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Physiology and development of the M and S molecular forms of *Anopheles gambiae* in Burkina Faso (West Africa)

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Abstract. In West Africa, M and S molecular forms of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) Giles, frequently occur together, although with different population bionomics. The S form typically breeds in rain-dependant water collections and is present during the rainy season only whereas the M form can thrive all year long in areas with permanent breeding opportunities. In the present study, we explored physiological and developmental trade-offs at play in laboratory colonies and field populations of the M and S forms that originated from an area of sympatry in Burkina Faso, where M and S larvae exhibit such habitat segregation. In the laboratory, larvae of the M form developed slower than the S form (mean values 9.51 and 8.85 days, respectively, Wilcoxon's test, $P < 0.001$).

Although wing length and dry weight at emergence showed large variations, M females were on average 8% heavier than S females of similar wing length. Higher nutritional reserves (proteins and lipids) in teneral adults explained part of this weight difference, reflecting a better ability of the M form to garner resources at the larval stage. Furthermore, a higher rate of ovarian maturation was observed in the M form after a single bloodmeal. The relevance of these findings for parasite transmission is discussed.

Key words. *Anopheles gambiae*, emergence, nutritional reserves, ovarian development, size-corrected weight, trade-offs.

Introduction

The mosquito *Anopheles gambiae sensu stricto* Giles (hereafter *An. gambiae*) is the major vector of human malaria throughout sub-Saharan Africa. Its central role in *Plasmodium* transmission relies on its high anthropophily and widespread distribution throughout the continent, owing to its remarkable ability to cope with man-made environmental changes (Costantini *et al.*, 2009; Simard *et al.*, 2009; Cohuet *et al.*, 2010). This ecological plasticity is mirrored in high levels of genetic polymorphisms at the chromosomal and molecular levels (Pombi *et al.*, 2008; White *et al.*, 2010). Non-random distribution of molecular polymorphisms in natural *An. gambiae* populations led to the description of two 'molecular forms', known as the M and S forms (della Torre *et al.*, 2001, 2002) which are nowadays considered incipient species, based on molecular and phenotypic divergence (Turner *et al.*, 2005; Lehmann & Diabaté, 2008; White *et al.*, 2010; Sanford *et al.*, 2011). The S form is presumed ancestral and is found throughout sub-Saharan Africa where it typically breeds in small temporary rainwater collections (e.g. puddles and road ruts). The distribution range of the M form is restricted to western and

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central Africa (della Torre *et al*., 2005) where it frequently occurs together with the S form, sharing larval development sites and adult resting and biting habits (Edillo *et al.*, 2002; Lehmann & Diabaté, 2008). However, larvae of the M form also colonize more permanent water collections such as dams and irrigated agricultural areas, where the S form is not found (Gimonneau *et al.*, 2012). Accordingly, the two forms show different temporal patterns of abundance in West African savannas: the S form is highly seasonal, is present in great numbers during the rainy season and virtually disappears at the onset of the dry season, whereas the M form remains reproductively active all year round, hence potentially extending parasite transmission to the whole year.

Recent experimental and field studies conducted in and around an irrigated rice-cultivation area in south-western Burkina Faso (West Africa) have shown that the M and S forms exhibited phenotypic differences in their larval ecology and development. Habitat segregation was demonstrated in these settings, with M form larvae being the only ones thriving in irrigated rice-fields paddies whereas temporary water collections in surrounding savannas were colonized by both, M and S form larvae (Gimonneau *et al*., 2012). Transplantation experiments in which larvae of both forms were reared in typical temporary and permanent water collections showed that S form larvae developed faster than M form larvae in both types of habitat (Diabaté *et al*., 2005). However when predators are present, the M form outcompetes the S form in terms of developmental success, reflecting better predator avoidance in the former (Robert *et al.*, 1989; Gimonneau *et al.*, 2010). The consequences of these phenotypic differences in larval biology on the fitness of emerging adults have not been assessed, in spite of the strong relationship between larval developmental time, adult body size and nutritional status at emergence (Briegel *et al.*, 2001; Briegel & Timmermann, 2001; Fernandes & Briegel, 2005). These parameters are important components of adult reproductive success and longevity (Ameneshewa & Service, 1996; Takken *et al.*, 1998; Aboagye-Antwi & Tripet, 2010), which determine mosquito fitness and their capacity to vector disease agents (Lyimo & Koella, 1992; Aboagye-Antwi *et al.*, 2010; Cohuet *et al.*, 2010).

In the present study, we compared the reproductive physiology and resource accumulation in laboratory colonies and field mosquitoes of the M and S forms of *An. gambiae* from western Burkina Faso. Larval development time was monitored and wing size, body weight, and nutritional reserves were measured on emerging adults. We further assessed the ability of young females to develop and mature eggs from a single bloodmeal. Important phenotypic differences in the physiology and development of the two forms are described and their consequences on adult fitness and vector competence are discussed.

Materials and methods

Mosquitoes

Mosquito colonies. Experiments were conducted using two *An. gambiae* colonies recently established from wild gravid females collected in human dwellings in two villages near Bobo-Bioulasso in south-western Burkina Faso. The M form colony was established in August 2008 from gravid females of the M form collected in the village VK7 (11°23′N, 4°24′W), bordered by a 1.200-ha irrigated rice cultivation area. The S form colony was established in 2009 from females of the S molecular form collected in Soumousso (11°00′N, 4°02′W). Both villages belong to a humid savannah biotope and are located ∼50 km apart.

The two colonies are maintained in separate rooms in the insectaries of the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo-Dioulasso under controlled conditions (27 ± 1 °C, 80 ± 10% relative humidity with LD 12 : 12 h cycle). Adult females of both colonies are routinely fed on restrained rabbits. Colonies are checked monthly using PCR (Santolamazza *et al*., 2008) to control for the absence of contamination between the two molecular forms.

Rearing and harvesting protocols

We used three experimental settings to explore and compare physiological and developmental traits in laboratory and field mosquitoes of the M and S forms of *An. gambiae.*

In a first set of experiments, thereafter referred to as Experiment 1, 2 batches of 100 first instars larvae of the M and S forms were randomly picked within the first hours after hatching from colonies rearing pans in the IRSS insectaries (two pans containing egg batches from a pool of at least 50 caged females were used per molecular form). Each larva was placed individually into a plastic cup (diameter 7 cm × height 8.5 cm) filled with 30 mL of spring water. All larvae were reared at a density of 1 larva per cup and at the same time, on insectaries shelves which maintained the water temperature at 27 ± 1 °C with a LD 12 : 12 h cycle. They were fed daily with 0.15 mg of ground fish food (Tetramin® Baby fish Food; Tetra GmbH, Melle, Germany), which was previously found to be an optimal feeding diet for *An. gambiae* larvae (Djogbenou *et al.*, 2010). Adult emergence was monitored daily, allowing for determination of the mean duration of larval development in each colony. Males were discarded and were not taken into account in any analysis. Upon emergence, adult females were randomly assigned to one of three groups, depending on the trait to be measured: (a) teneral body weight and wing length, (b) the volume of blood ingested upon feeding and (c) ovarian development after a single bloodmeal (below).

In a second set of experiments, thereafter referred to as Experiment 2, eggs laid by *An. gambiae* females from the M and S colonies were sent to the Institut de Recherche pour le Développement (IRD) in Montpellier, France, where larvae were reared in a programmable environmental chamber (Sanyo MLR 315H, Sanyo Electric Co., Osaka, Japan), reflecting temperature and humidity cycles as monitored on a hourly basis in the village VK7 during the rainy season (August) using a Vantage Pro 2 weather monitoring station (Weatherlink; Davis Instruments, Hayward, CA, U.S.A.).The LD cycle was set at 12 : 12 h with an average temperature of 28 °C during the day and 25 °C at night (range: 24–29 °C). Relative humidity was set at 80%. Eggs were hatched in plastic trays

(30 cm × 20.5 cm × 6.5 cm) containing deionized water and first instars were readily transferred into individual tubes (one larva per tube, diam. 25 mm × height 95 mm) containing 5 mL of deionized water. They were fed daily with 0.15 mg of ground fish food (Tetramin®). The tubes were arranged in racks and each day a random rotation of the racks was performed inside the environmental chamber to reduce positional effects. At pupation, tubes were covered with a fine net to prevent emerging adults from escaping. Emergence was controlled every hour in order to collect teneral adults within 1 h of emergence. Adults were immediately knocked down in a −40 °C freezer and females were individually stored in 1.5-mL plastic vials and stored at −80 °C, pending body weight, wing length and nutritional reserves assessment (below).

The third set of experiments (i.e. Experiment 3) involved wild *An. gambiae* mosquitoes collected as pupae from a natural temporary larval development site in Natéma (11°23′N; 04°29′W) where both molecular forms are known to occur. The village is located 10 km west of the VK7 rice fields, in a humid savannas background. Collections were conducted in October 2009. Larval densities were determined as low using the dipping technique according to Sattler *et al*. (2005). Pupae were reared on site, in transplantation cages (see Diabaté *et al*., 2005, 2008 for a description of the cages) so that they remained within the same abiotic and climatic conditions. Emerging adult mosquitoes were collected within 24 h of emergence. A leg was dissected out from all *An. gambiae* complex females and preserved for further molecular identification (Santolamazza *et al.*, 2008). Adults were flash frozen upon collection using liquid nitrogen and stored at −80 °C until transportation to the IRD in Montpellier, France, where their body weight and nutritional reserves were quantified (below). In this experiment, all mosquitoes were harvested within a unique period of 48 h. PCR identification of emerging females showed that each day, M and S specimens were collected in similar proportions.

Morphological and physiological traits

Wing length. For each adult female, the right wing was dissected out on a refrigerated table (−2 °C) and mounted on a microscope slide under a cover slip. A photograph was taken under a dissecting microscope (×40, Leica S6D) and the wing length was measured as described previously by Charlwood (1996) to an accuracy of ±0.001 mm using the ImageJ1.41.0 software (Wayne Rasband, National Institute of Health, Rockville, MD, U.S.A.).

Body weight. Body weight was measured for the females after they were freeze-dried for 72 h. Dry weight was measured immediately after the freeze-drying process to an accuracy of ±1 μg using a Mettler Toledo MX5 balance (Mettler-Toledo GmbH, Greifensee, Switzerland).

Nutritional reserves. Total soluble proteins, lipids and glycogen contents were determined on single mosquito specimens using colorimetric techniques according to the methods developed by Van Handel (1985a,b; 1988) and modified by Rivero & Ferguson (2003). Each metabolite concentration was obtained from the corresponding standard curve. Sugar contents were not considered in this study, being negligible in newly emerged unfed females (Clements, 2000). Furthermore, the glycogen content of females was not analysed because many estimates could not be reliably assessed. Protein and lipid contents were converted to their caloric value: 1 mg of protein and 1 mg of lipids represent an energetic value of 4 and 9 calories, respectively (in Clements, 2000).

Bloodmeal size and ovarian development. Newly emerged females in Experiment 1 were kept in individual cups (diam. 7 cm × height 8.5 cm) covered with a net. The night after their emergence, the females were individually offered a bloodmeal on a volunteer's arm for 30 min. Females of the two forms were fed simultaneously, on both arms and the same volunteer was used throughout. Only fully engorged females were considered for monitoring. They were randomly assigned to one of two groups dedicated either to ingested blood volume quantification or to the monitoring of their ovaries development. For the quantification of ingested blood, females were kept for 4 days after blood feeding in individual plastic tubes (diameter 25 mm × height 95 mm) in the insectary and provided with water only. The haematin present in faecal material was eluted in 1.0 mL of 1% lithium carbonate and absorbance was read at 400 nm. The amount of haematin was determined by comparison to a haematin standard curve and reflects the amount of haemoglobin a female excreted, considered as a proxy of the ingested blood volume (Briegel, 1980; Hogg *et al.*, 1996). For the observation of ovarian development, females were kept in plastic cups (diameter 7 cm × height 8.5 cm) for 4 days and were provided with water only. They were killed by freezing at −20 °C, and their ovaries were dissected out and observed under a microscope (×100 and ×200, Leica ICC 50) to determine their Christophers' developmental stage as described in Detinova (1962). Data on the ovarian development of additional females from the group devoted to haematin quantification were added, after controlling for an absence of significant differences between groups.

Statistical analysis. Development time of the two molecular forms in Experiment 1 was not normally distributed and was compared between molecular forms using the non-parametric Wilcoxon's test based on rank values.

Each of the traits related to size and nutritional reserves in teneral females was assessed with a two-way analysis of variance (ANOVA) testing the effect of molecular form, experiment and their interaction. Response variables were log-transformed to normalize their distribution when necessary. Residuals from each analysis were confirmed as not departing from a normal distribution using the Shapiro–Wilk *W* test (not shown). Only females with a full set of data were included in the analyses, i.e. wing length and dry weight for Experiment 1

and wing length, dry weight, protein content and lipid content for Experiment 2 and Experiment 3.

The volume of haematin taken during blood feeding was also analysed by ANOVA.

Differences in the proportion of ovaries found at the different Christophers' stages were assessed using the Cochran–Mantel–Haenszel test in a contingency table analysis of 'stage' and 'form', where data were stratified by experiment.

Analyses were performed using the JMP (v. 5.1.2) statistical software (SAS Institute Inc., Cary, NC, U.S.A.).

Results

Development time

Larval developmental time between hatching and emergence of teneral adults was successfully monitored for females of the M ($N = 90$) and S ($N = 99$) forms in Experiment 1. Larvae that died before emergence as well as males were not taken into account in the analysis. Larval development time was significantly shorter in the S (mean = 8.85 days) than in the M form (mean = 9.51 days, Wilcoxon's test, $Z = 3.164$, $P < 0.001$). The first adult females emerged on day 8 post-hatching and peaked on day 9 for both the M and S forms (Fig. 1). By day 10, all S form females had emerged, whereas emergence of M form females continued for up to two subsequent days, with 23% of M form females emerging on days 10 and 11 post-hatching (Fig. 1).

Body weight and wing length

Wing length and dry weight in teneral females varied significantly between molecular forms and between experiments within forms, with a significant interaction between form and experiment resulting in no clear underlying pattern (Tables 1 and 2). However, when the two traits were combined into a ratio of body weight per wing size unit, interesting trends emerged. The main effects for molecular form and experiment were significant, indicating that the ratio value varied between forms and between experiments but the interaction term was no more significant (Table 2). Across the three experiments, the teneral weight of M females was on average ~8% greater than that of S females of a similar wing length, and the same trend was observed for field and colony collected females [*overall mean*, M = 114.5 µg (109.4–119.9), S = 105.7 µg (103.0–108.4)]. Females emerging from the individual tubes of Experiment 2, were generally the smallest and lightest; they also had the lowest size-corrected weight (Table 1).

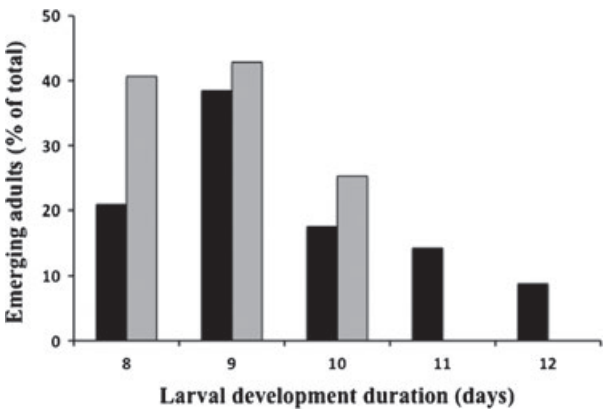

Fig. 1. The distribution of larval development time for females of the M (black bars adding up to 100%, $N = 90$) and S (grey bars adding up to 100%, $N = 99$) molecular forms of *Anopheles gambiae* reared in individual cups.

Nutritional reserves

There were significant differences in nutritional reserves accumulated by teneral females of the M and S forms in the different experiments (Table 3), and interaction terms between experiment and molecular form were statistically significant in all cases (Table 4). These interactions were mainly as a result of teneral females of the S form having less of either resource, particularly after developing in the individual tubes of Experiment 2. In each experimental condition, teneral females of the S form consistently showed 20–90% lower protein and lipid content than their M form counterparts, reflecting significantly lower overall nutritional reserves accumulated through larval life. This trend was observed in both laboratory-reared mosquitoes (Experiment 2) as well as field mosquitoes (Experiment 3), and was also statistically significant when energetic data were standardized to wing size (Tables 3 and 4).

Blood feeding and ovarian development in adult females

No difference was found in the amount of excreted haematin between the two molecular forms (ANOVA, $F_{1,42} = 0.959$, $P = 0.33$). Because all mosquitoes fed on the same volunteer at the same time, this suggested that females of both molecular forms ingested a bloodmeal of a similar size.

Ovarian development was monitored by recording the Christophers' stage of the oocytes 4 days after females had taken a first bloodmeal. Complementary observations were performed on females originating from larvae reared in trays at low densities and receiving 0.30 mg of Tetramin® per larva and per day (i.e. twice the amount used in Experiment 1 and 2). In each case, the proportion of females with ovaries at the different Christophers' stages differed between the M and S forms (Fig. 2, Cochran–Mantel–Haenszel test stratified by experiment, d.f. = 4, $\chi^2 = 266.22$, $P < 0.001$). Although mean wing length was not statistically different between forms (M: 2.39 mm ± 0.22, S: 2.47 mm ± 0.19, ANOVA: $F_{1,88} = 2.917$, $P = 0.09$), females of the S form were unable to develop their ovaries beyond Christophers' stage 2, whereas 55.1% of M females (49/89) did so, with up to 22% (20/89) reaching the final oocyte maturation stage (Fig. 2). This result demonstrated a better reproductive potential in the M form than in the S form after a single bloodmeal ingested within the first day after emergence.

Table 1. Mean wing length, body weight and size-corrected weight in teneral females of the M and S molecular forms of *Anopheles gambiae* from Burkina Faso reared under different experimental conditions.

		n	Wing length (mm) ± SE*	Dry weight (μg) (95% CI)†	Dry weight per wing size unit (μg/mm) (95% CI)†
Experiment 1	M	17	2.240 ± 0.039	272.8 (247.4–300.7)	121.9 (111.3–133.6)
	S	18	2.347 ± 0.038	289.0 (262.9–317.8)	123.2 (112.7–134.6)
Experiment 2	M	14	2.017 ± 0.043	170.8 (153.4–190.2)	84.7 (76.6–93.7)
	S	17	2.013 ± 0.039	154.0 (139.7–169.8)	76.6 (69.9–83.9)
Experiment 3	M	26	2.261 ± 0.032	328.3 (303.4–355.3)	145.3 (135.0–156.5)
	S	21	2.256 ± 0.035	282.7 (258.9–308.7)	125.4 (115.5–136.1)

*Standard error.
†95% confidence interval (CI): means, lower and upper bounds are back-transformed values (see text).

Table 2. ANOVA for wing length, body weight and size-corrected weight in teneral females of the M and S molecular forms of *Anopheles gambiae* from Burkina Faso reared under different experimental conditions.

Response	Source	d.f.	Sums of squares	*F*	P
Wing length (mm)	Experiment	2	1.512	113.314	<0.001
	Form	1	0.029	4.344	0.040
	Interaction	2	0.075	5.585	0.005
	Error	107	0.714	—	—
Dry weight (μg)*	Experiment	2	1.515	97.472	<0.001
	Form	1	0.022	2.791	0.098
	Interaction	2	0.042	2.729	0.070
	Error	107	0.832	—	—
Size corrected	Experiment	2	0.985	72.297	<0.001
Weight (μg/mm)*	Form	1	0.032	4.762	0.031
	Interaction	2	0.024	1.763	0.177
	Error	107	0.729	—	—

*Log_{10}-transformed values.

Discussion

When reared under a range of common experimental conditions, the M and S molecular forms of *An. gambiae* from Burkina Faso exhibited significant phenotypic differences in their physiology and development, which might reflect different adaptive strategies to cope with a highly seasonal environment.

Dry weight and wing length

We found evidence that M form females were consistently heavier than S form females of a similar wing size and reared under the same experimental conditions, which was at least partially as a result of their greater accumulation of proteins and lipids. M form females had between 20% and 90% more calories than S females of the same size. Thus, upon emergence, M form females would have more of these resources to readily allocate towards their survival and reproduction than S form females of the same size.

The wing length and body weight of teneral females and the ratio between these traits varied considerably according to the rearing conditions they experienced as larvae, as previously shown (Lyimo *et al*., 1992; Koella & Lyimo, 1996). Field specimens were larger and heavier than colony mosquitoes reared in the laboratory, as reported previously by Huho *et al.* (2007). However, in all cases, when reared in the same conditions, the ratio of dry body weight to wing length at emergence was greater in females of the M form than in the S form. Consistency of this result across experiments involving colony as well as field-collected specimens strongly suggests this represents genuine differences between *An. gambiae* molecular forms. However, generalization of this finding requires additional studies to be conducted in other ecological settings where the M and S forms co-exist. Indeed, recent investigations of wing morphometrics (i.e. length and width) in natural *An. gambiae s.s.* populations suggested geographical variation in the extent of morphological differentiation between the M and S form, which might reflect the strength of pre-mating barriers between them (Sanford *et al*., 2011).

The dry weight of teneral mosquitoes reflects the critical mass they attained before pupal commitment. In holometabo lous insects, critical mass represents a mass (and nutritional status) threshold at which hormonal mechanisms that ultimately determine metamorphosis are triggered (Telang *et al.*, 2007). Hence, the S form could have adapted to the unpredictability of its larval habitat and/or to its ill adaptation to the predator pressures, by evolving a smaller critical mass allowing earlier metamorphosis and escape from aquatic habitats. Emerging mosquitoes are unable to sustain flight towards a sugar source until 12 h after emergence and blood feeding is unlikely to occur before the first 24 h of an adult female's life (Foster & Takken, 2004). Teneral reserves are therefore crucial to insure survival during the first day of life, which might be jeopardized by lower caloric contents in S form females. Moreover, the smaller body weight to wing length ratio of S form females suggests their ratio of surface area to volume could be greater than for M females. This might further impact on survival, especially in arid environments, because individuals with a higher surface to volume ratio are expected to have a greater mass specific rate of water loss (Hadley, 1994). Evidence indeed suggests that S form mosquitoes are less resistant to desiccation challenges than sympatric mosquitoes of the M form (Lee *et al.*, 2009; Aboagye-Antwi *et al.*, 2010).

Table 3. Metabolic reserves and their corresponding energetic values in teneral females of the M and S molecular forms of *Anopheles gambiae* from Burkina Faso reared under different experimental conditions.

		n	Proteins (μg) (95% CI)*†	Lipids (μg) (95% CI)*†	Calories (× 1000) (95% CI)*	Calories (×1000) per wing size unit (95% CI)*
Experiment 2	M	14	15.26 (12.17–19.13)	10.45 (9.28–11.77)	159.0 (139.9–178.1)	78.70 (±8.37)
	S	17	6.12 (4.98–7.51)	6.00 (5.39–6.68)	81.9 (±17.3)	40.63 (±7.59)
Experiment 3	M	26	10.42 (8.83–12.30)	13.58 (12.44–14.82)	171.8 (±14.0)	75.91 (±6.14)
	S	21	10.11 (8.40–12.15)	11.41 (10.35–12.57)	149.7 (±15.6)	66.37 (±6.83)

*95% confidence interval (CI).
†Means, lower and upper bounds are back-transformed values (see text).

Table 4. ANOVA for metabolic reserves, their corresponding energetic values, and their energetic value corrected for wing size in teneral females of the M and S molecular forms of *Anopheles gambiae* from Burkina Faso reared under different experimental conditions.

Response	Source	d.f.	Sums of squares	*F*	P
Protein content (μg)*	Experiment	1	0.013	0.374	0.543
	Form	1	0.779	22.927	<0.001
	Interaction	1	0.680	20.027	<0.001
	Error	74	2.514	—	—
Lipid content (μg)*	Experiment	1	0.714	75.963	<0.001
	Form	1	0.463	49.266	<0.001
	Interaction	1	0.126	13.436	0.001
	Error	74	0.695	—	—
Calories	Experiment	1	30 014	23.338	<0.001
	Form	1	45 446	35.338	<0.001
	Interaction	1	14 015	10.898	0.002
	Error	74	95 168	—	—
Calories/mm	Experiment	1	2435	9.865	0.002
	Form	1	10 479	42.443	<0.001
	Interaction	1	3763	15.243	<0.001
	Error	74	18 270	—	—

*Log_{10}-transformed values.

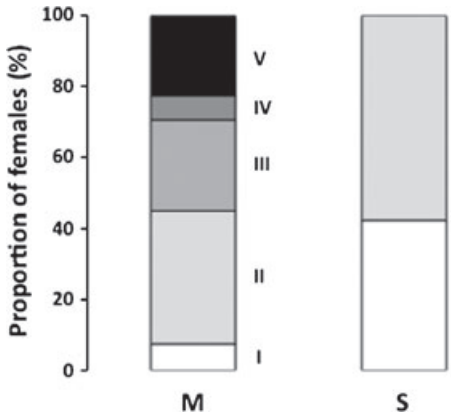

Fig. 2. Ovaries development in females of the M and S molecular forms of *Anopheles gambiae* after complete digestion of the first bloodmeal. Roman symbols are Christophers' stages. $N = 89$ for M and $N = 98$ for S.

Ovarian development

Field and laboratory experiments have repeatedly shown species-specific body size (wing length) thresholds for a mosquito female to develop her eggs after a single bloodmeal: small females reared under crowded and/or resource-poor settings have to make up for their teneral resource deficiencies before being able to allocate bloodmeal components to ovarian development (Takken *et al.*, 1998; Fernandes & Briegel, 2005). In such resource-deprived females, ovary development is arrested at Christophers' stage II, known as a 'resting stage' (Clements, 2000). By contrast with gravid females, females found at the resting stage are called 'pre-gravid' (Gillies, 1954) and a second bloodmeal usually allows them to complete ovarian development. Pre-gravid *Anopheles* females are found in various proportions in the field, according to the species, locality and season of sampling (Gillies, 1954; Lyimo & Takken, 1993; Charlwood *et al.*, 2003). In our experiment, the S form never developed ovaries beyond the resting stage with a single bloodmeal, although no difference in the size of the bloodmeal was found between the two molecular forms. Development of ovarian follicles is under the control of a juvenile hormone (JH) secreted by the *corpora allata* within the first day after emergence (Clements, 2000). Experimental evidence showed that JH secretion in *Aedes aegypti* is modulated by teneral nutritional status, with small undernourished females having limited amounts of JH and being unable to fully develop their ovaries without the resources contained in a subsequent bloodmeal (Noriega, 2004). The direct role of JH in ovary maturation was proven by ectopic application of an analogue of this hormone to small females, which restored follicle development (Feinsod & Spielman, 1980). Regulation of JH secretion by nutritional status probably also exists in *Anopheles gambiae* (Clements, 2000; Charlwood *et al.*, 2003) and could partially explain why S form females never developed their ovaries and remained pre-gravid. We infer the first bloodmeal is primarily and completely used to compensate for metabolic or nutritional deficiencies carried over from larval life in the S form, thus preventing JH secretion and ovaries maturation. By contrast, the first bloodmeal seems readily directed towards eggs development in M females, highlighting different innate reproductive potential between molecular forms.

Impact on Plasmodium *transmission*

The intensity of *Plasmodium* transmission is largely determined by the vectorial capacity of the mosquito population (Macdonald, 1957). The two most influential parameters in this

model are the rate at which humans are bitten and mosquito longevity (Cohuet *et al.*, 2010). Here we showed that females of the S molecular form may need to feed more than once during their first gonotrophic cycle to mature their ovaries. This may increase the chance of S form females becoming infected early in life in comparison with M form females, although the overall impact on parasite transmission by the S molecular form might be balanced by lower survival rate, especially in arid environments (Lee *et al.*, 2009; Aboagye-Antwi *et al.*, 2010). To date, field investigations comparing vectorial capacity in the two molecular forms did not detect differences in parasite prevalence between the M and S forms (Wondji *et al.*, 2005; Aboagye-Antwi *et al.*, 2010, but see Ndiath *et al.*, 2011). Comparative studies exploring mosquito population age structure in the field might provide further relevant information towards further unravelling the epidemiological impact of different trade-offs between longevity and reproduction in the M and S forms of *An. gambiae*.

Conclusion

Our results showed the importance of considering how body weight and wing length are related to other life history traits when it comes to comparing the M and S forms of adult female *An. gambiae*. A similar wing length did not accurately reflect how each form accumulates different nutritional reserves as larvae, or the energetic resources they would have available as teneral adults. Our results showed the M and S molecular forms differed in this respect, and did so in laboratory as well as in natural conditions. Furthermore, although the reproductive physiology of females from the two forms was only examined at emergence and after the first bloodmeal, the present study provided evidence that the two molecular forms of *An. gambiae* exhibit different reproductive strategies which could have arisen because human hosts are probably not a limiting resource, whereas breeding site desiccation is a real threat for the S form.

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