Theor Appl Genet (1990) 79:748–752

THEORETICAL AND APPLIED GENETICS

Intraspecific variation in nuclear DNA content among world populations of a mosquito, *Aedes albopictus* (Skuse)

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Received January 23, 1990; Accepted February 9, 1990
Communicated by F. Mechelke

Summary. *Aedes albopictus* is commonly distributed in most parts of the Oriental region and on many islands in the Indian and the Pacific Oceans. The species was recently introduced into the United States and Brazil. Feulgen cytophotometric quantitation of haploid nuclear DNA content was carried out for 37 populations of *Ae. albopictus* to determine the extent of intraspecific variation in nuclear DNA content and whether the range expansion of the species has coincided with an increase in DNA content. The haploid nuclear DNA content varied nearly three-fold. The minimum DNA content was 0.62 pg in Koh Samui from Thailand, and the maximum DNA content was 1.66 pg in Houston-61 from the United States. Statistical comparisons of populations revealed significant differences in DNA contents. No geographic clustering of populations was noted with respect to DNA content. In general, populations from the United States and Brazil had higher DNA contents, but there was no indication that the range expansion had occurred hand in hand with an increase in DNA content. Each population had a specific amount of DNA that is probably imposed by the microenvironment.

Key words: Feulgen cytophotometry – Intraspecific – DNA content – *Aedes albopictus*

Introduction

Interspecific variation in nuclear DNA content is well documented in the literature (for references, see Bachmann et al. 1972; Sparrow et al. 1972; Rees and Jones 1972; Hinengardner 1976; Cavalier-Smith 1985 b). These studies revealed an astonishing level of variation in nuclear DNA content among animals. The DNA content per haploid genome ranges from 0.05 picogram (pg) in tube sponge to 142 pg in *Lepidosiren* (Rees and Jones 1972; Cavalier-Smith 1985 b). Even closely related species of the same genus vary in their DNA content by as much as 2.5-fold among species of *Drosophila* (Laird 1973), and by three-fold among species of *Aedes* (Rao and Rai 1987). Sherwood and Patton (1982) found variation as high as 2.3-fold between some congeneric species of the *Thomomys* pocket gophers. Quantitative changes in DNA content are associated with divergence and evolution among species in many genera of vertebrates and invertebrates (Bachmann et al. 1972; Sparrow et al. 1972; Rees and Jones 1972; Hinengarden 1976; Sherwood and Patton 1982; Rao and Rai 1987). Rees and Jones (1972) suggested that DNA content increases with the increasing complexity of an organism. However, Cavalier-Smith (1985 a) has shown recently that increased organismic complexity is not necessarily associated with an increase in DNA content. This has led to a general biological problem known as C-value paradox.

Studies on the intraspecific variation in nuclear DNA content in animals are rare in the literature (Gold and Price 1985; Sherwood and Patton 1982; Gold and Amemiya 1987; Johnson et al. 1987; Burton et al. 1989). These studies have shown significant differences in DNA content between populations of the same species. In some cases, the variation in DNA content within species exceeds variation between species. Therefore, to evaluate the meaning of DNA content variation between species, our knowledge on the extent of variation within the species is essential.

The mosquito, *Aedes albopictus* (Diptera: Culicidae), is widespread and has been reported from most of the islands in the Indian Ocean westward to Madagascar, virtually all countries on mainland Asia, and most of the islands in the Pacific Ocean eastward to Hawaii (Hawley

1988). The mosquito was recently introduced into the United States and Brazil (Forratini 1986; Hawley 1988). Soon after its introduction, the species quickly spread to about 20 states in the United States and over broad areas of Brazil (Hawley 1988). Recently Rao and Rai (1987) investigated DNA content in a few native populations of *Aedes albopictus*. The haploid DNA content ranged from 0.87 picogram (pg) in the Calcutta strain (India) to 1.32 pg in the Mauritius strain. The recent introduction of *Ae. albopictus* prompted us to undertake a detailed investigation to determine nuclear DNA content in its native as well as in newly colonized populations to address two questions. (1) What is the level of variation in nuclear DNA content among different populations that were collected throughout its distribution range? (2) Has the range expansion of this species coincided with an increase in DNA content? The findings are presented in this paper.

Materials and methods

The origin and history of populations employed in the present study are given in Table 1. The eggs were hatched in boiled tap water with a small quantity of beef liver powder in a paper cup. The following day, approximately 150 larvae were transferred into an enamel pan with 2 l of tap water. The larvae were fed on beef liver powder. The larvae were raised in an insectary maintained at 27 °±1 °C and 80%±10% relative humidity (Rao and Rai 1987).

Chromosome squash preparations were made from 12- to 24-h-old male pupae on the slides, with previously drawn fine smears of chicken blood on one end (Rao and Rai 1987). The Feulgen staining was carried out according to Rao and Rai (1987). Thirty to fifty primary spermatocytes at Metaphase I were scored from a total of two to six pupae for each strain.

The two-wavelength method of Patau (1952) was employed to calculate relative Feulgen units using a Zeiss Microscope Photometer 01 (Mode T, HV 3, Damping 1, Spot 6, and Gain 20). The two wavelengths (505 nm and 550 nm) were determined by a spectral curve (Berlyn and Miksche 1976). The chicken RBCs stained on the same slide served as an internal standard for day-to-day variation (Rao and Rai 1987). Periodically the instrument was tested for reliability, using the methods of Berlyn and Miksche (1976).

A computer program was used to calculate absolute haploid nuclear DNA content (1C-value) using 2.50 pg per nucleus as the value for the diploid chicken genome. Statistical analyses were carried out using an SPSS program available at the University of Notre Dame computer center.

Results

The haploid DNA content and Duncan's groupings are presented in Table 2. The haploid DNA content ranged from 0.62 pg in Koh Samui (Thailand) to 1.66 pg in Houston-61 (USA). Statistical comparisons of populations revealed significant differences in DNA contents (ANOVA, all $P<0.05$).

Table 1. Origin of *Ae. albopictus* strains

Geographic strain	Collector(s)	Country, year of collection
Kent Ridge	T. J. Lam	Singapore, 1988
Amoy	L. S. Jieno	Singapore, 1988
Sri Lanka	F. Amerasinghe	Sri Lanka, 1986
Santa Tereza	F. Antunanao	Brazil, 1987
Cariacica	F. Antunanao	Brazil, 1986
Seburi	M. Mogi	Japan, 1988
Kabeshima	M. Mogi	Japan, 1988
Saga	M. Mogi	Japan, 1986
Ebina	T. Tadano	Japan, 1986
Zama	T. Tadano	Japan, 1986
Nagasaki	M. Mogi	Japan, 1988
Tananareve	R. Subra	Madagascar, 1974
Sabah	L. Munstermann	Malaysia, 1986
Malaysia	B. Knudsen	Malaysia, 1973
Perak Road	H. Yap	Malaysia, 1985
Gertak Sanguul	H. Yap	Malaysia, 1985
Koh Samui	D. Gould	Thailand, 1970
Korea	J. C. Lien	Korea, 1979
Saigon	D. Do-Van-Quay	Vietnam, 1966
Taipei	J. C. Lien	Taiwan, 1977
Shalimar Bagh	V. Sharma	India, 1988
Hardwar	V. P. Sharma	India, 1989
Ndo Ndo Creek	D. D. Pashley	Solomon Island, 1982
Manoa	D. Shroyer	Hawaii Island, 1984
Makiki	D. Shroyer	Hawaii Island, 1981
Savannah/GA	J. D. Miller	USA, 1988
Chambers County/TX	D. Sprenger	USA, 1986
Brazoria County/TX	D. Sprenger	USA, 1986
Milford/DE	C. Stachecki	USA, 1987
Houston-61/TX	D. Sprenger	USA, 1986
Houston-203/TX	D. Dickerson	USA, 1988
Chicago/IL	D. C. Arroyo	USA, 1987
Jacksonville/FL	W. Hawley	USA, 1987
New Orleans/LA	M. Andes and E. Bordes	USA, 1986
Indianapolis/IN	M. Sinko	USA, 1986
Evansville/IN	M. Dunn	USA, 1986
Memphis/TN	W. C. Black and J. A. Ferrari	USA, 1986

The haploid DNA contents estimated from two populations each from Singapore (Kent Ridge and Amoy), Brazil (Santa Tereza and Cariacica), India (Shalimar Bagh and Hardwar), and Hawaii (Manoa and Makiki) were significantly different from each other (ANOVA, $P<0.05$). Among six populations examined from Japan, the haploid DNA content ranged from 0.76 pg in Nagasaki to 1.16 pg in Zama. DNA contents of Kabeshima, Saga, and Ebina were not significantly different from each other, but were significantly different from Seburi and Zama (ANOVA, $P<0.05$). The latter two populations were not significantly different from one another. Among four populations from Malaysia, the lowest DNA content was 0.64 pg in Gertak Sanguul, and the highest DNA

Table 2. Haploid nuclear DNA contents in geographic strains of *Ae. albopictus*

Strain	N[a]	DNA (pg) ±SE	Duncan's grouping[b]
Kent Ridge	42	0.75±0.02	A
Amoy	42	1.29±0.06	D
Sri Lanka	36	0.92±0.05	B
Santa Tereza	44	1.18±0.02	D
Cariacica	44	0.98±0.04	B
Seburi	44	1.11±0.04	C
Kabeshima	57	0.82±0.03	B
Saga	51	0.80±0.02	B
Ebina	43	0.85±0.03	B
Zama	43	1.16±0.05	D
Nagasaki	48	0.76±0.03	A
Tananareve	45	0.78±0.03	A
Sabah	55	0.85±0.02	B
Malaysia	46	0.81±0.03	B
Perak Road	38	0.83±0.03	B
Gertak Sanguul	48	0.64±0.02	A
Koh Samui	54	0.62±0.02	A
Korea	59	0.69±0.03	A
Saigon	45	1.36±0.04	E
Taipei	57	1.48±0.05	E
Shalimar Bagh	47	1.42±0.05	E
Hardwar	41	0.96±0.02	B
Ndo Ndo Creek	38	1.12±0.06	C
Manoa	43	1.47±0.06	E
Makiki	51	0.75±0.03	A
Savannah/GA	34	1.65±0.07	F
Chambers County/TX	41	1.03±0.03	C
Brazoria County/TX	53	1.50±0.05	E
Milford/DE	48	1.46±0.05	E
Houston-61/TX	61	1.66±0.08	F
Houston-203/TX	24	1.33±0.08	D
Chicago/IL	12	1.11±0.09	C
Jacksonville/FL	26	1.13±0.10	C
New Orleans/LA	10	1.48±0.26	E
Indianapolis/IN	16	1.34±0.09	D
Evansville/IN	11	1.59±0.11	E
Memphis/TN	22	1.23±0.13	D

[a] Number of cells scored
[b] At $P<0.01$

content was 0.85 pg in Sabah. Only Gertak Sanguul differed significantly from the others (ANOVA, $P<0.05$).

Among 12 populations from the USA, the haploid DNA content ranged from 1.03 pg in Chambers County to 1.66 pg in Houston-61. DNA contents of Chambers County, Chicago, Jacksonville, Indianapolis, and Memphis were not significantly different from one another (ANOVA, $P<0.05$). Similarly, DNA contents of Savannah, Brazoria, Milford, Houston-61, Houston-203, New Orleans, and Evansville were not significantly different from one another. DNA contents of Chambers Country was significantly different from Savannah, Brazoria, Milford, Houston-61, Houston-203, New Orleans, and Evansville. DNA content of Chicago differed significantly from Savannah and Houston-61; that of Jacksonville differed significantly from Savannah, Brazoria, Milford, Houston-61, and Evansville. Further, DNA content of Memphis was significantly different from Savannah and Houston-61.

Duncan's multiple range test established six significantly different groupings ($P<0.01$). No definite pattern of geographic clustering emerged among populations. For example, 12 populations from the USA were distributed into four groupings that also contained populations from other geographic areas (Table 2).

Discussion

The early studies on the intraspecific variation in nuclear DNA content in animals report values determined from a few individuals of a population or a few populations (Gold and Price 1985; Sherwood and Patton 1982; Gold and Amemiya 1987; Johnson et al. 1987; Burton et al. 1989; Rao and Rai 1987). This is the first study that reports intraspecific variation in DNA content among geographic populations of *Ae. albopictus* collected across its distribution range. Populations from the same geographic area differed significantly in their DNA contents. Alternatively, populations from different areas have similar DNA content. There is thus no apparent correlation between geographic area and DNA content. Instead, each population has a specific amount of DNA that is probably imposed by the microenvironment. In general, populations from the USA and Brazil have higher DNA contents, but the range expansion of this species is not simply correlated with an increase in DNA content; some populations from its native distribution range have as much DNA content as those from the USA and Brazil.

Ae. albopictus is thought to have originated somewhere in the Indo-Malayan region (Smith 1956). Rao and Rai (1987) observed that the populations from this region have relatively lower DNA contents than those from the various islands. They suggested that the expansion of the species to various islands is associated with an increase in DNA content. However, the present data do not support this conclusion.

Cullis (1983) suggested that the nuclear DNA is organized into constant and fluid domains. The amount of the latter changes rapidly in response to a changing environment, as well as to both developmental and physical stimuli (Walbot and Cullis 1985; Cionini 1990). As a result, the amount of nuclear DNA may increase or decrease by the mechanisms of DNA amplification, elimination of certain DNA sequences (Bassi et al. 1984; Natali et al. 1986; Altamura et al. 1987; Cionini 1990), unequal crossing-over, or intragenomic drift (Cavalier-Smith 1985 b). The fluid domain of nuclear DNA is mainly composed of repetitive DNA sequences (Cavallini et al. 1986). The variation in the amount of repetitive DNA sequences

has been shown to be associated with changes in DNA content (Flavell et al. 1974; Hutchinson et al. 1980; Cavallini et al. 1986; Black and Rai 1988). Using the DNA-reassociation kinetics, Black and Rai (1988) found that the intraspecific variation in DNA content in two strains of *Ae. albopictus* is due mainly to highly repetitive DNA sequences; the amount of all classes of repetitive DNA sequences increased linearly with genome size. Further, the frequency of different classes of highly repetitive DNA sequences varied extensively among different populations of *Ae. albopictus* that were significantly different in DNA content (MacLain et al. 1987). We speculate that the variation in DNA content among populations of *Ae. albopictus* observed in the present study is due mainly to repetitive DNA sequences that are under rapid change in response to the microenvironment.

Finally, there has been much debate regarding the organismic functions of DNA content variation. DNA content has been found to be correlated with a variety of cellular and organismic properties such as total volume and length of the metaphase chromosomes, nucleus size, cell-cycle length, development rate, seed weight, flowering time, body size, climal or general habitat differences, and certain life history traits (Miksche 1971; Ebeling et al. 1971; Bennett 1976; Mazin 1980; Shuter et al. 1983; Cavalier-Smith 1985 a). Since many of these properties have adaptive significance to the organisms, it is believed that DNA content itself genetically controls most or all of these quantitative characters, and is subject to natural selection so as to optimize them (Cavalier-Smith 1985 a). An alternative explanation is that DNA content is a selectively neutral trait, and the variation in DNA content results by the spread of noncoding DNA sequences in the genome (Doolittle and Sapienza 1980; Orgel and Crick 1980). Ferrari and Rai (1989) found significant correlations between DNA content and two phenotypic traits, developmental time and body length, in different strains of *Ae. albopictus*. This indicates that the intraspecific variation in DNA content in *Ae. albopictus* is not consistent with the 'selfish DNA hypothesis' (Doolittle and Sapienza 1980; Orgel and Crick 1980) in that much of the variation in eukaryotic DNA content reflects gain or loss of phenotypically inconsequential DNA. Strains of *Ae. albopictus* with higher DNA contents have slow development and vice versa (Ferrari and Rai 1989). As stated above, strains from the newly colonized areas both in the USA and Brazil have higher DNA contents. The most pertinent question as to how the slow development assists in colonization of the species remains to be investigated.

In conclusion, the present study has shown a nearly three-fold variation in DNA content among geographic populations of *Ae. albopictus*. This corresponds to three-fold variation observed in DNA content among 23 species of *Aedes* (Rao and Rai 1987). As pointed out by Burton et al. (1989) this makes the determination of a single value for DNA content of a species difficult. It thus necessitates the use of large sample size and possibly multiple populations to measure the DNA content of a species (Johnson et al. 1987; Burton et al. 1989). Until the significance of intraspecific DNA content variation is properly elucidated, understanding the meaning of C-value paradox is difficult.

Acknowledgements. We thank Dr. S. Kambhampati for various suggestions and help in statistical analyses, and Dr. W. C. Black IV for writing the PCCALC computer program. This work was financially supported by an NIH research grant 5RO1 AI 21443 to KSR.

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