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Larval competition between *An. coluzzii* and *An. gambiae* in insectary and semi-field conditions in Burkina Faso

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Abstract

Competition in mosquito larvae is common and different ecological context could change competitive advantage between species. Here, larval competition between the widely sympatric African malaria mosquitoes, *Anopheles coluzzii* and *Anopheles gambiae* were investigated in controlled insectary conditions using individuals from laboratory colonies and under ambient conditions using wild mosquitoes in a semi-field enclosure in western Burkina Faso. Larvae of both species were reared in trays at the same larval density and under the same feeding regimen in either single-species or mixed-species populations at varying species ratios reflecting 0, 25, 50 and 75% of competitor species. In the insectaries, where environmental variations are controlled, larvae of the *An. coluzzii* colony developed faster and with lower mortality than larvae of the *An. gambiae* colony (8.8 ± 0.1 days and $21 \pm 3\%$ mortality vs. 9.5 ± 0.1 days and $32 \pm 3\%$ mortality, respectively). Although there was no significant effect of competition on these phenotypic traits in any species, there was a significant trend for higher fitness of the *An. coluzzii* colony when competing with *An. gambiae* under laboratory conditions (i.e., lower development time and increased wing length at emergence, Cuzik's tests, $P < 0.05$). In semi-field experiments, competition affected the life history traits of both species in a different way. Larvae of *An. gambiae* tended to reduce development time when in competition with *An. coluzzii* (Cuzick's test,

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Conflict of interests

The authors of this study have no conflict of interest concerning the work reported in this study.

Authors' contributions

GG and FS conceived and designed the study. FS and RD supervised its implementation. GG conducted the experiments and analyzed the data, with support from LB, WM, FS and RD. LB and WM dissected and mounted mosquito's wings. LB measured wing length. GG and FS drafted the manuscript, which was critically revised by LB, WM and RD.

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P=0.002) with no impact either on mortality or size at emergence. On the other hand, *An. coluzzii* showed a significant trend for reduced larval mortality with increasing competition pressure (Cuzick's test, P=0.037) and production of smaller females when grown together with *An. gambiae* (Cuzick's test, P=0.002). Our results hence revealed that competitive interactions between larvae of the two species are context dependent. They further call for caution when exploring ecological processes using inbred laboratory colonies in this system of utmost medical importance.

Keywords

An. coluzzii; *An. gambiae*; larvae; insectary; semi-field; asymmetrical competition

1. Introduction

Biotic interactions play an important role in regulating populations and structuring communities (Chase et al., 2002; Diamond and Case, 1986; Schoener, 1983). Interactions between species through interference or indirectly through resource exploitation often leads to negative effects on survival and reproduction (Blaustein and Chase, 2007) that impacts species abundance and distribution and hence their demographic and evolutionary history (Denno et al., 1995; Duyck et al., 2004; Reitz and Trumble, 2002). Understanding the role of these biotic interactions and their consequences on species distribution and individual fitness is of great importance when it comes to species of particular interest, such as endangered species or pests of economic or health importance (Shennan, 2008).

In mosquitoes, ecological interactions such as predation and both inter- and intra-specific competition have been largely studied and result principally in life history trait modifications (e.g. growth rate, size, lifespan) (Denno et al., 1995; Juliano, 2009; Reitz and Trumble, 2002). Interspecific competition has multiple consequences on mosquito development and impacts all life stages. It affects egg hatching rate (Edgerly et al., 1993), larval development time (Juliano et al., 2004; Paaïjmans et al., 2009), larval survivorship (Juliano et al., 2004; Kirby and Lindsay, 2009; Paaïjmans et al., 2009; Schneider et al., 2000), sex ratio (Lowrie, 1973), adult size and fecundity (Ho et al., 1989). Other factors such as temperature (Kirby and Lindsay, 2009; Paaïjmans et al., 2009; Russell, 1986; Teng and Apperson, 2000), larval density (Lowrie, 1973; Schneider et al., 2000) and the availability of a suitable food source (Barrera, 1996) also affect life history traits. In addition, species may express a different intrinsic ability to compete (Abrams, 1995). For example, several authors have assumed the existence of chemical factors produced by some mosquito species in competitive situations that “weaken” the competing species although the nature and the role of these chemicals have not been identified (Moore and Whitacre, 1972; Roberts, 1998). Competition outcomes on life history traits are generally density-dependent (Juliano, 2009; Kweka et al., 2012) and result in asymmetrical consequences for competing species which could be reversed by different environmental conditions (i.e. such as along an ecological gradient) (Juliano, 2009; Wellborn et al., 1996; Williams, 1996). Context-dependent reversal of competitive asymmetry was highlighted for some abiotic factors such as temperature or food quantity/quality, both in insectary and natural conditions (Ho et al.,

1989; Juliano, 2009; Lounibos et al., 2002; Teng and Apperson, 2000) but also in relation to mosquitoes' origin (laboratory strain vs. field mosquitoes) (Kesavaraju et al., 2012). Laboratory mosquitoes are easily used for experiments because of lack of readily available wild caught individuals, but it is likely that the ability to compete and subsequent outcomes on life history traits were selected for laboratory rearing conditions (Kesavaraju et al., 2012; Lorenz et al., 1984; Wallis et al., 1985). Therefore, extrapolations to the field situation, such as is required for ecological niche modelling or assessments of the outcomes of control interventions may be compromised, making evaluations of the extent of differences among laboratory and field strains essential (Lyons et al., 2012). However, there are few studies aiming to compare laboratory vs. field conditions and/or mosquito strains for different outcomes such as competitive interactions (Kesavaraju et al., 2012; Lyons et al., 2012).

The African malaria mosquitoes, *Anopheles coluzzii* (formerly *An. gambiae* M form) and *Anopheles gambiae* (formerly, *An. gambiae* S form) are major vectors of human malaria throughout sub-Saharan Africa (Coetzee et al., 2013). The two species are morphologically indistinguishable and largely sympatric throughout West and Central Africa (della Torre et al., 2001; della Torre et al., 2005) however they show widespread molecular divergence throughout their genome (Lawniczak et al., 2010; Neafsey et al., 2010; White et al., 2010). In the field, reproductive isolation operates through strong positive assortative mating, although hybridization might occur in a context-dependent way (Caputo et al., 2011; Dabire et al., 2013; Diabate et al., 2009). At the adult stage, both species share the same resources such as vertebrate host and indoor resting sites, although they were shown to diverge in some of their ecological requirements (Costantini et al., 2009; Lehmann and Diabaté, 2008; Simard et al., 2009). In the dry savannahs of West Africa, *An. coluzzii* and *An. gambiae* breed in ephemeral, rain-dependent freshwater aquatic habitats (e.g., puddles, road ruts and quarries). However, *An. coluzzii* larvae also thrive in permanent freshwater habitats, such as rice irrigation schemes, whereas *An. gambiae* larvae do not develop successfully in such habitats (Gimonneau et al., 2012a).

Differential adaptation to fundamentally different aquatic habitats in terms of size and temporal stability has been shown to foster ecological niche specialization in reproductively isolated populations (Schneider and Frost, 1996; Wellborn et al., 1996; Wiggins et al., 1980; Williams, 2006). Such ecological divergence in larval habitat preference has been hypothesized to play a pivotal role in the speciation process and recent radiation within the *An. gambiae* complex (Ayala and Coluzzi, 2005; Coluzzi et al., 2002). In freshwater communities, ecological predictions suggest that competition in small ephemeral habitats controls community structure whereas predation does so in larger and more long-lived habitats (Juliano, 2009; Wellborn et al., 1996; Williams, 1996). However, this hypothesis has only recently been investigated in the *An. gambiae* complex (Diabaté et al., 2008; Manoukis et al., 2008). We have previously shown that *An. coluzzii* exhibits behavioural adaptations to survive in predator-rich environments (Gimonneau et al., 2010; Gimonneau et al., 2012b; Roux et al., 2013). Yet, the role of competitive interactions in shaping the distribution of *An. coluzzii* and *An. gambiae* has not been explored in depth.

This study aims to analyze the effect of competitive interactions between *An. coluzzii* and *An. gambiae* on some life history traits of relevance to local adaptation and vector

competence, including larval development time, mortality rate during the aquatic phase, sex ratio at emergence and adult size (Alto et al., 2008). Because *An. gambiae* breeds in temporary habitats (Costantini et al., 2009; Gimonneau et al., 2012a) and theory predicts that species which exploit such habitat express traits that enhance developmental rate and competitive ability (Wellborn et al., 1996; Williams, 1996), we hypothesized that *An. gambiae* will show specific adaptations such as a faster larval development when exposed to competitors, as it was previously observed in East Africa between *An. gambiae* and *An. arabiensis* (Paaijmans et al., 2009). Hence, we examined the effect of competition between *An. coluzzii* and *An. gambiae* using different species ratios in an experimental arena exposed to controlled laboratory conditions and in a semi-field setting exposed to natural environmental fluctuations in Burkina Faso (West Africa) in order to depict outcomes of competitive interactions under these two different environments.

2. Materials and methods

2.1 Mosquitoes

Mosquito larvae reared under insectary conditions were obtained from newly established colonies (Mouline et al., 2012). Colonies derived from eggs laid by wild gravid females collected during the 2008 rainy season (more than 500 females per species were used to set up colonies). The *Anopheles coluzzii* colony originated from females collected while resting in human dwellings in Bama (11°23'14"N, 4°24'42"W), a village of the Kou Valley, situated 30 km north of Bobo-Dioulasso, Burkina Faso. The village is surrounded by 1.200 hectares of irrigated rice fields where the species predominates throughout the year (Baldet et al., 2003; Diabaté et al., 2002). The *Anopheles gambiae* colony was obtained from females collected indoors in Soumouso (11°00'46"N, 4°02'45"W), a village located 50 km southwards of Bama within the Guinean savannah domain, where surface waters dry out during the long (i.e., 6–7 months) dry season and *An. gambiae* is abundant during the rainy season only (Dabiré et al., 2009a; Dabiré et al., 2009b). Individuals from the F8 generation (~5 months in our laboratory conditions) of both colonies were used in the experiments.

Mosquito larvae used in semi-field experiments were F1 individuals obtained from females collected while resting in human dwellings in Bama and Soumouso during the 2009 rainy season. Wild gravid and/or blood-fed females (n=150 for each location) were placed individually in oviposition cups under standard insectary conditions (temperature 28°C ± 1°C, 80 ± 10% relative humidity and 12:12h light:dark cycle) and provided with 5% glucose solution *ad libitum*. After oviposition, females were killed and identified by PCR-RFLP assays performed on a single leg (Santolamazza et al., 2008). Eggs were pooled according to their species and newly hatched larvae were used in the experiments.

2.2. Experimental procedure

Laboratory experiments were conducted in the insectaries of the Institut de Recherche en Science de la Santé (IRSS) in Bobo-Dioulasso in December 2008 under controlled abiotic conditions (Table 1). Semi-field experiments were carried out in September and October 2009 in a screened greenhouse (Knols et al., 2002) located in Bama and exposed to ambient environmental conditions. Temperature and relative humidity were recorded inside the

greenhouse (Table 1) using a data logger (Fourier System, MicroLogPro EC750) and water temperature was measured directly in the experimental cups using a waterproof data logger (Scantronik, Event Fox mini). Photoperiod was calculated on the basis of incoming solar radiation from a weather station (Davis, Vantage Pro 2) positioned outside the greenhouse.

We used the same experimental procedure for both insectaries and semi-field experiments as follows: forty-eight newly hatched (~ 1 hour old) first instars larvae were distributed in 400ml circular plastic cups (11.4 cm in diameter) filled with 250ml of spring water. Different ratios of *An. coluzzii*:*An. gambiae* were used reflecting 0, 25, 50 and 75% of competitor species (i.e. 0, 12, 24 and 36 competing larvae with the remaining made up of the other species to a total of 48) with three replicates for each ratio (Paaijmans et al., 2009). Cups were placed randomly on a table and covered with a net. Spring water was added daily to compensate for evaporation, dead larvae were removed and larvae were fed *ad libitum* with 0.3mg of Tetramin® Baby fish food per larva per day (Koenraadt et al., 2004). Water was renewed every other day to prevent scum formation and the accumulation of metabolites that may be toxic to the larvae (Bayoh and Lindsay, 2003) and cups were replaced randomly on the table.

2.3. Data collection

Emerged mosquitoes were collected twice a day at 8 am and 5 pm using a mouth aspirator, sexed and stored at -20°C in individual tubes with silica gel. Individuals from mixed species treatments were identified by PCR-RFLP assays performed on a single leg (Santolamazza et al., 2008). Different life history traits were studied: larval development time, immature mortality, sex ratio and wing-length at emergence (used as a proxy of body size). The duration of immature development for each treatment and species was determined as the period from eggs hatching to adult's emergence. Larval mortality was calculated by subtracting the number of emerged mosquitoes from the initial number of larvae introduced per species and expressed as a proportion of this initial number of L1 larvae used in the experiment. The sex ratio was calculated as the number of emerging females divided by the total number of emerging adult mosquitoes (males and females) for each treatment and species.

Wing-length measurements were obtained for each experiment from a random subsample of 10–20 females per species and per treatment. The left wing was removed and mounted on a microscope slide under a cover slip. Digital images of wings were taken through a binocular microscope (Leica Z16 APOA, 24x) and wings were measured using the software “Leica application suite”. Length was measured from the distal part of the allula to the wing tip (Alto et al., 2008). All measurements were taken by the same person for consistency (Bookstein, 1991).

2.4. Data analysis

For each experiment, the effect of “species”, “competition” and their interaction on the larval development time, mortality and adult's wing-length were assessed using a two-way analysis of variance (ANOVA) followed when required with Tukey's *post hoc* test among levels of significant factors ($P < 0.05$). To meet normality assumptions, development time

was log transformed and mortality rates were arcsine transformed. The non parametric Cuzick's test for trend was used to assess whether there was any significant trend in trait variation across species ratios for each species (Cuzick, 1985).

The sex ratio deviation from 0.5 was assessed using tests of equal proportion for each species. All statistical analysis were performed with the R software v.2.12.1 (R_Development_Core_Team, 2009).

3. Results

3.1. Competition in insectary conditions

Development time in the insectary conditions was significantly different between species across competition classes ($F=37.144$; $ddl=1$, $P<0.001$; Table 2A). Indeed, overall mean development time was shorter for *An. coluzzii* than for *An. gambiae* (8.8 ± 0.1 days vs. 9.5 ± 0.1 days, respectively; Figure 1A). No competition effect or interaction was statistically significant in the ANOVA (Table 2A). However, Tukey's *post hoc* tests revealed statistically significant differences between species along the competition gradient (Figure 1A). Non-parametric Cuzick's test further revealed a significant trend for reduced larval development time in *An. coluzzii* with increasing competitors ratio (Cuzick's test, $P=0.022$). This was not the case in *An. gambiae* (Cuzick's test, $P=0.888$).

Overall, larval mortality also differed significantly between the two species ($F=5.887$; $ddl=1$, $P=0.027$; Table 2A). In all treatments and overall, *An. gambiae* consistently suffered higher mortality than *An. coluzzii* ($32\pm3\%$ vs. $21\pm3\%$, respectively; Figure 1C). However, Tukey's *post hoc* tests did not detect any statistically significant difference between species along the competition gradient ($P>0.05$, Figure 1C) and there was no trend in the dataset (Cuzick's test, $P>0.4$).

Wing size was not different between species but was significantly affected by competition ($F=5.744$, $ddl=3$, $P=0.001$, Table 2A), with a significant interaction term in the ANOVA ($F=12.642$, $ddl=3$, $P<0.001$; Table 2A). Competition seemed to have a beneficial effect on *An. coluzzii*, with a significant trend for wing size to increase with the proportion of competing species (Cuzick's test, $P<0.001$; Figure 1E). On the other hand, there was no significant trend in wing size variation across competitor species ratio and replicates in *An. gambiae* (Cuzick's test, $P>0.181$).

There was no effect of interspecific competition on sex ratio at emergence in any of the two species (Table 3).

3.2. Competition under semi-field conditions

Overall, development time in the semi-field enclosure differed between species ($F=26.655$, $ddl=1$, $P<0.001$) and presence of a competitor in the assay affected both species in a different way (Interaction term, $F=3.950$, $ddl=3$, $P=0.008$; Table 2B). Tukey's *post hoc* tests revealed statistically significant differences between species all along the competition gradient (Figure 1B, note that there is no difference between species in larval development time in single species populations) and there was a significant trend for decreased larval

development time in *An. gambiae* as the proportion of *An. coluzzii* increased from 0 to 75% (Cuzick's test $P=0.002$; Figure 1B). No such trend was observed in *An. coluzzii* ($P=0.201$).

There was no detectable effect of species nor competition on the mortality rate during development in semi-field conditions (Table 2B) although there was a significant trend for reduced mortality in *An. coluzzii* with an increasing competition pressure (Cuzick's test, $P=0.037$; Figure 1D). However, this is accompanied, in *An. coluzzii*, by a significant trend for decreasing adult size (as measured by wing length) with increasing competition (Cuzick's test, $P=0.002$; Figure 1F). No effect of competition was observed on *An. gambiae* larval mortality or wing-length variation across the competitor's ratios (Cuzick's tests, $P>0.2$).

Sex ratio for both species was not statistically different from 0.5 in all but one treatment: only *An. coluzzii* reared in single-species population presented a sex ratio significantly lower than 0.5, which favoured males when reared under our semi-field conditions (Table 3).

4. Discussion

Discrepancies between laboratory and field studies have been already pointed out to affect mosquito traits in different ways (Kesavaraju et al., 2012; Lyons et al., 2012; Tripet et al., 2008). Such different results could lead to inaccurate or biased inferences about biological and ecological issues (Juliano, 2009). In this study, we assessed the effect of competition at the larval stage between *An. coluzzii* and *An. gambiae* under laboratory and semi-field conditions on some life history traits. Our results present asymmetrical competitive outcomes in relation to the environmental context and mosquito origin. Overall, there are two main findings stemming from our study: first, the results of the semi-field study are in agreement with our hypothesis of a higher competitive ability of *An. gambiae* larvae over *An. coluzzii* and second, discrepancies between results obtained with wild specimens exposed to semi-field conditions and those obtained with laboratory mosquito colonies reared in insectaries warn against the use of artificial lab-based experimental systems to explore ecological interactions in this highly plastic biological model.

In insectary, strong founder effect at the onset of the colony together with directional selection for adaptation to laboratory conditions might reduce variance in certain phenotypic traits whereas relaxed environmental pressure on other traits (e.g. predator vigilance, immune system) might result in more trait variance for captive species (McPhee, 2004; McPhee and McPhee, 2012). In our case, both the *An. coluzzii* and *An. gambiae* colonies were seeded at the same time, and adapted to the same insectary conditions, being reared at the same place and according to the same maintenance protocol. However, overall higher fitness of *An. coluzzii* over *An. gambiae* colony mosquitoes was observed after 8 generations of unintentional selection to the lab environment suggesting alternative selective issues in the two genetic backgrounds. Such colony-specific genetic drift probably contributed to the phenotypic differences observed when exploring competition between the two species using our experimental settings. In addition to strain selection for insectary conditions, abiotic factors absent in insectary may have prompted differences in immature development. Daily water temperature variations in semi-field conditions (from 22.8 to 37°C, leading to 14.2°C

thermal amplitude) and/or direct sunlight exposure, which are absent or limited in insectaries conditions, may have impacted *An. gambiae* development (i.e., increased development time and larval mortality) and competitive outcomes in these conditions.

Results from the semi-field experiment better matched general ecological hypothesis, suggesting that species which exploit temporary aquatic habitat express traits that enhance their developmental rate and competitive ability (Wellborn et al., 1996; Williams, 1996). *An. gambiae* breeds in temporary aquatic habitats (Gimonneau et al., 2012a) and reduces its larval development time in the presence of competitors. Such trend has already been observed in East Africa, where *An. gambiae* was exposed to larval competition with its sibling species, *An. arabiensis* (Paaïmans et al., 2009). Faster development of *An. gambiae* over *An. coluzzii* was also reported from a field study conducted in Burkina Faso (Diabaté et al., 2008). Such competitive abilities of *An. gambiae* over *An. coluzzii* have implications in species distribution and aquatic habitat colonization. Faster development time in temporary aquatic habitat is a plastic phenotypic trait of preliminary importance to exploit such kind of habitat as species reduce their exposure to negative effects such as desiccation (Benedict et al., 2010), predation risk (Gimonneau et al., 2010), cannibalism (Koenraadt and Takken, 2003; Muturi et al., 2010), pathogens (Ward and Savage, 1972) and flushing due to rainfall (Paaïmans et al., 2007). Because reduced development time in competition was not accompanied by a concomitant reduction in adult body size at emergence nor an increase in larval mortality, this biological response to competition might further encompass differences in larval behavioural foraging strategies and efficiency in converting food into biomass (Gimonneau et al., 2012b) as it has been already demonstrated for *Aedes* species (Ho et al., 1989; Rashed and Mulla, 1989). Therefore, *An. gambiae* presents phenotypic abilities explaining its higher competitive success than *An. coluzzii* in temporary aquatic habitat, which are probably important in sustaining their sympatric distribution in nature. Competitive outcomes may also affect malaria transmission, especially for *An. coluzzii*, which presents significant body size reduction. Because mosquito body size is correlated to adult's survival (Ameneshewa and Service, 1996; Lyimo and Takken, 1993), the most important factor in malaria transmission (Lunde et al., 2013), such size decrease in *An. coluzzii* may potentially affect negatively its vectorial capacity in sympatric area.

4.1 Conclusions

In conclusion, our results suggested that competition between *An. coluzzii* and *An. gambiae* has some effects on their life history traits. However, we also showed that insectary studies conducted with laboratory-reared mosquitoes did not adequately match the outcomes of similar experiments conducted with field mosquitoes maintained in a semi-field setting. These results revealed that competitive issues between the two mosquito species are context dependent and highlight the importance of studying ecological processes under natural conditions. Future research using these two sibling species should be aware of a strong insectary effect in this system, which could lead to asymmetrical outcomes.

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Figure 1.

Boxplot of life history traits for *An. coluzzii* (grey) and *An. gambiae* (white) in insectary and semi-field conditions. A) and B) time to emergence (in days); C) and D) larval mortality (in %); E) and F) female's wing size (in mm). Boxes extend between the 25th and 75th percentile, a thick line denotes the median and a red cross denotes the mean. The whiskers extend up to the most extreme values. Tukey's *post hoc* test probability is indicated above bars as: 'ns', not significant; * P<0.05.

Table 1

Meteorological variables and water temperature (mean $\pm$ SEM) during experiments.

	Insectary (2008)	Semi-field (2009)
	1 – 14 December	23 September – 4 October
Water temperature		
Daily average (°C)	28.0 $\pm$ 1.0*	28.2 $\pm$ 4.5
Daily average minimum (°C)	27.6 $\pm$ 0.3	22.8 $\pm$ 1.0
Daily average maximum (°C)	28.2 $\pm$ 0.2	37.0 $\pm$ 1.8
Air temperature		
Daily average (°C)	28 $\pm$ 1.0	28.8 $\pm$ 4.5
Relative humidity		
Daily average (%)	80 $\pm$ 5.0	75.9 $\pm$ 14.7
Photoperiode L:D		
	12:12	12.75:11.25

* Water temperature variations were not assessed directly during the experiment. However, under our experimental settings, water temperature was shown to strictly follow air-temperature variation in our insectary. Data from air temperature fluctuation during the experiment are therefore provided.

Anova for time to emergence, mortality and wing-length under insectary (A) and semi-field (B) conditions for *Anopheles coluzzii* and *Anopheles gambiae* reared under different proportions of competing species.

Table 2

A - Insectary conditions									
Effect	Time to emergence			Mortality			Wing-length		
	d.f.	F-value	P-value	d.f.	F-value	P-value	d.f.	P-value	
Species	1	37.144	<0.001	1	5.887	0.027	1	0.737	0.393
Competition	3	1.416	0.237	3	0.085	0.967	3	5.744	0.001
Interaction	3	1.233	0.297	3	0.627	0.608	3	12.642	<0.001

B - Semi-field conditions									
Effect	Time to emergence			Mortality			Wing-length		
	d.f.	F-value	P-value	d.f.	F-value	P-value	d.f.	P-value	
Species	1	26.655	<0.001	1	1.680	0.213	1	0.206	0.651
Competition	3	2.072	0.103	3	1.536	0.244	3	1.492	0.222
Interaction	3	3.950	0.008	3	1.649	0.218	3	5.519	0.001

Table 3

Sex ratio for *An. coluzzii* and *An. gambiae* reared under different density of competitor (in %). Sex ratio was calculated as the number of emerging females divided by the total number of emerging adult mosquitoes (indicated in brackets) for each treatment and species. Sex ratio significantly different from 0.5 (test of equal proportion) is indicated by: * $P < 0.05$.

Competitors (%)	Insectary conditions		Semi-field conditions	
	<i>An. coluzzii</i>	<i>An. gambiae</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>
0	0.47 (111)	0.49 (105)	0.38 (98)*	0.60 (88)
25	0.53 (86)	0.46 (67)	0.57 (82)	0.47 (82)
50	0.50 (58)	0.50 (50)	0.51 (53)	0.50 (56)
75	0.65 (29)	0.57 (21)	0.50 (32)	0.40 (25)