Recent Appearance and Molecular Characterization of Mitochondrial DNA Deletions Within a Defined Nematode Pedigree

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Manuscript received July 12, 1989
Accepted for publication December 8, 1989

ABSTRACT

The mitochondrial genome of Romanomermis culicivorax, a parasitic nematode of mosquitoes, contains an amplified 3.0-kilobase (kb) locus organized as direct repeats and as noncontiguous, inverted copies. These amplified sequences are actively undergoing rearrangement. One recent event has resulted in a 1133-base pair (bp) deletion located entirely within a single amplified segment. The deletion junction occurs between two imperfect 58-bp repeats, implicating strand pairing in this alteration. A second event has generated mitochondrial DNA (mtDNA) forms differing by a single, intact 3.0-kb repeating unit. By analyzing molecules derived from independently reared subcultures, it appears these new mtDNA forms arose within the last 170 nematode generations. Our results indicate that the occurrence and selection of novel animal mitochondrial genomes can now be studied in this experimentally manipulable nematode system.

MITOCHONDRIAL genomes of animal cells are strikingly conserved in molecular size (14–19 kilobase pairs, kb), circular geometry, gene content, and maternal mode of inheritance (ATTARDI 1985; BROWN 1985). Length differences among these compact mitochondrial DNAs (mtDNAs) often occur in noncoding sequences comprising the replication and transcriptional control region, and are primarily a consequence of homopolymer tract size variation, differences in reiteration number among short tandem repeats, and sequence deletion or insertion (MORITZ, DOWLING and BROWN 1987).

Recently, several types of genetic rearrangements have been reported that dramatically alter mitochondrial genome size. mtDNAs with sequence deletions ranging from 1.3 to 7.6 kb have been isolated from mouse (BOURSOT, YONEKAWA and BONHOMME 1987) and human (HOLT, HARDING and MORGAN-HUGHES 1988; SCHON et al. 1989) cells. Because a significant amount of coding sequences has been eliminated, these molecules are necessarily propagated in a heteroplasmic state with wild-type mitochondrial genomes. Unexpectedly large molecules approaching 34 kb have been described in several vertebrates (KESLER and AVISE 1985; MORITZ and BROWN 1986, 1987; WALLIS 1987; BENTZEN, LEGGETT and BROWN 1988) and in scallops (SNYDER et al. 1987), and result from lengthy (0.8–8 kb) tandem duplication of specific mtDNA sequences. Collectively, these mtDNA rearrangements have been described within individuals randomly sampled from natural populations, and without defined pedigrees. Consequently, these investigations could not address whether rearranged mitochondrial genomes are of recent appearance or are relics of past genetic activity.

We have been studying large, polymorphic mtDNAs derived from the nematode Romanomermis culicivorax, an obligate mosquito parasite. These mitochondrial genomes contain a 3.0-kb locus amplified to different copy number within individual mtDNAs (BECK and HYMAN 1988). Unlike previously characterized mtDNA sequence duplications, this repeating unit is present within each molecule, both as head-to-tail tandem reiterations and as noncontiguous inverted copies (HYMAN, BECK and WEISS 1988). This unique arrangement of repeating units has also been observed within mtDNA derived from a second R. culicivorax laboratory population (BECK and HYMAN 1988). Both cultures were derived from the same original field isolate and have now been propagated as separate populations for 170 generations. In this report, we describe two new, independently generated, derivative mitochondrial genomes that appeared since these populations were separated. These results indicate that the appearance and fixation of novel nematode mtDNA forms are of recent occurrence and that the molecular mechanisms governing these events can be studied in this experimentally manipulable nematode.

MATERIALS AND METHODS

R. culicivorax strains and culture conditions: Two different sources of nematodes were used in these studies. We have previously characterized three polymorphic mtDNA molecules derived from a laboratory population propagated...
at the University of California (U.C.) Riverside, since 1972 (Beck and Hyman 1988). 3B4 is one isofemale lineage derived from this mixed nematode population (Powers, Platzer and Hyman 1986), and is morphomorphic for a 26-kb mitochondrial genome. The “Yolo” strain represents an independently maintained R. culicivorax laboratory population reared at the Yolo County (California) Mosquito Abatement District. Preliminary analysis of Yolo mtDNA (Beck and Hyman 1988) had indicated size polymorphism among these molecules similar to that observed in the U.C. Riverside culture.

Both the U.C. Riverside and Yolo nematodes are direct descendents of the original field population which was isolated in 1968 from Lake Charles, Louisiana (Petersen, Chapman and Woodward 1968), and subsequently maintained as a laboratory stock. The U.C. Riverside isolate was subcultured from the Petersen strain in 1972; the Yolo nematodes were split from Petersen’s culture in 1980. A detailed description of the relationship between the U.C. Riverside, Yolo and Petersen populations is available (Beck and Hyman 1988).

The propagation of R. culicivorax cultures has been previously described (Powers, Platzer and Hyman 1986). Contamination of laboratory cultures with wild strains harboring variant mtDNA forms is highly improbable. R. culicivorax is not endemic to California and attempts to establish and maintain wild populations by introduction of this nematode into controlled field settings within this region have been unsuccessful (E. G. Platzer, personal communication).

**Bacterial strains and plasmids**: The multipurpose cloning vectors pUC118 and pUC119 were used for molecular cloning (Vieira and Messing 1987). Escherichia coli strains DH5α (F−gt85dlacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK− m+)) supE44 thi−1 gyrA relA1) and MV1193 (Δ (lac-proAB thi supE44)) Δ (sr1-recA306::Tn10(Tc5)) ([F−traD36, proAB, lacZΔM15]) were rendered competent for DNA-mediated transformation (Maniatis, Fritsch and Sam brook 1982).

**DNA isolation and manipulation**: R. culicivorax mtDNA was purified from disrupted post-parasite stage nematodes by Hoechst 33258-CsCl isopycnic centrifugation or from individual post-parasites using a nematode mini-lysat procedure (Powers, Platzer and Hyman 1986). Plasmid DNAs were prepared by ethidium bromide-CsCl isopycnic centrifugation (Maniatis, Fritsch and Sambrook 1982). Single-stranded DNA was prepared from E. coli strain MV1193 harboring pUC118 (or 119) based recombinant plasmids (Maniatis, Fritsch and Sambrook 1982) using the debilitated M13-derived helper bacteriophage M13K07 system (Vieira and Messing 1987).

**DNA transfer-hybridization**: DNAs were 32P-labeled in vitro by nick translation (Rigby et al. 1977), annealed to electrophoretically fractionated DNA preparations transferred to nitrocellulose filters (Maniatis, Fritsch and Sambrook 1982) and visualized by autoradiography.

**Densitometric quantitation of cellular mtDNA populations**: Intensities of bands appearing on serial exposures of autoradiograms were measured using an LKB Ultrascan XL laser densitometer with integrative software to calculate peak areas.

**DNA sequencing and nucleotide sequence analysis**: Nucleotide sequences of sequential exonuclease III-generated deletions (Henikoff 1987) were determined using the “di-deoxy” chain termination method (Sanger, Nicklen and Coulson 1977).

Nucleotide sequence analysis was carried out using the

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**Figure 1.**—Physical analysis of R. culicivorax mtDNA. Restriction enzyme-cleaved mtDNA was fractionated on a 0.7% agarose gel and visualized by ethidium bromide staining. Lanes 1–3: EcoRI digested 3B4, Yolo (mixed population) and NAC6 mtDNAs, respectively. A 470-bp EcoRI fragment common among these mtDNAs is not visualized in this reproduction. □ denotes 2.3-kb deleted form of EcoRI-C. Migration position and molecular size (in kb) of relevant fragment size standards are noted.

University of Wisconsin Genetics Computer Group software package installed on a VAX 8700 mainframe computer.

**RESULTS**

**Recent appearance of a deletion derivative mitochondrial genome**: EcoRI digestion of mtDNA purified from R. culicivorax isofemale lineage 3B4 normally produces nine easily resolvable fragments (Figure 1, lane 1). Within a mtDNA sample prepared in October 1985, a 2.3-kb EcoRI mtDNA fragment present at substoichiometric amounts was detected on ethidium bromide stained gels (Figure 1, lane 1). This band has been visible in all EcoRI-cleaved 3B4 mtDNA samples isolated subsequent to this date, but cannot be observed in earlier preparations, either by ethidium bromide staining or increasing the sensitivity of detection with transfer-hybridization using 32P-labeled total mtDNA (Figure 2). The 2.3-kb EcoRI fragment was not detected in the original U.C. Riverside mixed population nor in any additional isofemale lineages derived from it.

To confirm that the new band was derived from the R. culicivorax mitochondrial genome, EcoRI-digested 3B4 mtDNA was transferred to nitrocellulose paper and hybridized with a 32P-labeled 672-bp EcoRI-BamHI cloned fragment derived from the EcoRI-C fragment of the 3B4 mitochondrial genome (probe A, Figure 3). Both the 3.4-kb wild-type EcoRI-C band and the 2.3-kb derivative fragment are detected (Figure 4, lane 1, both autoradiograms). This result sug-
Figure 2.—The deleted form of EcoRI-C is not detectable when the 3B4 isofemale lineage was initially generated. Purified mtDNAs were prepared from two R. culicivorax isofemale lineages established during 1985 (POWERS, PLATZER and HYMAN 1986). EcoRI-cleaved mtDNAs were fractionated on a 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized with 32P-labeled total mtDNA purified from the mixed U.C. Riverside culture. Lane 1: mtDNA from isofemale lineage NAC6. Lane 2: mixed NAC6 and 3B4 mtDNAs. Lane 3: 3B4 mtDNA. Differences in fragmentation patterns between NAC6 and 3B4 mtDNAs are due to previously characterized restriction fragment size polymorphism among these mtDNAs (BECK and HYMAN 1988). (--) anticipated migration position for the 2.3-kb deleted form of EcoRI-C. mtDNAs were prepared from nematode cultures harvested on 8/5/85.

gested that a 1.1-kb deletion had occurred within the 3B4 EcoRI-C region to generate a novel derivative mtDNA with all other portions of the mitochondrial genome remaining intact.

Both the 3.4-kb wild-type EcoRI-C fragment and the 2.3-kb cognate DNA segment were isolated by molecular cloning into pUC118 plasmid DNA. Fine structure restriction mapping of the two cloned mtDNA fragments positioned a continuous 1.1-kb deletion entirely within a 1.35-kb region of EcoRI-C delimited by TaqI and HincII sites (Figure 3). Upon establishing the complete nucleotide sequence of the 3459-bp EcoRI-C fragment, two 58-bp repeats were defined within this same TaqI-HincII fragment, beginning at nucleotides 1702 (upstream copy) and 2835 (downstream copy) (Figure 5). These two elements share 86% nucleotide sequence similarity (50 out of 58 positions) and are spaced 1133 bp apart within the 1.35-kb TaqI-HincII region.

The spacing between the two short imperfect repeats coincides with the measured size of the mtDNA segment excised from EcoRI-C, suggesting that these elements may have participated in the deletion event. Nucleotide sequencing of the 215-bp TaqI-HincII mtDNA fragment derived from the deleted EcoRI-C form reveals a hybrid organization containing a single repeat copy precisely matching the downstream element (that begins at nucleotide 2835, Figure 5), flanked on the 5' side by sequences through nucleotide 1701 (upstream information) and on the 3' side with base pairs normally adjacent to the downstream repeat (nucleotides 2888 and beyond). This structure indicates that all 1075 nucleotides residing between the two imperfect 58-bp repeats have been deleted, along with one complete repeat copy. Precise excision of this type suggests that pairing between these short repeats was involved in the deletion event.

**Heteroplasmy:** We next addressed the question of whether individual nematodes contained both the wild-type and deletion forms of 3B4 mtDNA. EcoRI-cleaved total cellular DNA was prepared from single nematodes and the DNA electrophoretically fractionated. After transfer to nitrocellulose, the filter was annealed with 32P-labeled probe A (Figure 3), which shares homology with both EcoRI-C and the 2.3-kb deletion derivative. By employing this hybridization assay (as exemplified in Figure 4), 20 out of 98 3B4 nematodes surveyed (20.4%) carried both types of mitochondrial genomes. The remaining individuals were homoplasmic for the wild-type mtDNA form, with the exception of one nematode, which appeared homoplasmic for the deletion derivative mtDNA form.

Densitometric analysis was used to determine relative amounts of the two mtDNA types within the 3B4 population and within heteroplasmic individuals. Molecules containing the 1133-bp deletion represent 16.1 ± 3% (four independent trials) of the total mtDNA present in purified preparations derived from the 3B4 lineage (Figure 4, lane 1 of either panel). Because only 20% of these same nematodes are heteroplasmic, a substantial fraction of their mtDNA must be maintained as the deleted form to achieve the elevated levels (16%) within the overall population. The percentage of total mtDNA comprised by the deleted form within heteroplasmic individuals ranges from 5.3% to near 100%, with 40% of the nematodes assayed containing a majority of their cellular mtDNA as the deletion derivative.

**Characterization of the “Yolo” mtDNA population:** We recently reported the presence of polymorphic mitochondrial genomes within nematodes derived from the “Yolo” culture (BECK and HYMAN 1988). The Yolo mtDNA mixture is composed of two major mitochondrial genome types that also populate our U.C. Riverside isolate, as judged by EcoRI digestion products of purified mtDNAs prepared from the previously characterized isofemale lineages 3B4 and NAC6 (BECK and HYMAN 1988) and from the Yolo strain (Figure 1, lanes 1–3). Based on relative fragment intensities, the predominant molecule in the Yolo population appears to be a 3B4-like mtDNA molecule.

**Yolo mtDNA contains one less tandem repeating unit than the U.C. Riverside cognate molecule:** We have previously mapped copies of an amplified 3.0-kb mtDNA segment to several locations within the 3B4
mitochondrial genome (Hyman, Beck and Weiss 1988). Based on the similarity of the EcoRI digestion pattern observed for the major mtDNA component of the Yolo population and for U.C. Riverside 3B4 mtDNA (Figure 1, lanes 1 and 2), we infer that this same segment is also amplified in Yolo mtDNA and the repeating units are distributed about the mitochondrial genome in the same fashion. Unexpectedly, when restriction enzymes that do not cleave within the amplified repeat unit are used to digest 3B4 and Yolo mtDNA preparations, one band is identified that exhibits a 3.0-kb size difference in each reaction pair. For example, HindIII-digested Yolo and 3B4 mtDNAs were fractionated by gel electrophoresis and transferred to nitrocellulose paper. The filter was annealed with 32P-labeled probe B (Figure 3), which represents a single, intact 3.0-kb repeating unit and therefore increases the hybridization signal that can be obtained from sequences within repeated mtDNA. The largest HindIII fragment (band A) in 3B4 mtDNA (Figure 6, lane 1) is measured to be 11.3 kb and cannot be detected within the Yolo population. Instead, this band is replaced by the 8.3-kb A' fragment diagnostic for Yolo mtDNA (Figure 6, lane 2).

Based on the physical map of the 3B4 mitochondrial genome (Hyman, Beck and Weiss 1988), bands exhibiting this 3.0-kb size differential encompass a specific 3B4 mtDNA region containing amplified DNA organized as two intact repeating units and a portion of a third copy, collectively present in a tandem array (e.g., HindIII-A, Figure 3). These results indicate that the size difference between HindIII-A in 3B4 mtDNA and HindIII-A' in the Yolo mitochondrial genome could be due to the presence of one additional intact direct repeat at this location in the 3B4 mitochondrial genome. This interpretation was confirmed by XhoI cleavage of 3B4 and Yolo mtDNA. A 3.0-kb fragment

**Figure 3.**—Composite physical representation of the *R. culicivorax* 3B4 mitochondrial genome and regions undergoing rearrangement. The restriction map (top line) has been linearized at a convenient HindIII site. Dark arrow: transcriptional orientation of cytochrome oxidase subunit I (COI) coding sequences. Dark boxes with overlined arrows: 58-bp short direct repeats. Stippled areas: 3.0-kb amplified repeating units. Endpoints of each intact repeat copy are arbitrarily positioned by vertical lines. Wavy line delimits endpoint of an incomplete repeating unit (Hyman, Beck and Weiss 1988). B, BamHI; E, EcoRI; H, HindIII; Hc, HincII; T, TaqI; X, XhoI. Probes A and B are described in the text.

**Figure 4.**—Distribution of the deletion derivative mitochondrial genome within *R. culicivorax* populations. Total cellular DNA was prepared from individual nematodes comprising either the 3B4 or Yolo populations. EcoRI-cleaved DNA was fractionated on a 0.7% agarose gel, transferred to nitrocellulose and hybridized with probe A (Figure 3). Upper panel: lane 1, purified 3B4 mtDNA; lanes 2–19, 3B4 individuals; lane 20, purified Yolo mtDNA. Lower panel: lane 1, purified 3B4 mtDNA; lanes 2–20, Yolo individuals. Migration position of the wild type and deletion EcoRI-C forms are labeled 3.4 and 2.3 (kb), respectively. A faint band migrating between the 3.4- and 2.3-kb fragments (lane 1, both panels) represents the EcoRI-C region derived from NAC6 mtDNA (Beck and Hyman 1988) and is included here for reference purposes. Note that this mtDNA form is a significant component of the mixed Yolo mtDNA population (lane 20, upper panel; 9 individual Yolo nematodes, lower panel). Individual 3B4 (lanes 6 and 10) and Yolo (lane 6) nematodes contain as yet uncharacterized polymorphic forms of EcoRI-C.
Using the nematode mini-lysate procedure, individuals from the Yolo population were assayed for the presence of the 2.3-kb EcoRI-C derivative (Figure 4, lower panel). We were unable to detect this fragment in 32 independent preparations, consistent with our inability to detect the deleted mtDNA form in purified Yolo mtDNA preparations.

Under optimum laboratory conditions, R. culicivorax traverses a 5-week reproductive cycle. The U.C. Riverside population has been propagated independently from the lineage leading to the Yolo culture since 1972 (Beck and Hyman 1988). Therefore, these two isolates have been reared in reproductive isolation for a maximum of 170 culture generations, and probably less. This suggests that major structural alterations resulting in both repeat copy number variation and appearance of a deletion derivative mtDNA have occurred during this abbreviated time period.

**DISCUSSION**

Comparison of the mtDNA constituency derived from two R. culicivorax laboratory populations related by shared ancestry but reared in isolation has revealed the presence of independently generated, novel mitochondrial genomes. We have characterized a deleted mtDNA form present in the 3B4 mtDNA population but not detected in Yolo nematodes. In addition, mtDNA isolated from the U.C. Riverside-derived 3B4 strain contains one additional 3.0-kb repeat copy relative to the major Yolo mitochondrial genome. Assuming random sampling when splitting the U.C. Riverside and Yolo cultures from the original Petersen isolate, our results indicate that the two rearrangements are of recent occurrence, appearing within the last 170 generations. Nonidentical breakpoints delimit independent deletions within the human mtDNA D-loop region, promoting the suggestion (Zeviani et al. 1989) that deletion derivative mitochondrial genomes can be generated de novo within individuals. Our observations provide confirmation of
this possibility, although the mechanisms sponsoring deletion formation may require secondary structures unique to the human D-loop region, rather than mtDNA strand pairing between direct repeats (as discussed below).

Extensive deletions within animal mtDNA have previously been observed in mouse (BOURSOT, YONENKAWA and BONHOMME 1987) and human (HOLT, HARDING and MORGAN-HUGHES 1988; SCHON et al. 1989; ZEVIANI et al. 1989) cells. The 1133-bp deletion that we have characterized within the R. culicivorax 3B4 mitochondrial genome includes the entire expanson of mtDNA (1075 bp) residing between two imperfect 58-bp direct repeats, along with one of the two repeat copies. A similar finding was observed within defective mtDNAs isolated from patients suffering from neuromuscular disorders (SCHON et al. 1989). Common among these mutant human mitochondrial genomes was a 4977-bp deletion with breakpoints occurring within two 13-bp direct repeats that bordered this region.

These findings collectively indicate that nucleotide pairing between short direct repeat copies contributes to the generation of mtDNA deletions, as has been well-documented in bacterial systems (ALBERTINI et al. 1982). Deletion may then occur by intramolecular homologous recombination between these sequences (Figure 7, upper panel) resulting in two daughter molecules, each containing but one repeat copy. Within R. culicivorax mtDNA, exchange must have occurred within the conserved 19 bp that comprise the 5' end of each short repeat (Figure 7, upper panel) resulting in the single downstream-like copy that is retained in the deleted DNA form (Figure 5). Alternatively, slipped-strand mispairing (LEVINSON and GUTMAN 1987) between these short reiterations would result in an unpaired single-stranded loop containing one repeat copy that would not participate as template during subsequent DNA replication. Figure 7 (lower panel) displays such a configuration as envisioned for the generation of the 1133-bp deletion characterized in R. culicivorax mtDNA.

Either mechanism could account for the observed nucleotide sequenceorganization at the deletion breakpoints in nematode and human mtDNA. SCHON et al. (1989) argue that the continuous, asymmetric synthesis of animal mtDNA would result in occupation of target parental strands by newly replicated complementary chains, thereby preventing mispairing after strand slippage. Therefore, they considered homologous recombination the likely mechanism in forming the mtDNA deletion. This reasoning necessarily requires that single-stranded regions with the potential to mispair would be generated exclusively during mtDNA replication.

Although several gene rearrangements within animal mtDNA have been attributed to sequence inversion and transposition (WOLSTENHOLME et al. 1985; MORITZ, DOWLING and BROWN 1987; JACOBS et al. 1988), recombination has yet to be documented within animal cell mitochondria (CLAYTON, DODA and FRIEDBERG 1974; HAYASHI, TAGASHIRA and YOSHIDA 1985). This implicates slipped-strand mispairing followed by mtDNA replication as a more probable participant in the formation of animal mtDNA deletions. Many animal mitochondrial genomes, including that of R. culicivorax, contain regions of elevated A + T content that may be susceptible to localized denaturation without the requirement of replication-sponsored melting. In this regard, the A + T content of the regions encompassing the 58-bp repeats in 3B4 mtDNA range from 80 to 85%. Invoking strand slippage rather than homologous recombination as a mechanism likely to generate mtDNA deletions eliminates the requirement for DNA replication (except for resolution of mispaired strands) or for recombinations in this process. We have not observed additional rearranged mtDNA forms resulting from exchange among other available copies of the 58-bp repeat that are present within each copy of the 3.0-kb amplified sequence. In addition, mtDNA multimers were not detected in our preparations (POWERS, PLATZER and HYMAN 1986) or in those of SCHON et al. 1989).

Collectively, this information suggests that any enzymes promoting generalized mitochondrial recombination within these two cell systems must act infrequently.

Our present data cannot discriminate between either a recent amplification or an excision event that would generate the repeat copy number differences between the Yolo and 3B4 mtDNAs. Lengthy exposure of the autoradiogram presented in Figure 6 reveals molecules within the U.C. Riverside 3B4 population containing HindIII-A' and therefore a repeat number characteristic of Yolo mtDNA. It is unlikely these mtDNA forms are “holdovers” from the original Petersen population, as they were not detected in our early characterization of the U.C. Riverside population (POWERS, PLATZER and HYMAN 1986). In addition, any differences in undefined selective pressures between the two populations are minimized, as both cultures were propagated using standardized techniques requisite for rearing this fastidious organism (PETERSEN and WILLIS 1972). We speculate that rare molecules containing one less tandem repeat in the U.C. Riverside isolate might result from infrequent excision events among these lengthy (3.0 kb) duplications, perhaps mediated by the mechanisms as discussed above.

Because 1.1-kb has been deleted from but one copy of redundant information defined by the 3.0-kb amplified mtDNA segment, we anticipated that individ-
Figure 7.—Possible mechanisms for mtDNA deletion. Upper panel: intramolecular recombination. Boxes represent short direct repeats. Vertical lines in boxed areas denote regions of nucleotide divergence (see Figure 5). Numbers refer to nucleotide positions within the EcoRI-C mtDNA fragment. Lower panel: slipped-strand mispairing. Boxed areas encompass nucleotide sequences of upstream and downstream 58-bp direct repeat copies. Nucleotide positions within EcoRI-C are numbered.
uals homoplasmic for the deleted mtDNA form might be identified, provided sufficient reproductive cycles have occurred to allow assortment of the two different mitochondrial genomes. We have found one individual homoplasmic for the deletion among the 98 3B4 individuals analyzed to date, representing the first documented homoplasmosis of an animal mitochondrial genome containing an extensive sequence deletion.

Using random sampling of offspring comprising successive generations of a heteroplasmic female, LAIPIS, VAN DE WALLE and HAUWSWIRTH (1984) demonstrated that segregation of polymorphic bovine mitochondrial genomes to achieve homoplasmic progeny occurred within 250 germ cell divisions (five animal generations). In contrast, genetic drift modeling has revealed that fixation of the deleted mtDNA form as required to generate the measured egg numbers. Berger, R. and W. 1987 required several thousand germ cell generations.

R. culicivorax adult females contain approximately 2500 eggs (POINAR 1979), an estimate similar to the number of gametes observed within Caenorhabditis elegans hermaphrodites (HIRSH, OPPENHEIM and KLAAS 1976). Germ-line development in R. culicivorax has not been studied. However, if oogenesis in R. culicivorax is comparable to that documented in C. elegans (5 stem cell cleavages followed by approximately 10 germ line divisions to generate the observed number of gametes; VON EHRENSTEIN and SCHIERENBERG 1980), 10–20 total cell divisions would be required to generate the measured egg numbers. Because homoplasmosis has occurred sometime within the last 170 nematode generations, a maximum of several thousand germ cell divisions would also be required for fixation of the deleted mtDNA form as judged from our random sampling of individuals derived from each generation of our laboratory population. These estimates suggest that mtDNA fixation may be achieved much more rapidly in cows (up to tenfold) than in nematodes and insects.

mtDNA heteroplasmy has now been observed in many animal cell systems (MONNEROT, MOUNOLOU and SOLIGNAC 1984; SOLIGNAC et al. 1984; HARRISON, RAND and WHEELER 1985; DENSMORE, WRIGHT and BROWN 1985; BERMINGHAM, LAMB and AVISE 1986; WALLIS 1987; BOURSOT, YONEKAWA and BONHOMME 1987; SNYDER et al. 1987; HOLT, HARDING and MORGAN-HUGHES 1988; LAIPIS, VAN DE WALLE and HAUWSWIRTH 1988). The smallest mitochondrial genome within the cellular population is often the most predominant form, which is what we have observed in a high percentage of nematodes heteroplasmic for wild-type and the deletion derivative mitochondrial genomes. Smaller mtDNAs may have a replicative or segregational advantage over larger molecules that populate cellular mtDNA pools (SOLIGNAC et al. 1984; RAND and HARRISON 1986; BOURSOT, YONEKAWA and BONHOMME 1987), thereby providing an additional factor contributing to rules governing vegetative segregation of mtDNAs. Given the short generation time of R. culicivorax and the ease with which it can be propagated, our results indicate that molecular mechanisms promoting the generation, selection and fixation of polymorphic mtDNA types within controlled nematode laboratory populations can now be investigated.

We wish to thank E. PLATZER for the use of his facilities to propagate nematodes, T. O. POWERS (University of Nebraska-Lincoln) for sharing early R. culicivorax mtDNA data, D. BIRD and M. WILSON for advice on DNA sequencing procedures and JOYCE L. BECK for useful discussions and critical reading of this manuscript. This work was supported by U.S. Department of Agriculture Competitive Grant 85-CRCR-1-1629, U.S. Agency for International Development Grant DPE-5542-4-55-7059-00, U.S. Public Health Service Biomedical Research funds, the Agricultural Experimentation and Research Committee of the University of California, Riverside, and a State of California Mosquito Research Student Mini-Grant (to T.M.S.).

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Communicating editor: J. R. Powell.