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Reproductive compatibility of several East and West African *Cotesia sesamiae* (Hymenoptera: Braconidae) populations and their crosses and backcrosses using *Sesamia calamistis* (Lepidoptera: Noctuidae) as the host

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The relative importance of the braconid *Cotesia sesamiae*, a gregarious larval parasitoid of lepidopteran stem borers, varies greatly with region in Africa; while the most common parasitoid of noctuid stem borers in eastern Africa, it is rare in western Africa. Thus, several strains of *C. sesamiae* from Kenya are envisaged for introduction into western Africa. The present study investigates the reproductive compatibility between four populations of *C. sesamiae* from West Africa and Kenya with the noctuid *Sesamia calamistis* as the host using reciprocal crosses as well as backcrosses of hybrid females with males of the parental populations. Searching time of the male for the female and mating period varied significantly with couple and ranged between 0.78–1.9 min and 3.4–12.8 s, respectively. Crosses that involved females from inland Kenya (KI) did not yield any female offspring. However, backcrosses of hybrid female bearing a KI male genome with a KI male yielded both female and male offspring. Thus, there was a partial reproductive incompatibility between KI and West African populations which suggested that the latter were infected with *Wolbachia* sp. However, this should not affect the efficacy of a population introduced from East into West Africa, as there is a high degree of sib-mating in this gregarious parasitoid species. It was concluded that the regional differences in the relative importance of *C. sesamiae* was due to differences in the insect and plant host range of the different populations.

Keywords: West African and Kenyan *Cotesia sesamiae* population; *Sesamia calamistis*; reproductive compatibility

Introduction

In West and Central Africa, lepidopteran stem borers such as the noctuids *Sesamia calamistis* Hampson, *S. botanephaga* Tams & Bowden, and *Busseola fusca* (Fuller), and the pyralid *Eldana saccharina* Walker, are the most injurious pests of cereals (see overview in Polaszek 1998). In areas with chronic pest problems, yield losses, usually due to multiple species attack, vary between 10 and 70% (Cardwell, Schulthess,

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Ndemah, and Ngoko 1997; Bosque-Pérez and Schulthess 1998; Ndemah and Schulthess 2002; Buadu et al. 2003).

Schulthess et al. (1997) proposed the exchange of natural enemy species and strains between African regions to control indigenous cereal stemborers. During country-wide surveys in West Africa and Cameroon, Gounou et al. (1994), Bosque-Pérez, Ubeku, and Polaszek (1994), Ndemah, Schulthess, Poehling, and Borgemeister (2001), Conlong (2001), and Gounou and Schulthess (2006) found that several parasitoid species, common in other regions in Africa, were very scarce or absent in western Africa. For example, the gregarious braconid *Cotesia sesamiae* (Cameron) is exceedingly rare in western Africa, while in East and southern Africa, it is the most common larval parasitoid recovered from *B. fusca* and *S. calamistis*. In East Africa, parasitism is mostly below 7% (Jiang, Zhou, Overholt, and Schulthess 2006). However, in western Kenya particularly in the Kitale area, parasitism of *B. fusca* can be as high as 70% (C. Gitau, unpublished data). Similarly in South Africa, parasitism of *B. fusca* on sorghum was as high as 75% and *C. sesamiae* is thought to keep *S. calamistis* under control (Kfir 1995).

The reasons for the general scarcity of *C. sesamiae* in western as compared to eastern Africa are not well understood. In Kenya, *C. sesamiae* exists as two biotypes that differ in their ability to parasitise *B. fusca*. *Cotesia sesamiae* from western Kenya completes development in *B. fusca* larvae, while in the coastal *C. sesamiae* biotype the eggs that are oviposited are encapsulated by haemocytes in *B. fusca* larvae (Gitau, Dupas, Ngi-Song, Mbugi, and Schulthess 2006). It was suggested that this is due to differences in polyDNA viruses (PDV) (Dupas, Gitau, and Silvain 2006; Gitau et al. 2006) that are injected together with the egg at oviposition. The PDV genes are expressed in the host larvae, causing the destruction of haemocytes, thus preventing encapsulation of the eggs (Beckage and Gelman 2004). Furthermore, there appear to exist several populations of *C. sesamiae* in Africa, which differ in insect and plant host range (A. Branca, unpublished data). For example, while in East and southern Africa *C. sesamiae* is mostly recovered from borers attacking sorghum or maize, in recent surveys in Cameroon it was only obtained from the noctuids *Poenoma serrata* Hampson and *Busseola quadrata* Bowden feeding on *Pennisetum purpureum* (Moench) and *Setaria megaphylla* (Steud) Dur. & Schinz, respectively, and never from *B. fusca* on maize (Ndemah, Schulthess, Le Rü, and Bame 2007). Similarly, during earlier surveys in the humid forest zone in Cameroon only two *C. sesamiae* cocoon masses were obtained from *B. fusca* on maize (Ndemah et al. 2001). Strain occurrence has also been reported for *Cotesia flavipes* Cameron (Potting, Vet, and Overholt 1997). In addition, differences in the regional importance of *C. sesamiae* may be due to differences in the suitability of the host; for example, *B. fusca* and *E. saccharina* exist in several different populations in Africa, which vary in host range and climatic requirements (Assefa, Mitchell, and Conlong 2006; Sezonlin et al. 2006). Furthermore, Ngi-Song, Overholt, and Stouthamer (1998), Ngi-Song and Mochiah (2001), and Mochiah, Ngi-Song, Overholt, and Stouthamer (2002a) reported unidirectional incompatibility, caused by *Wolbachia* sp. infection, whereby crosses between males from an infected population from the Kenya coast and females from an uninfected population from inland Kenya did not yield any female offspring. Using an antibiotic treatment, Mochiah, Ngi-Song, Overholt, and Stouthamer (2002b) established that *Wolbachia* sp. infection caused cytoplasmic incompatibility of the female mortality type in *C. sesamiae*.

Thus, the efficacy of an introduced strain of *C. sesamiae* may be affected by the parasitoid and host strain, as well as incompatibility problems caused by *Wolbachia* sp. infection, when outcrossing with locally occurring strains.

The objectives of the present study were: (i) to provide information on the biology and mating compatibility of East and West African populations of *C. sesamiae* and (ii) to evaluate the performance of F1 and F2 of crosses and back-crosses of the various populations using *S. calamistis* from Benin as a host. The results should also provide indications about *Wolbachia* infections occurring in Nigerian *C. sesamiae* populations.

Material and methods

Borer and parasitoid rearing

Sesamia calamistis larvae were obtained from laboratory colonies at the International Institute of Tropical Agriculture (IITA), Benin, which were maintained on an artificial diet developed by Bosque-Pérez and Dabrowski (1989). The experiments were conducted with four populations of *C. sesamiae*. They were obtained from *S. calamistis* collected from maize in East (NE) and northern Nigeria (NN), as well as from coastal Kenya (KC), and from *B. fusca* larvae collected at Kitale in inland Kenya (KI). The latter two were provided by the International Centre of Insect Physiology and Ecology, Nairobi, Kenya, where they had been reared on *S. calamistis*. At IITA, all populations were reared on *S. calamistis* following the method by Hailemichael (1997). To prevent mixing of populations, the parasitoid rearing as well as the parasitisation of the larvae were carried out in separate rooms kept at $27 \pm 1^\circ\text{C}$, a photoperiod of 12:12 day/light, and relative humidity of $75 \pm 5\%$.

Fourth instar larvae of *S. calamistis*, which are the most suitable host stage for the development and reproduction of *Cotesia* species (Hailemichael 1997), were selected from the stock culture to infest 8-cm pieces of maize stem. One larva was introduced into a 4-cm long tunnel bored into the stem segments using a cork borer. The infested stems were kept separately in transparent vials of 9×4 cm (height \times diameter), tightly covered with a plastic lid with a central hole of 2.5-cm diameter. The hole was plugged with cotton wool. The larvae were allowed to feed and produce frass for 24–30 h prior to the introduction of a mated female *C. sesamiae*. A droplet of honey mixed with water (ca. 10% concentration) served as food for the parasitoid. Insertion of the ovipositor could be indirectly determined by frass thrown out of the entrance hole by the defending larvae; as shown by Takasu and Overholt (1997), the attacked larvae displays an aggressive behaviour towards the parasitoids. The parasitoid was left in the vial for 24 h. Thereafter, the stem was dissected and larvae reared individually on artificial diet.

Cross-mating experiments

Cotesia sesamiae were allowed to emerge singly by separating individual dark cocoons from a cocoon mass. Immediately after emergence, individual males and females were paired and were allowed to mate for about 2 h in a transparent vial (9×4 cm, height \times diameter). The behavioural pattern of the couple was observed for 30 min and compared with the behavioural sequence of *Cotesia* spp. outlined by Kimani and Overholt (1995). Hence, successful mating was defined here as females attaining the

quiescent stage followed by copulation. Only females that mated were used for the experiment. Both heterogamic and homogamic crosses were carried out. In the homogamic crosses, a male and female of the same populations were allowed to mate, while in the heterogamic crosses, males of each population were allowed to mate with females of each of the other populations and vice versa, making it four homogamic and 12 heterogamic crosses. In addition, the F1 females from these crosses were also backcrossed with a male from the parent population and then offered *S. calamistis* larvae using the same procedure as above. In both experiments, larvae were reared on artificial diet until cocoon formation or pupation of larvae. One hundred females were tested for each cross-mating combination. The vials were inspected daily for pupation of larvae or cocoon formation. The mean duration of searching of the male for the female was from release of the male into the vial to mounting of the female, and mating was timed from mounting until dismounting. Mean number of F1 as well as of backcross (F2) progenies per female was determined for each coupling. The percentage of hosts from which parasitoid cocoons emerged, brood size (i.e. number of cocoons), number of emerging adults, sex ratio as the proportion of female offspring, parasitoid immature mortality as the percentage of adults emerging over number of cocoons, and developmental time of the parasitoid were monitored.

Data analysis

Observations on searching and mating time, and percent females that mated successfully in the simple cross experiment were carried out as a time series in three to four sets depending on availability of a parasitoid population. Thus, the data were analysed by analysis of variance (ANOVA), using the mixed model procedure of SAS with repeated measures (SAS 1997). The treatments were considered as fixed effects, while insect number within a set was considered as a random factor. Least square means (LSM) were separated using the *t*-test. The significance level was set at $P = 0.05$. For the assessment of the reproductive potential of the different crosses and all observations in the backcross experiment, only one set of time series above was used. Thus, differences in the variables measured were analysed by ANOVA using the general linear model (GLM) procedure of SAS for PC (SAS Institute 2000). Means were separated using the Student–Newman–Keuls (SNK) test. The significance level was set at $P = 0.05$. Correlation analyses using the PROCOR procedure of SAS (SAS Institute 2000) were done to detect relationships between the different variables measured. For this, data were pooled across crossing treatments. Insect counts were log transformed and percentages and proportions arcsine transformed before being subjected to ANOVA (Sokal and Rohlf 1981). Back-transformed data are presented.

Results

Mating behaviour of the parasitoids

The same behavioural steps leading to copulation as described by Kimani and Overholt (1995) were observed for all intra- and interpopulation couplings and details are not presented here.

Mean searching and mating time varied significantly with coupling (Table 1). The shortest searching time was found for three out of four Kenyan pairs, followed by Nigerian pairs, while Nigerian \times Kenyan pairs tended to have the longest searching

time. In two crosses involving the KI population, searching time was longer when the male was KI, except for the KC × KI pair where it was shorter with a KI male. No differences in mating period were found between homogamic crosses and the average period for intrapopulations crosses was 2.3 times lower compared to interpopulation crosses (Table 1). For the KC × KI cross, the mating period was considerably shorter when the male was KI. The percent of females that mated varied from 19.7 to 30.7% but it did not vary significantly among most crosses; a significantly negative correlation was found with both searching and mating time (Table 2). Searching time was highly positively correlated with the mating period (Table 2).

Reciprocal crosses

Results of the reciprocal crosses showed that all four populations were partially to fully compatible among themselves (Table 3). Percent larvae with cocoons ranged from 27.7 to 74.5%, but there were no clear trends regarding population origin of the male or female. A negative relationship was found between larvae producing cocoons and the mating period (Table 2). The same trends were found for number of cocoons and adults obtained which ranged from 26.7–59.5 to 16.4–55.0, respectively, as well as for sex ratio, which varied from 0.0 to 0.8. Crosses that involved a female KI did not yield any female offspring. Development time of the parasitoid offspring did not vary with cross and ranged from 17.7 to 18.2 days (data not shown in the table). Number of larvae producing cocoons was positively related to percent females that

Table 1. Searching and mating period of male parasitoids with females, and percent females that mated from different populations of *C. sesamiae*.

Crossing (♀ × ♂)	Search period (min)	Mating period (s)	% Females mated
NE × NE	1.30b	4.8a	25.4 b
NN × NN	1.30b	4.3a	27.6 b
KC × KC	0.90a	3.4a	28.8b
KI × KI	0.78a	3.4a	30.7c
NE × NN	1.30b	8.4b	21.3a
NN × NE	1.50bc	10.0bc	20.2a
NE × KC	1.90c	12.4c	28.9bc
KC × NE	1.70c	10.8c	20.7a
NE × KI	1.35b	5.8ab	19.7a
KI × NE	1.70c	8.4b	20.2a
NN × KC	1.80c	12.8c	19.8a
KC × NN	1.50bc	11.5c	24.0b
NN × KI	1.35b	4.8a	22.1a
KI × NN	0.90a	5.8ab	26.7bc
KI × KC	1.85c	11.0c	19.7a
KC × KI	0.85a	3.4a	28.9bc
<i>t</i>	2.76	16.5	12.5
S.E.	0.32	3.4	0.8
<i>P</i> value	0.039	0.0001	0.0001

Within column, means followed by the same letter are not significantly different ($P < 0.05$), *t*-test; NN, NE, KC and KI stand for *C. sesamiae* population from northern Nigeria, eastern Nigeria, coastal Kenya and Kitale in inland Kenya, respectively.

Table 2. Correlations between searching and mating time of the male *C. sesamiae* and some reproductive parameters of the female parasitoid in cross experiments involving *C. sesamiae* populations from East and West Africa.

	1	2	3	4	5	6	7
1. Searching period (min)	–						
2. Mating period (s)	0.86*	–					
3. % Females that mated	–0.64*	–0.55*	–				
4. % Larvae with cocoons	–0.44	–0.54*	0.81*	–			
5. Number of cocoons	–0.49*	–0.38	0.69*	0.55*	–		
6. Number of adults	–0.16	–0.02	0.51*	0.42	0.74*	–	
7. Sex ratio	–0.17	–0.17	0.22	0.19	0.56*	0.51*	
8. Parasitoid mortality	–0.44	–0.59*	0.11	–0.20	–0.06	–0.60*	–0.35

r values ≥ 0.48 are significant at $P \leq 0.05$.

mated, and the number of cocoons per larvae was positively correlated with both percent females that mated and with larvae producing cocoons. Immature mortality ranged between 2.5 and 64.9% (Table 3). Sex ratio was positively related with number of cocoons and numbers of emerging adult parasitoids, and negatively with parasitoid mortality, while the latter was negatively correlated with duration of mating.

Backcrosses

Backcrosses of hybrid females with the parent male showed full mating compatibility (Table 4). The percent of females that mated varied from 20.1 to 57.6%. There were no clear trends with regard to origin of the hybrid. The percent larvae yielding cocoons was between 20.8 and 65% and tended to be higher with KC/KI or NE/KI hybrids than with other pairs. A similar trend was observed for numbers of cocoons per larvae, varied between 19.1 and 56.6, and for the numbers of adult parasitoids per larvae as well as for sex ratio, which ranged from 0.56 to 0.73. Development time of the parasitoid offspring did not vary with backcross and ranged from 17.0 to 18.4 days (data not shown in the table). Immature mortality varied between 10.9 and 55%. As in the reciprocal cross experiment, number of cocoons per larvae was positively correlated with percent females that mated ($r = 0.49^*$) and sex ratio ($r = 0.55$). Development time was negatively related with numbers of cocoons ($r = -0.57^*$) and sex ratio ($r = -0.89^*$).

Discussion

The sequence of events observed in the mating behavior of all *C. sesamiae* populations tested in the reciprocal and backcross experiment were the same as reported for *C. sesamiae* and other *Cotesia* species (Kimani and Overholt 1995). Thus, when placed in close vicinity, males and females from different populations appear to recognise each other as potential mates. The significance of acoustic signals produced by wing fanning has been documented in Braconidae by Sivinski and Webb (1989). In parasitoids, wing fanning may also be used to disperse pheromones from the male or to increase airflow over the male, improving male

Table 3. Reproductive potentials (means \pm S.E.) of crossings of different *C. sesamiae* populations.

Crossing (♀ × ♂)	% Larvae with cocoons	No. cocoons	No. adults	% Immature mortality	Sex-ratio (♀/total)
NE × NE	69.5 \pm 0.8d	44.4 \pm 9.1c	34.9 \pm 7.0b	21.4 \pm 0.1b	0.80 \pm 0.3b
NN × NN	64.1 \pm 1.1d	47.9 \pm 9.1c	41.6 \pm 8.6bc	13.3 \pm 0.5ab	0.70 \pm 0.5a
KC × KC	69.9 \pm 0.9d	52.4 \pm 4.9c	52.3 \pm 7.0c	2.5 \pm 0.8a	0.80 \pm 0.5b
KI × KI	74.5 \pm 0.9d	59.5 \pm 9.1c	55.0 \pm 8.6c	7.1 \pm 0.1a	0.70 \pm 0.4a
NE × NN	27.6 \pm 1.2b	48.9 \pm 9.1c	40.3 \pm 9.1bc	17.9 \pm 1.2b	0.70 \pm 0.1a
NN × NE	31.2 \pm 0.9bc	45.1 \pm 9.1c	33.4 \pm 8.6b	26.4 \pm 1.5b	0.70 \pm 0.1a
NE × KC	60.1 \pm 0.9d	54.6 \pm 12.9c	52.5 \pm 8.6c	5.2 \pm 1.6a	0.70 \pm 0.2a
KC × NE	32.8 \pm 0.6bc	26.7 \pm 9.9b	25.5 \pm 9.1b	4.2 \pm 0.6a	0.80 \pm 0.2b
NE × KI	30.5 \pm 1.0bc	42.7 \pm 9.1b	33.1 \pm 8.6b	11.9 \pm 1.3ab	0.75 \pm 0.1ab
KI × NE	51.6 \pm 7.5c	34.9 \pm 7.0ab	16.4 \pm 0.3a	52.1 \pm 3.2d	0.00 \pm 0.0c
NN × KC	46.3 \pm 1.4c	38.1 \pm 9.1bc	29.9 \pm 12.1b	23.2 \pm 4.9b	0.70 \pm 0.1a
KC × NN	46.2 \pm 0.5c	51.4 \pm 12.9b	49.3 \pm 4.0c	10.5 \pm 2.6ab	0.70 \pm 0.1a
NN × KI	51.8 \pm 7.5c	44.2 \pm 8.6 b	17.0 \pm 0.2a	60.8 \pm 2.6d	0.80 \pm 0.1b
KI × NN	46.8 \pm 4.3c	34.0 \pm 4.0a	18.4 \pm 0.1a	45.5 \pm 2.2c	0.00 \pm 0.0c
KI × KC	34.4 \pm 4.3ab	28.9 \pm 12.1a	17.9 \pm 0.1a	34.1 \pm 9.1bc	0.00 \pm 0.0c
KC × KI	54.6 \pm 12.9c	52.5 \pm 8.6c	18.2 \pm 0.1a	64.9 \pm 2.0d	0.75 \pm 0.1b
<i>F</i>	2.2	2.2	4.9	40.8	18.8
<i>P</i>	0.0304	0.0300	0.0001	0.0001	0.0001

Within column, means followed by the same lower case letter(s) are not significantly different ($P < 0.05$), Student–Newman–Keuls (SNK) test.

orientation to female odors. Among Hymenoptera, that use them, female-produced pheromones tend to be released in small quantities and are used for short-range attraction, while male-produced pheromones are released in larger quantities and are used for long-range attraction (Ayasse, Paxton, and Tengö 2001). In olfactometer studies by Kimani and Overholt (1995) *C. sesamiae* did not appear to react to volatile conspecific odours, in contrast to *Cotesia flavipes* Cameron, where both the male and female parasitoid seemed to produce pheromones. They suggested that the lack of response in *C. sesamiae* might be because the odours from this species are not highly volatile and may only be perceived at a very short distance. In the present study, searching time was on an average longest for Nigerian \times Kenya pairs and shortest for intrapopulation pairs. Thus, it appears that visual or chemical clues produced by the parasitoids vary with parasitoid strain.

The longer the male searched for the female, the lower the percentage of successful mating, and the longer the couple took to copulate. This negatively affected the percent larvae that produced cocoon, i.e. successful parasitism. Thus, intrapopulation crosses tended to be reproductively more successful than inter-population crosses. Similarly, Kimani and Overholt (1995), reported lower cocoon production in inter- compared to intraspecific crosses. They suggested that this could be due to mortality of eggs that fused with incompatible or less compatible sperm, the union of gametes producing a lethal lesion. In the present study, parasitoid mortality appeared to be negatively related to sex ratio suggesting that mortality was higher among male than female immatures.

Table 4. Mating success and reproductive potentials of backcrosses.

Backcross ($\text{♀} \times \text{♂}$)	% Females mated	% Larvae with cocoons	No. cocoons	No. adults	% Immature mortality	Sex-ratio ($\text{♀}/\text{total}$)
NE/NN \times NE	20.1 \pm 0.5a	38.6 \pm 2.1ab	19.1 \pm 2.8c	8.6 \pm 1.2d	55.0 \pm 1.1e	0.58 \pm 0.02c
NE/NN \times NN	21.6 \pm 0.5a	38.6 \pm 1.1ab	19.1 \pm 2.8c	8.6 \pm 1.2d	55.0 \pm 1.1e	0.58 \pm 0.02c
NE/NN \times KC	27.7 \pm 0.5b	48.5 \pm 2.6bc	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 1.1d	0.56 \pm 0.04c
NE/NN \times KI	27.7 \pm 0.5b	56.7 \pm 1.1bc	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 1.1d	0.59 \pm 0.01c
NN/KC \times NN	21.6 \pm 0.5a	48.5 \pm 0.8bc	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 1.1d	0.59 \pm 0.03c
NE/KC \times NE	27.7 \pm 0.5b	20.8 \pm 0.6a	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 0.8d	0.57 \pm 0.04c
NE/KC \times KC	27.7 \pm 0.6b	20.8 \pm 1.1a	20.8 \pm 3.1c	11.5 \pm 1.6cd	44.7 \pm 0.2d	0.58 \pm 0.02c
NN/KC \times KI	29.6 \pm 0.6b	28.6 \pm 2.0a	28.6 \pm 5.5b	16.4 \pm 2.7c	42.5 \pm 0.6d	0.56 \pm 0.02c
NN/KI \times NN	28.6 \pm 0.5b	48.8 \pm 5.5b	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 1.1d	0.67 \pm 0.02b
NN/KI \times NE	28.6 \pm 0.5b	35.9 \pm 5.2ab	20.6 \pm 4.7c	11.5 \pm 1.6cd	43.6 \pm 1.1d	0.70 \pm 0.02a
NN/KI \times KC	27.7 \pm 0.6b	50.3 \pm 1.1bc	26.7 \pm 3.9b	16.4 \pm 2.7c	38.7 \pm 0.4d	0.67 \pm 0.02b
NN/KI \times KI	27.7 \pm 0.6b	34.3 \pm 0.7ab	26.7 \pm 3.9b	16.4 \pm 2.7c	38.7 \pm 0.4d	0.67 \pm 0.02b
NE/KI \times NN	21.8 \pm 0.5a	40.9 \pm 5.7b	20.8 \pm 3.1c	17.6 \pm 3.3c	12.7 \pm 1.1a	0.67 \pm 0.04b
NE/KI \times NE	21.6 \pm 0.5a	56.3 \pm 3.6c	26.7 \pm 3.9b	18.8 \pm 4.5c	30.1 \pm 2.4c	0.71 \pm 0.01a
NE/KI \times KC	29.6 \pm 0.7b	40.2 \pm 5.6b	28.6 \pm 5.5b	25.5 \pm 5.2b	10.9 \pm 0.4a	0.71 \pm 0.03a
NE/KI \times KI	31.6 \pm 0.7bc	38.6 \pm 5.5ab	30.6 \pm 3.8b	23.8 \pm 3.6bc	22.4 \pm 0.8bc	0.57 \pm 0.04c
KC/KI \times NN	39.6 \pm 0.8bc	56.6 \pm 5.7c	32.3 \pm 4.2b	27.4 \pm 3.9a	15.2 \pm 0.4ab	0.71 \pm 0.01a
KC/KI \times NE	33.3 \pm 0.9bc	58.9 \pm 5.7c	38.6 \pm 5.5b	28.8 \pm 3.5a	25.3 \pm 0.6bc	0.67 \pm 0.01b
KC/KI \times KC	44.4 \pm 0.8c	62.5 \pm 5.7c	41.4 \pm 2.1b	29.2 \pm 2.2a	29.5 \pm 0.6c	0.73 \pm 0.01a
KC/KI \times KI	57.6 \pm 1.0c	65.0 \pm 5.7c	56.6 \pm 2.4a	36.5 \pm 2.5a	35.5 \pm 0.6cd	0.72 \pm 0.02a
<i>F</i>	39.7	2.5	2.4	2.9	123.8	6.4
<i>P</i>	0.0001	0.0322	0.040	0.028	0.0001	0.0120

Within column, means followed by the same lower case letter(s) are not significantly different ($P < 0.05$). Student–Newman–Keuls (SNK) test.

When KI males were crossed with other *C. sesamiae* populations, they produced both male and female progenies while the reciprocal crosses did not yield any female offspring. Ngi-Song et al. (1998), Ngi-Song and Mochiah (2001), and Mochiah et al. (2002a) also reported unidirectional incompatibility, caused by *Wolbachia* sp. infection, whereby crosses between males from the KC infected coastal population and females from the uninfected KI population did not yield any female offspring. Subsequent crosses carried out using progeny reared on an antibiotic-supplemented medium indicated that antibiotic treatment restored compatibility (Mochiah et al. 2002b). Bidirectional incompatibility also occurs when the two populations are infected with different *Wolbachia* variants (Stouthamer, Breeuwer, and Hurst 1999). Vavre, Fleury, Varaldi, Fouillet, and Bouletreau (2000, 2002) reported three examples of *Wolbachia*-mediated cytoplasmic incompatibility, resulting either in haploidisation of fertilised eggs, which develop into viable males, or in their death. The present findings strongly suggest that both Nigerian populations were infected with *Wolbachia* sp.

The results of the present study do not explain regional differences in the relative importance of this parasitoid in Africa. Ndemah et al. (2007) obtained *C. sesamiae* only from non-crop borers feeding on wild grasses though in earlier surveys, *C. sesamiae* was also obtained from two *B. fusca* larvae feeding on maize (Ndemah et al. 2001). It is suggested that in West Africa and Cameroon, the occasional *C. sesamiae* obtained from noctuids feeding on maize or sorghum is a result of a spillover of the parasitoid from the natural into the agricultural habitat. This opens the opportunity of introducing *C. sesamiae* populations from East Africa, which are adapted to cultivated habitats. Various populations collected in the different regions of Kenya are being released for control of *B. fusca* in Cameroon, which is neighbouring Nigeria. Successful populations will later be introduced into other West and Central African countries. Ngi-Song and Mochiah (2001) and Mochiah et al. (2002b) suggested that differences in mating compatibility is an important factor that should be considered when planning for biological control of stemborers with *C. sesamiae*. However, with gregarious parasitoid species like *C. sesamiae*, where the males emerge first and then mate with their siblings, and where pheromones appear to play a minor role in mate finding (Kimani and Overholt 1995), sib-mating would strongly dominate but to which extent is not known for *C. sesamiae*; in *Cotesia glomerata* (L.), for example, around 60% of the females breed with their brothers (Kitano and Tagawa 1981). However, the male *C. sesamiae* lives as long as, or, depending on the temperature, longer than the females (Mbapila and Overholt 2001) suggesting that after sib-mating the male is actively searching for females outside its brood. Furthermore, outcrossing could also occur in case of superparasitism, where the same larva is parasitised by two different female parasitoids. Thus, although interpopulation crossing in Cameroon is probably rare because of the scarcity of *C. sesamiae* (Ndemah et al. 2007), it is only prudent to examine cross-mating compatibility since there is no field evidence of the importance of outcrossing of *C. sesamiae*.

Mochiah et al. (2002a) using a simple recurrent equation model showed that the mixing of *Wolbachia* infected with uninfected populations could reduce population growth rates and recommends adjusting the population to be introduced to the infection status of the native population. However, Branca and Dupas (2006) using two modelling approaches, which, among others, also included population size

concluded that if a *Wolbachia*-free *C. sesamiae* population is introduced into an infected population, it may have reduced growth rate during the early stages of invasion, when its population is low, however high sib-mating would reduce this impact. However, this outcome may be affected by the existence of different variants of *Wolbachia*, which may cause bidirectional incompatibility. In collaboration with ICIPE, of the scientists Institut de Recherche pour le Développement, France, are currently doing a phylogenetic study of both *C. sesamiae* and *Wolbachia* in Africa. In this context, uni- and bidirectional incompatibility will be investigated.

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