clusters are not active during embryogenesis.

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