



## Competitive interactions between larvae of the malaria mosquitoes *Anopheles arabiensis* and *Anopheles gambiae* under semi-field conditions in western Kenya

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### ABSTRACT

The present paper reports the occurrence of competition between larvae of the malaria mosquito sibling species *Anopheles arabiensis* and *An. gambiae* under ambient conditions in western Kenya. Larvae of both species were reared at the same density and under the same food conditions outdoors in single-species and mixed-species populations (species ratio 1:1) in transparent cups that floated in small and large semi-natural pools, which experienced different diurnal variations in water temperature. In a second experiment, both species were reared at similar densities and under the same food conditions in trays in either single-species or mixed-species populations at different proportions (species ratio 1:1, 1:3 or 3:1). Competition affected the development rate of both species in an opposite way: the development time of larvae of *An. arabiensis* increased whereas the development time of larvae of *An. gambiae* decreased in the presence of its sibling species. In small pools larvae developing in mixed-species populations experienced a higher mortality than larvae reared in single-species populations, whereas no such effect was observed in the large pools. In both species the time to pupation was longer and emerging females were larger in the small pools. Larval mortality of *An. arabiensis* was lower in the small pools compared to the large pools, whereas *An. gambiae* showed the opposite trend. Overall *An. arabiensis* showed reduced development rates, higher mortality rates and emerged with a larger body size compared to *An. gambiae*. The implication of these competitive interactions between larvae of *An. arabiensis* and *An. gambiae* under semi-field conditions needs to be considered in the design and implementation of programmes that aim to reduce malaria transmission as competition may alter the species composition in the field.

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### 1. Introduction

The two sibling malaria mosquito species *Anopheles arabiensis* Patton and *An. gambiae* Giles sensu stricto (hereafter referred to as *An. gambiae*), both belonging to the *An. gambiae* sensu lato complex (hereafter referred to as *An. gambiae* s.l.), are widespread throughout sub-Saharan Africa. Although *An. gambiae* is usually the predominant species in environments with high humidity and *An. arabiensis* is more common in zones with less rainfall, both species occur sympatrically across a wide range of tropical Africa (Petrarca et al., 1998; Coetzee et al., 2000).

Their immature stages require an aquatic environment to develop and are found in transient, sunlit and small pools (Gillies

and DeMeillon, 1968; Gimnig et al., 2001). A clear difference in breeding site preference of the two species has not been observed, but the requirements of both species for their larval habitats are subject of discussion. Several studies suggest these preferences are similar (Charlwood and Edoh, 1996; Gimnig et al., 2001), others think these are different but were unable to show that explicitly (Minakawa et al., 1999). As both species are often found to share larval habitats (Charlwood and Edoh, 1996; Minakawa et al., 1999; Gimnig et al., 2001; Edillo et al., 2002; Chen et al., 2006) and *An. gambiae* s.l. larvae tend to aggregate (Service, 1971; Koenraadt et al., 2004), there will be frequent contact between individuals. However, the effects of interspecific competition between larvae of *An. arabiensis* and *An. gambiae* have rarely been studied. Laboratory studies by Schneider et al. (2000) and Kirby and Lindsay (submitted) showed a competitive disadvantage of *An. arabiensis* over *An. gambiae*, which was expressed in a higher mortality rate in mixed-species populations. In a separate study, Koenraadt and Takken (2003) demonstrated the occurrence of intra- and interspecific competition within the *An. gambiae* s.l. complex, where older larvae preyed on younger ones.

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The existence of interspecific competition between immatures of certain culicine mosquito species (mainly *Aedes* species) has been well established and a wide range of effects has been observed. Interspecific competition may affect larval development rate (Ho et al., 1989; Barrera, 1996; Juliano et al., 2004), larval survivorship (Black et al., 1989; Barrera, 1996; Braks et al., 2004; Juliano et al., 2004), the resistance to larval starvation (Barrera, 1996), sex-ratios (Lowrie, 1973a,b) and adult mosquito size (Ho et al., 1989). Moreover, it may alter mosquito–virus interactions as shown by Alto et al. (2005) for *Aedes albopictus* Skuse and therefore affect pathogen transmission.

These studies further showed that the extent of interspecific competition may depend on a variety of factors, including temperature (Russell, 1986; Ho et al., 1989), larval densities (Lowrie, 1973a,b; Russell, 1986) and food quality and quantity (Russell, 1986; Novak et al., 1993; Barrera, 1996; Daugherty et al., 2000). Furthermore, interspecific differences in larval foraging behaviour may result in a difference in resource acquisition (Yee et al., 2004) and one species might obtain more energy from the substrate than the other (Barrera, 1996). This may be explained by morphological differences of the mouth brushes (Widahl, 1992), different speed of movement of the mouth brushes and therefore different food quantity consumed per unit time (Ho et al., 1989, 1992; Widahl, 1992), a difference in feeding activity (Grill and Juliano, 1996) or by a different efficiency of the digestive system (Ho et al., 1992).

Various mosquito control methods, such as the use of insecticide-treated bednets (ITNs) and the larvicide *Bacillus thuringiensis israeliensis* (*Bti*), are currently being applied and technologies like genetically modified mosquitoes and the sterile-male technique are under development. These methods may not only result in decreasing mosquito abundance but also in a shift in the

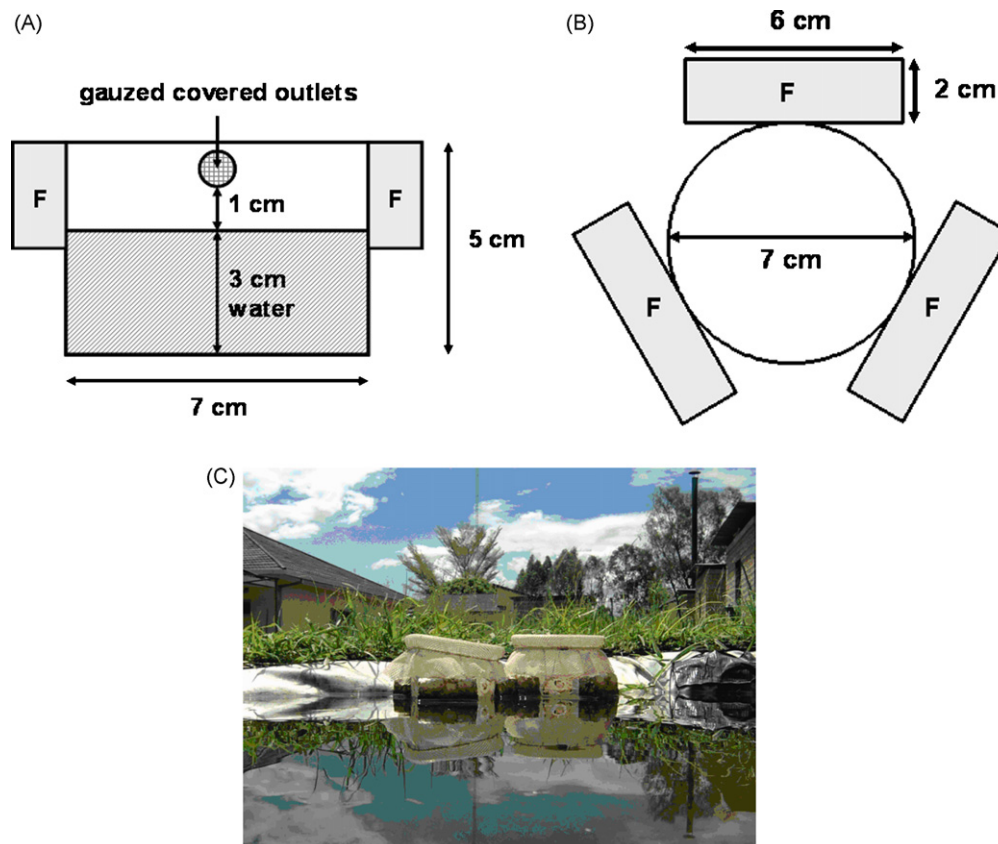
local species composition. This has recently been shown in an ITN study by Lindblade et al. (2006), which overall reduced the number of vectors in the area, but also changed the *An. arabiensis* to *An. gambiae* ratio from 1:1 to proportionally more *An. arabiensis*.

As both species are frequently found to co-exist in the same larval habitats, a better understanding of the competitive interactions between *An. arabiensis* and *An. gambiae* is needed. These interactions may alter the distribution and abundance of adult mosquitoes and hence the risk of malaria. Therefore we studied whether competitive interactions occur between larvae of *An. arabiensis* and *An. gambiae* under ambient and semi-field conditions in western Kenya. In a first experiment we examined the effects of competition in habitats of different sizes, which experienced different diurnal temperature variations. In a second experiment the effect of various species ratios within a population was assessed. We analyzed the effect of competition and habitat temperature on the development time and mortality of the immature stages and on the adult sex-ratio and female wing length.

## 2. Materials and methods

### 2.1. Mosquitoes

Experiments were carried out at the Kenya Medical Research Institute (KEMRI) in Kisian, Western Kenya. We used larvae of *An. gambiae*, maintained at the Centre for Vector Biology and Control Research (CVBCR) at KEMRI and larvae of *An. arabiensis*. As there was no *An. arabiensis* available in culture, wild-caught gravid females were collected from houses near the Ahero rice fields, approximately 30 km from Kisumu, prior to each experimental series. These females were allowed to oviposit in the insectaries



**Fig. 1.** (A) Schematic drawing of the side view of an experimental cup. F indicates a polystyrene float. (B) Schematic drawing of the top view of an experimental cup. F indicates a polystyrene float. (C) Experimental cups floating in a large-sized semi-natural pool.

and a large subset of the offspring was identified to species, as described below, which confirmed a homogeneous *An. arabiensis* population. The *An. gambiae* colony at the CVBCR was frequently replenished with field-caught mosquitoes.

## 2.2. Experiment 1: effects of interactions in different sized semi-natural pools

The experiment was carried out in April and May 2006 in two experimental series with, per series, three replicates for each treatment (single-species *An. arabiensis* or *An. gambiae* and mixed-species populations) in each habitat size (small-sized or large-sized pool). In a third series, two replicates for each treatment were used in the small-sized pool only, as two cups with mixed-species populations were lost in the second series.

Fifteen newly hatched (~1 h old) first instar larvae (hereafter referred to as L1 larvae) of *An. arabiensis* and 15 newly hatched L1 larvae of *An. gambiae* were combined in a transparent plastic cup (0.78 larvae/cm<sup>2</sup>). The control groups consisted of 30 newly hatched L1 larvae of either *An. arabiensis* or *An. gambiae* in identical cups.

Each cup (Fig. 1A and B) was provided with three polystyrene floats and three screened (0.20 mm mesh size) openings (Ø6 mm) in the cup wall to drain excess rainwater. The cup was filled with 3 cm water and was covered with netting (1-mm mesh size) throughout the experiment.

The cups floated in small (Ø16 cm, water depth 4 cm) or large (Ø96 cm, water depth 32 cm) semi-natural pools (Fig. 1C). The pools were created by digging holes in the ground and lined with transparent plastic (0.13 mm). The plastic was pressed tightly against the soil to optimize plastic–soil contact and filled with clear tap water up to 10 mm under the brim. The plastic of the large pools was kept in place by metal rings (50 mm wider in diameter than the pool). Water was added daily in the early morning to compensate for evaporation and to maintain a uniform water level. The setup was cleaned and refilled on a weekly basis.

By using cups that floated in pools of different size, larvae were reared in the absence of predators and with a constant density-dependent and food-resource factor. Therefore, we were able to study the effects of different daily water temperature dynamics in the different pools on the competitive interactions between larvae of *An. arabiensis* and *An. gambiae*.

## 2.3. Experiment 2: effects of interactions at disproportionate species ratios

This second experiment was carried out in May 2006 in an open greenhouse with a semitransparent roof. Circular plastic trays (Ø12 cm, 5 cm deep) were filled with 3 cm of tap water and 60 newly hatched (~1 h old) L1 larvae were placed in each tray (0.53 larvae/cm<sup>2</sup>). The following *An. arabiensis* to *An. gambiae* ratios were tested: 1:0, 3:1, 1:1, 1:3 and 0:1, with three replicates for each ratio. All trays were covered with netting (1-mm mesh size) throughout the experiment. Water was added or removed daily in the early morning to compensate for evaporation or precipitation, respectively, and to maintain a uniform water level.

## 2.4. Data collection

In both experiments, larvae were counted, categorized to larval stage and fed 0.3 mg Tetramin® Baby fish-food (TetraWerke, Melle, Germany) per larva every day. Every 2 days the cups or basins that were used for rearing were cleaned and water was replaced to prevent scum formation and accumulation of metabolites that may be toxic to the larvae (Bayoh and Lindsay, 2003). Dead larvae

and pupae were discarded daily. Living pupae were collected and reared to adults in paper cups that contained 2 cm of tap water and were covered with netting. The tap water that was used for larval and pupal rearing originated from a well at KEMRI. Before use, water was stored in closed basins (100 L) for several days to allow sediments and other inorganic particles to settle.

Gender of the emerged mosquitoes was recorded and females were stored in vials with some silica gel, whereby females from the mixed-species treatments were stored in separate vials to prevent contamination. Wing length of each female adult (one wing per female) was measured with a dissecting microscope as described by Lyimo et al. (1992).

All females from the mixed-species populations as well as a large subset (approximately 80%) of the *An. arabiensis* progeny that was reared in the single-species populations, were identified using the polymerase chain reaction (PCR) for species differentiation within the *An. gambiae* complex (Scott et al., 1993).

## 2.5. Meteorological data

No water temperature data were recorded at the time of the experiments, due to the failure of the equipment. Over a period of 40 days during the same months in the previous year (Paaijmans et al., 2008), continuous water temperature measurements showed that the mean water temperatures of the small-sized and large-sized pools were similar (27.4 ± 0.2 and 28.1 ± 0.2 °C, respectively). However, considerably larger daily variations in temperatures occurred in the small-sized pool, where the mean difference between the average daily minimum (22.4 ± 0.1 °C) and maximum (36.8 ± 0.3 °C) water temperature was 14.4 °C. This difference was 7.1 (25.1 ± 0.1 – 32.1 ± 0.2) °C in the large-sized pool.

To give an indication of the climatic conditions during our experiments, Table 1 shows some meteorological variables during both experiments. An automated meteorological station at KEMRI measured the incoming short wave radiation at 1.5 m above ground (pyranometer; Kipp & Zonen, The Netherlands), temperature and relative humidity at 2 m above ground (ventilated probe; Vaisala, Finland), wind speed at 2 m above ground (cup anemometers; homemade) and rainfall (rain gauge; Eijkelkamp, The Netherlands). Totaled (rainfall) or averaged (other meteorological variables) data were stored every 15 min on a 21 × Microdatalogger (Campbell Sci-

**Table 1**  
Summed (rainfall only) or mean (±S.E.M.) meteorological variables during experiments 1 (n = 45 days) and 2 (n = 19 days)

	Experiment 1	Experiment 2
	9 April–25 May	8–26 May
Air temperature		
Daily average (°C)	22.3 ± 0.1	22.2 ± 0.1
Daily average minimum (°C)	18.1 ± 0.1	18.0 ± 0.2
Daily average maximum (°C)	28.1 ± 0.1	27.8 ± 0.2
Precipitation		
Total during experiment (mm)	167.8	72.6
Maximum recorded (mm day <sup>-1</sup> )	18.9	17.7
Incoming solar radiation		
Average daily total (MJ m <sup>-2</sup> day <sup>-1</sup> )	21.0 ± 0.4	20.0 ± 0.6
Relative humidity		
Daily average (%)	77.4 ± 0.6	78.0 ± 1.0
Daily average minimum (%)	51.1 ± 1.0	51.6 ± 1.9
Daily average maximum (%)	95.6 ± 0.2	95.5 ± 0.4
Wind speed		
Daily average (m s <sup>-1</sup> )	0.9 ± 0.0	0.9 ± 0.0
Daily average minimum (m s <sup>-1</sup> )	0.3 ± 0.0	0.3 ± 0.0
Daily average maximum (m s <sup>-1</sup> )	2.5 ± 0.5	2.4 ± 1.2

entific Inc., UK). There was no significant difference between the climatic conditions of experiments 1 and 2.

2.6. Statistical analysis

Per experimental unit (cup or basin) and per treatment (rearing of single-species or mixed-species populations), the development time (mean time to pupation), mortality (percentage of larvae that did not reach adult stage), sex-ratio (number of male mosquitoes divided by number of females) and wing length (mean female wing length) were determined per species. To establish the development time, only pupae that developed into adults were taken into account.

Data were analyzed using SPSS software (Version 15.0.1, SPSS Inc., Chicago, IL). Experiment 1: a GLM was used to test for the effect of ‘competition’, ‘species’, ‘temperature’ and for an interaction between them on the development time, mortality, sex-ratio and wing length. Experimental series was included in the GLM as a random factor. Maximal models were fitted first and, beginning with higher order interactions, non-significant terms were sequentially removed in a process of backward elimination to generate minimal models. To meet normality assumptions, mortality data were square root transformed and sex-ratio data were double square root transformed. Experiment 2: a GLM was used to test for the effect of ‘proportion of competitive species’, ‘species’ and for an interaction between them on the development time and mortality. Experimental series was included in the GLM as a random factor. Again maximal models were fitted first and, beginning with higher order interactions, non-significant terms were sequentially removed in a process of backward elimination to generate minimal models. Mortality data were square root transformed to meet normality assumptions. Comparison of sex-ratios and wing lengths between treatments was done by a non-parametric tests (Mann–Whitney *U*), using a Bonferroni correction for multiple tests.

3. Results

3.1. Experiment 1: effects of interactions in different sized semi-natural pools

3.1.1. Effect on development time

Larvae of *An. arabiensis* took significantly longer to develop into pupae than *An. gambiae* larvae, regardless of habitat size and thus temperature. However, this effect was competition-dependent (Fig. 2 and Table 2): in small pools the development time of *An. arabiensis* increased from 9.3 (±0.1) days in the absence to 10.8 (±0.3) days in the presence of the sibling species, whereas the development time of *An. gambiae* decreased from 9.0 (±0.2) to 8.3 (±0.2) days, respectively. The same phenomenon was observed in the large pools, whereby the development time of *An. arabiensis* increased from 8.5 (±0.1) days in the absence to 9.0 (±0.3) days in the presence of the sibling species and the development time of *An. gambiae*

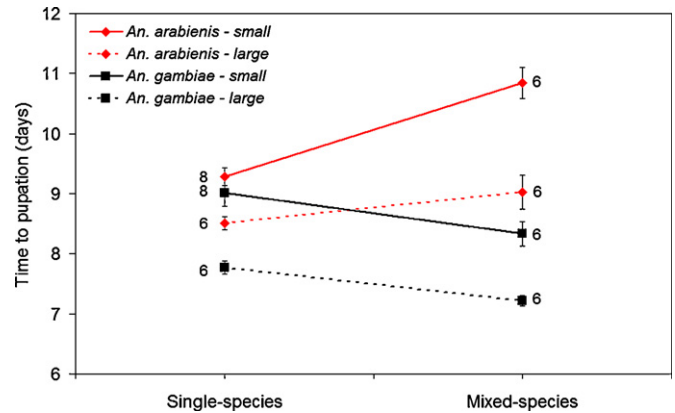


Fig. 2. Interaction plot for the effect of habitat sharing by larvae of *An. arabiensis* (diamonds) and *An. gambiae* (squares) under ambient conditions on the mean time to pupation in small pools (solid lines) and large pools (dotted lines). Error bars represent the standard error of the mean. The number near each data point indicates the number of replicates available for the analysis.

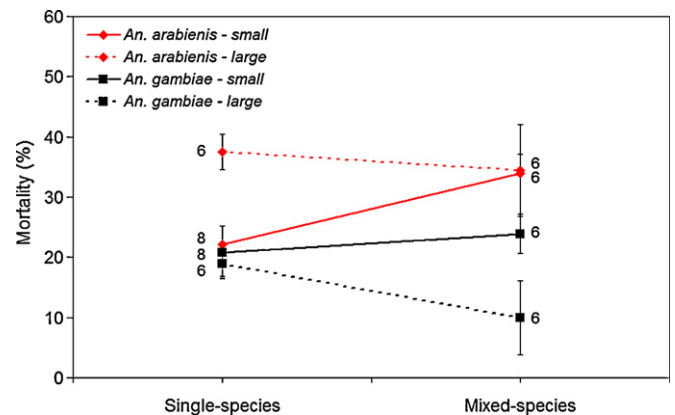


Fig. 3. Interaction plot for the effect of habitat sharing by larvae of *An. arabiensis* (diamonds) and *An. gambiae* (squares) under ambient conditions on the mortality of the immature stages in small pools (solid lines) and large pools (dotted lines). Error bars represent the standard error of the mean. The number near each data point indicates the number of replicates available for the analysis.

decreased from 7.8 (±0.1) to 7.2 (±0.1) days, respectively. Development time was significantly affected by temperature: in small pools, the time to pupation was longer for both species in both single-species and mixed-species populations, compared to large pools (Fig. 2 and Table 2).

3.1.2. Effect on mortality

Competition did alter the observed mortality rates for both species, but the effect was temperature-dependent (Fig. 3 and Table 2): larvae that were in competition in small pools experienced

Table 2

GLM analyses of the effects of competition (presence/absence of sibling species), species and habitat temperature dynamics on the time to pupation and mortality of larvae of *Anopheles arabiensis* and *An. gambiae*

Effect	Time to pupation			Mortality		
	d.f.	F	P	d.f.	F	P
Competition	1,44	2.27	0.139	1,46	0,28	0,598
Species	1,44	93.46	<0.001	1,46	19.15	<0.001
Temperature	1,44	80.83	<0.001	1,46	0.67	0,416
Competition × species	1,44	35.98	<0.001			>0.05
Competition × temperature	1,44	2.73	0.105	1,46	7.98	0,007
Species × temperature	1,44	0.18	0.671	1,46	7.76	0,008
Competition × species × temperature	1,44	4.50	0.040			>0.05

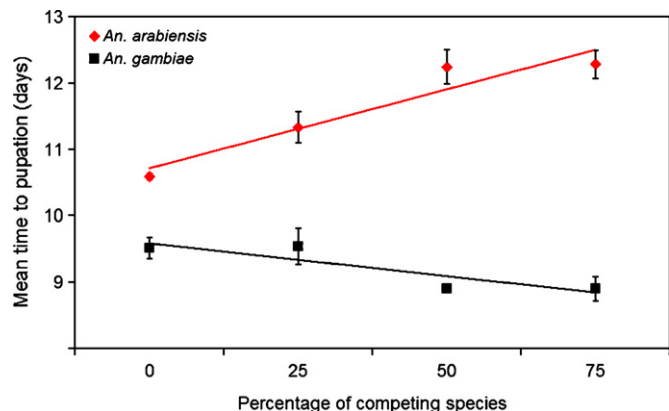


Fig. 4. Interaction plot for the effect of an increased proportion of the competing species present on the development time for larvae of *An. arabiensis* (diamonds) and *An. gambiae* (squares). The error bars represent the standard error of the mean. The number of replicates per data point is three.

a higher mortality than larvae reared in single-species populations, whereas no such observation was made in the large pools. Overall, the mortality of the immatures of *An. arabiensis* was significantly higher than that of *An. gambiae*. However, both species responded differently to habitat size: the mortality of the immature stages of *An. arabiensis* was lower in the small pools than in the large pools, whereas *An. gambiae* showed the opposite trend (Fig. 3 and Table 2).

### 3.1.3. Effect on sex-ratio

Competition and temperature had no effect on the sex-ratio of both species. The average male/female ratio was significantly lower ( $F_{1,48} = 12.97$ ;  $P < 0.001$ ) for *An. arabiensis* ( $0.8 \pm 0.1$ ) than for *An. gambiae* ( $1.4 \pm 0.2$ ).

### 3.1.4. Effect on female size

Temperature affected female size ( $F_{1,50} = 4.17$ ;  $P = 0.047$ ) and the average wing length of females of *An. arabiensis* (small pool:  $2.99 \pm 0.02$  mm; large pool:  $2.93 \pm 0.04$  mm) was significantly larger ( $F_{1,50} = 15.36$ ;  $P < 0.001$ ) than that of females of *An. gambiae* (small pool:  $2.88 \pm 0.03$  mm; large pool:  $2.83 \pm 0.02$  mm). Competition had no effect on the mean wing length of the female mosquitoes for both species.

## 3.2. Experiment 2: effects of interactions at disproportionate species ratios

### 3.2.1. Effect on development time

Larvae of *An. arabiensis* took significantly (species:  $F_{1,16} = 300.2$ ;  $P < 0.001$ ) longer to develop into pupae than larvae of *An. gambiae*. However, this effect was competition-dependent: when a higher proportion of the competing species was present in the population, the development time of *An. arabiensis* increased (from  $10.6 \pm 0.0$  days when reared alone to  $12.3 \pm 0.2$  days when 75% of the population consisted of its sibling species) whereas the development time of *An. gambiae* decreased (from  $9.5 \pm 0.2$  days in single-species to  $8.9 \pm 0.2$  days when 75% of the population consisted of its sibling species) (Fig. 4; 'proportion competitive species'  $\times$  'species' interaction:  $F_{3,16} = 17.4$ ;  $P < 0.001$ ).

### 3.2.2. Effect on mortality

A higher proportion of the sibling species present in the population had no significant effect on the mortality of the immatures of both species. No difference was observed between the percentages of larvae of *An. arabiensis* (mortality:  $15.6 \pm 2.1\%$ ) and *An. gambiae* (mortality:  $16.4 \pm 1.6\%$ ) that did not reach adult stage.

### 3.2.3. Effect on sex-ratio

There was no effect of the proportion of the sibling species present in the populations on the sex-ratio for both species. The average male/female ratio was  $1.0 (\pm 0.2)$  for *An. arabiensis* and  $1.2 (\pm 0.1)$  for *An. gambiae*.

### 3.2.4. Effect on female size

Sharing a habitat with different percentages of the other species present in the populations had no effect on the wing length for both species. Overall, females of *An. arabiensis* ( $3.34 \pm 0.02$  mm) had significantly larger wings (Mann–Whitney  $U$ :  $P < 0.001$ ) than females of *An. gambiae* ( $3.22 \pm 0.02$  mm).

## 4. Discussion

We demonstrated the existence of a competitive interaction between larvae of *An. arabiensis* and *An. gambiae* under ambient conditions, which increased the time to pupation for *An. arabiensis* by 1.6 days in small pools. By contrast, the time to pupation for *An. gambiae* was decreased by 0.7 days. A similar trend (an increase of 0.5 days for *An. arabiensis* and a decrease of 0.6 days for *An. gambiae*) was observed in the large pools. Further investigations established that the effect became more prominent with an increasing proportion of the competing species present in the population.

Considering the time it took the larvae to pupate, the detrimental effect of habitat sharing for *An. arabiensis* was greater than the advantageous effect for *An. gambiae*. A possible reason might be that the development rates of the single-reared populations of *An. gambiae* were close to the highest rate possible under the given circumstances and therefore a further increase in development rate was not possible, as the species had reached its biological maximum. A shorter development time can be advantageous for the survival of the immature mosquitoes, as they are exposed for a shorter period to threats such as cannibalism, predation, pathogens and climatic variables such as rainfall resulting in flushing of breeding sites (Paaijmans et al., 2007) or periods of drought that result in desiccation of the larval habitat (Koenraadt et al., 2003).

Competition did alter the mortality rates for both species, but the effect was temperature-dependent. In the small pools, sharing of a habitat caused reduction of the survivorship of both species. Both Schneider et al. (2000) and Kirby and Lindsay (submitted) observed the same competitive disadvantage, but for *An. arabiensis* only. There was no effect of competition on the sex-ratio or female body size for both species.

The immediate cause of the different effects of habitat sharing between *An. arabiensis* and *An. gambiae* remains unknown. Both experiments were designed such that competitive interactions could be studied with a constant density-dependent and food-resource factor. We propose some factors that may have caused a competitive interaction between the two species.

First, competition could be driven by chemical or physical cues. Chemical cues have been observed in *Ae. aegypti* (L.), where larvae release growth retardants to the water (Moore and Whitacre, 1972; Kuno and Moore, 1975; Dye, 1984) and pollute the water, probably with nitrogenous waste (Bédhomme et al., 2005). Besides chemical interference, physical interaction has also been proposed as a factor of importance (Dye, 1984). In theory, chemical cues could cause intraspecific as well as interspecific competition. If *An. gambiae* larvae produce growth retardants, fewer individuals of *An. gambiae* at the same larval density may result in the faster development of this species, while this growth retardant may inhibit the development of *An. arabiensis*.

A second explanation for the occurrence of competitive interactions may be the difference in foraging success between *An. arabiensis* and *An. gambiae*. Schneider et al. (2000) proposed that the larger size of females of *An. arabiensis* could mean that this species requires a larger quantity of food. The species that is more efficient in food detection, ingestion and digestion may have a competitive advantage over the other species. Part of the food particles provided to the larvae sank to the bottom of the cups. *An. gambiae* might benefit from these particles, as this species crawls more often along the bottom substrate than *An. arabiensis* does (Tuno et al., 2007). Other explanations for differences in foraging success, that are shown to be of importance in culicine mosquitoes, are morphological differences of the mouth brushes (Widahl, 1992), difference in stroke frequency of these mouth brushes and therefore different food quantity taken per unit time (Ho et al., 1989, 1992; Widahl, 1992), a more active feeding behaviour with more time browsing and less time resting (Grill and Juliano, 1996) or by a more efficient digestive enzyme system (Ho et al., 1992).

Considering the effect of habitat size, both *An. arabiensis* and *An. gambiae* took longer to develop into pupae in the small pools. This probably explains the larger body size of females that resulted from the smaller pools. On the first hand, our data seem to contradict the general assumption that insects experience an increased growth rate and grow to smaller sizes at higher temperatures (Atkinson, 1994), which has indeed been observed for *An. gambiae* as well (Lyimo et al., 1992; Bayoh and Lindsay, 2003). As food quantity and larval densities were kept constant in our experiments and the mean water temperature in the different sized pools was comparable, 27.4–28.1 °C (see Section 2), we suggest that the difference in larval development time between the different-sized pools may be explained by a different magnitude and duration of the alternating phases. The difference between the daily minimum and maximum water temperature was 14.4 ( $\pm 0.3$  S.E.M.) °C in the small-sized and 7.1 ( $\pm 0.2$ ) °C in the large-sized pools. Small-sized pools experience higher temperatures during daytime compared to larger ones, which will result in higher development rates of the larvae (Depinay et al., 2004). These higher temperatures, however, last only for a relatively short period during the day. Therefore, we suggest that the longer exposure to lower temperatures during the evening and at night (Paaijmans et al., 2008) contributed to the longer larval development time in the small pools. As the larger pools experience less variation in water temperature, larvae will have more equivalent hourly growth rates throughout the day, which appears advantageous.

The effect of habitat size on larval mortality differed for both species: compared to the larger pools, *An. arabiensis* expressed a higher survivorship in the small pools, whereas *An. gambiae* had a higher mortality. It is therefore concluded that *An. arabiensis* can tolerate pools that are warmer during day-time and cooler during night-time, such as small puddles, better than larger pools, which exhibit less variation in temperature during the day. This may in part explain some of the differential variations that are observed in sibling species composition and temporal dynamics of sympatric adult populations. Recent work by Kirby and Lindsay (submitted) who observed a greater production of *An. arabiensis* at extremely high water temperatures and of *An. gambiae* at lower temperatures, supports this conclusion.

Overall *An. arabiensis* had lower development rates, higher mortality rates (experiment 1 only) and grew into larger females compared to *An. gambiae*. Similar data have now been reported by Kirby and Lindsay (submitted). Schneider et al. (2000) also found that the mortality of *An. arabiensis* was significantly higher than that of *An. gambiae*, but they observed a shorter development time for *An. arabiensis* larvae compared to *An. gambiae*. Similar size differences between both species have previously been reported in the

field and are considered to have a genetic base (Hogg et al., 1996; Petrarca et al., 1998).

Interestingly, females of both *An. gambiae* and *An. arabiensis* that were reared in the greenhouse of experiment 2 were larger than the females of experiment 1, which were reared outdoors, even though the weather conditions during both experiments were similar. Also, both the survival and the mean time to pupation of *An. arabiensis* larvae increased when larvae were reared in the more protective environment of the greenhouse. Apparently, natural conditions such as direct sunlight and natural daily variations in water temperature affect the life-history traits of the immature mosquitoes, which are easily overlooked in laboratory studies and cannot be easily simulated.

Competitive suppression of *An. arabiensis* by *An. gambiae* may result in a behavioural change of *An. arabiensis*, causing females to avoid oviposition in breeding sites that are occupied by *An. gambiae*. However, this has not been observed in the field where larvae of both species are often found together in puddles and pools (Charlwood and Edoh, 1996; Minakawa et al., 1999; Gimnig et al., 2001; Edillo et al., 2002; Chen et al., 2006). Further studies should focus on the factors that govern competitive interactions and explore the existence of these interactions in the field. Oviposition experiments can provide information about female behaviour concerning habitat choice with presence or absence of the competing species at varying densities.

Future research should also aim at the effects of competition when early instars are competing with late instars of opposite species, as in a field situation all four larval instars can be found together in one habitat (Koenraadt et al., 2004). It has been demonstrated for *An. arabiensis* that the presence of older larvae may reduce the development rate of younger ones (Koenraadt and Takken, 2003). Furthermore, the effect of food quantity and quality and various lower larval densities on competition needs further investigation. Densities of the immature mosquitoes encountered in the field seem lower than those used in our experiment (e.g. 0.13 larvae/cm<sup>2</sup> (Gimnig et al., 2002), 0.016 larvae/cm<sup>2</sup> (Koenraadt and Takken, 2003) and 0.009 larvae/cm<sup>2</sup> (Koenraadt et al., 2004)).

Understanding the exact mechanisms underlying the competitive interactions between *An. arabiensis* and *An. gambiae* will be required when proper mosquito control methods are applied. For example, in areas with a high ITN use and a proportionally higher number of *An. arabiensis* in the area (Lindblade et al., 2006), the *An. gambiae* larvae present may develop faster when they occur sympatrically with *An. arabiensis*, than they would on their own. Furthermore, understanding the competitive traits of malaria vector species would be an important step in the development of genetically modified mosquito populations (Takken and Scott, 2003). The goal of such *Plasmodium*-resistant mosquito lines is to replace the current vector population and, therefore, the genetically modified mosquito needs to be a better competitor than current malaria vectors.

In conclusion, a competitive interaction between larvae of *An. arabiensis* and *An. gambiae* was observed, with *An. gambiae* being the better competitor. Larvae of *An. arabiensis* had a significant extension of their development time, whereas *An. gambiae* developed faster into pupae. These effects were more pronounced when a higher proportion of the sibling species was present in the population. Furthermore, competition altered the mortality rates of both species, but the effect was temperature-dependent. Under natural conditions, when they are vulnerably exposed to other biotic and abiotic factors, such competitive interactions are likely to affect the distribution and abundance of the resultant adult populations and can therefore affect the transmission of malaria, as *An. arabiensis* and *An. gambiae* have a differential vectorial competence (White, 1974).

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