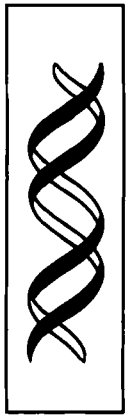

Volume 41

Advances in Genetics

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Academic Press
San Diego London Boston New York Sydney Tokyo Toronto

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Academic Press
A Harcourt Science and Technology Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

Academic Press
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

International Standard Book Number: 0-12-017641-6

PRINTED IN THE UNITED STATES OF AMERICA
99 00 01 02 03 04 EB 9 8 7 6 5 4 3 2 1

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Mosquito Genomes: Structure, Organization, and Evolution

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I. OVERVIEW

The family Culicidae is composed of more than 3,400 mosquito species, many of which are major vectors of arboviruses, malaria, and filariasis. In view of their

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Advances in Genetics, Vol. 41

0065-2660/99 $30.00

importance as vectors, many mosquito genera and species have been the subject of extensive cytological and genetic investigations over the last 40 years (Kitzmiller 1953, 1976; White, 1980; Rai *et al.*, 1982; Rai, 1991). As a result, there is a voluminous literature on mosquito genomes scattered in various entomological and genetics journals. The purpose of this review is to highlight the salient features of mosquito genomes and their evolution. It is indeed surprising that, except for a couple of minireviews (Besansky and Collins, 1992; Kumar and Rai, 1993), the various facets of this work have not been reviewed earlier.

We begin with a general review of mosquito systematics, highlighting and summarizing recent studies that employed modern cladistic analysis of morphological and molecular characters to estimate phylogenetic relationships among sister families to Culicidae and among Culicidae subfamilies, tribes, genera, subgenera, and species. We next review the extensive literature on karyotypes, emphasizing that the number of chromosomes has remained at a constant $2n = 6$ despite a relatively ancient origin for Culicidae, the evolution of both homomorphic and heteromorphic sex chromosomes, and evidence of extensive translocations and inversions. The literature on the evolution of genome size and organization in Culicidae is summarized and considered in light of current phylogenetic relationships. Genome evolution is also reviewed in the context of the now-extensive studies on heterochromatin distribution and in terms of the linkage maps that are beginning to arise through various recent intensive genome mapping projects in Culicidae.

II. MOSQUITO TAXONOMY, EVOLUTION, AND THE FOSSIL RECORD

The family Culicidae, which includes all mosquitoes, is divided into three subfamilies, Anophelinae, Toxorhynchitinae, and Culicinae (Knight and Stone, 1977; Knight, 1978; Ward, 1984, 1992; Service, 1993). Anophelinae includes three genera, the neotropical *Chagasia* (4 species), the Australasian *Bironella* (9 species in 3 subgenera), and the nearly cosmopolitan *Anopheles* with some 422 species grouped in 6 subgenera. Toxorhynchitinae includes a single genus, *Toxorhynchites* with 76 species. Culicinae is by far the largest subfamily: it is subdivided into 10 tribes, 33 genera, and 117 subgenera and includes about 2,925 described species. Although mosquito systematics is in a state of flux (Munstermann, 1995), the total numbers of genera, subgenera, and species in Culicidae currently stand at 37, 129, and 3,436, respectively (Service, 1993). The genus *Aedes*, which includes some 962 species grouped in 43 subgenera, is one of the best studied cytogenetically (Rai *et al.*, 1982).

Based on the fossil record, scanty through it is, and zoogeographic evidence involving past intercontinental connections and faunistic composition, it has been suggested that mosquitoes had evolved by the Jurassic, approx-

imately 210 million years ago (MYA) (Edwards, 1932). This is about the time continental drift began (Wilson, 1963). The continental breakup led to fragmentation and geographical isolation of populations. This may have been accompanied by great ecological flux that promoted rapid speciation (McClelland, 1967). Ross (1951) proposed that a burst of Culicinae lineages arose approximately 120 MYA. By the end of the Cretaceous, some 65 MYA, the generic composition of family Culicidae was well established (Belkin, 1968; Rohdendorf, 1974).

New Zealand has been in its present position of isolation for approximately the last 50 million years (Rick, 1970). With the exception of three species, *Aedes notoscriptus*, *Aedes australicus*, and *Culex quinquefasciatus*, the present-day mosquito fauna of New Zealand is relict and endemic. This provides circumstantial evidence that the genus Aedes existed prior to the island's separation from Australia and that it was probably widely dispersed during the Cretaceous, which began 145 MYA (Belkin, 1968). Fossils of family Culicidae (*Culex*, *Aedes*) and its sister family Chaoboridae are well known from the Eocene (Tertiary) and Oligocene, which began 60 and 55 MYA, respectively (Rohdendorf, 1974).

III. CLADISTIC ANALYSIS OF CULICIDAE

The phylogenetic relationship of Culicidae relative to other nematocerous dipteran families has been evaluated using modern cladistic analysis. Munstermann and Conn (1997) have reviewed the impact of molecular biology and cladistic analysis on systematics of selected taxa of Culicidae with particular emphasis on the *Aedes* and *Anopheles* species. Phylogenies have been estimated with suites of morphological characters (Oosterbroek and Courtney, 1995) and nucleotide sequences from the 18S and 5.8S nuclear ribosomal DNA (rDNA) (Miller *et al.*, 1997) and 28S rDNA (Pawlowski *et al.*, 1996). The morphological and 18S datasets are congruent in identification of Chaoboridae (phantom midges) as a sister group to Culicidae and in placement of Corethrellidae as a basal clade to Chaoboridae–Culicidae. The 28S dataset supported monophyly of these three families but consistently indicated Chaoboridae–Corethrellidae as sister taxa. Phylogenies of high-order relationships among these three families and Chironomidae, Ceratopogonidae, Dixidae, Psychodidae, and Simulidae are incongruent in all three studies. Each study cites several independent lines of support for the higher-order relationships derived from their respective phylogenies but all studies also indicate that these relationships were supported by few characters or lack strong bootstrap support. The rDNA papers use different species in each family, obviating a combined analysis as a means to resolve this conflict. The rDNA studies also suffer from sampling of single species in most families,

preventing identification of synapomorphies for each family. These relationships should be explored further with more complete taxon sampling and an examination of single-copy nuclear genes.

The relationship of Culicidae subfamilies has been examined with nucleotide datasets using rDNA genes (Pawlowski *et al.*, 1996; Miller *et al.*, 1997) and the single-copy nuclear gene *white* (Besansky and Fahey, 1997): All three studies were congruent in placement of Anophelinae as the basal clade in Culicidae. Furthermore, the 18S and *white* genes were consistent in placing Toxorhynchitinae as basal to the Culicinae.

Relationships among tribes, genera, and species in Culicinae have also been evaluated using modern cladistic analysis. Judd (1996) examined 59 morphological characters in 37 taxa within the tribe Sabethini. Cladistic analysis, using *Eretmapodites quinquevittatus* and *Haemagogus spegazzinii* as outgroups, supported Sabethini as a monophyletic group but strongly suggested paraphyletic relationships among species in at least three genera (*Runchomyia*, *Tripteroides*, and *Wyeomyia*). Wesson *et al.* (1992) sequenced the 5.8S–28S half of the internal transcribed spacer of the rDNA cistron (ITS2) to examine phylogenetic relationships among seven species in three genera (*Aedes*, *Haemagogus*, and *Psorophora*) of Aedini. Their analyses suggested paraphyletic relationships among species in the *Aedes* subgenus *Stegomyia* and suggested that *Haemagogus* and *Psorophora* arose within *Aedes*. The resolved phylogeny also provided evidence for biogeographical relationships among Aedini species: one clade contained Old World species (*Ae. aegypti*, *Ae. simpsoni*, *Ae. vexans*, and *Ae. albopictus*); a second clade contained the New World taxa *Ae. triseriatus*, *Haemagogus mesodentatus*, and *Psorophora verox*. Besansky and Fahey (1997) performed a thorough taxon sampling of variation in the *white* gene among taxa in tribes Culicini, Sabethini, and Aedini in the Culicinae. Their analysis supported placement of Sabethini as basal to Culicini and Aedini. Like the analysis of Wesson *et al.* (1992), this analysis of the *white* gene placed old World Aedini (*Ae. aegypti* and *Ae. albopictus*) in a separate clade from the New World species (*Ae. triseriatus*, *Haemagogus equinus*) with high bootstrap support. The rDNA genes (Pawlowski *et al.*, 1996; Miller *et al.*, 1997) and *white* gene all support a monophyletic relationship between Culicini and Aedini.

Miller *et al.* (1996) examined sequence divergence in the entire internal transcribed spacer (ITS) among 14 species in four subgenera of the genus *Culex*. Species in the subgenera *Culex*, *Lutzia*, and *NeoCulex* were monophyletic. There was low bootstrap support for monophyly of species in the subgenus *Culex* but only single species were examined in the subgenera *Lutzia* and *NeoCulex*. Some relationships among species and species complexes were also examined.

Kumar *et al.* (1998) constructed restriction maps of the rDNA cistron of 12 species of mosquitoes in six genera of the subfamily Culicinae using eight

6-bp recognition restriction enzymes. *Anopheles albimanus* was used as an outgroup. Clades within the RFLP (restriction fragment length morphism) phylogeny were not well supported and were incongruent with the morphology character based and molecular phylogenies previously discussed. The lack of resolution in the RFLP dataset was probably due to homoplasy arising from frequent independent loss or possibly, though less likely, from gain of restriction sites among unrelated taxa. Studies by Kumar *et al.* (1998) showed that only relationships among closely related taxa were well supported. As in Besansky and Fahey (1997), *Ae. triseriatus* and *Ha. equinus* were monophyletic. The sister species, *Ae. epactius* and *Ae. atropalpus*, were also monophyletic. Species in the *Aedes albopictus* and the *Aedes scutellaris* subgroups of the *Aedes scutellaris* group were monophyletic in the (RFLP) phylogeny. Based on a correlation of the allozyme differentiation among certain species and their geological histories and calibration of a well-established geologic event in the South Pacific, Pashley *et al.* (1985) concluded that the *Ae. albopictus* and the *Ae. scutellaris* subgroups diverged relatively recently.

In summary, modern cladistic analyses of morphological and molecular characters consistently support Chaoboridae–Corethrellidae as sister taxa to Culicidae. All analyses support Anophelinae as the basal clade in Culicidae and are consistent in placing Toxorhynchitinae as basal to the Culicinae. Within Culicinae, the tribe Sabethini is basal to Culicini and Aedini. All datasets support a monophyletic relationship between Culicini and Aedini. Many subgeneric relationships within Sabethini, Culicini, and Aedini may be paraphyletic and warrant taxonomic revision.

These studies do not address the key question of whether Toxorhynchitinae arose within Anophelinae or as a separate lineage from a common ancestor with Anophelinae. This becomes a pivotal issue in discussing the origins of some major genetic differences between anopheline and culicine mosquitoes later in this chapter. This issue may become resolved in the future through examination of additional gene sequences and intensive sampling of primitive and derived members of both Toxorhynchitinae and Anophelinae. However, it is also quite possible that ancestral taxa are extinct in either or both subfamilies and that the issue will never be adequately resolved.

IV. CHROMOSOME NUMBER IS CONSERVED IN CULICIDAE

Chromosomal karyotypes have been established for "no less than" 19 genera, 35 subgenera, and 200 species in family Culicidae (White, 1980). Over the last several years, additional species have been cytologically examined (Rao and Rai, 1987a, 1990). One of the most remarkable findings of this karyotypic survey is that, despite the ancient origin of the group and despite extensive repattern-

ing of the genome involving translocations and inversions (Matthews and Munstermann, 1994; Mori *et al.*, 1998), the basic chromosome number ($2n$ = 6) has remained unchanged. The only exception, *Chagasia bathana* ($2n$ = 8) of the subfamily Anophelinae, possesses three autosome pairs and a heteromorphic pair of sex chromosomes (Kreutzer, 1978).

All other anophelines possess two pairs of generally metacentric chromosomes of unequal size and one pair of heteromorphic sex chromosomes that often show extensive polymorphism in overall length and of the quantity and quality of heterochromatin differentiation among various species (White, 1980). The position of the centromeres in the heteromorphic X and Y chromosomes in Anophelinae varies from subtelocentric or acrocentric to submetacentric and metacentric (Baimai *et al.*, 1993a, b, 1995).

In contrast, species of the subfamilies Toxorhynchitinae and Culicinae all possess three pairs of homomorphic metacentric and/or slightly submetacentric chromosomes: a pair of small chromosomes, a pair of large chromosomes, and a pair of intermediate-sized chromosomes (Rai, 1963; McDonald and Rai, 1970; Rai *et al.*, 1982, Rao and Rai, 1987a). In culicine mosquitoes, sex is determined by a gene at a single locus. Females are homozygous recessive at this locus, and males are heterozygous for a dominant allele (Gilchrist and Haldane, 1947; McClelland, 1962). In species in which linkage group–chromosome correlations have been made, the shortest chromosome contains the sex locus and is therefore sex determining (McDonald and Rai, 1970; Baker *et al.*, 1971; Dennhöfer, 1972). Differences clearly exist in overall lengths and arm ratios of individual chromosomes, both within and between species, but can be easily overlooked if careful measurements of each arm of a chromosome are not made (Rai, 1980; Rai *et al.*, 1982). Total chromosomal length varies almost fivefold, from 8.2 μm in *Anopheles quadrimaculatus* to 39.3 μm in *Aedes alcasidi*. Within the genus *Aedes*, there is a threefold variation in chromosome length (Table 1.1)

Conservation of chromosome number in Culicidae does not indicate synteny. Matthews and Munstermann (1994) and Severson *et al.* (1995) clearly document that groups of allozyme loci have remained linked and colinear in a variety of culicine taxa but that these linkage groups have translocated and are inverted extensively across the three culicine chromosomes. The extensive variation in chromosome number in most diptera taxa studied does not predict the extreme conservation found in Culicidae. For example, the chromosome number ranges from n = 3 to 7 in the genus *Drosophila* (see White, 1973) and from n = 3 to 8 in the genus *Glossina* (Mauldin 1970). In family Muscidae, most species possess six pairs of chromosomes; however, six species have only five pairs each (Boyes, 1967). Nevertheless, certain other dipteran families such as Simulidae (Rothfels, 1979) and Sarcophagidae also show extensive conservation of chromosome number, although some exceptions do occur (White,

1973). No logical explanation exists for the extraordinary conservation of the haploid chromosome number in Culicidae. The chromosomal karyotype data from Culicidae in general support White's (1973) suggestion that there may be some kind of barrier that maintains chromosome number in the Diptera. Nevertheless, we know nothing about the actual nature of such a barrier.

V. SEX CHROMOSOME EVOLUTION IN CULICIDAE

Current dogma suggests that heteromorphic sex chromosomes evolved from virtually identical homologues. Both theoretical considerations (Charlesworth, 1978) and considerable experimental evidence suggest that it is the gradual accumulation of repetitive sequences on the Y chromosome followed by loss of recombination between the heteromorphic pair that leads to the differentiation of X and Y chromosomes. Theory predicts eventual loss of function and eventual extinction of the Y chromosome (Steinemann *et al.*, 1993; Morell, 1994; Rice, 1994, 1996). This directionality is generally referred to as the "rise and fall of the Y chromosome" (Morell, 1994). Evolution of a heteromorphic Y chromosome may have occurred only once or possibly may have been reversed in the evolution of sex chromosomes in Culicidae. The primitive Nematocera families Tipulidae and Dixidae possess homomorphic sex chromosomes. However, the sister families Chaoboridae–Corethrellidae contain genera with homomorphic (*Eucorethra, Corethrella, Chaoborus*) and heteromorphic (*Mochlonyx*) sex chromosomes (Rao and Rai, 1987a). If homomorphy was ancestral in Culicidae, then it was retained in the lineages leading to Toxorhynchitinae and Culicinae, while heteromorphy probably evolved early in the evolution of Anophelinae and was retained in all taxa. This scenario is supported by the current dogma concerning the evolution of sex chromosomes (Rice, 1996). Alternatively, if, as proposed by Rao and Rai (1987a), Culicidae arose from a Mochlonyx-like ancestor, then Anophelinae retained heteromorphic sex chromosomes, while homomorphic sex chromosomes evolved through euchromatinization or loss of the Y in Toxorhynchitinae and Culicinae.

VI. GENOME SIZE AND GENERAL GENOME ORGANIZATION

A. Interspecific variation and genome organization

Considerable effort has been expended in recent years to determine haploid nuclear DNA amounts in the superfamily Culicoidea (Jost and Mameli, 1972; Rao and Rai, 1987b, 1990; Black and Rai, 1988; Kumar and Rai, 1990). This has been done through quantitative cytophotometry of Feulgen-stained primary

Table 1.1. Mean Chromosomal Lengths in 30 Representative Species Belonging to 8 Genera of Mosquitoes and Related Taxa in Superfamily Culicoidae

Family	Genus/species	Mean chromosome length (μm)			TCL[a]	References
		I	II	III	(I + II + III)	
Chaoboridae	*Mochlonyx velutinus*	2.2(X); 1.3(Y)	4.6	5.4	12.2	Rao and Rai, 1987a
	Chaoborus americanus	2.3	3.1	3.3	8.7	Rao and Rai, 1987a
Culicidae	*Anopheles quadrimaculatus*	1.4(x); 0.9(y)	3.0	3.8	8.2	Rai, 1963
	Culex pipiens	2.4	4.2	5.0	11.6	Rai, 1963
	Culex territans	2.6	4.1	5.4	12.1	Rai, 1963
	Culex restuans	3.0	5.4	6.2	14.6	Rai, 1963
	Toxorhynchites splendens	3.4	4.7	5.0	13.1	Rao and Rai, 1987a
	Wyeomyia smithii	4.6	5.8	6.2	13.0	Rai, 1963
	Haemoagogus Equinus	6.6	9.6	10.7	26.9	Rao and Rai, 1987a
	Aedes togoi	3.0	4.6	5.4	13.0	Rai, 1963
	Ae. metallicus	5.2	6.3	7.8	19.3	Rao and Rai, 1987a
	Ae. hebrideus	5.1	6.3	7.9	19.3	Rao and Rai, 1987a
	Ae. aegypti	5.4	6.9	7.6	19.9	Rai, 1963
	Ae. heischii	6.3	7.4	8.0	21.6	Rao and Rai, 1987a
	Ae. kesseli	6.3	7.8	9.4	23.5	Dev and Rai, 1984
	Ae. atropalpus	6.2	8.4	9.2	23.8	Rai, 1963
	Ae. pseudoscutellaris	6.9	9.6	9.9	24.6	Dev and Rai, 1984
	Ae. unilineatus	6.4	9.1	10.0	25.5	Rao and Rai, 1987a

Ae. cooki	6.9	8.8	9.4	25.6	Dev and Rai, 1984
Ae. seatoi	7.3	9.3	10.5	27.1	Rao and Rai, 1987a
Ae. polynesiensis	7.4	9.4	11.1	27.9	Dev and Rai, 1984
Ae. katherinensis	7.5	9.6	12.6	29.7	Rao and Rai, 1987a
Ae. stimulans	7.6	10.7	11.5	29.8	Rai, 1963
Ae. pseudoalbopictus	7.9	10.3	11.8	30.0	Rao and Rai, 1987a
Ae. malayensis	7.9	10.3	12.1	30.4	Dev and Rai, 1984
Ae. flavopictus	8.3	11.5	13.5	33.3	Rao and Rai, 1987a
Ae. triseriatus	9.9	10.7	15.2	35.8	Rao and Rai, 1987a
Ae. zoosophus	9.1	14.4	14.8	38.3	Rao and Rai, 1987a
Ae. alcasidi	9.9	13.6	15.8	39.3	Dev and Rai, 1984
Ae. albopictus					
Oahu, Hawaii	6.0	6.3	8.9	21.2	Rao and Rai, 1987b
Calcutta, India	6.5	8.1	9.3	23.9	Rao and Rai, 1987b
Kolar, India	6.6	7.9	9.5	24.0	Rao and Rai, 1987b
Mauritius	7.2	9.3	10.4	26.9	Rao and Rai, 1987b
Tananareve, Madagascar	8.3	11.0	11.8	31.1	Rao and Rai, 1987b
Pune, India	8.0	11.2	12.8	32.0	Rao and Rai, 1987b
Delhi, India	9.2	11.4	12.2	32.8	Rao and Rai, 1987b

[a]TCL: Total chromosomal length in micrometers.

spermatocytes and in a few cases through analyses of renaturation kinetics of nuclear DNA (Black and Rai, 1988; Warren and Crampton, 1991; Besansky and Powell, 1992). As a result, haploid genome sizes have been established for 44 species belonging to 13 genera of mosquitoes and related Culicoidea families (Table 1.2).

Genome size is generally small in Anophelinae (0.23–0.29 pg/haploid genome) (Jost and Mameli, 1972; Black and Rai 1988; Besansky and Powell, 1992). The single species *Toxorhynchites splendens*, examined in subfamily Toxorynchitinae possesses an intermediate-size genome of 0.62 pg as do *Sabethes cyaneus* and *Wyeomyia smithii* (Sabethini).The haploid genomes of *Culex* species examined ranged from 0.54 to 1.02 pg and those of *Culiseta* species (Culicini) from 0.92 to 1.25 pg. *Armigeres subalbatus* and *Haemagogus equinus* (Aedini) contained 1.24 and 1.12 pg, respectively. At the generic level, the cosmopolitan genus *Aedes* showed more than threefold variation in nuclear DNA amounts, with the Polynesian species *Ae. pseudoscutellaris* and *Ae. cooki* (belonging to the *Ae. scutellaris* subgroup in the subgenus *Stegomyia*) possessing the lowest genome size of 0.59 pg and *Ae. zoosophus*(subgenus *Protomacleaya*) possesing the highest genome size of 1.9 pg among the 23 species examined (Rao and Rai, 1987b, 1990).

Placed in the context of phylogenetic relationships discussed earlier, these figures suggest a general increase in genome size during the evolution of Culicidae. Black and Rai (1988) demonstrated that all classes of repetitive DNA sequences increased linearly in amount with total genome size. Furthermore, linear regression analysis of a fairly large dataset involving 28 species belonging to 11 genera of the superfamily Culicoidea showed a highly significant positive correlation ($r = 0.87$; $p = 0.0001$) between total chromosomal length and haploid genome size (Rao and Rai, 1987b). Nevertheless, eightfold variation in haploid genome size was accompanied by only an approximate fivefold variation in the total chromosomal length, indicating that DNA amounts have increased almost twice as much as the increase in chromosomal size.

Studies using reassociation kinetics have provided information on genome organization in Anophelinae and Culicinae (Black and Rai, 1988; Warren and Crampton, 1991; Besansky and Powell, 1992). Genome organization refers to the amounts, complexity, and dispersion of repetitive elements in a genome. Two basic forms of genome organization have been described in eukaryotes (Davidson *et al.*, 1975). The first type is termed "short period interspersion" and describes a pattern in which single-copy sequences, 1000–2000 bp in length, alternate regularly with short (200–600 bp) and moderately long (1000–4000 bp) repetitive sequences. This characterizes genome organization in the majority of animal species and was found in the culicine species *Culex pipiens*, *Ae. aegypti*, *Ae. albopictus*, and *Ae. triseriatus* (Black and Rai, 1988). The second type of genome organization is termed "long-period interspersion"

and describes a pattern of long (> 5600 bp) repeats alternating with very long (> 13,000 bp) uninterrupted stretches of unique sequences. Repeats in *An. quadrimaculatus* (Black and Rai, 1988) and *An. gambiae* (Besansky and Powell, 1992) follow a long-period interspersion pattern.

Genome organization is of the long-interspersion type in *Chironomus tentans* (Wells *et al.*, 1976) but has not been determined in sister families Chaoboridae–Corethrellidae. However, haploid DNA amounts of 0.47, 0.55, and 0.40 pg were observed in the three principal genera *Corethrella*, *Mochlonyx*, and *Chaoborus*, respectively (Table 1.2; Rao and Rai, 1990). In insects, long period interspersion is characteristic of most species with small genome sizes (0.1–0.5 pg/haploid genome), while short-period interspersion tends to be associated with larger genomes with larger amounts of repetitive DNA (Palmer and Black, 1997). It is difficult to predict genome organization in Chaoboridae–Corethrellidae based on genome size, because they fall into the upper limit for long-interspersed species. Thus there remain two competing hypotheses for ancestral genome evolution in Culicidae. It is possible that long-period interspersion was ancestral in Culicidae and was retained in the lineage leading to Anophelinae, while larger genomes developed through accumulation of short-period interspersed repetitive elements in Culicinae. The alternative hypothesis is that Culicidae arose from a short-period interspersed species and, while that organization was retained in the Culicinae, repetitive elements were shed and became organized into a long-period interspersion pattern in the Anophelinae. This is the scenario considered by Rao and Rai (1990), who proposed a phylogeny of the superfamily Culicoidea based on genome sizes (Figure 1.1). They suggested that the line that possibly gave rise to Anophelinae from a Mochlonyx-like ancestor underwent many deletions of highly repetitive DNA. However, this scenario lacks any empirical evidence from evolution of genome size in other systems.

Cullis (1983) suggested that nuclear DNA is organized into constant and fluid domains. The fluid domain, which is composed mainly of repetitive DNA sequences (Cavallini *et al.*, 1986), shifts in response to changing environments and developmental and physical stimuli (Walbot and Cullis, 1985). Genome size shifts dynamically as a result of DNA amplification, bursts of transpositions, unequal crossing-over that can simultaneously cause elimination and gain of certain DNA sequences (Bassi *et al.*, 1984; Natali *et al.*, 1986; Altamura *et al.*, 1987), and intragenomic drift (Cavalier-Smith, 1985a). However, these mechanisms generally cause genomes to accumulate repetitive elements, and very few mechanisms have been proposed for genomewide "shedding" of repetitive elements. Considering these arguments, it is most parsimonious to suggest that long-period interspersion was ancestral in Culicidae. However, it is critical to determine genome organization in Chaoboridae or Corethrellidae to test this hypothesis. Furthermore, analysis of genome orga-

Table 1.2. Haploid Genome Size (Picogram DNA) in 44 Species Belonging to 13 Genera of Mosquitoes and Related Taxa

Family	Subfamily	Tribe	Genus	Subgenus	Species	pg DNA/haploid genome ± SE	References
Dixidae			*Dixa*		*obscura*	0.156	Jost and Mameli, 1972
Chaoboridae	Corethrellinae		*Corethrella*		*brakeleyi*	0.47 ± 0.02	Rao and Rai, 1990
	Chaoborinae		*Mochlonyx*		*velutinus*	0.55 ± 0.02	Rao and Rai, 1990
	Chaoborinae		*Chaoborus*	Chaoborus	*americanus*	0.40 ± 0.02	Rao and Rai, 1990
Culcidae	Anophelinae		*Anopheles*	Anopheles	*labranchiae*	0.234	Jost and Mameli, 1972
			An.		*atroparvus*	0.242	Jost and Mameli, 1972
			An.		*quadrimaculatus*	0.245 ± 0.01	Rao and Rai, 1990
			An.		*freeborni*	0.294	Jost and Mameli, 1972
			An.	Cellia	*stephensi*	0.242	Jost and Mameli, 1972
			An.		*gambiae*	0.27	Besansky and Powell, 1992
	Toxorhynchitinae		*Toxorhynchites*	Toxohynchites	*splendens*	0.618 ± 0.019	Rao and Rai, 1990
	Culicinae	Sabethini	*Sabethes*	Sabethes	*cyaneus*	0.786 ± 0.02	Rao and Rai, 1990
			Wyeomyia	Wyeomyia	*smithii*	0.855 ± 0.011	Rao and Rai, 1990
		Culicini	*Culex*	Culex	*pipiens*	1.02 ± 0.19	Jost and Mameli, 1972
			Cx.		*pipiens*	0.540 ± 0.012	Rao and Rai, 1990
			Cx.		*quinquefasciatus*	0.54 ± 0.01	Rao and Rai, 1990
			Cx.		*restuans*	1.02 ± 0.04	Rao and Rai, 1990
		Culisetini	*Culiseta*	Culicella	*litorea*	0.92	Jost and Mameli, 1972
			Cu.		*morsitans*	1.21 ± 0.04	Rao and Rai, 1990
			Cu.	Climacura	*melanura*	1.25 ± 0.005	Rao and Rai, 1990
		Aedini	*Haemagogus*	Haemagogus	*equinus*	1.120 ± 0.023	Rao and Rai, 1990
			Armigeres	Armigeres	*subalbanus*	1.124 ± 0.027	Rao and Rai, 1990
			Aedes	Stegomyia	*pseudoscutellaris*	0.591 ± 0.012	Rao and Rai, 1987b

Ae.		*cooki*	0.594 ± 0.027	Rao and Rai, 1987b
Ae.		*polynesiensis*	0.725 ± 0.018	Rao and Rai, 1987b
Ae.		*aegypti*	0.812 ± 0.031	Rao and Rai, 1987b
Ae.		*aegypti*	0.83	Warren and Crampton, 1991
Ae.		*malayensis*	0.943 ± 0.025	Rao and Rai, 1987b
Ae.		*hebrideus*	0.965 ± 0.031	Rao and Rai, 1987b
Ae.		*seatoi*	0.971 ± 0.023	Rao and Rai, 1987b
Ae.		*alcasidi*	0.974 ± 0.016	Rao and Rai, 1987b
Ae.		*unilineatus*	1.064 ± 0.04	Rao and Rai, 1987b
Ae.		*metallicus*	1.093 ± 0.033	Rao and Rai, 1987b
Ae.		*heischii*	1.121 ± 0.039	Rao and Rai, 1987b
Ae.		*katherinensis*	1.277 ± 0.02	Rao and Rai, 1987b
Ae.		*pseudoalbopictus*	1.29 ± 0.028	Rao and Rai, 1987b
Ae.		*flavopictus*	1.33 ± 0.024	Rao and Rai, 1987b
Ae.	Aedes	*cinereus*	1.210 ± 0.03	Rao and Rai, 1987b
Ae.	Howardina	*bahamensis*	1.375 ± 0.03	Rao and Rai, 1987b
Ae.	Ochlerotabus	*canadensis*	0.904 ± 0.02	Rao and Rai, 1987b
Ae.		*communis*	1.013 ± 0.05	Rao and Rai, 1987b
Ae.		*caspius*	0.988	Jost and Mameli, 1972
Ae.		*stimulans*	1.439 ± 0.039	Rao and Rai, 1987b
Ae.		*excrucianus*	1.500 ± 0.03	Rao and Rai, 1987b
Ae.	Protomaclyeaya	*triseriatus*	1.520 ± 0.062	Rao and Rai, 1987b
Ae.		*zoosophus*	1.902 ± 0.062	Rao and Rai, 1987b

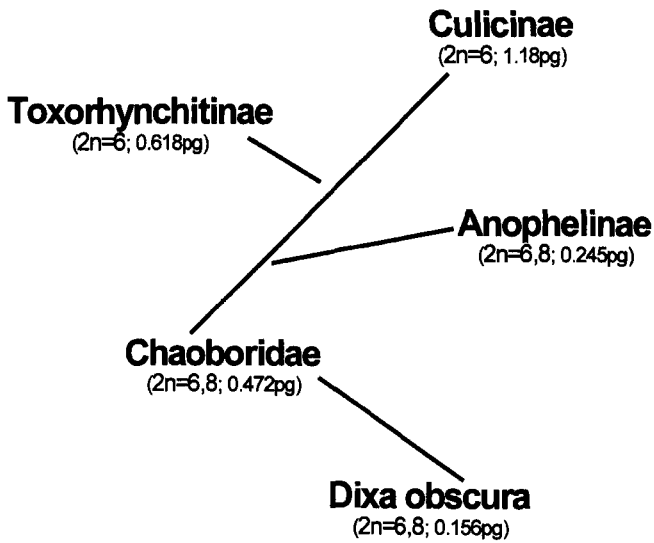

Figure 1.1. Genome sizes in some members of Culicoidea and the proposed phylogeny. (Distances between members are arbitrary.) After Rao and Rai (1990).

nization in Sabethini and Toxorhynchitinae is imperative to determine when short-period interspersion arose in culicine evolution.

B. Intraspecific genome size variation

Genome studies in Rai's laboratory have also focused on intraspecific variation in genome size and have indicated unequivocally that DNA amounts are not fixed within species (Ferrari and Rai, 1989; Rao and Rai, 1987b; Kumar and Rai, 1990). An analysis of 47 geographic populations of *Ae. albopictus* from 18 countries showed a 2.5-fold variation in DNA amounts, ranging from 0.62 pg in the Koh Samui population from Thailand to 1.66 pg in a population from Houston, Texas recently introduced to the continental United States (Table 1.3). Furthermore, extensive variation existed among and within populations from contiguous geographic locations. For example, the haploid DNA amounts of two populations each of *Ae. albopictus* from Singapore (Kent Ridge and Amoy) and Brazil (Santa Tereza and Cariacica) were significantly different from each other. Six Duncan's groupings of genome sizes were observed among the 37 populations of *Ae. albopictus* studied by Kumar and Rai (1990). Genome size was independent of geographic origin in the various populations examined. For example, 12 populations from the United States belonged to four groupings that also contained populations from other geographic areas (Kumar and Rai, 1990).

Using DNA-reassociation kinetics, Black and Rai (1988) showed that

Table 1.3. Haploid Genome Size (Picogram DNA) in 47 Geographic Populations of *Aedes albopictus* from 18 Countries

Genus	Species	pg DNA/haploid genome ± SE	References
Aedes	*albopictus*		
	Geographic populations		
	Koh Samui, Thailand	0.62 ± 0.02	Kumar and Rai, 1990
	Korea	0.69 ± 0.03	Kumar and Rai, 1990
	Tananareve, Madagascar	0.78 ± 0.03	Rao and Rai, 1987b
	Sri Lanka	0.92 ± 0.05	Kumar and Rai, 1990
	Pontianak, Indonesia	1.07 ± 0.044	Rao and Rai, 1987b
	Ndo Ndo Creek, Solomon Island	1.12 ± 0.06	Kumar and Rai, 1990
	Tananareve, Madagascar	1.15 ± 0.026	Kumar and Rai, 1990
	Hong Kong	1.26 ± 0.026	Rao and Rai, 1987b
	Mauritius	1.32 ± 0.035	Rao and Rai, 1987b
	Saigon, Vietnam	1.36 ± 0.04	Kumar and Rai, 1990
	Taipei, Taiwan	1.48 ± 0.05	Kumar and Rai, 1990
	Malaysia		
	Gertak Sanguul	0.64 ± 0.02	Kumar and Rai, 1990
	Malaysia	0.81 ± 0.03	Kumar and Rai, 1990
	Perak Road	0.83 ± 0.03	Kumar and Rai, 1990
	Sabah	0.85 ± 0.02	Kumar and Rai, 1990
	Singapore		
	Kent Ridge	0.75 ± 0.02	Kumar and Rai, 1990
	Amoy	1.29 ± 0.06	Kumar and Rai, 1990
	India		
	Calcutta	0.86 ± 0.03	Rao and Rai, 1987b
	Kolar	0.94 ± 0.025	Rao and Rai, 1987b
	Hardwar	0.96 ± 0.02	Kumar and Rai, 1990
	Delhi	1.02 ± 0.008	Rao and Rai, 1987b
	Pune	1.07 ± 0.62	Rao and Rai, 1987b
	Shalimar Bagh	1.42 ± 0.05	Kumar and Rai, 1990
	Hawaii		
	Makiki	0.75 ± 0.03	Kumar and Rai, 1990
	Oahu	1.24 ± 0.032	Rao and Rai, 1987b
	Manoa	1.47 ± 0.06	Kumar and Rai, 1990
	Japan		
	Nagasaki	0.76 ± 0.03	Kumar and Rai, 1990
	Saga	0.80 ± 0.02	Kumar and Rai, 1990
	Kabeshima	0.82 ± 0.03	Kumar and Rai, 1990
	Ebina	0.85 ± 0.03	Kumar and Rai, 1990
	Seburi	1.11 ± 0.04	Kumar and Rai, 1990
	Zama	1.16 ± 0.05	Kumar and Rai, 1990
	Tokyo	1.29 ± 0.032	Rao and Rai, 1987b
	Brazil		
	Cariacica	0.98 ± 0.04	Kumar and Rai, 1990
	Santa Tereza	1.18 ± 0.02	Kumar and Rai, 1990

continues