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# Structure of rDNA in the mosquito *Anopheles gambiae* and rDNA sequence variation within and between species of the *A. gambiae* complex

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**The structure of the rDNA repeating unit of *Anopheles gambiae* (Diptera: Culicidae) was determined by restriction endonuclease mapping and hybridization analyses on four independent clones obtained from a genomic library of a colony (G3) from the Gambia (West Africa). rDNA gene coding sequences are conserved, but much intragenomic and intraspecific (geographic) variation occurs in the intergenic spacer. Hybridization of subclones from spacer and coding sequences to genomic DNA that was isolated from single mosquitoes from laboratory colonies of four other *A. gambiae* complex species reveals conservation of coding sequences but concerted evolution in the intergenic spacers.**

## INTRODUCTION

The structure of the rDNA repeating unit is known for only a few groups of insects. In these, the unit is composed of genes for 28S, 5.8S (within an internal transcribed spacer, ITS), and 18S ribosomal RNAs and an intergenic spacer (IGS) separating tandem units (Beckingham, 1982). Transcription of the 28S gene may extend into or even through the IGS (Tautz and Dover, 1986). In *Drosophila melanogaster* the nucleolus organizers contain 200–250 tandemly repeated units (Gillings *et al.*, 1987). The length of the repeating unit in different insect species varies from less than 10 kilobases (kb) to over 20 kb depending primarily on the length of the intergenic spacer and whether or not the 28S gene is interrupted by introns (Beckingham, 1982).

In *Drosophila* spp. approximately half of the rDNA repeating units contain one of several classes of intron within the 28S gene (Kidd and Glover, 1980). Each class, for example T1 or T2, has a unique insertion point (Roiha and Glover, 1980). Genes containing an intron produce functional transcripts at a reduced rate if at all (Long *et al.*, 1981).

Base sequences within rDNA genes are highly conserved, although there are regions of relative variability (Tautz *et al.*, 1986; Gerbi, 1985; Dutton and Krider, 1984; Endow and Glover, 1979). However, total length and base sequence within the IGS evolve rapidly (Boncinelli *et al.*, 1983; Dover and Flavell, 1984; Arnheim, 1983; Coen *et al.*, 1982*a*, *b*) while still maintaining the integrity of possible sequence-independent functional domains (DeWinter and Moss, 1986; 1986; Reeder, 1984; Coen and Dover, 1982; Kohorn and Rae, 1982).

Intra- and interspecific heterogeneity in IGS length is common in *Drosophila* spp. and may be largely or entirely attributable to the number of tandemly repeating subunits within the spacer (Williams *et al.*, 1985; Erickson and Schmickell, 1985; Dover *et al.*, 1982; Kunz *et al.*, 1981). Length variation is apparently a consequence of unequal crossing-over between subrepeats within the intergenic spacers (Tautz *et al.*, 1987; Coen and Dover, 1983; Treco *et al.*, 1982; Fedoroff, 1979).

While any given genome will possess variant rDNA repeating units, the degree of variation between closely related species is usually much greater than that within a species (Coen *et al.*, 1982a; Dover, 1982). The observation that within a genome members of a multigene family evolve in concert, concerted evolution (*e.g.*, Pichersky *et al.*, 1986; Strachan *et al.*, 1985; Schimenti and Duncan, 1985; Morzycka-Wroblewska *et al.*, 1985; Hayashida *et al.*, 1984; reviewed in Arnheim, 1983), suggests that cellular processes, such as gene

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conversion or stochastic gain/loss through unequal recombination, can rapidly homogenize genomes with respect to certain variants (Ohta and Dover, 1984; Ohta and Dover, 1983; Ohta, 1983).

This study examines the structure of the rDNA repeating unit in the mosquito *Anopheles gambiae* through restriction endonuclease mapping of four rDNA clones. Restriction fragments containing rRNA genes were identified using heterologous probes. Subclones of intron, ITS, and IGS sequences were hybridized to restriction endonuclease digests of DNA extracted from single mosquitoes from laboratory colonies of *A. gambiae* complex species to identify relatively rapidly evolving regions and to assess intragenomic variation.

The *A. gambiae* complex consists of six sibling species distributed in sub-Saharan Africa (Service, 1985). Two species, *A. arabiensis* and *A. gambiae*, are widely distributed. *A. merus* and *A. melas* are restricted to, respectively, the eastern and western coasts. The other species employed in this study, *A. quadriannulatus*, is found primarily in south-eastern Africa.

## METHODS

### Species and strains

Species and strains employed in this study and their geographic origin and laboratory source are listed (table 1). A map of Africa shows the locations of countries from which strains were derived (fig. 1).

### Library construction

Construction of the *A. gambiae* (strain G3) library utilizing whole genomic DNA ligated to EMBL3 arms (Stratagene Inc.: Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services) has been described previously (Collins *et al.*, 1987). All four of the clones analyzed in the present study originated from this library.

### Heterologous probes

Localization of 18S and 28S genes involved hybridization of enzyme cleaved and membrane bound phage DNA to several heterologous *Calliphora erythrocephala* probes (plasmids pKB-7 containing 18S and 28S genes, pKB-42 and pKB-33 containing 18S and 28S genes, respectively; Beckingham and White, 1980; Beckingham and Smith, 1981). *C. erythrocephala* probes were supplied by K. Beckingham.

**Table 1** Designations, geographic origins, and laboratory source of species and colonies* employed in the present study

| Species *Anopheles* | Geographic origin | Source* | Designation | |
|---|---|---|---|---|
| | | | Prior | In figures |
| *melas* | Gambia | 5 | BREFET | ml |
| *merus* | Kenya | 5 | — | mr |
| *guadriannulatus* | Zimbabwe | 4 | CHIL | q |
| *arabiensis* | Burkina Faso | 2 | ARZAG | a BF |
| | Sudan | 1 | SEN | a SUDs |
| | Sudan | 1 | GMAL | a SUD |
| | Kenya† | 3 | KISU | a KENw |
| | Kenya† | 5 | — | a KENe |
| | Zimbabwe | 4 | LUNDI | a ZIM |
| *gambiae* | Gambia | 1 | G3 | g GAM |
| | Burkina Faso | 2 | GMMK6 | g BF |
| | Nigeria | 1 | BAD | g NIG |
| | Kenya† | 5 | AS46 | g KENw |
| | Zanzibar (Kenya) | 1 | ZANU | g ZAN |

*Sources*: 1. London School of Hygiene and Tropical Medicine, 2. Istituto di Parassitologia, Universita di Roma. 3. Kenya Medical Research Institute, 4. Blair Research Laboratory, Zimbabwe. 5. Collected by F. H. Collins.

* These designations have appeared in the previous publications of numerous researchers. Figure labels in the designations of this study are based on the geographic origin of colonies of *A. gambiae* and *A. arabiensis*.

† a KENw and g KENw were collected near Kisumu in south-western Kenya. a KENe was collected near Mombasa in south-eastern Kenya.

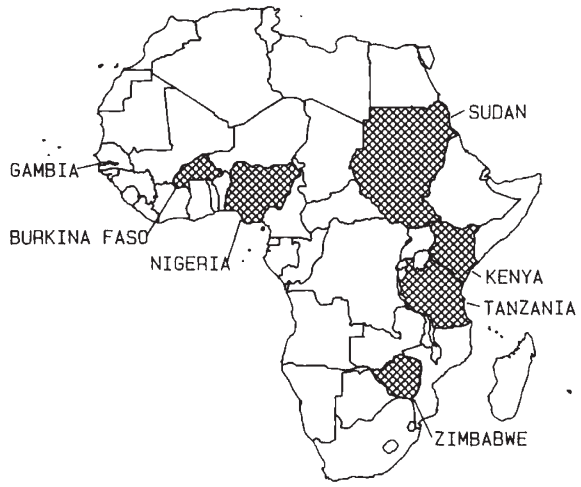


**Figure 1** Map of Africa showing the countries of origin of colonized *A. gambiae* complex species and strains.

### *Restriction mapping and hybridization*

Restriction endonuclease digestions were performed on 1 μg of purified phage DNA in a volume of 40 μl containing 10–50 units of restriction endonuclease. Digestions proceeded overnight, 12–16 h. Restriction enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, MD) or New England Biolabs (Beverly, MA) and used with the buffers supplied or recommended by the manufacturers.

Digested DNA was fractionated by size on 0·75% agarose gels. DNA was transferred to GeneScreen Plus membrane (NEN Research Products, Boston, MA) using the Southern (1975) blotting procedure.

Radio-labelling of probes for hybridization to membrane-bound DNA was accomplished through nick-translation with a BRL kit and $^{32}$P-deoxycytidine. Hybridizations were done under stringent conditions (50% formamide, 6×SSC at 42°C; Maniatis *et al.*, 1982; Strachan *et al.*, 1982) followed by washing 3 times for 45 min each at 50°C in 0·1% SDS, 0·2×SSC).

Membrane filters were frequently stripped of one probe and reprobed with another. Stripping entailed soaking with gentle agitation at 42°C for 30 min. in 0·4 M NaOH. Stripped filters were neutralized in 0·1 M tris, 0·1×SSC, 0·1% SDS (*p*H 7·5) for 30 min at 42°C.

### *Subcloning*

Subclones were made from one of the four rDNA clones (λAGr12). Whole phage DNA was digested with XhoI, XhoI and EcoRI, or EcoRI and BamHI and ligated into either pUC-12 (Vieira and Messing, 1979: in the SalI site of the polylinker for XhoI digests) or into pBluescript (Stratagene, Inc.).

The following subclones are used in this study (Figure 1): (1) pAGr12C, which contains the XhoI bounded ITS, (2) pAGr12B, consisting of IGS sequences flanking the 18S gene, (3) pAGr12D, consisting of IGS sequences, (4) pAGr12E, consisting of IGS sequences, and (5) pAGr12A, a short segment at the junction of the IGS and 28S sequences (Collins, *et al.* 1987). In addition, the intron subclone pAGr23A, a 1·1 kb EcoRI/SalI fragment in pBluscript from λAGr23, was supplied by A. Mendez (Mendez *et al.*, 1988). The following naming convention was adopted. AGr indentifies each subclone as part of a rDNA repeating unit (thus r) from *A. gambiae* (thus AG). The λ clone number follows. The remaining letter distinguishes the different plasmid subclones derived from same λ clone.

### *Single mosquito DNA extraction*

DNA was extracted from single female mosquitoes according to the procedure of Livak (1984) except that all volumes were doubled. All of the DNA extracted from one mosquitoe was digested in a single volume of 40 μl, as described, and electrophoresed in a single agarose gel lane. Verification of the completeness of digestion for single restriction digests involving EcoRI, XhoI, or HindIII was made by reprobing filters with pAGr12A, pAGr12C, or pAGr12B, respectively, which should give a single band of hybridization. These expectations were empirically derived.

Transfer to membrane, hybridization, and subsequent washing and stripping of filters was as described above for phage DNA.

## RESULTS

### *Cistron structure in A. gambiae*

Seven-enzyme restriction maps of the four rDNA clones are presented in fig. 2. Two of the clones contain 28S genes interrupted by relatively long sequences not homologous to 28S gene. These putative introns are both inserted in the same region of the 28S gene even though their sequences are apparently very different (the intron in λAGr23 contains 3–4 EcoRI, 3–4 SalI, and 1 HindIII sites while the intron in λAGr24 contains no EcoRI or SalI but three HindIII sites).

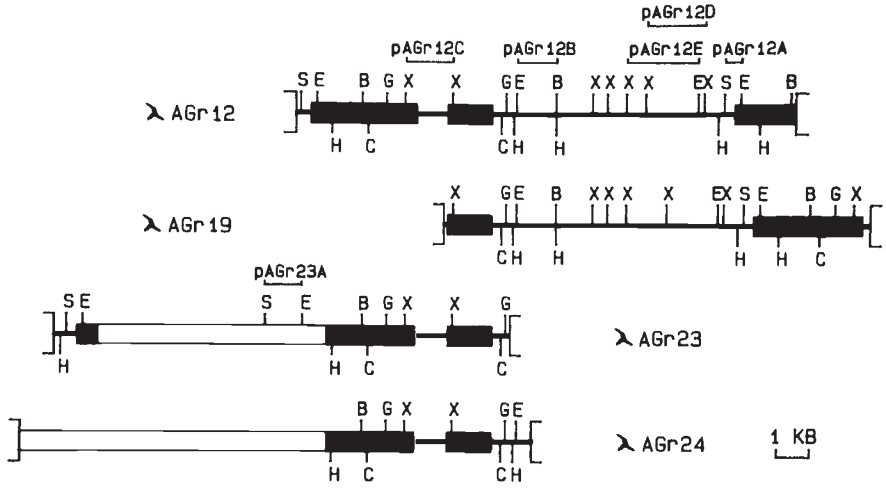


**Figure 2** Restriction maps of *A. gambiae* rDNA clones. B = BamHI, C = SacII, E = EcoRI, G = BglII, H = HindIII, S = SalI, X = XhoI. No XbaI or PvuII sites were detected.

A series of restriction enzyme digests on whole genomic DNA from single mosquitoes was done to determine if the intron insertion point observed in the clones was typical for the genome. The location of intron insertion sites within the 28S gene was determined as follows: DNA extracted from females of the G3 strain was digested with a series of two restriction enzymes. One enzyme, SalI, cleaving the DNA at a known conserved site at or near the terminus of the 28S gene (Collins *et al.*, 1987) was used with one of a series of enzymes cleaving the DNA in a sequence extending from the terminus toward the ITS. By hybridizing with a probe from the conserved terminus (pAGr12A), we identified a series of restriction fragments representing a known and ever greater fraction of the 28S gene. Introns or sequence variants appear as secondary restriction fragments for any given double digest. Intron insertion points are inferred as occurring between adjacent restriction sites whose double digestion profiles reveal a new series of secondary fragments.

Since the intron in λ AGr23 contains numerous SalI sites and since there is a conserved SalI site near the terminus of the 28S gene but not elsewhere in the gene, the intron insertion point can be identified as between the EcoRI and HindIII sites in the gene. An EcoRI/SalI double digest probed with the conserved sequences in pAGr12A reveals only the expected 0·5 kb fragment. However, a HindIII/SalI double digest reveals numerous secondary bands. These secondary bands are present in all other double digests and, therefore, probably represent SalI or HindIII sites within the introns (fig. 3(a)). This is confirmed for some bands when the filter is reprobed with the intron subclone that hybridized to some of the same secondary bands (fig. 3(b)). The same digestions and hybridizations were repeated substituting XhoI for SalI with the same results.

### *Conserved and variable sequences*

The restriction maps of the four rDNA clones are identical, excluding introns, in the coding regions

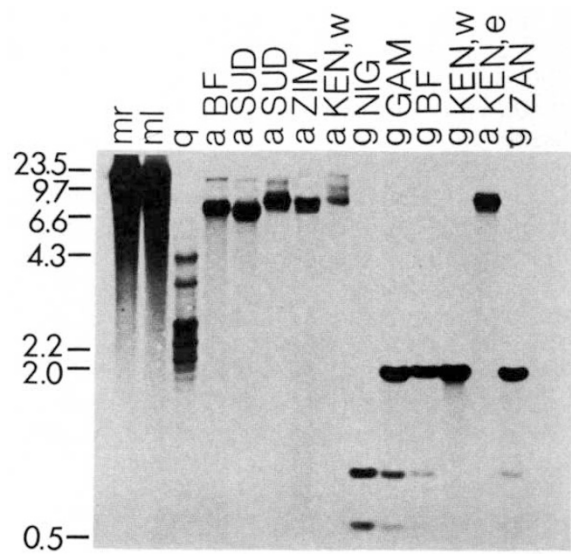


**Figure 4** Hybridization profiles of XhoI-digested single-mosquito DNA from *A. gambiae* complex species probed with pAGr12D, containing intergenic spacer sequences. Numbers are the length (kilobases) of a size standard (HindIII digested phage lambda DNA).

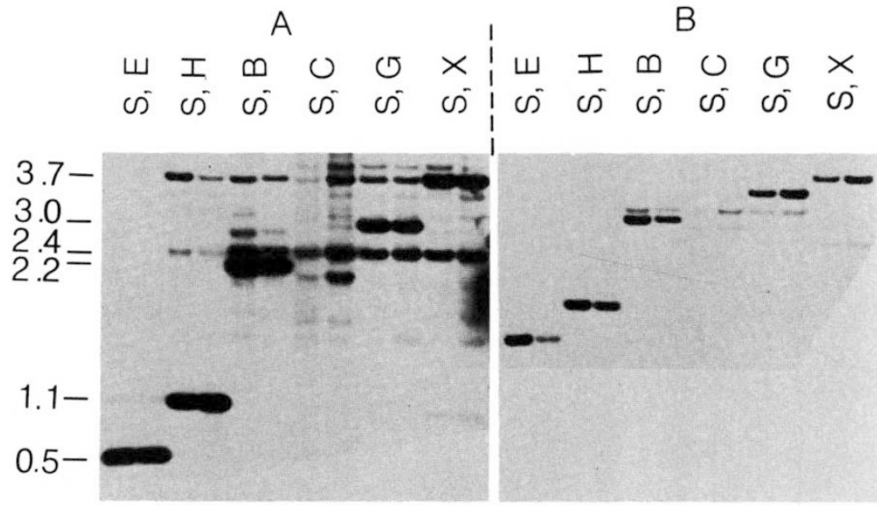


**Figure 3** Hybridization profile of *A. gambiae* (strain G3) single mosquito DNA probed with either pAGr12A, containing a conserved sequence (fig. 3(a)), or pAGr23A, a putative intron subclone from λAGr23 (fig. 3(b)). DNA from two individuals was digested with each enzyme set. Numbers on the left are the size (kilobases) of adjacent restriction fragments. Figs. 3(a) and 3(b) are from the same filter. Restriction endonucleases: B = BamHI, C = SacII, E = EcoRI, G = BglII, H = HindIII, S = SalI, X = XhoI.

(18S, 28S, and ITS). Only two of the clones, λAGr12 and λAGr19, contain whole IGS sequences. Here, the IGS sequences differ in the internal region characterized by abundant XhoI sites.

However, the small amount of variation in the IGS revealed in the rDNA clones is not representative of variation within the genome. For instance, when XhoI digests of mosquitoes from various *A. gambiae* strains are probed with IGS subclones pAGr12D and pAGr12E, restriction fragment length polymorphisms reveal spacer sequence variation (figs. 4 and 5).

Sequence variation in the IGS is especially evident when EcoRI or HindIII digests of single females are probed with IGS subclones, such as pAGr12D (fig. 6). Here, numerous bands reflecting IGS length polymorphism or restriction site gain/loss are revealed. The intensity of the bands is not uniform, suggesting differences in the relative abundance of variants.

It does appear, however, that the borders of the IGS are conserved. EcoRI digests of DNA from single mosquitoes of various strains of *A. gambiae* all reveal only a 1·3 kb fragment when probed with pAGr12A, demonstrating the conservation of two EcoRI sites in the IGS near the 28S gene (fig. 7, and Collins *et al.* 1987). Similarly, a single band of hybridization is observed when HindIII digests of single mosquitoes of various

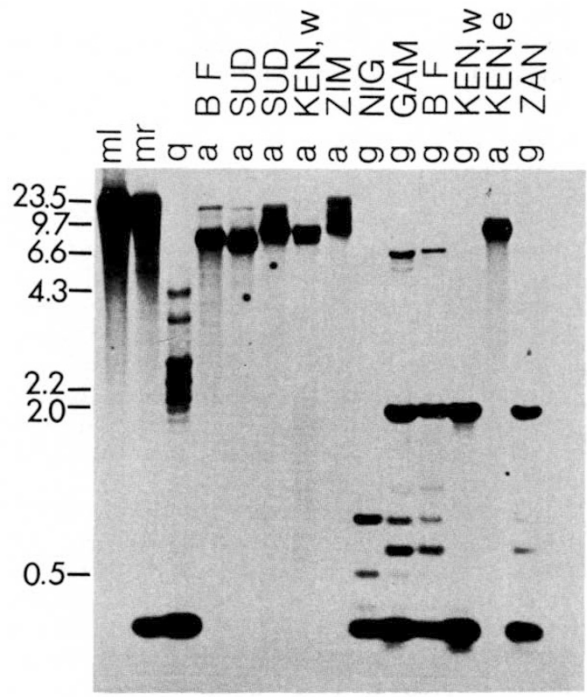


**Figure 5** Hybridization profiles of XhoI-digested single-mosquito DNA from *A. gambiae* complex species probed with pAGr12E, containing intergenic spacer sequences. Numbers are the length (kilobases) of a size standard (HindIII digested phage lambda DNA).

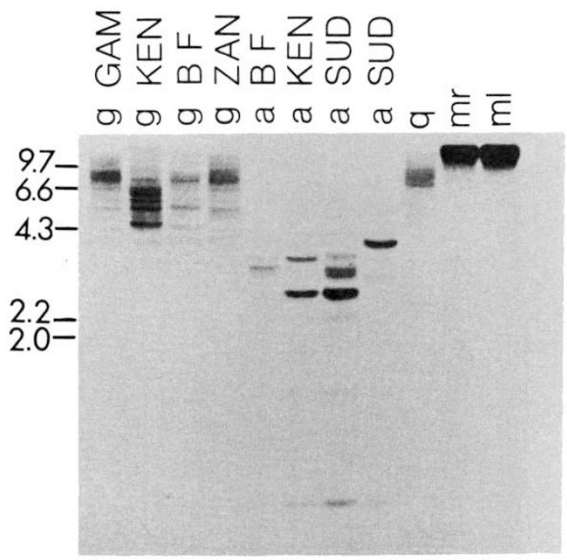


**Figure 6** Hybridization profiles of HindIII-digested single-mosquito DNA from *A. gambiae* complex species probed with pAGr12D, containing intergenic spacer sequences. Numbers are the length (kilobases) of a size standard (HindIII digested phage lambda DNA).

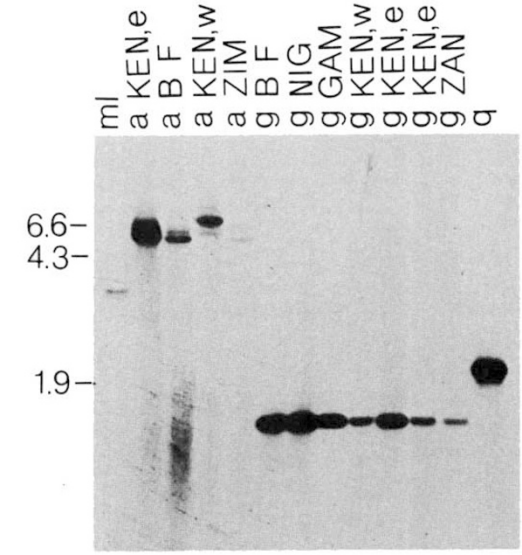


**Figure 7** Hybridization profiles of EcoRi-digested single-mosquito DNA from *A. gambiae* complex species probed with pAGr12A, containing intergenic spacer sequences flanking the 28S gene. Numbers are the length (kilobases) of a size standard (HindIII digested phage lambda DNA).

strains are probed with pAGr12B, demonstrating the conservation of HindIII sites in the IGS at the margin of the 18S gene (fig. 8).

### *Interspecific variation*

DNA extracted from single females of *A. melas*, *A. merus*, *A. quadriannulatus*, *A. arabiensis* (five strains), and *A. gambiae* (six strains) was cut with single digests of EcoRI, HindIII, and XhoI. After transfer of DNA, the filters were probed with gene, intron, or spacer sequences to determine if there is: (1) interspecific variation in rDNA sequence, (2) greater variation in noncoding versus coding sequences, and (3) intraspecific variation in some rDNA sequences. All digestions were tested for completeness as described above.

XhoI digests when probed with pAGr12C, which contains a small part of the 28S and 18S genes and all of the ITS, yield a single 1·4 kb band of hybridization for all species and strains. However, reprobing these filters with internal IGS sequences reveals both intraspecific and interspecific variation in the hybridization pattern (figs 4 and 5). Each species reveals a unique pattern of restriction fragment length polymorphisms.

The different *A. gambiae* strains may or may not share particular bands of hybridization. However, the distribution of spacer variants is not consistent with the geographic origins of the strains. For instance, the strains from geographically separated Zanzibar and Burkina Faso show

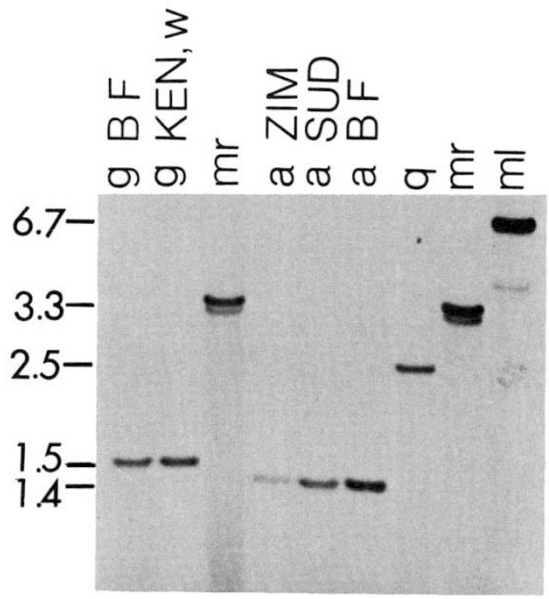


**Figure 8** Hybridization profiles of HindIII-digested single-mosquito DNA from *A. gambiae* complex species probed with pAGr12B, containing intergenic spacer sequences flanking the 18S gene. Numbers are the length (kilobases) of a size standard (HindIII digested phage lambda DNA).

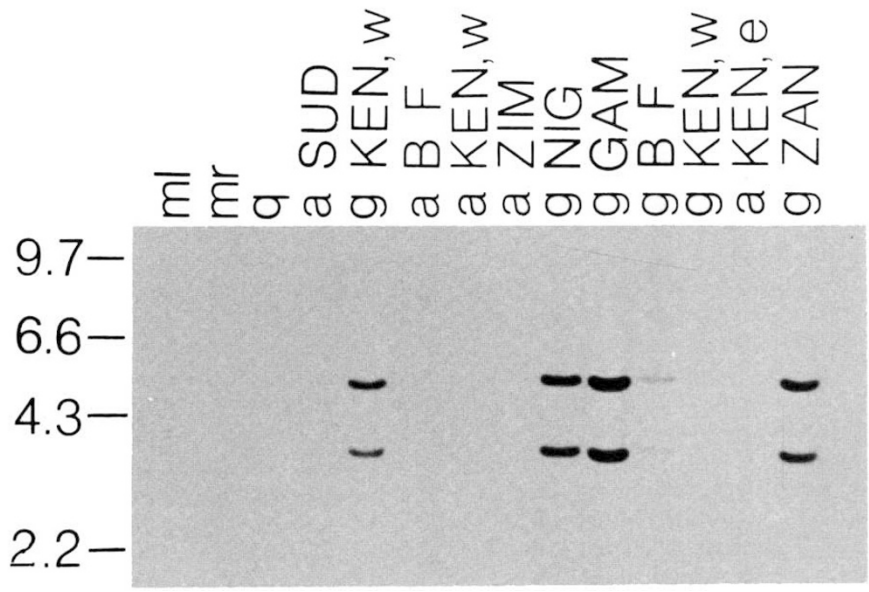


**Figure 9** Hybridization profiles of XhoI-digested single-mosquito DNA from *A. gambiae* complex species probed with pAgr23A, a putative intron sequence from the *A. gambiae* clone λAGr23.

the same hybridization profile when probed with pAGr12D. However, strains from geographically more proximate Nigeria and Gambia are dissimilar (fig. 4). At least six mosquitoes from each strain have been used to verify the consistency of these patterns.

Similar evidence of intraspecific variation is obtained with EcoRI and HindIII digests when probed with internal IGS sequences.

Interspecific but not intraspecific variation is revealed when EcoRI or HindIII digests are probed with sequences from the margins of the IGS flanking the 18S and 28S genes (Figs. 7 and 8) (see also Collins *et al.*, 1987).

When filters containing DNA from single individuals of *A. gambiae* and other complex members are probed with the intron sequence from *A. gambiae*, only *A. gambiae* strains reveal sequence homology (fig. 9). All of the *A. gambiae* strains have this sequence (Mendez *et al.*, 1988).

## DISCUSSION

The restriction maps of the four rDNA repeating units and hybridization profiles in whole genomic DNA demonstrate that the gene coding sequences are relatively conserved in *A. gambiae*. This study detected no restriction site polymorphism in the coding sequences except when interrupted by introns. Hybridization of the 1·4 kb ITS sequence to XhoI digests of other *A. gambiae* complex members further attests to the conservation of coding sequences since the pattern of hybridization was the same for all species. Conservation of the coding sequences, in contrast to the variation in IGS sequences within and between species, may suggest the action of selection to maintain sequence-dependent function. Since a reduction in the number of repeating units transcribed can have a large deleterious effect (DeSalle *et al.*, 1986), the apparent high frequency of introns in gene coding sequences suggests that they may be spliced out during subsequent processing of transcripts (see Tautz and Dover, 1986).

The intron sequences appear to evolve rapidly since there is no detectable homology (under stringent hybridization) between an *A. gambiae* intron sequence and the DNA of other sibling species. Rapid evolution of introns is also observed in *Drosophila* spp. (Roiha *et al.*, 1983). The concerted evolution of intron sequences suggests the rapid turnover of a very abundant class of sequences. Since unequal crossing-over will eliminate introns from repeating units (Gillings *et al.*, 1987) and at a fast rate (Maddern, 1981), it appears that this process and transposition into the 28S gene (Peacock *et al.*, 1981; Roiha *et al.*, 1981) of new

variants account for the concerted evolution (see Dover and Coen, 1981, for a discussion of this mechanism of converted evolution).

It is not possible from the present results to tell if intron-bearing clones are clustered as they are in *Drosophila* spp. (Kalumuck and Procunier, 1984; Salzano and Malva, 1984). However, as in *Drosophila* spp. the introns in *A. gambiae* insert at a specific point in the 28S gene. At the present level of resolution, it appears that different intron classes may insert at the same point, unlike the situation in *Drosophila* spp.

The intergenic spacer in the *A. gambiae* complex evolves very rapidly but at different rates in different regions. The boundaries of the spacer do not vary intraspecifically but do possess interspecific restriction site differences. In contrast, the interior of the spacer varies intraspecifically as well as interspecifically. Together these observations may reflect selective constraint on sequence variation in parts of the intergenic spacer (Williams *et al.*, 1987; Williams and Strobeck, 1985) or indicate that gene conversion or unequal crossing-over rates vary with location (Tautz *et al.*, 1987). Since unequal crossing-over and gene conversion occur between relatively short sequences (Michelson and Orkin, 1983; Amstutz *et al.*, 1985) it is possible that rates of gene conversion (*e.g.*, Jubier-Maurin *et al.*, 1985) could vary within the relatively long (>6 kb) intergenic spacers, perhaps as a consequence of the DNA sequence (Tautz *et al.*, 1987; Keil and Roeder, 1984).

Although concerted evolution in the rDNA multigene family is fast, selection on spacer sequence may be ineffective since between-individual variation in the abundance of variant repeating units within a population is expected to be low as a consequence of the low rates of unequal exchange or gene conversion relative to the rate of reshuffling of chromosomes attending sexual reproduction (Ohta and Dover, 1984). Consequently, spacer variation between populations should be high as molecular drive homogenizes populations for different variants (Dover, 1982, 1986). Unequal crossing-over occurring at two levels may account for the population and interspecific differences in the IGS. First, unequal crossing-over between spacer subrepeats may generate length polymorphism. Second, unequal crossing-over between whole rDNA repeating units may account for differences in the abundance of spacer variants between populations and species (see also Coen *et al.*, 1982*a*).

The rapid evolution of internal IGS sequences and, perhaps, introns in the *A. gambiae* complex suggests that these sequences may be useful in exploring population structure in natural situations. Presumably, molecular drive, mediated by gene conversion and unequal exchange rates, homogenizes genomes and populations for particular variants without the generation of much between-individual variation. Therefore, in the absence of strong sequence-dependent selection, local demes will rapidly diverge in some of these sequences.

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## REFERENCES

AMSTUTZ, H., MUNZ, P., HEYER, W.-D., LEUPOLD, U. AND KOHLI, J. 1985. Concerted evolution of transfer RNA genes: intergenic conversion among three unlinked serine transfer RNA genes in *Schizosaccharomyces pombe*. *Cell*, *40*, 879–886.

ARNHEIM, N. 1983. concerted evolution of multigene families. In Nei, M. and Koehn, R. K. (eds) *Evolution of Genes and Proteins*, Sinauer, Boston, pp. 38–61.

BECKINGHAM, K. 1982. Insect rDNA. *Cell Nucl.*, *10*, 205–263.

BECKINGHAM, K. AND SMITH, V. L. 1981. The ribosomal DNA of *Calliphora erythrocephala*: the cistron classes of total genomic DNA. *J. Mol. Biol.*, *149*, 141–169.

BECKINGHAM, K. AND WHITE, R. 1980. The ribosomal DNA of *Calliphora erythrocephala*: an analysis of hybrid plasmids containing ribosomal DNA. *J. Mol. Biol.*, *137*, 349–373.

BONCINELLI, E., BORHESE, A., GRAZIANI, F., LAMANTIA, A., MANZI, A., MRIANI, C. AND SIMEONE, A. 1983. Inheritance of the rDNA spacer in *Drosophila*. *Mol. Gen. Genet.*, *189*, 370–374.

COEN, E. S. AND DOVER, G. A. 1982. Multiple Pol I intiation sites in rDNA spacers of *Drosophila melanogaster*. *Nucl. Acids Res.*, *10*, 7017–7026.

COEN, E. S. AND DOVER, G. A. 1983. Unequal exchanges and the coevolution of X and Y chromosomal rDNA arrays in *Drosophila melanogaster*. *Cell 33*, 849–854.

COEN, E., STRACHAN, T. AND DOVER, G. A. 1982*a*. Dynamics of concerted evolution of ribosomal DNA and histone gene families in the *melanogaster* subgroup of *Drosophila*. *J. Mol. Biol.*, *158*, 17–35.

COEN, E. S., THODAY, J. M. AND DOVER, G. A. 1982*b*. The rate of turnover of structural variants in the ribosomal gene family of *Drosophila melanogaster*. *Nature*, *295*, 564–568.

COLLINS, F. H., MENDEZ, A., RASSMUSEN, M. O., MEHAFFEY, P. C., BESANSKY, N. J. AND FINNERTY, V. 1987. A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. *Am. J. Trop. Med. Hyg.*, *37*, 37–41.

DESALLE, R., SLIGHTORN, J. AND ZIMMER, E. 1986. The molecular through ecological genetics of abnormal abdomen. II. Ribosomal DNA polymorphism is associated with the abnormal abdomen syndrome in *Drosophila mercatorum*. *Genetics*, *112*, 861-875.

DEWINTER, R. F. J. AND MOSS, T. 1986. Spacer promoters are essential for efficient enhancement of *X. laevis* ribosomal transcription. *Cell*, *44*, 313-318.

DOVER, G. A. 1982. Molecular drive: a cohesive mode of species evolution. *Nature*, *299*, 111-117.

DOVER, G. A. 1986. Molecular drive in multigene families: how biological novelties arise, spread and are assimilated. *Trends Genet.*, *2*, 159-165.

DOVER, G. A., BROWN, S., COEN, E. S., DALLAS, J., STRACHAN, T. AND TRICK, M. 1982. The dynamics of genome evolution and species differentiation. In Dover, G. A. and Flavell, R. B. (eds) *Genome, Evolution*, Academic Press, New York, pp. 343-372.

DOVER, G. A. AND COEN, E. 1981. Spring cleaning ribosomal DNA: a model for multigene evolution? *Nature*, *290*, 731-732.

DOVER, G. A. AND FLAVELL, R. B. 1984. Molecular coevolution: DNA divergence and the maintenance of function. *Cell*, *38*, 622-623.

DUTTON, F. L. AND KRIDER, H. M. 1984. Ribosomal RNA cistrons of X-chromosomes clonally derived from *Drosophila melanogaster* laboratory populations: redundancy, organization and stability. *Genetics*, *107*, 405-421.

ENDOW, S. A. AND GLOVER, D. M. 1979. Differential replication of ribosomal gene repeats in polytene nuclei of *Drosophila*. *Cell*, *17*, 597-605.

ERICKSON, J. M. AND SCHMICKELL, R. D. 1985. A molecular basis for discrete variation in human ribosomal DNA. *Am. J. Hum. Genet.*, *37*, 311-325.

FEDEROFF, N. V. 1979. On spacers. *Cell*, *16*, 697-710.

GERBI, S. A. 1985. Evolution of ribosomal DNA. In MacIntyre, R. (ed.) *Molecular Evolutionary Genetics*, Plenum, New York, pp. 419-517.

GILLINGS, M. R., FRANKHAM, R., SPIERS, J. AND WHALLEY, M. 1987. X-Y exchange and the coevolution of the X and Y rDNA arrays in *Drosophila melanogaster*. *Genetics*, *116*, 241-251.

HAYASHIDA, H., MIYATA, T., YMAWAKI-KATOOKA, Y., HONJO, T., WELS, J. AND BLATTNER, F. 1984. Concerted evolution of the mouse immunoglobulin gamma chain genes. *EMBO J.*, *3*, 2047-2054.

JUBIER-MAURIN, V., DOD, B. J., BELLIS, M., PIECHACZYK, M. AND ROIZES, G. 1985. Comparative study of the L1 family in the genus Mu: possible role of retroposition and conversion events in its concerted evolution. *J. Mol. Biol.*, *184*, 547-564.

KALAMUCK, K. E. AND PROCUNIER, J. D. 1984. Non-selective amplification of ribosomal DNA repeat units in compensating genotypes of *Drosophlia melanogaster*. *Biochem. Genet.*, *22*, 453-465.

KEIL, R. L. AND ROEDER, G. S. 1984. Cis-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *Saccharomyces cerevisiae*. *Cell*, *39*, 377-386.

KIDD, S. J. AND GLOVER, D. M. 1980. A DNA segment from *Drosophila melanogaster* which contains five tandemly repeating units homologous to the major rDNA insertion. *Cell*, *19*, 103-119.

KOHORN, B. D. AND RAE, P. M. M. 1982. Non-transcribed spacer sequences promote *in vitro* transcription of *Drosophila melanogaster* ribosomal DNA. *Nucl. Acids Res.*, *10*, 6879-6886.

KUNZ, W., PETERSEN, G., RENKAWITZ-POHL, R., GLATZER, K. H. AND SCHAFER, M. 1981. Distribution of spacer length classes and the intervening sequence among different nucleolus organizers in *Drosophila hydei*. *Chromosoma*, *83*, 145-158.

LIVAK, K. 1984. Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics*, *107*, 611-634.

LONG, E. O., COLLINS, M., KIEFER, B. I. AND DAWID, I. B. 1981. Expression of the ribosomal DNA insertions in bobbed mutants of *Drosophila melanogaster*. *Mol. Gen. Genet.*, *182*, 377-384.

MADDERN R. H. 1981. Exchange between ribosomal RNA genes of the X and Y chromosomes of *Drosophila melanogaster*. *Genet. Res.*, *38*, 1-7.

MANIATIS, T., FRITSCH, E. F. AND SAMBROOK, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory.

MENDEZ, A. M., COLLINS, F. H. AND FINNERTY, V. 1988. Organization of the 28S gene intron of rDNA in *Anopheles gambiae*. (Manuscript in preparation).

MICHELSON, A. M. AND ORKIN, S. H. 1983. Boundaries of gene conversion within the duplicated human a-globin genes: concerted evolution by segmental recombination. *J. Biol. Chem.*, *258*, 15245-15254.

MORZYCKA-WROBLEWSKA, E., SELKER, E. U., STEVENS, J. N. AND METZENBERG, R. L. 1985. Concerted evolution of dispersed Neurospora crassa 58 RNA genes: pattern of sequence conservation between allelic and nonallelic genes. *Mol. Cell Biol.*, *5*, 46-51.

OHTA, T. 1983. On the evolution of multigene families. *Theor. Pop. Biol.*, *23*, 216-240.

OHTA, T. AND DOVER, G. A. 1983. Population genetics of multigene families that are dispersed into two or more chromosomes. *Proc. Natl. Acad. Sci. (USA)*, *80*, 4079-4083.

OHTA, T. AND DOVER, G. A. 1984. The cohesive population genetics of molecular drive. *Genetics*, *108*, 501-521.

PEACOCK, W. J., APPELS, R., ENDOW, S. AND GLOVER, D. M. 1981. Chromosomal distribution of the major insert in *Drosophila melanogaster* 28S rRNA genes. *Genet. Res.*, *37*, 204-214.

PICHERSKY, E., BERNATZKY, R., TANKSLEY, S. D. AND CASHMORE, A. R. 1986. Evidence for selection as a mechanism in the concerted evolution of *Lycopersicon esculentum* (tomato) genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Proc. Natl. Acad. Sci. (USA)*, *83*, 3880-3884.

REEDER, R. J. 1984. Enhancers and ribosomal gene spacers. *Cell*, *38*, 349-351.

ROIHA, H. AND GLOVER, D. M. 1980. Characterization of complete type II insertions in cloned segments of ribosomal DNA from *Drosophila melanogaster*. *J. Mol. Biol.*, *140*, 341-355.

ROIHA, H., MILLER, J. R., WOODS, L. C. AND GLOVER, D. M. 1981. Arrangements and rearrangements of sequence flanking the two types of rDNA insertion in *Drosophila melanogaster*. *Nature*, *290*, 749-753.

ROIHA, H., READ, C. A., BROWNE, M. J. AND GLOVER, D. M. 1983. Widely differing degrees of sequence conservation of the two types of rDNA insertion within the *melanogaster* species subgroup of *Drosophila*. *EMBO J.*, *2*, 721-726.

SALZANO, G. AND MALVA, C. 1984. Non-random loss of uninterrupted ribosomal DNA repeating units upon induction of a bobbed mutation. *J. Mol. Biol.*, *177*, 189-200.

SCHIMENTI, J. C. AND DUNCAN, C. H. 1985. Concerted evolution of the cow beta-globin genes. *Mol. Biol. Evol.*, *2*, 505–513.

SERVICE, M. W. 1985. *Anopheles gambiae*: Africa's principal malaria vector, 1902–1984. *Bull. Entomol. Soc. Am.*, *Fall*, 8–12.

SOUTHERN, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, *98*, 503–517.

STRACHAN, T., COEN, E., WEBB, D. AND DOVER, G. A. 1982. Modes and rates of change of complex DNA families of *Drosophila*. *J. Mol. Biol.*, *158*, 37–54.

STRACHAN, T., WEBB, D. AND DOVER, G. A. 1985. Transition stages of molecular drive in multi-copy DNA families in *Drosophila*. *EMBO J.*, *4*, 1701–1708.

TAUTZ, D. AND DOVER, G. A. 1986. Transcription of the tandem array of ribosomal DNA of *Drosophila melanogaster* does not terminate at any fixed point. *EMBO J.*, *5*, 1267–1273.

TAUTZ, D., TAUTZ, C., WEBB, D. AND DOVER, G. A. 1987. Evolutionary divergence of promoters and spacers in the rDNA family of four *Drosophila* species. *J. Mol. Biol.*, *195*, 425–542.

TAUTZ, D., TRICK, M. AND DOVER, G. A. 1986. Cryptic simplicity in DNA is a major source of genetic variation. *Nature*, *322*, 652–656.

TRECO, D., BROWNELL, E. AND ARNHEIM, N. 1982. The ribosomal gene nontranscribed spacer. *Cell Nucl.*, *12*, 101–126.

VIEIRA, J. AND MESSING, J. 1982. The pUC plasmids, an Ma13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene*, *19*, 259–268.

WILLIAMS, S. M., DESALLE, R. AND STROBECK, C. 1985. Homogenization of geographical variants at the nontranscribed spacer of rDNA in *Drosophila mercatorum*. *Mol. Biol. Evol.*, *2*, 338–346.

WILLIAMS, S. M., FURNIER, G. R., FUOG, E. AND STROBECK, C. 1987. Evolution of ribosomal DNA spacers of *Drosophila melanogaster*: different patterns of variation on X and Y chromosomes. *Genetics*, *116*, 225–232.

WILLIAMS, S. M. AND STROBECK, C. 1985. Sister chromatid exchange and the evolution of rDNA length. *J. Theor. Biol.* *116*, 625–636.