A Dark Unspotted Phenotype of *Anopheles (Cellia) maculatus* Theobald, with Notes on Its Inheritance (Diptera: Culicidae)

Supat Sucharit[1], Bruce A. Harrison[2] and Rampa Rattanarithikul[2]

ABSTRACT. A dark unspotted phenotype of *Anopheles (Cellia) maculatus* Theobald, is described for adult males and females found in a colony of wild type (spotted) *maculatus* (Kuala Lumpur Strain). Morphological differences are tabulated for separating the melanistic phenotype from the wild phenotype. The prevalence of the unspotted phenotype throughout 5 colony generations and data from preliminary crossing experiments between the dark and wild phenotypes are tabulated. Attempts to colonize the melanistic mutant were not successful.

INTRODUCTION

Anopheles (Cellia) maculatus Theobald 1901, is a very colorful and variable member of the Neocellia series, subgenus *Cellia*. As indicated by its name, this species has very distinct white and black spots on the wings and legs and a bright white banding pattern on the tarsomeres and palpus. Considerable phenotypic variations in those patterns and a wide geographical distribution have resulted in the description of a number of currently recognized synonyms (Knight and Stone 1977).

This species is a primary vector of human malaria parasites in Malaysia, but is apparently of less or no importance as a vector throughout the remainder of its distribution. This biological difference has prompted several authors (Christophers 1931, 1933; Reid et al. 1966; Reid 1968) to search for morphological traits that can be used to identify the vector strain. Their analyses of the various palpal and tarsal banding, wing spotting, abdominal scaling and also larval traits, have all resulted in the same conclusion, i.e., these variants belong to one species.

Early in 1977, the senior author detected 2 different adult phenotypes in a colony of *maculatus* at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Besides the normal wild phenotype, a number of adults were darkly pigmented without palpal, wing or leg markings. Initially, a mixed colony of 2 different species was suspected, and preliminary morphological examinations using published keys indicated the

[1]Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

[2]Department of Medical Entomology, U.S. Army Medical Component-AFRIMS, APO San Francisco 96346 (or International mail - Rajvithi Road, Bangkok 4, Thailand).

dark phenotype was similar to certain unicolored members (e.g. *sintonoides*) of the subgenus *Anopheles*. Subsequently, selection and hybridization studies were conducted with the 2 phenotypes until the entire colony of *maculatus* unexpectedly expired in late 1977. Based on these experiments the senior author reported the dark specimens as mutants of *maculatus* (1977 Mahidol University Annual Research Abstracts). Fortunately, melanistic specimens had been preserved and were available for a closer morphological examination by the junior authors in 1978. This examination confirmed the opinion of the senior author, that these specimens belong in the subgenus *Cellia*, the Neocellia series (see Taxonomy section), and are melanistic representatives of *maculatus*. Since genetically abnormal specimens of anophelines are rarely encountered and/or recognized, and then reported in the literature, a description of these melanistic specimens of *maculatus* and notes on their inheritance are provided below.

MATERIALS AND METHODS

The colony of *maculatus* giving rise to the dark phenotype was located at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. This colony originated from a colony at the Institute for Medical Research, Kuala Lumpur, Malaysia, and represented the Malaysian and vector strain of *maculatus*. The colony was maintained by the artificial mating technique as described by Ow Yang et al. (1963), and was in the 173rd generation when the melanistic forms were detected. Late in 1977, during the inheritance part of this study, this colony expired because of technical problems. Early in 1978 another colony was initiated at the Faculty of Tropical Medicine from eggs received from the colony at I.M.R., Kuala Lumpur, Malaysia.

Pinned melanistic specimens (7♀, 1♂) have been preserved and are deposited in the collections of the: Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Medical Entomology Project (MEP), Smithsonian Institution, Washington, D.C. 20560; and British Museum (Natural History), London, England, Immature specimens were not preserved.

TAXONOMY

Excellent descriptions of the adult and immature stages of *maculatus* were made by Reid (1968) and will not be repeated here. The adult female and male of the melanistic mutant are described below, and their differences from wild type adults are summarized in Table 1.

FEMALE (Fig. 1). *Head.* Vertex with several narrow erect pale scales just above interocular space, remaining erect scales on vertex and occiput light to dark brown; interocular space dorsally with several long brown setae, patch of very long white sinuous scales on each side forming frontal tuft, laterally with short white ocular scales; clypeus dark and bare; pedicel with integument brown, with brown scales in dorsolateral patch; antennal flagellomere 1 with brown scales on basomesal and basolateral aspects, cream scales on distomesal aspect; flagellomere 2 usually with several pale scales;

proboscis with dark brown decumbent scales; palpus equal to or slightly shorter than proboscis, with erect or partially erect scales at base and on segment 2, remaining scales decumbent; palpus entirely dark brown or brown with mesal patch of paler scales on segment 3 and pale yellow scales on tip of segment 5. *Thorax.* Integument tan to dark brown, scutal integument gray-brown, fossa, scutal angles, supraalar and prescutellar areas darker than central area of scutum; anterior promontory with long erect pale scales, shorter darker scales laterally in front of dorsocentral setal rows and fossa; scutum covered with short widely spaced white flat scales except at bare spots on fossa, supraalar, and prescutellar areas; scutal setae long tan to brown, in acrostichal, dorsocentral, prescutal, fossal, antealar and supraalar groups; scutellum with anterior row of short white flat scales, posterior row of long dark brown setae; midlobe of scutellum with dark brown integument, lateral lobes tan; anterior pronotum with long black setae and several dark brown scales; posterior pronotum bare; pleural sclerites light to dark brown, post-spiracular sclerite darkest; upper sternopleural scales brown, prealar scales creamish white; pleural setae: 0 propleural, 2-5 spiracular, 2-6 prealar, 3-4 upper and 3-5 lower sternopleural, 5-9 upper and 0 lower mesepimeral. *Wing.* Veins brown scaled except remigium with basal and apical brown scales and median creamish white scales. *Halter.* Stem pale, knob with dorsal and ventral brown scales. *Legs.* Integument tan to brown; coxae with brown scales, midcoxa with upper and lower scale patches; basal half of forefemur swollen; legs entirely dark brown scaled without pale spots or bands except pale ventral scales on distal half of forefemur, entire venter of midfemur and narrow line on basal 0.8 of hindfemur; venter of tibiae and tarsomeres may appear yellow when illuminated at certain angles. *Abdomen.* Integument light brown, segments covered with long brown setae; terga with yellow to brown scales, scales increasing posteriorly on each tergum and on most distal segments; terga II-IV with 3-20 scales, while terga V-VIII with more than 20 scales; tergal scales paler on midline, darker laterally, most distal segments with posterolateral patches of dark brown scales; sterna with few brown scales near posterior margin of segments VI-VIII; cerci covered with dark brown scales.

MALE. Like female except: *Head.* Antennal setae of whorl longer, more numerous than those on female; pedicel enlarged, dark brown, without scales; antennal flagellomere 1 with several light brown scales on mesal surface and pale scales on mesal and dorsal surface; antennal flagellomere 2 with several slender pale scales on mesal surface; proboscis long slender bent downward in distal half, with dark brown decumbent scales; palpus with 2 apical segments flattened clublike, entirely brown scaled except large mesal patch of pale scales on segment 2 and large mesal patch of pale scales on segment 3. *Thorax.* 4 - 5 upper mesepimeral setae. *Wing.* More slender than female wing, with fewer scales. *Abdomen.* Terga II-VIII with scales near posterior margin; terga II-V with 5-10 light brown or cream scales concentrated near midline; terga VI-VII with brown scales, heaviest concentration on posterior half, particularly posterolateral corners; sternum and tergum VIII with dark brown scales. *Genitalia.* Basimere with dark brown scales dorso- and ventrolaterally, with 5 parabasal spines; claspette without lobes, with ventromesal spicules, 1-2 long large apical setae, stout lateral club and 2 small more mesal setae, apical seta(e) much longer than club, small mesal setae nearly length of club, lateral club fused from 3 - 4 basal stems; aedeagus narrow, curved dorsally, with 5-7 leaflets on each side of apex; largest leaflets

TABLE 1: Phenotypic characters distinguishing melanistic adults from wild type adults of *Anopheles maculatus* (Kuala Lumpur Strain).

External Structure	Color patterns of 2 phenotypes of *Anopheles maculatus*	
	Wild Phenotype	Dark Unspotted Phenotype
Palpus	3 distinct white bands	brown
Antennal pedicel scales	white	brown
Antennal flagellomere 1 scales	white	brown and cream
Coxal scales	forecoxa brown mid-and hindcoxa white	brown
Legs	black with white spots and white tarsal bands	brown without spots or bands (paler on venter)
Thorax (anterior pronotal and sternopleural) scales	white	brown
Wings (except remigium)	black with very distinct white spots	brown
Halter scales	white dorsally brown ventrally	brown
Abdominal terga scales	white with dark posterolateral scales on segments VI-VIII	cream scales mesally remainder brown
Male basimere scales	white except dorsolateral patch of black scales on distal half	brown

broad, curved, with large serrations on concave edge; proctiger conical, membraneous and wrinkled, without spicules.

The melanistic specimens have the general appearance of a member of the subgenus *Anopheles*. Using most keys to the subgenera of *Anopheles* (except ♂ keys) they would key to the subgenus *Anopheles* due to the absence of pale wing spots. However, using the following combination of non-color characters, the specimens are easily identified as belonging to the subgenus *Cellia* and the Neocellia series: (1) 4,5 parabasal spines on the male basimere; (2) absence of propleural setae; (3) broad scales on the scutum extending caudad to the scutellum; (4) broad scales on the scutellum; (5) anterior pronotum with at least a few scales; and (6) at least some abdominal terga with scales.

The only apparent abnormal modification of this mutant is a change in scale color, and even then, not all scales on the body are involved. The color of the scales on the frontal tuft, scutum, scutellum and the remigium on the wing apparently are not affected by this mutation. The melanistic scales are slightly lighter than the dark scales found on the wild phenotype. However, the total absence of pale spots and bands and the rich brown color of the specimens definitely results in a much more melanistic habitus than previously described for this species. Otherwise, no structural modifications or abnormalities were observed on the 8 preserved specimens. Furthermore, intermediate specimens were not preserved and apparently were not observed, as all specimens were recorded as either the wild phenotype or the melanistic phenotype. Only two minor color variations were noticed on the melanistic specimens, these are: (1) palpus entirely brown scaled (5 females) or brown with a mesal patch of pale brown scales on segment 3 and pale yellow scales on the apex of segment 5 (2 females); and (2) variation in the amount of cream scales versus brown scales on the abdominal terga. Similar variations also occur in the wild phenotype of *maculatus* (see Discussion). Other variations of the melanistic specimens, such as differences in the number of setae in certain thoracic pleural groups, all fall within the normal range observed in the wild phenotype.

INHERITANCE

Melanistic adults were first observed in the 173rd generation of the colony and observations were started with the 174th generation. Table 2 summarizes the numbers and sexes of both phenotypes examined in the colony from generation 174 to 180.

During the 175th generation several crossing experiments using artificial mating were conducted (Table 3). The initial cross (1 dark ♀ x 1 dark ♂) resulted in only dark F_1 progeny. An additional cross between these F_1 progeny failed to produce F_2 progeny, however, the dark specimens occurring in generations 174, 175, 176, 179 and 180 almost certainly represented progeny (to include F_2) from dark parents. Reciprocal crosses between dark and wild type adults produced progeny of both phenotypes (no intermediates), with wild to dark ratios of 2.88:1 and 2.29:1. Back crosses were not attempted, and the F_1 males and

TABLE 2. The ratio of 2 adult phenotypes in a colony of *Anopheles maculatus* during 5 generations.

	Wild Type (Spotted)				Dark Unspotted				Ratio
Generation	♀	♂	♀/♂	Total	♀	♂	♀/♂	Total	Wild:Dark
174	147	78	1.88	225	5	3	1.6	8	28:1
175	28	4	7	32	3	1	3	4	8:1
176	141	114	1.2	255	27	12	2.3	39	6.5:1
177	---------- NO OBSERVATIONS ----------								
178	---------- NO OBSERVATIONS ----------								
179	111	72	1.54	183	1	0	-	1	183:1
180	106	115	0.92	221	3	1	3	4	55:1
Totals	533	383	1.39	916	39	17	2.29	56	16.4:1

TABLE 3. Crossing experiments for 2 phenotypes of *An. maculatus* from generation 175.

Exp.	Parents (Phenotype)	F_1 Progeny (Phenotype)						
		Wild Type (Spotted)			Dark Unspotted			Ratio Wild:Dark
		♀	♂	Total	♀	♂	Total	
1	1 dark ♀ x 1 dark ♂	0	0	0	15	7	22	0:22
2	1 dark ♀ x 1 spotted ♂	26	23	49	10	7	17	2.88:1
3	1 spotted ♀ x 1 dark ♂	15	17	32	8	6	14	2.29:1

females were not dissected to determine the development of the internal reproductive organs, or the presence or absence of sperm in the spermatheca.

Although the dark specimens obviously represent a mutant of *maculatus*, the genetic mechanism of inheritance for this trait cannot be established on the basis of the above data.

DISCUSSION

Abnormal specimens of *Anopheles* sp. are probably not too uncommon, although they are only infrequently collected and preserved. Such aberrant specimens are due to some physical abnormality or variation that may or may not be genetically inherited. A growing number of heritable traits have been described and confirmed for anophelines in recent years, e.g., short palps, long palps, wartoid or warted palps, bent or semi-beaked proboscis, and beaked proboscis (Kitzmiller and Mason 1967). These traits are modifications of a single body structure and are usually recognized as abnormalities of a given species by collectors. However, traits that change the general adult habitus of a well known species usually result in that species being misidentified, and can result in the description of new species and/or subspecies. At least 2 such traits are known, i.e., albinism and melanism. Examples of these occurring in anophelines are: (1) albinism in *An. (Cel.) vagus* Doenitz 1902, with the albino specimens originally described as the following distinct species, *immaculata* James 1902, *flava* Swellengrebel 1917 and *albino* Stoker and Koesoemawinangoen 1949; (2) albino specimens originally described as *An. (Cel.) ludlowae* var. *flavescens* Swellengrebel 1927, which are currently not definitely associated with a particular species, but probably apply to *vagus* (Reid 1968); (3) hypomelanic specimens of *An. (Cel.) pallidus* Theobald 1901, reported from India by Bhatnagar et al. (1958); (4) albinoid specimens of *An. (Cel.) gambiae* Giles 1902 sensu lato, from Africa reported by Service (1964), Gilles and de Meillon (1968) and van Someron (1969); (5) partial melanism in *An. (Ano.) montanus* Stanton and Hacker 1917, based on one female (Harrison and Scanlon 1975); and (6) probable partial melanism in *An. (Cel.) fluviatilis* James 1902, originally described as a separate species, *leptomeres* Theobald 1903.

Previously described variations of *maculatus* usually involved palpal and tarsal banding patterns, or the distribution of scales on the abdominal terga (Reid 1968). However, Wattal et al. (1960) described a partially melanistic female from India with the sector and subapical pale spots missing on the wing. These authors also described several females with median pale spots or a pale stripe on palpal segment 3. Reid (1963) discussed 2 males of *maculatus* from Malaysia, that had broad pale marks on the wings, and unusually pale tarsi. These 2 specimens were previously identified (Gater 1935) as a species near *An. jamesii* Theobald 1901.

Aside from the novelty of albino, melanistic and other aberrant traits, such phenotypic characters are extremely useful to researchers working on the cytogenetics of mosquitoes, if they can be successfully established in colonies. Chromosomal differences correlated with such traits can then be mapped and thereafter used as markers for identifying and locating other

characters. With the use of phenotypic markers, gene loci for other non-phenotypic traits, such as resistance to pesticides, refractiveness to infection with pathogens, sterility, etc., may eventually be located on chromosomes of vector species, and be used to alter vector or pest populations for the benefit of man.

A standard chromosome map has been prepared for specimens of *maculatus* from the Center for Disease Control, U.S. Public Health Service Stock (Narang et al. 1973). Marker strains of *maculatus* are now needed to identify particular landmarks (usually bands) on this map. The genetically inherited dark mutant described here for *maculatus* probably could have served as an excellent genetic marker, however, the entire *maculatus* colony expired before the inheritance of the trait could be completely worked out, and before it could be isolated and established as a separate colony. Although a new colony is now established, additional melanistic specimens have not been encountered. Apparently this dark mutant has also been detected by Mr. W.H. Cheong, Institute for Medical Research, Kuala Lumpur, Malaysia, but a colony has not been established.

ACKNOWLEDGMENTS

We gratefully acknowledge Mr. Kamhang Surathin, Mrs. Somjai Viraboonchai and Mr. Watanasak Tumrasrin, Faculty of Tropical Medicine, Mahidol University, Bangkok, and Mrs. Prasertsri Rohitaratana, U.S. Army Medical Component - AFRIMS, Bangkok, for their assistance in rearing and preparing the specimens. Thanks are due Mr. W.H. Cheong, Institute for Medical Research, Kuala Lumpur, Malaysia, for providing the Kuala Lumpur (Malaysian) strain of *maculatus* for colonization. We are indebted to Dr. Visut Baimai, Faculty of Science, Mahidol University, Bangkok, and Dr. Michael E. Faran, Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C., for reviewing the manuscript. The helpful comments of Dr. A. Ralph Barr, School of Public Health, University of California, Los Angeles CA, are also appreciated. Special thanks are also due Mrs. Prachong Panthusiri, U.S. Army Med. Comp., - AFRIMS, for the illustrations, and Mrs. Nipaporn Nimsomboon, U.S. Army Med. Comp., - AFRIMS, for typing the manuscript.

LITERATURE CITED

Bhatnagar, V.N., M.L. Bhatia and K.S. Krishnan. 1958. On certain morphological abnormalities noted in *A. pallidus* Theobald, 1901 and *A. stephensi stephensi* Liston, 1901. Indian J. Malariol. 12: 39-42.

Christophers, S.R. 1931. Studies on the anopheline fauna of India (Parts I-IV). Rec. Malaria Surv. India 2: 305-32.

Christophers, S.R. 1933. The fauna of British India, including Ceylon and Burma. Diptera. Vol. IV. Family Culicidae. Tribe Anophelini. Taylor and Francis, London. 371 p.

Gater, B.A.R. 1935. Aids to the identification of anopheline imagines in Malaya. Malaria Advis. Bd., Federated Malay States, Singapore. 242 p.

Gilles, M.T. and B. de Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region), 2nd ed., Publ. S. Afr. Inst. Med. Res. 54: 1-343.

Harrison, B.A. and J.E. Scanlon. 1975. Medical entomology studies-II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). Contrib. Am. Entomol. Inst. 12(1): 1-307.

Kitzmiller, J.B. and G.F. Mason. 1967. Formal genetics of anophelines. pp. 3-15. in Wright, J.W. and R. Pal (ed.). Genetics of insect vectors of disease. Elsevier Publ. Co., New York. 794 p.

Knight, K.L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). 2nd Edition. Thomas Say Found., Entomol. Soc. Am. 6: 1-611.

Narang, N., S. Narang, J.B. Kitzmiller, G.P. Sharma and O.P. Sharma. 1973. Evolutionary changes in the banding patterns of salivary gland chromosomes in the genus *Anopheles*, subgenus *Cellia*. J. Med. Entomol. 10: 13-22.

Ow Yang, C.K., F.L. Sta Maria and R.H. Wharton. 1963. Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosq.News 23: 34-5.

Reid, J.A. 1963. Notes on anopheline mosquitoes from Malaya, with descriptions of three new species. Ann. Trop. Med. Parasitol. 57: 97-116.

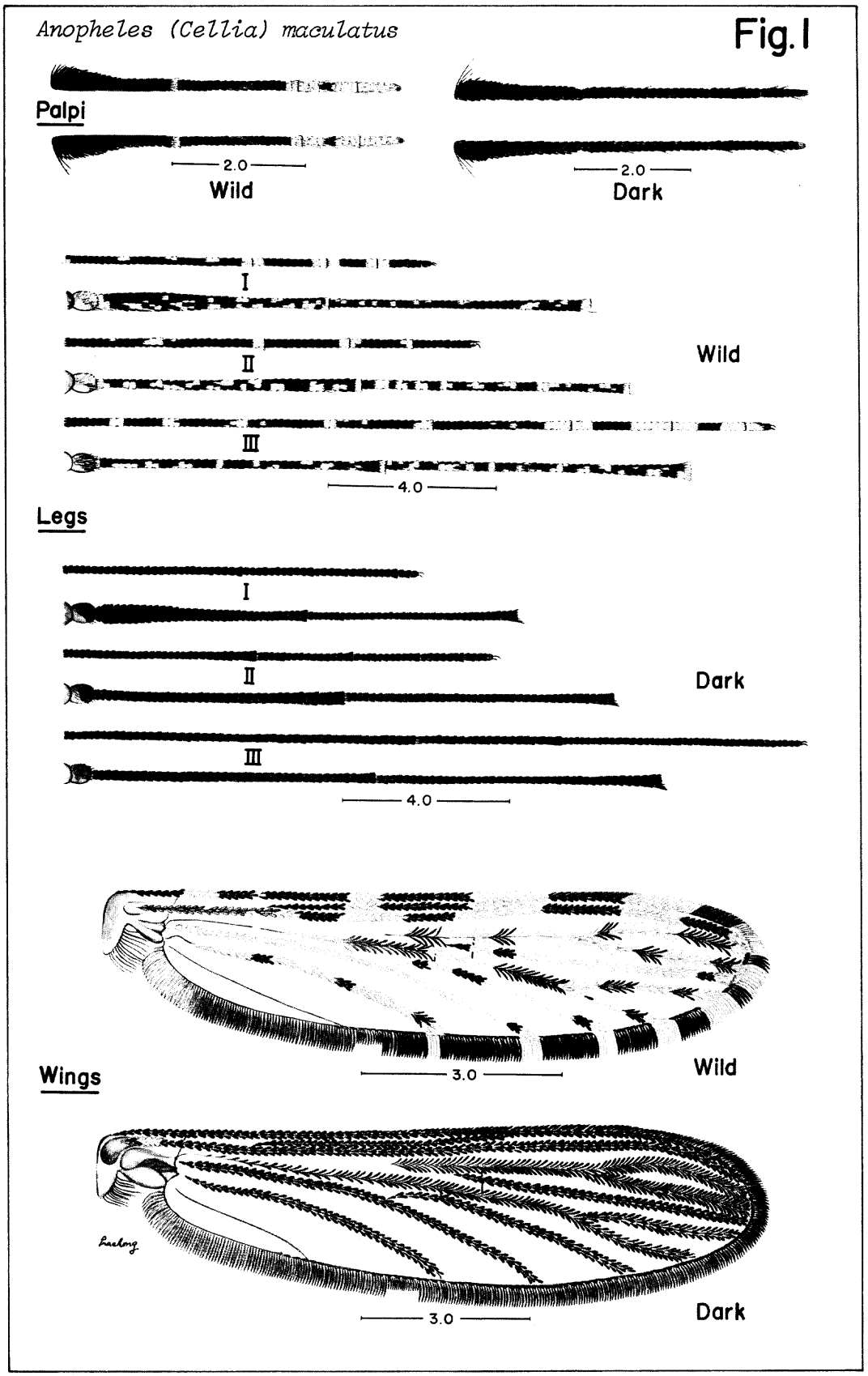
Reid, J.A. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res. Malaya 31: 1-520.

Reid, J.A., B.L. Wattal and W. Peters. 1966. Notes on *Anopheles maculatus* and some related species. Bull. Indian Soc. Malaria Comm. Dis 3: 185-97.

Service, M.W. 1964. Two albinoid females of *Anopheles gambiae* Giles (Diptera: Culicidae) from northern Nigeria. Proc. R. Entomol. Soc. Lond. (B) 33: 101-2.

van Someron, E.C.C. 1969. Some interesting mosquitoes from Kenya. Mosq. Syst. Newsletter 1: 17-8.

Wattal, B.L., N.L. Kalra and M.L. Mammen. 1960. Observations on certain morphological abnormalities in twenty species of Indian *Anopheles*. Indian J. Malariol. 14: 291-310.


Anopheles (Cellia) maculatus
Fig.I
Palpi
2.0
Wild
2.0
Dark
I
II
III
Wild
4.0
Legs
I
II
III
Dark
4.0
3.0
Wild
Wings
3.0
Dark