elucidated the contribution of each variable in morphometric variation. Finally, we explored the effect of chromosomal inversion polymorphisms on wing morphology. Our findings revealed significant effect of local environmental conditions on wing morphology. Chromosomal polymorphism was associated to wing shape variation across populations. These outcomes are discussed in a context of environmental adaptations and their impact on malaria epidemiology and vector control strategies.

2. Materials and methods

2.1. Study sites and mosquito sampling

Wing morphometric traits were assessed and compared in *A. funestus* mosquitoes collected in nine ecological zones belonging to five different bioclimatic domains of Cameroon (Olivry, 1986), encompassing most of the bio-geographic diversity of the country, ranging from the northern arid savannas to the evergreen rainforest in the south (Fig. 1, Table 1). To reduce possible local effects and increase the number of specimens, mosquitoes were collected from 2 to 7 villages in each zone (average distance between villages within zone = 7.83 km). Adult females *A. funestus* were captured by day-time spraying aerosols of pyrethroid insecticides inside human dwellings (Service, 1993). Anopheline mosquitoes were identified using morphological identification keys (Gillies and de Meillon, 1968). Ovaries from half-gravid *A. funestus* females were dissected and stored in Carnoy fixative solution (3 parts of 100% ethanol: 1 part glacial acetic acid by volume) for subsequent cytogenetic analysis. Carcasses were stored individually in labeled tubes containing a desiccant and kept at −20 °C.

2.2. Mosquito PCR identification and karyotyping

Genomic DNA was extracted from the body of adult mosquito females using the protocol described in Morlais et al. (2004). DNA was then resuspended in sterile water in individual tubes. Morphological identification of *A. funestus* s.s. (hereafter *A. funestus*) was confirmed by molecular identification (Cohuet et al., 2003; Koekemoer et al., 2002). Polytene chromosomes obtained from the ovaries of half-gravid females *A. funestus*, were squashed and stained according to standard protocols (della Torre, 1997). The preparations were examined under a phase-contrast microscope, and paracentric chromosomal inversions were scored according to the *A. funestus* cytological map (Guelbeogo et al., 2005; Sharakhov et al., 2004).

2.3. Environmental data

A set of seven eco-geographical variables (EGVs) was used to describe the average environmental conditions in each zone (source: *LocClim* database developed by the Food Agriculture Organization – FAO, http://www.fao.org/sd/2002/EN1203a_en.htm): elevation (in m), rainfall (in mm), temperature (in °C), evapotranspiration (in mm), relative humidity (water vapor pressure in %), mean number of hours of sunlight per day (h), and wind speed (in ms^{-1}). Climate data are yearly means, averaged over the past 30 years, obtained from interpolation of field stations data. Computational operations linked to geo-analysis requirements were performed using the software ArcGIS 8.3 (http://www.esri.com/software/arcgis/index.html).

2.4. Morphometric analysis

2.4.1. Sample processing

A digital image of each mosquito female wing, left and right (dorsal view) was taken through a binocular microscope (Leica MZ6). Both wings were removed and mounted on microscope slides under cover slips. Morphometric measurements were taken from both wings of each female, except when only one undamaged wing was available. Twelve morphometric measurements, as recommended by Bookstein (1991), were scored from the digital images of each wing (Fig. 2) using the free software COOw (http://www.mpl.ird.fr/morphometrics/). All measurements were taken by the same person for more consistency (Bookstein, 1991).

2.4.2. Repeatability

Random measurement error is common in morphometric analysis, and it can cause serious statistical problems (Arnqvist and Martensson, 1998). To detect this kind of error, we repeated measures of all individuals twice, and we quantified their repeatability by the ratio between the individual variance and the total variance. For this purpose, we used the free software VAR 1.4 (http://www.mpl.ird.fr/morphometrics/).

2.4.3. Size and shape

For comparing overall wing size between zones, we used the isometric estimator known as "centroid size" (CS) derived from coordinates data: it is defined as the square root of the sum of squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein, 1991). Variation in shape was examined using geometric morphometrics based on generalized least squares Procrustes superim-

Table 1
Chromosomal inversion frequencies and mean wing size in *Anopheles funestus* mosquitoes sampled from a range of ecological zones in Cameroon.

Zone[a]	Bioclimatic domain[b]	Temperature[c] (°C)	Rainfall[c] (mm)	Elevation[c] (m)	Number of villages	Number of mosquitoes	Inversion frequency (%) 3Ra	3Rb	3La	Wing size (mm)
A	Tropical dry	27	878	249	3	27	0.0	0.0	0.0	2.66 ± 0.12
B	Tropical dry	28	989	284	2	33	1.5	0.0	3.0	2.67 ± 0.15
C	Tropical dry	26	1223	335	5	59	63.6	68.6	66.9	2.70 ± 0.16
D	Tropical dry	24	1348	590	3	24	87.5	97.9	95.8	2.76 ± 0.15
E	Adamaoua Highlands	23	1509	1073	3	21	76.2	73.8	100.0	2.90 ± 0.14
F	Adamaoua Highlands	23	1680	969	2	26	71.2	80.8	100.0	2.84 ± 0.12
G	Western Highlands	22	1874	779	7	22	63.6	72.7	100.0	2.93 ± 0.12
H	Central Plateau	25	1633	493	2	35	100.0	100.0	100.0	2.70 ± 0.13
I	Central Plateau	24	1853	708	2	18	100.0	100.0	100.0	2.63 ± 0.10

Source: Climatic data are yearly means averaged across the past 30 years. http://www.fao.org/sd/2002/EN1203a_en.htm.

[a] Zone denomination refers to Fig. 1.
[b] Bioclimatic domains are as defined by Olivry (1986).
[c] Climatic data are yearly means averaged across the past 30 years.