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Seasonal variation in wing size and shape between geographic populations of the malaria vector, *Anopheles coluzzii* in Burkina-Faso (West Africa)

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Highlights

- Developmental and morphological plasticity is demonstrated among populations,
- Wing shape chiefly varies among the geographic origin of mosquitoes,
- Wing shape conformation thus link with local adaptation,
- Wing size and surface area changes depend on the environmental cues experienced.

Abstract

The mosquito, *Anopheles coluzzii* is a major vector of human malaria in Africa with widespread distribution throughout the continent. The species hence populates a wide range of environments in contrasted ecological settings often exposed to strong seasonal

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fluctuations. In the dry savannahs of West Africa, this mosquito population dynamics closely follows the pace of surface water availability: the species pullulates during the rainy season and is able to reproduce throughout the dry season in areas where permanent water bodies are available for breeding. The impact of such environmental fluctuation on mosquito development and the phenotypic quality of emerging adults has however not been addressed in details. Here, we examined and compared phenotypic changes in the duration of pre-imaginal development, body dry mass at emergence and wing size, shape and surface area in young adult females *An. coluzzii* originated from five distinct geographic locations when they are reared in two contrasting conditions mimicking those experienced by mosquitoes during the rainy season (RS) and at the onset of the dry season (ODS) in Burkina-Faso (West Africa). Our results demonstrated strong phenotypic plasticity in all traits, with differences in the magnitude and direction of changes between RS and ODS depending upon the geographic origin, hence the genetic background of the mosquito populations. Highest heterogeneity within population was observed in Bama, where large irrigation schemes allow year-round mosquito breeding. Further studies are needed to explore the adaptive value of such phenotypic plasticity and its relevance for local adaptation in *An. coluzzii*.

Key words

Mosquito; development; phenotypic plasticity; dry season; geometric morphometrics.

LIST OF ABBREVIATIONS

RH = Relative Humidity

RS = Rainy Season

ODS = Onset of the Dry Season

LM = landmarks

CS = Centroid Size

GPA = Generalized Procrustes Analysis

PCA = Principal Component Analysis

PC1 = First axis of the Principal Component Analysis

PC2 = Second axis of the Principal Component Analysis

ΔD = vector length

θ = angular difference

MD = Metric Disparity

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INTRODUCTION

Species living in dry savannahs of West Africa, including the malarial mosquito *Anopheles coluzzii* (Diptera, Culicidae), have to face high seasonality in their environment, with the occurrence of an adverse dry season during which mean temperatures rise, relative humidity decreases, and water collections dry up. In such environment, the population dynamics of anopheline species follow the pace of water collections availability (Adamou *et al.*, 2011; Lehmann *et al.*, 2014, 2010; Yaro *et al.*, 2012). However, larval instars of *An. coluzzii* can be found in both temporary (*i.e.* rain-filled) and permanent (*i.e.* generally man-made) water collections (Costantini *et al.*, 2009; Gimonneau *et al.*, 2012). Hence, in areas where large anthropogenic (*e.g.* dams, ricefields, etc.) or natural (*e.g.* ponds, river edges, etc.) surface water collections are available all year round, mosquitoes persist and reproduce all year long, whereas in areas where only temporary waters are available, local mosquito populations virtually disappear during the dry season. In such area, *An. coluzzii* females might enter a state of quiescence/diapause and persist throughout the dry season by aestivation (Dao *et al*., 2014; Lehmann *et al.*, 2010; 2014). In this context, mosquitoes may have developed local behavioural, physiological and/or morphological adaptations to survive and persist during the adverse conditions of the dry season. There is indeed evidence in the recent literature for seasonal physiological adjustments in some anopheline species, including *An. coluzzii* (Adamou *et al.*, 2011; Hidalgo *et al.*, 2014; Huestis *et al.*, 2012, 2011; Huestis and Lehmann, 2014; Lehmann *et al.*, 2010; Mamai *et al.*, 2014; Yaro *et al.*, 2012). Whether these physiological changes sustain variation in other fitness traits of the mosquito remains unknown and their evolutionary and adaptive value has yet to be assessed.

Environmental conditions (*i.e.* biotic and abiotic factors) perceived by larvae during development are known to pilot insect's developmental rate (Couret *et al.*, 2014; Damos and Savopoulou-Soultani, 2012; Lyons *et al.*, 2013; Mouline *et al.*, 2012) with consequences on adults' overall phenotypic quality, including their morphometric properties (*i.e.* shape, size, asymmetry) (Aboagye-Antwi and Tripet, 2010; Atkinson, 1994; Czarnoleski *et al.*, 2013; Kingsolver *et al.*, 2009; Pétavy *et al.*, 2004). Morphological approaches have recently received increasing attention mainly with the advent of new applications and current developments in geometric morphometrics (Ayala *et al.*, 2011; Sadeghi *et al.*, 2009; Zimmermann *et al.*, 2012). Although morphometric methods were traditionally used at the upper taxonomic level (*i.e.* at the genus/species level), geometric morphometrics now offers

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powerful analytical and graphical tools for quantitative assessment and visualisation of morphological variations within and among species. Currently, geometric morphometric approaches are increasingly applied to a wide range of research fields including systematics, phylogeny and population genetics, ontogeny and developmental stability (Debat *et al.*, 2011; Klingenberg and Marugán-Lobó, 2013; Klingenberg and McIntyre, 1998; Morales Vargas *et al.*, 2013; Savriama *et al.*, 2012). One of the main added values of geometric morphometrics is its ability to consider the variation of both size and shape of individuals and/or organs separately. As such, wings have been the subject of many geometric morphometric analyses in insects (Baylac and Daufresne, 1996; Rohlf and Slice, 1990), and many of these studies characterised populations within species among geographic and climatic variations (Haas and Tolley, 1998; Hoffman and Shirrifs, 2002; Morales Vargas *et al.*, 2013; Roggero and d'Entrèves, 2005). Indeed, it was shown that wing shape variation can inform on current or recent population events and contains a great deal of information on genetic variation among populations (Dujardin, 2011, 2008). On the other hand, insect wing size, often used as a proxy of whole-insect size, has been shown to vary chiefly according to larval growth conditions (Koella and Lyimo, 1996; Lyimo *et al.*, 1992; Mouline *et al.*, 2012). Recently, Andersen *et al.* (2005) showed that both size and shape of the wing of *Drosophila mercatorum* (Diptera, Drosophilidae) changed as a response to the maternal and developmental temperature. Similarly, Ayala *et al.* (2011) further argued that changes observed in wing morphometric properties of *Anopheles funestus* mosquitoes are the result of natural selection and may contribute to local adaptation in wild populations of this mosquito. Accordingly, developmental plasticity expressed under various environmental conditions may result in morphological changes in adults that contribute to local adaptation.

To test this hypothesis, we monitored larval development and assessed adult phenotypic variation in five ecologically and geographically distinct populations of *An. coluzzii* exposed to contrasted environmental cues mimicking the rainy and dry season conditions in Northern Burkina-Faso. Desiccation has been proposed as a major threat when larvae develop in temporary waters. It is therefore expected that mosquito populations adapted to breed in rain-dependant collections will speed-up their development in response to increased desiccation threat in dry season conditions (Diabaté *et al.*, 2008). On the other hand, because drying-out of the breeding site is not a threat when larvae develop in permanent waters, we predicted that the duration of larval development will increase in the dry season conditions, reflecting suboptimal growth conditions (*e.g.* exposure to extreme temperatures

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and fluctuations thereof, see Colinet *et al.,* 2015). The impact of changing developmental duration on the overall adult fitness should vary according to the level of local adaptation of the population under scrutiny. Here, we used wing geometric morphometrics and we monitored dry weight at emergence to explore phenotypic plasticity in emerging adult females when larvae were reared under contrasted environmental conditions.

MATERIALS AND METHODS

1. Sample populations

In Burkina-Faso (West Africa), annual rainfalls and duration of the rainy and dry seasons vary along a latitudinal gradient. Although, rainfalls do not exceed 300 mm during the 2-3 months of the rainy season in the northernmost regions (*i.e.* 9-10 months of dry season), it can be as high as 1,200 mm during the 5-6 months of the rainy season in the southernmost regions (*i.e.* 6-7 months of dry season; see Figure 1). The experiments were conducted using four mosquito populations derived from the progeny of wild-caught *An. coluzzii* females from North and South Western Burkina-Faso. Gravid female mosquitoes were sampled from within human dwellings in localities where *An. coluzzii* mosquitoes are present all year long (*i.e.* presence of year-round mosquito breeding opportunities in Oursi [14°40'N, 0°27'W] and Bama [12°01'N, 04°23'W]) and in localities where no permanent breeding is possible and the mosquito populations are highly seasonal (*i.e.* Déou [14°35'N, 0°43'W] and Soumousso [11°01'N, 04°02'W]; Figure 1). Before experiments, all populations were reared for 6 generations in order to eliminate any potential trans-generational effect driven by the native environment and/or stress from transportation, while limiting acclimation to laboratory conditions and trait homogenization. All colonies were reared at the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo-Dioulasso under controlled conditions (27 ± 1 ºC, 80 ± 10% relative humidity with LD cycles of 12h:12h). Females were routinely blood fed on restrained rabbits, their eggs were collected on filter paper and larvae were reared in trays and fed grounded fish food (Tetramin®) *ad libitum*. For experiments, we included a fifth sample consisting of *An. coluzzii* mosquitoes from the Ngousso colony originated from equatorial South Cameroon [3°52'N, 11°31'E] in 2006. Mosquitoes from this last colony were further considered as a control colony because they were maintained in laboratory conditions for >50 generations (Harris *et al.*, 2010) and thus were not adapted to fluctuating environmental conditions.

2. Experimental rearing conditions

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Mosquitoes of the five populations were reared from eggs to adults in programmable climatic chambers (Sanyo MLR 315H, Sanyo Electric Co., Osaka, Japan) parameterised to reproduce ambient daily climatic variation (*i.e.* temperature, relative humidity [RH] and photoperiod duration) experienced by mosquitoes in Oursi, the northernmost region of Burkina-Faso, during the rainy season and at the onset of the dry season. Hourly temperature and RH, and photoperiod duration were set up using the climatic data available at http://www.gaisma.com/ for the Oursi area, averaged over the past 30 years for a period ranging from August 1st to 31th (*i.e.* rainy season, hereafter referred to as RS conditions) and from December 1st to 31th (*i.e.* at the onset of the dry season, hereafter referred to as ODS conditions) Detailed daily climatic data provided by Claude *et al.* (1981) were also included. Climatic conditions in the incubators (N=1 for RS conditions, N=2 for ODS conditions) mimicked those observed in the fields and were tightly monitored using MicroLog Pro monitors placed inside the incubators and inside one of the rearing pans to monitor larval rearing water temperature (EC750, Davis Instruments, Hayward, CA, USA; see Figure 2).

For each population, 3-4 independent sessions from fresh batches of eggs obtained from more than 50 caged gravid females were used to rear mosquitoes. For each session, two batches of eggs were merged in order to achieve large sample sizes and synchronous hatching. For each climatic chamber (N=3), the experimental conditions (RS, ODS) were switched between the sessions.

Upon collection in the population cages, eggs were transferred into independent plastic trays (30 cm × 20.5 cm × 6.5 cm) containing 1 L of deionised water, and immediately exposed to the RS or ODS experimental conditions in the climatic chambers. After hatching, first larval instars were readily transferred into new plastic trays filled with 1 L of deionised water at an optimal growing density of 200 larvae per tray to reduce variation in adult body size at emergence. Four to six plastic trays (*i.e.* 800-1,200 larvae) were used for each mosquito population and each experimental condition (RS, ODS). Every day, the position of the trays was randomly alternated to avoid positional effects within the incubators. Larvae were fed daily *ad libitum* with sprinkled ground fish food (Tetramin®) until pupation. Pupae were collected and immediately transferred into plastic cups (diameter 7 cm × height 8.5 cm) filled with 10 mL of deionized water, and maintained under RS or ODS conditions until adult emergence. Males were discarded and the right wing of adult females was dissected out and mounted onto microscope slides for further geometric morphometrics analyses.

3. Pre-imaginal development and body dry mass

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Duration of the pre-imaginal development was monitored in each mosquito population reared in both RS and ODS conditions. Every 12 hours, live larvae and pupae were counted and transferred into new plastic trays to prevent scum formation and accumulation of toxic waste metabolites. Duration of the pre-imaginal development was measured from egg hatching to adult emergence (Supplementary data 1).

For each anopheline population and each treatment (RS, ODS), a subset of freshly emerged (*i.e.* <1-h old) individuals issued from the different rearing sessions were randomly sampled and dried for 3 days at 60 °C. Dried females were then weighted using a microbalance (Sartorius SE2, d=1µg) to obtain the mosquito's dry mass.

4. Wing geometric morphometry

4.1 Sample preparation and data acquisition

Pictures of the detached right wing of female mosquitoes were captured using a Religa 2000R Qimaging Iast 1394 digital camera connected to a binocular microscope (x40). Pictures were calibrated, and the wing was put at the center of the visual field to allow accurate size comparisons, and to reduce the risk of optical distortion. As recommended by several authors a set of 12 landmarks (LM) per wing was digitised (Jirakanjanakit *et al.*, 2008, 2007; Morales Vargas, 2013; Figure 3). Damaged wings were not used to perform this assay.

4.2 Wing size, shape and surface area

Wing size variation between experimental conditions (RS, ODS) and among the five populations of *An. coluzzii* was investigated using the isometric estimator Centroid Size (CS) derived from coordinate's data (Jirakanjanakit *et al.*, 2008, 2007; Jirakanjanakit and Dujardin, 2005). CS is defined according the following equation: $CS = \sqrt{(\sum d^2)}$, where d represents the distance between the configuration centre of LM and each individual LM (Bookstein, 1997).

Wing shape conformations were obtained using the Generalized Procrustes Analysis (GPA; Rohlf and Slice, 1990) superimposition algorithm and the subsequent projection of the Procrustes residuals into Euclidian space. Both non-uniform (local variation) and uniform (global variation such as stretching and compression) components were examined to describe the differences in shape as deviation from an average configuration of LMs. The Collyer and Adams (2007) procedure was used to measure the wing shape vector phenotype changes occurring between the two experimental rearing conditions among the five populations, and defined in terms of the magnitude of change (vector length; ΔD) and the direction of change (angular difference; θ).

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Wing surface area of mosquitoes was measured using the area of the digitised polygon.

5. *Statistical analyses and software used*

5.1 Statistical analyses

Before analysis, normality distribution and homoscedasticity of the pre-imaginal development duration and dry mass datasets were verified using Shapiro-Wilk and Bartlett's tests, respectively. Then we tested the effect of the rearing session on both pre-imaginal development duration and body dry mass of female mosquitoes. Because no significant influence of the rearing session was found (ANOVA, *ddl*=1, F=20882, *P*=0.091), we used two-way ANOVAs to compare both pre-imaginal development duration and body dry mass between the two experimental rearing conditions (RS, ODS) and among the five mosquito populations. Tukey's *post-hoc* procedures were then used to perform comparisons among the levels of significant factors.

The relative geometric morphometric measurement error was first measured in both size and shape variables (see Supplementary data 2). Then, Procruste ANOVAs were used to test the effect of the rearing session on both size and shape variations in female *An. coluzzii*. Because, we did not find a significant effect of the rearing session, wing size variation between experimental conditions and among populations was then investigated using an ANOVA procedure followed with Tukey's *post-hoc* when required. Wing shape variables were analysed using a MANCOVA analysis with experimental conditions (RS, ODS), mosquito populations and CS as explicative factors. Because the scaling for centroid size does not remove the allometric changes of size and their influence on shape, a multivariate regression test was computed with size as independent variable and shape as dependent one. Statistical significance of the multivariate regressions was assessed using non-parametric permutation tests (10,000 permutations). A principal component analysis (PCA) was then used to graphically represent wing shape conformations between RS and ODS conditions, and among the five populations. The corresponding mean shape change along the two first component axes was examined showing configuration changes at a magnitude scale of 3.0. Statistical significance of the PCA was checked by permutation tests (10,000 permutations). A neighbour joining tree on the Mahalanobsis distances derived from the conformation variables was also examined. The significance of ΔD (vector length) and θ (angular difference) calculated using the procedure described in Collyer and Adams (2007) was tested using 5,000 random permutations.

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Wing surface area of mosquitoes was measured using a TclTk script (derived from the R. Suchenwirth's algorithm available at http://wiki.tcl.tk/12081) computing the area of the digitised polygon considered as an assemblage of triangles, each one defined by their corner coordinates (use of Heron's formula). Because there is expectation that CS and surface area are redundant information, we then tested such correlation using a Pearson test and a linear regression model.

An analysis of the metric disparity of the wing conformation was performed and compared between populations and among experimental rearing conditions using Student's t tests.

5.2 Software used

The CLIC90 software developed by Dujardin and Slice (2007) and Dujardin (2008) was used (i) to perform the LM digitisation, (ii) calculate the superimposition coordinates, the wing CS and surface area, and (iii) perform the Collyer and Adams (2007) procedure. CLIC90 was also used to build the neighbour joining tree based on the Mahalanobsis distances and the analysis of the metric disparity, both derived from the conformation variables.

We used the R 2.15.0 software (R Development Core Team, 2008) to perform the different permutation tests, Tukey *post-hoc* tests, PCA and statistical variance analyses.

RESULTS

1. Pre-imaginal development and body dry mass

A total of 4,005 female mosquitoes (*i.e.* 266 to 626 individuals for each population and treatment) were used to conduct the assay. Mortality during larval development varied greatly across populations and environmental conditions, ranging from 20% in the Soumousso population reared under RS conditions, to 80% in the Déou population reared under ODS conditions. Unreliable monitoring of mortality in several replicates unfortunately prevented us from analysing further survival data. When considering only those larvae that successfully developed to the adult stage, pre-imaginal development duration significantly changed between the rearing conditions and these changes differed among the mosquito populations considered, as shown by the significant interaction term (ANOVA, *ddl*=4, F=2.65, $P<0.05$). Indeed, although pre-imaginal development duration increased significantly in three of the four *An. coluzzii* populations from Burkina Faso under ODS conditions (Déou, Oursi and

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Bama), this trend was not statistically significant in the Soumousso population nor in the laboratory population from Cameroon (Figure 4A).

Body dry mass was assessed for a total of 220 female mosquitoes (*i.e.* 10 to 34 individuals for each population and treatment). Analyses showed that the dry mass of female mosquitoes changed significantly between the rearing conditions and that these changes differed among mosquito populations, as shown by the significant interaction term (ANOVA, *ddl*=4, F=31.80, $P<0.001$). Indeed, whereas body dry mass decreased in females from Déou and Oursi (Northern locations) when they were reared in ODS conditions, it increased sharply in females from Bama (Southern location, permanent population; Figure 4B). The trend for decreased dry mass in ODS conditions was not statistically significant in females from Soumousso and Ngousso.

2. Wing geometric morphometrics

A total of 489 wings from all experimental groups (*i.e.* populations and rearing treatments) were used as template to perform geometric morphometric analyses. Wings were randomly sampled in females issued from the different rearing sessions

2.1 Wing size

Significant variation in wing Centroid Size (CS) was found between the two experimental rearing conditions and these changes differed among the five mosquito populations, as supported by a statistically significant interaction term (ANOVA, *d.f.*=1, F= 2.65, $P<0.05$). Although CS increased significantly when mosquitoes were reared under the ODS conditions in females from Oursi, Soumousso, Bama and Ngousso, no such change was observed in those from Déou (Northern location, temporary population; Figure 5).

2.2 Wing shape

The MANCOVA analysis showed that wing shape differed significantly between RS and ODS conditions and that these changes differed among the five mosquito populations, as supported by a statistically significant interaction term (MANCOVA, $F_{4,96}=1.64$, $P<0.001$; Figure 6). A PCA analysis was thus performed to graphically assess the magnitude and direction of shape variation among experimental groups (Figure 6). The first axis of the PCA (PC1) accounted for 45.8% of the total inertia. This axis depicted differences in several components of the wing, including the proximal-distal axis, and the posterior-distal compartment between the Burkina-Faso populations and the laboratory colony originating

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from Cameroon, and to a lesser extent between the two populations from southern Burkina-Faso (*i.e.* Bama and Soumousso). The second axis (PC2) accounted for 10.3% of the total inertia, and was mainly constructed by shape variation between RS and ODS conditions. Changes along PC2 involved differences in the proximal-posterior compartment, and the anterior-posterior axis of the distal region. In particular ODS-reared mosquitoes sampled from Déou, Oursi, Soumousso and Ngousso showed decreased length of the anterior-posterior axis of wings. A reverse pattern was observed in ODS-reared females *An. coluzzii* from Bama (Figure 6). Analysis of the neighbour joining tree on the Mahalanobsis distances supported PCA results (Figure 7). Accordingly, wing shape conformation differed more among geographic populations than between experimental rearing conditions within populations.

The Collyer and Adams (2007) procedure performed on wing shape conformation changes between RS and ODS showed that the magnitude of changes did not differ among geographic populations (Table 1). However, analyses highlighted significant changes of direction between the two southern populations (*i.e.* Bama *vs* Soumousso, θ=100.70°; P<0.001) and between the two permanent ones (*i.e.* Bama *vs* Oursi, θ=113.40°; P<0.001; Table 1).

MANCOVA analysis also highlighted that shape variables were not independent of allometric effects as supported by the significant effect of CS on wing shape variation between experimental rearing conditions (MANCOVA, $F_{4,24}$=1.77, P<0.05), and among the five populations (MANCOVA, $F_{4,96}$=1.31, P-value<0.05). Accordingly, multivariate regressions performed on mosquito populations showed a significant effect of wing CS on wing shape variation between RS and ODS conditions in females from Soumousso (R^2=0.53, P<0.01) and Ngousso (R^2=0.45, P<0.001), and their allometric models also differed between RS and ODS (Table 2) so that the greater the difference in size, the greater the difference in shape. However, no such relationship was observed in the three other populations.

2.3 Wing surface area

Analysis showed that the surface area of wings was positively correlated with its size (Pearson test, t_{486}=12, R^2=0.81, P<0.001; Figure 8). Therefore, both surface area and wing size presented a similar pattern of variation (Supplementary data 3).

2.4 Metric disparity of the wing conformation

The metric disparity (MD) in wing conformation across all treatments was significantly more important than within each treatment (Figure 9). Moreover, analysis

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showed that only females from Ngousso exhibited distinct MD according the experimental rearing conditions. Accordingly, females reared under ODS conditions expressed more diverse wing conformations than their counterparts reared under RS in this test population. Whatever the experimental rearing conditions, females from Bama exhibited more diverse wing conformations (i.e. higher MD) than the four other populations.

DISCUSSION

The present work provides new information about developmental and associated morphological plasticity in distinct geographic populations of *An. coluzzii* when they are reared under contrasted environmental conditions. Our data mainly support that (i) pre-imaginal development duration changes as a response to the environmental conditions that larvae experience during their development: development is slowed down in all populations under ODS conditions, (ii) body dry mass of emerging mosquitoes changes according to both the geographic origin of the mosquito population and the environmental conditions they experience during larval development, and (iii) larval rearing conditions and the geographic origin of population further influence wing size, shape and surface area of emerging females. Our work further highlighted the redundancy in information brought by both size and surface area of mosquito wings, as shown by similar patterns of variation and significant correlation among the variables.

In insects, thermal conditions experienced during ontogenesis pilots the duration of larval development. Generally, within a permissive range of temperature, development speeds up with increasing temperature, until an optimal is reached; it then slows down when temperatures increase further (Colinet *et al.,* 2015). Moreover, examples including the Asian malaria vector *An. stephensi* showed that larval development slows down when temperature fluctuates around hot thermal ranges (Paajimans *et al.*, 2010). Similarly, water temperature in larval breeding sites was shown to influence the duration of larval development in the mosquito *Culex pipiens* (Madder *et al.*, 1983; Rueda *et al.*, 1990) and in major African malaria vectors including *An. funestus* and members of the *An. gambiae* complex (Bayoh and Lindsay, 2004, 2003; Gimonneau *et al.*, 2013; Lyons *et al.*, 2013). Our results are in agreement with these findings and demonstrated significant impact of environmental rearing conditions on *An. coluzzii* pre-imaginal development duration. In particular, the results suggested that mosquitoes exposed to dry season conditions (*i.e.* ODS) with high mean temperature and strong daily fluctuations slowed down pre-imaginal development, suggesting

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the ODS conditions divert from the optimum thermal development range of this mosquito. Interestingly however, our results showed this effect was not observed in all of our test populations (*i.e.* Soumousso and Ngousso did not exhibited significant changes). Effects of thermal fluctuation on the development duration of pre-imaginal stages might be context-specific depending on the proximity of the experimental conditions to the thermal development thresholds of the different mosquito populations, rather than the drying-out characteristics of their breeding-sites (Colinet *et al.,* 2015*;* Kingsolver *et al.*, 2009).

Moreover, although extended larval development duration in ODS conditions resulted in larger emerging adults (*i.e.* higher wing size) in all our test populations, these adults were generally lighter than those obtained after rearing under RS conditions, except in the Bama population. This finding suggests that the ability of mosquito larvae to harvest, store and utilise resources is altered under strong thermal fluctuations, as demonstrated in other insect species (Lounibos *et al.*, 2002; Reiskind and Zarrabi, 2012). In *Drosophila* sp. re-allocation of resources under strong temperature fluctuation was explained by increased metabolic demands at the cellular level during development when insects are exposed to higher temperatures (Czarnoleski *et al.*, 2013).

Moreover, the Bama population behaved at odds compared to the other test populations, showing increased body weight when reared under ODS conditions. Maintenance of the dry weight-wing length allometric relationship in Bama as opposed to other *An. coluzzii* geographic populations hence suggests intra-species plasticity in foraging efficiency under fluctuating temperatures and possible shifts in thermal reaction norms between geographic and or permanent *versus* temporary populations of *An. coluzzii*, as suggested for other Diptera species (Huey *et al.*, 2000; Lyons *et al.*, 2013; Reiskind and Zarrabi, 2012). More collections from a wider area representative of the distribution range of *An. coluzzii* in Africa would be needed to draw strong conclusions about local adaptation to thermal environment in this species.

Interpreting pattern of variation of wing shape conformation is obviously tentative and hypothetic. Indeed, the incidence of geometric morphometric changes of insect's wing in flight aerodynamic remains poorly understood (Debat *et al.*, 2008; Fry *et al.* 2003; Gilchrist *et al.* 2000), and it remains very difficult to connect such geometric variations with functional constraints for organisms. Notwithstanding, there is evidence that the changes we observed in the anterior-posterior axis of the distal compartment of mosquito wings influence the wing

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aerodynamic lift during flight (Vogel, 1981). Changes in wing size and surface area were also observed as a response to the environmental conditions experienced by mosquitoes during larval development. Accordingly, results showed that both size and surface area of wings, which were highly correlated and therefore redundant, increase in ODS-reared mosquitoes, expect in Déou females, suggesting that these two parameters also depend upon the geographic origin of mosquitoes. One hypothesis to explain such a wing variations could be based on air conditions variations at the onset of the dry season. Air density (*d*) is known to depend on both ambient temperature and RH conditions ($d = \frac{P}{R.T}$, with P the air pressure, R a constant heat capacity dependent of the RH, and T the ambient temperature). Air density might thus change from one season to the next in dry savannahs of West Africa. Incidences of air density changes on flight performance of insects have been already studied under high altitude (Dillon *et al.*, 2006). Accordingly, authors showed that insects with greater wing area relative to body size decrease the induced velocity required for sustaining flight at high altitude, therefore minimizing the energetic cost of flight in low air density conditions. For instance, the mountain honeybees have longer wings and greater wing surface area but invariant body mass relative to their congenerics from lowlands (Hepburn *et al.*, 1998). We observed similar patterns of variation in female *An. coluzzii* from Oursi, Soumousso and Ngousso when they were reared under ODS conditions. Females increased wing size and surface area but did not change body dry mass. No such pattern was observed in ODS-reared females from Déou. Indeed, these females exhibited a decrease in body dry mass and no change in wing size or surface area. Again, because the population from Déou is present only during the rainy season in the wild, this outcome might result from exposure to stressful conditions during development that are outside of the usual temperature range the species is exposed to in its natural habitat.

In a recent study, Yaro *et al.* (2012) observed a range of phenotypes expressed by Sahelian populations of *An. coluzzii* in Mali with respect to their reproductive behaviour. These authors proposed that local mosquito populations might group together specimens with different aestivation abilities, including “strong aestivators” programmed to engage into a state of diapause (*e.g.* gonotrophic dissociation) at the onset of the dry season, and “weak aestivators” that will depend upon surface water availability to terminate their gonotrophic cycle and lay their eggs. Heterogeneity along similar lines has also been described in overwintering *Culex* mosquitoes in temperate environments (Reisen *et al.*, 2010; Tsuda and

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Kim, 2008). It is noteworthy in our study, that higher heterogeneity in wing shape conformation and changes thereof between RS and ODS conditions compared to other test populations was demonstrated for the Bama population of *An. coluzzii*, as revealed by the Collyer and Adams (2007) procedure (Table 1) and our disparity analysis (Figure 9). Such developmental and morphological heterogeneity might reflect the existence of higher phenotypic plasticity and different survival strategies (*e.g.* aestivation abilities) within the local *An. coluzzii* population from Bama. Further studies might shed light on local phenotypic diversity of *An. coluzzii* in such environment where large scale irrigation schemes and surrounding temporary surface water collections offer diverse opportunities for mosquito breeding during the rainy season and expose immature stages to various biotic and abiotic factors that might differentially influence the set-up of alternative dry season survival strategies in adults (Adamou et al., 2011; Huestis and Lehmann, 2014; Lehmann et al., 2014, 2010).

CONCLUSION

Overall results showed that wing size, shape and surface area of female *An. coluzzii* depend upon both the genetic background (geographic origin) and rearing conditions experienced by mosquitoes during their larval development. In particular, results highlighted that wing shape conformation mainly depend on the geographic and climatic conditions of the mosquito's habitat, whereas both size and surface mainly change as a response to the environmental conditions perceived during pre-imaginal development. Further investigations are however needed to understand the functional roles of these geographic and seasonal adjustments and to link such plasticity with the mosquito phenology observed in the fields. Additional work is also required to examine phenotypic plasticity locally, and especially in areas where permanent mosquito breeding is possible in large irrigated areas (*e.g.* Bama) and mosquito phenotypic plasticity is highest.

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TABLES AND FIGURES CAPTIONS

Figure 1. Geographical localisation of the four localities where wild *An. coluzzii* mosquitoes were collected in northern and south-western regions of Burkina-Faso (West Africa). Squares represent permanent mosquito

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populations and circles represent temporary populations (see text). Blue lines represent mean annual rainfall in mm (derived from Clavel *et al.*, 2009).

Figure 2. Daily fluctuations in air (solid red lines) and rearing water (dashed red lines) temperature (°C) and relative humidity (% – solid blue lines) used in the climatic chambers to mimic environmental variations in Oursi (A) during the rainy season (RS), and (B) at the onset of the dry season (ODS). Photoperiod duration is represented by the black bars below.

Figure 3. Position of the different landmarks (LM) digitised on the dorsal face of the right wing of females *Anopheles coluzzii*. LM1 to 11 are type I landmarks, whereas LM12 is type II landmark defined as the transition between the alula and the posterior margin of the wing.

Figure 4. A/ Mean pre-imaginal development duration (in days) ±SE for the five *An. coluzzii* populations reared under RS (white) and ODS (grey) experimental conditions. B/ Mean body dry mass (in mg) ±SE for the five *An. coluzzii* populations reared under RS (white) and ODS (grey) experimental conditions. Letters in superscript indicate statistical significance at the threshold of α=5%.

Figure 5. Mean wing centroid sizes (pixel) ±SE for the five *An. coluzzii* populations reared under RS (white) and ODS (grey) experimental conditions. Letters in superscript indicate statistical significance at the threshold of α=5%.

Figure 6. Projection on the first plane of the Principal Component Analysis (PCA) of 489 individual wings from five *An. coluzzii* populations reared under RS (white) and ODS (grey) conditions. Only the centroids of each group are plotted for ease of interpretation. The direction and magnitude of wing shape variation between RS and ODS are represented by arrows. Both positive and negative mean shape changes along the two first component axes (PC1, PC2) are represented by sketches positioned along each axis, and corresponded to a 3.0 unit of change. Circles represent temporary mosquito populations, squares represent permanent populations, and triangles represent the control laboratory population.

Figure 7. Neighbour joining tree on the Mahalanobsis distances derived from the wing conformation variables

Figure 8. Linear regression between centroid size (CS) and surface area of mosquito wings.

Figure 9. Metric disparity of the wing shape conformation ±SE for the five *An. coluzzii* populations reared under RS (white) and ODS (grey) experimental conditions. The total metric disparity is presented in the black bar. Letters in superscript indicate statistical significance at the threshold of α=5%.

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Table 1. Results of the Collyer and Adams (2007) procedure performed on wing shape conformations of North (*i.e.* Déou and Oursi), South (*i.e.* Soumousso and Bama), permanent (*i.e.* Oursi and Bama) and temporary (*i.e.* Déou and Soumousso) populations of mosquitoes. Both magnitude (ΔD) and direction (θ) of the wing shape variation from RS to ODS are presented with the associated *P*-value (P<0.05). ns: not significant; ***: *P*<0.0001.

Table 2. Result of the multivariate regression analysis (column 2) testing the incidence of wing size variation from RS to ODS conditions on the wing shape variations for each *An. coluzzii* population. When a significant allometric incidence is found for a population, a different slopes model analysis (column 3) between RS and ODS rearing conditions is computed. 'ns': not significant; *: *P*<0.05; **: *P*<0.0.1; ***: *P*<0.0001. 'NA': Not applicable because of non significant multivariate regression.

Supplementary data 1. Mean larvae survivorship estimation for each anopheline colony exposed to ODS or RS conditions

Supplementary data 2. Methodology and results of the relative geometric morphometric measurement error.

Supplementary data 3. Barplot of the wing surface area means (in pixel) ±SE of the five mosquito colonies reared under RS (white) or ODS (grey) experimental conditions. Separate letters indicate significant content differences (*P*-value<0.05).

Table 1.

Population	Magnitude ΔD	*P*-value	Direction (θ)	*P*-value
North	9.0e-03	ns	60.02	ns
South	2.7e-03	ns	100.70	***
Permanent	4.1e-03	ns	113.4	***
Temporary	4.6e-03	ns	34.36	ns

Table 2.

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Population	Multivariate regression	Different slopes model
Déou	ns (R^2=0.48)	NA
Oursi	ns (R^2=0.22)	NA
Soumousso	** (R^2=0.53)	***
Bama	ns (R^2=0.25)	NA
Ngousso	*** (R^2=0.45)	*

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