VARIATION IN ABDOMINAL COLOR PATTERN IN EIGHT POPULATIONS OF *AEDES AEGYPTI* FROM THE PHILIPPINES

MOTOYOSHI MOGI,[1] TAKAO OKAZAWA,[1] ICHIRO MIYAGI[2] AND LILIAN A. DE LAS LLAGAS[3]

ABSTRACT. Scale patterns of abdominal tergites of 8 populations of *Aedes aegypti aegypti* from the Philippines were quantified (pattern values from 1 to 15 in ascending order of paleness) following McClelland's (1974) system. Many interpopulation variations existed in frequency distributions of pattern values (PV) and mean pattern values (MPV). Females were paler and showed clearer interpopulation differences in coloration than males. Darker (smaller MPV) populations included dark (PV 1–5), medium (6–10), and pale (11–15) specimens in this order of abundance. Paler (larger MPV) populations included as many as or even more pale specimens than dark or medium ones. In the intermediate populations, pale specimens were apparently fewer than dark ones but more than medium ones. In paler and intermediate populations, frequency distributions of PV were more or less bi- or trimodal. The absence of a pale basal band on tergite 2 was common in specimens of PV 1–6 (females) and 1–7 (males).

INTRODUCTION

Aedes aegypti (Linn.) has long been the subject of research due to its importance as a vector of yellow fever and dengue. Mattingly (1957) first recognized that the species is composed of 2 almost allopatric subspecies, *Ae. a. aegypti* and *Ae. aegypti formosus* (Walker), differing from one other in color and ecology at least at the population level. This view was reinforced by McClelland (1974) who studied abdominal color variations for 69 isolates from all parts of the world. The subspecies *formosus* is darker, feral and confined to Africa south of the Sahara; while the nominal subspecies is paler, domestic and distributed widely over the other continents with penetration into the coast of East Africa.

In the last area where the 2 subspecies are sympatric, the intermediate population occurs in the peridomestic habitat (Trpis and Hausermann 1975). This situation fits well with "a tendency for populations closely associated with man to be relatively paler than those less associated with man (McClelland 1974)." However, except for some old observations such as Hill (1921), the tendency has not been substantiated in the geographical regions where only the nominal subspecies is present.

McClelland (1974) found an isolate from Manila, the Philippines, the second palest among 69 isolates examined (the palest one was from Saudi Arabia). Earlier, Machado-Allison and Craig (1972) and Crovello and Hacker (1972) measured desiccation resistance and life table characteristics of an isolate from Cebu, the Philippines, but did not report color variations. This paper describes the color variation in the adults of 8 populations of *Ae. a. aegypti* from the Philippines, and examines whether a correlation can be found between coloration and selected habitat characteristics.

MATERIALS AND METHODS

Table 1 shows the origin of the 8 populations studied. Selected habitat characteristics are indoor versus outdoor, large city versus town, and presence versus absence of dry season. Indoor larval habitats are filled with water by man, while outdoors the water levels are subject mainly to rainfall. With a population in excess of 1.5 million, Manila forms a striking contrast to the 3 other towns (populations less than 100,000). This distinction was supported by observation of the sympatric species of *Stegomyia*, *Ae. albopictus* (Skuse). In Manila, *Ae. a. aegypti* was found exclusively in small container habitats, while the same niche was also inhabited by *Ae. albopictus* in the towns. Climatically, the whole of the Philippines belongs to the tropical zone, and the annual average temperature is ca. 27°C except for the highlands. The rainfall pattern is varied and during the dry season which lasts for ca. 3 months, the breeding of *Ae. a. aegypti* is almost confined to water receptacles filled by man.

Each laboratory population was established from larvae collected in November and December 1981 from a single container or a single type of container located in the same microhabitat except LUMA-1. The last one was established from adult females biting man on the second floor of a large building located in the office quarter near the port. Although larval habitats of this population were not specified, it is highly probable that such

[1] Division of Parasitology, Department of Microbiology, Saga Medical School, Nabeshima, Saga 840–01, Japan.

[2] Laboratory of Medical Zoology, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, Naha, Okinawa 902, Japan.

[3] Department of Parasitology, Institute of Public Health, University of the Philippines System, Manila, The Philippines.

Table 1. Origin of *Aedes aegypti* populations studied.*

Code	Place	Sample size	Breeding place	Micro-habitat	Macro-habitat	Dry season
LUMA-1	Manila, Luzon	25	—	indoors	city	present
LUMA-4	Manila, Luzon	30	flower vase	indoors	city	present
LUMA-2	Manila, Luzon	50	drum	outdoors	city	present
LUMA-3	Manila, Luzon	60	coconut shell	outdoors	city	present
LUTU-1	Tuguegarao, Luzon	25	water pot	outdoors	town	absent
LUTU-2	Tuguegarao, Luzon	20	car battery	outdoors	town	absent
MIKI-1	Kidapawan, Mindanao	50	tire	outdoors	town	absent
PAPU-1	Puerto Princesa, Palawan	100	drum	outdoors	town	present

* All collected as larvae except LUMA-1 as adults.

water receptacles as flower vases and saucers were the main source. The other 3 populations from Manila were collected at a residential quarter ca. 3 km away from the collection site of LUMA-1. Collection sites of LUTU-1 and 2 were located with a distance of ca. 1 km. Adult females emerging from wild larvae were fed on human hands and allowed to oviposit on wet cardboard. In the case of LUMA-1, collected females were exposed to oviposition substrates. Eggs thus obtained were dried, then sent via airmail to Japan and hatched after arrival in tap water. The resultant larvae were reared with dry yeast at 25°C and 15-h photophase.

The first generation adults were anesthetized with chloroform 2–5 days after emergence and examined under stereoscopic microscopes. Scale patterns of abdominal tergites were recorded following McClelland's (1974) system of classification. Each specimen was put into one of 30 pattern grades covering the whole range of abdominal scale pattern variations arranged in alphabetical order from the darkest (Grade F), satisfying the definition of *Ae. a. formosus*, to palest (Grade Q). Pattern grades can be converted into pattern values ranging from 1 (Grade F) to 15 (Grade Q) by McClelland's (1974) scoring system. The absence of pale basal band on abdominal tergite 2, which was recognized by McClelland (1974) for some specimens of PV 1, was also recorded but not considered in the classification and scoring. Sample size was ca. 50 except LUMA-2, for which only 11 (female) and 6 (male) specimens could be examined due to the low hatch rate. Because of this small sample size, LUMA-2 was excluded from statistical tests in the below.

RESULTS

'l the populations could be regarded as *Ae. ypti* on the basis of absence or rarity of iens with pattern value (PV) 1 (Fig. 1). er, interpopulation and between-sex differences in mean pattern value (MPV) and frequency distributions of PV existed.

The mean pattern value (MPV) of males correlated well with that of females ($r = 0.72, P < 0.05$ by t test). Males were darker than females as indicated by smaller MPV and smaller proportion of pale specimens. In females, PV 1 and 15 were encountered in one and 6 populations, respectively. In males, PV 1 was found in as many as 5 populations, while the highest PV was 13 and PV's above 11 were found in only 3 populations. Between-sex differences of frequency distribution of PV were tested (χ^2 test), after grouping PV into 3 major classes, each including PV of 1–5, 6–10, and 11–15. For convenience, these classes are hereafter referred to as dark, medium and pale, respectively. Chi-square was calculated following Brandt and Snedecor's method (Fisher 1970). Between-sex differences in frequency distributions of PV thus proved significant ($P < 0.05$ for MIKI-1, $P < 0.01$ for the rest) except PAPU-1.

Table 2 shows the results of the χ^2 test for interpopulation differences in frequency distributions of PV. In females, 3 groups were recognized, that is, 2 darker populations (MIKI-1, PAPU-1), 3 paler ones (LUMA-3, LUTU-1, LUTU-2), and the intermediates (LUMA-1,

Table 2. Results of χ^2 test for between population differences in frequency distributions of pattern values.*

Strain code	Female	Male
LUMA-1		b
LUMA-4		b
LUMA-3	a	a
LUTU-1	a	a
LUTU-2	a	b
MIKI-1	b	b
PAPU-1	b	b

* Populations indicated by common letters are not significantly different at the level of $P=0.05$.

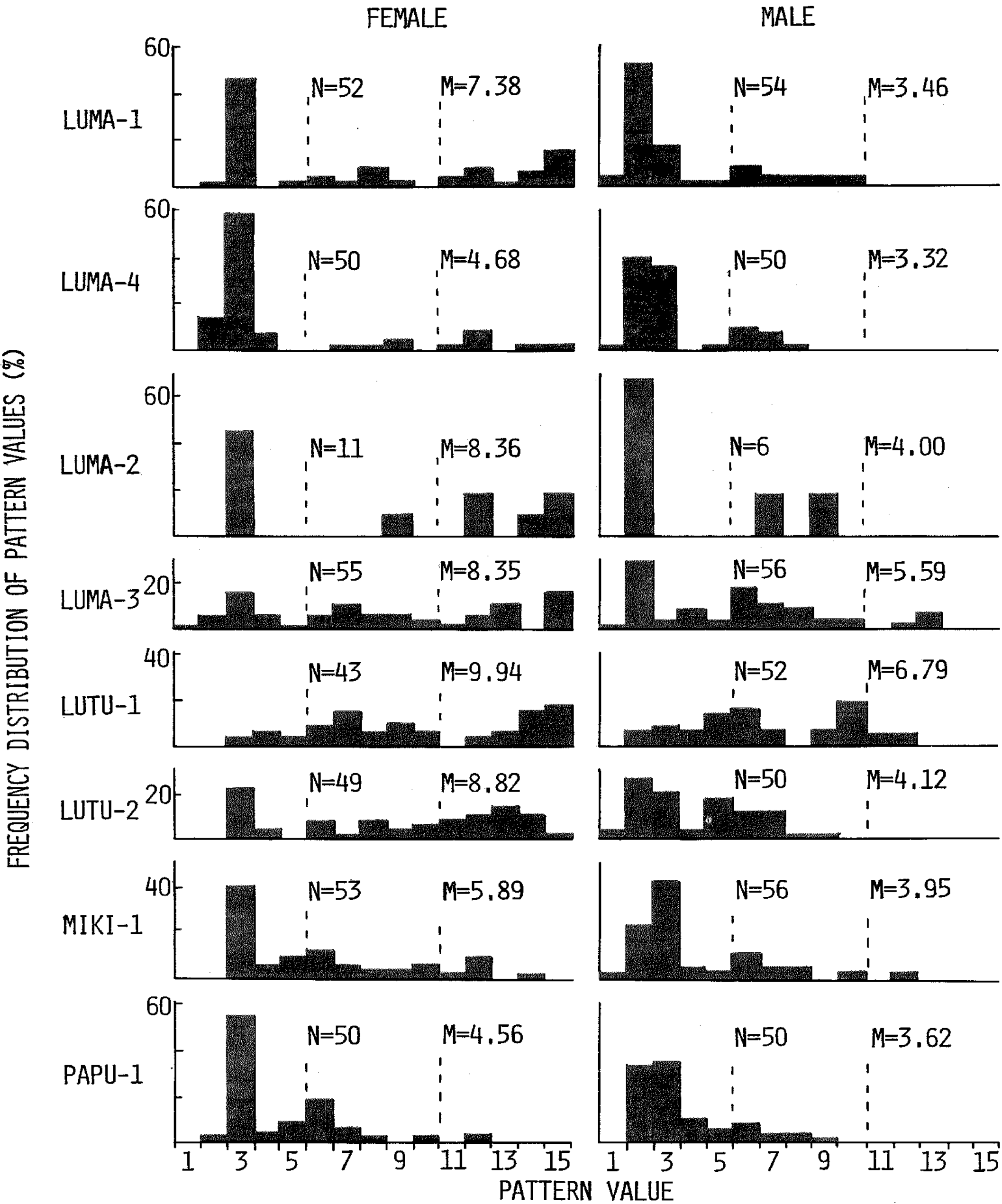

Fig. 1. Frequency distributions (%) of pattern values in 8 populations of *Aedes aegypti* from the Philippines. N: Number of specimens examined. M: Mean pattern value.

LUMA-4). The 2 darker populations, each from Mindanao and Palawan, included dark, medium and pale specimens in this order of abundance without specimens assigned to PV 15. Three paler populations, all from outdoor habitats in Luzon, were characterized by the inclusion of as many as or even more pale specimens than dark or medium ones. Frequency distributions of PV lacked a single distinct peak but were bi- or trimodal. The 2 indoor populations (LUMA-1, LUMA-4) were intermediate between the above 2 types; pale specimens were apparently fewer than dark ones but more than medium ones. Thus, these populations were distinctly bimodal in frequency distributions of PV, a large peak in the dark range and a smaller peak in the pale range. It can be concluded that bi- or trimodality in frequency distribution of PV is usual for *Ae. a. aegypti* in Luzon. As a result of this bi- or trimodality, MPV often fell into troughs of frequency distributions of PV. Thus, MPV or the variation range alone is insufficient for describing color characteristics of such populations.

In males, interpopulation differences were not as clear as in females due to fewer or no pale individuals in all the populations, and 2 groups alone could be recognized. LUMA-3 and LUTU-1 included significantly larger proportions of pale or medium specimens than the others, among which interpopulation differences were not demonstrated. Thus females were better indicators for detecting interpopulation differences in scale pattern of abdominal tergites.

Interpopulation differences in coloration of females could not be correlated with any of selected habitat characteristics. Both of the 2 indoor populations were intermediate, but the outdoor populations were represented by either paler (LUMA-3, LUTU-1, LUTU-2) or darker (PAPU-1, MIKI-1) populations. Manila populations were paler and intermediate, while populations from smaller towns were paler (LUTU-1, LUTU-2) or darker (PAPU-1, MIKI-1). In the regions with a dry season, paler (LUMA-3), intermediate (LUMA-1, LUMA-4) or darker (PAPU-1) populations occurred. From the region without a dry season, paler (LUTU-1, LUTU-2) or darker (MIKI-1) populations were found.

Specimens lacking a pale basal band on tergite 2 were not restricted to PV 1 (Grade F), prevailing from 1 through 7 in males and from 1 through 6 in females (Table 3). However, this phenotype appeared restricted to grades from F through J. According to McClelland's (1974) system, PV 5 includes Grade J_2 and K_0, 6 includes J_3 and K_1, and 7 includes J_4, K_2 and L_0, (alphabets with larger suffixes are paler than the same letter with smaller suffixes); the concerned phenotype did not appear in specimens of K_0, K_1, K_2 and L_0. The total occurrence rate (based on the total number excluding K_0, K_1, K_2 and L_0) was higher in males (78.3%) than in females (11.6%) ($P < 0.01$ by χ^2 test). We interpreted the phenotype as including specimens with a few pale scales never forming a pale band, in addition to those without pale scales at the base of the segment. The former included 21.7% male and 50.0% female specimens lacking a basal pale band on tergite 2. Under the narrow definition which excluded specimens with a few pale scales, the total occurrence was 61.5% in males and 5.7% in females.

DISCUSSION

Some subjectivity is unavoidable in the description of such a continuous color variation as the abdominal scale pattern of *Ae. aegypti*. However, McClelland's (1974) system is clear enough to minimize the observer error, thus permitting comparison between data of various observers. This system was adopted in the

Table 3. Numbers of specimens with tergite 2 lacking a pale basal band/numbers of specimens examined in 8 populations of *Aedes aegypti* from the Philippines.

	Pattern value (and pattern grade)												
	Female						Male						
Strain code	1(F)	2(G)	3(H)	4(J_1)	5(J_2)	6(J_3)	1(F)	2(G)	3(H)	4(J_1)	5(J_2)	6(J_3)	7(J_4)
LUMA-1		1/1	2/24		0/1	0/2	2/2	26/28	8/9	1/1	1/1	2/4	
LUMA-2			1/5					4/4					
LUMA-3	1/1	0/3	0/9	0/3	1/1	0/3	1/1	13/16	2/2	3/5	0/2	2/10	1/4
LUMA-4		0/7	0/29	0/3			1/1	15/20	12/18	0/3			
LUTU-1			1/2	1/3	0/1	1/4		3/3	4/4	3/3	6/6	4/7	1/2
LUTU-2			3/11	1/2		1/4	2/2	12/13	8/10	2/2	9/9	4/6	
MIKI-1			3/21	0/3			1/1	11/13	18/23	2/4			
PAPU-1		0/1	3/27	0/2				15/16	17/17	1/5			

further analysis of color variations of *Ae. aegypti* in Africa (VandeHey et al. 1978), but has not been applied to *Ae. a. aegypti* outside Africa.

The mean pattern value of the Manila females examined by McClelland (1974) reads ca. 11.3 with the range from 4 (Grade J_4) to 15 (Q), paler than all the 8 populations here examined. This difference may partly be due to the fact that he examined specimens from a laboratory colony. The MPV may increase during the laboratory maintenance without intended selection (T. Okazawa and M. Mogi, unpublished data).

McClelland (1974) illustrated frequency distributions of PV for 24 isolates, which he considered are representative of various types of PV distributions. Of the 24 isolates, 16 are from subsaharan Africa and mostly typical *formosus* populations. For the 8 collections of the type subspecies examined from outside Africa, 3 types of frequency distributions were recognized; the one composed exclusively of dark (PV 1–5) specimens (Fiji, Hawaii, Miami), that composed almost exclusively of pale (PV 11–15) specimens (Saudi Arabia) and that including dark, medium and pale specimens in this order of abundance (Colombo, Vellore, Kuala Lumpur, Miami Airport). The last one corresponds to the 2 darker populations from Mindanao and Palawan, the Philippines. Therefore, paler and intermediate populations from Luzon, which show distinct bi- or trimodality in frequency distributions, represent another type.

The genetic basis of abdominal color pattern of *Ae. a. aegypti* is not simple. At least 2 loci, *s* in the linkage group II (McClelland 1962, Craig and VandeHey 1962, Craig and Hickey 1967) and *pa* in I (Craig and Hickey 1967), control the development of pale scaling. It has been considered that a series of alleles exist at both loci. Therefore, it is difficult to calculate gene frequencies from phenotypic frequencies. The frequency distribution of phenotypes is also influenced by temperatures experienced during the larval stage (Craig and VandeHey 1962). It is not known whether the temperature influences the process of pigmentation or acts through differential mortality among cohorts with differential genetic backgrounds. This extrinsic factor, however, was controlled in our study.

We have demonstrated the presence of wide variation in abdominal color pattern among local populations of *Ae. a. aegypti* in the Philippines, but failed to show a correlation between coloration and any of the habitat characteristics examined. Multiple environmental factors other than those examined here may influence the determination of coloration of local *Ae. a. aegypti* populations. Such factors as history of introduction may also be involved. Studies with ample material collected from more localities with various combinations of habitat conditions are essential before any conclusion about the correlation between adult coloration and habitat characteristics can be deduced. Until the completion of such studies, it is felt that a correlation between coloration and domesticity is an interesting but unproved hypothesis insofar as the regions outside Africa are concerned.

Recent electrophoretic studies of allozymes revealed that Asian populations of *Ae. a. aegypti* (India, Indonesia, and Taiwan) are genetically different from those of the New World and East Africa (Powell et al. 1980). Intensive studies on adult coloration might support this view.

ACKNOWLEDGMENTS

The authors are indebted to Dr. D. G. Rivera, Director, Malaria Eradication Service of the Philippines, Drs. M. Tsukamoto, M. Horio and Ms. T. Toma of the overseas research team, and Ms. M. Legaspi, University of the Philippines System, for their cooperation in collecting the material. Their thanks are also due to Dr. Y. Eshita, Teikyo University, for his aid in the literature search, and Dr. B. D. Cabrera, University of the Philippines System, for his review of the manuscript.

Part of this study was supported by the overseas research grant of the Ministry of Education, Science and Culture of Japan in 1981 (56041048, Leader, Prof. I. Miyagi).

References Cited

Craig, G. B. and W. A. Hickey. 1967. Genetics of *Aedes aegypti*, pp. 67–131. *In* Wright, J. W. and R. Pal (eds.). Genetics of insect vectors of diseases. Elsevier, New York.

Craig, G. B. and R. C. VandeHey. 1962. Genetic variability in *Aedes aegypti* (Diptera: Culicidae) I. Mutations affecting color pattern. Ann. Entomol. Soc. Am. 55:47–58.

Crovello, T. J. and C. S. Hacker. 1972. Evolutionary strategies in life table characteristics among feral and urban strains of *Aedes aegypti* (L.). Evolution 26:185–196.

Fisher, R. A. 1970. Statistical methods for research workers. Morikita Publ., Tokyo. 326 pp. (Japanese transl.)

Hill, G. F. 1921. Notes on some unusual breeding places of *Stegomyia fasciatus* Fabr. in Australia. Ann. Trop. Med. Parasitol. 15:91–92.

Machado-Allison, C. E. and G. B. Craig. 1972. Geographic variation in resistance to desiccation in *Aedes aegypti*, and *A. atropalpus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 65:542–547.

Mattingly, P. F. 1957. Genetical aspects of the *Aedes aegypti* problem. I. Taxonomy and bionomics. Ann. Trop. Med. Parasit. 51:392–408.

McClelland, G. A. H. 1962. Genetical control of abdominal scale color in *Aedes aegypti*. Bull. Entomol. Soc. Am. 8:163.

McClelland, G. A. H. 1974. A worldwide survey of variation in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). Trans. R. Entomol. Soc. Lond. 126:239–259.

Powell, J. R., W. J. Tabachnick, and J. Arnold. 1980. Genetics and the origin of a vector population: *Aedes aegypti*, a case study. Science 208:1385–1387.

Trpis, M. and W. Hausermann. 1975. Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark-release-recapture. Bull. Entomol. Res. 65:199–208.

VandeHey, R. C., M. G. Leahy, and K. S. Booth. 1978. Analysis of color variations in feral, peridomestic and domestic populations of *Aedes aegypti* (L.) (Diptera: Culicidae). Bull. Entomol. Res. 68:443–453.