Mosquito Systematics, 25(3):192–197, 1993

VARIATION IN THE HINDTARSAL MARKINGS OF *ANOPHELES DARLINGI* (DIPTERA: CULICIDAE) IN BELIZE

RALPH E. HARBACH[1], DONALD R. ROBERTS[2] AND SYLVIE MANGUIN[2]

ABSTRACT. Aberrant phenotypes of *Anopheles darlingi* with basal dark scaling on either one or both of hindtarsomeres 3 and 4 are reported from Belize. Based on wild-caught females and adults reared from wild-caught larvae, it appears that approximately 8% of the natural population bears some degree of basal dark scaling on these hindtarsomeres. The occurrence of similar variants in other species of the subgenus *Nyssorhynchus* is summarized, and their significance in terms of inaccurate species identification is noted.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *darlingi* Root has not been reported or collected from Belize since Komp (1940, 1941), Kumm and Ram (1941), and Walker (unpublished[3]). In fact, we thought this species had disappeared from Belize, perhaps due to agricultural and control practices, because we did not encounter it during intensive larval surveys conducted throughout the country in September 1990, April 1991, and September 1992 (Rejmankova et al. 1993, Roberts et al. 1993). In May 1993, however, we repeatedly collected adult females of this species from human bait both inside and outside houses located near rivers between Middlesex in Stann Creek District and Belmopan in Cayo District. Larvae were subsequently collected from riverine habitats near St. Thomas and Belmopan. *Anopheles darlingi* undoubtedly contributes in a major way to the increasing numbers of malaria cases in Belize (PAHO 1992).

Faran and Linthicum (1981) and Linthicum (1988) used the absence of dark scaling on hindtarsomeres 3 and 4 as the primary character for distinguishing adults of the subgenus *Nyssorhynchus* from those belonging to the other subgenera of *Anopheles* in the Neotropical Region. They indicated that hindtarsomeres 3 and 4 are entirely white in this group, except in "unusual variants," which apparently refers to the so-called "mutants" and "anomalous specimens" of *An. albimanus* Wiedemann, *An. aquasalis* Curry, *An. rondoni* (Neiva and Pinto), *An. strodei* (Root), and *An. triannulatus* (Neiva and Pinto) discussed by Faran (1980). Linthicum (1988) specifically listed *An. rondoni* and *An. nigritarsis* (Chagas) as exceptions, but these species differ from the others in having basal dark bands constantly present on hindtarsomere 3 (*An. rondoni*) or both hindtarsomeres 3 and 4 (*An. nigritarsis*).

Faran (1980) divided the subgenus *Nyssorhynchus* into two sections, the Albimanus and Argyritarsis sections, but excluded four poorly known species of the "*Myzorhynchella* group," which was raised only recently to sectional status (Peyton et al. 1992). He distinguished adults of the Argyritarsis Section from those of the Albimanus Section primarily by the absence of a dark basal band on hindtarsomere 5. *Anopheles darlingi* is a member of the Argyritarsis Section, which is characterized by having hindtarsomeres 3–5

[1] Department of Entomology, Museum Support Center, MRC 534, Smithsonian Institution, Washington, DC 20560. Current address: Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

[2] Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814.

[3] Four females of *An. darlingi* from Sierra de Agua (17° 30′ N 88° 50′ W), Orange Walk District, collected by A.J. Walker in June 1946 are deposited in the National Museum of Natural History, Smithsonian Institution. These specimens were examined and listed by Linthicum (1988).

entirely white-scaled (Faran 1980, Linthicum 1988). Of the eight species belonging to the Argyritarsis Section, dark markings on hindtarsomeres 3 and 4 have been observed only in *An. darlingi.* Most of the species mentioned above belong to the Albimanus Section. *Anopheles nigritarsis* belongs to the Myzorhynchella Section.

Komp (1942:37) mentioned that he had "specimens of *darlingi* from British Honduras [= Belize] with additional black hind tarsal bands," but this observation apparently went unnoticed by later authors because Komp never included it in a species description. We first noticed the presence of basal dark scaling on hindtarsomeres 3 and 4 in a few specimens of *An. darlingi* while identifying mosquitoes collected during malaria vector ecology studies involving the use of remote sensing. These mosquitoes were all frozen for *Plasmodium* detection and identification. The purpose of this paper is to bring attention to the presence of basal dark markings observed in adults of *An. darlingi* reared from wild-caught larvae and the progeny of wild-caught females that were frozen for isozyme analysis. There are no published reports of aberrant hindtarsal markings in populations of *An. darlingi* from any other country in Central or South America.

MATERIALS AND METHODS

Hindtarsal markings were examined in adults reared from wild-caught larvae and progeny broods obtained from females captured on human bait on May 25, 1993. Larvae were collected in shaded masses of floating plant debris and patches of *Cabomba* sp. along the edges of the Sibun River near St. Thomas (17° 09′ N 88° 37′ W) and Roaring Creek near Belmopan (17° 15′ N 88° 48′ W). The specimens were transported the next day to the Smithsonian Institution Museum Support Center in Suitland, MD. Larval collections and progeny broods were reared separately in plastic pans in an air-conditioned room held at 21 ± 1°C. Each pan was provided with straw for floatage and gently aerated (through a sandstone) by means of an aquarium pump. Fourth-instar larvae and pupae were removed from the pans and reared individually in plastic vials. Most of the adults reared from wild-caught larvae and a portion of those reared from each progeny brood were mounted on points on pins and examined for hindtarsal markings. All of these specimens, along with their associated larvae and/or pupal exuviae, were deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, for future study and reference. A number of adults (12%) obtained from progeny broods died upon emergence and could not be saved as voucher specimens. These specimens were examined for hindtarsal markings, then discarded along with their associated larval and pupal exuviae. The remainder of the adults (24% from wild-caught larvae and 39% from progeny broods) were examined while alive and subsequently frozen at −70°C for later biochemical studies. We retained larval and pupal exuviae from the frozen adults reared from wild-caught larvae. Two or three first-, second-, third-, and fourth-instar larvae from each progeny brood were preserved in 80% ethanol for future morphological study.

OBSERVATIONS

The variation observed in the hindtarsal markings of *An. darlingi* from Belize is illustrated in Fig. 1. Figure 1A shows the normal condition where the distal portion of hindtarsomere 2 and all of hindtarsomeres 3–5 are white-scaled. The other drawings in Fig. 1 illustrate variation in basal dark scaling sometimes present on the third and fourth hindtarsomeres: (1) dark scaling at the base of hindtarsomere 3 (Fig. 1B), (2) dark scaling at the base of hindtarsomere 4 (Fig. 1C), and (3) dark scaling at the bases of both hindtarsomeres 3 and 4 (Fig. 1D,E). The amount of dark scaling on hindtarsomeres 3 and 4 is variable, ranging from a narrow or incomplete ring to a broad, distinct band. The dark scaling, when present, usually forms complete bands. In general, when dark scaling is well developed on hindtarsomere 3, there is a corresponding reduction in the amount of

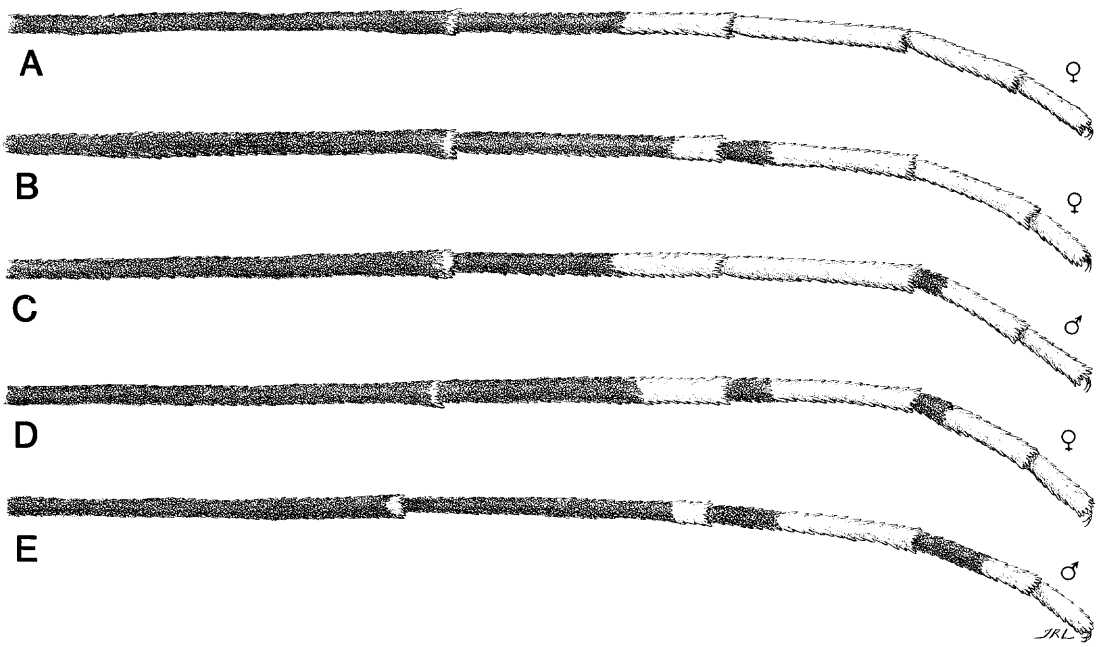

Fig. 1. Hindtarsal markings observed in specimens of *Anopheles darlingi* from Belize. A, Prevalent form, hindtarsomeres 3–5 entirely white-scaled; B–E, aberrant forms showing variation in basal dark scaling that sometimes occurs on one or both of hindtarsomeres 3 and 4.

white scaling on the distal portion of hindtarsomere 2 (Fig. 1B,E). In such cases, the extent of basal dark scaling on hindtarsomere 2 exceeds the range of "basal 0.35–0.55 dark" given by Linthicum (1988).

Ninety-four adults were reared from wild-caught larvae. Seven of these had basal dark scaling on one or both of hindtarsomeres 3 and 4. Six specimens (4♂, 2♀) exhibited the condition shown in Fig. 1C, i.e., basal dark scaling on hindtarsomere 4, and one female had basal dark scaling on both hindtarsomeres 3 and 4. If this sample is representative of the natural population of *An. darlingi* in Belize, then 7.4% (7/94) of the wild population would be expected to have dark markings on one or both hindtarsomeres 3 and 4. Of the seven individuals, 85.7% (6/7) had dark scaling at the base of hindtarsomere 4, and 14.3% (1/7) had dark scaling on both hindtarsomeres 3 and 4. Of the overall sample, 6.4% (6/94) had dark scaling at the base of hindtarsomere 4, and 1.1% (1/94) had dark scaling on the bases of both hindtarsomeres 3 and 4.

Progeny broods were obtained from 11 females. One female had a prominent dark band at the base of hindtarsomere 3, another had prominent dark basal bands on both hindtarsomeres 3 and 4, and the remainder (9) had normal hindtarsi. If these wild-caught females are grouped with the 94 adults reared from wild-caught larvae, then a total of 8.6% (9/105) of the natural population of *An. darlingi* in Belize would be expected to have some degree of dark scaling on hindtarsomeres 3 and 4. Two females among these individuals (1.9% of 105) had dark scaling on the bases of both hindtarsomeres 3 and 4, one female (1.0%) had dark scaling only at the base of hindtarsomere 3, and six individuals (4♂, 2♀) (5.7%) had dark scaling only on the base of hindtarsomere 4.

Of the progeny obtained from the female with dark scaling at the base of hindtarsomere 3, only nine survived to adulthood. One female was exactly like the mother in having dark scaling at the base of hindtarsomere 3, and one male differed in having dark scaling at the bases of both hindtarsomeres 3 and 4.

Three males and four females exhibited the normal condition shown in Fig. 1A. A total of 22.2% (2/9) of the individuals that survived from this brood had dark bands on the hindtarsomeres.

Only three offspring (2♂, 1♀) of the female with prominent dark bands at the bases of hindtarsomeres 3 and 4 survived to adulthood. Unfortunately, the single female emerged from the pupal exuviae without hindtarsi. These were left within the exuviae and could not be examined. The two males, however, had basal dark bands on hindtarsomeres 3 and 4 exactly like their mother. It is possible that this brood may have bred true, i.e., all of the progeny may have exhibited the maternal phenotype.

Of the progeny obtained from the other nine females, 119 (57♂, 62♀) survived to adulthood, and all but one of these were normal with respect to hindtarsomeres 3 and 4. A single female from a brood of 12 surviving adults (6♂, 6♀) had dark markings on the bases of hindtarsomeres 3 and 4. Therefore, 8.3% (1/12) of this brood exhibited dark hindtarsal markings. This is nearly the same ratio seen in wild-caught specimens.

DISCUSSION

Accurate identification of mosquitoes depends on a thorough knowledge of morphological variation within species. Variation in ornamentation sometimes leads to erroneous identification. In fact, lack of knowledge of variation in hindtarsal markings has led to the naming of a number of varieties and species that are conspecific, e.g., the *bisignatus* and *trisignatus* varieties of *An. albimanus* (Hoffmann 1938), the *guarauno* and *delta* varieties of *An. aquasalis* (Anduze 1948), *An. deltaorinoquensis* Cova Garcia, Pulido F. and Amanista M., which is a synonym of *An. aquasali* (Faran 1980), and *Cellia cuyabensis* (Neiva and Pinto), which is a synonym of *An. triannulatus* (Pinto 1939). Because morphological keys are designed by trained taxonomists primarily for the use of non-taxonomists and inexperienced identifiers, it is important that keys account for intraspecific variation as well as interspecific differences. In most published keys, specimens of *An. darlingi* with dark markings on hindtarsomeres 3 and 4 will key properly to the correct subgenus and species, but this is not so with the keys of Faran and Linthicum (1981) and Linthicum (1988), where reliance on the first or primary key character would cause these specimens to be misidentified at the subgeneric level. Fortunately, *An. darlingi* is a very distinct species in Belize, where it usually should be recognizable in both the adult and larval stages.

Among the subgenera of *Anopheles* in the Neotropical Region, hindtarsomeres 3 and 4 are not entirely white in *Anopheles, Lophopodomyia, Kerteszia,* and *Stethomyia.* As indicated above, these hindtarsomeres are entirely white in species of the *Nyssorhynchus* except for *An. nigritarsis* (Myzorhynchella Section), *An. rondoni* (Albimanus Section), and "unusual variants" (Faran and Linthicum 1981, Linthicum 1988). *Anopheles nigritarsis* is characterized by the constant presence of basal dark bands on hindtarsomeres 3 and 4 and *An. rondoni* by the constant presence of a dark basal band on hindtarsomere 3. Aberrant dark bands sometimes occur variously on one or more of hindtarsomeres 3–5 in certain populations of *An. albimanus, An. aquasalis, An. strodei,* and *An. triannulatus* of the Albimanus Section and *An. darlingi* of the Argyritarsis Section. A summary of additional dark bands observed in these species is given in Table 1. These are the variants that are likely to cause problems for non-taxonomists, particularly those who place too much emphasis on primary key characters, use them out of habit, or use them because they are more discrete and easier to observe than secondary key characters.

The phenotypes with aberrant hindtarsal bands observed in species of *Nyssorhynchus* have been called "mutants" and "anomalous specimens" by various authors (e.g., Rozeboom 1963, Kitzmiller and Mason 1967, Faran 1980). Indications are that these phenotypes are fairly common, especially in *An. albimanus, An. triannulatus,* and *An. darlingi.* From this study, it appears that approx-

Table 1. Summary of aberrant hindtarsal markings observed in species of the subgenus *Nyssorhynchus* of *Anopheles*.

Section	Species	Variants	Populations from	Principal references
Albimanus	*albimanus*	Basal dark bands on: 1) hindtarsomere 3 2) hindtarsomeres 3,4	Costa Rica, El Salvador, Guatemala, Texas (U.S.A.)	Hoffmann 1938, Rozeboom 1963, Faran 1980
	aquasalis	Apical dark bands on: 1) hindtarsomere 4 2) hindtarsomeres 3,4 Basal dark bands on: 3) hindtarsomeres 3,4	Venezuela	Anduze 1948, Faran 1980
	strodei	Basal dark bands on: 1) hindtarsomeres 3,4 2) hindtarsomere 4 3) hindtarsomeres 3,4 but absent on 5[1]	Brazil	Rachou and Ferraz 1951
	triannulatus	Basal dark bands on: 1) hindtarsomere 4 2) hindtarsomeres 3,4	Brazil	Pinto 1939, Galvão and Lane 1941
Argyritarsis	*darlingi*	Basal dark bands on: 1) hindtarsomere 3 2) hindtarsomere 4 3) hindtarsomeres 3,4	Belize	present study

[1] Species of the Albimanus Section are characterized by the presence of a basal dark band on hindtarsomere 5.

imately 8% of the *An. darlingi* in Belize have basal dark scaling on either one or both of hindtarsomeres 3 and 4, and the data suggest that a heritable genetic basis exists for the expression of this trait. Consequently, we prefer to characterize these phenotypes as "aberrant forms" or "normal variants," which imply deviation from the usual or prevalent form rather than a rare individual or strain resulting from mutation.

ACKNOWLEDGMENTS

We are grateful to Jorge Polanco, Director, Vector Control Program, Ministry of Health, Belize City, Belize, for in-country support; to Eugene Lisby and Gustavo Escalante, Malaria Vector Control Office, Cayo District, Belize, for assistance in making adult and larval collections; to Taina Litwak, Walter Reed Biosystematics Unit (WRBU), Washington, DC, for preparing the illustration; to Eliska Rejmankova, University of California, Davis, for identifying the aquatic plant sometimes found in association with *An. darlingi* larvae; to Jim Pecor and E.L. Peyton, WRBU, for assistance in setting up collections and rearing specimens; to Ronald A. Ward, Walter Reed Army Institute of Research, Washington, DC, and E.L. Peyton for commenting on the manuscript; and to Jayson Glick, Manager, WRBU, for allowing us the use of laboratory space and equipment needed to rear and process specimens.

REFERENCES CITED

Anduze, P.J. 1948. Notas entomologicas I. Dos variedades nuevas de *A. aquasalis*. Bol. Med. (Caracas) 1:17–19.

Faran, M.E. 1980. Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. (Ann Arbor) 15(7):1–215.

Faran, M.E. and K.J. Linthicum. 1981. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Mosq. Syst. 13:1–81.

Galvão, A.L.A. and J. Lane. 1941. Obser-

vações sôbre alguns anofelinos de Salôbra, Mato Grosso (Diptera, Culicidae). Rev. Biol. Hyg. 11:10–18.

Hoffmann, C.C. 1938. La formacion de razas en los *Anopheles* mexicanos. II. *Anopheles albimanus* y sus variedades en la Republica Mexicana. An. Inst. Biol. Univ. Nac. Auton. Mex. 9:167–180.

Kitzmiller, J.B. and G.F. Mason. 1967. Formal genetics of anophelines, pp. 3–15. *In*: J.W. Wright and R. Pal (eds.), Genetics of insect vectors of disease. Elsevier Publishing Company, New York, NY.

Komp, W.H.W. 1940. The occurrence of *Anopheles darlingi* Root in British Honduras and Guatemala. Science (Washington, DC) 91:522–523.

Komp, W.H.W. 1941. The occurrence of *Anopheles darlingi* Root in Central America. Am. J. Trop. Med. 21:659–670.

Komp, W.H.W. 1942. The anopheline mosquitoes of the Caribbean Region. Natl. Inst. Health Bull. 179. U.S. Gov. Printing Office, Washington, DC.

Kumm, H.W. and L.M. Ram. 1941. Observations on the *Anopheles* of British Honduras. Am. J. Trop. Med. 21:559–566.

Linthicum, K.J. 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq. Syst. 20:98–271.

PAHO. 1992. Status of malaria programs in the Americas. XL report. CD36/INF/1 (Eng.). Pan American Health Organization, Washington, DC.

Peyton, E.L., R.C. Wilkerson and R.E. Harbach. 1992. Comparative analysis of the subgenera *Kerteszia* and *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq. Syst. 24:51–69.

Pinto, C. 1939. Disseminação da malaria pela aviação; biologia de *Anopheles gambiae* e outros anofelinos dos Brasil. Mem. Inst. Oswaldo Cruz 34:293–430, 61 pl.

Rachou, R.G. and D.M. Ferraz. 1951. Contribuição ao conhecimento da distribuição geográfica dos anofelinos no Brasil: estado de Santa Catarina. Rev. Bras. Malariol. Doenças Trop. 3:540–554.

Rejmankova, E., D.R. Roberts, R.E. Harbach, J. Pecor, E.L. Peyton, S. Manguin, R. Krieg, J. Polanco and L. Legters. 1993. Environmental and regional determinants of *Anopheles* (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22 (in press).

Roberts, D.R., O. Chan, J. Pecor, E. Rejmankova, S. Manguin, J. Polanco and L. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456–459.

Rozeboom, L.E. 1963. Mutant forms of *Anopheles albimanus* Wiedemann (Diptera: Culicidae). Proc. Entomol. Soc. Wash. 65:110–114.