Reproductive Output of Female *Anopheles gambiae* (Diptera: Culicidae): Comparison of Molecular Forms

A. S. YARO,[1] A. DAO,[1] A. ADAMOU,[1] J. E. CRAWFORD,[2] S. F. TRAORÉ,[1] A. M. TOURÉ,[1] R. GWADZ,[2] AND T. LEHMANN[2, 3]

J. Med. Entomol. 43(5): 833–839 (2006)

ABSTRACT Knowledge of ecological differences between the molecular forms of *Anopheles gambiae* Giles (Diptera: Culicidae) might lead to understanding of their unique contribution to disease transmission, to better vector control, and to identification of the forces that have separated them. We compared female fecundity measured as egg batch size in relation to body size between the molecular forms in Mali and contrasted them with their sibling species, *Anopheles arabiensis* Patton. To determine whether eggs of different egg batches are of similar "quality," we compared the total protein content of first-stage larvae (L1s), collected <2 h after hatching in deionized water. Egg batch size significantly varied between *An. gambiae* and *An. arabiensis* and between the molecular forms of *An. gambiae* (mean batch size was 186.3, 182.5, and 162.0 eggs in *An. arabiensis* and the M and the S molecular form of *An. gambiae*, respectively). After accommodating female body size, however, the difference in batch size was not significant. In the S molecular form, egg protein content was not correlated with egg batch size ($r = -0.08$, $P > 0.7$) nor with female body size ($r = -0.18$, $P > 0.4$), suggesting that females with more resources invest in more eggs rather than in higher quality eggs. The mean total protein in eggs of the M form (0.407 μg per L1) was 6% higher than that of the S form (0.384 μg per L1), indicating that the M form invests a greater portion of her resources into current (rather than future) reproduction. A greater investment per offspring coupled with larger egg batch size may reflect an adaptation of the M form to low productivity larval sites as independent evidence suggests.

KEY WORDS *Anopheles gambiae*, body size, egg batch size, egg size, fecundity

Sub-Saharan Africa bears the brunt of malaria with >70% of the 500 million cases estimated to occur annually over the world and an even higher percentage of the malaria-related mortality (Snow et al. 2005). Malaria transmission is driven by the mosquito vector system, which in most of Sub-Saharan Africa consists of three primary species, namely, *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton, and *Anopheles funestus* Giles (Diptera: Culicidae). Both *An. gambiae* and *An. funestus* are further subdivided into semi-isolated populations, typically referred to as forms (Coluzzi et al. 1979, Bryan et al. 1982, Coluzzi et al. 1985, Lochouarn et al. 1998, Toure et al. 1998, Costantini et al. 1999, della Torre et al. 2001, Favia et al. 2001, Gentile et al. 2001). The extent of genetic isolation between forms was studied extensively (Lanzaro et al. 1998, Taylor et al. 2001, Tripet et al. 2001, Lehmann et al. 2003, Cohuet et al. 2004, Michel et al. 2005), but the mechanisms of isolation (Tripet et al. 2001, 2004; Diabate et al. 2006), and the driving forces are poorly understood. The body of knowledge that has been recently accumulating on the genetics and molecular biology of *An. gambiae* must be interpreted in relation to phenotypic variation, but such knowledge is surprisingly meager.

Spatial and temporal associations between abundance of certain species and forms in relation to aridity and to rice cultivation (Coluzzi et al. 1979, Coluzzi et al. 1985, Toure et al. 1998) lead to the notion that the molecular forms are diverging ecologically (della Torre et al. 2005). Field studies failed to detect adaptive differences between the larvae of the molecular forms in different habitats (Edillo et al. 2002, Diabate et al. 2005). If the molecular forms have adapted to different niches (see above), they must have diverged in key adaptive (phenotypic) traits. Fecundity and female's investment per egg are key determinants of fitness and are expected to diverge rapidly in response to selection for different optima in allocating resources in different ecological environments (Bell 1980, Fox and Czesak 2000, Armbruster et al. 2001, Hatle et al. 2002). To our knowledge, no information is available on comparing the reproductive output of the molecular forms of *An. gambiae* in natural settings.

Laboratory and field studies have shown that larger mosquitoes produce larger egg batches (Kittayapong et al. 1992, Ameneshewa et al. 1996, Hogg et al. 1996). Low temperature, low nutrient availability, and high

[1] Malaria Research and Training Center, 1805, Point G. Bamako, Mali.
[2] Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 12735 Twinbrook Pkwy., Rockville, MD 20852.
[3] Corresponding author, e-mail: tlehmann@niaid.nih.gov.

density result in longer larval development and smaller adult size (Briegel 1990, Lyimo et al. 1992, Gimnig et al. 2002), but density can interact with temperature to produce unexpected results (Lyimo et al. 1992). Intraspecific genetic variation also contributes to the variation in adult mosquito size (Lehmann et al. 2006) and indirectly affects egg batch size. Studies that evaluated the relationship between female size and egg batch size in anophelines assumed that female investment in all eggs is equal or at least that the mean egg "quality" per egg batch is the same. To our knowledge, this assumption has not been tested in anophelines.

We compared the molecular forms of *An. gambiae* in terms of female reproductive output based on 1) the number of eggs laid (egg batch size) in relation to her body size and 2) the total protein content of the eggs measured on 2-h-old first-stage larvae (L1s). This comparison is a prerequisite to test the hypothesis that these species and molecular forms have adapted to different conditions, in which the optimal strategy of allocating female resources to various components of its reproduction is not the same. Such differences can affect the role the forms play in disease transmission

Materials and Methods

Indoor resting mosquitoes were collected (using an aspirator) during the end of October and early November 2004 from three villages in Mali: Donéguébougou (7° 59′5″ W, 12° 48′38″ N), located 15 km north of Bamako, Pimperena (5° 42′ W, 11° 28′ N), located 280 km southwest of Bamako, and Selingué (8° 17′ W, and 11° 42′ N), located 120 km south of Bamako. An additional collection was done on 8–9 September 2005 from Donéguébougou by using the same methods to obtain sympatric populations of the molecular forms for the comparison of egg total protein (see below). Mosquitoes were transported to the insectary at the Malaria Research and Training Center, Bamako, where they were kept at 27°C and 75–85% RH. They were housed in 1-gal cages and provided with fresh sugar solution daily. On the third day after collection, each female was placed in a 50-ml Falcon tube containing 15 ml filtered water for oviposition. A strip of filter paper (2 cm in width) surrounded the water edge, providing a wet surface to collect the eggs. The next morning, females that laid eggs were removed and preserved by desiccation. The species and the molecular form of each female were determined using polymerase chain reaction (PCR) assays (Scott et al. 1993, Favia and Louis 1999). The eggs were counted (including eggs that remained in the tube) and placed in a labeled petri plate (10 cm in diameter by 2 cm in height) containing filtered laboratory water. The eggs were inspected every 2 h, beginning in the second day postoviposition for hatching. To quantify the female's investment in individual eggs, newly hatched L1s of each family (<2 h old) were removed from the deionized water and preserved separately in 85% ethanol for total protein analysis.

Total protein content of individual L1s was measured using the Micro BCA protein assay (Pierce Chemical, Rockford, IL) as described previously (Yaro et al. 2006). Briefly, individual larvae were dried, and protein was extracted by 12 min of vortexing at medium speed after the samples underwent two freeze-thaw cycles in 115 μl of 50 mM NaOH. After centrifugation for 7 min at 14,000 rpm, 110 μl of the supernatant was removed and loaded into individual microplate wells with replicate standard curves (including NaOH blanks) prepared with bovine serum albumin (provided with the Micro BCA kit) in the same solution according to the manufacturer's directions. The logarithm of the protein concentration was regressed against the logarithm of the absorbance values to predict the protein concentration of individual larvae based on the standard curve of each plate. The total larval protein was calculated based on the volumes used in the colorimetric reaction and in the extraction.

Female body size was measured by her wing length defined as the distance from the alular notch to the wing's distal tip by using a dissecting scope at 20× magnification fitted with a millimeter ruler. A single wing was removed from each female, spread over a small drop of water placed on a microscope slide, and covered with coverslip. All wings were measured to the nearest 0.1 mm by using the same microscope and settings.

Infection with *Plasmodium falciparum* was determined in individual females by using enzyme-linked immunosorbent assay to detect the circumsporozoite protein (CSP), which is secreted by the parasite during development in the mosquito. Briefly, protein extraction was performed and tested in 96-well microtitration plates. A colorimetric reaction was used to visually recognize positive samples by comparisons with positive and negative controls.

Analysis of variance (ANOVA) and covariance (ANCOVA) including a priori comparisons (contrasts) were performed using SAS (SAS Institute 2002). Least-square means were used in comparing the body size "adjusted" means of egg batch size as part of the ANCOVA. Lack of fit analysis was used to determine whether the regression model provided adequate fit to the data.

Results

Egg batches were collected from a total of 262 females, consisting of 90, 119, and 53 of *An. arabiensis* and S and M molecular forms of *An. gambiae*, respectively. In each location one species or molecular form predominated. Thus, 88 of 90 *An. arabiensis* females were collected in Doneguebougou, 102 of 119 of S form were collected in Pimperena, and 49 of 53 M form were collected in Selingue. Overall prevalence of *P. falciparum* infection was 12.6%, and differences between the species and molecular forms (9.4, 16.0, and 10.0% of the M, S, and *An. arabiensis*, respectively) were not statistically significant ($\chi^2 = 2.3$, df = 2, $P > 0.3$). Infected mosquitoes were excluded from the

Table 1. Comparison of female reproductive output of the molecular forms of *An. gambiae* (M and S) and *An. arabiensis* (A) in terms of egg batch size (egg) given female body size (wing length, WL), and total protein content per egg

Response	ANOVA results				Means and contrast significance[a]				
	Source	df	F/MS[b]	*P*	A	$P_{a\text{-}g}$	M	$P_{m\text{-}s}$	S
Egg	Model	2							
R^2 = 6%	Pop	2	7.2/15,026	0.001	186.3	0.03	182.5	0.011	162.0
	Error	226							
WL mm	Model	2							
R^2 = 8%	Pop	2	15.5/0.558	0.0001	3.19	0.0001	3.11	0.025	3.03
	Error	226							
Egg/WL	Model	5	9/16,853	0.0001					
R^2 = 17%	Pop	2	0.6/1,067	0.57	180[c]	0.73	182[c]	0.37	169[c]
	WL	1	23/43,361	0.0001					
	P × WL	2	0.4/817	0.65					
	Error	223							
Prot/L1[d]	Model	44	3.2/1.23	0.0001					
(μg/L1)	Family(pop)	43	50/1.16	0.0001			0.407	0.023	0.384
R^2 = 27%	Pop	1	6.4	0.023					
	Error	392							

Only the molecular forms were compared in terms of total protein content per egg (see text for details).

[a] $P_{a\text{-}g}$ is the statistical significance of the contrast between *An. arabiensis* and both molecular forms of *An. gambiae*. $P_{m\text{-}s}$ is the statistical significance of the contrast between the molecular forms of *An. gambiae*.

[b] F test value and the mean squares term associated with the source. For one-way ANOVA, it is the same as that of the model.

[c] Least-square means estimate mean egg batch size given the same WL (see Fig. 1). The relationship between female body size and egg batch size.

[d] Based on the second collection from Donegebougou (September 2005). Only M and S were analyzed because of insufficient numbers of *An. arabiensis* families (n = 9). Family nested within population was treated as a random factor and the test of the effect of the molecular form was performed accordingly.

analysis because their numbers were not large enough to evaluate the effect of infection on female reproductive output.

The mean number of eggs per batch was 186.3, 182.5, and 162.0 in *An. arabiensis* and the M and S molecular forms of *An. gambiae*, respectively (Table 1). The differences in egg batch size were significant. However, variation in body size measured by wing length was also significant (Table 1), with the mean wing length of *An. arabiensis* and the M and S molecular

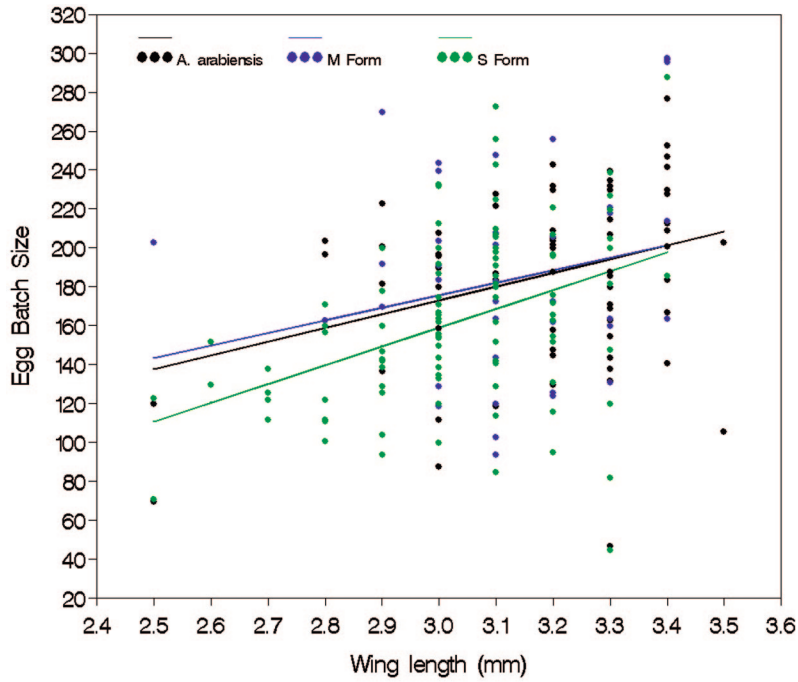

Fig. 1. Relationship between egg batch size and body size (wing length) of the molecular forms of *An. gambiae* and *An. arabiensis*. Lines represent predicted egg batch size for each species and form based on a covariance analysis (see Table 1 and text for details).

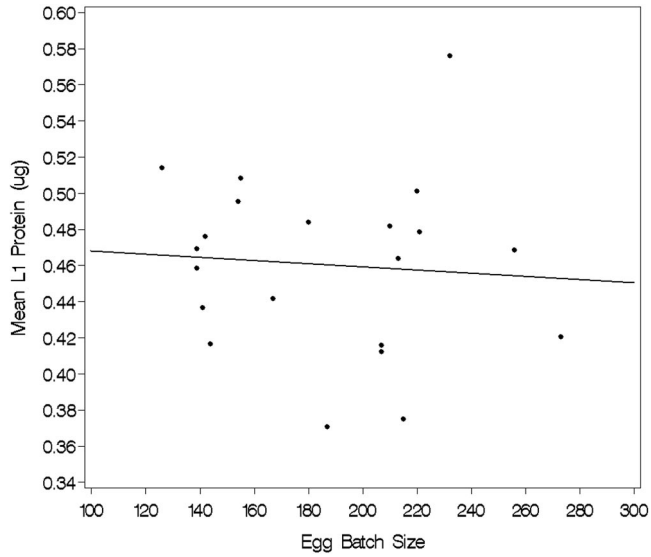

Fig. 2. Variation in the mean protein content per 2-h-old L1s against variation in egg batch size. Regression line show insignificant trend (slope = −0.0009; df = 1, 19; $P > 0.7$).

forms of *An. gambiae* being 3.2, 3.1, and 3.0, respectively (Table 1). Because body size is an important determinant (covariate) of egg batch size in mosquitoes (see Introduction), we compared the female egg production between species and forms, accommodating variation in body size by using a covariance analysis (Table 1; Fig. 1). Lack-of-fit test indicated no systematic deviation from linear relationships between egg batch size and wing length ($P > 0.11$; data not shown). After accommodating female size, the differences among the species and the molecular forms were not significant and the interaction between species/form and body size was insignificant, indicating that females of the same size are predicted to lay the same number of eggs regardless of their molecular form or species. These results suggest that females of the same size of the molecular forms of *An. gambiae* and their sibling species *An. arabienisis* similarly invest in egg batch size, although the expected egg batch size may differ among them because of differences in their mean body size.

Female's reproductive output consists of the number of eggs and her investment in each egg. The total protein in individual L1s <2 h old that hatched in deionized water is a reasonable measure of the female investment in her eggs, although comparing it with other measures would help future studies of mosquito fitness. The relationship between egg batch size, female body size, and the mean protein content per egg was evaluated in 701 L1s representing 21 families of the S molecular form from Pimperena. Egg protein content was not correlated with egg batch size ($r = -0.08$, df = 20, $P > 0.7$; Fig. 2) nor with female body size ($r = -0.18$, df = 19, $P > 0.4$), suggesting that females with more resources invest in a greater number of eggs of the same quality rather than in higher quality eggs.

To compare the nutritional content of eggs of the molecular forms, we used an additional collection of mosquitoes from Doneguebougou (8–9 September 2005), representing both forms in the same location and time. We measured protein content of individual L1s comprising 203 M form and 204 S form respectively representing 22 and 23 families by sampling 8–10 L1s per family (except one M family sampled by four L1s). The mean total protein in eggs of the M form (0.407 μg per L1; Table 1) was higher than that of the S form (0.384 μg per L1; Table 1). No heterogeneity was found in the variances of the molecular forms with respect to protein content (Levene's test for homogeneity of variance: df = 1, 43; MS = 0.0329, 0.0211; $F = 1.56$, $P > 0.21$).

Discussion

Surprisingly little is known about the ecological differences between the molecular forms of *An. gambiae* and between them and *An. arabiensis* except that the M form and *An. arabiensis* extend their distribution into drier environments (Coluzzi et al. 1985, Toure et al. 1998) and that their larvae predominate in rice (*Oryza* spp.) fields (Githeko et al. 1996, Toure et al. 1998). Ecological differences between the molecular forms can reveal the basis for the selection that "pulls apart" these incipient species. However, field surveys in areas of sympatry revealed that they cohabit the same larval sites (Minakawa et al. 1999, Gimnig et al. 2001, Edillo et al. 2002), and field experiments revealed no adaptive difference that explain the spatial segregation between rice fields and puddles or quarries (Diabate et al. 2005). Phenotypic divergence due to adaptation to different habitats is expected to be especially rapid on components of fitness assuming different optima in resource allocation between hab-

itats (Stearns 1992, Hatle et al. 2002). We compared the females of *An. arabiensis* and the molecular forms of *An. gambiae* in their reproductive output, including both 1) female's investment in individual eggs and 2) total egg batch size given female size. Differences between the molecular forms in resource allocation between egg number and egg quality as well as between current and future reproduction can affect their abundance and longevity, hence their role in disease transmission (Stearns 1992, Sheldon 2000).

Evaluating different measures of female investment in individual eggs would help future studies of mosquito fitness. As measured by the total protein in recently hatched larvae, female investment per egg remained constant regardless of the size of her egg batch or her own body size, confirming that mean egg size is equal within the S molecular form. However, mean investment per egg was not equal between the molecular forms, indicating that the total reproductive output between forms (and possibly species) can be misleading unless it includes the mean investment per egg. The difference in the mean egg protein content between forms amounted to 6%, indicating that the M form invests more resources in current reproduction (rather than in future reproduction). A greater investment per offspring of the M form is consistent with the lower productivity (per first instar) of rice fields, where the M form predominates compared with puddles in the surrounding areas, where the S form predominates, but not with the lower developmental success of the M form in both habitats (Diabate et al. 2005). A greater investment in current reproduction is usually associated with smaller future reproduction, including shorter longevity (Stearns 1992, Sheldon 2000). Hence, it suggests a shorter longevity for the M form, which in turn may reduce its vectorial capacity. This conjecture is consistent with the M form lower rate of infection with *P. falciparum,* its shorter longevity near rice cultivation areas (Robert et al. 1988, Diuk-Wasser et al. 2005), and the trend (although not statistically significant) of lower prevalence reported here.

The mean egg batch size was largest in *An. arabiensis* and smallest in the S molecular form of *An. gambiae,* as were the differences in the mean body size. Importantly, the expected egg batch size of females of the same size was similar regardless of their species and molecular form, suggesting a similar resource allocation to current and future reproduction with respect to offspring number. However, if the difference in body size between the molecular forms reported here is stable, then the females of the molecular forms differ in their egg batch size. Conditions in larval sites such as temperature, larval density, and abundance of nutritional resources play a major role in determining adult size (Briegel 1990, Kittayapong et al. 1992, Lyimo et al. 1992, Gimnig et al. 2002) and such factors vary considerably among larval sites and time points of the same site; suggesting that the differences in body size between the molecular forms (and hence in egg batch size) are not stable. Despite the large environmental variation, independent studies consistently found that *An. arabiensis* is larger than *An. gambiae* (Hogg et al. 1996, Petrarca et al. 1998) and high heritability for body size was measured in the M molecular form (Lehmann et al. 2006). Testing the stability of the difference in body size between the molecular forms requires additional studies.

The source of the bloodmeal is known to affect fecundity (Briegel 1990). In West Africa, however, previous studies found very high human biting rate from indoor collected mosquitoes across the species and molecular forms (Diatta et al. 1998, Dao 2003). Presumably, the species and forms in our study all fed on people. Variation in bloodmeal size due to interrupted feeding probably contributed to the large unaccounted variation in our analysis (Hogg et al. 1996). It is possible that some females retained some eggs after oviposition, but preliminary data suggest that this is exceedingly rare in field-collected *An. gambiae* and their F_1s (T.L. et al., unpublished data). Our interpretation of these results assumed that these factors did not differ between the species and forms or that their effects were minimal.

The variation in egg batch size and body size between species and forms may represent not only taxonomic units, but possibly geographical difference between populations (of the same form) because most *An. arabiensis* (88 of 103) were collected in Doneguebougou, whereas most of the M form (49 of 53) and the S form (102 of 119) were collected in Selingue and Pimperena, respectively. Despite including an additional source of variation between species and forms, the relationship between egg batch size and female size was similar across populations, suggesting a similar reproductive strategy where differences are mediated by variation in body size. Elucidating whether variation in body size and its corollaries represent rapid adaptive response to divergent conditions experienced by the molecular forms requires additional studies.

Acknowledgments

We thank the villagers of Donéguébougou, Pimpérena, and Sélingué for their kindness and generosity by accepting the mosquito collection in their homes. We thank Bill Hawley, Abdoulaye Diabate, Philip McCall, Randy DeJong, and Richard Sakai for helpful comments and suggestions on this manuscript. We also thank all the Malian team at Malaria Research and Training Center for support. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases.

References Cited

Ameneshewa, B., M. W. Service, V.-B. National Organization for Control of Malaria and Other, and A.A.E. Diseases. 1996. The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia. Med. Vet. Entomol. 10: 170–172.

Armbruster, P., W. E. Bradshaw, K. Ruegg, and C. M. Holzapfel. 2001. Geographic variation and the evolution of

reproductive allocation in the pitcher-plant mosquito, *Wyeomyia smithii.* Evolution 55: 439–444.

Bell, G. 1980. The costs of reproduction and their consequences [population models, plants and animals]. Am. Nat. 116: 45–76.

Briegel, H. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. J. Med. Entomol. 27: 839–850.

Bryan, J. H., M. A. Di Deco, and V. Petrarca. 1982. Inversion polymorphism and incipient speciation in *Anopheles gambiae* s.s in the Gambia, West Africa. Genetica 59: 167–176.

Cohuet, A., I. Dia, F. Simard, M. Raymond, and D. Fontenille. 2004. Population structure of the malaria vector *Anopheles funestus* in Senegal based on microsatellite and cytogenetic data. Insect Mol. Biol. 13: 251–258.

Coluzzi, M., V. Petrarca, and M. A. Di Deco. 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae.* Boll. Zool. 52: 45–63.

Coluzzi, M., A. Sabatini, V. Petrarca, and M. A. Di Deco. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Trans. R. Soc. Trop. Med. Hyg. 73: 483–497.

Costantini, C., N. Sagnon, E. Ilboudo-Sanogo, M. Coluzzi, and D. Boccolini. 1999. Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. Parassitologia 41: 595–611.

Dao, A. 2003. Etude comparative des paramètres entomologiques de la transmission du paludisme dans différents villages de la préfecture de Kati En zone de savane soudanienne au Mali. M.S. thesis. University of Bamako, Mali, Bamako.

della Torre, A., C. Fanello, M. Akogbeto, J. Dossou-yovo, G. Favia, V. Petrarca, and M. Coluzzi. 2001. Molecular evidence of incipient speciation within *Anopheles gambiae* s.s. in West Africa. Insect Mol. Biol. 10: 9–18.

della Torre, A., Z. Tu, and V. Petrarca. 2005. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. Insect Biochem. Mol. Biol. 35: 755–769.

Diabate, A., R. K. Dabire, E. H. Kim, R. Dalton, N. Millogo, T. Baldet, F. Simard, J. E. Gimnig, W. A. Hawley, and T. Lehmann. 2005. Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: a transplantation experiment. J. Med. Entomol. 42: 548–553.

Diabate, A., R. K. Dabire, P. Kengne, C. Brengues, T. Baldet, A. Ouari, F. Simard, and T. Lehmann. 2006. Mixed-swarms of the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in sympatric area from Burkina Faso. J. Med. Entomol. 43: 480–483.

Diatta, M., A. Spiegel, L. Lochouarn, and D. Fontenille. 1998. Similar feeding preferences of *Anopheles gambiae* and *A. arabiensis* in Senegal. Trans. R. Soc. Trop. Med. Hyg. 92: 270–272.

Diuk-Wasser, M., M. B. Toure, G. Dolo, M. Bagayoko, N. Sogoba, S. F. Traore, N. Manoukis, and C. E. Taylor. 2005. Vector abundance and malaria transmission in rice growing villages in Mali. Am. J. Trop. Med. Hyg. 72: 725–731.

Edillo, F. E., Y. T. Toure, G. C. Lanzaro, G. Dolo, and C. E. Taylor. 2002. Spatial and habitat distribution of *Anopheles gambiae* and *Anopheles arabiensis* (Diptera: Culicidae) in Banambani village, Mali. J. Med. Entomol. 39: 70–77.

Favia, G., and C. Louis. 1999. Molecular identification of chromosomal forms of *Anopheles gambiae* sensu stricto. Parassitologia 41: 115–118.

Favia, G., A. Lanfrancotti, L. Spanos, I. Siden-Kiamos, and C. Louis. 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. Insect Mol. Biol. 10: 19–23.

Fox, C., and M. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annu. Rev. Entomol. 45: 341–369.

Gentile, G., M. Slotman, V. Ketmaier, J. R. Powell, and A. Caccone. 2001. Attempts to molecularly distinguish cryptic taxa in *Anopheles gambiae* s.s. Insect Mol. Biol. 10: 25–32.

Gimnig, J. E., M. Ombok, L. Kamau, and W. A. Hawley. 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. J. Med. Entomol. 38: 282–288.

Gimnig, J. E., M. Ombok, S. Otieno, M. G. Kaufman, J. M. Vulule, and E. D. Walker. 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. J. Med. Entomol. 39: 162–72.

Githeko, A. K., M. W. Service, C. M. Mbogo, and F. K. Atieli. 1996. Resting behaviour, ecology and genetics of malaria vectors in large scale agricultural areas of Western Kenya. Parassitologia 38: 481–489.

Hatle, J., M. Crowley, A.A.L., and S. Juliano. 2002. Geographic variation of reproductive tactics in lubber grasshoppers. Oecologia (Berl.) 132: 517–523.

Hogg, J. C., M. C. Thomson, and H. Hurd. 1996. Comparative fecundity and associated factors for two sibling species of the *Anopheles gambiae* complex occurring sympatrically in The Gambia. Med. Vet. Entomol. 10: 385–391.

Kittayapong, P., J. D. Edman, B. A. Harrison, D. R. Delorme, and B. T. Mahidol University. 1992. Female body size, parity, and malaria infection of *Anopheles maculatus* (Diptera: Culicidae) in peninsular Malaysia. J. Med. Entomol. 29: 379–383.

Lanzaro, G. C., Y. T. Toure, J. Carnahan, L. Zheng, G. Dolo, S. Traore, V. Petrarca, K. D. Vernick, and C. E. Taylor. 1998. Complexities in the genetic structure of *Anopheles gambiae* populations in west Africa as revealed by microsatellite DNA analysis. Proc. Natl. Acad. Sci. U.S.A. 95: 14260–14265.

Lehmann, T., R. Dalton, E. H. Kim, E. Dahl, A. Diabate, R. Dabire, and J. P. Dujardin. 2006. Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of *Anopheles gambiae.* Infect. Genet. Evol. 6: 410–416.

Lehmann, T., M. Licht, N. Elissa, B. T. Maega, J. M. Chimumbwa, F. T. Watsenga, C. S. Wondji, F. Simard, and W. A. Hawley. 2003. Population structure of *Anopheles gambiae* in Africa. J. Hered. 94: 133–147.

Lochouarn, L., I. Dia, D. Boccolini, M. Coluzzi, and D. Fontenille. 1998. Bionomical and cytogenetic heterogeneities of *Anopheles funestus* in Senegal. Trans. R. Soc. Trop. Med. Hyg. 92: 607–612.

Lyimo, E. O., W. Takken, and J. C. Koella. 1992. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae.* Entomol. Exp. Appl. 63: 265–271.

Michel, A. P., W. M. Guelbeogo, O. Grushko, B. J. Schemerhorn, M. Kern, M. B. Willard, N. Sagnon, C. Costantini, and N. J. Besansky. 2005. Molecular differentiation between chromosomally defined incipient species of *Anopheles funestus.* Insect Mol. Biol. 14: 375–387.

Minakawa, N., C. M. Mutero, J. I. Githure, J. C. Beier, and G. Yan. 1999. Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. Am. J. Trop. Med. Hyg. 61: 1010–1016.

Petrarca, V., G. Sabatinelli, Y. T. Toure, and M. A. Di Deco. 1998. Morphometric multivariate analysis of field samples of adult *Anopheles arabiensis* and *An. gambiae* s.s. (Diptera: Culicidae). J. Med. Entomol. 35: 16–25.

Robert, V., B. Ouari, V. Ouedraogo, and P. Carnevale. 1988. [An ecologic study of adult and larval Culicidae in a rice field of Kou Valley, Burkino Faso]. Acta Trop. 45: 351–359.

SAS Institute. 2002. SAS for Windows version 9.0. SAS Institute, Cary, NC.

Scott, J. A., W. G. Brogdon, and F. H. Collins. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49: 520–529.

Sheldon, B. 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol. Evol. 15: 397–402.

Snow, R. W., C. A. Guerra, A. M. Noor, H. Y. Myint, and S. I. Hay. 2005. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature (Lond.) 434: 214–217.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford, United Kingdom.

Taylor, C., Y. T. Toure, J. Carnahan, D. E. Norris, G. Dolo, S. F. Traore, F. E. Edillo, and G. C. Lanzaro. 2001. Gene flow among populations of the malaria vector, *Anopheles gambiae*, in Mali, West Africa. Genetics 157: 743–750.

Toure, Y. T., V. Petrarca, S. F. Traore, A. Coulibaly, H. M. Maiga, O. Sankare, M. Sow, M. A. Di Deco, and M. Coluzzi. 1998. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. Parassitologia 40: 477–511.

Tripet, F., Y. T. Toure, C. E. Taylor, D. E. Norris, G. Dolo, and G. C. Lanzaro. 2001. DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae.* Mol. Ecol. 10: 1725–1732.

Tripet, F., G. Dolo, S. Traore, and G. C. Lanzaro. 2004. The "wingbeat hypothesis" of reproductive isolation between members of the *Anopheles gambiae* complex (Diptera: Culicidae) does not fly. J. Med. Entomol. 41: 375–384.

Yaro, A. S., A. Dao, A. Adamou, J. E. Crawford, J. M. Ribeiro, R. Gwadz, S. F. Traore, and T. Lehmann. 2006. The distribution of hatching time in *Anopheles gambiae.* Malar. J. 5: 19.

Received 14 March 2006; accepted 20 June 2006.