Heterochromatin Condensation and Evolution of Unique Satellite-DNA Families in Two Parasitic Wasp Species: *Diadromus pulchellus* and *Eupelmus vuilleti* (Hymenoptera)[1]

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Large quantities of satellite DNA families (15%–25% of the genome) were found in the DNA of two species of parasitic wasps, *Diadromus pulchellus* and *Eupelmus vuilleti*. In both species the satellite DNA was found to consist wholly or largely of a single family unique to that species. Several clones of each family were obtained and sequenced. Palindromes in each consensus sequence suggest the formation in vivo of hairpin structures that may play a role in the mode of heterochromatin condensation in these insects. The ancestral repeating motifs were determined from the consensus sequences. Plausible scenarios are presented for the evolution of the two satellite DNAs. The occurrence of only one family of satellite DNAs in both species may indicate that, in male haploids, such families have shorter persistence times than necessary for the origins of new duplicated sequences.

Introduction

The order Hymenoptera is divided into two suborders, Symphyta and Apocrita, and contains >200,000 species. The Parasitica superfamily of parasitic wasps is one of the largest in the Apocrita and contains ~100,000 species. Its members share several distinctive features, including a highly perfected ecological and ethological parasitic strategy (Askew 1971), a very low level of enzyme polymorphism (Graur 1985; Sheppard and Heydon 1986; Woods and Guttman 1987), and male haploidy, which may cause loss of the most deleterious mutations (Hartl and Brown 1970; Hartl 1972). The genomic organization of Hymenoptera has so far been studied in only two species, *Diadromus pulchellus* and *Eupelmus vuilleti* (Bigot 1989), revealing the presence of a large quantity of highly repeated DNA component. To further define the nature of this component, the structure of satellite DNA in these two parasitic species has been studied.

Satellite DNAs are composed of well-conserved, tandemly repeated sequences, usually <1,000 bp long, with $>10^5$ copies/haploid genome. These sequences are often concentrated in substantial blocks at the centromeric and telomeric regions of a chromosome and form the principal component of constitutive heterochromatin. The different satellite-DNA families within a species may vary greatly in length, repetitive frequency, and nucleotide sequence. The proportion of the genome composed of satellite DNAs may also vary widely (range 0%–60%) between species, as can the number

1. Key words: terebra, highly repetitive DNA, intrastrand dyad structure, ancestral motif.

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of satellite-DNA families (Ehrlich et al. 1973; Cordeiro-Stone and Lee 1976; Gaillard et al. 1981; Miklos 1985; Beridze 1986; Willard and Waye 1987).

The large intermonomeric DNA sequence divergence in satellite DNA is generated by base changes, deletions, insertions, and, probably, by chromosome rearrangements. However, these changes do not all seem to be randomly distributed throughout the chromosomes. Walker (1971) and MacGregor and Sessions (1986) have proposed an evolutionary model for satellite DNA. According to this model, the DNA sequences appear in the centromeric regions, and some satellite sequences are duplicated in tandem-repeat blocks concentrated around the centromere. In the course of evolution these blocks would migrate by saltation toward the telomeres and would accumulate sequence variations by mutation. During the saltation process the satellites evolve into multiple families within one evolutionary lineage.

No specific physiological role has been attributed to satellite DNA. In the calf, the amount of 5-methylcytosine in satellite DNA has been correlated with the affinity of H1 histone for methylated satellite DNA (Davie and Delcuve 1988; Pages and Roizes 1988). The results suggest that DNA methylation plays a role in modulating the structure of chromatin. However, methylated nucleotides have not been detected in Diptera (Adams and Burdon 1985), raising the question of the modulation of the structure chromatin in insects.

In the present study, we report that the genomes of two species of parasitic wasps, *D. pulchellus* and *E. vuilleti,* each contain a unique satellite-DNA family which accounts for a large part of the genome. The evolutionary relationships of these satellite-DNA families have been determined and used to develop a hypothesis for their heterochromatic role. The evolution of the parasitic satellite DNA is discussed in terms of the Walker (1971) and MacGregor and Sessions (1986) model.

Material and Methods

Material

Diadromus pulchellus is an endoparasitoid of *Acrolepiopsis assectella* (Lepidoptera), which infests *Allium* species. *Eupelmus vuilleti* is an ectoparasitoid of several species of the family Bruchidae (Coleoptera), which infest different plant species of the family Phaseolinae. The two wasp species were bred, in mass, under standard conditions for *D. pulchellus* (Rojas-Rousse 1977) and *A. assectella* (Arnault 1979, 1982) and for *E. vuilleti* and its host *Callosobruchus maculatus* (Terrasse and Rojas-Rousse 1986).

DNA Isolation

DNA was isolated from 1–5-d-old imagos (~5,000–10,000 insects) according to the method of Junakovic et al. (1984). The extracts were treated with pancreatic RNAse A (DNase free), and traces of ribonucleic acids and phenol were eliminated on hydroxylapatite in sodium phosphate buffer pH 7 (NaP) at 60°C, were dialyzed and were precipitated with absolute ethanol. The pellets were washed with 70% ethanol, were resuspended in sterile water, and were stored at 4°C.

Detection of Methylated Bases

DNA was hydrolyzed by the "four-hour-two-enzyme" technique (Gehrke et al. 1984), which treats denaturated nucleic acid successively with nuclease P1 and bacterial alkaline phosphatase for 4 h. The obtained nucleosides were separated by high-speed isocratic high-performance liquid chromatography (HPLC) separation with a Super-

cosil LC18DP column, eluted with 0.05 M K_2HPO_4, pH 4.4, 8% methanol at 25°C, flow rate 2.5 ml/min, detection 254 nm. Nucleosides were identified by comparing their elution with those of synthetic deoxynucleosides (Sigma).

Restriction Digests, Gel, and Southern Blots

The satellite DNAs of both species were searched for orderly ladders of multimeric repeated units by agarose-gel and Southern-blot analyses using genomic DNA as probe. Twenty-two restriction enzymes were used: *Acc*I, *Alu*I, *Ava*I, *Ava*II, *Bam*HI, *Bgl*II, *Cla*I, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hpa*II, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Rsa*I, *Sau*3A, *Sma*I, *Taq*I, and *Xho*I (Boehringer-Mannheim). To ensure complete digestion, incubations were performed for 4 h at 37°C with 10 units/μg of DNA in the conditions described by the supplier. Standard conditions were used for gel electrophoresis and Southern blots (Maniatis et al. 1982).

Purification of DNA Fragments

The fragments of genomic DNA produced by each restriction enzyme were separated on Sigma type IX agarose gels and stained with ethidium bromide. The DNA fragments were eluted using a Quiagen kit, and the concentration of each fragment was estimated on agarose gels.

Probes and Hybridization

Satellite-DNA fragments, which contained equal quantities of monomers and dimers, and total genomic DNA were labeled with dATP [α^{32}P] (Amersham) by primer extension using the Klenow fragment of *Escherichia coli* DNA polymerase (Boehringer Mannheim) and were used as probes.

Hybridizations were performed in 0.45 M NaCl, 0.045 M trisodium citrate (3 × SSC), 0.2% Ficoll 400, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.1% lauryl sulfate (SDS) overnight at 65°C. The final washings were done in 2 × SSC, 0.1% SDS at 65°C, with account being taken of an internal sequence variability of ~20% for a DNA with a GC content of ~40%.

Dot-Blot Estimation of the Proportion of Satellite DNA in the Genome

Dot-blot analysis of total genomic DNA and of isolated monomers and dimers was performed on Hybond C. The concentration of genomic DNA was determined assuming that $1A_{260}$ = 50 μg/ml. Both hybridization with monomeric and dimeric satellite-DNA probe and final washing were done in the same conditions as were used for Southern blots. Hybridized DNA was estimated by liquid scintillation counting.

Cloning, Sequencing, and Alignment

Monomeric and dimeric fragments were cloned with the phage vector M13mp18 (Norrander et al. 1983; Perbal 1984) and were sequenced by the Sanger method with a Boehringer-Mannheim sequencing kit and dATP [α^{35}S] (Amersham). Alignment was done by eye.

Results

Detection of Satellite DNA

Southern blots of *Diadromus pulchellus* DNA probed with total genomic DNA showed orderly ladders of multimeric repeated units, after digestion with *Alu*I, *Ava*I, *Dde*I, *Hae*III, *Hpa*II, *Sau*3A, and *Taq*I (fig. 1a). The *Taq*I enzyme defined a repeated

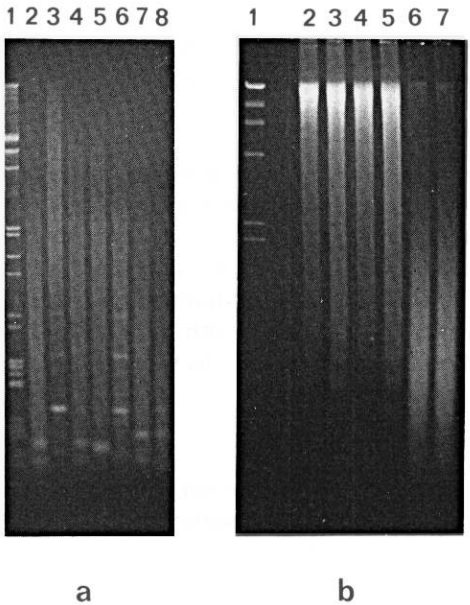

FIG. 1.—Detection of ladder pattern in total genomic DNA digests on 1.2% agarose gels stained with ethidium bromide. a, *Diadromus pulchellus:* lane 1, lambda DNA *Hin*dIII-*Eco*RI + pBr 322 *Hae*III; lanes 2–8, female DNA digested, respectively, by *Alu*I, *Ava*I, *Dde*I, *Hae*III, *Hpa*II, *Sau*3A, and *Taq*I. b, *Eupelmus vuilleti:* lane 1, lambda DNA *Hin*dIII; lane 2, male DNA *Kpn*I; lane 3, female DNA *Kpn*I; lane 4, male DNA *Sal*I; lane 5, female DNA *Sal*I; lane 6, male DNA *Hpa*II; lane 7, female DNA *Hpa*II.

unit of ~320 bp. An additional *Taq*I site frequently cut this unit into two fragments of 110 bp and 210 bp. The uniqueness of the *Taq*I satellite DNA was tested by Southern blotting of genomic DNA digested with *Alu*I, *Ava*I, *Dde*I, *Hae*III, *Hpa*II, and *Sau*3A and probed either with the *Taq*I monomer and dimer DNA fragments or with total genomic DNA. The same ladder patterns were obtained with both probes. This single satellite DNA accounted for ~15% of the genome in both sexes. The isoschizomers *Hpa*II and *Msp*I produced the same pattern with male and female DNA (data not shown), indicating the absence of methylation at these sites.

Agarose gels of *Eupelmus vuilleti* genomic DNA showed ladder patterns after digestion with *Kpn*I and *Sal*I (fig. 1b and fig. 2). Southern blots probed with total genomic DNA gave the same kind of patterns with *Alu*I, *Hae*III, *Hpa*II, *Rsa*I, and *Taq*I. *Kpn*I defined a repeated unit of ~110 bp. The uniqueness of this satellite DNA was demonstrated by Southern blots of the hybridizations between the *Kpn*I monomer-and-dimer DNA probe and the total genomic DNA probe. This satellite DNA accounted for ~25% of the genome in both sexes.

The *Hpa*II and *Msp*I digests (data not shown) of DNA showed the same pattern in both sexes. However, the *Kpn*I and *Sal*I ladder patterns of males and females differed in intensity (fig. 1b), showing the presence of uncut sites in males. These sites might be methylated (see Consequence Sequence section following).

The Southern blot of a NewsieveGTG agarose gel showed units of different lengths (fig. 2). A fragment of ~80–85 bp was interspersed among the 110-bp units. An identical result was obtained by electrotransfer from identical DNA digests separated on a denaturing gel (8 M urea, 6% acrylamide-bis-acrylamide 19/1, 55 mM Tris-borate, 55 mM boric acid, 2 mM ethylenediaminetetraacetate; gel 400 mm long, 150

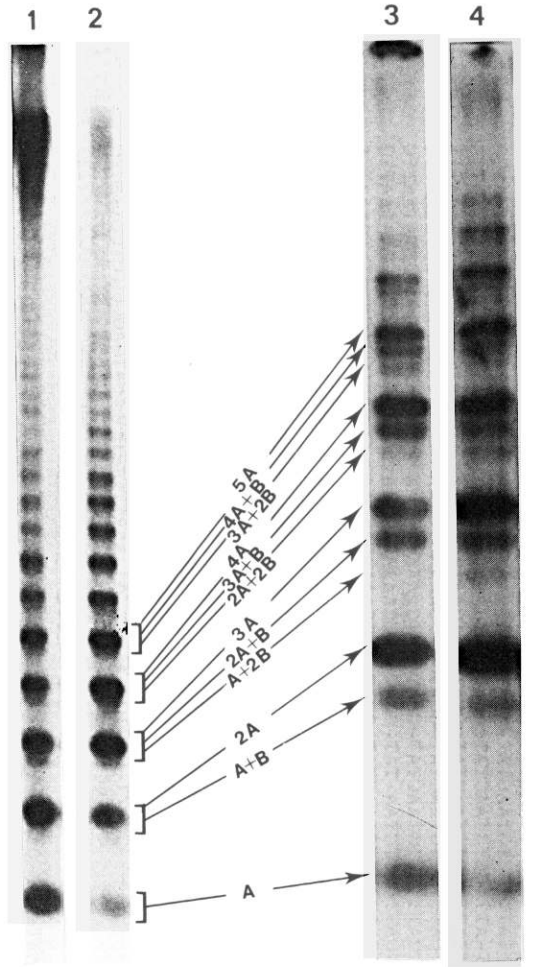

FIG. 2.—Southern blot analysis of *Eupelmus vuilleti* satellite DNA, using a 1.5% agarose gel (lanes 1 and 2) and a 4% Nusieve GTG agarose gel (lanes 3 and 4). *Kpn*I (lanes 1 and 3) and *Sal*I (lanes 2 and 4) digests of female DNA were probed with radiolabeled *Kpn*I monomers and dimers. A = the major tandem repeat of 108 bp; B = a minor sequence of ~80–85 bp and apparently interspersed between A repeats.

mm wide, 0.9 mm thick) and tested with the same probe. However, the data at this step are not sufficient to determine whether this heterogeneity indicates the existence of a minor satellite-DNA subfamily. If such a minor subfamily is present, it accounts for ~2%–3% of the total genome. The method used will detect a ribosomal fragment repeated ~100 times and can therefore detect ladder patterns representing ≥0.1% of the genome.

Consensus Sequence

Eight *Taq*I monomeric sequences were obtained from *D. pulchellus* DNA. Only one clone was entirely sequenced. In the other cases, T-and-A clustering led to problems that were not solved by using dITP, deazadGTP, reverse transcriptase, or Sequenase. However, the seven other monomers were reconstituted from 14 partial sequences in both orientations and the presence of a well-conserved *Taq*I site at position 212. The

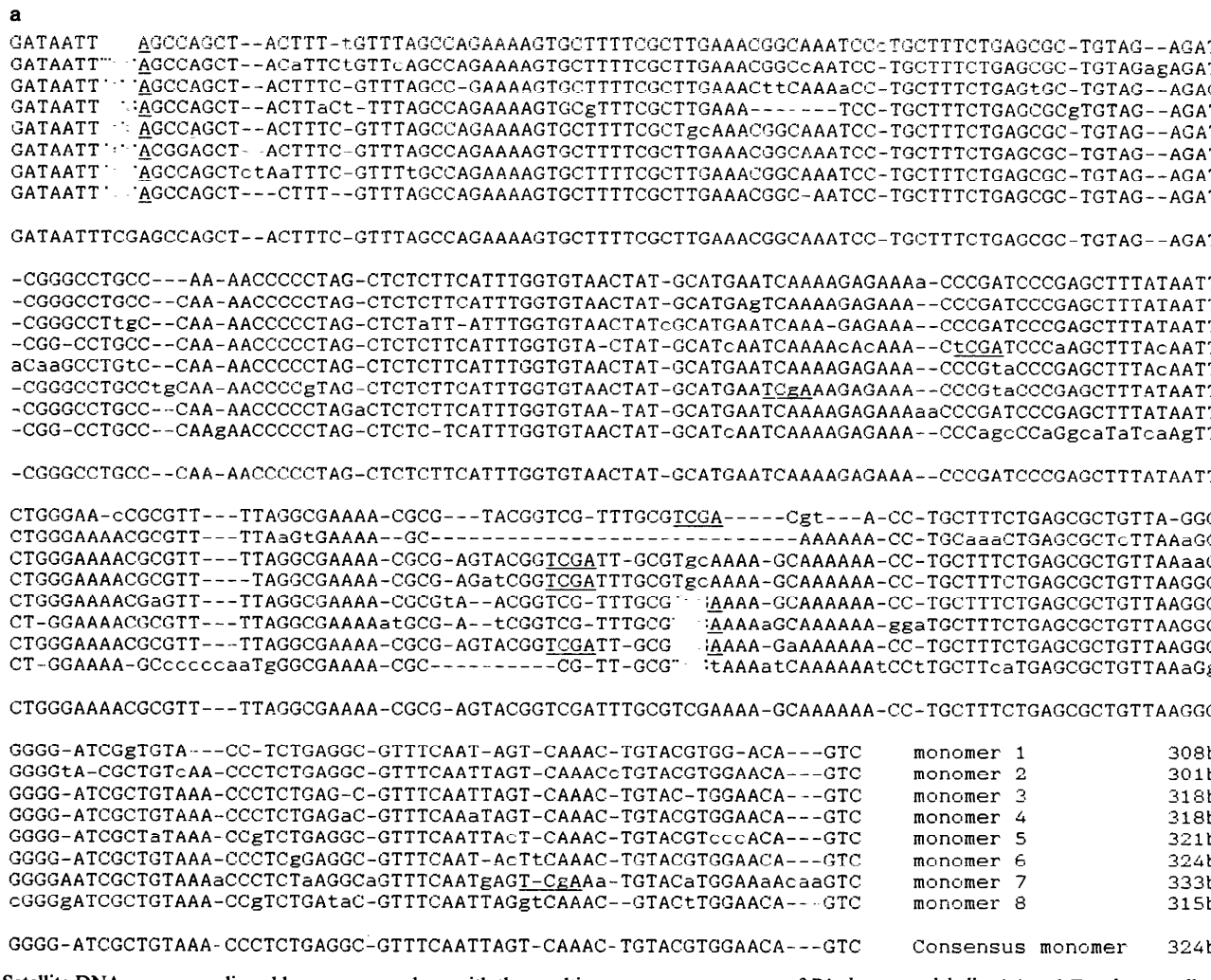

FIG. 3.—Satellite DNA sequences aligned by monomer, along with the resulting consensus sequences of *Diadromus pulchellus* (a) and *Eupelmus vuilleti* (b). Major bases are in uppercase letters; minor bases are in lowercase letters. The unexpected *TaqI* sites in the sequenced monomers of *D. pulchellus* are underlined.

b

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ACCGATTGAAATaTt-ATAAAA-T-GgCCG--AA-AATCCCT-GTA-GACgGA-TGTC-tAGGTG-TtA-GGgaTGA
ACCGATTGAAAca-aCATAAAA-TtGCCCG--AA-AATtCCT-GTA-GACgGA--GTC-CAGGTG-cCA-GGA-TGA
ACCGATTGAAATTTaCATAAAA-TgtCCCG--AA-AATCCCT-GTA-GAa-GA-TGT-gtAGGTG-TCA-G-A-TGA
ACCGATTaAAAgTTtCATAAAAgT-GCCtG--AA-AAT-CCT-GTA-GACgaA-gGTCgCAGaTG-TCA-GGAgTGA
ACCGATTGAAATaTa-ATAAAA-T-GCCaa--AA-AATCCCT-GTAgGACgGA-TGTC-CAGGTG-aCA-GGA-TGA
ACCaATTGtAATTT-gATAAAAaT-GCCCG--AA-AATtCCT-GTA-GAa-GA-TGTC-aAGGTG-TCA-GGA-TGA
ACCGATTGAAATTT-CATAAAA-T-GcCCG--ActAATatCT-GTA-GAC-GA-TGTC-CAGGTG-TCA-GGA-TGA
ACCGATaaAAATT------------CCC---AA-AtTCCC--aT--GAC--AaTa-C-CAGGTG-TCA-GGA-TGA
ACCaATTGAAgaTTgAgAAgAaT-GCCCG--AA-AATtCC-gGTA-GAC-GAaTGTC-CAGGTG-TCA-GGt-TGA
ACCGATTtAAATTT-CATAAAA-TaG-CCGctAAtAATttCctGTAcGtAtGA-TGTC-tAGGTGaTCAcGGA-TGA
ACCaATTGAAATTT-CATAAAA-T-GCCCG--AA-AATtaCTc-TA-GAC-GA-TGTC-CAGGTG-TCA-GGA-TcA
ACCGATTGAAATTT-CATAAAA-T-GCCCG--AA-gtTtaCTccTA-GAC-GA-TGTC-CAGGTG-TaA-GGA-TGA
ACCGATTGAAATTT-CATAAAA-T-GaCCG--AA-AATt-CTtGcAtGAC-GA-TGTC-CAGaTG-TCA-GGA-TG-
ACtaccTGAAtcTacCg-AAAA-T-GgCCG--AA-AcTCaCT-GaA-GAC--AaTGTC-CAGGTG-TCA-G-AgTGA
ACCGA-TGAAATTT-CATAAAAgT-GCCCG--AA-AATCCCT-GTt-GAC-GAgTGTC-CAG-TG-TCA-GGA-TGA
AgtGAcTGtAATTacCg-AAAA-TcG-CCG--AA-AcTCgCT-GaA-GAC-GA-TaTC-CAGGTG-TCA-GGA-TGA

ACCGATTGAAATTT-CATAAAA-T-GCCCG--AA-AATCCCT-GTA-GAC-GA-TGTC-CAGGTG-TCA-GGA-TGA

CCCCCA--TtTCGA-C-aTAAgACg--TC--TACGAGGAAGTGCA-CCAGGT   monomer  1         107bp
CagCCA--GGTCGA-C-GTAACACtA--C--TACGAG-AAGTGCc-atAGGT   monomer  2         106bp
CCCCCA--GaTCGA-C-GTAACAC---TC--TACGAGGAAGTGCA-CCAGGT   monomer  3         107bp
CCCCCA--GaTCGg-C-aTAACACCA-TC--TACGAGGAAcTtCA-CCAGGT   monomer  4         112bp
CCCCCA--GGTCGA-C-GTA-tA-CA-TC--TACGcGG---TGCA-CCAGGT   monomer  5         105bp
CCaCCA--GGTaGA-C-GTAACAtCA-TC--TACGAGGAAGTGCA-CCAGGT   monomer  6         108bp
CCtCCA--GGTCGA-C-GTAACACtA-TC--TACGAGGAAGTGCA-CCAGGT   monomer  7         108bp
gCCCCA--GcTCGA-C-GTAACAtCA-TC--TAtGAGGAAGTtCA-CCAGGT   monomer  8          94bp
CCCCCA--GGTCGA-C-tTAACgCCA-TC--TACGAGGAAcTGCA-CCAGGT   monomer  9         110bp
-CCCCAacGtaCGAaCaGTAACgCCAcTgcgTACGAGGA-GTGCAtCCAGGT   monomer 10         125bp
tCCCCA--GGTCGA-C-GTAACACCA-TC--TACGAtGAAaTG-AaCC-GcT   dimer 1;unit 1     106bp
tCCCCA--GGTCGA-C----ACA----TC--TAtGAGGAA-TGCA-CCAGGT   dimer 1;unit 2     100bp
aCCCCA--GGaCGA-C-GTAACACCA-TC--TACGAGGAAGcGCAtCCAGaT   dimer 2;unit 1     109bp
-CCCCA--GGTCGA-C-GTAACACCA-TC--atCGAGGAAGTGCA-CCAGGT   dimer 2;unit 2     106bp
CCCaCA--GGTCGA-C-GTAACACCA-TC--TACGAGGAAGcGCA-CCAGaT   dimer 3;unit 1     107bp
CCCCCAa-GGaCGA-a-tTAACACCA-TC--TACGAGGAA-TGCA-CCAGGT   dimer 3;unit 2     107bp

CCCCCA--GGTCGA-C-GTAACACCA-TC--TACGAGGAAGTGCA-CCAGGT   Consensus sequence 107bp
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ACCGATTGAAATTTCATAAAATGTCCGCAAATCCCTGTAGACGATGTCCAGGTGTCAGTA

TGACCCTCAGGTCGACGTAACACCATCTACAAAGAATCGCACTAGGTATTATTCCAGTAC

ACGTTGTCCAGGTATCAGGGTAACCTCCAGATCGACGTAACATCATCAACGAGGAAGTTC

ACCAGGT (A+B)= 187 bp

FIG. 4.—Sequence of one of the two sequenced clones, showing the association (A+B) between a 10-bp unit and a 80–85-bp unit (underlined).

consensus sequence (fig. 3a) was 324 bp long, contained 47% GC, and had a mean intermonomer DNA sequence divergence of 9.1%.

Other unexpected *Taq*I sites (underlined in fig. 3a) were found in the sequenced monomers, in spite of the fact that the different satellite-DNA polymers were cloned

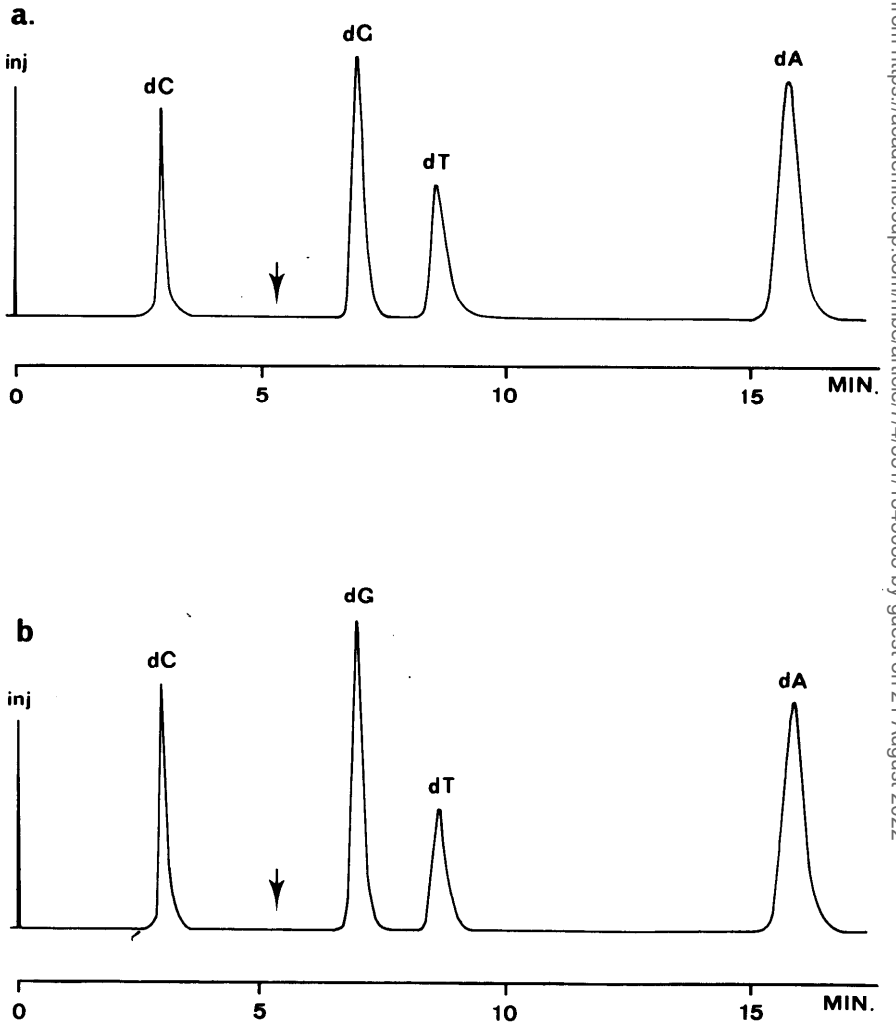

FIG. 5.—HPLC analysis of the nucleoside of DNA from *Diadromus pulchellus* (a) and *Eupelmus vuilleti* (b). Arrows indicate the expected elution position of 5-methyl-cytosine.

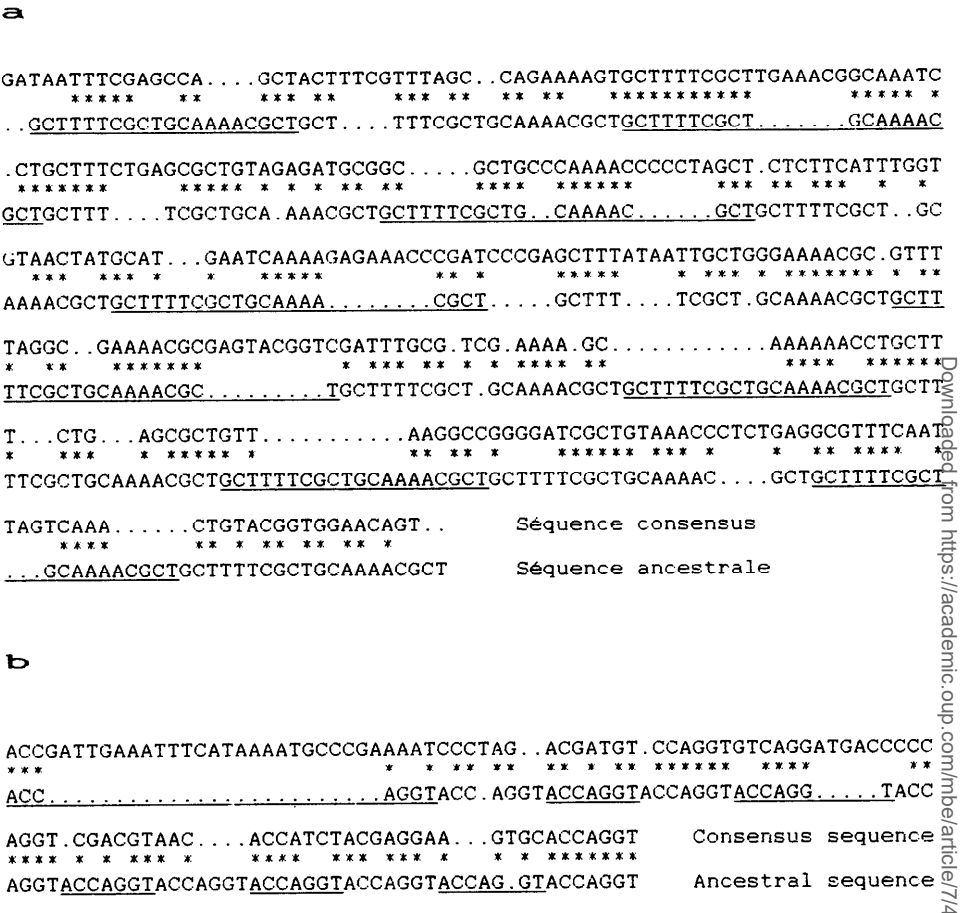

FIG. 6.—Comparison of the consensus sequence of satellite DNA monomer and its putative ancestral sequence in *Diadromus pulchellus* (a) and *Eupelmus vuilleti* (b). To distinguish them in the ancestral sequences, the asterisks (*) indicate the regions of strong conservation of the palindromic motifs. Each motif is also underlined alternately in the ancestral sequence. These regions might be the basis for the formation of dyad intrastrand DNA structure in the present-day satellite DNAs of *D. pulchellus* and *E. vuilleti*.

with purified fragments from completed *Taq*I digestion. Consequently, *D. pulchellus* satellite DNA is somewhat resistant to digestion with *Taq*I. These uncut *Taq*I sites might be methylated (see Search for Methylated Bases section following).

The consensus sequence for *E. vuilleti* (fig. 3b) was obtained from 11 *Kpn*I monomers (fig. 2, A) and from three *Kpn*I dimers (fig. 2, A). The sequence of an 80–85-bp fragment plus a 110-bp unit (fig. 2, A+B) was obtained from two clones (fig. 4). This 80–85-bp sequence corresponded to the 3′ part of the 110-bp unit. The consensus sequence (fig. 3b) has a 107-bp unit length, a 49.1% GC content, and a mean intermonomeric DNA sequence divergence of 13.9%. The intermonomeric DNA sequence divergence (13%) of the 80–85-bp fragment was similar to that of the consensus sequence; this sequence is therefore not a minor subfamily.

In conclusion, the two parasitic species both contain large amounts of satellite DNA (15%–25% of the total genome), and each species has a unique satellite-DNA family.

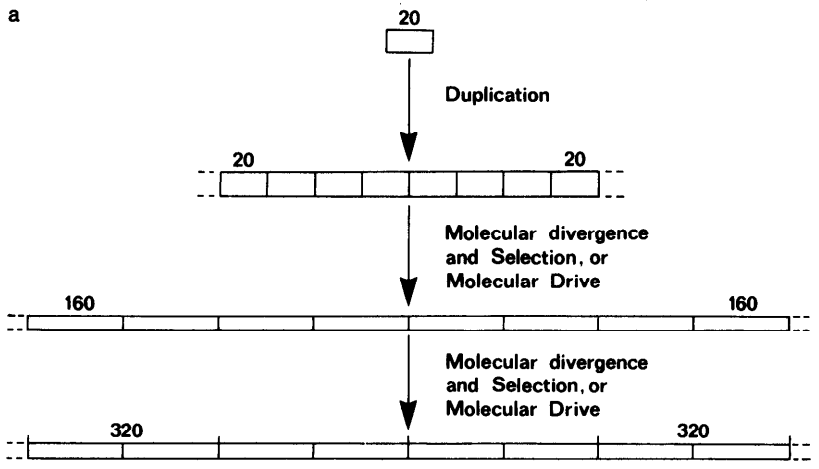

FIG. 7.—Schematic representation of the putative evolution of the 324-bp unit in *Diadromus pulchellus* (a) and of the 107-bp unit in *Eupelmus vuilleti* (b).

Search for Methylated Bases

No methylated base was found by HPLC analysis, either in the total DNA or in purified satellite monomers (fig. 5). Therefore, the undigested *Taq*I (*D. pulchellus*), *Kpn*I, and *Sal*I (*E. vuilleti*) sites cannot be due to methylated bases.

Search for an Ancestral Motif

Screening of the eight individual monomers of *D. pulchellus* for a conserved motif revealed a 20-bp sequence (GCTTTTCGCTGCAAAACGCT) which was repeated 16 times in the consensus unit (fig. 6a). The sequence alignment showed an internal average identity of 69% between positions 35–140 and 195–319, which could be the relic of a 160-bp intermediate evolutionary motif. The 324-bp *Taq*I unit would therefore originate from a 20-bp motif duplicated during evolution, which, either by molecular divergence and selection or by molecular drive (Dover 1982), gave rise to a larger, 160-bp motif. This intermediate motif gave rise to the present 324-bp monomer (fig. 7a).

Analysis of 10 monomers from *E. vuilleti* produced a 7-bp motif with the sequence ACCAGGT. Thus, the 106-bp consensus sequence (fig. 2, A) would have originated from a dodecamer of the 7-bp motif (fig. 6b). Both the irregular distribution of the 80–85-bp fragment (fig. 2, B) and the internal sequence similarity between the fragment (fig. 2, A+B) with the 106-bp consensus sequence could be due to insertion of a 28-bp fragment in position 3, which then invaded the whole satellite DNA either by selection and conversion or by molecular drive (fig. 7b).

Potential Dyad Intrastrand DNA Structure

The palindromic structure of the 20-bp motif of *D. pulchellus* and the 7-bp motif of *E. vuilleti* are the most striking features of these satellite DNAs. These sequences could form dyad intrastrand DNA structures, with or without a loop, as illustrated in figure 8. The same structures may be formed from the highly conserved regions in the present satellite-DNA sequences (fig. 6), as far as thermodynamic parameters permit.

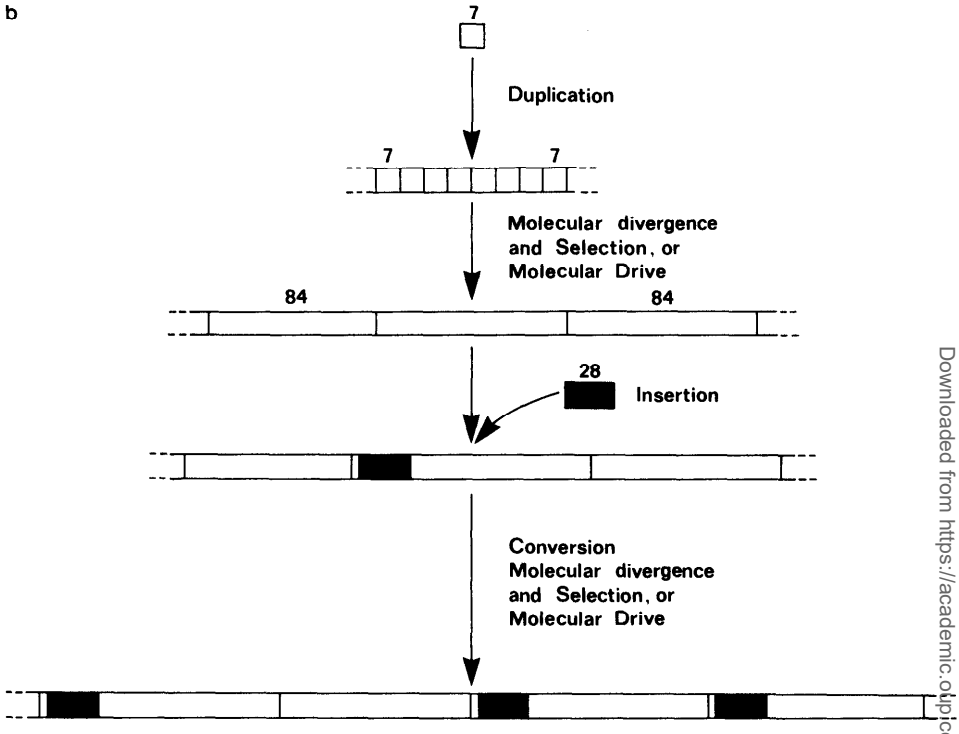

FIG. 7 (*Continued*)

Discussion

Diadromus pulchellus and *Eupelmus vuilleti* satellite DNAs have several common structural and organizational features which can be used to reconstruct their evolutionary history and to propose a role for them. The potential capacity of satellite DNA to form dyad intrastrand DNA structures may be used to develop two hypotheses.

The first hypothesis is that satellite DNAs might be involved in heterochromatin condensation. Dyad intrastrand DNA structures, induced by the palindromes in the satellite-DNA sequence, would be formed and maintained by specific proteins. In the calf, it has been proposed that chromatin regions rich in methylated DNA would have an increased affinity for H1 histone and that DNA sequences would be assembled and maintained as compact chromatin structure through the action of the H1 histone (Davie and Delcuve 1988; Pages and Roizes 1988). This type of heterochromatin condensation cannot occur in parasitic wasps because their DNA contains no methylated bases. Certain regions of the satellite-DNA sequences in *Drosophila melanogaster* (Miklos 1982) and in the crab *Gecarcinus lateralis* (Fowler et al. 1988) lack methylated bases and contain potential dyad intrastrand DNA structures. These dyad structures might be the proper mechanism of heterochromatin condensation. However, it is not necessary to assume that the mechanism of chromatin condensation is completely different in species with and without methylation. It can be proposed that the role played by methylation signals in vertebrate genomes would be performed by the presence of specific dyad structure distributed along the satellite in insects.

The uncut *Kpn*I and *Sal*I sites in *E. vuilleti* males and the partially "inhibited" activity of *Taq*I in *D. pulchellus* are not due to methylated bases. The lack of digestion

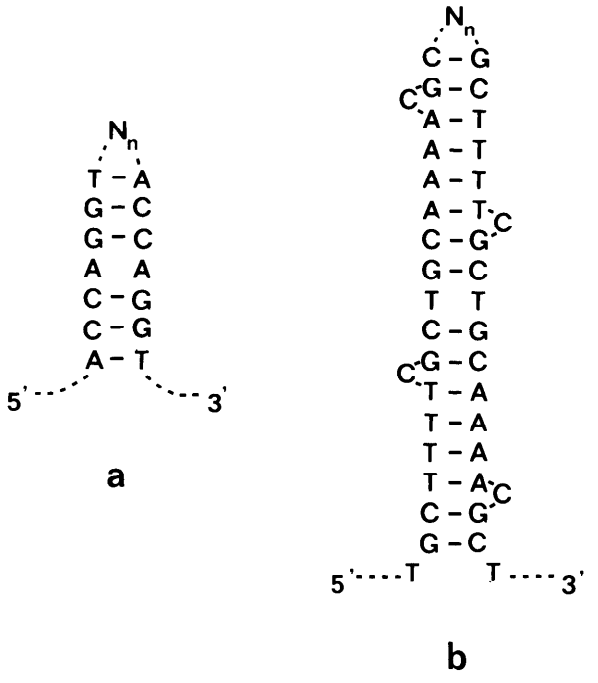

FIG. 8.—Possible dyad structures generated by the ancestral motif of *Eupelmus vuilleti* (a) and *Diadromus pulchellus* (b).

must be due to chemical or structural changes in these restriction sites. These changes might be due to dyad intrastrand DNA structures which would denature the structure of restriction sites necessary for the activity of the enzyme. The differences in the restriction patterns of male and female *E. vuilleti* suggest that the presence of dyad intrastrand DNA structures might also be sexually regulated.

However, the fact that a satellite DNA has a particular sequence and that this sequence forms dyad intrastrand DNA structures does not mean that it has a function. A second hypothesis is therefore that the formation of dyad intrastrand DNA structures in a sequence may be the cause of its duplication in tandem repeats, by interacting, e.g., with DNA-replication processes.

The other main features of the two parasitic-wasp genomes are the size and the uniqueness of their satellite-DNA families. Satellite DNA accounts for 15% of the *D. pulchellus* genome and for 25% of that of *E. vuilleti*. Other eukaryotic genomes—e.g., those of *Drosophila*, crab, and calf (Ehrlich et al. 1973; Cordeiro-Stone and Lee 1976; Gaillard et al. 1981; Miklos 1985; Beridze 1986; Willard and Waye 1987)—contain similar or greater proportions of satellite DNA. However, in contrast to these species, *D. pulchellus* and *E. vuilleti* satellite DNAs are formed by only one satellite-DNA family. These results suggest that the effects of the turnover processes for satellite DNA in Parasitica are different from those in vertebrates and other arthropods. The uniqueness of the satellite-DNA family would indicate that the evolutionary period of satellite-DNA degeneration must be less than or equal to the time necessary for the appearance of a new satellite-DNA family. Satellite-DNA turnover has its own evolutionary rate, which would be fast or slow, and the species might be devoid of satellite-DNA sequences during certain evolutionary periods. These features are not in disagreement with the

evolutionary model of Walker (1971) and MacGregor and Sessions (1986). In fact, the molecular processes are probably identical in Parasitica, vertebrates, and other arthropods. The principal peculiarities of the parasitic wasps may be due to features of the Hymenoptera at the organismal level. In these species, the speed of the processes concerning the amplification rate of a new motif of satellite DNA, its evolution, and its disappearance would be modified by the special constraints imposed by the haplo-diploid system.

Our hypothesis for the role and evolution of satellite DNA is now being tested in studies on the satellite DNAs of *D. collaris* and *E. orientalis,* two sibling species of *D. pulchellus* and *E. vuiletti,* respectively.

Sequence Availability

The DNA sequences have been deposited in EMBL under reference number M31306 for *Epelmus vuilleti* and M31307 for *Diadromus pulchellus.*

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