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A taxonomic and genetical study of the Anopheles coustani group of mosquitoes in Southern Africa.

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Species	Locality	no. families bred	no. adults pinned	Collection methods
coustani B	Johannesburg, Tvl.	1	13	Inside house
	Benoni, Tvl.	3	12	Biting man outdoors
		4	25	
ziemanni A	Thankerton, Tvl.	1	6	Pit collection
	Potgietersrus, Tvl.	10	79	Biting man outdoors
	Grey Stones, Tvl.	3	25	" " " , man-baited net
	Jaffray, Tvl.	2	19	" " "
	Pusela, Tvl.	3	20	" " "
		19	149	
ziemanni B	Mahongo, Namibia	34	203	Cattle kraal
		34	203	
	TOTAL:	114	743	

APPENDIX II

DETAILED RECIPES FOR THE STAINS AND BUFFERS USED IN THE ELECTROPHORETIC ANALYSIS.

Electrode buffer: 6.2g boric acid, 20g Tris, 2g EDTA made up to 1 litre with distilled water and adjusted to pH8.6 with saturated boric acid (modified Barlow & Ridgway, 1971).

Gel buffer: 90ml electrode buffer made up to 300ml with distilled water and adjusted to pH8.2 with saturated boric acid.

Tris/Mal buffer: 1M Tris adjusted to pH6.4 with 1M maleic acid.

Tris/Hcl buffer: 1M Tris adjusted to pH8.5 with concentrated hydrochloric acid.

Phosphate buffer: 0.2M Sodium hydrogen phosphate adjusted to pH7.3 with 0.2M hydrochloric acid.

Esterase (Est): 50mg Fast Blue RR, 50ml 0.1M Tris/Mal buffer pH6.4, 4ml 1% alpha-naphthylacetate in acetone. Refridgerate for 1 hour or until bands appear.

Octanol dehydrogenase (Odh) and superoxide dismutase (Sod): 25mg NAD, 20mg NBT, 100ml 0.05M Tris/Hcl buffer pH8.5, 1.0ml ethanol, 0.2ml octanol. Incubate for 1 hour at 37°C. Add 5mg PMS. Blue in sunlight. Leave overnight (modified Mahon et al, 1976).

Glutamic-oxaloacetic transaminase (Got): 60mg 2-oxoglutaric acid, 100mg L-aspartic acid, 120mg Fast Blue RR, 50ml o.2M phosphate buffer pH7.3. Warm buffer to 37°C. Make a paste

of the dry ingredients then add buffer. Incubate at 37°C for 20 minutes or until brown bands appear (modified Nichols & Ruddle, 1973).

Lactic dehydrogenase (Ldh): 20mg NAD, 12 mg NBT, 1mg PMS, 40ml substrate solution (40g sodium lactate, 1.2g sodium cyanide, 119mg magnesium chloride, 250ml 0.05M phosphate buffer pH7.5), 10ml distilled water. Incubate at 37°C until dark bands appear.

Xanthine dehydrogenase (Xdh): 20mg NAD, 12mg NBT, 1mg PMS, 20mg hypoxanthine, 50ml 0.05M Tris/Hcl buffer pH7.1. Incubate at 37°C until dark bands appear (Shaw & Prasad, 1970).

Alpha-glycerophosphate dehydrogenase (∝Gpdh): 20mg NAD, 12mg NBT, 1mg PMS, 90mg di-sodium EDTA, 100mg ∝glycerophosphate, 50ml 0.05M Tris/Hcl buffer pH8.5. Incubate at 37°C until bands appear.

Isocitric dehydrogenase (Idh): 75mg isocitric acid, 20mg NADP, 12mg NBT, 1mg PMS, 10 drops 10% magnesium chloride, 50ml 0.05M Tris/Hcl buffer pH8.5. Incubate at 37°C until bands appear.

Malic dehydrogenase (Mdh): 20mg NAD, 12mg NBT, 1mg PMS, 5ml 2M neutralised malic acid, 50ml 0.05M Tris/Hcl buffer pH8.5. Incubate at 37°C until bands appear.

Phosphoglucomutase (Pgm): 20mg NADP, 12mg NBT, 1mg PMS, 10 drops 10% magnesium chloride, 25mg glucose-1-phosphate containing glucose 1.6 diphosphate, 25 units glucose-6-phosphate dehydrogenase, 50ml 0.05M Tris/Hcl buffer pH7.1 Incubate at 37°C until bands appear (modified Ayala et al,

1972).

Abbreviations:

NAD - nicotinamide adenine dinucleotide

NADP - nicotinamide adenine dinucleotide phosphate

NBT - nitro-blue tetrazolium

PMS - phenazine methosulphate

EDTA - ethylenediamine tetraacetic acid.

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APPENDIX III

A SUMMARY OF THE STUDIES DONE ON THE IMMATURES OF A.COUSTANI, A.ZIEMANNI AND A.TENEBROSUS, USING COMPUTER TECHNIQUES *

No species-specific differences in the immature stages of A."coustani", A."ziemanni" and A.tenebrosus had been previously reported (Gillies & De Meillon, 1968), so it was decided to study the larvae and pupae using the Belkin (1962) system and computer analysis.

Eight characters for 300 pupae and 11 characters for 100 larvae, from many families of the three species, were measured and entered into the computer. The programme used for the discriminant function analysis was SPSS (Nie, et al, 1975) utilizing the stepwise method and running all three groups at once. 99.33% discrimination was obtained from the pupal data and 99% from the larval data. Figs. 28 and 29 are exa les of the computer printouts showing the grouping of the three species.

The heavily branched antennal shaft hair of the larva and the relatively few ribs on the pupal trumpets, make tenebrosus quite distinctive, "coustani" and "ziemanni" being more difficult to separate (table 6).

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Table 6. Keys to the larvae and pupae of A."coustani", A.tenebrosus and A."ziemanni", whereby 91 and 89% correct identification respectively was obtained.

Larva.

1. The sum of seta 1-A, 23 or more branches.....tenebrosus
   The sum of this seta with 22 or less branches.....2

2. The sum of seta 2-I with 14 or more branches...."coustani"
   The sum of seta 2-I with 13 or less branches...."ziemanni"

Pupa.

1. The sum of seta 11-C with 8 or more branches...tenebrosus
   The sum of seta 11-C with 7 or less branches.....2

2. Seta 6-IV simple....."coustani"
   Seta 6-IV with either or both hairs split....."ziemanni"

1 - Anopheles constani

2 - Anopheles tenebrosus * indicates a group centroid

3 - Anopheles ziemanni

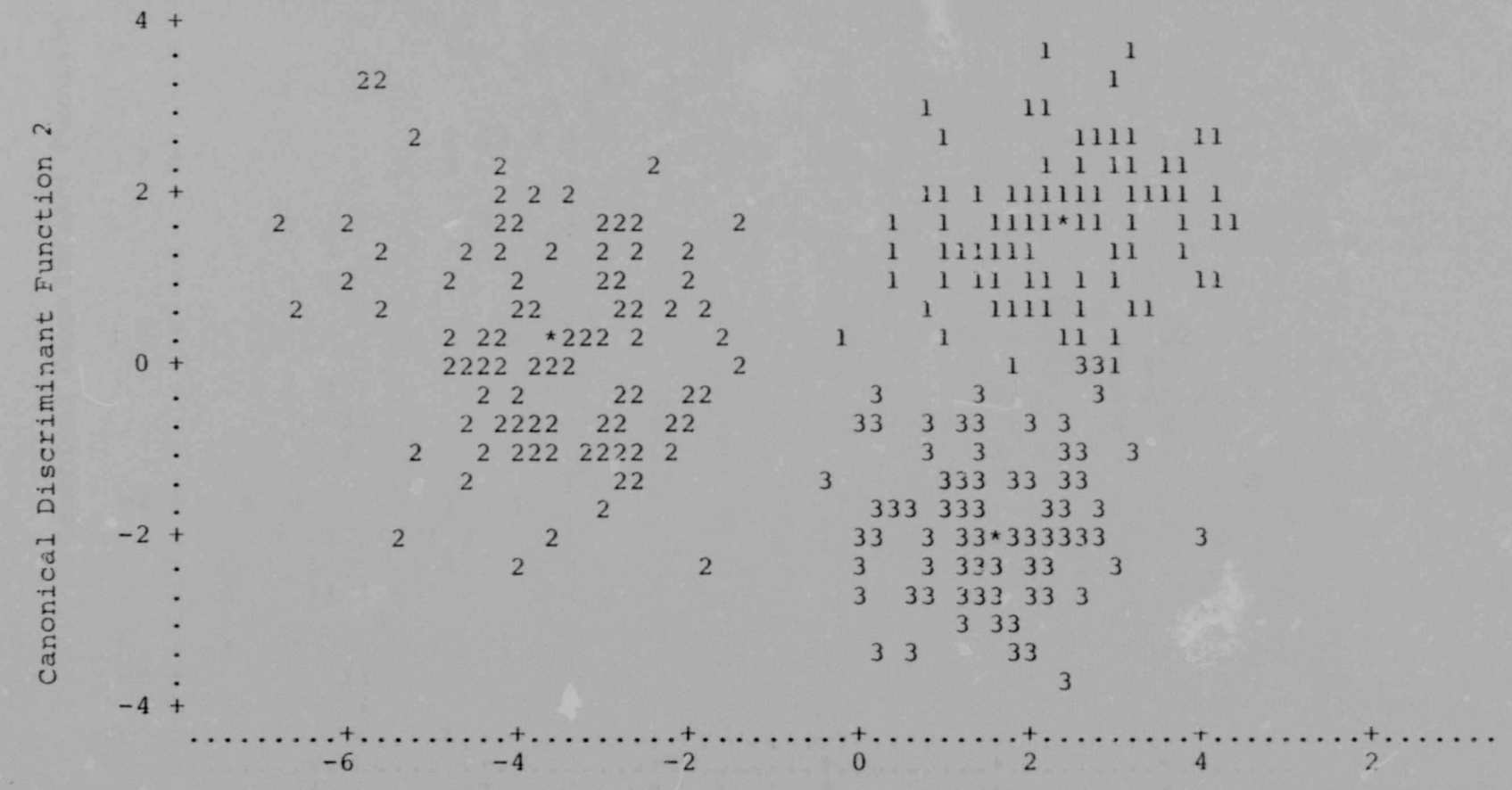
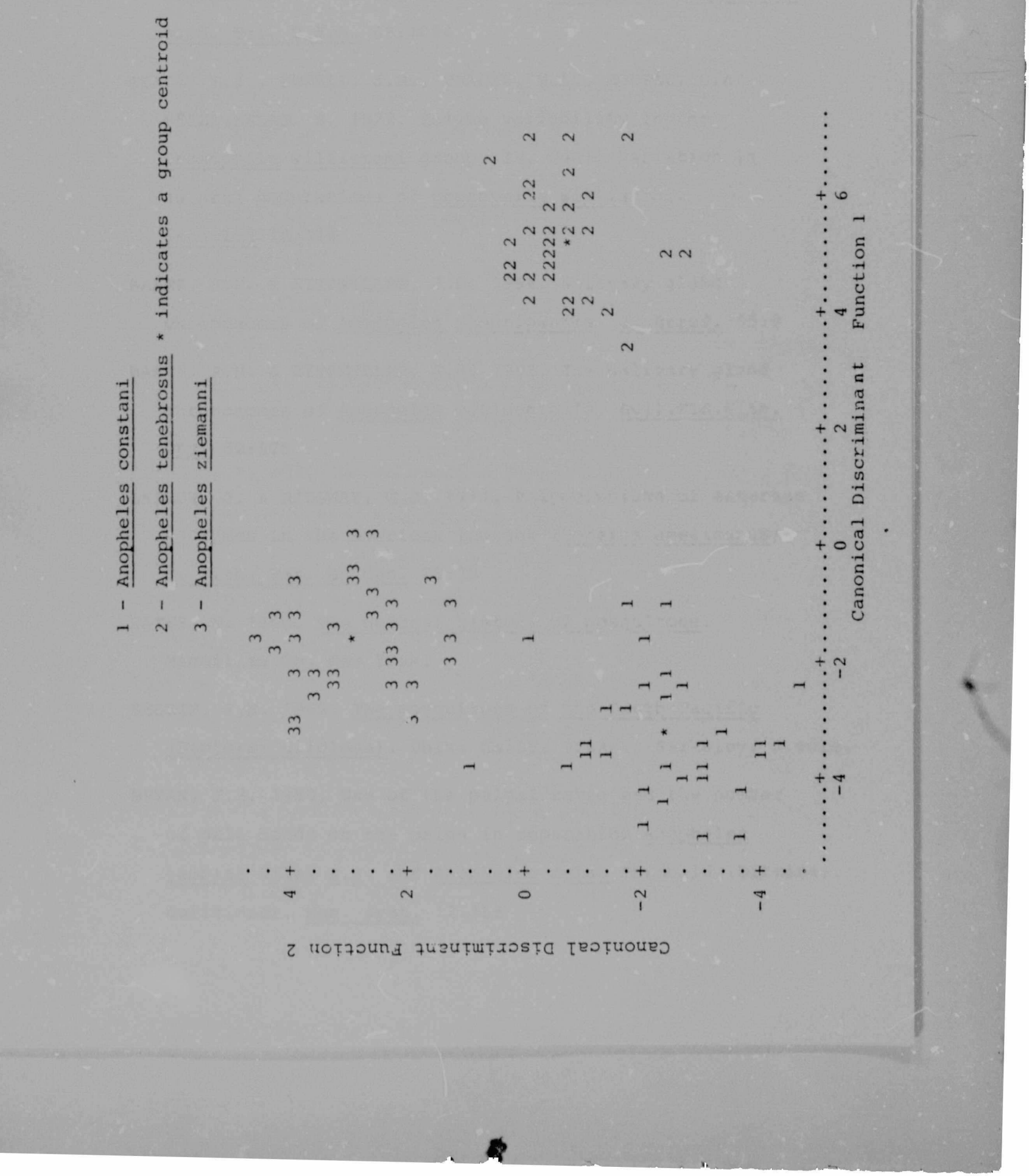


Fig. 28. Scatterplot of the computer analysis of the pupae.

Fig. 29. Scatterplot of the computer analysis of the larvae.



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