EVIDENCE FOR MICROGEOGRAPHIC GENETIC SUBDIVISION OF *ANOPHELES QUADRIMACULATUS* SPECIES C

S. K. NARANG, J. A. SEAWRIGHT AND P. E. KAISER

U. S. Department of Agriculture, Agricultural Research Service, Insects Affecting Man and Animals Research Laboratory, P.O. Box 14565, Gainesville, FL 32604

ABSTRACT. Species C of the *Anopheles quadrimaculatus* complex has a patchy distribution and has been found principally near the coast in river and springs systems of northwest Florida (*GULF, SR, SFR*—designated as C_1, and *CHOC*—designated as C_2) and the Ogeechee River (*OGE*—C_2) near Savannah, Georgia. We have documented significant differences in allelic frequencies at 5 loci (malic enzyme, aconitase, aldehyde oxidase, esterase-5 and esterase-6), and genetic substructuring (F_{st}) between C_1 and C_2. The *GULF* and *CHOC* populations occurred in apparently similar environments, but were separated by about 160 km. Both these populations are separated from OGE by over 160 km (with low to undetectable distribution of species C in the transect regions). The higher genetic similarity of the *CHOC* with *OGE* populations indicated either selective pressures or genetic drift or both as being responsible for the differences between C_1 and C_2 populations.

INTRODUCTION

Studies on allozyme and chromosomal variability in geographical or spatially subdivided populations of different insect species have revealed geographical structure in genetic variation (King 1987). The ecological and genetic variations have been attributed to selective pressures operating in different environments or subenvironments (ecological niches). The species with extensive distributions are expected to contain a large pool of spatially distributed genetic variability, which allows the whole species to persist longer by the "spreading of risk" (Den Boer 1968). Both selection and genetic drift play important roles in maintaining polymorphism (Chesser et al. 1980). In species inhabiting patches of habitats with similar environment, drift will be expected to play a major role in maintenance of genetic variation among populations. Although the effect of spatial subdivision on genetic substructuring of species is generally accepted among geneticists, inadequate empirical evidence of this effect exists for anopheline mosquitoes.

Anopheles quadrimaculatus is an important pest species widely distributed over the eastern part of the United States. Until recently, it was considered to be a single species (Darsie and Ward 1981); however, cytogenetic, hybridization and electrophoretic studies revealed a species complex and the identification of sibling species A and B (Lanzaro 1986[1], Lanzaro et al. 1988, Kaiser et al. 1988a), species C (Kaiser et al. 1988b, Narang and Seawright 1988; Narang et al. 1989a, 1989c) and species D (Narang et al. 1989b). We have analyzed the sibling species from the southeastern USA (from Florida to Texas north to Kentucky) and a few sites in northern states (Wisconsin, New York and New Jersey) for variability at 29 loci. Species C was found to have a patchy distribution and was collected in northern Florida in swamps and hardwood hammocks within 8 km of the Gulf Coast, along the Choctawhatchee River in the panhandle and along the Suwannee and Sante Fe rivers and associated springs, and in southern Georgia along the Ogeechee River near Savannah. This species seemed to be an ideal target for measuring the effect of micro- and macrogeographic spatial subdivision on population structure in nature. In this paper we present the results of electrophoretic studies that show the effect of spatial subdivision on the population structure in *An. quadrimaculatus* species C.

MATERIALS AND METHODS

Collection sites: Adult mosquitoes from 5 areas (4 in Florida and one in Georgia, Fig. 1) were analyzed by starch-gel electrophoresis. We have designated collections from these areas as follows: Florida Gulf Coast within 8 km of the coast (*GULF*), Suwannee River (*SR*), Santa Fe River (*SFR*), Choctawhatchee River (*CHOC*) and the Ogeechee River near Savannah, GA (*OGE*). Adults were aspirated primarily from tree holes from hardwood swamps in the *GULF*, *SR*, *SFR* and *CHOC* areas, and *OGE* adults were collected from tree holes and underneath bridges. In addition to the sites shown in Fig. 1, we sampled adult populations in the vicinity of hardwood hammocks and tidal creeks along the the Gulf Coast from the Choctawhatchee River in the northwest to Hernando County in the southwest (25 miles south of *CRI* in Fig. 1). Species C, which is usually sympatric with species A and less frequently with species B and D,

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[1] Lanzaro, G. C. 1986. Use of enzyme polymorphism and hybridization crosses to identify sibling species of the mosquito, *Anopheles quadrimaculatus* (Say). Ph.D. dissertation, University of Florida, Gainesville, FL.

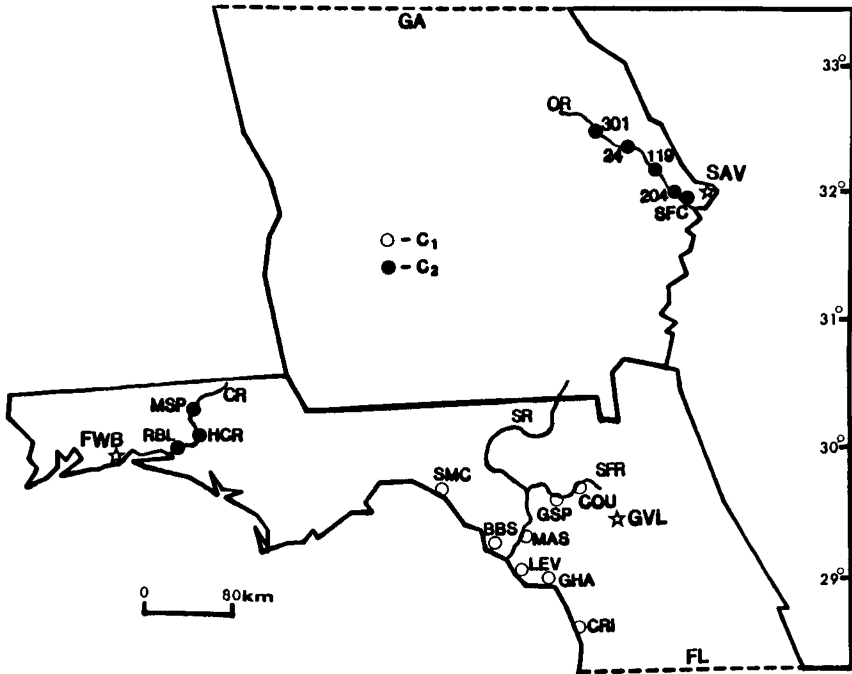

Fig. 1. Collection sites of *Anopheles quadrimaculatus* species C in Florida and Georgia. BBS—Bear Bay Swamp, Dixie Co.; CoU—Canoe outpost, Alachua Co.; CRI—Chassahowitzka River, Citrus Co.; GHA—Gulf Hammock, Levy Co.; GSP—Ginnie Spring, Gilchrist Co.; HCR—Holmes Creek, Washington Co.; LEV—Levy, Levy Co.; MAS—Manatee Springs, Levy Co.; MSP—Morrison Springs, Walton Co.; RBL—Rooks Bluff, Walton Co.; SFC—Shed's Fish Camp, Catham Co.; SMC—Smith McCullen Creek, Taylor Co.; 24—Hwy 24 Bridge, Screven Co.; 19—Hwy 119 Bridge, Effingham Co.; 204—Hwy 204 Bridge, Catham Co.; 301—Hwy 301 Bridge, Screven Co. Cities: FWB—Fort Walton Beach; GVL—Gainesville; SAV—Savannah. Rivers: CR—Choctawhatchee River; OR—Ogeechee River; SFR—Sante Fe River; SR—Suwannee River.

predominated most of these populations; frequencies of C varied from 7 to 90%. These samples were similar to *GULF* mosquitoes in that they had certain alleles of aconitase-1 (*Acon-1*) and malic acid (*Me-1*), which were absent in samples from *CHOC* and *OGE*.

Initially, collections within each of the 5 large areas were treated separately for the purpose of testing possible differentiation within each area. After statistical analysis, all populations collected from north Florida, i.e., *GULF*, *CHOC*, *SR* and *SFR*, were treated as microgeographic populations. The *OGE* collections have been considered as macrogeographic with respect to the Florida populations.

Identification of sibling species C: Species C was identified by the electrophoretic taxonomic key of Narang et al. (1989a, 1989b).

Electrophoresis: Starch-gel electrophoresis was conducted primarily according to Steiner and Joslyn (1979) with a few modifications (Narang et al. 1989a, 1989c). Electromorphs of 29 presumptive loci in 18 enzyme systems were studied. The enzyme systems, including the number of loci analyzed (see Narang et al. 1989a for buffer systems), were: aconitase (ACON, 1 locus), adenylate kinase (ADK, 3 loci), aldehyde oxidase (AO, 1 locus), esterases (EST, 5 loci), glutamate oxaloacetate transaminase (GOT, 2 loci), glucose oxidase (Go, 1 locus), alpha-glycerophosphate dehydrogenase (GPDH, 1 locus), phosphoglucose isomerase (PGI, 1 locus), hexokinase (HK, 2 loci), hydroxy acid dehydrogenase (HAD, 1 locus), isocitrate dehydrogenase (IDH, 2 loci), malate dehydrogenase (MDH, 1 locus), malic enzyme (ME, 1 locus), mannose phosphate isomerase (MPI, 2 loci), peptidase (PEP, 2 loci), 6-phosphogluconate dehydrogenase (6PGD, 1 locus), phosphoglucomutase (PGM, 1 locus) and xanthine dehydrogenase (XDH, 1 locus).

A laboratory stock, Q2, of species A, inbred over 100 generations (Lanzaro 1986[1]) and homozygous for 29 loci included in this study, was used as a reference standard for the identification of alleles (electromorphs). The electromorph coded by each locus of Q2 was given a mobility value (*Rf*) of 100. The mobility values of electromorphs of the corresponding locus in natural populations were calculated relative to the reference standard band of Q2.

Analysis: Statistical analysis of electrophoretic variability data was performed by using the computer program, BIOSYS-1 (Swofford and Selander 1981). Each polymorphic locus was tested for conformance of observed electromorph frequencies to those expected under Hardy-Weinberg equilibrium, with expected frequencies for small samples at some loci corrected by using Levene's (1949) formula. Genotypic fixation index "F" and Selander's (1970) "D" statistics were calculated for those loci where alternative alleles were either fixed or the frequencies were considerably different in separate populations. Negative and positive values of Selander's "D" indicate deficiencies and excesses, respectively, of heterozygotes and indicate the degree of substructuring in each population. The genotypic fixation index "F" was used to measure the amount of genetic variation in the population that is attributable to subpopulations. "F" was calculated as the difference between the frequency of heterozygotes observed and those expected under the Hardy-Weinberg law of squares,

$$F(\text{estimate}) = 1 - (H_o/H_e)$$

with H_e and H_o as expected and observed frequencies of heterozygotes, respectively.

The breeding structure of species C was estimated by Wahlund's formula (refer to Speiss 1977, pp. 351–354). The variance (V) in alleles and genotype frequencies for populations (assuming equal size of each, as subdivisions of a large population) was calculated as the difference between the average homozygote frequency among subpopulations and the square of the average allelic frequency of all subgroups,

$$V = \overline{q^2} - \bar{q}^2$$

The increment for homozygotes and deficiency in heterozygotes can be recognized in parallel. The Wahlund "equivalent of inbreeding" (F) resulting from division of a total population into subpopulations (isolates or demes) was calculated as

$$F = \text{Variance}/\bar{p}\,\bar{q}$$

In order to better understand the populational structure and interpret the Wahlund effect, the probability of homozygosity in isolates compared with that of a panmictic population, we calculated 3 levels of genetic differentiation by using F-statistics (Nei 1977). With the assumption that a total population (t) is divided into isolated subpopulations (s) or demes (e.g., *GULF*, *CHOC* and *OGE*), the inbreeding coefficients, F_{it} and F_{is}, refer to the fixation indices (homozygosities) of individual mosquitoes relative to the total population and its subpopulations, respectively. The fixation index, F_{st}, measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation. When subpopulations differ in allelic frequencies (p, q above), the F_{st} is the "equivalent of inbreeding," due to the Wahlund effect, as if the entire population were propagated by mating among sibs (Speiss 1977, pp. 351–354). Significance of gene frequency differences among populations was tested for each locus by the chi-square,

$$\chi^2 = 2NF_{st}(K - 1)$$

with (K − 1) (s − 1) degrees of freedom, where N is the total sample size, K is the number of alleles for the locus and s is the number of populations (Workman and Niswander 1970). The significance of the F_{is} values was tested by chi-square (Nei 1977),

$$\chi^2 = N(F_{is})^2$$

Genetic similarity and distance (Nei, 1978) among population pairs were used to perform hierarchical cluster analysis using arithmetic averages (UPGMA; Sneath and Sokal 1973, pp. 230–234), and relationships were summarized in the form of a dendrogram.

RESULTS

Micro and macrogeographic variation: Table 1 shows differences in frequencies of electromorphs of 6 loci, *Acon-1*, *Ao-1*, *Est-5*, *Est-6*, *Me-1* and *Pep-4*. The electromorph frequencies for the remaining 23 loci are not relevant to documentation of the subdivision in species C, but these data are available upon request. The *OGE* and *CHOC* populations differed from *GULF*, *SR* and *SFR* at the *Me-1* and *Acon-1* loci. *Me-1*, *Rf* 92 was the predominant allele with frequencies of 0.97 to 1.00 in *OGE* and *CHOC*. This allele ranged from 0.16 to 0.37 in the *GULF*, *SR* and *SFR* populations. *Acon-1*, *Rf*, 109, which was the most common allele in *OGE* and *CHOC* (frequency ranged from 0.83 to 0.94) was absent in *SR* and *SFR* populations (small sample sizes) but occurred at low frequencies in *GULF* collections: 0.10 in both LEV and BBS. Similarly, frequencies of the common alleles of polymorphic loci in *CHOC* populations such as *Ao-1* (*Rf*, 91), *Est-5* (*Rf*, 100) and *Est-6* (*Rf*, 100) differed significantly from those of *GULF*, *SR* and *SFR*. The fast allele of *Pep-4* (*Rf*, 165) present in *CHOC* and *OGE* populations was very rare in *GULF*, *SR* and *SFR* populations. There was no gradient (cline) of allelic frequencies of these loci either within or among collections from 5 areas. No significant differences between riverine (*CHOC*, *OGE*, *SR* and *SFR*) and Gulf Coast

Table 1. Differences in frequencies of electromorphs of some polymorphic loci among populations of species C of the *Anopheles quadrimaculatus* species complex.

	Populations								
	GULF		SR	SFR		CHOC			OGE
Locus	BBS	LEV	MAS	GSP	COU	RBL	HCR	MSP	119
Acon-1									
n	76	41	10	6	4	79	15	21	17
86	0.01	0	0	0	0	0	0	0	0
100	0.07	0	0	0	0	0.02	0.07	0.07	0.06
109	0.10	0.10	0	0	0	0.83	0.93	0.86	0.94
118	0.82	0.90	1.00	1.00	1.00	0.15	0	0.07	0
Me-1									
n	230	19	31	21	5	143	49	70	101
85	0.01	0				0	0	0.02	0.01
92	0.37	0.16	0.29	0.31	0.20	0.99	1.00	0.97	0.99
100	0.62	0.84	0.71	0.69	0.80	0.01	0	0.01	0
Ao-1									
n	110	107	11	30	10	61	16	29	18
76	0.02	0.02	0	0	0	0	0	0	0
78	0.09	0.07	0.18	0.05	0.05	0	0.03	0	0
85	0.19	0.15	0.18	0.18	0.20	0	0	0	0
88	0.42	0.44	0.23	0.32	0.60	0.01	0.22	0.02	0.06
91	0.15	0.31	0.32	0.45	0.15	0.64	0.69	0.66	0.83
96	0.01	0	0	0	0	0.27	0	0.22	0.06
100	0.08	0	0.09	0	0	0.08	0.06	0.10	0.05
103	0.03	0.01	0	0	0	0	0	0	0
108	0.01	0	0	0	0	0	0	0	0
Est-5									
n	45	40	11	22	11	44	*	13	*
94	0.13	0.03	0.23	0.14	0.14	0.23		0.15	
98	0	0	0	0	0	0.11		0.08	
100	0.33	0.17	0.46	0.32	0.36	0.66		0.77	
102	0.13	0	0.14	0.11	0.14	0		0	
106	0.36	0.56	0.14	0.34	0.27	0		0	
120	0.05	0.24	0.05	0.09	0.09	0		0	
Est-6									
n	108	26	15	55	9	47	*	21	*
77	0.03	0	0	0.04	0	0.06		0.07	
83	0.02	0	0	0.01	0	0.04		0.05	
88	0.03	0.03	0	0.03	0	0.04		0.05	
90	0.06	0.10	0.19	0.06	0	0.22		0.21	
94	0.72	0.87	0.81	0.74	1.00	0.10		0.12	
100	0	0	0	0	0	0.48		0.43	
104	0.14	0	0	0.12	0	0		0	
118	0	0	0	0	0	0.06		0.07	
Pep-4									
n	329	186	35	25	14	111	48	69	89
50	0.01	0.01	0.01	0.02	0	0.02	0.03	0.09	0.19
59	0	0	0	0	0	0	0	0	0.04
68	0	0	0	0	0	0.07	0	0.06	0
100	0.96	0.96	0.99	0.98	1.00	0.69	0.67	0.65	0.60
135	0.02	0.03	0	0	0	0.06	0.06	0.04	0.04
165	0.01	0	0	0	0	0.13	0.23	0.15	0.07
190	0	0	0	0	0	0.03	0	0	0.06
215	0	0	0	0	0	0	0	0.01	0

* No data.

(*GULF*) populations were observed for allelic frequencies of 23 loci, of which *Est-2*, *Est-4*, *Est-7*, *Got-2*, *Had-3*, *Idh-1*, *Mpi-1*, *Pep-2*, *Pgi-1* and *Xdh-1* were polymorphic in two or more populations. *Adk* (3 loci), *Go*, *Got-1*, *a-Gpdh*-1, *Hk* (2 loci), *Idh-2*, *Mdh-1*, *Mpi-3* and *6Pgd-3* were monomorphic in all populations. The *GULF* collections revealed a moderately higher level of genetic variability than those of *CHOC* and *OGE* (Table 2). The data from 301 (*OGE*) were not

Table 2. Genetic variability at 29 loci in natural populations of species C of the *Anopheles quadrimaculatus* complex (standard errors in parentheses).

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Direct count	Mean heterozygosity Hdywbg[2] expected
GULF					
BBS	178.5 (27.0)	4.5 (0.5)	51.9	0.13 (0.03)	0.27 (0.06)
LEV	83.4 (16.4)	3.1 (0.3)	66.7	0.11 (0.02)	0.23 (0.04)
CHOC					
MSP	45.6 (5.3)	2.7 (0.4)	44.4	0.10 (0.03)	0.18 (0.04)
HCR	35.2 (3.1)	2.3 (0.3)	40.7	0.10 (0.02)	0.17 (0.04)
RBL	68.5 (6.9)	2.9 (0.4)	51.9	0.12 (0.03)	0.20 (0.05)
OGE					
24	21.9 (5.0)	2.0 (0.3)	46.4	0.13 (0.03)	0.19 (0.05)
204	23.3 (4.9)	2.0 (0.3)	46.4	0.10 (0.03)	0.18 (0.04)
119	52.4 (6.7)	2.4 (0.3)	39.3	0.12 (0.03)	0.17 (0.04)
SFC	17.1 (3.8)	1.9 (0.3)	35.7	0.13 (0.04)	0.16 (0.05)
SR					
MAS	20.4 (2.6)	2.6 (0.3)	51.9	0.12 (0.03)	0.22 (0.05)
SFR					
GSP	25.3 (2.7)	2.4 (0.3)	37.0	0.10 (0.02)	0.22 (0.06)
COU	10.8 (0.9)	2.0 (0.3)	44.4	0.11 (0.04)	0.22 (0.05)

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
[2] The unbiased estimate based on conditional expectation (see Nei 1978).

Table 3. Coefficients for heterozygote deficiency or excess in populations of *Anopheles quadrimaculatus* species C.

	Acon-1					Me-1				
Population	(No)	Obs.	Exp.	F	D[a]	(No)	Obs.	Exp.	F	D[a]
1. *GULF + CHOC*	232	41	118	0.65	−0.65*	525	77	226	0.66	−0.66*
2. *GULF + CHOC + OGE*	266	46	136	0.66	−0.66*	649	80	247	0.68	−0.68*
3. *GULF*										
BBS	76	14	14	−0.02	0.01	230	69	109	0.37	−0.37*
LEV	41	8	7	−0.11	0.10	19	6	5	−0.19	0.16
BBS + LEV	117	22	21	−0.05	0.04	249	75	116	0.35	0.35
4. *SR*										
MAS	8	0	0	0	0	31	6	13	0.53	−0.54*
5. *SFR*										
GSP	6	0	0	0	0	21	3	9	0.67	−0.67*
COU	4	0	0	0	0	5	2	2	0	0
6. *SR + SFR*	18	0	0	0	0	57	11	24	0.53	−0.53*
7. *CHOC*										
MSP	21	2	2	−0.04	0.01	84	0	0	0	0
HCR	15	1	1	−0.03	0	49	0	0	0	0
RBL	79	16	20	0.21	−0.21	143	0	0	0	0
MSP + HCR + RBL	115	19	23	−0.19	−0.20	276	0	0	0	0
8. *OGE*										
119	16	2	2	−0.06	0.03	101	1	1	−0.01	0
MISC.	18	3	3	−0.09	0.06	23	2	2	−0.04	0.02
119 + MISC.	34	5	5	−0.08	0.06	124	3	3	−0.01	0.01

[a] Significant heterozygote deficiency based on χ^2 test using allele pooling method for loci with multiple alleles (Swofford and Selander 1981).

included in Table 2 due to small sample size and lack of data on many loci.

Genetic substructuring: The evidence for genetic substructuring in species C is shown in Tables 3, 4 and 5 and Fig. 2. When samples from Florida were treated as a single, panmictic population, significant heterozygote deficiencies were observed for *Me-1* and *Acon-1*. These deficiencies were even more pronounced when samples from Georgia were included (Table 3).

Table 4. Results of the F-statistics (Nei, 1977) for some polymorphic loci in species C of the *Anopheles quadrimaculatus* complex. Significance of F_{is} and F_{st} are indicated with the chi-square value. Calculations based on allelic frequencies of 29 loci (populations listed in Table 1).

Locus	F_{is}	Chi-square value	F_{it}	F_{st}	Chi-square value	d.f.
Acon-1	0.612	85.0	0.873	0.673	916.6	15
Ao-1	0.680	150.7	0.783	0.322	1,679.6	40
Est-5	0.863	171.3	0.887	0.175	402.5	25
Est-6	0.712	107.0	0.784	0.248	837.2	40
Me-1	0.565	161.2	0.844	0.642	1,296.8	10
Pep-4	0.460	160.0	0.500	0.075	793.8	35

Table 5. Estimates of Nei's (1978) unbiased genetic identity (above diagonal) and genetic distance (below diagonal) for natural populations of sibling species C of the *Anopheles quadrimaculatus* species complex.

Population	1	2	3	4	5	6	7	8	9	10
1. *OGE*-119	***	0.98	0.93	0.92	0.92	0.87	0.83	0.86	0.86	0.84
2. *OGE*-Misc[1]	0.02	***	0.91	0.91	0.91	0.86	0.82	0.84	0.86	0.83
3. *CHOC*-MSP	0.07	0.09	***	0.97	0.99	0.92	0.88	0.91	0.91	0.88
4. *CHOC*-HCR	0.09	0.09	0.03	***	0.97	0.92	0.87	0.92	0.91	0.90
5. *CHOC*-RBL	0.08	0.09	0.01	0.03	***	0.92	0.88	0.91	0.91	0.88
6. *GULF*-BBS	0.14	0.15	0.09	0.08	0.09	***	0.98	0.99	0.99	0.99
7. *GULF*-LEV	0.18	0.20	0.13	0.14	0.13	0.02	***	0.97	0.98	0.97
8. *SR*-MAS	0.16	0.17	0.10	0.08	0.10	0.02	0.03	***	0.99	0.99
9. *SFR*-GSP	0.15	0.16	0.09	0.10	0.13	0.01	0.02	0.01	***	0.99
10. *SFR*-COU	0.18	0.18	0.13	0.11	0.13	0.01	0.03	0.01	0.01	***

[1] Samples of 24, 204, 301 and SFC were pooled due to small sample size from each locality.

A large proportion of the heterozygote deficiency and fixation index in the total population (*GULF* + *CHOC* or *GULF* + *CHOC* + *OGE*) was due to near-fixation of alternate alleles of *Acon-1* and *Me-1* in *CHOC* and *OGE* as compared with those in the *GULF* collections. With the exception of deficiencies for heterozygotes for *Me-1* in the *GULF*, *SR* and *SFR* collections, all of the other loci in site collections from Florida and Georgia were in Hardy-Weinberg equilibrium. Two possible explanations, viz. null alleles and distinguishing phenotypes, for the *Me-1* heterozygote deficiency were discounted because of 230 adults of BBS analyzed for *Me-1*; all had a band, and the 3 alleles of *Me-1* separated well and heterozygotes were easily identifiable. During this study we did find difficulties in scoring electromorphs at *Pep-2*, *Pep-4*, *Had-3* and *Mdh-1* from preblood-fed wild females. Data from these females were not used. We also observed alleles of *Ao-1*, *Pgm-1*, *Pep-4* and esterases, for which the close migration of bands could cause confusion in identifying the heterozygotes. But for those loci the difficulty in recognizing heterozygotes was anticipated, and this problem was solved by including a reference standard after every 5 field samples, in addition to running the gel for 18 hours instead of 16 hours.

Analysis of the breeding structure by Wahlund' s formula, and genetic relationships (Fig. 2) showed that species C is subdivided into at least 2 subpopulations, designated as C_1 (collections from *GULF*, *SR* and *SFR*) and C_2 (*CHOC* and *OGE*). The variance in alleles and genotype frequencies of *Me-1* and *Acon-1* between C_1 (*GULF*-BBS) and C_2 (*CHOC*-RBL) isolates were 0.10 and 0.11, respectively. Similarly, the Wahlund "equivalence of inbreeding" coefficients, F, were 0.45 and 0.44 for *Me-1* and *Acon-1*, respectively.

Analysis of the standardized variance of allele frequencies (F_{st}) at *Acon-1*, *Ao-1*, *Me-1*, *Est-5*, *Est-6* and *Pep-4* (Table 4) indicated significant differentiation, and hence significant substructuring in species C, both at the micro- (*GULF*, *SR* and *SFR* vs. *CHOC*) and macrogeographic level (*GULF*, *SR* and of Florida vs. Georgia populations). For example, 64% of the variance of allele frequencies at *Me-1* (F_{st} = 0.642) and 67% at *Acon-1* (F_{st} = 0.673) were due to genetic differences among isolates at 2 loci leaving about 36% and 33% of respective gene diversity to be found in mosquitoes within any given isolate (1-F_{st}). The positive values for F_{it} at the 6 loci in Table 4 indicated a lower number of heterozygous individuals relative to that expected in pooled samples from *GULF*, *CHOC* and *OGE*. A low heterozygote population was also evident from Wright's D statistic in Table 3. F-statistics for 6 loci (listed in Table 4) among samples from

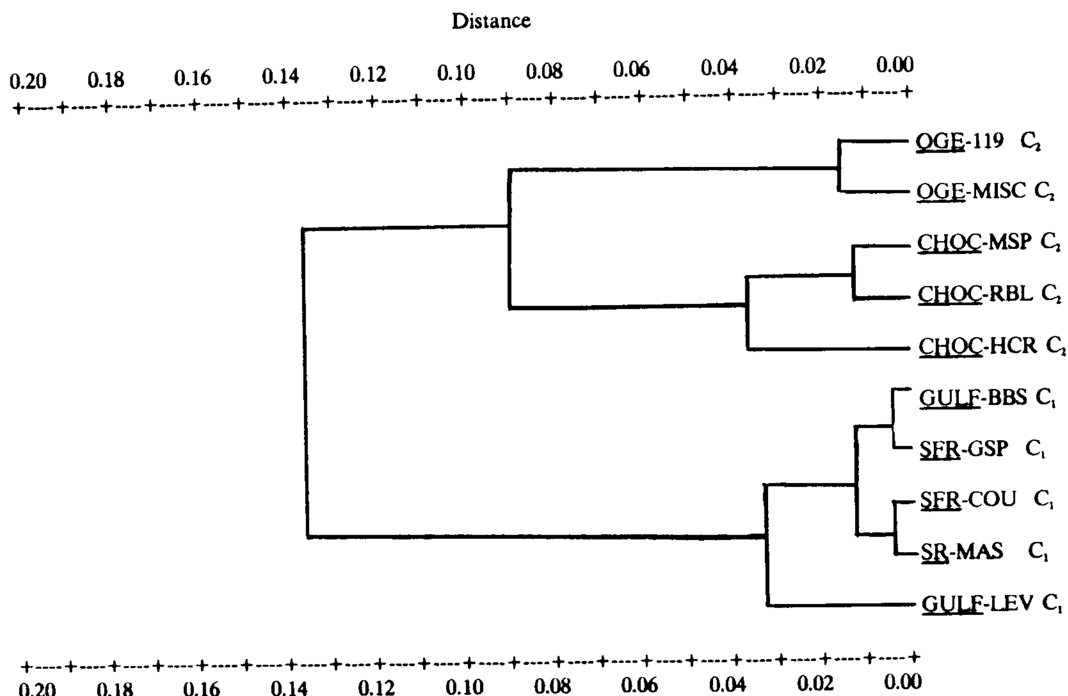

Fig. 2. Phenogram of genetic relationships among population isolates of *Anopheles quadrimaculatus* species C (C_1 and C_2) using unweighted pair group (with arithmetic means) clustering of Nei's (1978) distances. Locations of populations are shown in Fig. 1.

GULF showed lack of differentiation (substructuring) within this group. The same was true for *CHOC* and *OGE* collections (data not shown).

Table 5 lists values of genetic similarities and distances (Nei 1978) among populations of *GULF*, *CHOC*, *SR*, *SFR* and *OGE*. The mean genetic identities ranged from 0.87 to 0.92 between Florida Panhandle collections (*CHOC*) and those of *GULF*, *SR* and *SFR*; and 0.82 to 0.87 between *GULF* and *OGE*; and 0.91 to 0.93 between *CHOC* and *OGE*. The identities within population of an isolate were consistently high (0.99 in *SFR*, 0.98 in *GULF*, 0.97 to 0.99 in *CHOC* and 0.98 in *OGE*). A phenogram of genetic relationship among isolates (Fig. 2), using unweighted pair-group (with arithmetic means) clustering of Nei's (1978) distances, shows that *CHOC* and *OGE* are closely related and can be differentiated from *GULF*, *SR* and *SFR*. A similar relationship among the five groups was obtained using modified Rogers distance (Wright 1978).

Table 6 shows diagnostic values of some polymorphic loci. Though none of these loci can be used as single locus diagnostic to differentiate an individual mosquito of C_1 subpopulation from that of C_2, eleven 2-locus diagnostics can be used to distinguish C_1 from C_2.

Table 6. Diagnostic values[1,2] of some polymorphic loci, which can be used in pairs to differentiate C_1 from C_2 subpopulation of the *Anopheles quadrimaculatus* species C.

Locus/loci in pairs	Diag. value	Locus/loci in pairs	Diag. value
Acon-1	89.27	Acon-1: Est-5	98.87
Me-1	92.16	Acon-1: Est-6	99.46
Ao-1	96.05	Me-1: Ao-1	99.69
Est-5	89.42	Me-1: Est-5	99.17
Est-6	94.92	Me-1: Est-6	99.60
Pep-4	74.28	Ao-1: Est-5	99.58
Acon-1: Me-1	99.16	Ao-1: Est-6	99.80
Acon-1: Ao-1	99.58	Est-5: Est-6	99.46
		Ao-1: Pep-4	98.90

[1] Diagnostic values were calculated by the method of Ayala and Powell (1972).

[2] A locus or a pair of loci is considered diagnostic if the probability of correct diagnosis of an individual to a given taxon is 99% or higher.

DISCUSSION

The characteristic allelic frequencies at 5 loci (*Me-1*, *Acon-1*, *Ao-1*, *Est-5* and *Est-6*) in natural populations and analysis of standardized variance of allele frequencies indicate the division of species C into subpopulations (isolates) of

possibly large geographically structured populations. The coastal and riverine collection sites in Florida studied herein are representative of contiguous ecological zones. In general, large populations of species C occur throughout these zones. The frequency of C_1 was > 50% in 11 of 15 Gulf Coast populations. The riverine populations of the Suwannee and Santa Fe contained 17.8 and 21% species C_1, respectively. Six sites along the Choctawhatchee River averaged 61.9% species C_2 and 4 collections from the Apalachicola River (not included in these analyses) had a mean of 38.1% C_2. Therefore, our collections indicated the presence of a continuous population of species C from the Choctawatchee River in the Florida panhandle to the coastal hammocks north of Pinellas County, and throughout the major river systems breeching this coastal zone.

Our collections provided evidence that species C occurs in sympatry with the other 3 sibling species, with one possible exception. We did not find a population that contained both C_1 and species D, although this is probably due to sampling error. Of the 15 coastal populations sampled, all had species A and C (C_1 or C_2) and 4 sites contained species B (and C_1 or C_2). Nineteen locations on Florida rivers were studied, and all included species C_1 or $C_2$with species A and 15 with species B; 6 panhandle sites had C_2 and species D.

The differences between C_1 and C_2 populations of *Ae. quadrimaculatus* species C in Florida indicates a relatively low dispersal of these isolates from their breeding sites. The dissimilarities in variability patterns for polymorphic loci are perhaps due to drift and limited dispersal among neighboring demes and by subenvironmental (ecological niches) factors affecting each locus differently. If the latter is true, the microgeographic isolates C_1 and C_2 offer an ideal oppurtunity to test the possible occurrence of distinct ecologically adapted genetic structures. The role of causing Founders effect in causing allelic differences between in C_1 and C_2 was ruled out because of the lack of significant differences in the level of mean genetic variability (H and P in Table 2) among *GULF* (BBS, $n = 329$), *CHOC* (RBL, $n = 69$; MSP, $n = 46$) and *OGE* (119, $n = 52$).

This situation of semi-isolation among populations has obviously produced variation in allelic frequencies and considerable genetic divergence indicated by the significant F_{st} value (0.219; calculated for 29 loci). Differentiation among populations of species C is relatively high compared with values reported for other insect species that have been extensively sampled. Pamilo (1983) reported F_{st} values of 0.074 and 0.090 for 2 island species of *Formica* ants in Finland, compared with 0.072 and 0.046 for continuously distributed species. However, genetic differentiation was relatively higher in spatially subdivided species. In other studies, F_{st} values were 0.009 for monarch butterfly (Eanes and Koehn 1978), 0.192 for 12 macrogeographic populations of beetle species, *Collops georgianus*, endemic to Georgia, occupying patchily distributed habitats of granitic rock outcrop (King 1987), and 0.109 among 5 subpopulations of *Drosophila equinoxialis* (Nei 1975).

Studies on hybridization crosses by induced matings, larval salivary gland chromosomes (polytene chromosomes of adult ovarian nurse cells not suitable for analysis, Kaiser et al. 1989c) and mtDNA RFLPs of the 2 isolates, C_1 and C_2, from different regions are in progress. This information may be useful for research on the development of efficient genetic strategies for control of anopheline species.

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