Homogenization of Geographical Variants at the Nontranscribed Spacer of rDNA in *Drosophila mercatorum*[1]

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rDNA nontranscribed spacer (NTS) lengths of *Drosophila mercatorum* have been measured in individuals from several geographic regions. Individuals from the different geographic subpopulations share some length fragments but are in general distinct. The length differences, both within and between individuals, arise from different copy numbers of a 250-bp repeating unit that is localized to one part of the NTS. In addition to the length differences caused by the 250-bp repeat, there is a Y chromosome (male)–specific length variant elsewhere in the NTS that is ~70 bp shorter than the NTS fragment from the X chromosome. Sexual dimorphism seems to be present in all *Drosophila*. Also, *D. mercatorum* has fewer NTS length variants per individual than does *D. melanogaster* while possessing comparable levels of restriction-site polymorphism. The mechanisms that may cause this pattern of variation are selection, gene conversion, and unequal recombination.

Introduction

The recent characterization of molecular variation in repeated genes has led to the realization that the members of gene families are more homogeneous in nucleotide sequence than would be expected if they had been evolving independently (Hood et al. 1975; Arnheim et al. 1980; Zimmer et al. 1980; Dover 1982; Dover et al. 1982). This observation has led to the description of a new mode of evolution called either coincidental evolution (Hood et al. 1975) or concerted evolution (Arnheim et al. 1980; Zimmer et al. 1980). The underlying mechanisms that are hypothesized to be responsible for this homogeneity include unequal crossing-over (Black and Gibson 1974; Ohta 1976; Smith 1976; Perelson and Bell 1977) and gene conversion (Fogel and Mortimer 1969; Hood et al. 1975 [and ref. therein]; Fogel et al. 1978). Recently, these two mechanisms, along with biased gene conversion and transposition, have been called molecular drive (Dover 1982).

Evidence for molecular drive comes from work on the *melanogaster* subgroup of the genus *Drosophila,* where the nucleotide sequences in the rDNA and histone gene families are highly conserved within species but are quite distinct between species (Coen et al. 1982*a;* Strachan et al. 1982). The rDNA consists of approximately 200 tandemly repeated copies of the genes encoding the 18S and 28S rRNA separated by a nontranscribed spacer (NTS). The nucleotide sequences of several NTS clones from *D. melanogaster* reveal exceptionally low levels of sequence

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variation cooccurring with large amounts of length variation for this region (Coen et al. 1982*a*, 1982*b*; Simeone et al. 1982), the latter arising from varying numbers of an internal repeat ~250 bp long.

We have initiated a series of studies on the NTS of rDNA in another distantly related species, *Drosophila mercatorum*, to determine the general patterns present in multigene families. This paper reports on the amount and geographic origin of length variants at the NTS of *D. mercatorum*, as well as on the lack of restriction-enzyme polymorphisms in one geographic region.

Material and Methods

Most (15 of 19) of the *Drosophila mercatorum* strains studied were collected from Kamuela on the island of Hawaii over a period of 6 years. All Kamuela stocks collected in 1974 (strains I–VIII; table 1) except one (strain VIII) are isofemale lines; the other Kamuela stocks are pooled isofemale lines made at later dates (1976, 1978, and 1980). One stock (strain VI; table 1) is parthenogenetic and therefore an all-female line. Single stocks from Oahu, Colombia, El Salvador, and Rochester, New York, as well as those from Kamuela were provided by A. R. Templeton. The flies used for the assessment of restriction polymorphism were wild-caught males and male offspring of wild-caught females collected at Kamuela from 1980 to 1984 and therefore represent a survey of natural variants. The NTS was cloned from *D. mercatorum*, and a restriction map of it is shown in figure 1. A more complete description of the clone will be given elsewhere (R. DeSalle, unpublished data).

All DNA samples were taken from single flies. The DNA was extracted by the method of Coen et al. (1982*b*), except for the following modifications: (1) 3M sodium acetate was used instead of potassium acetate before the sample was put on ice, and (2) the pellets were resuspended in only 0.2% diethyl pyrocarbonate after the first ethanol precipitation.

The total DNA from individual flies was digested in a final volume of 30 μl with the reaction conditions defined by the manufacturer (Boehringer Mannheim and Bethesda Research Labs) except that 1 mg/ml of gelatin was added to a final

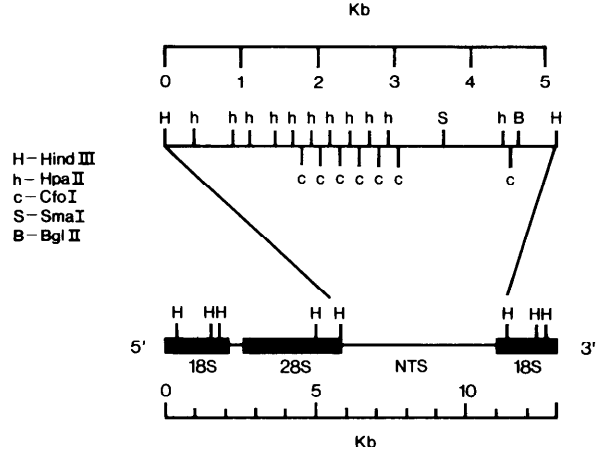

FIG. 1.—Fine-structure map of clone 9HH2-16, the NTS of *Drosophila mercatorum*. The NTS was subcloned from a λCharon 30 clone of the entire ribosomal repeat of *D. mercatorum* from Kamuela females (R. DeSalle, unpublished data).

concentration of 10% (v/v). For determination of length variation, the DNA was digested with *Hin*dIII; for the localization of the length variation, the DNA was digested with both *Hin*dIII and *Cfo*I; and for the determination of restriction-site polymorphism, the DNA was digested with *Eco*RI. The cut DNA was then electrophoresed on agarose gels in 1 × Tris-borate or 1 × Tris-acetate buffer (Maniatis et al. 1982). The agarose concentration was either 0.6% or 0.8% for the *Hin*dIII-digested DNA and the *Eco*RI-digested DNA, and 1.2% for the *Hin*dIII/*Cfo*I-digested DNA. Length standards were lambda DNA cut with either *Hin*dIII or *Hin*dIII and *Eco*RI.

After electrophoresis DNA was transferred to nitrocellulose; the gel was denatured in 0.5 N NaOH, 1.5 M NaCl; neutralized in 0.5 M Tris-HCl (pH 7.0), 3 M NaCl; and placed on a sheet of Saran Wrap on a flat surface. The nitrocellulose was wetted in distilled water and submerged in 3 M NaCl, 0.3 M citric acid (20 × SSC) and placed on top of the gel. Three layers of Whatman 3-mm filter paper were put on the nitrocellulose after being submerged in distilled water and 20 × SSC. Approximately 1 inch of paper towels were placed on top and weighted down. The nitrocellulose was removed after at least 12 h and baked for 2 h at 65 C.

The blots were hybridized to a plasmid containing a clone for *D. mercatorum* rDNA NTS labeled with 32-P by nick translation. The blots were presoaked for at least 30 min in 10 ml of 5 × SSC, 1% N-lauroyl sarcosine, 10 mM KH_2PO_4, 10 mM K_2HPO_4, and 1 × Denhardt's solution at 65 C (Maniatis et al. 1982). This prehybridization solution was discarded, and a second aliquot of the presoak solution containing boiled, nick-translated probe (2–5 × 10^6 counts per blot) was added. The hybridization reaction was performed at 65 C for 12–24 h. Blots were washed twice for 15 min, each time in 500 ml of 1 × Denhardt's solution containing 0.2% lauryl sulfate and 2 × SSC. Two quick rinses with 250 ml of 0.1% lauryl sulfate, 2 × SSC followed. The blots were then washed in the second solution for 1 h at 37 C with agitation. This was repeated for a second hour. Two final rinses were done in 0.2 × SSC, and then the blots were air dried before autoradiography. Autoradiography was done for 1–4 days at −76 C.

Nick translations were done as follows: 1 μl of 1 mg/ml DNase was mixed with 9 μl of DNase activation buffer (10 mM Tris (pH 7.5), 5 mM $MgCl_2$, 1 mg/ml bovine serum albumin) for 1–3 h on ice. Afterwards this was diluted 1:15,000 with DNase activation buffer. The final reaction mixture contained ~1 μg plasmid DNA, 10 μl nick-translation buffer (20 μM each of dATP, dTTP, dGTP, 40 mM Tris [pH 7.5], 20 mM $MgCl_2$, 0.1 mg/ml BSA), 1 μl DNA polymerase I (from Boehringer Mannheim), 0.5 μl diluted DNase, and ~10 μl labeled dCTP. The reaction was stopped after 2–3 h at 15 C by adding 30 μl stop buffer (0.2 M EDTA [pH 7.5], 1% lauryl sulfate, 40 mg/ml blue dextran, and 0.1 mg/ml bromphenol blue) and putting the solution on ice for 10 min. The solution was run on a Sephadex G-100 column, and the blue dextran fraction was collected.

Results

Individual flies from the different geographic regions have characteristic lengths of the NTS (figs. 2 and 3). They share most of their major bands but may differ with respect to less intense bands. For example, all the strains from Kamuela (except one, which will be discussed later) have NTSs of 4.8 kb and 5.1 kb. In almost all individuals, only these two bands were present. This represents large within-

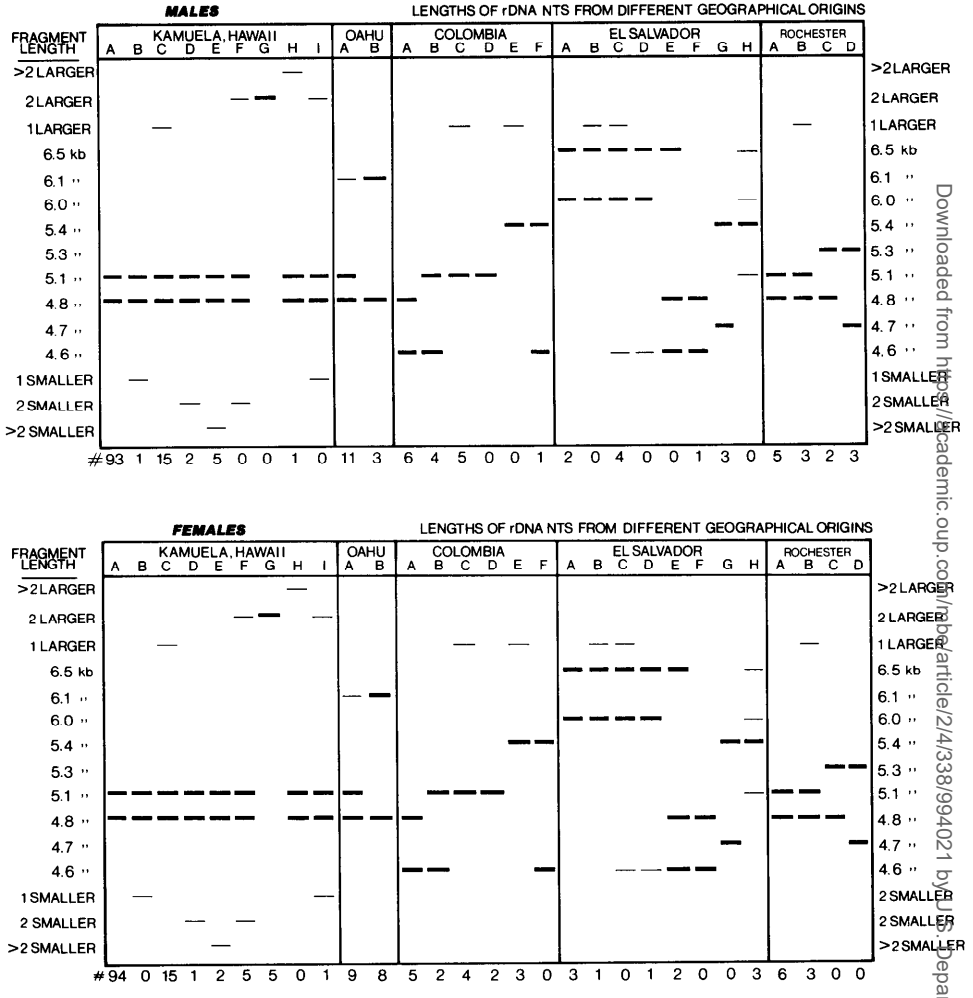

FIG. 2.—Male and female NTS length patterns from the five geographic subpopulations. The letters at the top of the table designate the pattern of NTS lengths for each region. Each bold line represents a major band, and each thin line represents a minor band. The fragment length designation *1 LARGER* indicates that one band present in this pattern is larger than those given by the sizes. The same is true for *2 LARGER, >2 LARGER,* and the categories for *SMALLER.*

individual and within-population invariance because individuals with only two bands visible must have only a few or no other length variants. Flies from the other geographic regions are more polymorphic at the population level.

The small number of NTS length variants per individual of *Drosophila mercatorum* distinguishes this species from *D. melanogaster.* Single flies of *D. melanogaster* have more minor but not more major NTS bands than do those of *D. mercatorum,* and the isofemale lines of *D. melanogaster* studied are more polymorphic than the isofemale lines of *D. mercatorum* (Coen et al. 1982*b;* Boncinelli et al. 1983). This indicates that the amount of natural variation for NTS length is greater in *D. melanogaster* than in *D. mercatorum.*

The Kamuela and Oahu strains share most of their major bands. All individuals from sexual stocks and from both locations have a 4.8-kb band, and all from

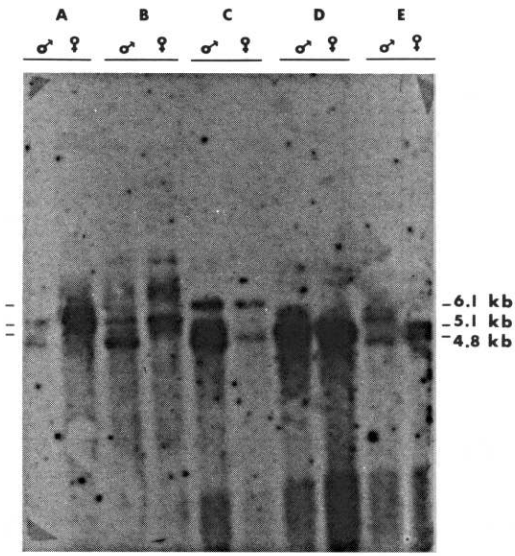

FIG. 3.—Hybridization patterns of single flies from different geographic regions. The DNA was *Hin*dIII digested, run on a 0.6% agarose gel, and hybridized to the *Drosophila mercatorum* NTS clone, 9HH2-16, described in the legend to fig. 1. *A,* Colombia; *B,* El Salvador; *C,* Oahu; *D,* Kamuela; and *E,* Rochester.

Kamuela and most from Oahu have the 5.1-kb band. Oahu flies, however, have a major band 6.1-kb long never observed in the Kamuela flies. The similarity between these two regions may be explained by a small founder group being responsible for colonizing all the islands (Templeton and Johnston 1982).

There is one Kamuela strain (table 1; number VI, designated K28-0-IM and the only impaternate line) that has neither the 4.8-kb nor the 5.1-kb bands. Unlike the other strains examined, it is relatively resistant to mating with any *D. mercatorum* males (Carson et al. 1977; S. M. Williams, personal observation). K28-0-IM also differs in that its mode of reproduction (parthenogenesis) produces completely homozygous individuals by undergoing meiosis and then gamete duplication (Carson 1973).

The similarity between Oahu and Kamuela strains is greater than that between strains of other geographic regions. Approximately half of the individuals from Colombia, El Salvador, and Rochester have 4.8-kb NTSs, but in most cases there is a second major band that distinguishes flies from the different regions (4.6 kb in Colombia, 6.5 kb in El Salvador, and 5.3 kb in Rochester). The non-Hawaiian stocks are also more polymorphic for NTS length, but again individuals are very limited in the number of variants that they carry. Only two individuals from El Salvador have more than two major bands. The small number of NTS length variants per individual appears to be a species characteristic of *D. mercatorum.*

There are also differences between the males and females of the same region and, in Kamuela, between sexes of the same strain (table 1 and figs. 4 and 5). This result might be expected because the rDNA is on both the X and Y chromosomes, as in *D. melanogaster,* and because the exchange of genetic material between these chromosomes is probably infrequent in natural populations (Neuhaus 1937; Tartof and Dawid 1976). Such sex differences imply that the rate of X-Y exchange is less

Table 1
Length Patterns of the Kamuela Strains

Strain	Pattern[a] A	B	C	D	E	F	G	H	I
Males:									
I. K23-0-bi	5		3						
II. K43-0-bi	3		3						
III. K48-GA3PD-F	6		4						
IV. K65-0-bi	6			2	2				
V. K54-PK-SLOW	11								
VI. K28-0-Im									
VII. K31-3-ADH-S	9								
VIII. K74	9								
IX. K76	7								
X. K78A	12	1	1		2				
XI. K78B	3				1				
XII. K80A	8								
XIII. K80B	5								
XIV. KI 1 80	3							1	
XV. KI 2 80	6		4						
Total	93	1	15	2	5			1	
Females:									
I. K23-0-bi	8								
II. K43-0-bi	3		3						
III. K48-GA3PD-F	8		3						
IV. K65-0-bi	11					1			
V. K54-PK-SLOW	11		1						
VI. K28-0-Im							5		
VII. K31-3-ADH-S	7								
VIII. K74	6								
IX. K76	7								
X. K78A	14				2	1			
XI. K78B	3			1					
XII. K80A	7		1						
XIII. K80B	5								
XIV. KI 1 80			1			3			1
XV. KI 2 80	4		6						
Total	94		15	1	2	5	5		1

[a] Patterns are those shown in fig. 2.

than that of X-X exchange. A similar rDNA X-Y difference has been described in *D. melanogaster* at the nucleotide sequence level (Yagura et al. 1979).

Although the regions differ considerably in their NTS length patterns, the observed variation within each sex is localized in one region of the NTS represented by repeating *Cfo*I/*Hpa*II recognition sites (fig. 1) separated by ~250 bp (R. DeSalle, unpublished data). Variation in the number of these repeats accounts for the observed length differences within individuals and among strains. This is seen in the *Cfo*I/*Hin*dIII double-digest autoradiographs. All flies surveyed of each sex (at least three males and three females from each geographic region) have the same double-digest pattern (fig. 5). The exception to this pattern is that males have an extra band. This band is the 5′ *Hin*dIII/*Cfo*I fragment that has two lengths in males (fig. 5). The two bands differ by ~70 bp (1680 bp vs. 1750 bp).

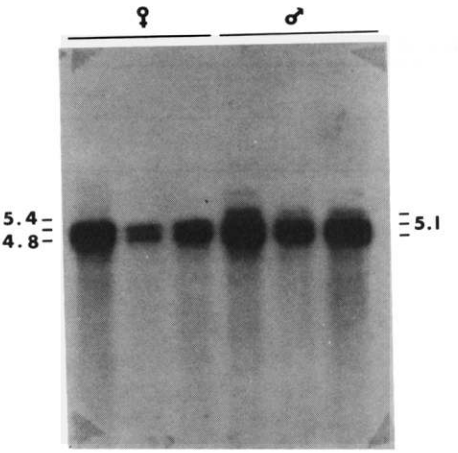

FIG. 4.—Hybridization pattern of strain I (K23-0-bi) digested with *Hin*dIII and hybridized to 9HH2-16. Note that the males have an extra band.

Although the pattern of NTS length variation for *D. mercatorum* differs significantly from that of *D. melanogaster,* the level of restriction-site polymorphism within each species is similar. No restriction-site polymorphisms have been found within the NTS for either *Eco*RI or *Hin*dIII in more than 700 flies collected between 1980 and 1984. The only polymorphisms found, from a collection made in 1982, are *Hin*dIII sites in the transcribed region of the 28S gene. These findings represent a high degree of homogeneity for NTS nucleotide sequence.

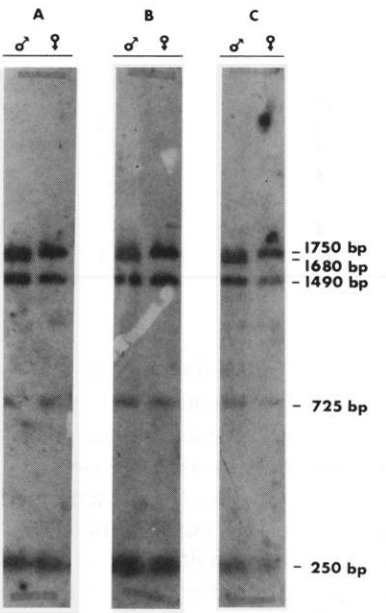

FIG. 5.—Autoradiograph of single flies DNA digested with both *Hin*dIII and *Cfo*I and then hybridized to 9HH2-16 (NTS clone). Note that males have an extra band ~70 bp smaller than the females'. This is at the 5′ end of the NTS. *A,* Kamuela; *B,* Oahu; and *C,* Rochester. Individuals from other regions have identical patterns.

Discussion

The amount of NTS length variation within individuals of *Drosophila mercatorum* is less than that observed within individuals of *D. melanogaster,* but the within-population variation is comparable in the two species. That is, although an individual of *D. mercatorum* has fewer NTS length variants than an individual of *D. melanogaster,* there is variability within subpopulations of both species. Also, the pattern of NTS length variation within an individual of *D. mercatorum* is associated with its geographic origin in that the variants in an individual from one geographical region rarely have complete overlap with those carried by individuals from other regions. The patterns may serve as markers for determining the geographic origin of individuals. This among-population variation has not been demonstrated in any other species and gives an idea of the kind and amount of variation present in multigene families in natural populations. In addition, a restriction-enzyme study of the Kamuela population revealed a high degree of within-population nucleotide sequence homogeneity similar to that in *D. melanogaster* (Coen et al. 1982).

The fact that the variation is localized to one part of the NTS implies that the number of internal *Cfo*I/*Hpa*II repeats is limited for each locale and that new variants are either not produced or are eliminated/incorporated rapidly. The mechanisms that may cause this type of pattern are (1) selection, (2) gene conversion, and (3) unequal crossing-over—between sister chromatids and/or homologous chromosomes. The latter two mechanisms have been incorporated into the molecular-drive hypothesis and can influence both the creation of new variants and their subsequent homogenization (Strausberg et al. 1978; Fedoroff 1979; Baltimore 1981; Dover 1982).

Although we cannot determine the mechanism(s) that has caused the observed patterns of NTS length variation, we demonstrate a new kind of pattern in multigene families. We find length differences among regions with relatively little within-individual variation, implying a strong homogenizing force of some kind. This homogenizing force is also evident from the lack of restriction-site polymorphisms within the NTS.

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