5. Title: Developmental changes in the protein constitution of mosquitoes as revealed by disc electrophoresis

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Objective It has been shown that many parasitic invertebrates alter their protein constitution during their successive developmental stages (Desowitz, 1962)*. It was of interest to determine whether mosquitoes exhibit a similar phenomenon in that protein patterns might conceivably be employed as an adjunctive taxonomic aid. Polyacrilamide gel electrophoresis (disc electrophoresis) was selected as the method of analysis since it resolves the protein pool into many more fractions than does the conventional zone technique.

Description Armigeres subalbatus from a laboratory bred colony were used in this study. Various developmental stages were obtained, washed by centrifugation and then thoroughly homogenized in an all—glass tissue grinder. The homogenate was centrifuged and the supernatant collected for disc electrophoretic analysis.

Progress Changes in disc electrophoretic protein patterns from the 2nd instar larva to the adult stage are illustrated in figure 9. It will be seen that the 2nd and 3rd instar larvae contained little of heavier protein of low electrophoretic mobility, the main component had a mobility similar to that of albumin. The proteins of the 4th instar larva were of both the mobile and immobile types. The immbile fraction was composed of two main components with the slightly more mobile component predominating. There was also an increase in the "pre—albumin" fractions. At the pupal stage there was a marked decrease in the albumin—like and "pre—albumin" proteins while the immobile fractions were similar to those of the 4th instar larva. The main change in proteins from pupa to adult was in the immobile fraction. There was a decrease in the more mobile component with a concomittant increase in the immobile protein. A protein that migrated about halfway between the albumin—like protein and immobile component was also increased in the adult stage.

Studies are in progress comparing the patterns of various mosquitoes at their different stages of development. Preliminary results have revealed differences in pattern between adult Armigeres subalbatus and Anopheles stephensi.

* Ann. Trop Med Parasit. 56: 161.

Summary A large number of specimens were added to the mosquito collection during the year, including a new and previously undescribed species of Anopheles belonging to the aitkenii group, An. tigertti, and several species of Aedes not previously known from Thailand. Much additional information on the distribution and ecology of several species has been accumulated. Detailed studies of specific groups including the Anopheles minimus complex and the Mansonia and Coquillettidia species of Thailand were begun.

The susceptibility status of Aedes aegypti and Aedes albopictus from Surat Thani province to various insecticides including the organophosphate, Abate, were established. While resistance to both DDT and dieldrin were observed in these mosquitoes, the complete susceptibility of larvae to low concentrations of Abate was established. Anopheles subpictus adults from Rayong province were found to be susceptible to both DDT and dieldrin, while An. vagus adults from Bang Khen showed an increase in vigour tolerance to DDT and complete resistance to dieldrin.

Tests of the gut contents of eight species of Culicine mosquitoes from the Bang Phra arbovirus study site, utilizing the agar–gel diffusion technique, indicated that the majority had fed on either horses or cattle.

Studies of the ecology and control of Aedes aegypti and Aedes albopictus were carried out on the island of Ko Samui as part of an investigation into the epidemiology of dengue hemorrhagic fever. Each of these two species utilized separate but overlapping habitats; the breeding of A. aegypti was confined largely to houses, while A. albopictus oviposited in a wide variety of natural sites outdoors. Fluctuations in population densities of both species were recorded by a variety of techniques over an eight month period during and after the course of an epidemic of dengue hemorrhagic fever. A field trial for the control of A. aegypti was carried out in one village on the island utilizing malathion fog for destruction of adult mosquitoes and treatment of domestic water containers with Abate for larval control. Complete control of A. aegypti was obtained with these methods for approximately 10 weeks.

Distinctive changes in protein patterns between various developmental stages of Armigeres subalbatus were observed by disc–electrophoresis studies

Table 1. Source of blood meals of mosquitoes collected at Bang Phra during 1966–67 as determined by the agar–gel diffusion techniques.

Species	Total number tested	Number positive reactions	Per Cent positives with antiserum	
			Buffalo/Cow*	Horse
		Dairy Farm		
Aedes mediolineatus	10	10	100	–
Aedes vexans	19	19	100	–
Anopheles aconitus	8	8	100	–
Culex fuscocephala	503	496	99	–
Culex gelidus	71	71	100	–
Culex tritaeniorhynchus	380	380	100	–
Mansonia annulifera	24	24	100	–
Mansonia uniformis	43	43	100	–
		Red Cross Horse Farm		
Aedes mediolineatus	13	10	–	100
Aedes vexans	4	4	–	100
Culex fuscocephala	132	128	–	100
Culex gelidus	653	630	–	100
Culex tritaeniorhynchus	478	478	–	100
Mansonia annulifera	11	11	–	100
Mansonia uniformis	62	62	–	100

* Positive ractions with both anti–cow and anti–buffalo sera.

Table 2. Larval habitats of Stegomyia species collected on Koh Samui, 1966–68.

Habitat	Number of times larvae collected from habitat				
	Aedes albopictus	Aedes aegypti	Aedes albolineatus	Aedes vittatus	Aedes annandalei
Artificial containers*	20	42	—	—	—
Coconut shells, husks, bracts	50	6	2	—	—
Tree holes	5	2	2	—	—
Bamboo internodes	8	—	4	—	1
Pandanus axils	6	—	—	—	—
Rock pools**	8	—	—	21	—
Total	97	50	8	21	1

* — Water jars, cans, fuel drums, etc.
** — sea coast — A. albopictus (6), A. vittatus (15)
waterfall — A. albopictus (2), A. vittatus (6)

Table 3. Results of dissections of A. aegypti collected biting man on Ko Samui, 1967–68

Month	No. Dissected	Per Cent Nulliparous[1])	Per Cent Gravid[2])	Per Cent Parous[3])
August	21	14	33	53
September	187	16	52	32
October	239	17	47	36
November	111	16	58	26
December	41	20	51	29
January	124	10	51	39
February	143	11	56	33
March	101	14	36	50

Table 4. Results of dissections of A. albopictus collected biting man on Koh Samui, 1967–68

Month	No. Dissected	Per Cent Nulliparous[1])	Per Cent Gravid[2])	Per Cant Parous[3])
August	62	13	32	55
September	438	11	34	55
October	393	32	12	56
November	578	19	18	63
December	389	27	15	58
January	345	12	14	74
February	102	27	19	54
March	34	16	6	79

1) Females with ovarian follicles in Stage I; no dilatations on follicle pedicels.
2) Females with stage IV or V eggs in ovaries.
3) Females with ovarian follicles in Stage II; one or more dilatations on follicle pedicels.

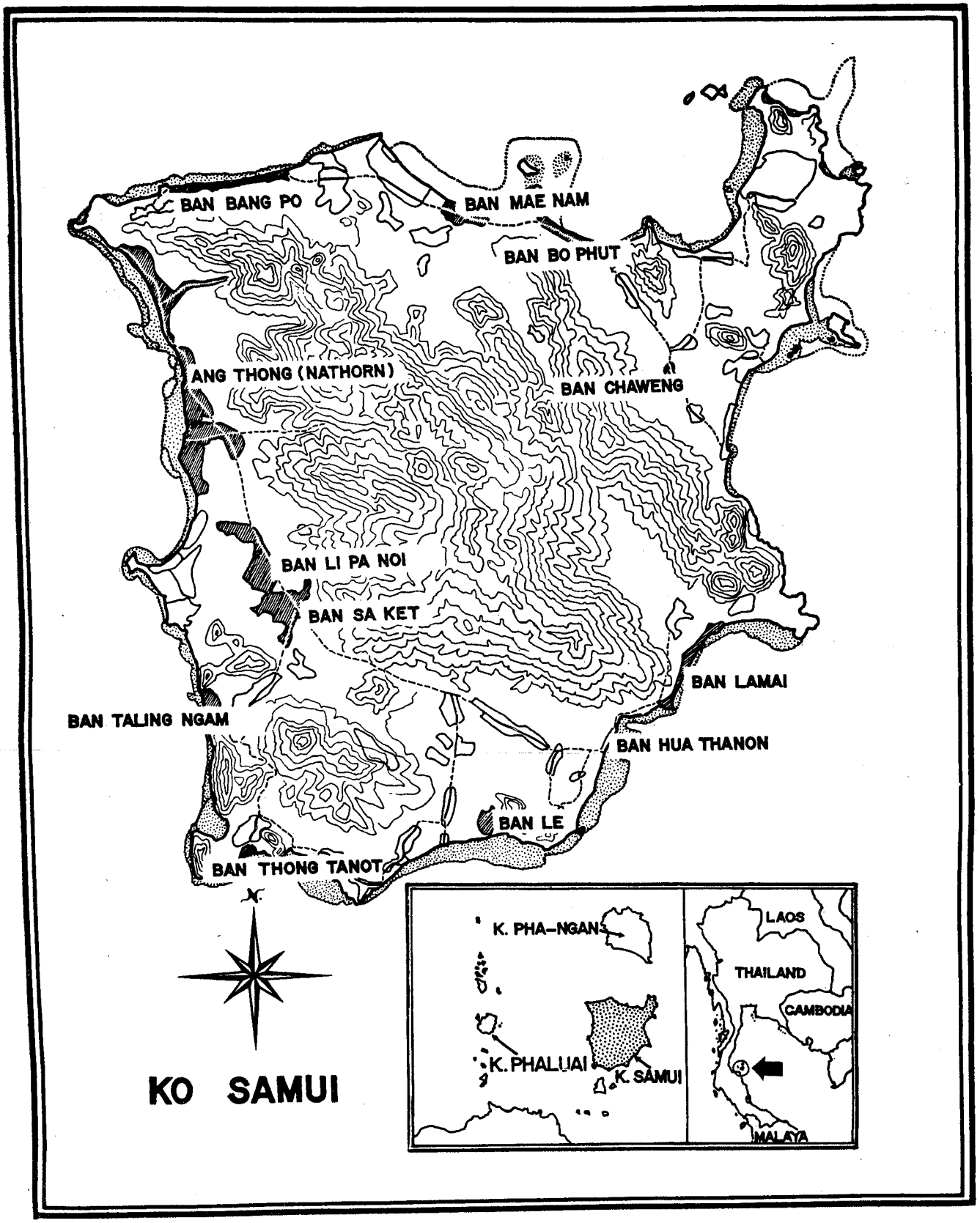

Fig 1. Map of Ko Samui showing location of principal villages.

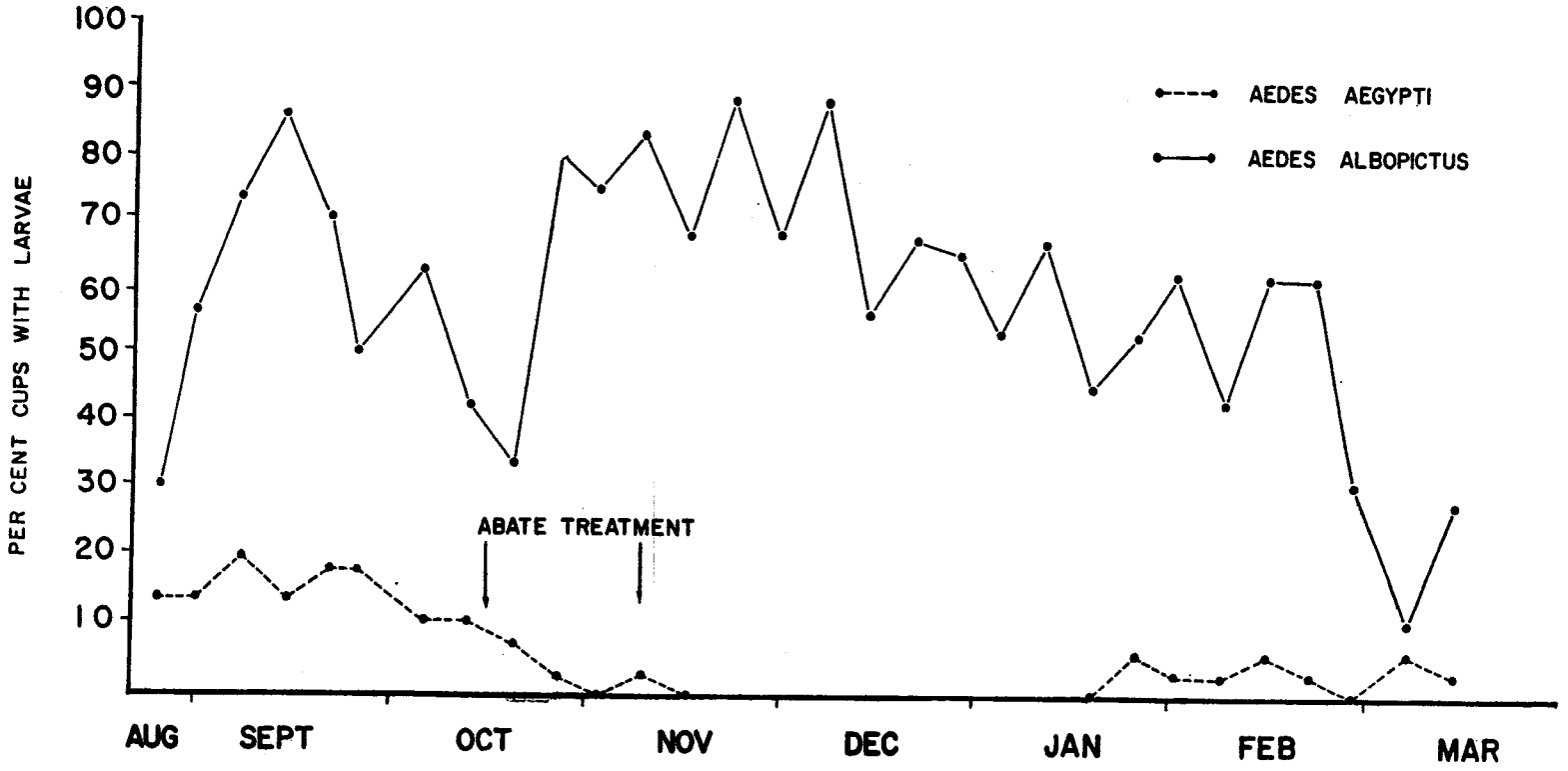

Fig 2. Results of weekly collections of Stegomyia larvae from bamboo cups at Baw Phut, 1967–68.

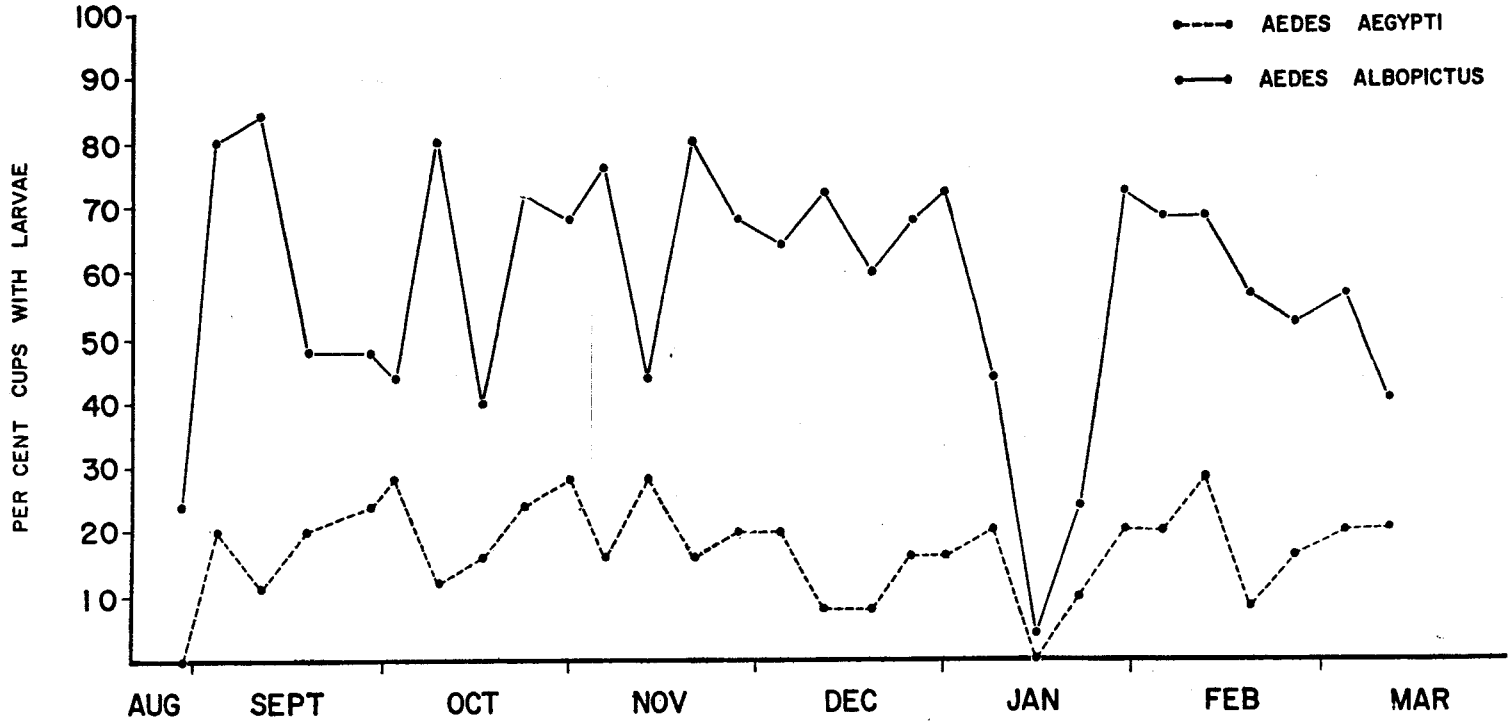

Fig 3. Results of weekly collections of Stegomyia larvae from bamboo cups at Mae Nam, 1967–68.

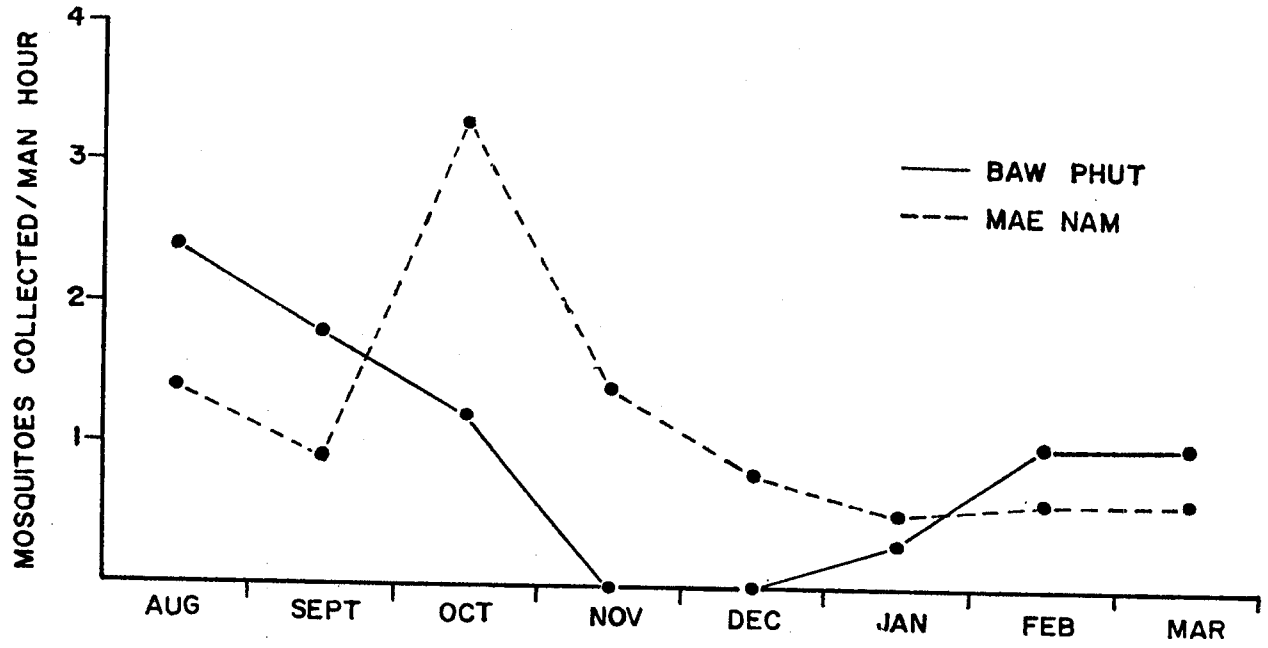

Fig 4. Results of weekly collections of Aedes aegypti biting man at Baw Phut and Mae Nam, 1967–68.

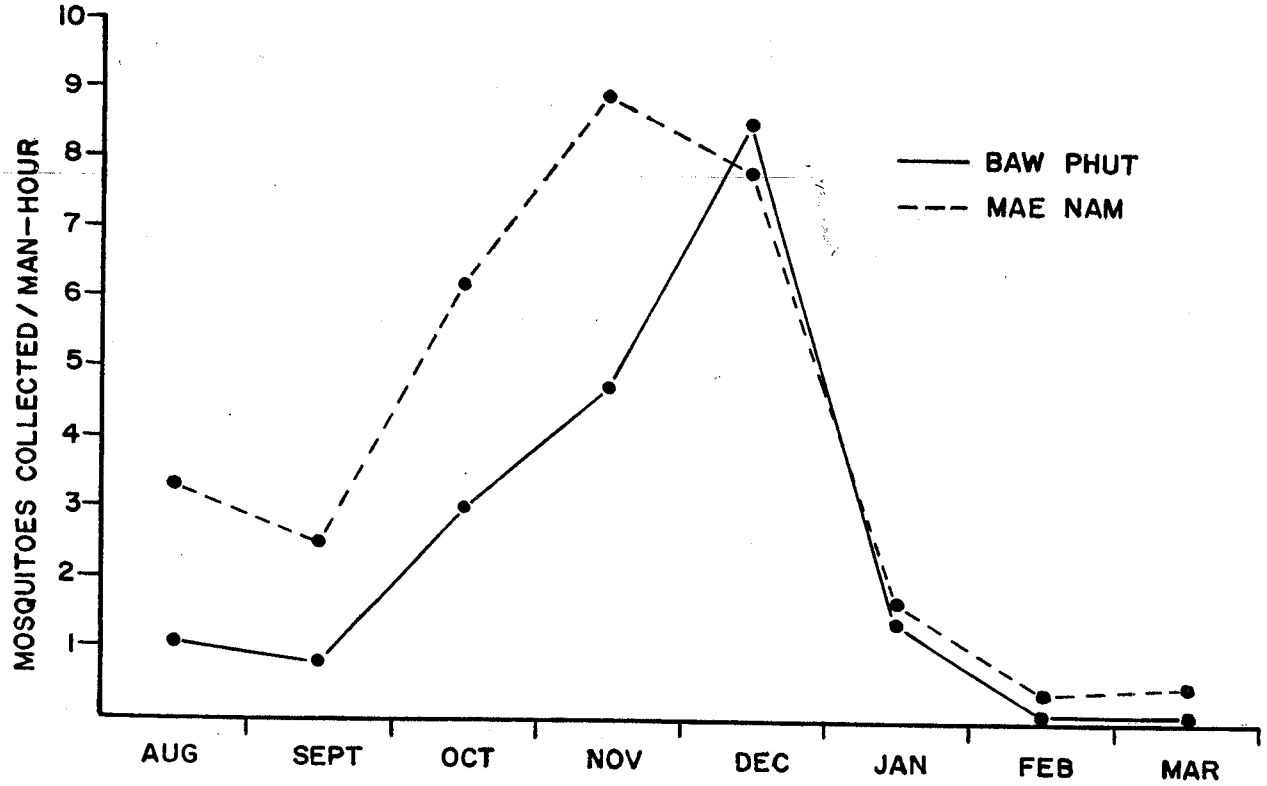

Fig 5. Results of weekly collections of Aedes albopictus biting man at Baw Phut and Mae Nam, 1967–68.

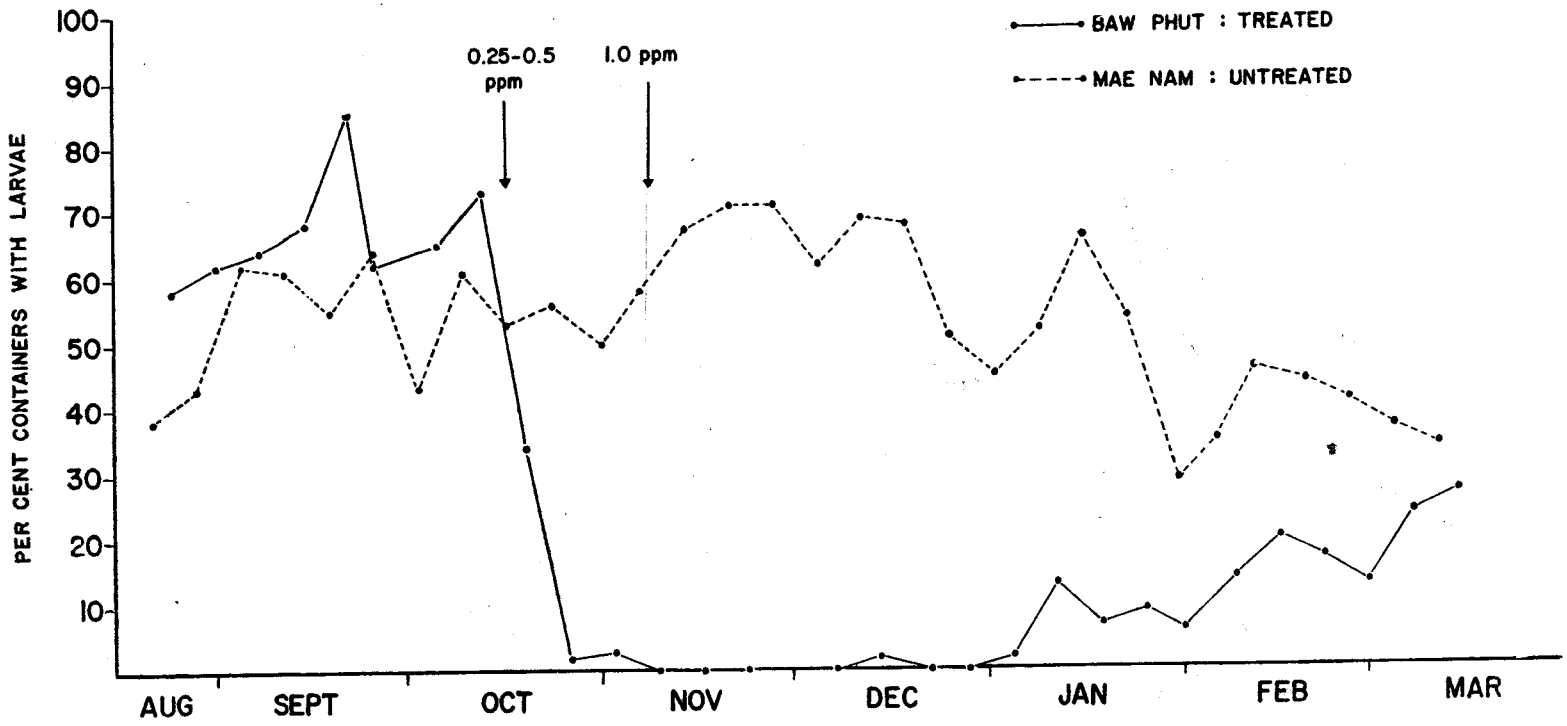

Fig 6. Results of collections of A. aegypti larvae from domestic water containers in Baw Phut and Mae Nam 1967—68.

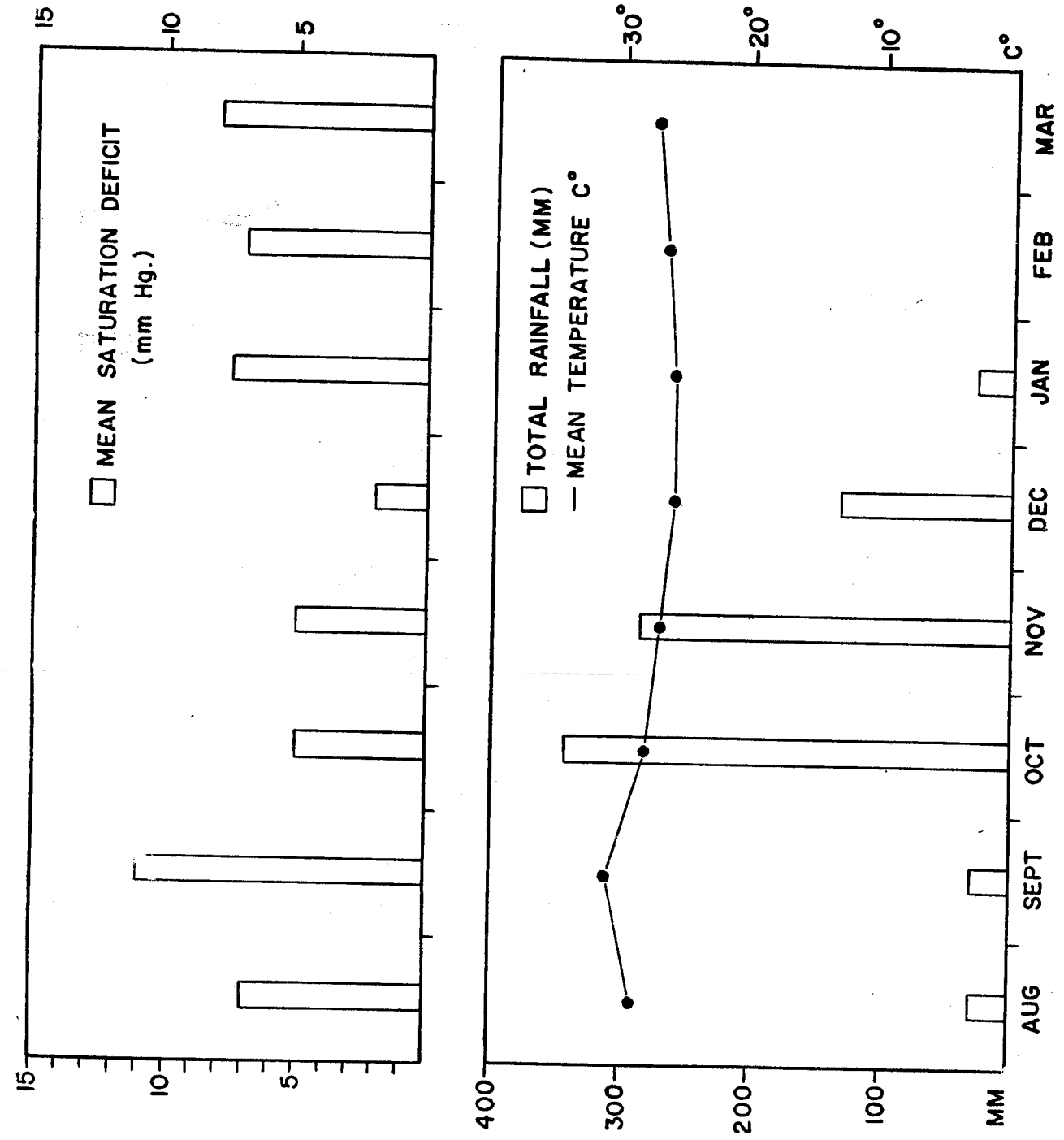

Fig 7. Climatological observations made at Baw Phut, 1967–68.

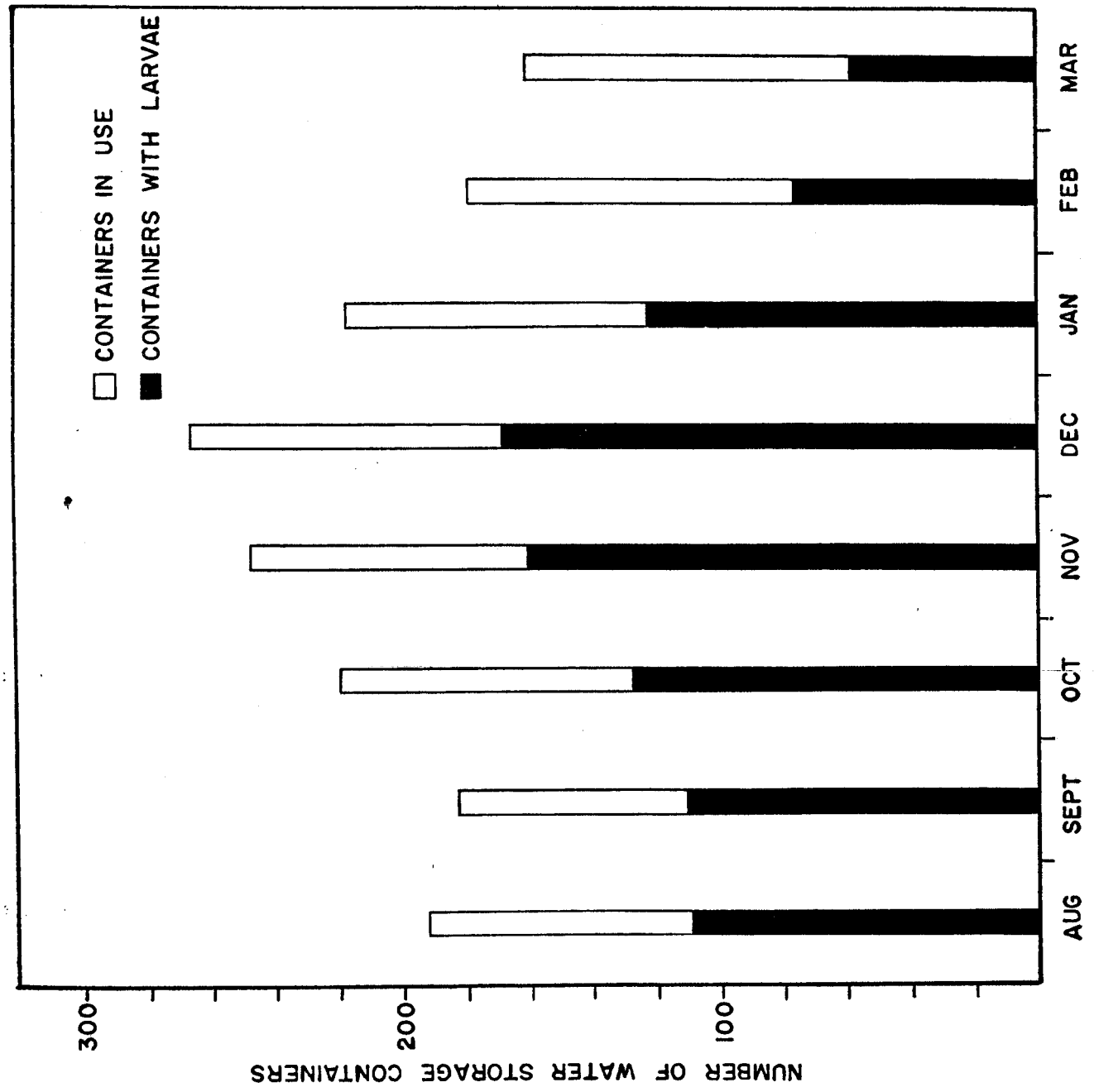

Fig 8. Number of water containers in use and infested with A. aegypti in 60 households on Ko Samui, 1967–68.

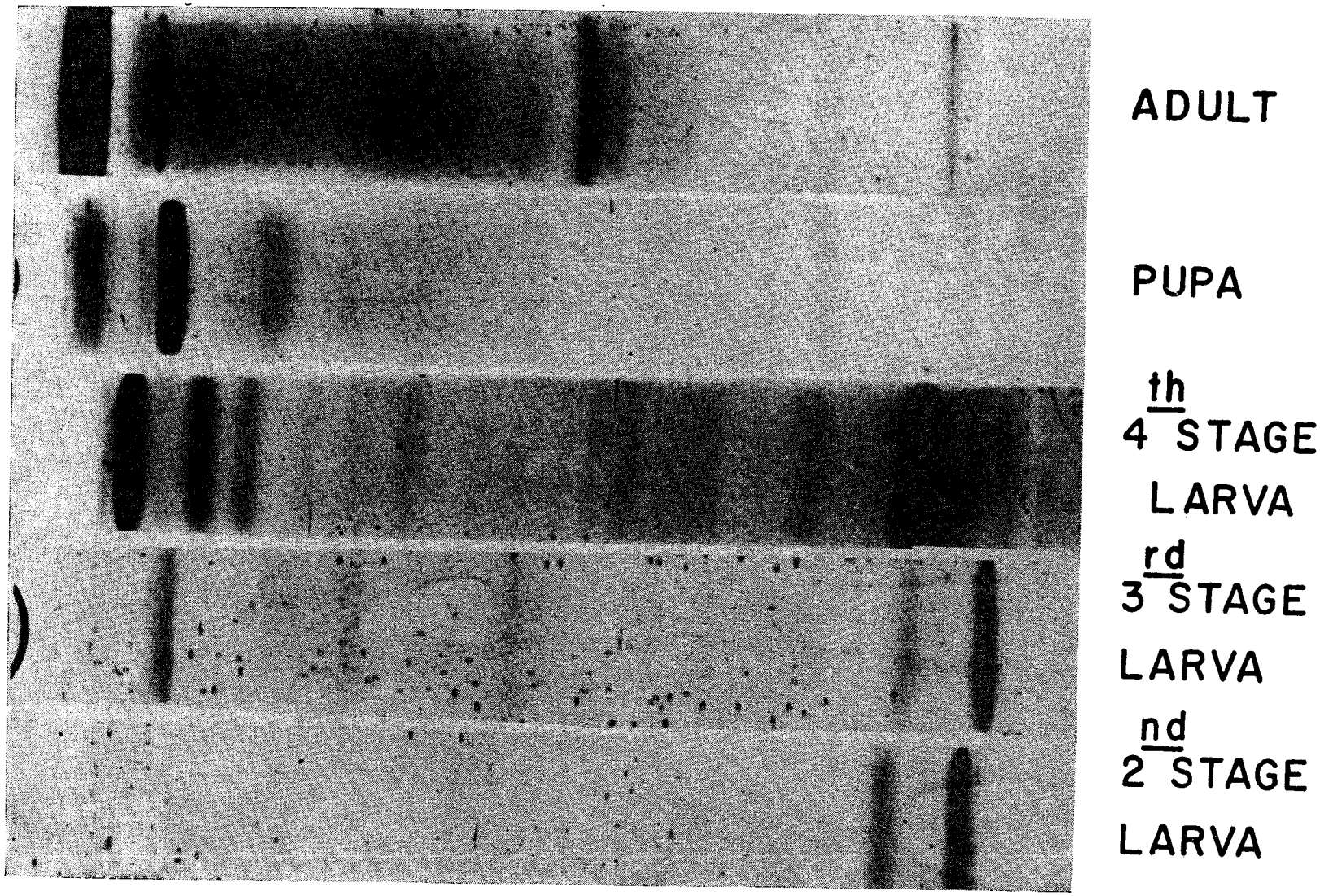

Fig 9. Developmental changes in the protein constitution of Armigeres subalbatus as revealed by disc electrophoresis