Protein Variation and Systematics in the *Culex pipiens* Group of Species

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ABSTRACT. Electrophoretically-detected variation at 18 enzyme protein structural loci in members of the *Culex pipiens* group of species is summarised using Rogers' genetic distance algorithm and presented as a similarity dendrogram. The two native Australian species *C. australicus* Dobr. & Drum. and *C. globocoxitus* Dobr. are more similar to each other than either is to *C. quinquefasciatus* Say, *C. molestus* Forsk., *C. pallens* Coq. and European *C. pipiens L.* Within this latter group of species *C. quinquefasciatus* separates from the other three. The two basic clusters represent those species with rickettsial endosymbiotes, and those without these organisms. It is argued that the symbiote-free condition is the ancestral one, and that the two clusters of species represent separate evolutionary lines that diverged during the Cretaceous.

INTRODUCTION

Electrophoretically-detectable variation at protein loci (electromorphs) represents a source of data that can be usefully included in studies of the relationships within a group of species. These electromorphs are essentially taxonomic characters, but with an advantage over most other characters in that they show simple Mendelian inheritance. Several studies covering a variety of organisms, e.g. protozoa (Borden *et al.* 1977), *Drosophila* (Yang *et al.* 1972); fish (Utter *et al.* 1973); lizards (Gorman and Shochat 1972); rodents (Johnson and Selander 1971), have demonstrated that classifications based on electromorph data are in general agreement with those derived independently from other sources.

As a prelude to a study to determine the biological species status of members of the *Culex pipiens* complex in Western Australia (Miles 1974) genic variation at 18 enzyme protein structural loci in natural populations was measured in a search for taxonomically diagnostic electromorphs suitable as simple genetic markers. This paper presents an analysis of these data using the concept of genetic distance (see Chakraborty and Tateno 1976), and compares the pattern of genetic similarities with various aspects of the physiology of the different species.

MATERIALS AND METHODS

Collection and identification of material

Natural populations were sampled at the localities given in Table 1. Egg rafts were collected from breeding sites and from wild caught blood fed

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or gravid females which were allowed to oviposit in the laboratory. The samples from England (localities 36 and 37) were obtained as hibernating females; feeding and oviposition followed the artificial interruption of hibernation. The material from Japan was obtained as gravid F1 females reared from pooled egg rafts collected from a rural breeding site. Each egg raft was reared individually in an insectary at 23 ± 1°C and 50 ± 5% relative humidity, with a 16 hr photoperiod (LD 16:8). Larvae were fed on a suspension of powdered yeast tablets, and adults had access to a 10% sucrose solution. Ten larvae from each egg raft were reared through to adults for identification, and the remainder electrophoresed. Adult identification was based on the structure of the male aedeagus (see Fig. 1) with the additional characters of oocyte development beyond Stage IIb in virgin females (*molestus*) and the presence of the larval colour mutant gene *yellow larva*, which occurs at a high frequency in Australian populations of *C. quinquefasciatus*. The *pallens* material was identified by the absence of autogeny, and distinct genitalia comparable to the type material illustrated by Iltis (1966).

If less than 4 families of any species were collected from the localities given in Table 1 no electromorph frequencies were calculated.

Electrophoresis

Late fourth instar larvae were electrophoresed as described by Miles (1976). Larvae from different egg rafts were run on the same gel, together with one larva from a reference colony. Gels were stained for the following enzyme systems: alcohol dehydrogenase (ADH), alpha-naphthyl acetate esterases (EST), malic dehydrogenase (MDH) (Miles 1976, 1977a); "leucine" amino-peptidase (LAP) (Beckman and Johnson 1964); hexokinase (HK) (Madhavan *et al.* 1972); alphaglycerophosphate dehydrogenase (aGPD) (Grell 1967); xanthine dehydrogenase (XDH) (Glassman and Saverance 1963); glutamic dehydrogenase (GDH) (Shaw and Koen 1968); superoxide dismutase (SOD) (Bauer and Schorr 1969); fructose-1, 6-diphosphatase (F-1, 6-DIP), lactic dehydrogenase (LDH), 6-phosphogluconic dehydrogenase (6-PGD) (Shaw and Prasad 1970); and malic enzyme (ME) (as for MDH but substituting NADP for NAD and adding 4 drops of 10% magnesium chloride). The staining methods followed those of the authors cited, with minor modifications as to quantities.

A series of *quinquefasciatus*, *globocoxitus*, *molestus* and *pallens* stocks were selected for homozygosity at presumptive larval loci by sib-mating. The normal system of crosses and backcrosses between stocks of the same and different species established the genetic basis of the enzyme phenotypes and the allelic relationships between electromorphs with identical mobilities but in different species. Standard X^2 tests were used to test observed against expected phenotypic proportions in each cross. The possibility of linkage between any larval enzyme locus and the autosomal sex-determining factors could not be tested. The enzyme phenotypes of 2nd, 3rd and 4th instar larvae were compared to assess qualitative and quantitative ontogenetic variation.

Electromorphs identified at each locus were designated alphabetically in order of decreasing mobility from the origin, with the assumption of homology for those species that could not be colonised (*australicus* and *pipiens*). In cases where an enzyme phenotype was the product of electromorphs

at more than one locus, these loci were numbered in order of decreasing mobility from the origin.

Genetic similarity

The parental enzyme genotypes, or more accurately the genotype of that part of the parental genome expressed as an active enzyme during the fourth larval instar, was inferred from the enzyme phenotypes of the larvae from each egg raft. The assumption was made that all phenotypes were equally fit under the particular laboratory conditions. Electromorph frequencies at each locus were calculated from the parental genotypes for each locality and species.

A matrix of genetic distance coefficients (D) was generated from the electromorph frequency data using the algorithm of Rogers (1972), and a genetic similarity dendrogram (where S=1-D) derived from this matrix using an unweighted pair-group cluster analysis (Sokal and Sneath 1963).

RESULTS

Electromorph frequency data for the 14 polymorphic loci for each species and locality are given in Tables 2-5. No intra- or inter-specific variation was detected at the presumptive monomorphic loci *Gdh, Ldh, Me-2* and *Sod*. The scoring of larval genotypes at all loci was limited by larval mortality, particularly for the two native Australian species *australicus* and *globocoxitus*, and the small samples from some localities.

The genetic similarity dendrogram derived from Rogers' D coefficients is presented in Fig. 2. Two basic clusters are apparent: *quinquefasciatus/pipiens/pallens/molestus*, and *australicus/globocoxitus*. Within the first group, *quinquefasciatus* clusters separately from the other three species. There is a further sub-division within the *quinquefasciatus* cluster which may be associated with the localities 11,14,15,17,20 and 21 being on the route from Perth to the goldmining areas.

It is interesting to note that the discrete clustering of localities from which the same species was collected holds for those localities from which more than one species was sampled (localities 2,3,5,7,9,11,12,14,15, 16,23A, 23C, 24, 26A, 26B and 32). Assuming independent assortment of electromorphs at each locus, and that the females which oviposited in the laboratory represented a random sample of the females contributing to each larval generation in the field, then this is additional evidence of positive assortative mating between *australicus, quinquefasciatus, globocoxitus* and *molestus* (see Miles 1976, 1977 a,b).

DISCUSSION

An important characteristic of the *Culex pipiens* group of species is the presence of rickettsial endosymbiotes in some species, e.g. *C. quinquefasciatus, C. molestus, C. pallens* and European *C. pipiens*, and their absence

in others, e.g. *C. australicus, C. globocoxitus* and Southern African *C. pipiens* (Irving-Bell 1977). The continued existence of these symbiotes in the former group of species indicates a degree of coadaptation between the symbiote and host genomes, a system taking considerable geological time to evolve. In addition, where the symbiote is responsible for intraspecific cytoplasmic incompatibility, as in *C. molestus* (Yen and Barr 1973), it can be demonstrated (e.g. Fine 1978) that, once acquired, these organisms are unlikely to be lost. The symbiote-free condition is therefore considered to be the ancestral one.

The two major groupings resulting from the larval electromorph frequency analysis contain the symbiote-positive and symbiote-negative species respectively. An estimate of the length of time that has elapsed since these two evolutionary lines diverged can be made from the timing of the rifting of the African and Antarctic/Australian Plates (see Cracraft 1973). The presence of a symbiote-negative species in Southern Africa and two symbiote-negative species in Australia suggests that the ancestral symbiote-free taxon was in existence prior to the rifting of the two plates, during the late Cretaceous. This is not unlikely, as the major mosquito genera are recognisable in their present-day form at least by the Oligocene (reviewed by Marks 1972). Major genetic similarities still exist between the two evolutionary lines, as crosses between *C. globocoxitus* and European *C. pipiens* will produce a fertile F1 generation (Miles 1974).

Further analysis of the group *C. quinquefasciatus/C. molestus/C. pallens/* European *C. pipiens* is difficult as the literature on these species generally refers to *"C. pipiens"*, and often deals with characteristics expressed in laboratory colonies rather than in nature. However the grouping is supported by characters such as mating in swarms, e.g. *C. quinquefasciatus, C. pallens* and European *C. pipiens*.

The advantages and disadvantages of using electrophoretic data in systematic studies have been discussed, e.g. Avise (1974), Lakovaara *et al.* (1976). One disadvantage is the low resolving power of conventional electrophoresis. A combination of electrophoretic techniques (e.g. Coyne 1976), or the use of methods to detect differences between protein molecules other than charge differences (e.g. Johnson 1976) generally results in lower estimates of genetic similarity between species. This is of significance when genetic distance dendrograms alone are used to estimate divergence times between species or species groups. In the present study the genetic similarities between the members of the *C. pipiens* group should be taken as overestimates, but the pattern of clustering nevertheless appears to confirm the general evolutionary relationships within this group of species.

Measurements of genetic distance or similarity based on electromorph frequency data should be treated with caution, and not as a systematist's panacea. While there is a general correlation between geological time and genetic distance (e.g. Carson 1976), these values alone should not be used to define taxonomic categories, as in the cases of Ayala *et al.* (1974) and Schopf and Murphy (1973). These values can only be of use if they are derived from taxa whose individual biological species or subspecies status has already been established.

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List of Tables

Table 1. Location and composition of samples from natural populations of members of the *Culex pipiens* group of species. Abbreviations: a = *australicus*, q = *quinquefasciatus*, g = *globocoxitus*, m = *molestus*, pal = *pallens*, pip = European *pipiens*.

	Localities		Species
1.	Ajana	(Western Australia)	g
2.	Albany	"	a,q,g,m
3.	Bodallin	"	a,g
4.	Cannington	"	m
5.	Carnarvon	"	q,g
6.	Chidlow	"	a
7.	Clontarf	"	q,m
8A.	"Doorawarrah"	"	g
8B.	"Doorawarrah"	"	q
9.	Geraldton	"	q,m
10.	Gidgegannup	"	g
11.	Guildford	"	a,q,g
12.	Irwin River	"	a,g
14.	John Forrest National Park	"	a,g,m
15.	Kalgoorlie	"	q,m
16.	Kununurra	"	a,g
17.	Leonora	"	q
18.	Mandurah	"	m
19.	Manjimup	"	m
20.	Menzies	"	q
21.	Merredin	"	q
22.	Mobrup	"	g
23A.	Narrogin	"	*q,g,m
23B.	Narrogin	"	g
23C.	Narrogin	"	q,g,m
24.	Nedlands	"	*a,q,g,m
25.	Quindanning	"	g
26A.	Popanyinning	"	a,m
26B.	"Glencoe"	"	a,g
27.	Southern Cross	"	a
29.	York	"	g
30.	Werribee	(Victoria)	m
31.	Brisbane	(Queensland)	q
32.	St George	"	a,g
33.	Darwin	(Northern Territory)	q
34.	Auckland	(New Zealand)	q
35.	Tokyo	(Japan)	pal
36.	Tonbridge Wells	(England)	pip
37.	West Kirby	"	pip

*one egg raft m/q hybrid

Table 2. Larval electromorph frequencies in *Culex australicus*. (N) = number of genomes sampled.

Enzyme locus	E'morph	Localities*					
		6	11	24	26A	27	32
Est-1	a	0.714	0.842	0.761	0.727	0.750	
	e	0.286	0.158	0.239	0.273	0.250	
Est-2	b	0.571	0.563	0.548	0.500	0.875	
	f	0.429	0.437	0.452	0.500	0.125	
Adh	d	0	0	0.047	0	0.075	0
	e	1.000	1.000	0.922	0.929	0.925	0.900
	f	0	0	0.031	0.071	0	0.100
Mdh	a		0.524	0.412	0.438	0.583	
	d		0.476	0.582	0.562	0.417	
Lap-1	d		0.450	0.500		1.00	0
	e		0.550	0		0	1.000
	f		0	0.500		0	0
Lap-2	a		1.000	0.500		1.000	0.500
	c		0	0.500		0	0.500
Lap-4	a		0.500	1.000		0.500	0.500
	b		0.500	0		0.500	0.500
Me-1	a		0.136	0.125			
	b		0.000	0.063			
	c	0	0.591	0.500			
	d		0.273	0.312			
Hk	a			0.654			
	c			0.346			
a-Gpd	a		0.625	0.300			
	b		0.375	0.700			
6-Pgd	a			1.000			
	b			1.000			
	c			0			
Xdh	b		0.639	0.800	0.624		
	c		0.361	0.200	0.376		
F1,6diP	b			0.524			
	c			0.476			
	(N)	16	48	40	8	22	10

*see Table 1

Table 3. Larval electromorph frequencies in *Culex quinquefasciatus*. (N) = number of genomes sampled

Enzyme locus	E'morph	Localities*																
		2	5	8B	9	11	14	15	16	17	20	21	23A	23C	24	31	33	34
Est-1	d	0.692	0.398	0.220	0.412	0.500	0.654	0.329	0.587	0.491	0.417	0.500	0.531	0.500	0.457	0.348	0.167	
	f	0	0.213	0.020	0.193	0	0	0.427	0.130	0	0	0	0	0	0.067	0.161	0	
	g	0.308	0.389	0.760	0.395	0.500	0.346	0.244	0.283	0.509	0.583	0.500	0.469	0.500	0.476	0.491	0.833	
Est-2	e	0.625	0.294	0.396	0.528	0.500	0.692	0.298	0.652	0.481	0.306	0.633	0.571	0.583	0.448	0.526	0.364	
	f	0.375	0.706	0.604	0.472	0.500	0.308	0.702	0.348	0.519	0.694	0.367	0.429	0.417	0.552	0.474	0.636	
Adh	a	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0	0
	b	0.573	0.618	0.576	0.292	0.339	0.476	0.440	1.000	0.554	0.750	0.467	0.409	0.612	0.624	0.562	0.435	0.091
	c	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0	0
	d	0.427	0.382	0.424	0.708	0.661	0.524	0.560	0	0.418	0.250	0.533	0.591	0.388	0.376	0.438	0.565	0.909
Mdh	a		0.588	0.258	0.438	0.459		0.526		0.602		0.438	0.595	0.200	0.500	0.500	0.409	
			0.412	0.742	0.562	0.541		0.474		0.398		0.562	0.405	0.800	0.500	0.500	0.591	
Lap-1	a						0	0.278		0.293	0.500		0.096		0.353	0	0.045	
	c						0	0.389		0.586	0.500		0.808		0.471	0	0.705	
	e						1.000	0.333		0.121	0		0.096		0.088	0.125	0.250	
	f						0	0		0	0		0		0.088	0.875	0	
Lap-2	a						0.500	0.769		0.934	1.000		0.643		0.500	1.000	0.500	
	c						0.500	0.231		0.066	0		0.357		0.500	0	0.500	
Lap-3	a							0.250		0.750	0.250		0.250		0.289			
	b							0.750		0.250	0.750		0.750		0.711			
Lap-4	a						0.500	1.000		0.660	1.000		0.881		0.844	0.500	1.000	
	b						0.500	0		0.340	0		0.119		0.156	0.500	0	
Me-1	a		0.111	0.031	0.147		0.500	0.079		0.083		0.214			0.040	0.500	0.250	
	c		0.889	0.969	0.853		0.500	0.921		0.917		0.786			0.960	0.500	0.750	
Hk	a		0		0.227			0.083		1.000	0.500				0.437	0.500	0.500	
	c		1.000		0.773			0.917		0	0.500				0.563	0.500	0.500	
a-Gpd	a		0.417	0.833	0.500		0.643	0.500		0.400	0.533	0.500			0.417	0.375		
	b		0.583	0.167	0.500		0.357	0.500		0.600	0.467	0.500			0.583	0.625		
6-Pgd	a		0.729							0.867	0.621				0.500	0.525	0.333	
	b		0.271							0.133	0.379				0.500	0.475	0.667	
Xdh	a		0.333	1.000	0.083			0.500		0.342	0.269	0.458	0.682	0.612	0.545	0.238	0.361	0.351
	b		0.667	0	0.917			0.500		0.658	0.731	0.542	0.318	0.388	0.455	0.762	0.639	0.649
F1,6diP	b				0.474					0.517					0.552	0.718		
	c				0.526					0.483					0.448	0.282		
	(N)		44	20	22	58	8	60	12	62	14	24	24	28	48	22	12	20

*see Table 1

Table 4. Larval electromorph frequencies in *Culex globocoxitus*. (N) = number of genomes sampled

Enzyme locus	E'morph	Localities*													
		1	2	3	8A	10	11	12	22	23B	23C	24	25	26A	29
Est-1	a	0.271	0.518	0.046	0.342	0.400	0.208	0.250	0	0	0	0.186	0.111	0.125	0.024
	c	0.729	0.482	0.909	0.658	0.600	0.792	0.750	1.000	1.000	1.000	0.814	0.889	0.854	0.786
	e	0	0	0.045	0	0	0	0	0	0	0	0	0	0.021	0.190
Est-2	a	0.719	0.543	0.227	0.457	0	0.418	0.429	0.389	0.881	0.500	0.275	0.471	0.603	0.333
	f	0.281	0.457	0.773	0.543	1.000	0.582	0.571	0.611	0.119	0.500	0.725	0.529	0.397	0.667
Adh	c	0.012	0.067	0	0.077	0.012	0	0.029	0.197	0.200	0.080	0.049	0.206	0.056	0.200
	d	0.012	0	0	0	0.297	0.625	0	0.013	0	0	0.037	0	0	0
	e	0.976	0.933	1.000	0.923	0.691	0.375	0.971	0.790	0.800	0.920	0.914	0.794	0.944	0.800
Mdh	b	0.333	0.500	0.500	0.333	0.700		0.417	0.579	0.200		0.611	0.400	0.500	1.000
	e	0.667	0.500	0.500	0.667	0.300		0.583	0.421	0.800		0.389	0.600	0.500	0
Lap-1	c	0.333			1.000	0.450			0.595	0		0.133	0.656		0.565
	e	0.667			0	0.550			0.405	1.000		0.867	0.344		0.435
Lap-2	a	0.500			1.000	0.500			0.524	0		0.500	0.875		1.000
	b	0.166			0	0			0			0	0		0
	c	0.168			0	0.500			0.476			0.500	0.125		0
	d	0.166			0	0			0			0	0		0
Lap-3	a	0.500				0			0	0		0	0		1.000
	b	0.500				1.000			1.000	1.000		1.000	1.000		0
Lap-4	a	0.429				1.000			0.881	0.500		1.000	1.000		0.682
	b	0.571				0			0.119	0.500		0	0		0.318
Me-1	a	0.147	0.278	0.250	0.417	0.087		0.375	0.062	0		0.300	0.050	0.250	0.214
	b	0	0	0	0.042	0		0.125	0	0		0.100	0	0.250	0
	c	0.853	0.722	0.750	0.416	0.913		0.375	0.938	1.000		0.500	0.950	0.250	0.786
	d	0	0	0	0.125	0		0.125	0	0		0.100	0	0.250	0
Hk	a	0.500	0.500		0.917			0.250				0.333		0.083	
	c	0.500	0.500		0.083			0.750				0.667		0.917	
a-Gpd	a	0.583			0.417			0.667				0.643	0.591	0.417	
	b	0.417			0.583			0.333				0.357	0.409	0.583	
6-Pgd	b	0.500	0.525											0.500	
	c	0.500	0.475											0.500	
Xdh	b	1.000			0.813			0.861		0	0.653	0.667	0.250	0.725	
	c	0			0.187			0.139		1.000	0.347	0.333	0.750	0.275	
f1,6diP	b	0.506			1.000							0.581			
	c	0.494			0							0.419			
	(N)	14	14	18	16	8	22	22	14	28	8	22	10	30	14

*see Table 1

Table 5. Larval electromorph frequencies in *Culex molestus*, *C. pallens* and European *C. pipiens*. (N) = number of genomes sampled

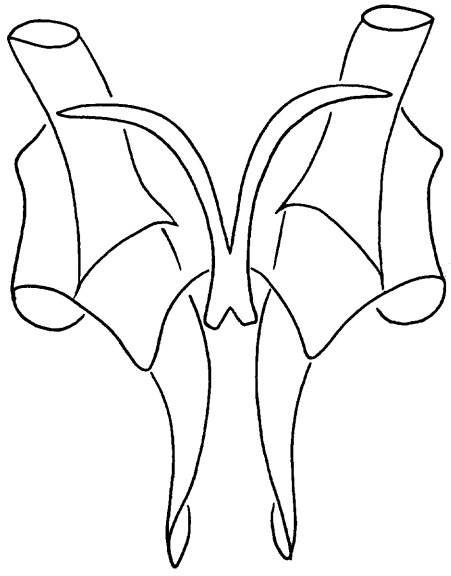
Enzyme locus	E'morph	Localities*													
		2	4	7	9	14	15	18	19	24	26B	30	35	36	37
Est-1	b	0.792	0.559	0.633	0.655		1.000	0.639	0.853	0.233	0.808	0.391	0	0.625	0.708
	c	0	0	0	0		0	0	0	0	0	0	0.540	0	0
	d	0.083	0.441	0	0.242		0	0.167	0.059	0	0	0.609	0	0.375	0.292
	f	0.125	0	0.367	0.103		0	0.194	0.088	0.767	0.192	0	0.460	0	0
Est-2	b	0	0	0	0		0	0	0	0	0	0	0	0.400	0.583
	c	0.750	0.861	0.813	0.517		0.292	0.591	0.647	0.500	0.458	0.548	0	0	0
	d	0	0	0	0		0	0	0	0	0	0	0.380	0	0
	f	0.250	0.139	0.187	0.483		0.708	0.409	0.353	0.500	0.542	0.452	0.620	0.600	0.417
Adh	b	0	0	0	0	0	0	0	0	0	0	0	0.144	0	0
	d	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.681	0.605	0.600
	e	0	0	0	0	0	0	0	0	0	0	0	0.074	0.395	0.400
	f	0	0	0	0	0	0	0	0	0	0	0	0.101	0	0
Mdh	a		0		0		0		0			0	0.6100	1.000	1.000
	c		0.727		0.306		0.455		0.591			0.344	0	0	0
	d		0		0		0		0			0	0.390	0	0
	e		0.273		0.694		0.545		0.409			0.656	0	0	0
Lap-1	a	0	0	0		0.189	0	0	0			0	0	0.167	0.083
	b	0	0	0		0	0	0	0			0	0	0.606	0.717
	c	0.385	0.387	0.454		0.534	0	0	0.500			0.500	0.238	0	0
	d	0	0	0		0	0	0	0			0	0	0.227	0.200
	e	0.615	0.613	0.546		0.277	0	1.000	0.500			0.283	0.762	0	0
	f	0	0	0		0	1.000	0	0			0.217	0	0	0
Lap-2	a		0.431		0.929	0.500	1.000	0.438	0.711			0.975	0.738	0.655	0.586
	b		0		0	0	0	0.062	0			0	0	0	0
	c		0.569		0.071	0.500	0	0.500	0.289			0.025	0.262	0.345	0.414
Lap-3	a		0.167		1.000		0.438	0.206	0.333			0.167	0.381	0	0.615
	b		0.833		0		0.562	0.794	0.667			0.833	0.619	1.000	0.385
Lap-4	a		0.444		1.000	0.500	0.333	0.647	0.922			0.733	0.738	0.917	0.833
	b		0.556		0	0.500	0.667	0.353	0.078			0.267	0.262	0.083	0.167
Me-1	a		0.364		0.167		0.167	0.250				0	0.083	0.429	0.200
	b		0		0		0	0				0	0.125	0.071	0
	c		0.636		0.833		0.833	0.750				1.000	0.792	0.429	0.800
	d		0		0		0	0				0	0	0.071	0
Hk	a				0.708		0.708					0.500	0.654		0
	b				0		0					0	0		1.000
	c				0.292		0.292					0.500	0.346		0
α-Gpd	a		0.833				1.000	0.667					0.542	0.375	0.688
	b		0.167				0	0.333					0.458	0.625	0.312
6-Pgd	a						0.618						0.612		0.556
	b						0.382						0.388		0.444
Xdh	a		0		0		0.167	0.125	0.207				0.364	0.215	0.381
	b		1.000		1.000		0.833	0.875	0.793				0.636	0.785	0.619
F1,6diP	a		0.592		0.635		0.427						0.500		0.424
	b		0.408		0.365		0.573						0.500		0.576
	(N)	12	18	20	6	4	4	16	12	8	4	4	82	32	14

*see Table 1

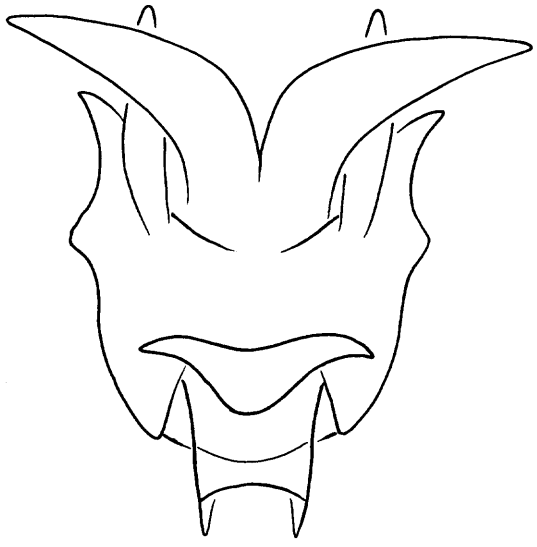
Captions to Figures

Fig. 1. Male terminalia characteristic of each taxon within the *Culex pipiens* group of species. Type I = *molestus* and European *pipiens*; Type II = *quinquefasciatus*; Type III = *australicus*; Type IV = *globocoxitus* (from Dobrotworsky 1967)

Fig. 2 Genetic similarity dendrogram based on Rogers' coefficient for members of the *Culex pipiens* group of species. Abbreviations: austr. = *australicus*; quinque = *quinquefasciatus*; globo = *globocoxitus*; mol = *molestus*; pal = *pallens*; pip = European *pipiens*.



I



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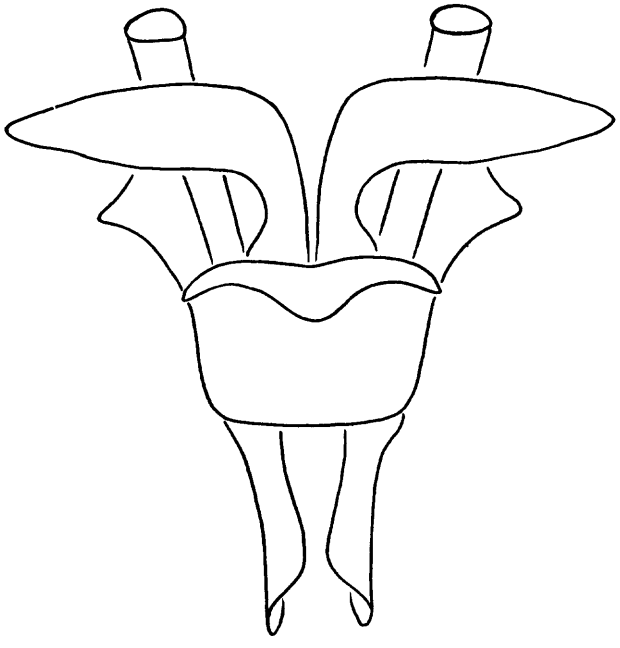

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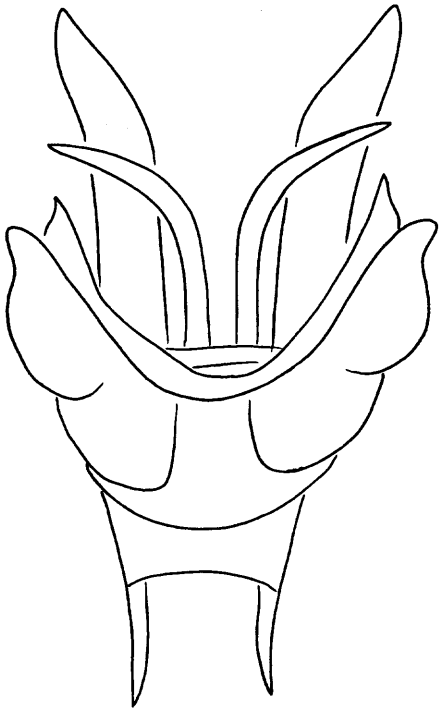

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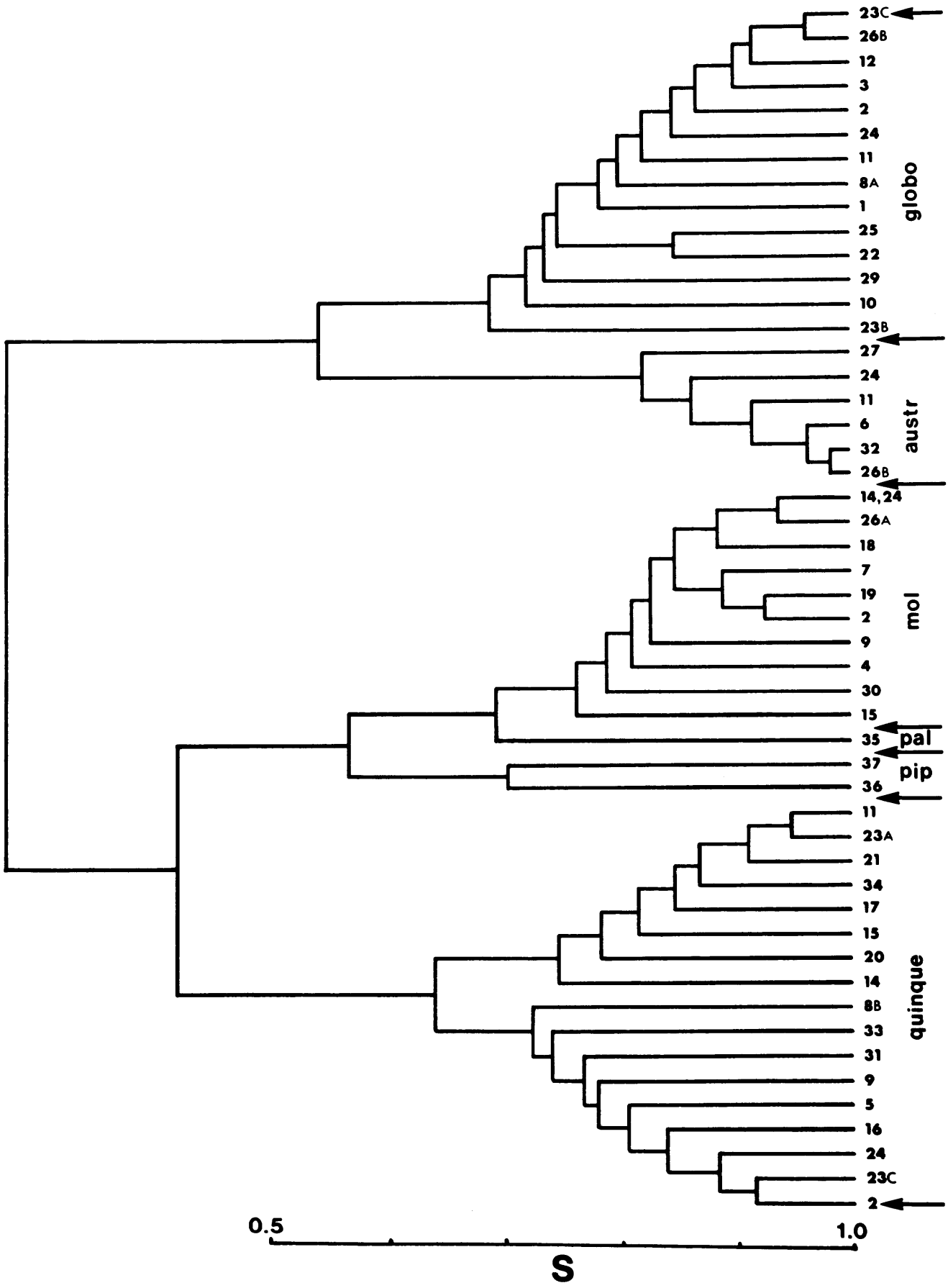
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8B
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16
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23C
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globo
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0.5
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S