Differences in mate acceptance and host plant recognition between wild and laboratory-reared *Busseola fusca* (Fuller)

P.-A. Calatayud[1], G. Juma[1], P. G. N. Njagi[2], N. Faure[3], S. Calatayud[1], S. Dupas[3], B. Le Rü[1], G. Magoma[4], J.-F. Silvain[3] & B. Frérot[5]

1 IRD, UR 072, c/o ICIPE, Nairobi, Kenya
2 ICIPE, Behavioural and Chemical Ecology Department, Nairobi, Kenya and Université Paris-Sudii, Orsay, France
3 IRD, UR 072, c/o CNRS, Laboratoire Evolution, Génomes et Spéciation, Gif-sur-Yvette, France and Université Paris-Sudii, Orsay, France
4 Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
5 INRA UMR 1272, PISC, Versailles, France

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Correspondence
P.-A. Calatayud (corresponding author), IRD, UR 072, c/o ICIPE, PO Box 30772-00100, Nairobi, Kenya. E-mail:pcalatayud@icipe.org

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Abstract

The present study was aimed at characterizing differences in mate acceptance and host plant recognition between *Busseola fusca* (Lep.: Noctuidae) reared for several generations under laboratory conditions and wild conspecifics, directly collected from maize stems in the field. The mating success was significantly higher in laboratory reared when compared with the wild *B. fusca* population. Oviposition on artificial stems was significantly higher for laboratory-reared insects than for the wild ones. Moreover, unlike adults of the wild strain, laboratory-reared *B. fusca* showed no preference to oviposit on surrogate stems impregnated with maize extracts. Long-range attraction to the host plant was significantly lower for laboratory-reared insects. Furthermore, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of mitochondrial DNA showed that the wild type and laboratory populations belonged to the same genetic strain, indicating that these differences between the populations were mainly phenotypic.

Introduction

Regular availability and adequate numbers of insects for experimental purposes can be easily obtained by mass rearing in the laboratory. However, rearing of insects under laboratory or artificial conditions for several generations can cause drastic changes both phenotypically and genotypically. Phenotypic changes generally occur in host plant acceptability/recognition, pheromonal responses and in reproductive parameters respectively (Tingey 1986; Masson et al. 1987). In addition, some insect species may lose their ability to successfully develop on their original host (Guthrie and Carter 1972). In other cases, they develop a tendency to accept plants outside their known natural host range (Schoonhoven 1967). Genetic alterations can include loss of genes, decreases in heterozygosity, and shifts in allele frequencies (Masson et al. 1987; Norris et al. 2001). The latter may be caused by random genetic drift, founder effects, and non-random mating and mate selection.

The stemborer, *Busseola fusca* (Fuller) (Lep.: Noctuidae), is an important pest of maize and sorghum in sub-Saharan Africa (Kfir et al. 2002). At the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya), *B. fusca* has been successfully reared under laboratory conditions for several generations on a meridic diet developed by Onyango and Ochieng'-Odero (1994). Wax papers rolled helicoidally to form cylindrical artificial stems were used as oviposition supports for the female moths.

A colony originating from specimens collected from maize in the field in Western province of Kenya in 1998 has been maintained at the Animal Rearing and Containment Unit (ARCU) of ICIPE.

In the present study, possible phenotypic changes in mate acceptance and host plant recognition in insects reared under laboratory conditions since 1998 were investigated by comparing them with wild insects collected in maize fields in the same area from which the original laboratory-reared insects had been collected.

To check if the wild population from which the laboratory-reared colony was initiated and maintained at ICIPE was similar to the wild population used for this study, a genetic assessment by comparing a fragment of the mitochondrial cytochrome *b* of the two populations was made. This assessment was based on the analysis of the population genetics of *B. fusca* done by Sezonlin et al. (2006).

Materials and Methods

Insects

For laboratory-reared population, pupae were directly provided by ARCU, from a colony that was initially started with about 200 individuals of both sexes, collected from maize stems in Western province of Kenya in 1998. To limit the risks of inbreeding about 200 new individuals (both males and females) collected from the same province were added to the colony thrice yearly (Onyango FO, ICIPE, personal communication). About five to six generations of *B. fusca* are produced in the insectary per year.

For the wild population used, fourth to fifth larval instars were collected from maize fields in the same region. They were then reared for 5–10 days on artificial diet described by Onyango and Ochieng'-Odero (1994) until pupa formation. Pupae were sexed and males and females kept separately in plastic boxes (21 cm long × 15 cm wide × 8 cm high) until adult emergence. A cotton pad moistened with water maintained relative humidity (RH) at >80% in the boxes. Insects were kept in a rearing room maintained at 25.9 ± 0.05°C, 58.5 ± 0.4% RH and a 12 : 12 h [light : dark (L : D)] reverse photoperiod with the scotophase lasting from 07:00 to 19:00 hours. This allowed all experiments to be carried out during day-time.

To obtain gravid females for each *B. fusca* population, the following procedure was followed. Minimum ten 1-day old females were released in a mosquito-net cage (40 × 40 × 63 cm) at the onset of scotophase. The first females started to call 6 h thereafter. Similar numbers of 1-day old males from the same population were introduced 1 h later. During the ensuing 1-h period, mating pairs were taken out of the cage and transferred individually into plastic containers (8 cm high, 5 cm diameter). At the end of copulation, the insects were separated and the females used on the following night for oviposition experiments.

Plants

Maize (*Zea mays* L., cv. 511) seeds were provided by Simlaw, Kenya Seed Company (Nairobi, Kenya). Plants were grown in a greenhouse at ICIPE in individual plastic pots (13 cm diameter, 12 cm high) containing peat. Mean temperatures were approximately 31/17°C (day/night) with a 12 : 12 h (L : D)photoperiod. The plants were used 3 weeks after planting, corresponding to the fifth to sixth leaf stage.

Mate acceptance

Mating and calling experiments were started at the onset of scotophase and stopped with the onset of photophase according to Calatayud et al. (2007). Except for the wind tunnel observations, all experiments were carried out under the same ambient conditions used in rearing of the insects.

Mating treatments

Naive virgin moths were used on the first night after emergence. Four possible female (F) : male (M) combinations of laboratory-reared (LR) and wild (W) populations were investigated (designated $F_{LR} \times M_{LR}$, $F_W \times M_W$, $F_{LR} \times M_W$, $F_W \times M_{LR}$ respectively) (table 1).

For each treatment, 32–38 pairs were used. Each pair was introduced into a transparent plastic jar (16 cm high, 9 cm diameter), containing a wet piece of cotton wool that maintained RH at around 80%. Three cylindrical surrogate stems made from three rectangular pieces of nylon cloth (15 long, 5 cm wide) rolled helicoidally from top to bottom were placed in each jar. These supports were found to elicit a good ovipositional response in *B. fusca* (Juma 2005). After four nights, the total number of eggs laid was counted. Thereafter, the female moths were dissected to assess the number of spermatophores in the *bursa copulatrix*, which in lepidopterans indicate the number of successful matings (Lum 1979).

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Table 1 Oviposition rates and spermatophore transfer in a no-choice test in laboratory-reared and wild *Busseola fusca* females

Mating treatments[1]	N	No. of eggs laid/female after 4 nights (means ± SE)[2]	No. of spermatophores/female after 4 nights (means ± SE)[2]	Percentage[3] of females with 0–3 spermatophore(s)			
				0	1	2	3
$F_{LR} \times M_{LR}$	38	155.9 ± 31.0 b	1.37 ± 0.14 c	15.8	39.5	36.8	7.9
$F_{W} \times M_{W}$	38	53.1 ± 21.2 a	0.32 ± 0.08 a	68.4	31.6	0.0	0.0
$F_{LR} \times M_{W}$	36	130.5 ± 36.6 a	0.61 ± 0.11 ab	50.0	38.9	11.1	0.0
$F_{W} \times M_{LR}$	32	170.5 ± 30.3 b	0.87 ± 0.12 b	31.2	50.0	18.8	0.0

[1]F, female; M, male; LR, laboratory-reared; W, wild population.

[2]Means in a column followed by the same letter are not significantly different at 5% level (Student–Newman–Keuls test).

[3]Chi-squared test results (contingency tables [2 × 6]): 0 spermatophore: d.f. = 3, $\chi^2 = 24.117$, $P < 0.0001$; 1 spermatophore: d.f. = 3, $\chi^2 = 2.477$, $P = 0.4794$; 2 spermatophores: d.f. = 3, $\chi^2 = 19.637$, $P = 0.0002$; 3 spermatophores: d.f. = 3, $\chi^2 = 8.546$, $P = 0.0360$.

Calling time of female moths

Female moths began calling by extruding the ovipositor, usually to its full length, and ceased calling by retracting it slowly. Calling was defined as 50% of full extension of the ovipositor. The calling females remained still with no wing vibrations (Calatayud et al. 2007). To determine the periodicity of the calling behaviour, naive females were placed in a mosquito-net cage (30 × 30 × 30 cm) the night succeeding emergence.

Calling postures were observed at hourly intervals for the two moth populations to determine the onset of calling. Calling observations were made under a red 80-W fluorescent tube.

Male response to sex pheromone

Attraction of *B. fusca* males to a synthetic sex pheromone blend was tested in a Plexiglass™ (Nairobi, Kenya) wind tunnel (184 cm long × 60 cm wide × 40 cm high). The wind tunnel was equipped with a fan that pushed air through the working section of the tunnel and an extractor on the opposite site of the tunnel extracted the air thereby generating an air flow of about 40 cm/s. Illumination was provided by a 40-W incandescent red light bulb mounted 70 cm above the midsection of the wind tunnel. The pheromone source was placed on a 27-cm-high metal platform (source platform) located 20 cm away from the upwind end of the tunnel. Males were released individually from the top of a 15-cm-high carton platform (release platform), 124 cm downwind from the source platform. The synthetic pheromone blend was formulated at the Institut National de la Recherche Agronomique (France) according to the published pheromone identification of *B. fusca* by Nesbitt et al. (1980). One rubber septum cap (Sigma-Aldrich, St Louis, MO, USA), impregnated with 1 mg mixture of the pheromone [(*Z*)-11-tetradecen-1-yl acetate, (*E*)-11-tetradecen-1-yl acetate and (*Z*)-9-tetradecen-1-yl acetate (69 : 17 : 14)] was placed vertically on the source platform, at least 1 h before start of the experiment. Males were then introduced into the dark wind tunnel room. The behaviour of the male moths was observed for 10 min. Behavioural activities recorded included time spent on the release platform, initiation of oriented flight (upwind zigzag flight towards the pheromone plume) and making contact with the pheromone source. The latency time taken by males before take-off and the time taken to fly and to contact the source were recorded. This experiment was carried out with 1-day old virgin males in room temperatures between 22°C and 25°C.

Host plant recognition for oviposition

As oviposition in *B. fusca* does not occur during photophase and start after onset of scotophase (Calatayud et al. 2007), all experiments started at the onset of scotophase and stopped with onset of photophase. After each experiment, the females were dissected to check the presence of spermatophore(s). Only females bearing spermatophore(s) were considered. Both *B. fusca* females and males can mate only once per night. Most of the females mate one time but some of them can mate two even three times over the course of their life. In general, the females oviposit the succeeding night after the first mating (Unnithan 1987; Unnithan and Paye 1990).

Short-range host plant recognition

Two-day-old gravid females were used. The experiments were carried out during the first three nights

succeeding the mating night because *B. fusca* females were found to lay 90% of the eggs during this period, and moths mortality occur after the fourth and fifth night (Juma 2005; Calatayud et al. 2007).

A two-choice experiment was set up using a maize plant and a helicoidally rolled rectangular piece of wax paper (60 cm long, 7 cm wide). This oviposition substrate is commonly used when rearing *B. fusca* (Onyango FO, ICIPE, personal communication). Females from each population were placed individually in mosquito-net cages (40 × 40 × 63 cm). The ovipositional response was recorded as the total number of eggs laid per female on each support after three nights and expressed as percentage of the total number of eggs laid per female on either substrate.

To determine the role of maize surface chemicals on oviposition response, females were placed individually in transparent plastic jars (16 cm high, 9 cm diameter, RH over 80%) and offered two aforementioned nylon surrogate stems (15 long, 5 cm wide): one stem was imbibed with 1 ml of chloroform (control) and another with 1 ml of maize chloroform extracts. Maize extracts were obtained following the method described by Derridj et al. (1996) for collecting waxes from the maize cuticle, which are known to stimulate oviposition of *B. fusca* (Juma 2005). Forty entire plants were collected around 18:00 hours and individually dipped into 500 ml of chloroform for 2 s. After filtration, the extract was then concentrated in rota-vapour (boiling point at 40°C, vacuum at 474 mbar) up to an equivalent of one plant per ml and stored at −20°C before use.

The total number of eggs laid on each substrate was recorded after 8 h of the first night after mating occurred, and expressed as percentage of the total number of eggs laid on either substrate. All experiments were carried out under the same ambient conditions as those used in rearing the insects.

Long-range host plant recognition

Attraction of *B. fusca* females towards maize plants was tested in the Plexiglas wind tunnel described above, under the same ambient conditions. Two pots each containing five maize plants were placed 20 cm from the upwind end of the tunnel. The observation began after placing a single gravid female moth (2 days old) on a 15-cm-high carton platform (release platform) located 124 cm downwind from the plants. After release, the behaviour of the female moth was observed for 10 min. Behavioural activities recorded included time spent on the release platform, initiation of oriented flight (upwind zigzag flight towards the plants) and making contact with the plants (landing on a plant and ovipositing). The latency time of females before take-off and the time to reach the source were also recorded. The experiment was carried out at room temperature between 22°C and 25°C.

Molecular analyses

For the laboratory-reared population, male and female adult moths were randomly sampled from the laboratory colony three times during the year of conducting the study. For the wild population, adult moths of the both sexes were also randomly sampled from the field each time they were required for experimental work. A total of 108 and 126 insects with the same male : female ratio from both laboratory-reared and wild populations, respectively, were analysed.

For each insect population, total DNA was extracted from insect thoraxes, using the DNeasy tissue kit (Qiagen GmbH, Hilden, Germany). The same protocol described by Sezonlin et al. (2006) was used with some modifications. A 1000 bp fragment of the gene encoding cytochrome *b* was amplified with the primers CP1 (modified from Harry et al. 1998): 5′GATGATGAAATTTTGGATC3′ and TRs (Simon et al. 1994): 5′TATTTCTTTATTATGTTTTCAAAAC3′. PCR cycling conditions were as follows: initial denaturation for 5 min at 94°C; 40 cycles at 94°C for 1 min, 46°C for 1.5 min elongation, 72°C for 1.5 min extension and a final extension period of 5 min at 72°C. The 20 μl reaction mixture contained 3 mM $MgCl_2$, 0.5 μM primers, 0.25 μM δNTPs and 100 ng of DNA plus 1 U of Promega *Taq* polymerase (Pleasanton, CA, USA). Amplified products were then digested by an enzymatic restriction method as follows. The 20 μl reaction mixture containing 100 μg/ml bovine serum albumin (BSA), 0.5 U promega *Xho*II restriction enzyme and amplified DNA was incubated for 4 h at 37°C and the digested fragments were separated on a 1.5% electrophoresis agarose gel.

Data analysis

The numbers, times and percentages were log $(x + 1)$ and arcsine transformed respectively. For multiple-mean comparisons, means were separated by Student–Newman–Keuls test when ANOVA was significant ($P < 0.05$). For two-mean comparisons, means were separated by Student's *t*-test. Linear regression was used to determine the relationship between the

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number of spermatophores per female and the number of eggs laid. Chi-squared test (contingency tables [2 × 2] and [2 × 6]) was used to compare the percentages.

In two-choice oviposition experiments, a Monte Carlo test procedure, based on 199 simulations (the number of simulations generally used for this test) based on the hypothesis of neutrality in preference between two oviposition supports for each individual, was used (Vaillant and Derridj 1992). For any individual i, a pair $[n_1(i), n_2(i)]$ was reduced to a single observation by considering the variate $d_i = n_1(i) - n_2(i)$ instead of the bivariate $[n_1(i), n_2(i)]$. Three criteria (C_1, C_2 and C_3) providing information on the tendency to prefer a given host were then calculated:

$$C_1 = (d_1 + d_2 + \cdots + d_N)/N,$$

$$C_2 = (|d_1| + |d_2| + \cdots + |d_N|)/N,$$

$$C_3 = m/N,$$

where m is the number of d_i's greater than zero.

The first criterion, C_1, represents the difference between the mean numbers corresponding to both choices. When the rank of the observed C_1 is very low or very high when compared with the simulated ones, a significant general preference can be assumed. The second criterion, C_2, is similar to C_1 but takes into account the absolute values of the d_i's. Criterion C_3 gives a crude idea of the similarity in preference as it corresponds to the proportion of individuals preferring one oviposition support. Strong preference for a single support for most of the insects tested is indicated when all the three criteria are significant. The statistics were carried out using software made freely available by Vaillant and Derridj (1992).

Differentiation between laboratory-reared and wild populations based on one locus analysis (mitochondrial cytochrome *b*) was tested by an exact test based on an unbiased estimate of the log-likelihood P-value, using the web version 3.4 of Genepop software (Raymond and Rousset 1995).

Results

Mate acceptance

Mating treatments

The number of eggs laid per mated female varied significantly between crosses (F = 9.275; d.f. = 3; P < 0.0001). Two groups were characterized based on Student–Newman–Keuls test, with the lowest number of eggs obtained with $F_W \times M_W$ and the highest number with $F_{LR} \times M_{LR}$ (table 1). Egg numbers increased when wild females were mated with laboratory-reared males.

The number of spermatophores per female also varied significantly between crosses (F = 14.454; d.f. = 3; P < 0.0001). The highest mean number of 1.37 spermatophores/female was obtained with $F_{LR} \times M_{LR}$ (table 1). The number of spermatophores per female decreased significantly when either of the sexes sourced from the wild population. In the $F_W \times M_W$ cross, about 68.4% of females had no spermatophore while 31.6% had one spermatophore, and none had been mated more than once. In contrast, in the $F_{LR} \times M_{LR}$ cross, 84.2% of females were successfully mated, with 36.8% and 7.9% of them having been mated twice and thrice respectively. However, the percentage of successfully mated females increased significantly to 68.8% when wild females were mated with laboratory-reared males (chi-squared result between $F_W \times M_W$ vs. $F_W \times M_{LR}$, contingency table [2 × 2]: d.f. = 1, $\chi^2 = 8.178$, P = 0.0042).

Regardless of the crosses, female moths that mated successfully laid more eggs when compared with unmated ones (d.f. = 142; t = 7.986; P < 0.0001). However, for mated females there was no significant relationship between the number of eggs laid and the number of spermatophores per female (n = 84; $R^2 = 0.001$; P = 0.746).

Calling time of female moths

The calling period of female moths varied significantly between the two *B. fusca* populations. The onset of calling occurred about 1 h earlier for laboratory reared than for the wild females. The calling time (hours) of laboratory-reared females was 6.2 ± 0.2 (n = 38) and was significantly lower when compared with the calling time of wild females, 7.2 ± 0.2 (n = 45) (means ± SE) (Student's *t*-test result: d.f. = 81; t = 3.031; P = 0.0033).

Male response to sex pheromone

There was no significant difference in the attraction of males towards the synthetic pheromone blend between the two *B. fusca* populations for any of the behavioural steps (fig. 1). The latency times before take-off as well as the times to contact the pheromone source were similar between the two moth populations. The latency times before take-off (seconds) were 76.2 ± 11.9 (n = 27) and 87.0 ± 9.7

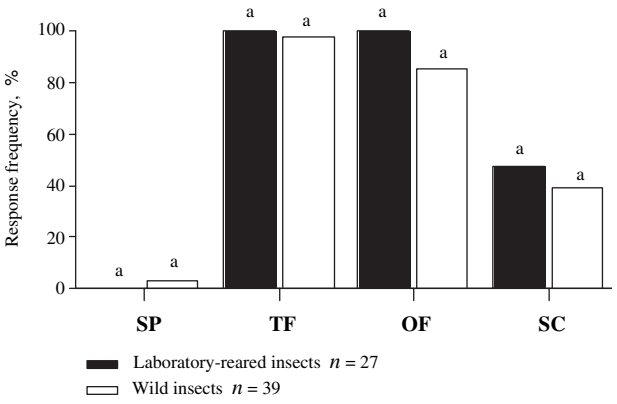

Fig. 1 Response of *Busseola fusca* males from laboratory-reared and wild populations responding to a synthetic pheromone blend in a wind tunnel. Bars with the same letter were not significantly different at 5% level (chi-squared test). Comparisons were made only within each behavioural step. SP, stay on the platform; TF, take flight; OF, orientation upwind towards the source; SC, source contact.

(n = 34) (means ± SE) for laboratory-reared and wild males respectively. The flight times (seconds) taken to contact the source after being release in the wind tunnel were 104.4 ± 29.9 (n = 13) and 133.8 ± 29.8 (n = 16) (means ± SE) for laboratory-reared and wild males respectively.

Host plant recognition for oviposition

Short-range host plant recognition

In two-choice tests, the wild female moths laid significantly more eggs on maize than on paper while there was no significant difference for laboratory-reared females (table 2).

The Monte Carlo procedure indicated a strong preference of most females in the wild population for maize; the values of the test criteria were $C_1 = 181.55$; $C_2 = 181.55$; $C_3 = 1.00$, with all values being significant ($P < 0.05$). In contrast, only a few laboratory-reared females showed preference for maize; the values of the test criteria were $C_1 = 88.15$; $C_2 = 129.45$; $C_3 = 0.60$, and only C_1 and C_2 were significant ($P < 0.05$).

Similarly, wild female moths laid significantly more eggs on surrogate stems imbibed with maize extracts than on those imbibed with solvent alone; there was no significant difference in case of laboratory-reared females (table 3).

Similarly, the Monte Carlo procedure indicated a strong preference of most wild females for stems imbibed with maize extracts. The values of the test criteria were $C_1 = -64.65$; $C_2 = 82.41$ and $C_3 = 0.29$. All these values were significant ($P < 0.05$). Again, few laboratory-reared females showed a preference for maize extracts; the values of the test criteria were $C_1 = -9.12$; $C_2 = 148.76$ and $C_3 = 0.53$. In this case, only C_1 and C_2 were significant ($P < 0.05$).

Long-range host plant recognition

In wind tunnel experiments, the percentages of females that oriented upwind towards the maize plants with subsequent plant contact (including landing and oviposition) were significantly higher for wild than laboratory-reared females (d.f. = 1,

Table 2 Oviposition on maize and paper surrogate stems by laboratory-reared and wild *Busseola fusca* females during three consecutive nights in a two-choice experiment

Population origin	Support	No. of eggs laid/female after 3 nights (mean ± SE)
Laboratory-reared insects, n = 20	Maize	153.9 ± 37.1 a (56.3%)
	Paper surrogate stem	65.7 ± 16.3 a (43.7%)
Wild insects, n = 20	Maize	199.2 ± 49.2 b (91.2%)
	Paper surrogate stem	17.7 ± 8.8 a (8.8%)

The values in parentheses represent the percentage of eggs laid per female on each support.
Means in a column followed by the same letter are not significantly different at 5% level (Student's *t*-test).

Table 3 Oviposition on nylon surrogate stems imbibed with plant surface extracts or solvent alone (=control) by laboratory-reared and wild *Busseola fusca* females 8 h after the onset of scotophase in a two-choice experiment

Population origin	Support	No. of eggs laid/female after 8 h of night (mean ± SE)
Laboratory-reared insects, n = 17	Maize extracts	122.6 ± 34.3 a (42.5%)
	Control	113.5 ± 26.3 a (57.5%)
Wild insects, n = 17	Maize extracts	122.5 ± 37.8 b (70.2%)
	Control	35.8 ± 10.6 a (36.2%)

The values in parentheses represent the percentage of eggs laid per female on each support.
Means in a column followed by the same letter are not significantly different at 5% level (Student's *t*-test).

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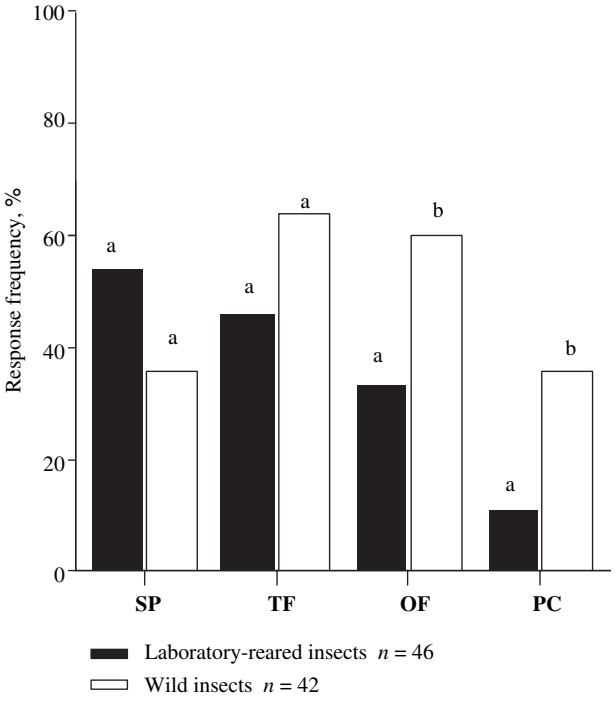

Fig. 2 Response of *Busseola fusca* females from laboratory-reared and wild populations responding to the maize plant in a wind tunnel. Bars with the same letter were not significantly different at 5% level (chi-squared test). Comparisons were made only within each behavioural step. SP, stay on the platform; TF, take flight; OF, orientation upwind towards the plants; PC, plant contact including landing on the plant and ovipositing.

$\chi^2 = 6.415$, P = 0.0113, and d.f. = 1, $\chi^2 = 7.716$, P = 0.0055 respectively) (fig. 2). However, there was no significant difference between the two populations for the percentage of females that stayed stationary on the release platform and those that took-off. The latency times before take-off as well as the times to contact the plant were similar between the two moth populations. The latency times before take-off (seconds) were 178.6 ± 37.5 (n = 15) and 160.3 ± 28.5 (n = 25) (means ± SE) for laboratory-reared and wild females respectively. The flight times (seconds) taken to reach the plant for oviposition after being release in the wind tunnel were 327.2 ± 31.3 (n = 5) and 314.1 ± 26.7 (n = 15) (means ± SE) for laboratory-reared and wild females respectively.

Molecular analyses

Molecular analyses performed on individuals from the two colonies, showed the presence of two mitochondrial clades for each populations, i.e. KI and KII, characterized by three DNA fragments (600, 300 and 100 bp) and two DNA fragments (880 and 100 bp) respectively (fig. 3). Individuals from KII were more abundant than those from KI regardless of the population source and sex. Moreover, the continuing addition of wild individuals into the laboratory-reared strain does not show any significant

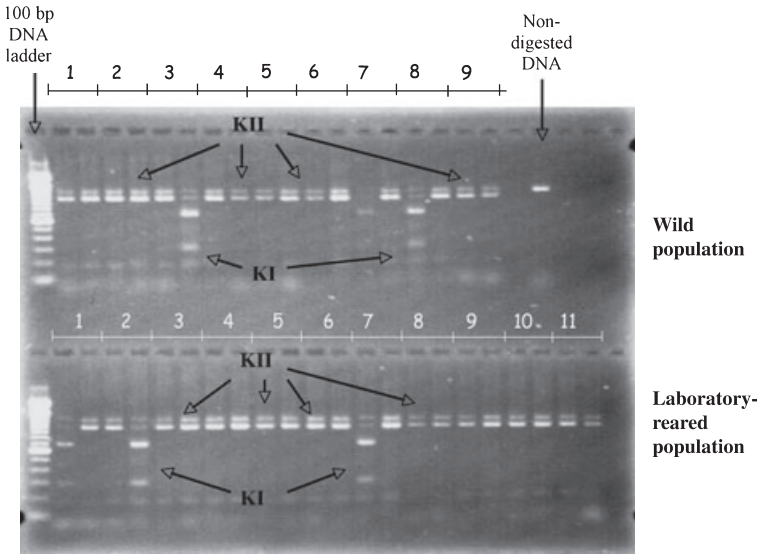

Fig. 3 A representative agarose gel electrophoresis plate of DNA products of several pairs of individuals collected randomly noted from one to nine or 11 (first lane = female, second lane = male for each number) of *Busseola fusca* from wild and laboratory-reared populations following PCR amplification of a cytochrome *b* DNA fragment. Results showed the presence of two mitochondrial clades labelled KI and KII.

drift in the region where the insects came from. They displayed the same variability of frequencies of KI and KII clades during the year of conducting the study, and therefore the data were pooled for each population for statistical analysis. There was no significant difference in the frequencies of KI and KII clades for either sex between the wild and laboratory-reared populations [P = 0.495, according to log-likelihood (*G*) based exact test].

Discussion

Phenotypic changes occurred when *B. fusca* is reared for several generations under laboratory conditions. The mating success for laboratory-reared moths was greater than for wild insects. Moreover, female moths, that successfully mated, generally laid a larger number of eggs as unmated ones. However, as shown by Calatayud et al. (2007), the number of eggs laid did not relate to the number of times a female mated.

This increased mating success for laboratory-reared insects has been previously reported in *Musca domestica* (L.) (Dipt.: Muscidae), *Heliothis virescens* (Fabricius) (Lep.: Noctuidae) and *Dacus oleae* (Gmelin) (Dipt.: Tephritidae) (Fye and Labrecque 1966; Raulston 1975; Economopoulos et al. 1976). *Busseola fusca* males and females exhibit a simple and rapid courtship behaviour without any particular characteristic behavioural events and, as shown by Frérot et al. (2006), males do not produce pheromones. These authors also observed that *B. fusca* females seem to select the males based on their landing behaviour and probably based on other cues (e.g. visual, acoustic) and they may refuse to copulate by either escaping or ceasing to call. Both wild and laboratory-reared males responded similarly to the pheromone blend in the wind tunnel. Thus, the low mating performance of males in the treatment $F_w \times M_w$ was not because of the inability of males to locate the female, but because of a rejection by the females or simply a lower performance of males to copulate successfully. The rearing condition (i.e. superior nutrition, lower stress conditions) may have enhanced the vigour of the males with subsequent increase in the mating success. Moreover, the high mating success for laboratory-reared insects could also indicate a loss of female choice in this population.

Female moths reared in the laboratory for several generations showed a reduced host plant recognition than wild insects, and most of them had partially lost their discriminatory acuity for the host plant in the presence of other oviposition substrate. The loss in host plant specificity/recognition was also reported by Schoonhoven (1967) for *Manduca sexta* (L.) (Lep.: Sphingidae), whereby laboratory-reared insects accepted host plants that were rejected by wild ones.

This is further supported by the results on the long-range host plant location behaviour, which indicate that insects reared under artificial conditions for a long time have reduced host-finding capacity. As suggested by Schoonhoven (1967) for laboratory-reared *M. sexta,* the lower percentage of laboratory-reared female moths that oriented toward the plants could be attributed to a change in their overall behaviour to another subset of very specific cues emanating from the host plant. This author hypothesized that such a behavioural modification could be attributed to a change in processing of the incoming chemosensory information by the central nervous system (CNS), a change in the information relayed by the chemoreceptors to the CNS, or a combination of both. Moreover, insects reared on artificial diet, which is superior to plants in quality and quantity, are generally heavier than wild insects (Shanower et al. 1993; Jiang N, ICIPE, personal communication). This may affect their flight ability as reported for *D. oleae* (Fletcher and Economopoulos 1976; Remund et al. 1977) and may also explain the lower percentage of laboratory-reared female moths that oriented and flew towards the plants.

The change in ovipositional response to plants for laboratory-reared insects may be explained by the selection pressure exerted through mass rearing that allowed selection of individuals with low discriminatory ability. A similar phenomenon has been reported in laboratory-reared codling moth, *Cydia pomonella* (Lep.: Tortricidae), for which rearing for only one generation was sufficient for selection of such a population (Witzgall et al. 2005).

Based on the analyses of the mitochondrial cytochrome *b* DNA, wild and laboratory-reared population of *B. fusca* were found to be genetically identical, indicating they belonged to the same mitochondrial genetic strain. However, this does not rule out natural selection on nuclear genes. Even if the laboratory-reared population continuously received wild individuals, at least three generations of each newly introduced individuals were maintained under laboratory conditions. Therefore, it is probable that a genetic change might have occurred in the laboratory strain as a result of non-random mating and selection, which consist mostly in a reduction of allele frequency. Numerous cases have documented

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such genetic change in laboratory insects (Nei et al. 1975; Hedrick et al. 1976; Pashley et al. 1985; Pashley 1986; Mukhopadhyay et al. 1997; Norris et al. 2001).

In conclusion, although artificially reared insects may be readily available in adequate quantities for research work, our study showed that these insects undergo phenotypic changes with regard to host plant recognition and acceptability. Experimental results generated using these insects may therefore not be a true reflection of the attributes of the species in the wild. This is particularly the case for studies aimed at investigating the role of plant chemistry on host plant location and selection for oviposition in *B. fusca*.

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