

# Satellite DNA Repeat Sequence Variation is Low in Three Species of Burying Beetles in the Genus *Nicrophorus* (Coleoptera: Silphidae)

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Three satellite DNA families were identified in three species of burying beetles, *Nicrophorus orbicollis*, *N. marginatus*, and *N. americanus*. Southern hybridization and nucleotide sequence analysis of individual randomly cloned repeats shows that these satellite DNA families are highly abundant in the genome, are composed of unique repeats, and are species-specific. The repeats do not have identifiable core elements or substructures that are similar in all three families, and most interspecific sequence similarity is confined to homopolymeric runs of A and T. Satellite DNA from *N. marginatus* and *N. americanus* show single-base-pair indels among repeats, but single-nucleotide substitutions characterize most of the repeat variability. Although the repeat units are of similar lengths (342, 350, and 354 bp) and A+T composition (65%, 71%, and 71%, respectively), the average nucleotide divergence among sequenced repeats is very low (0.18%, 1.22%, and 0.71%, respectively). Transition/transversion ratios from the consensus sequence are 0.20, 0.69, and 0.70, respectively.

## Introduction

Burying beetles, genus *Nicrophorus* (Silphidae), derive their name from their habit of utilizing carcasses of small vertebrates for food and larval breeding sites. A male–female pair find a carcass and process it by removing fur or feathers, rolling it into a ball, consuming and regurgitating some of the flesh, and burying the processed carcass. The buried carcass serves as both the developmental environment and the food source for their offspring. *Nicrophorus* species are generally distributed throughout North America and occupy a number of habitats where suitable carrion can be found (Peck and Kaulbars 1987). One species, *N. americanus*, the largest species in North America, has undergone a substantial decline in distribution and is listed as an endangered species (Hecht 1989). *Nicrophorus* species have been the subject of study in ecology/natural history (e.g., Kozol, Scott, and Traniello 1988; Trumbo 1990; Creighton, Vaughn, and Chapman 1993; Lomolino et al. 1995), behavior (e.g., Fetherston, Scott, and Traniello 1990, 1994; Scott 1994a, 1994b), and, recently, genetics using molecular markers (Scott and Williams 1993; Kozol, Traniello, and Williams 1994). Here we describe unique satellite DNA sequences isolated from three *Nicrophorus* species.

Tandemly repeated DNA sequences, or satellite DNA in particular, are a major component of most eukaryotic genomes and are characteristically located in constitutive heterochromatin in centromeric and telomeric regions of chromosomes. Most highly repetitive sequences have a buoyant density that differs from that of the majority of nuclear DNA because they are AT-rich and thus form “satellite” bands during cesium chloride centrifugation. The role of satellite DNA in the genome is unclear, and satellite DNA is largely considered to be nonfunctional; however, there is evidence that

centromeric proteins may recognize and bind some families of satellite DNA (Muro et al. 1992; Masumoto et al. 1989).

There is considerable variation in the total amount and the number of classes of tandemly repeated DNA in eukaryotic genomes, and in the size and sequence variability of the repeats comprising the tandem arrays. Repetitive DNA has been classified as micro-, mini-, and satellite DNA according to the size of the repeat unit and the size of the array (Tautz 1993). Estimates of the proportion of a particular class of repeated DNA in the genome ranges from less than 1% to over half of the genomic DNA (e.g., Lohe and Roberts 1988; Davis and Wyatt 1989). In several insects, more than one class of satellite DNA has been identified (e.g., Miklos and Gill 1982; Lohe and Roberts 1988; Bachmann, Venanzetti, and Sbordoni 1996; Ugarković, Durajlija, and Plohl 1996). The size of the tandemly repeated units varies from 2 bp to more than 40 kb, and the total size of the tandem array varies from less than 100 bp to over 100 Mb (Miklos and Gill 1982; Benedum et al. 1986). In insects, many repeats have been described in the range of 100–500 bp. Some repeat units have identifiable substructures or conserved sequences embedded in the repeat (Rovira, Beermann, and Edström 1993; Tarès, Cornuet, and Abad 1993; Ugarković, Podnar, and Plohl 1996).

Satellite DNA, and tandemly repeated DNA in general, shows characteristic patterns of sequence variation, with high homogeneity within a repeat family (within a species) and rapid divergence between species (e.g., Petitpierre et al. 1995). The high degree of sequence similarity among repeats in satellite DNA is a characteristic pattern of concerted evolution described in multigene families (Arnheim 1983). Various mechanisms of homogenization, such as unequal crossing over, gene conversion, slippage replication, and differential replication or gene amplification, have been proposed to explain the total amount of satellite DNA, the sequence variability among repeated units, and the rate of interspecific divergence. Rates of mutation and recombination, the length of the tandem array, and the length and com-

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plexity of a repeat unit are considered to be involved in the evolutionary dynamics of tandemly repeated DNA (Walsh 1987; Stephan 1989; Charlesworth, Sniegowski, and Stephan 1994).

We are interested in characterizing sequence variability in the complex satellite DNA of *Nicrophorus* beetles. In this study, we describe repetitive DNA detected in the genomes of three species of burying beetles, *N. orbicollis*, *N. marginatus*, and *N. americanus*, with respect to size and sequence variability, and compare characteristics of complex satellite DNA described for other insects.

## Materials and Methods

### Specimens

The beetles used in this study were generously provided by M. P. Scott (University of New Hampshire) and A. Kozol (who holds a permit to collect *N. americanus*). *Nicrophorus orbicollis* beetles were from New Hampshire, and *N. marginatus* and *N. americanus* beetles were from Block Island, R.I.

### Cloning and Sequencing Satellite DNA Repeats

Total genomic DNA was extracted from individual beetles using a method described by Harrison, Rand, and Wheeler (1985) and was digested with several restriction enzymes during preliminary investigations. Three enzymes were chosen for repeat cloning because they produced single intensely staining bands on complete digestion of the genomic DNA: *Hind*III, *N. orbicollis*; *Hae* III, *N. marginatus*; and *Bam*HI, *N. americanus*. After electrophoresis on 1.8% agarose gels, bands at approximately 350 bp in each of the three species were excised and purified from the gel. The restriction fragments of *N. americanus* and *N. orbicollis* were cloned into pBSKS+ (Stratagene), and the *Hae* III fragments of *N. marginatus* were cloned into M13mp18 using standard procedures (Sambrook, Fritsch, and Maniatis 1989).

For manual sequencing, double-stranded DNA templates in pBSKS+ were purified using alkaline lysis (Sambrook, Fritsch, and Maniatis 1989) and extraction with acid phenol (pH 5.2). Sequencing was done by the dideoxy chain termination method (Sanger, Nicklen, and Coulson 1977) using Sequenase version 2.0 (U.S. Biochemical). For automated sequencing (ABI), double-stranded templates in pBSKS+ were prepared using the Wizard Miniprep DNA purification system (Promega Corp.). Single-stranded templates in M13mp18 were prepared using the QIAprep Spin M13 kit (Qiagen, Inc.). The following numbers of randomly cloned repeats were sequenced: 23 repeats from *N. orbicollis*, 17 repeats from *N. marginatus*, and 20 repeats from *N. americanus*. Complete and overlapping coverage in both directions was obtained for at least one repeat from each species. Automatic sequencing was performed in only one direction for the rest of repeats. Manually sequenced repeats had coverage in both directions for approximately 75% of the repeat and single coverage elsewhere.

### Southern Blots

DNA restriction enzyme digestions were fractionated on 0.9% agarose gels. Gel denaturing, neutralizing, and transfer to Zetabind nylon membrane (Cuno, Inc.) was carried out according to the membrane manufacturer's instructions. Prehybridization and hybridization were also carried out according to the membrane manufacturer's instructions: prehybridization for 2 h at 42°C in 5 × SSC (0.75 M sodium chloride, 0.075 M sodium citrate); 10 × Denhardt's solution (2 μg/ml bovine serum albumin, 2 μg/ml polyvinylpyrrolidone, 2 μg/ml ficoll); 0.05 M NaPO<sub>4</sub>, pH 6.7; 500 μg/ml sonicated denatured salmon sperm DNA; 5% dextran sulfate; and 50% formamide, and hybridization for 20 h at 42°C in 5 × SSC; 1 × Denhardt's solution; 0.02 M NaPO<sub>4</sub>, pH 6.7; 100 μg/ml sonicated denatured salmon sperm DNA; 10% dextran sulfate; and 50% formamide. The following washes were done to remove nonspecifically bound probe: one wash for 15 min with 2 × SSC, 0.1% sodium dodecyl sulfate (SDS) at room temperature; one wash for 15 min with 0.1 × SSC, 0.1% SDS at room temperature; and two washes for 30 min each with 0.1 × SSC, 0.1% SDS at 60°C. The hybridization probe used was the clone of the *N. orbicollis* 342-bp *Hind*III repeat labeled using the random primer method (Feinberg and Vogelstein 1983).

### Sequence Analysis

The sequences were aligned using CLUSTALW (Thompson, Higgins, and Gibson 1994). Dot plot analyses were performed using GCG (Devereux, Haebler, and Smithies 1984). Analysis of polymorphism was done using DnaSP (Rozas and Rozas 1995). BLAST (Altschul et al. 1990) searches were performed to identify sequence similarity with other sequences in the GenBank databases.

## Results

### Repeat Organization

Genomic DNA isolated from single individuals of each of the three beetle species was digested with several restriction enzymes. In *N. orbicollis*, digestion with *Hind*III and *Hae* III produced a single intensely staining band at approximately 350 bp, and digestion with *Dra*I produced a single band that appeared to be about 50 bp smaller than the *Hind*III band. The single intensely staining bands observed after enzyme digestion and agarose gel electrophoresis are evidence of the presence of a repeat unit in high copy number in the genome. We gel-isolated and cloned the *Hind*III fragments and sequenced 23 repeats (fig. 1).

A cloned *Hind*III monomer was radioactively labeled and used to probe a Southern blot of *N. orbicollis* genomic DNA digested to completion with *Eco*RI. Hybridization under stringent conditions revealed a ladder-like pattern that is characteristic of tandemly repeated DNA sequences. An identical pattern was observed in both males and females of the species. The ladder began with a band the same size as the 342-bp *Hind*III repeat, and the larger sized bands were multimers of this single

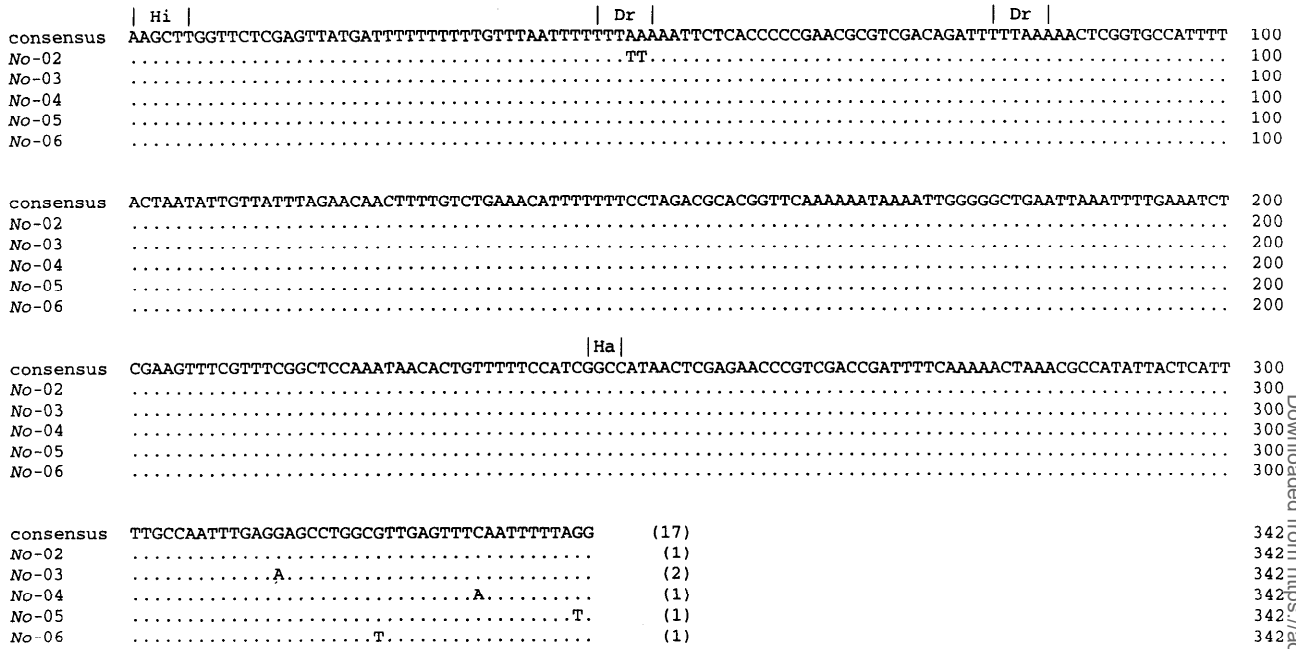


FIG. 1.—Sequence alignment of satellite DNA repeats in *N. orbicollis*. Dots denote nucleotides that are the same as the consensus; dashes denote gaps. Numerals at the end of lines represent cumulative sequence length, and numerals in parentheses represent the number of samples with that sequence. Restriction enzyme recognition sites are noted above the sequence: Dr, *Dra* I; Ha, *Hae* III; Hi, *Hind*III. GenBank accession numbers: AF016151–AF016156.

repeat unit. The ladder pattern results from tandemly repeated units, some of which lack *Eco*RI sites. Indeed, all of the sequenced *Hind*III fragments lacked *Eco*RI sites. The ladder pattern was visible up to 7.0 kb, which would represent approximately 20 tandemly repeated 342-bp monomeric units that are polymorphic for *Eco*RI restriction sites. This represents a tandem array of approximately 7 kb in length. *Eco*RI-digested genomic DNA of *N. marginatus* and *N. americanus* were also present on this Southern blot, and there was no evidence of any interspecific cross hybridization of the *N. orbicollis* repeat to any fraction of the genomic DNA of these two species.

Genomic DNA digestions of *N. marginatus* with various restriction enzymes showed that *Bam*HI produces a single intensely staining band after agarose gel electrophoresis, as does *Hae* III digestion of *N. americanus*. Similar to *N. orbicollis*, the presence of a single band indicates repeats in high copy number. These bands were excised from the gels and cloned. Although no Southern blots using the repeat as a probe were performed on *N. marginatus* and *N. americanus*, the arrangement of the repeats in these species, as in *N. orbicollis*, is most likely in tandem.

Repeat Sequence Characterization

For *N. orbicollis*, sequences of 23 repeats were determined, and these represent six different sequences, which are shown in figure 1. The consensus length of the repeat is 342 bp, and there are no positions exhibiting indels. This satellite DNA family is called “NOH342.” The mean proportion of A+T is 0.65. There are six polymorphic sites (1.8%), and the average number of nucleotide differences among the 23 repeats,

excluding gaps, is 0.60. The ratio of transitions to transversions from the consensus sequence is 0.20, and all of the nucleotide changes are to an A or T.

For *N. marginatus*, sequences of 17 repeats were determined, and these represent 11 different sequences, which are shown in figure 2. The consensus length of the repeat is 350 bp, and this satellite DNA family is called “NMH350.” Relative to the consensus sequence, three repeats have single-base-pair insertions, two repeats have single-base-pair deletions, and one position exhibits three character states that can be interpreted as two separate insertions, or an insertion and a subsequent substitution. In this family, excluding gaps, the mean proportion of A+T is 0.71, the number of polymorphic sites is 27 (7.7%), and the average number of nucleotide differences among all repeats is 4.28. The ratio of transitions to transversions from the consensus sequence is 0.69, and 16 of the 27 nucleotide changes are to an A or T.

For *N. americanus*, sequences of 20 repeats were determined, and these represent 10 different sequences, which are shown in figure 3. The consensus length of the repeat is 354 bp, and one repeat has a single-base-pair insertion relative to the consensus sequence. This satellite DNA family is called “NAB354.” In this family, the mean proportion of A+T is 0.71, the number of polymorphic sites is 17 (4.8%), and the average number of nucleotide differences among all repeats, excluding gaps, is 2.5. The ratio of transitions to transversions from the consensus sequence is 0.70, and 13 of the 17 sites represent a nucleotide change to an A or T.

Repeats from all three satellite DNA families are characterized by numerous runs of A, T, and A+T (figs.

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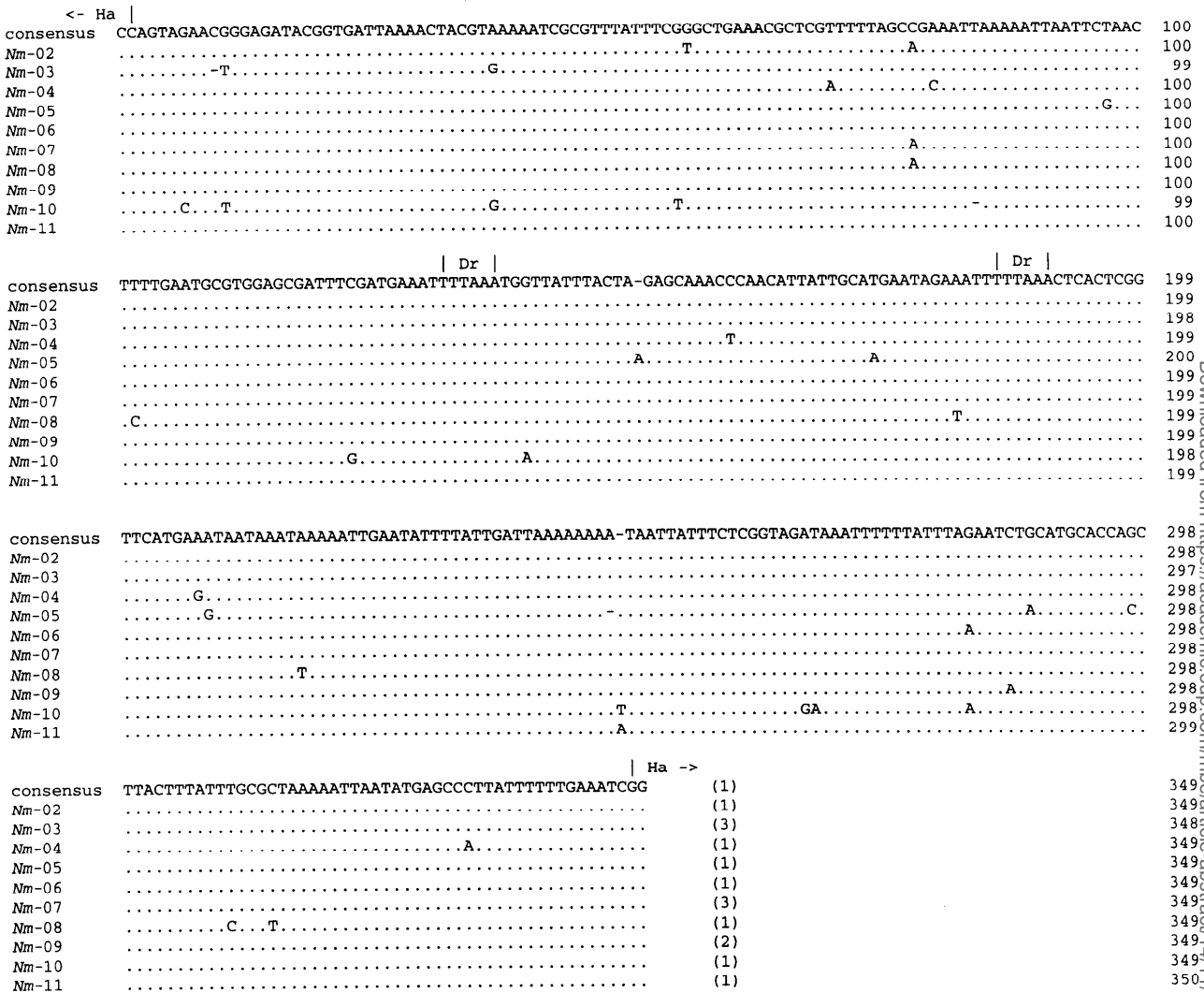


FIG. 2.—Sequence alignment of satellite DNA repeats in *N. marginatus*. Symbols and numerals are defined as in figure 1. Restriction enzyme recognition sites are noted above the sequence: Dr, *Dra* I; Ha, *Hae* III. GenBank accession numbers: AF016157–AF016167.

1–3). Other than the homopolymeric runs, no substructure consisting of direct or inverted repeat motifs could be detected by dot plot analysis (fig. 4), and no subelements or core sequences were identified that are similar in all three satellite DNA families. BLAST searches revealed no significant sequence similarity with other sequences in the GenBank databases.

**Discussion**

Individual repeats comprising satellite DNA were isolated from single females of three species of *Nicrophorus* burying beetles and were cloned and sequenced. The low interrepeat variability, the sizes of the repeats, and the proportions of A+T are strikingly similar for these three species. However, Southern hybridization, sequence analysis, and GenBank searches indicate that these satellite DNA sequences are species-specific and unique. The repeats do not show interspecific cross hybridization, and attempts at interspecific alignments show that most similarity is only in the poly A, T, or AT regions that are dispersed in the individual repeats.

Tandemly repeated genes or DNA sequences are characterized by concerted evolution, the observation that repeated sequences within species do not evolve independently. Concerted evolution is thought to result from various mechanisms of DNA turnover, such as unequal crossing over, DNA amplification, and gene conversion, that lead to the homogenization of repeated DNA. However, the amount of interrepeat variability in satellite DNA within and between species varies considerably, most likely as a consequence of variation in the rates of mutation, unequal crossing over, sexual recombination, and natural selection (Ohta and Dover 1984). Families of satellite DNA may be strain-specific (Lu et al. 1994) or species-specific (Lohe and Roberts 1988; Juan et al. 1993), or they may have considerable homology or conserved subelements across species (Lohe and Roberts 1988; Chen, Lin, and Hodgetts 1989; Bachmann and Sperlich 1993; Juan et al. 1993).

In the context of the mechanisms and evolutionary forces influencing the evolution of satellite DNA, there are several aspects of the *Nicrophorus* data that need to

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	Ba		
consensus	GGATCCAAATAAATCGT'TTTTGGCAAAGCCATATCTTCTGAACGCGTTCGTCGGATTTTGATGAACTAAGCGCCATATTAAT'AAATCGCAAATTTGAGA		100
Na-02	.....		100
Na-03	.....	A	100
Na-04	.....	T	100
Na-05	.....		100
Na-06	.....		100
Na-07	.....		100
Na-08	.....	T	100
Na-09	.....		100
Na-10	.....		100
		Dr	
consensus	TTTTTTGGAAGCATCCCTTACGAGAAAAAATATTTTTTAAGTAAC'TTATTAATCAAAGGGCTGTGCAGAAATTTTAAATAAAAAATTTT-ATTCCCT		199
Na-02	.....	TTTT	199
Na-03	.....	T	199
Na-04	.....		199
Na-05	.....		199
Na-06	.....		199
Na-07	.....	C	199
Na-08	.....		199
Na-09	.....	TTTT	200
Na-10	.....	T	199
consensus	GAACGCGAAGTCCGATTTGAAAAAATTCACAGTATTAATAATATTATAATTATAAGACACTTTTACTTGAAACTTTTTTCAGTTGGAAGTATCCCTG		299
Na-02	.....	T	299
Na-03	.....	A	299
Na-04	.....		299
Na-05	.....	G	299
Na-06	.....	A	299
Na-07	.....		299
Na-08	.....		299
Na-09	.....		300
Na-10	.....		299
consensus	AAGAAATATTTTGAGTTTGAATTCGATTTTTCAAATCTTCATTTAGCGTTTC	(10)	353
Na-02	.....	(1)	353
Na-03	.....	(2)	353
Na-04	.....	(1)	353
Na-05	.....	(1)	353
Na-06	.....	(1)	353
Na-07	.....	(1)	353
Na-08	.....	T	353
Na-09	.....	C.A	354
Na-10	.....	A	353

FIG. 3.—Sequence alignment of satellite DNA repeats in *N. americanus*. Symbols and numerals are defined as in figure 1. Restriction enzyme recognition sites are noted above the sequence: Ba, *Bam*HI; Dr, *Dra* I. GenBank accession numbers: AF016168–AF016177.

be explained. These include the low interrepeat sequence variability, the similar nucleotide compositions and repeat lengths observed in all three species, and the sequence divergence among species.

The amount of sequence variability among satellite DNA repeats in insects is approximately 1%–15%, although variability ranges from a low of 0.68% among repeats in the fall armyworm (*Spodoptera frugiperda*) (Lu et al. 1994) to about 30% sequence divergence in pairwise comparisons in subspecies of the tsetse fly (*Glossina morsitans*) (Trick and Dover 1984), which is similar to the level of divergence observed between different families of satellite DNA within a species (Plohl and Ugarković 1994a, 1994b). In *Nicrophorus*, interrepeat variability is low compared to that in other insects, with 0.18% in *N. orbicollis*, 0.71% in *N. americanus*, and 1.22% in *N. marginatus*.

The nucleotide composition and size of the *Nicrophorus* repeats are similar to classes of satellite DNA described in other species of insects. Overall, satellite DNA sequences tend to be A+T-rich. The proportion of A+T in the *Nicrophorus* repeats ranges from 65% to 71% and is similar to the proportion of A+T observed in 10 species of tenebrionid beetles, 58% to 73% (Pe-

titpierre et al. 1995); the *pDsPv400* repeats of the cave cricket *Dolichopoda schiavazzii*, 81% (Bachmann, Venanzetti, and Sbordoni 1996); the TRS188 repeats of the Australian sheep blowfly *Lucilia cuprina*, 66%–71% (Perkins, Bedo, and Howells 1992); and the grasshopper *Eyprepocnemis plorans*, 59% (López-León et al. 1995).

Satellite DNA can be described as simple or complex, depending on the length of the repeat unit. For example, in *Drosophila*, satellite DNA has been characterized extensively and falls into two groups. One group is composed of simple sequence tandem repeats of only 5, 7, or 10 bp, and the other group is composed of complex repeats 359 bp in length (Lohe and Roberts 1988). Although simple sequence satellite DNA has not been characterized as well in other insects, the size of complex satellite DNA described in insects generally falls into two size classes, one in the range of approximately 140–190 bp, and the other in the range of approximately 300–400 bp. The aphid *Megoura viciae* is an exception, with a 600-bp satellite repeat (Bizzaro, Manicardi, and Bianchi 1996).

The *Nicrophorus* repeats (consensus sizes 342, 350, and 354 bp) are members of the larger size class, which is represented by, for example, the 270-bp repeat of the

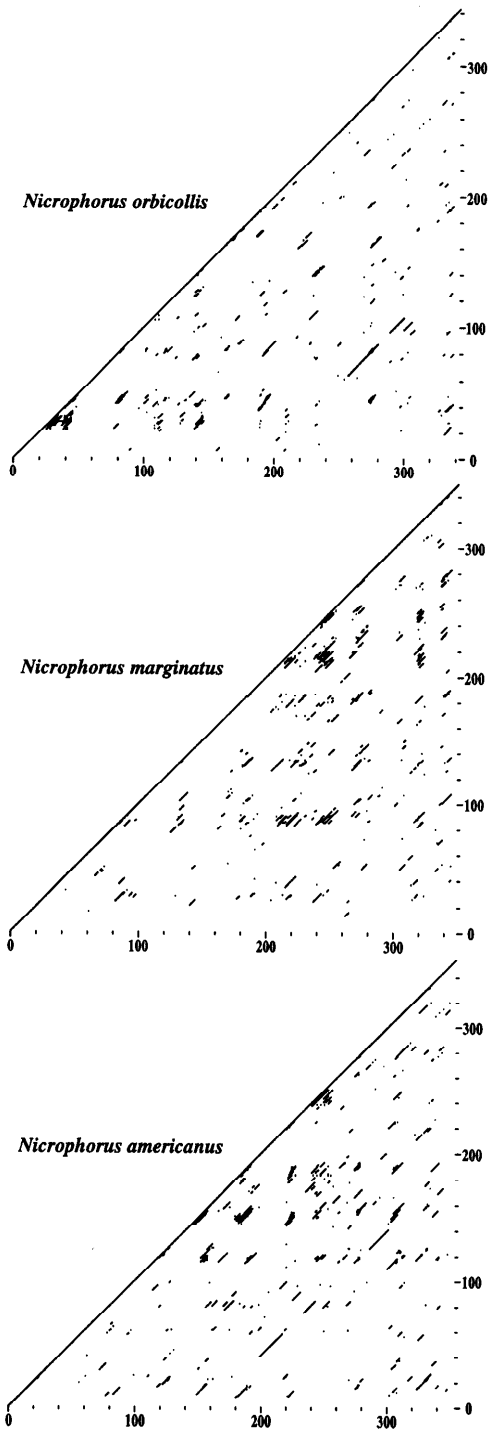


FIG. 4.—Dot plots of the satellite DNA repeat consensus sequence for each species compared to itself. Plots were generated using a 10-bp window with a stringency of 6.7.

fleshfly *Sarcophaga bullata* (Hershfield and Swift 1990), the 380–400-bp *pDsPv400* repeats of *Dolichopoda schiavazzii* (Bachmann, Venanzetti, and Sbordoni 1996), the 360-bp repeat of the flour beetle *Tribolium castaneum* (Ugarković, Podnar, and Plohl 1996), the 344-bp repeat of *Tenebrio obscurus* (Plohl and Ugarković 1994a), the 324-bp repeat of the parasitoid wasp *Diadromus pulchellus* (Bigot, Hamelin, and Periquet

1990), and the 359-bp repeat in *Drosophila melanogaster* and sibling species (Lohe and Roberts 1988). Although the *Nicrophorus* repeats are nearly twice the size of the smaller size class, they appear to be unique repeats which cannot be partitioned into two smaller repeat units (fig. 4). Although the functional significance of satellite DNA is unclear, the common repeat size and the similar A+T composition of this group of complex satellite DNA in insects suggests that length and composition may be selectively constrained.

If these complex repeat families characterized in *Nicrophorus* are homologous, then the considerable divergence in repeat sequence, in contrast to maintaining similar length and composition among *Nicrophorus* species, also suggests a constraint on overall length and composition but little or no apparent constraint on mutational divergence.

Models of the evolution of tandem-repetitive DNA (noncoding and nonfunctional) suggest that repeat length, variability, and substructure vary with the rate of recombination, with decreasing rates of recombination (relative to the mutation rate) leading to increasing repeat length and complexity (Smith 1976; Stephan 1989). Stephan and Cho (1994) extend Stephan's (1989) model, incorporating natural selection to constrain repeat length, and use computer simulations to examine allelic variation in copy number, interrepeat variability, and repeat length under different relative rates of recombination. One of their predictions is that changes in the relative rate of recombination (the rate of unequal crossing over relative to the rate of point mutation) in the range of intermediate to high values has a stronger influence on interrepeat variability than on repeat length.

According to this model, the low interrepeat sequence variability in *Nicrophorus*, especially *N. orbicollis*, may be explained by high rates of recombination, such as unequal crossing over, which is associated with homology-dependent recombination, relative to the rate of point mutation, or the repeats may be a result of recent amplification events, as compared to other species, and thus may not yet have accumulated many point mutations in the repeat family. It is possible that there is selective constraint on the repeats maintaining both length and sequence; however, the range of interrepeat variability observed in other organisms suggests that selection is less likely to explain low variability than are mechanisms associated with repeat homogenization.

The evolutionary forces and molecular mechanisms influencing the evolution of satellite DNA are not clear. Slippage replication, which is important in the evolution of microsatellite DNA sequences (e.g., Strand et al. 1993) may explain the repeat length variation in regions of homopolymeric runs. It is thought that unequal crossing over and various mechanisms of gene amplification (Smith 1976; Walsh 1987; Charlesworth, Sniegowski, and Stephan 1994; Stephan and Cho 1994) are important in the evolution of complex satellite DNA families. Other factors, such as A+T content and repeat substructure (Bachmann, Venanzetti, and Sbordoni 1996), are also considered to influence the evolutionary dynamics of satellite DNA.

In this paper, we have characterized major satellite DNA repeats from three species of burying beetle in the genus *Nicrophorus* and thus have extended our knowledge of repeat sequence evolution to representatives of the insect family Silphidae. Although no specific substructure or motifs are evident in *Nicrophorus* satellite DNA, there are several general sequence characteristics shared with satellite DNA in other insects and other organisms, including repeat length, high A+T composition, and numerous homopolymeric runs. These features, conserved across the three species examined here as well as many other organisms, suggest common evolutionary mechanisms influencing repetitive DNA, or some possible functional role for satellite DNA. In contrast, the *Nicrophorus* species show substantial interspecific sequence divergence and among the lowest values of interrepeat variability. The low interrepeat variability presumably occurs as a consequence of sequence homogenization by mechanisms such as unequal crossing over that occur at a relatively higher rate than in other insects, or as a consequence of relatively recent gene amplification events.

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