Comparative morphology and multivariate analysis for the discrimination of four members of the *Anopheles gambiae* group in southern Africa.

by

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ABSTRACT. The external morphology of four members of the *Anopheles gambiae* group occurring in southern Africa was studied. Wild-caught larvae or the progeny of wild-caught females were used. Colony material was not used, except for the egg measurements. All life stages of the four species *An. gambiae*, *An. arabiensis*, *An. quadriannulatus* and *An. merus* were examined and thirteen characters chosen for computer analysis. A multivariate discriminant function analysis was run using all four groups at once and 97% discrimination was obtained.

INTRODUCTION

The acceptance that *Anopheles gambiae* Giles was a complex of cryptic species (Paterson 1962, Davidson and Jackson 1962, Paterson et al. 1963) helped to explain the pronounced ecological and behavioral diversity of this taxon (see Gillies and DeMeillon, 1968). For example, *An. quadriannulatus* is a cattle-feeding, outdoor-resting member of the group and is not known to transmit malaria parasites. *Anopheles gambiae* and *An. arabiensis*, on the other hand, are highly efficient vectors of malaria parasites in Africa and it is against these two species that most control programs are directed. This makes it imperative that populations are identified correctly before control measures are formulated. Probably the most common method used today is chromosomal identification where banding sequences on the giant polytene chromosomes show specific differences between the species (Coluzzi and Sabatini 1967, 1968, 1969; Hunt 1972). The biochemical key for identification by electrophoresis of the soluble enzymes (Mahon et al 1976; Miles 1979) is adequate when studying large populations. This method should be correlated with chromosomal identification when the identity of individuals is required (Hunt and Coetzee 1986a).

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Both chromosomal and electrophoretic identification techniques require a high level of expertise and sophisticated laboratory equipment. The ideal method would still be identification by morphology. It is quick and can be carried out in the field with the minimum of equipment.

A comprehensive morphological study of *An. gambiae, An. arabiensis, An. melas* and *An. merus* was carried out by Coluzzi (1964). He revealed some characters for separating the salt-water breeders from the freshwater breeders. This had already been done to some extent by Ribbands (1944), Muirhead-Thomson (1951) and Paterson (1963, unpublished WHO/MAL document no. 421). Coluzzi (1964) was unable to find reliable characters for separating *An. gambiae* and *An. arabiensis* which are sympatric over a large area of their distribution. His samples originated mainly from colony material. Subsequently, many workers have attempted to find morphological differences between the three freshwater species (Ismail and Hammoud 1968; Zahar et al. 1970; Clarke 1971; White & Muniss 1972; Reid 1973; 1975a,b) without success. Ramsdale and Leport(1967), Green (1971), Bryan (1980) and Bushrod (1981) tested existing structural characters for separating the members of the group and found that they were not always reliable. Ribeiro et al. (1979) described a subspecies, *An. quadriannulatus davidsoni,* from the Cape Verde Islands based on morphological criteria only. Later work by Cambournac et al. (1982) showed that the Cape Verde populations had *An. arabiensis* polytene chromosome banding configurations and that inversion polymorphisms were at the same frequency as *An. arabiensis* on mainland Senegal. Using the morphological data from *An. quadriannulatus davidsoni* and published data for the other species, Ribeiro (1980) proposed a phylogeny for the group.

The present study compares morphological data from four members of the *An. gambiae* group occurring in southern Africa and presents the results of a computer analysis of the data.

MATERIALS AND METHODS

Wild-caught specimens were used exclusively in this study except for the egg measurements. Adults and larvae were collected by various means from numerous localities. The numbers, map references and collection methods for the four species *An. gambiae, An. arabiensis, An. quadriannulatus* and *An. merus,* are given in Table 1.

Methods for laboratory rearing and preparation of specimens for morphological study are given by Coetzee (1987). Identification of specimens was either by chromosomes (Green 1972; Hunt 1987) or enzyme electrophoresis (Miles 1979). These methods can also be found in Green and Hunt (1980) and Hunt and Coetzee (1986b).

Measurements of morphological characters were taken using an eye-piece micrometer at X40 magnification. Setal counts of the larvae and pupae followed Belkin's (1962) numbering system. Egg length measurements were taken from 36-hour old, unhatched eggs.

Most of the material studied has been deposited in the collection of the South African Institute for Medical Research. However, small representative samples have been deposited in the collections of the British Museum (Natural History), London, and the National Museum of Natural History, Smithsonian Institution, Washington D.C.

RESULTS

Adults. The following characters were examined: Palpus ratio (Coluzzi, 1964 - length segments IV +V/III); number of pale bands on palpus; number of coeloconic sensilla on antennal flagellomeres; size of pale band at the joint of hindtarsomeres 3 and 4. The results of these are given in Table 2 together with data from other sources where available. Many other characters were examined but showed no obvious differences, e.g., wing spots 1 to 8 on the costa and first vein were measured (Fig. 1); wing spots 9 to 24 were recorded for presence or absence; tarsal claws and male genitalia were examined using a scanning electron microscope.

Immature Stages. Full setal counts of larvae and pupae were recorded. The results for *An. quadriannulatus* appear in full in Coetzee (1987) and only those characters showing differences are presented here (Table 2). Table 2 also includes characters used by previous workers. Egg length measurements are given in Table 2.

A stepwise multivariate discriminant function analysis (software from SAS Institute Inc., Box 8000 Cary, North Carolina, USA 27511) was used in an attempt to maximize the separation of the four species. The aim of an analysis is to provide a method for predicting which group an unidentified specimen is most likely to fall into or to obtain a small number of useful discriminating variables. The characters used were: hindleg banding measurement; the number of coeloconic sensilla on antennal flagellomeres 5, 6, 9 and the total number on all flagellomeres; the palpus index; the sums of branches of pupal setae 10-C, 5-I, 4-II and 6-III; the sums of branches of larval setae 2-P and 10-II; and the egg length. These were chosen because of high "t" values when tested for differences between the means using Student's "t" test. A total of 100 specimens were used and 97% total discrimination was obtained. Figures 2 and 3 show the scatterplots obtained from the computer analysis.

DISCUSSION

In 1903 Theobald wrote about the hindleg bandings of *An. costalis (=gambiae)* "...in fact, I have seen fresh specimens in which it is nearly absent." Coluzzi (1964) states "Another character relates to the rings and spots of white scales on the tarsi which on the whole, are more extensive in *A. merus* than in *A. gambiae* populations examined. The ratio of the length of the white ring to length of tarsus usually gives definite discriminatory values." Indeed, the hindleg pale

band at the junction of tarsomeres 3 and 4 appears to be a good character for grouping *gambiae/arabiensis* and *quadriannulatus/merus* (Coetzee et al. 1982; Coetzee 1986) although *An. arabiensis* is known to be variable (Sharp et al. 1989). White (1985) gives results of measurements of ten hindleg bands for each of the six members of the *An. gambiae* group. The mean values for the four species *An. gambiae, An. arabiensis, An. quadriannulatus* and *An. merus* correspond well with the results presented in Coetzee (1986) and here (Table 2). More data are needed for *An. melas* and *An. bwambae* before the usefulness of this character for these species can be assessed, especially in areas of sympatry with other members of the group.

Bushrod (1981) plotted the total number of coeloconic sensilla on the female antennae against the palpus ratio and found this method to be effective in separating *An. merus* from the freshwater breeders *An. gambiae* and *An. arabiensis*. *Anopheles quadriannulatus* too, can be separated from *An. merus* using this method although some overlap does exist (Fig. 4).

The separation of individual *An. gambiae* from *An. arabiensis* females was not reliable. The following key, applied to a minimum of three females per family and using an average figure for each family, identified 100% of the *An. merus* families, 100% of the *An. quadriannulatus*, 94% of the *An. gambiae* and only 87.5% of the *An. arabiensis* families. The margin of error for the identification of *An. arabiensis* would presumably be even higher in light of recent work by Sharp et al. (1989) in Zululand who show that the character used in couplet 1 is ineffective for *An. arabiensis* in DDT sprayed areas.

1. Pale band at the joint of hindtarsomeres 3 and 4, 0.1mm or more 2
- This pale band 0.09mm or less 3

2. Palpus ratio of 0.85 or higher . . . . . *merus*
- This ratio 0.84 or lower . . . . . *quadriannulatus*

3. The sum of coeloconic sensilla on flagellomeres 5+6+9 of both antennae 13 or more . . . . . *arabiensis*

- This sum 12 or less . . . . . *gambiae*

The use of a multivariate discriminant function analysis of 13 variables from all life stages correlated for each individual entered into the program, has an obvious advantage over the above key as 97% of the *An. gambiae* and *An. arabiensis* individuals were correctly identified. As both methods call for the rearing of progeny from wild-caught females, the use of the discriminant analysis is only slightly more cumbersome. The availability of portable computers makes it quite feasible to carry out sophisticated statistical analyses in the field.

Comparison of larval and pupal characters with those found useful by Coluzzi (1964) and Reid (1973; 1975a, b) show differences in mean values which minimize the usefulness of these characters when applied to southern African populations.

The values for *An quadriannulatus* given by Ribeiro (1980) were obtained by assuming the standard deviations found in *An. quadriannulatus davidsoni* (Ribeiro et al. 1979) are the same as *An. quadriannulatus* and applying these values to the Coefficients of Difference given by White (1973) (Ribeiro, pers. comm.). There is one important flaw in making the above assumption. *Anopheles quadriannulatus davidsoni* is actually *An. arabiensis* (Cambournac et al. 1982). The values given by Ribeiro (1980) bear no resemblance to South African *An. quadriannulatus* and, in fact, show a marked similarity to the *An. melas* values quoted by Ribeiro (1980). This, of course, would also materially alter the phylogenies based on these morphological characters.

Studies of the external morphology, when applied in the traditional manner, are not applicable to groups of cryptic species identified by genetical markers, as in the case of the *An. gambiae* group. Morphological studies on anopheline species should be based on genetically identified wild females or their progeny. Adequate correlated data bases will enable one to test morphological characters for discrimination of genetically identified species. Furthermore, one should be able to establish which, if any, of the previously described and named synonyms of the taxon might be assigned to the genetic species concealed under the taxon name. This approach was followed by Lambert and Coetzee (1982) in their study of the *An. marshallii* group of species. The use of sophisticated statistical programs to achieve these ends is now almost obligatory but as most of the programs are available internationally, this should not present a problem in the exchange of data sets between interested workers in the same field.

A combination of all available techniques and their logical application is now essential for the understanding of the systematics of insect vectors of disease pathogens. The obvious limitations inherent in the current identification techniques may be minimized if a combined approach is used.

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REFERENCES

Belkin, J. N. 1962. The mosquitoes of the South Pacific. University of California Press. 2 Volumes. xii + 608pp., 412 figs.

Bryan, J. H. 1980. Use of palpal ratio and the number of pale bands on the palps in separating *Anopheles gambiae* Giles s.s. and *Anopheles melas* Theobald (Diptera: Culicidae). Mosq. Syst. 12: 155-163.

Bushrod, F. M. 1981. The *Anopheles gambiae* Giles complex and Bancroftian filariasis transmission in a Tanzanian coastal village. Ann. Trop. Med. Parasitol. 75: 93-100.

Cambournac, F.J.C., V. Petrarca, and M. Coluzzi. 1982. *Anopheles arabiensis* in the Cape Verde Archipelago. Parassitologia (Rome) 24: 265-267.

Clarke, J. L. 1971. Potential use of the spermatheca in the separation of species A and B females of the *Anopheles gambiae* complex in northern Nigeria. Bull. W.H.O. 45: 260-263.

Coetzee, M. 1986. Practical use of the hindleg banding patterns for identifying members of the *Anopheles gambiae* group of mosquitoes. Mosq. Syst. 18: 134-138.

Coetzee, M. 1987. A taxonomic description of *Anopheles quadirannulatus* Theobald (Diptera: Culicidae) from southern Africa. Syst. Entomol. 12: 305-312.

Coetzee, M., K. Newberry, and D. Durand. 1982. A preliminary report on a morphological character distinguishing important malaria vectors in the *Anopheles gambiae* Giles complex in southern Africa. Mosq. Syst. 14: 88-93.

Coluzzi, M. 1964. Morphological divergences in the *Anopheles gambiae* complex. Riv. Malariol. 43: 197-232.

Coluzzi, M. and A. Sabatini. 1967. Cytological observations on species A and B of the *Anopheles gambiae* complex. Parassitologia (Rome) 9: 73-88.

Coluzzi, M. and A. Sabatini. 1968. Cytological observations on species C of the *Anopheles gambiae* complex. Parassitologia (Rome) 10: 155-165.

Coluzzi, M. and A. Sabatini. 1969. Cytological observations on the saltwater species *Anopheles merus* and *Anopheles melas* of the *gambiae* complex. Parassitologia (Rome) 11: 177-187.

Davidson, G. and E. Jackson. 1962. Incipient speciation in *Anopheles gambiae* Giles. Bull. W.H.O. 27: 303-305.

Davidson, G., H. E. Paterson, M. Coluzzi, G. F. Mason, and D. W. Micks. 1967. The *Anopheles gambiae* complex. In: Genetics of Insect Vectors of Disease. (Eds. J. W. Wright and R. Pal) pp. 211-250. Elsevier Publishing Company, Amsterdam.

Gillies, M. T. and B. DeMeillon. 1968. The Anophelinae of Africa South of the Sahara. Publication of the South African Institute for Medical Research no. 54.

Green, C. A. 1971. The practical problem of identifying members of the *Anopheles gambiae* complex in autecological studies. Parassitologia (Rome) 13: 421-426.

Green, C. A. 1972. Cytological maps for the practical identification of females of the three freshwater species of the *Anopheles gambiae* complex. Ann. Trop. Med. Parasitol. 66: 143-147.

Green, C. A. and R. H. Hunt. 1980. Interpretation of variation in ovarian polytene chromosomes of *Anopheles funestus* Giles, *A. parensis* Gillies and *A. aruni?*. Genetica 51: 187-195.

Hunt, R. H. 1972. Cytological studies on a new member of the *Anopheles gambiae* complex. Trans. R. Soc. Trop. Med. Hyg. 66:532.

Hunt, R. H. 1987. Location of genes on chromosome arms in the *Anopheles gambiae* group of species and their correlation to linkage data in other members of the genus *Anopheles*. Med. Vet. Entomol. 1: 81-88.

Hunt, R. H. and M. Coetzee 1986a. Chromosomal and electrophoretic identification of a sample of *Anopheles gambiae* group (Diptera: Culicidae) from the island of Grand Comoros, Indian ocean. J. Med. Entomol. 23: 655-660.

Hunt, R. H. and M. Coetzee. 1986b. Field sampling of *Anopheles* mosquitoes for correlated cytogenetic, electrophoretic and morphological studies. Bull. W. H. O. 64: 897-900.

Ismail, I.A.H. and E. I. Hammoud. 1968. The use of coeloconic sensillae on the female antenna in differentiating the members of *Anopheles gambiae* Giles complex. Bull. W. H. O. 38: 814-821.

Lambert, D. M. and M. Coetzee. 1982. A dual genetical and taxonomic approach to the resolution of the mosquito taxon, *Anopheles (Cellia) marshallii* (Culicidae). Syst. Entomol. 7: 321-332.

Mahon, R. J., C. A. Green and R. H. Hunt. 1976. Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera, Culicidae). Bull. Entomol. Res. 66: 25-31.

Miles, S. J. 1979. A biochemical key to adult members of the *Anopheles gambiae* group of species (Diptera: Culicidae). J. Med. Entomol. 15: 297-299.

Muirhead-Thomson, R. C. 1951. Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast. Bull. Entomol. Res. 41: 487-502.

Paterson, H. E. 1962. Status of the East African saltwater-breeding variant of *Anopheles gambiae* Giles. Nature (Lond.) 195: 469-470.

Paterson, H. E. 1964. Direct evidence for the specific distinctness of forms A, B and C of the *Anopheles gambiae* complex. Riv. Malariol. 43: 191-196.

Paterson, H. E. 1968. Evolutionary and population genetical studies of certain diptera. PhD thesis, University of the Witwatersrand, Johannesburg.

Paterson, H. E., J. S. Paterson and G. J. Van Eeden. 1963. A new member of the *Anopheles gambiae* complex: a preliminary report. Med. Proc. 9: 414-418.

Ramsdale, C. D. and G. H. Leport. 1967. Studies of the *Anopheles gambiae* complex in West Africa. Bull. W. H. O. 36: 494-500.

Reid, J. A. 1973. Larval differences between sympatric populations from Kaduna, West Africa of species A and B of the *Anopheles gambiae* group. Parassitologia (Rome) 15: 87-98.

Reid, J. A. 1975a. Pupal differences between species A and B of the *Anopheles gambiae* group from Kisumu, East Africa. Mosq. Syst. 7: 1-7.

Reid, J. A. 1975b. Pupal differences between species A and B of the *Anopheles gambiae* group from Kaduna, West Africa. Mosq. Syst. 7: 229-302.

Ribbands, C. R. 1944. Differences between *Anopheles melas (A. gambiae* var. *melas)* and *Anopheles gambiae.* Ann. Trop. Med. Parasitol. 38: 85-99.

Ribeiro, H. 1980. A biometric study of the taxonomy of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). Garcia de Orta Ser. Zool. 9: 139-154.

Ribeiro, H., H. da C. Ramos, C. A. Pires and R. A. Capela. 1979. Description and biometric study of *Anopheles (Cellia) quadriannulatus davidsoni* ssp. n., a seventh member of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) endemic to the Cape Verde archipelago. Garcia de Orta Ser. Zool. 8: 75-88.

Sharp, B. L., D. LeSueur and F. Ridl. 1989. The value of hindleg banding patterns in the identification of species of the *Anopheles gambiae* complex in Natal, South Africa. Mosq. Syst. 21: 21-26.

Theobald, F. V. 1903. A Monograph of the Culicidae or Mosquitoes. British Museum (Natural History), London.

White, G. B. 1973. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae). III. The distribution, ecology, behavior and vectorial importance of species D in Bwamba County, Uganda, with an analysis of biological, ecological, morphological and cytogenetical relationships of Uganda species D. Bull. Entomol. Res. 63: 65-97.

White, G. B., 1985. *Anopheles bwambae* n. sp., a malaria vector in the Semliki valley, Uganda, and its relationships with other sibling species of the *An. gambiae* complex (Diptera: Culicidae). Syst. Entomol. 10: 501-522.

White, G. B. and J. N. Muniss. 1972. Taxonomic value of spermatheca size for distinguishing four members of the *Anopheles gambiae* complex in East Africa. Bull. W. H. O. 46: 793-799.

Zahar, A. R., M. Hills and G. Davidson. 1970. An attempt to group freshwater species of the *Anopheles gambiae* complex by some morphological larval and adult characters. Parassitologia (Rome) 12: 31-46.

TABLE 1. Details of collections of members of the Anopheles gambiae group of species.

Species	Locality	Map Reference	Collection Methods	No. wild Adults	No. wild Larvae	Total No. Adults
gambiae	Mahongo, Namibia	18°05'S, 21°45'E	Resting indoors	7		48
	Yaka Yaka, Congo	4°22'S, 15°09'E	" "	44		135
	Grand Comoros	11°40'S, 43°16'E	Larval collection		115	60
				51	115	243
arabiensis	Pelindaba, Zululand	27°05'S, 32°33'E	Man-baited net	17		29
			Cattle kraal	7		7
			Biting man outdoors	6		6
	Tetepan, Zululand	27°02'S, 32°15'E	Knockdowns	2		2
	Komatipoort, Transvaal	25°26'S, 31°56'E	Biting man outdoors	2		8
			Pit collection	2		9
	Jaffray, Transvaal	23°50'S, 30°20'E	Biting man outdoors	1		5
	Nsoro, Swaziland	26°40'S, 31°56'E	" " "	3		12
	Big Bend, Swaziland	26°49'S, 31°56'E	" " "	1		1
	Mahongo, Namibia	18°05'S, 21°45'E	Resting indoors	3		9
	Kanyemba, Zimbabwe	15°40'S, 30°20'E	Biting man outdoors	30		30
				74		118
merus	Kosi Bay, Zululand	26°55'S, 32°55'E	Cattle kraal	1		10
	Opansi, Zululand	27°34'S, 32°18'E	" "	39		105
			Biting man outdoors	3		13
	Makanis Drift, Zululand	27°02'S, 32°19'E	Pit collection	2		20
			Cattle kraal	9		9
	Shemula, Zululand	27°05'S, 32°17'E	Pit collection	2		20
			Cattle kraal	2		20
	Pelindaba, Zululand	27°05'S, 32°33'E	Man-baited net	1		1
	Tugela River Mouth, Zululand	29°20'S, 31°20'E	" " "	1		5
	Nkunduzi, Zululand	27°50'S, 32°10'E	Pit collection	14		18
	Soutini, Transvaal	23°26'S, 30°54'E	Biting man outdoors	3		22
			Larval collection		3	3
	Eiland, Transvaal	23°35'S, 30°31'E	" "		4	4
				77	7	250
quadriannulatus	Constantia, Transvaal	23°35'S, 30°35'E	Pit collection	4		37
	Hoogmoed, Transvaal	23°20'S, 30°10'E	Cattle kraal	1		10
	Komatipoort, Transvaal	25°26'S, 31°56'E	Man-baited net	5		39
			Pit collection	8		60
	Dzumeri, Transvaal	23°48'S, 30°10'E	Larval collection		2	2
	Soutini, Transvaal	23°26'S, 30°54'E	" "		6	6
	Masisi, Transvaal	23°10'S, 30°35'E	" "		9	9
	Shemula, Zululand	27°05'S, 32°17'E	Pit collection	2		20
	Opansi, Zululand	27°34'S, 32°18'E	Cattle kraal	4		11
			Pit collection	1		1
	Makanis Drift, Zululand	27°02'S, 32°19'E	" "	3		26
			Cattle kraal	5		5
			Larval collection		12	12
	Umfolozi River, Zululand	28°20'S, 32°20'E	" "		19	19
	Pelindaba, Zululand	27°05'S, 32°33'E	Cattle kraal	2		2
	Kanyemba, Zimbabwe	15°40'S, 30°20'E	Biting man outdoors	1		1
				36	48	260

Table 2. Average numbers and lengths of characters examined on four members of the Anopheles gambiae group. P = data from the present study. Sample size is in parentheses.

Character	gambiae	arabiensis	quadri-annulatus	merus	Source
Palp index	0.76(24)	0.79(30)	0.79(52)	0.88(21)	P
	0.76(517)	0.75(259)		0.87(263)	Coluzzi, 1964
	0.76(713)				Bryan, 1980
			0.79(29)		Paterson, 1968
			0.75(30)		Ribeiro, 1980
% 4-banded Palps	0 (164)	4.6(86)	26.9(115)	75.2(149)	P
			4.58(88)		Paterson, et al. 1963
				84.8(46)	Paterson, 1968
Coeloconic Sensilla					
Seg. 1	2.3(65)	2.6(41)	2.5(47)	4.3(44)	P
	2.73(224)	2.76(192)	3.18(33)	4.36(76)	Ismail & Hammoud 1968
Seg. 2	3.6	4.2	3.6	5.2	P
	3.92	4.02	4.24	5.11	Ismail & Hammoud 1968
Seg. 3	4.1	4.4	4.2	6.0	P
	4.43	4.56	4.61	5.62	Ismail & Hammoud 1968
Seg. 4	3.4	4.1	3.7	5.7	P
	3.78	3.9	3.85	5.05	Ismail & Hammoud 1968
Seg. 5	2.9	3.7	3.8	5.2	P
	3.41	3.48	3.06	4.55	Ismail & Hammoud 1968
Seg. 6	2.3	3.2	2.8	4.1	P
	2.62	2.98	2.52	3.76	Ismail & Hammoud 1968
Seg. 7	1.9	2.1	2.4	3.1	P
	2.06	2.24	2.18	2.62	Ismail & Hammoud 1968
Seg. 8	0.5	0.9	0.6	0.4	P
	0.51	0.27	0.42	0.46	Ismail & Hammoud 1968
Total no. Coeloconic Sensilla	21.2(65)	26.2(41)	24.8(47)	34.5(44)	P
	24.42(224)	25.44(192)	24.39(33)	32.12(76)	Ismael & Hammoud 1968
			24.39(27)		Paterson, 1968
Hind leg joint 3/4	0.07(299)	0.08(109)	0.14(155)	0.14(243)	P
	0.07(10)	0.08(10)	0.13(20)	0.11(10)	White, 1985
Pupal Setae					
10-C	2.3(91)	2.77(65)	2.42(60)	2.86(59)	P
4-I	6.08(83)	6.04(55)	6.67(51)	5.41(58)	P
5-I	2.29(85)	2.69(58)	2.82(56)	1.98(51)	P
7-I	4.37(79)	4.64(56)	5.09(53)	5.96(55)	P
4-II	5.19(93)	5.38(61)	6.34(58)	4.48(62)	P
6-III	2.29(87)	2.96(69)	2.78(50)	2.42(52)	P
6-IV	1.69(80)	1.54(67)	1.71(49)	1.62(52)	P
3-V	1.12(93)	1.28(68)	1.02(59)	1.15(60)	P
6-V	1.62(91)	1.58(69)	1.53(49)	1.2(55)	P
3-VI	1.09(94)	1.11(72)	1.1(62)	1.02(61)	P
7-VII	1.18(97)	1.28(69)	1.02(59)	1.02(58)	P

Table 2. (Cont.)

Character	gambiae	arabiensis	quadri-annulatus	merus	Source
Pupal Setae					
4+5, II	16.8(50)	17.03(34)	19.13(31)	14.42(31)	P
	17.84(140)	16.22(100)		13.33(40)	Coluzzi, 1964
			9.84(80)		Ribeiro, 1980
1, III+IV	14.6(50)	16.92(37)	15.39(31)	13.94(31)	P
	13.03(140)	16.32(100)		17.38(40)	Coluzzi, 1964
			6.3(80)		Ribeiro, 1980
2, I+II+III	32.02(49)	31.74(35)	29.74(31)	29.58(31)	P
	31.61(140)	30.51(100)		30.1(40)	Coluzzi, 1964
			15.34(80)		Ribeiro, 1980
2, IV+V+VI+ VII	28.9(49)	28.94(36)	27.35(31)	25.16(31)	P
	26.04(140)	27.5(100)		22.95(40)	Coluzzi, 1964
			13.58(80)		Ribeiro, 1980
4,II + 2,V	23.94(48)	24.61(33)	26.16(31)	20.55(31)	P
+ 10,VII	22.68(140)	23.23(100)		17.73(40)	Coluzzi, 1964
			12.52(80)		Ribeiro, 1980
4,II-2,VII	4.24(49)	4.37(35)	6.65(31)	3.0(31)	P
	5.5(43)	1.1(31)			Reid, 1975a
9,VII-4,II	16.52(50)	17.78(32)	13.48(31)	19.61(31)	P
	13.2(39)	21.0(28)			Reid, 1975a
	20.8(35)	8.7(34)			Reid, 1975b
3,III	2.96(50)	2.55(38)	2.8(30)	4.13(31)	P
	2.5(44)	4.3(27)			Reid, 1975a
Larval Setae					
2-C	9.17(29)	8.84(25)	8.14(36)	9.47(32)	P
	7.89(428)	9.91(292)		9.68(88)	Coluzzi, 1964
			3.31(30)		Ribeiro, 1980
5-C	17.67(60)	18.6(47)	18.73(55)	22.0(59)	P
	17.2(484)	18.56(333)		22.58(102)	Coluzzi, 1964
			13.73(30)		Ribeiro, 1980
6-C	18.86(37)	20.16(37)	20.0(37)	21.73(41)	P
	18.1(495)	19.31(377)		22.6(119)	Coluzzi, 1964
			12.99(30)		Ribeiro, 1980
7-C	21.6(35)	19.92(36)	22.97(32)	22.85(40)	P
	20.08(495)	20.62(383)		24.31(118)	Coluzzi, 1964
			16.29(30)		Ribeiro, 1980
9-C	3.26(34)	2.63(32)	3.71(35)	3.85(34)	P
	3.45(498)	3.06(379)		3.96(93)	Coluzzi, 1964
11-C	70.5(30)	73.06(33)	68.29(28)	71.34(32)	P
13-C	4.3(37)	4.11(38)	4.57(37)	3.98(41)	P
	4.39(488)	4.68(375)		4.81(136)	Coluzzi, 1964
1-P	8.61(61)	9.53(51)	10.42(55)	8.77(61)	P
	7.41(656)	10.74(405)		9.13(151)	Coluzzi, 1964
	6.16(70)	9.4(76)			Reid, 1973
	7.53(549)	9.71(1168)			Zahar et al. 1970
			4.61(30)		Ribeiro, 1980
2-P	13.35(60)	15.22(49)	14.36(58)	14.14(57)	P
	12.96(556)	14.97(391)		14.47(127)	Coluzzi, 1964
			15.21(30)		Ribeiro, 1980
4-P	22.94(31)	21.76(25)	22.59(27)	21.41(49)	P
	20.84(468)	20.41(360)		20.09(106)	Coluzzi, 1964

Table 2. (Cont.)

Character	gambiae	arabiensis	quadri-annulatus	merus	Source
Larval Setae					
1-M	32.54(61)	32.52(54)	33.16(57)	41.02(61)	P
	31.4(473)	33.34(383)		39.83(132)	Coluzzi, 1964
			28.29(30)		Ribeiro, 1980
13-M	7.18(34)	6.27(33)	8.48(33)	6.85(40)	P
1-I	6.44(39)	7.05(37)	6.7(40)	6.66(41)	P
	7.69(491)	9.64(394)		7.88(139)	Coluzzi, 1964
			4.38(30)		Ribeiro, 1980
4-I	6.22(37)	6.62(37)	7.2(40)	5.12(41)	P
9-I	4.82(39)	4.49(37)	4.87(39)	3.40(42)	P
13-I	5.49(39)	5.64(39)	6.06(36)	5.46(41)	P
5-II	4.85(39)	4.64(39)	4.88(41)	4.32(41)	P
9-II	10.53(60)	9.8(56)	10.37(60)	8.0(62)	P
10-II	4.64(56)	3.45(49)	3.97(58)	3.27(59)	P
13-II	6.47(38)	6.08(39)	7.64(42)	6.9(42)	P
2-III	3.18(39)	3.26(38)	3.43(42)	2.95(41)	P
6-III	33.57(23)	33.3(23)	31.82(28)	32.08(36)	P
7-III	4.81(36)	4.72(36)	5.26(42)	4.13(38)	P
9-III	9.4(57)	8.78(55)	8.98(61)	7.15(59)	P
9-IV	7.69(58)	7.98(49)	7.7(56)	5.23(61)	P
9-V	7.05(60)	6.8(50)	6.83(54)	5.16(61)	P
9-VI	6.61(31)	6.75(32)	7.24(34)	5.54(39)	P
	6.75(26)	4.53(28)			Reid, 1973
2-VII	4.87(38)	4.14(35)	4.83(40)	3.9(42)	P
	5.24(505)	5.2(400)		3.92(139)	Coluzzi, 1964
			3.13(30)		Ribeiro, 1980
9-VII	5.85(48)	5.82(44)	6.75(44)	4.95(56)	P
3-VIII	8.69(54)	9.71(41)	9.4(53)	7.7(57)	P
	9.14(73)	6.75(76)			Reid, 1973
5-VIII	4.52(33)	4.27(37)	4.7(40)	4.33(39)	P
	4.93(72)	4.22(77)			Reid, 1973
1-S	4.47(34)	5.29(34)	6.0(32)	4.53(38)	P
	4.85(67)	3.38(75)			Reid, 1973
Egg	0.52(26)	0.50(50)	0.48(40)	0.55(50)	P
Length	0.504(102)	0.499(62)		0.542(60)	Coluzzi, 1964
	0.49(100)			0.575(100)	Paterson, 1962
		0.487(100)	0.474(100)		Davidson *et al.* 1967
				0.557(385)	Paterson, 1964

LEGENDS TO FIGURES

Figure 1. Line drawing showing wing spots which were measured or recorded for presence or absence.

Figure 2. Computer printout of discriminant function analysis of four members of the *Anopheles gambiae* group, with *Anopheles merus* clearly separated on the right.

Figure 3. Computer printout of discriminant function analysis of the three freshwater breeding members of the *Anopheles gambiae* group.

Figure 4. Scatter diagram using the palpal index and the number of coeloconic sensilla showing the separation of *Anopheles merus* from the other three members of the *Anopheles gambiae* group.

Figure 1.

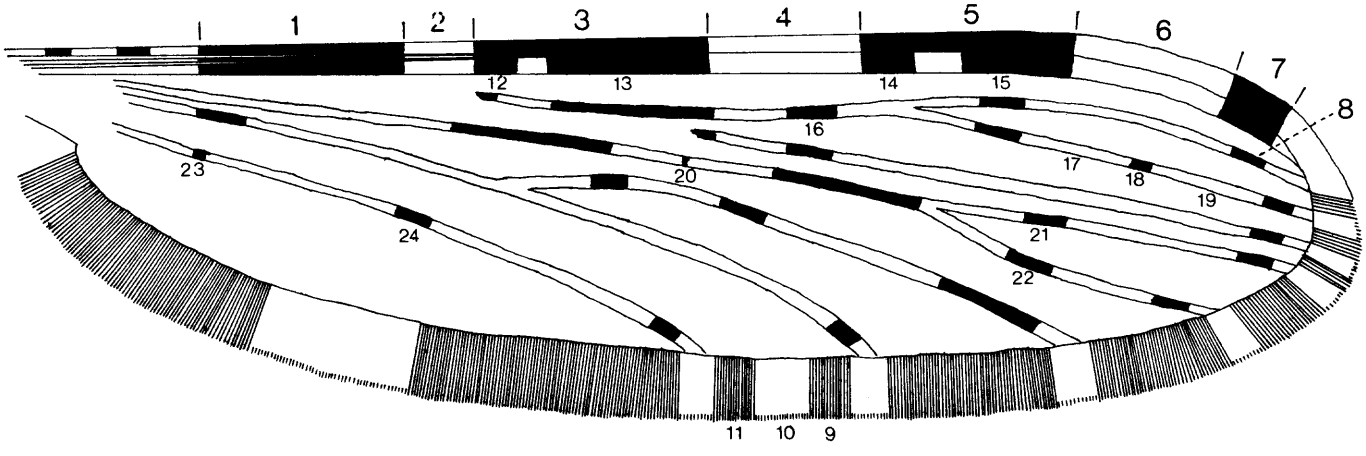


Figure 2.

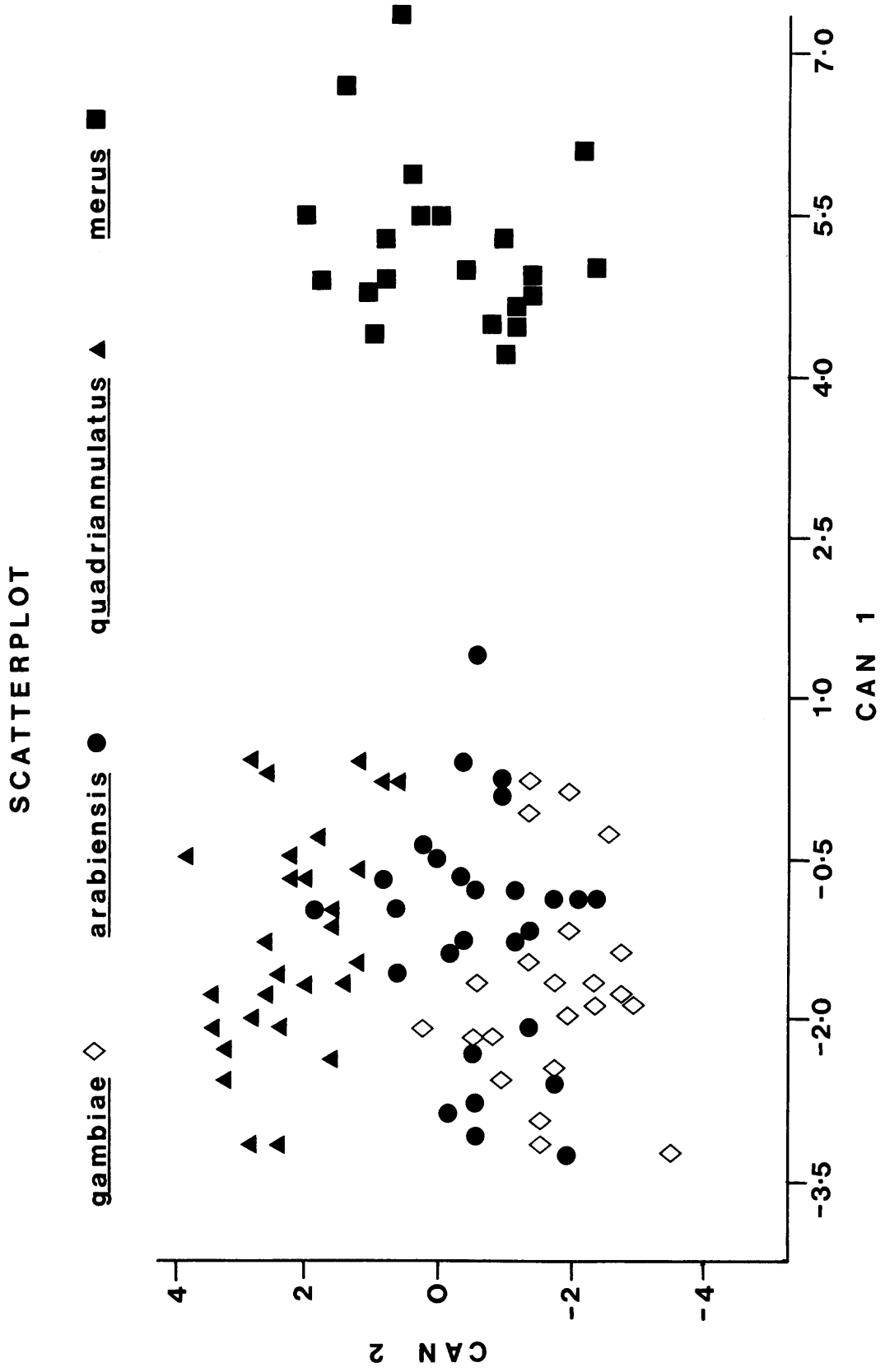

SCATTERPLOT
gambiae ◇ arabiensis ● quadriannulatus ▲ merus ■
CAN 2
4
2
0
-2
-4
CAN 1
-3·5
-2·0
-0·5
1·0
2·5
4·0
5·5
7·0

Figure 3.

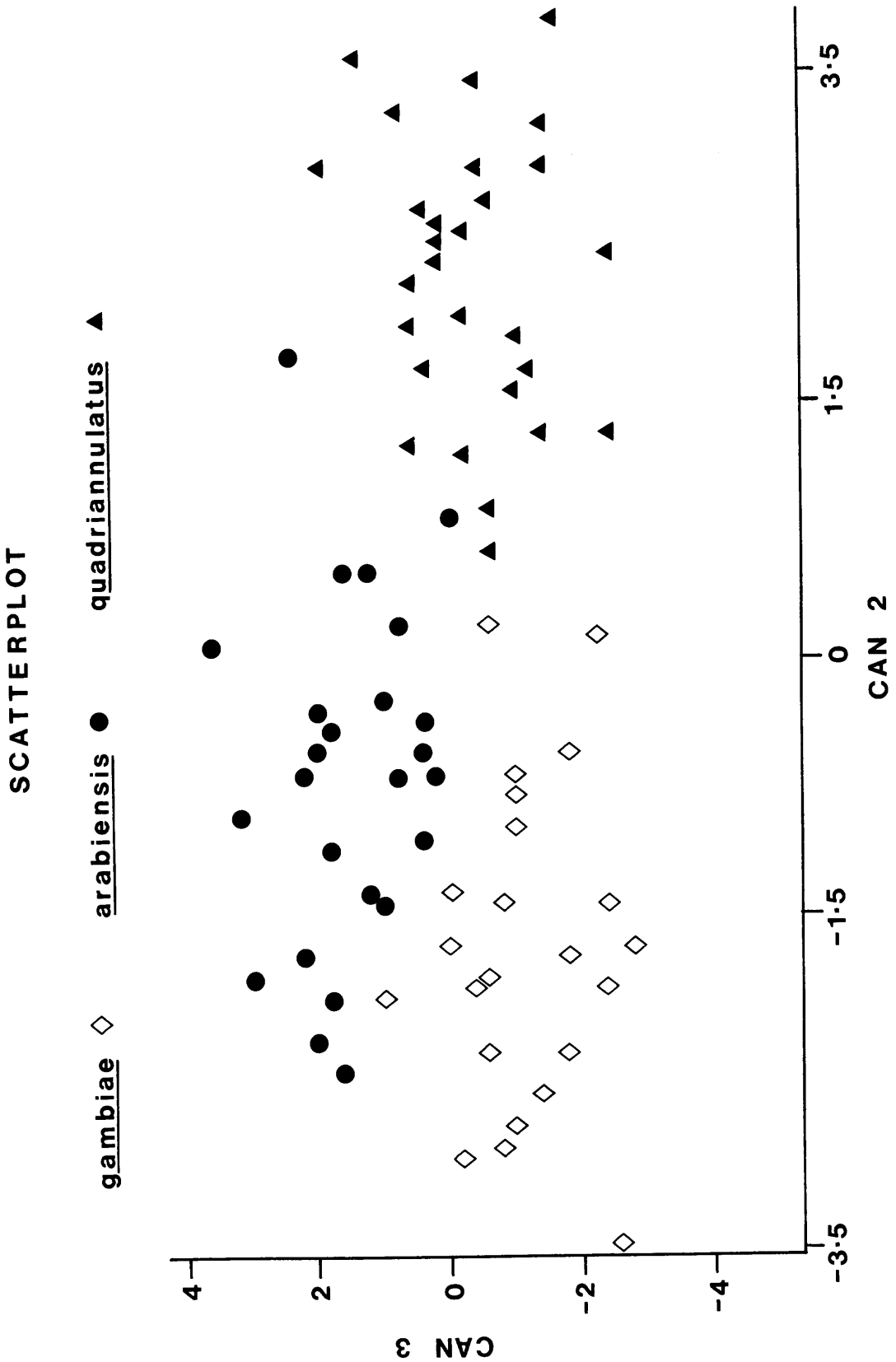
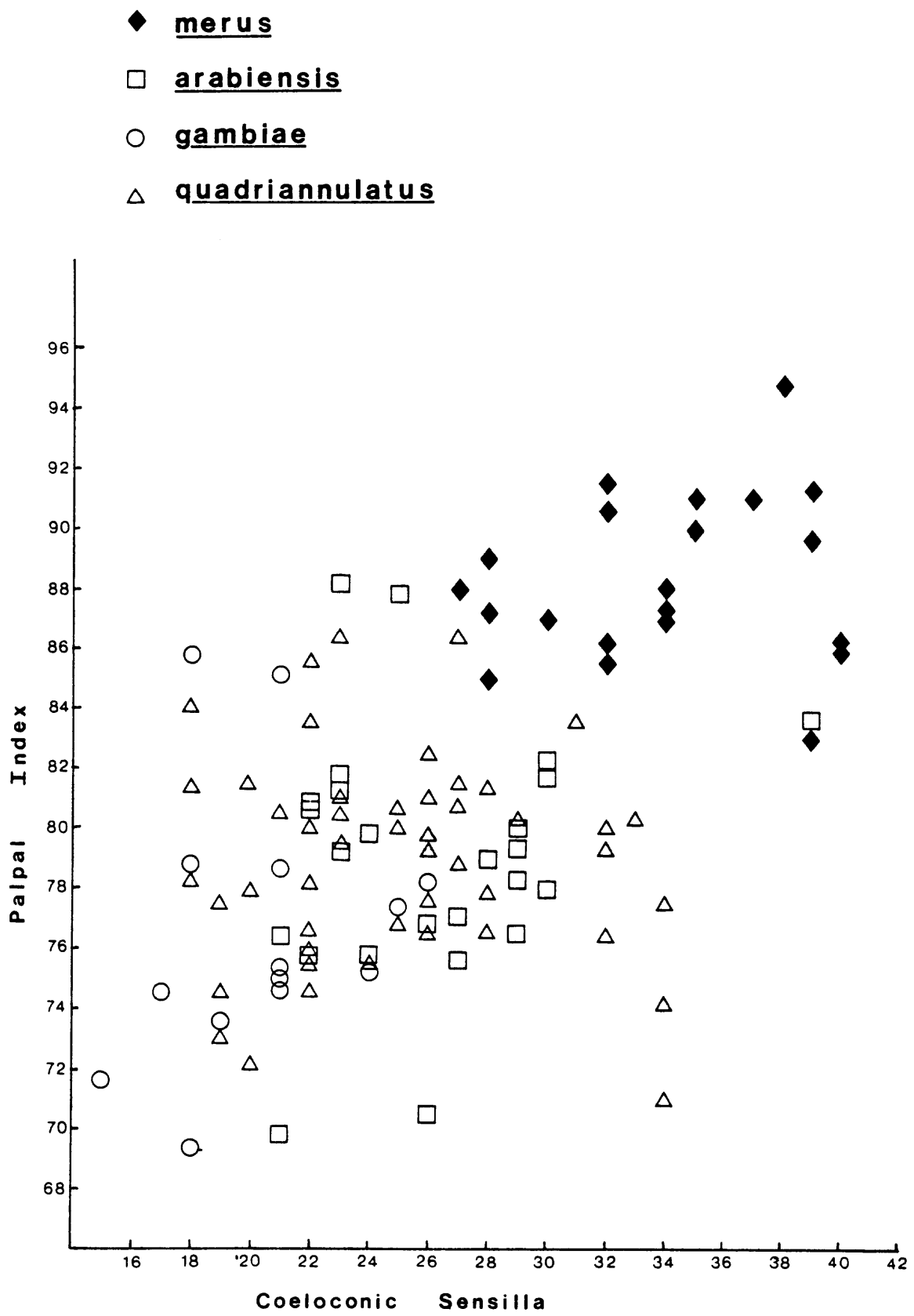

SCATTERPLOT
gambiae
arabiensis
quadriannulatus
CAN 3
CAN 2
4
2
0
-2
-4
3·5
1·5
-1·5
-3·5

Figure 4.


merus
arabiensis
gambiae
quadriannulatus
Palpal Index
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
16
18
20
22
24
26
28
30
32
34
36
38
40
42
Coeloconic Sensilla