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Inter and intraspecific variation in nuclear DNA content in *Aedes* mosquitoes

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Haploid nuclear DNA of 23 species of *Aedes*, as determined by Feulgen cytophotometry, was found to vary 3-fold. This was accompanied by a 2-fold variation in total chromosomal length. There was a significant correlation ($r = 0{\cdot}765$, $P < 0{\cdot}001$) between these two parameters. Genome size varied from 0·87 pg to 1·3 pg among 10 strains of *Aedes albopictus*, from wide geographic regions. Large scale differences in chromosomal DNA amounts have accompanied speciation and evolution in aedine mosquitoes.

INTRODUCTION

Amounts of nuclear DNA vary greatly among and within taxa (Bachman *et al.*, 1972; Sparrow *et al.*, 1972; Rees and Jones, 1972; Hinegardner, 1976; Sherwood and Patton, 1982). For example, the genome sizes of several *Drosophila* species show a 2·5-fold variation in C-values (Laird, 1973). Similarly Rees *et al.* (1978) observed a 3-fold variation in nuclear DNA content among species of acridid grasshoppers with uniform karyotype. Bier and Muller (1969) measured the genome sizes of a variety of insects and found that primitive groups have larger genomes than more recently evolved insect groups. In mosquitoes, very little work has been done in this field. Jost and Mameli (1972) and Spradling *et al.* (1974) have provided some data on relative and absolute amounts of DNA in two species of *Aedes* and six species of three other genera.

The genus *Aedes* (Culicidae) is subdivided into 38 subgenera (Knight and Stone, 1977) and includes more than 1000 species (White, 1980). One of the largest subgenera, *Stegomyia*, contains about 110 described species divided into seven groups including the *scutellaris* group which is subdivided into the *scutellaris* and *albopictus* subgroups. *Ae. scutellaris* subgroup is widely distributed in Southeast Asia and the South Pacific (Marks, 1954; Huang and Hitchcock, 1980), and has a predominantly allopatric distribution; on the other hand the *albopictus* subgroup has largely sympatric distribution over a wide range stretching from Madagascar in the west through the Indo-Malayan and the Oriental regions, China, Japan, the Pacific islands and extending as far east as Hawaii, and is recently reported to be in southern United States (Knight and Stone, 1977; Rai, 1987). Both these subgroups have been the object of extensive genetic studies with particular emphasis on the genetics of speciation (Rai, 1983; Rai, 1987).

A striking cytological feature of *Aedes* is the constancy in the chromosome number ($2n = 6$) and the lack of pronounced variation in chromosome morphology among various species (Rai *et al.*, 1982; Rao, 1985). Most species so far studied are characterised by a small pair of metacentric chromosome and two pairs of larger metacentric or submetacentric chromosomes. However, careful measurements of chromosomal arms, giemsa C-banding studies, meiotic analyses of species hybrids and linkage map comparisons have shown the existence of individual differences among the karyotypes of various species (Rai, 1980; Dev and Rai, 1984; Sherron and Rai, 1984; Rao and Rai, 1987). The uniformity in the chromosome number and yet the worldwide distribution and habitat diversity of various species, make *Aedes* an interesting genus to study the extent of changes in nuclear DNA amount and its possible evolutionary role.

Feulgen cytophotometry is an important investigative technique to determine quantitative genomic changes across and within species. This

paper presents data on nuclear DNA in (a) 23 species of *Aedes*, belonging to five subgenera with different distribution and habitat preferences, with particular emphasis on the *Aedes scutellaris* group, and (b) 10 geographic strains of the widespread species *Ae. albopictus*. Other objectives of this study were to correlate total chromosomal length to nuclear DNA amounts in order to ascertain the evolution of chromosomal DNA between and within species and to provide information on which to base future analyses of genome organisation.

MATERIALS AND METHODS

Table 1 provides the names of the 23 species with the sites and dates of their original collection. Field collected species were raised at 21°C in the laboratory. The rest of the species were reared in the insectary maintained at 25 ± 2°C and 80 ± 10 per cent relative humidity (Craig and Vande Hey, 1963).

Testes from 12–24 h old male pupae, were dissected in insect saline, fixed in formalin-acetic acid-ethanol (6:1:14) (Sharma and Sharma, 1980), and squashed in 45 per cent acetic acid. Each slide also contained on one side a fine smear of chicken red blood cells (Dhillon *et al.*, 1977).

For Feulgen staining, the tissue was hydrolysed in 5 N HCl for 30 min at room temperature and washed throughly in cold running water. Staining was done in Schiff's reagent for 1 h and excess stain was washed off with three rinses in aq. 10 per cent potassium metabisulphite solution (McLeish and Sunderland, 1961).

Relative feulgen units were calculated according to Patau (1952), using the two-wavelength method (505 m and 555 nm, determined through absorbance versus wavelength curve) (Berlyn and Mikshe, 1976), with a Zeiss Microscope Photometer 01. Thirty to fifty primary spermatocytes at

Table 1 Taxonomy and source of species and strains of *Aedes* examined

Subgenus	Species	Strains	Source, site, year collected
Stegomyia	*aegypti*	Rock	Rockfeller Inst., 1959
	heischii		Kenya, 1972
	metallicus		Kenya, 1972
	albopictus	Mauritius	Mauritius, 1972
		Tokyo	Tokyo, 1979
		Hongkong	Hongkong, 1978
		Hawaii	Oahu, 1971
		Tana	Madagascar, 1978
		Pontaniak	Indonesia, 1978
		Pune	India, 1984
		Delhi	India, 1984
		Kollar	India, 1984
		Calcutta	India, 1973
	flavopictus		Japan, 1981
	pseudalbopictus		Taiwan, 1982
	seatoi		Thailand, 1972
	unilineatus		Senegal, 1983
	katherinensis		Austrialia, 1982
	alcasidi		Taiwan, 1972
	malayensis		Thailand, 1968
	hebrideus		Vanvatu Is., 1982
	polynesiensis		Samoa Is., 1979
	pseudoscutellaris		Fiji, 1980
	cooki		Tonga Is., 1973
Ochlerotatus	*excrucians*		Mich., USA., 1984*
	stimulans		Ind., USA., 1984*
	communis		Mich., USA., 1984*
	canadensis		Mich., USA., 1984*
Aedes	*cinereus*		Mich., USA., 1984*
Howardina	*bahamensis*		Bahamas, 1970
Protomacleaya	*triseriatus*		Ind., USA., 1969*
	zoosophus		Texas, 1981

* Field collected

metaphase I, were scored from a total of three to five pupae. In most cases anaphase I cells were also measured from the same slide to confirm the values obtained for the metaphases. The chick erythrocytes stained simultaneously not only served as an internal standard for the day to day variations but the values were also used to convert relative mosquito DNA values to absolute amounts in picograms (pg), using 2·5 pg per nucleus as the value for the diploid chicken genome (Mirsky and Ris, 1951; Leslie, 1955; Rasch *et al.*, 1978). All DNA amounts reported are 1C values or values of the unreplicated haploid complement.

A One-Way ANOVA with absolute DNA values as the response variable and the different species and strains as the treatment was performed. Duncan's Multiple Range Test was used to compare mean DNA values of some species and to establish different groupings.

Total chromosomal length measurements at somatic metaphases were obtained according to the procedure described by Rao and Rai (1987).

RESULTS

Haploid DNA amounts (1C) of the 23 *Aedes* species, the total chromosomal length of 18 species, and the results of the Duncan's Test are shown in table 2. The ANOVA was significant at the $P < 0{\cdot}001$ level.

In the *aegypti* group, the rock strain of *Ae. aegypti* possessed $0{\cdot}812 \pm 0{\cdot}031$ pg of haploid DNA. Among the seven species examined in the *scutellaris* subgroup, *Ae. katherinensis* with $1{\cdot}277 \pm 0{\cdot}02$ pg had the highest 1C nuclear DNA per cell. It was approximately double the amount found in *Ae. pseudoscutellaris* and *Ae. cooki* which

Table 2 Absolute 1C nuclear DNA amounts and total chromosomal length

Species/strains	DNA (pg)	S.E.[a]	Duncan's groups[b]	TCL (u)[c]
aegypti	0·812	0·03		19·90[d]
heischii	1·121	0·03		21·62
metallicus	1·093	0·03		19·32
albopictus				
Mauritius	1·321	0·03	A	26·91
Tokyo	1·286	0·03	A	
Hong Kong	1·259	0·03	A	
Oahu	1·239	0·03	A B	21·23
Tana	1·148	0·02	B C	31·10
Pontaniak	1·068	0·04	C	
Pune	1·066	0·06	C	31·95
Delhi	1·021	0·01	C D	32.85
Kollar	0·944	0·03	D	24·07
Calcutta	0·865	0·03	D	23·99
flavopictus	1·330	0·02		33·34
pseudalbopictus	1·290	0·02		30·09
unilineatus	1·064	0·04		25·50
seatoi	0·971	0·02		27·11
katherinensis	1·277	0·02	A	29·51
alcasidi	0·974	0·02	B	21·44
malayensis	0·943	0·03	B	19·12
hebrideus	0·965	0·03	B	19·33
polynesiensis	0·725	0·02	C	20·88
cooki	0·594	0·03	D	
pseudocutellaris	0·591	0·01	D	16·24
excrucians	1·500	0·03		
stimulans	1·439	0·04		29·80[d]
communis	1·013	0·05		
canadensis	0·904	0·02		
cinereus	1·210	0·03		21·80[e]
bahamensis	1·375	0·03		
triseriatus	1·520	0·06		35·88
zoosophus	1·902	0·06		38·29

[a] S.E. = standard error; [b] $P < 0{\cdot}001$; [c] TCL = total chromsome length in microns; [d] Rai (1963); [e] Mukherjee *et al.* (1970).

possessed the smallest genome of all *Aedes* examined. *Ae. alcasidi*, *Ae. malayensis* and *Ae. hebrideus* were grouped together and did not differ significantly in their DNA amounts. The 10 strains of *Ae. albopictus* ranging from Hawaii to Mauritius had 1C DNA amounts ranging from 1·321 ± 0·035 pg to 0·865 ± 0·03 pg. Duncan's Test established six groupings based on genome sizes with some overlapping. Both *Ae. triseriatus* and *Ae. zoosophys* of the subgenus *Protomacleaya*, with 1·52 ± 0·062 pg and 1·902 ± 0·062 pg respectively have the highest DNA values of all aedine species studied here.

Linear regression analysis of the DNA amounts to the total chromosomal length of 18 species showed a significant correlation ($r = 0·765$; $P < 0·001$) (fig. 1).

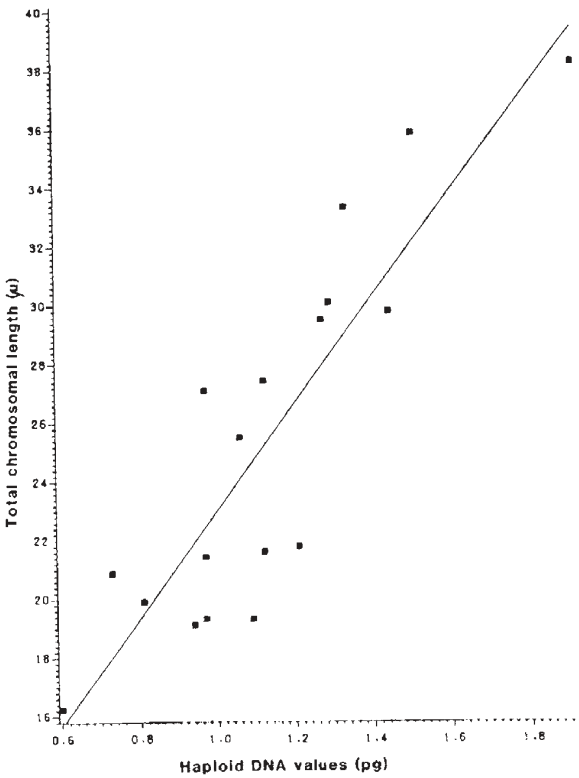

Figure 1 Linear regression of haploid DNA amounts to the total chromosomal length in 18 species of *Aedes*.

DISCUSSION

A three-fold variation in haploid DNA genome sizes was found among the 23 species of *Aedes*. In general, there was no correlation between DNA amounts and their systematic affinities. Also, this variation was accompanied by a two-fold range in the total chromosomal lengths at somatic metaphases. This increase in chromosomal size was often observed in all the three pairs of chromosomes, rather than one particular pair (Rai, 1963; Dev and Rai, 1984; Sherron and Rai, 1984; Rao and Rai, 1987). There was a good correlation between the DNA amounts and the total chromosomal lengths (fig. 1).

Based on biogeographical and morphological considerations, Belkin (1962) had originally suggested that speciation in the *Ae. scutellaris* subgroup proceeded from east to west with the southeast Asian species being of more recent origin and Polynesian species more primitive. This has been confirmed by other lines of research (Rai, 1983). Our results show that island dwelling species in Polynesia possessed low DNA amounts whereas the continental southeast Asian species had higher DNA values. This suggests that there has been a gradual amplification of chromosomal DNA in the evolution of species in this subgroup.

The mean nuclear DNA amount in the *albopictus* subgroup was higher than the *scutellaris* subgroup. *Ae. flavopictus* and *Ae. pseudalbopictus* had larger genomes and showed good pairing of chromosomes at metaphase 1 in their hybrids. In contrast, crosses between *Ae. seatoi*, with a relatively smaller genome and *Ae. flavopictus* showed chromosomal size heteromorphism in two of the three pairs (Rai and Herman, 1985). The same was observed between *Ae. seatoi* and *Ae. unilineatus*.

The actual patterns of quantitative change within the *Ae. albopictus* strains was interesting. The Indo-Malayan region, where *Ae. albopictus* is widely distributed, is presumably the center of its origin. Strains from this region possess relatively lower DNA amounts. The expansion of the species to the various island regions is apparently associated with increase in nuclear DNA amounts. Similar correlation exists between nuclear genome sizes and historical migration of the species in the *Ae. scutellaris* subgroup. The nearly two-fold variation in DNA amount observed in strains of *Ae. albopictus* demonstrates the presence of extensive populational difference in cellular DNA. The role it may play in its divergence is not well understood. Black and Rai (1987) showed that increase in DNA amounts in different strains was primarily due to higher amounts of highly repeated elements, although they found that in general all three classes of repeated DNA increased with genome sizes. Numerous studies have identified similar intraspecific variation in genome size in other taxa (Sherwood and Patton, 1982; Greenlee *et al.*, 1984). It

is possible that these differences arise within species and subsequently lead to or enhance divergence and speciation (Robertson, 1981). It is also well known that intrachromosomal addition of DNA within and between species have little effect on phenotypic or genotypic characters (Hutchinson *et al.*, 1979, Sherwood and Patton, 1982).

The four species in the *Ochleratus* subgenus, had larger genome sizes and exhibited a 66 per cent difference in DNA content. The total chromosomal length of *Ae. stimulans* was the largest among the species reported by Rai (1963). *Ae. caspius*, a related species of the same subgenus, found in saline waters, was reported to have an haploid amount of 0·99 pg (Jost and Mameli, 1972). *Ae. triseriatus* and *Ae. zoosophus* are somewhat specialised in their habitat preferences in that both are tree-hole mosquitoes.

There are numerous reports of large scale differences in the nuclear DNA content in related species (Hinegardner, 1976; Bennett and Smith, 1976). Britten and Davidson (1971), suggested that DNA increases are important in the origin of repetitive DNA which in turn may play a role in evolutionary diversification. In *Dermestes*, Fox (1969) reported significant differences in nuclear DNA despite very similar karyotypes. The variation in the total DNA content was mostly accounted for by variation in the fast fraction, that is made up largely of highly repetitive DNA (Rees *et al.*, 1976). Using dot-hybridisation for nine clones of highly repeated elements from species of *Ae. scutellaris* subgroup, McLain *et al.* (1986) found that these elements varied greatly in their relative abundance among closely related species. This suggests that there are indeed differences in the amounts of repetitive DNA in the different species.

Spradling *et al.* (1974) examined the annealing properties of ribosomal, hnRNA and messenger RNA to DNA in vast excess in mammalian and *Ae. albopictus* cell lines. They found that the mosquito cell line had 3·5-fold less DNA than the mammals. The range for mammalian haploid genome is 3–5·8 pg (Hinegardner, 1976). This compares with 0·86–1·66 pg for the *Ae. albopictus* cell line. The mean for *Ae. albopictus* obtained in our study is about 1·12 pg which fits very well with their results. The amount of DNA in the genome has been correlated with several variables, such as length of the mitotic cycle (Van 't Hof and Sparrow, 1963) and the minimum length of generation time (Smith and Bennett, 1975). When the generation time from the early larval stage to adult was studied in the *Ae. alobopictus* strains, there was a significant correlation between the haploid DNA amounts and the developmental time (Ferrari and Rai, unpublished).

Based on DNA amounts it was suggested that organisms (*Chironomus*, 0·2 pg/ haploid genome [Wells *et al.*, 1976]; *Apis mellifera*, 0·35 pg/haploid genome [Crain *et al.*, 1976*b*]), with low DNA amounts (less than 0·4 pg) would show the *Drosophila*-type of longterm interspersion of repeats while those with larger genomes (*Musca*, 0·89 pg/haploid genome [Crain *et al.*, 1976*b*], will exhibit quite typical short-term repeat pattern the *Xenopus*-type. With *Aedes* showing such extensive variation in DNA amounts it would be interesting to study the pattern of repeated elements in this large genus. Black and Rai (1987) found that genomic organisation in *Ae. albopictus* and *Ae. triseriatus* was of the short interspersed type.

In conclusion, large scale differences in chromosomal DNA amounts have accompanied speciation and evolution in *Aedes* besides structural changes brought about by chromosomal rearrangements (Munstermann, 1981; Rai *et al.*, 1982).

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