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Source: International Journal of Insect Science, 8(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/IJIS.S32481

Relationships of Reproductive Traits With the Phylogeny of the African Noctuid Stem Borers

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ABSTRACT: The display of the reproductive behavior in most noctuid Lepidoptera follows a diel periodicity and is limited to a precise period of either the day or the night. These behavioral traits and the sex pheromone chemistry can be species specific and thus might be linked to the phylogeny. The objective of this study was to test the relationship of these reproductive traits with phylogeny. The study was undertaken using eight closely related species of noctuid stem borers, which are easy to rear under artificial conditions, namely, *Busseola fusca*, *B. nairobica*, *B.* sp. nr. *segeta*, *Manga melanodonta*, *M.* sp. nr. *nubifera*, *Pirateolea piscator*, *Sesamia calamistis*, and *S. nonagrioides*. For each species, the adult emergence period, the mating time, and the oviposition period were estimated, referred as biological traits. The components of the sex pheromones emitted by the females of each species were also analyzed by gas chromatography–mass spectrometry. Among the biological traits measured, only those linked to the oviposition pattern (timing and egg loads per night) were significantly correlated with the phylogeny of these species. For the sex pheromone components, among the 13 components identified in all species, only four, namely, Z9-tetradecenyl acetate (Z9-TDA), Z11-TDA, E11-TDA, and Z11-hexadecenyl acetate (Z11-HDA), showed the highest significant correlations with the phylogeny. These results suggest that among the different reproductive traits evaluated, only few are phylogenetically constrained. Their involvement in the reinforcement of ecological speciation in noctuid stem borers is discussed.

KEYWORDS: Lepidoptera, adult emergence period, mating time, oviposition period, sex pheromone chemicals, Z9-tetradecenyl acetate (Z9-TDA), Z11-tetradecenyl acetate (Z11-TDA), E11-tetradecenyl acetate (E11-TDA), Z11-hexadecenyl acetate (Z11-HDA)

CITATION: Calatayud et al. Relationships of Reproductive Traits With the Phylogeny of the African Noctuid Stem Borers. *International Journal of Insect Science* 2016:8 95–103 doi:10.4137/IJIS.S32481.

TYPE: Original Research

RECEIVED: July 1, 2016. **RESUBMITTED:** September 13, 2016. **ACCEPTED FOR PUBLICATION:** September 13, 2016.

ACADEMIC EDITOR: Takumasa Kondo, Editorial Board member

PEER REVIEW: One peer reviewer contributed to the peer review report. The reviewer's report totaled 207 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Provenance: the authors were invited to submit this paper.

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Introduction

Reproductive activities in Lepidoptera follow a diel periodicity and are limited to a precise period of either the day or the night. They can differ among species and somehow can show some species' specificity. Most of the noctuid moths live for a few days and display a diel periodicity in emergence time, calling behavior, and oviposition period; this determines the onset of reproductive activities. These traits can affect the reproductive isolation between population variants feeding on different host plants as larvae, but sharing habitats as adults at their reproductive stage, and they can therefore be involved in the reinforcement of ecological speciation.

In addition to these behavioral parameters, the sex pheromone composition both within and between species is involved in the reproductive isolation of Lepidoptera species.[1,2] In Lepidoptera, sex pheromone compositions are mostly species specific and the pheromone evolution processes can support systematic positions,[3–8] and participate in the complex processes of reproduction and gene flow[9] by driving mate localization, recognition, and acceptance. Chemical cues play an essential role in specific mate recognition systems[10] and they maintain the reproductive isolation between closely related species. The reproductive isolation, preventing interbreeding between different species, is at the origin of the "biological species" concept developed by Mayr in 1942.[11] Sex pheromones consist of a precise blend of compounds with different chemical structures, and the resulting chemical signature of a species induces in the conspecific partner of the opposite sex a specific behavior.[12] Between closely related species, related or identical molecules are used, and the chemical signature is the result of different ratios of the same compounds. Males are very sensitive to both the ratio and the total amount of the pheromone components.[13,14] For the distinction of closely related species, sex pheromone chemistry can be used in addition to temporal patterns to confirm phylogeny. Such information is essential for all studies in chemical ecology.

Lepidopteran maize stem borers (including noctuids, pyralids, and crambids) are the most important pests in

sub-Saharan Africa.[15] Among the noctuids, *Busseola fusca* and six *Sesamia* spp. (including *S. calamistis* and *S. nonagrioides*) are considered economically important. Although information on the reproductive biology of *B. fusca*, *S. calamistis*, and *S. nonagrioides* is, eg, available,[16,17] there is none about the temporal pattern of the major events constituting the reproductive behavior in other related noctuid stem borer species such as *B.* sp. nr *segeta*, which have been recently reported from cultivated plants in East Africa.[18] This study investigates the timing of adult emergence, mating time, and oviposition period, as well as the sex pheromone compositions, of eight closely related species of noctuid stem borers feeding on Poaceae species in Eastern Africa. Furthermore, the phylogenetic signal of these temporal patterns and sex pheromone compositions were tested by phylogenetic linear regression tests using a phylogenetic Bayesian tree assembled on these noctuid species.

Materials and Methods

Lepidoptera species used. The following eight species of noctuid stem borers were used in this study:

- *Busseola fusca* (Fuller 1901) is an oligophagous species found on plant species belonging to the Poaceae family, including maize and *Sorghum* spp.[19–22] This species is common in all areas of sub-Saharan Africa. The *B. fusca* larvae were collected from stems of maize in Machakos (Eastern Kenya) (01°50′S, 37°26′E, 1665 m).
- *Busseola nairobica* Le Ru, recently identified by Félix et al,[8] is a specialist species mainly found on broadleaf panicum (*Panicum deustum* Thunb. 1794).[19–21,23] This species is known mainly from the eastern part of the Oriental Rift Valley in South Kenya and from Tanzania neighboring the Kenyan border. The larvae used were collected from stems of *P. deustum* Thunb. 1794 in the Ngong Forest (Nairobi, Kenya, 01°52′S, 37°24′E, 1754 m).
- *Busseola* sp. nr. *segeta* larvae were collected from *Pennisetum purpureum* Schumach. in Kakamega (Western Kenya, 00°22′N, 34°53′E, 1430 m). This insect is an undescribed species whose description is under way (Le Ru, B., personal communication). It is an oligophagous species found on plant species belonging to the Poaceae family including maize and *Sorghum* spp.[19–21,23] This species is known from the Democratic Republic of Congo, Uganda, Kenya, and Ethiopia, and it is an important pest of maize in the Kakamega region.[22,24]
- *Manga melanodonta* (Hampson 1910) is a specialist species; the larvae were collected from the stems of *Setaria megaphylla* (Steudel) Th. Durand & Schinz in Kisii town (Western Kenya: 00°39′S, 34°43′E, 1535 m).
- *Manga* sp. nr. *nubifera* is a specialist species; larvae were collected from stems of *Megathyrsus infestus* (Andersson) B. K. Simon & S. W. L. Jacobs (Little Guinea) in Mtito Andei (Eastern Kenya: 02°40′S, 38°11′E, 739 m).
- *Pirateolea piscator* (Fletcher) is a polyphagous species found on a large number of plant species from Poaceae and Cyperaceae families; larvae were collected from *P. purpureum* in Gatamayu forest (Kenya: 01°03′S, 36°43′E, 2297 m). This insect is also found on maize and sugarcane in different localities of Kenya;[23]
- *Sesamia calamistis* Hampson is a polyphagous species found on a relatively large number of plant species from different families such as Poaceae, Cyperaceae, and Typhaceae.[19–21] This species is common in sub-Saharan Africa. The specimens used in this study were sourced from the stems of *Sorghum bicolor* (L.) Moench in Kisumu (Western Kenya: 00°35′N, 34°27′E, 1283 m).
- *Sesamia nonagrioides* Lefebvre is polyphagous, found on a large number of plant species from Poaceae, Cyperaceae, and Typhaceae families.[19–21] This species is known from West, Central, and East Africa, as well as the Palearctic Region from Western Europe and from North Africa to Iran. The *S. nonagrioides* larvae were collected from the stems of *Phragmites mauritianus* Kunth in the Victoria Lake region (Western Kenya: 00°48′S, 34°29′E, 1148 m).

Another Lepidoptera species, *Brithys crini* F. (Apameina), having a semi-boring habit and collected from the stems of *Amaryllis* sp. (Amaryllidaceaea) in Nairobi, was used as the outgroup for phylogenetic analysis.

All insects collected in the field were maintained in the laboratory for two-to-three generations at 24.4 ± 0.03°C and 58.4 ± 0.2% Relative Humidity (means ± standard errors [SEs]) on an artificial diet developed by Onyango and Ochieng'-Odero.[25] The pupae and adults were maintained at 25.3 ± 0.9°C and 68.6 ± 12.8% h.r. (means ± SEs) and 12 hours light: 12 hours darkness cycle. One room was kept under a reversed photoperiod, with the scotophase lasting from 7.00 to 19.00 h, herewith referred to as night, and another under normal conditions with the photophase lasting from 7.00 hours to 19.00 hours. This allowed all observations to be carried out during the day time. For the reversed photoperiod, the observations were made using a red 80 W fluorescent tube as a light source.

Pupae were sexed and, males and females were kept separately until emergence in a plastic box (30 cm length, 12 cm width, and 10 cm height) for molecular analyses, experiments, and sex pheromone collection.

Molecular data sets. For all species, the DNA was extracted from the hind legs of the adult, experienced to be the muscle-rich part of the insect, using a Macherey-Nagel NucleoSpin® tissue kit (Macherey-Nagel GmbH & Co. KG). Polymerase chain reaction (PCR) amplifications were conducted for three mitochondrial genes (Table 1), *cytochrome oxidase c subunit I* (*COI*), *cytochrome b* (*Cytb*), and *12s rRNA* (*12S*). Two nuclear regions were also sequenced, *elongation factor 1-α* (*EF-1α*) and *28s rRNA* (*28S*). The PCR reactions were conducted using Promega GoTaq Flexi DNA

Table 1. Primers PCR conditions and Accession Number (AN) of the genes used for the phylogenetic study.

GENES	PRIMERS[1]	FRAGMENT SIZE (bp)	ANNEALING TEMPERATURE °C	AN *Busseola fusca*	AN *Busseola nairobica*	AN *Busseola* sp. nr. *segeta*	AN *Manga melanodonta*	AN *Manga* sp. nr. *nubifera*	AN *Pirateolea piscator*	AN *Sesamia calamistis*	AN *Sesamia nonagrioides*	AN *Brithys crini*
COI	F: HCO.2198 R: LCO.1490	658	60	KP019363	KP019365	KP019362	KX055400	KX055402	KX055403	KP019364	KP019367	KX055401
EF-1α	F: M46-1 R: rcM4	983	60	KP019375	KP019377	KP019374	KX055413	KX055415	KX055416	KP019376	KP019378	KX055414
Cytb	F: CP1 R: Tser	433	48	KX055405	KX055409	KX055410	KX055404	KX055411	KX055412	KX055408	KX055407	KX055406
28S	F: 28S01 R: 28SR01	834	60	KX055392	KX055396	KX055397	KX055391	KX055398	KX055399	KX055395	KX055394	KX055393
12S	F: SR-J-14233 R: SR-N-14588	359	56	KX055383	KX055387	KX055388	KX055382	KX055389	KX055390	KX055386	KX055385	KX055384

Notes: [1]HCO.2198: TAAACTTCAGGGTGACCAAAAAATCA from Folmer et al;[26] LCO.1490: GGTCAACAAATCATA AAGATATTGG from Folmer et al;[26] M46-1: CAGGAAACAGCTATGACCGAGGAAATYAARAAGGAAG from Cho et al;[27] rcM4: TGTAAAACGACGGCCAGTACAGCYACKGTYCTC ATRTC from Cho et al;[27] CP1: GATGATGAAATTTTGGATC modified from Harry et al;[28] Tser: TATTTCTTTATTATGTTTTCAAAAC from Simon et al;[29] 28S01: GAC TAC CCC CTG AAT TTA AGC AT from Choong-Gon et al;[30] 28SR01: GAC TCC TTG GTC CGT GTT TCA AG from Choong-Gon et al;[30] SR-J-14233: AAG AGC GAC GGG CGA TGT GT from Simon et al;[29] SR-N-14588: AAA CTA GGA TTA GAT ACC CTA TTA T from Simon et al.[29]

Polymerase with 1× Buffer, 3 mM $MgCl_2$, 0.4 mM deoxynucleoside triphosphates (dNTPs), 0.4 µM of each primer, 1 U DNA Polymerase, and ~10 ng of DNA. The conditions were five minutes at 95°C, followed by 40 cycles of one minute at 95°C, one minute 30 seconds at 60°C (Table 1 for annealing temperature for each gene), and one minute at 72°C, with a final cycle of five minutes at 72°C. Resulting PCR products were processed by the French sequencing center Genoscope using a BigDye v3.1 sequencing kit and Applied 3730 × l sequencers. Sequences were aligned manually with Mesquite 2.75 (available at: www.mesquiteproject.org). Accession numbers are given in Table 1.

Phylogenetic analyses. The data set consisted of a total of eight Lepidoptera stem borer species using 3267 bp (*COI* + *EF-1α* + *Cytb* + *28S* + *12S*). The software Partition Finder[31] was used to determine the best subset of partitions. The tested partitions were based on the different genes and on codon positions for coding genes. The best-fit model of substitution for each partition was determined using the Bayesian information criterion. The phylogenetic relationships were estimated with Bayesian inference using the program MrBayes v3.2.1.[32] The run consisted of two independent analyses with the following settings: four Markov chains of 20 million generations, random starting trees, default priors, and trees sampled every 100 generations. A burn-in period of four million generations was used. Node support was estimated by clade posterior probability.

Temporal patterns measured.

Adult emergence period. For each species and each sex, emergence was recorded hourly from 7.00 hours to 19.00 hours in both experimental rooms through the aforementioned plastic box. The experiment was run for 10 consecutive nights and days.

Mating time. To determine the mating time, one-day-old females were placed with one-day-old males in a mosquito net cage (30 × 30 × 30 cm). Mating postures were observed at hourly intervals. Because no mating was observed during the photophase, the experiments started at the onset of the scotophase and ended at the onset of the photophase.

Oviposition period. None of the females laid eggs the night it mated. Thus, all the females used for determining the temporal pattern of oviposition had mated the night before the observations started.

Gravid females were placed individually in transparent plastic jars (10 cm diameter × 20 cm height) together with an oviposition substrate consisting of a wax paper cut rectangularly (15 cm × 6 cm) and rolled helicoidally from top to bottom to form a cylindrical surrogate stem.[33] No eggs were laid on surrogate stems during the photophase. Thus, oviposition experiments started at the onset of the scotophase and ended with the onset of the photophase.

The oviposition time was estimated by monitoring at hourly intervals during the first two nights after mating. The surrogate stems were renewed every hour.

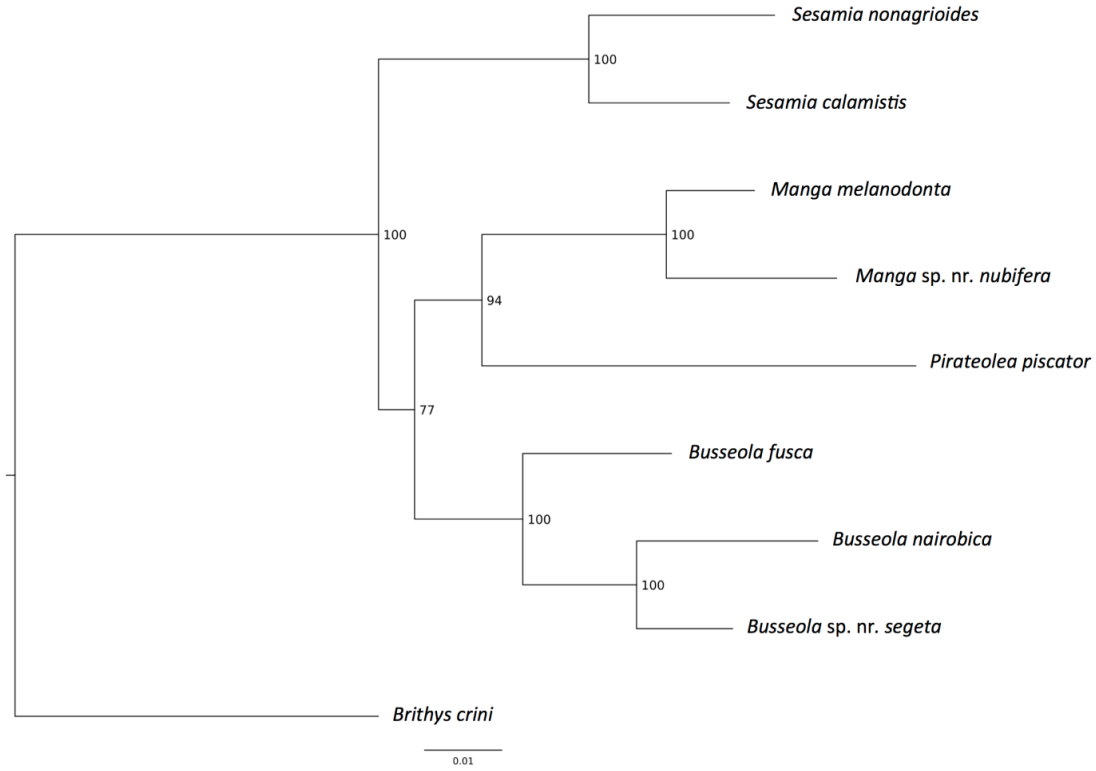

Figure 1. Phylogenetic Bayesian tree using 1896 bp (*COI* + *EF-1α*). Posterior probabilities are given at nodes. *Brithys crini* is used as the outgroup.

In a separate experiment, to evaluate the oviposition rate per night, the number of eggs was counted each night over five successive nights, renewing the surrogate stem each night.

After each experiment, each female was dissected to verify the presence of spermatophore(s) in the *bursa copulatrix*, indicating that they were mated. Only females with spermatophore(s) were included in the analysis.

Sex pheromone analysis. For all Lepidoptera species, the pheromone glands of calling virgin females were extruded with a thin forceps, and the gland tegument area was gently rubbed for five minutes with the adsorbent part of a Carbowax™–Divinylbenzene (Supelco) 65 µm solid-phase microextraction (SPME) fiber assembly.[34] The SPME collection of each female was analyzed by gas chromatography (GC) using a Varian 3400 CX gas chromatograph with a split–spitless injector heated at 240°C and a flame ionization detector detector at 260°C. The fiber was subjected to thermal desorption in the injector. A 30 m long ~0.32 mm internal diameter (i.d.) Rtx®-Wax column (Restek®, France) was used with the oven temperature being ramped from 60°C to 100°C at 15°C/min, then to 245°C at 5°C/min. Helium was the carrier gas (15 psi). GC–mass spectrometry (GC-MS) analyses were carried out on a Varian ion trap Saturn II mass spectrometer coupled to a 3400 CX gas chromatograph. The MS conditions were as following: electron impact (IE) mode, 70 eV, 40–330 amu. The GC injector split–splitless was held at 250°C, and the RTX-5 Sil-MS (Restek®), 30 m ~0.32 mm i.d. column temperature ramped from 50°C to 300°C at 8°C/min. Pheromone compounds were identified by comparing retention times and mass spectra of SPME samples collected from the gland with synthetic reference compounds. For each Lepidoptera species, at least three females were analyzed (n = 3). For *B. fusca*, *Busseola* sp. nr. *Segeta*, and *B. nairobica*, the values were extracted from Félix et al.[8]

Statistical analyses. To test the phylogenetic signal for temporal pattern parameters and sex pheromone compounds, a phylogenetic Bayesian tree was assembled based on the Lepidoptera species used in this study (Fig. 1). The data of this phylogenic tree were then transformed to an ultrametric form and a multivariate phylogenetic comparative method ('mvMORPH' package R of Clavel et al[35]) was used to test the phylogenetic inertia of the different variables evaluated (ie, the temporal pattern parameters, including the pheromone components). This method allows the testing of the phylogenetic signal using various statistics; Abouheif's C_{mean}, Pagel's λ (considered the most conservative test by Mûnkenmûller et al[36]), Moran's *I*, and Blomberg's *K* regression tests.[35]

Results

The phylogenetic analysis confirmed the monophyly of the genera, *Sesamia*, *Manga*, *Pirateolea*, and *Busseola*, as well as of each species within its respective genus (Fig. 1).

The different statistics of phylogenetic inertia produced congruent results (Table 2). Pagel's λ was significant only for oviposition time and the number of eggs during the second night, as well as for several pheromone components: tetradecenol

Table 2. P-values and significance of the different regression tests used to evidence the phylogenetic relatedness on the different parameters evaluated including the pheromone components (according to Clavel et al[35]).

PARAMETERS	ABOUHEIF'S C_{MEAN} TEST P-VALUE		PAGEL'S λ P-VALUE		MORAN'S *I* P-VALUE		BLOMBERG'S *K* P-VALUE	
Day-emerg. (female)	0.367	NS	1.000	NS	0.360	NS	0.487	NS
Day-emerg. (male)	0.293	NS	0.706	NS	0.271	NS	0.255	NS
Night-emerg. (male)	0.330	NS	1.000	NS	0.324	NS	0.328	NS
Night-emerg. (female)	0.236	NS	1.000	NS	0.202	NS	0.310	NS
Mating time	0.984	NS	1.000	NS	0.913	NS	0.985	NS
Oviposition time	0.096	NS	0.047	*	0.052	NS	0.011	*
Nbre eggs (1st night)	0.001	**	0.099	NS	0.011	*	0.006	**
Nbre eggs (2nd night)	0.001	**	0.034	*	0.002	**	0.012	*
TDol	0.384	NS	<0.0001	***	0.090	NS	0.138	NS
Z9-TDol	0.494	NS	<0.0001	***	0.076	NS	0.110	NS
Z11-TDol	0.063	*	0.384	NS	0.498	NS	0.129	NS
TDA	0.521	NS	1.000	NS	1.000	NS	0.642	NS
Z9-TDA	0.007	**	0.010	*	0.498	NS	0.034	*
E9-TDA	0.048	*	0.276	NS	0.489	NS	0.070	NS
Z11-TDA	0.021	*	<0.0001	***	0.019	*	0.011	*
E11-TDA	0.014	*	0.007	**	0.235	NS	0.016	*
Z11 HDol	0.085	NS	1.000	NS	0.498	NS	0.317	NS
HDA	0.105	NS	1.000	NS	0.106	NS	0.466	NS
Z9-HDA	0.384	NS	0.0002	***	1.000	NS	0.127	NS
Z11-HDA	0.007	**	<0.0001	***	0.371	NS	0.004	**
Z11-Hdal	0.250	NS	1.000	NS	0.275	NS	0.495	NS

Notes: *P < 0.05; **P < 0.01; ***P < 0.001.
Abbreviation: NS, non significant.

(TDol), Z9-TDol, Z9-tetradecenyl acetate (Z9-TDA), Z11-TDA, E11-TDA, Z9-hexadecenyl acetate (Z9-HDA), and Z11-HDA. However, TDol, Z9-TDol, and Z9-HDA were not significant for the other regression tests. The number of eggs during the first night was not significant for Pagel's λ but significant for all other tests (Morans' *I*, Abouheif's C_{mean}, Blomberg's *K*). Oviposition time was not significant for Abouheif's C_{mean} and Morans' *I* but significant for the two other tests. None of the other variables was significant for any of the tests used (Table 2). The oviposition time was shortest for the *Busseola* genus, highest in *S. calamistis* and *P. piscator*, and intermediate for the *Manga* genus (Fig. 2). *Sesamia* spp. oviposited significantly more eggs during the two nights following the mating night compared to the other genera (Fig. 3). By contrast, species of the *Manga* genus oviposited significantly less than the others.

For all species, males and females emerged at the last two hours of the photophase period and at the first 2–4 hours of the night (Fig. 4). In the context of the mating time, some species such as *Busseola* sp. nr. *segeta* mated early, while others such as *Manga* sp. nr. *nubifera* and *B. nairobica* mated later compared to the other species (Fig. 2), but this was not linked to phylogeny (Table 2).

For all stem borer species, 13 sex pheromone components were identified, and only four of them, Z9-TDA, Z11-TDA, E11-TDA, and Z11-HDA were significantly correlated with the phylogeny (validation by most of the regression tests used; Table 2). Z9-TDA was more abundant in the *Manga* genus and in *P. piscator* than in the *Sesamia* and *Busseola* genera. Z11-TDA and E11-TDA were mostly present in *Busseola* genus. Z11-HDA was more abundant in *Sesamia* genus, less in *Busseola* genus, and at the lowest level in *Manga* genus (Table 3). For the other pheromone components, no phylogenetic signal could be evidenced.

Discussion

As also reported for other noctuids, for all species used in this study, both males and females started to emerge at the last hours of the photophase period and emerged mostly at the beginning of the night.[17,37–39] As reported by the authors of these studies, light duration and transition play an important role in promoting the emergence of moths.

In many insects, there is a general tendency for males to emerge before the females, which is known as protandry. This was only observed for *B. nairobi*, whereby, during the night, the males emerged before the females (Fig. 4). By contrast,

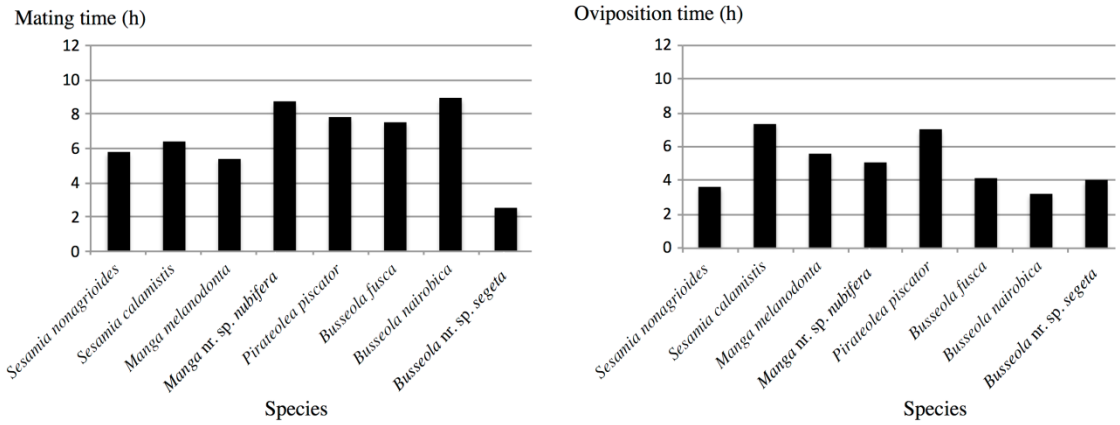

Figure 2. Mean mating times (left) (n = 20–50) and oviposition times (right) (n = 15–30) of different Lepidoptera species.

for *M. melanodonta* and *P. piscator*, the females emerged earlier during the night than the males. For the other stem borer species, even if the males emerged shortly before the females at the end of the days, they did not do so at the onset of night. The absence of protandry was already reported in *B. fusca* by Ratnadass et al[38] and Calatayud et al.[17]

Among the moth species that emerged earlier at night, such as *S. nonagrioides*, *S. calamistis*, *M. melanodonta*, *M. nubifera*, *P. piscator*, and *B. fusca*, mating took place shortly after emergence, whereas for the moths emerging later (such as *B. nairobica* and *Busseola* sp. nr. *Segeta*), mating occurred mostly the night following the emergence night (P.-A. Calatayud, personal observation). For most of the species, a minimum time before mating (similar to a minimum time for the adult's maturation) is required by the female moth,[17] indicating a synchrony between calling behavior and pheromone production in Lepidoptera.[37,40,41] Readiness to mate increases the efficiency of reproduction in insects with a short life span. No significant correlation was found between the mating time and phylogeny. However, this biological parameter was expected to be highly species specific. It was specifically earliest for *Busseola* sp. nr. *segeta* and latest for *Manga* sp. nr. *nubifera* and *B. nairobica* as compared to the other species. This parameter might evolve fast and should therefore not be correlated with the phylogeny.

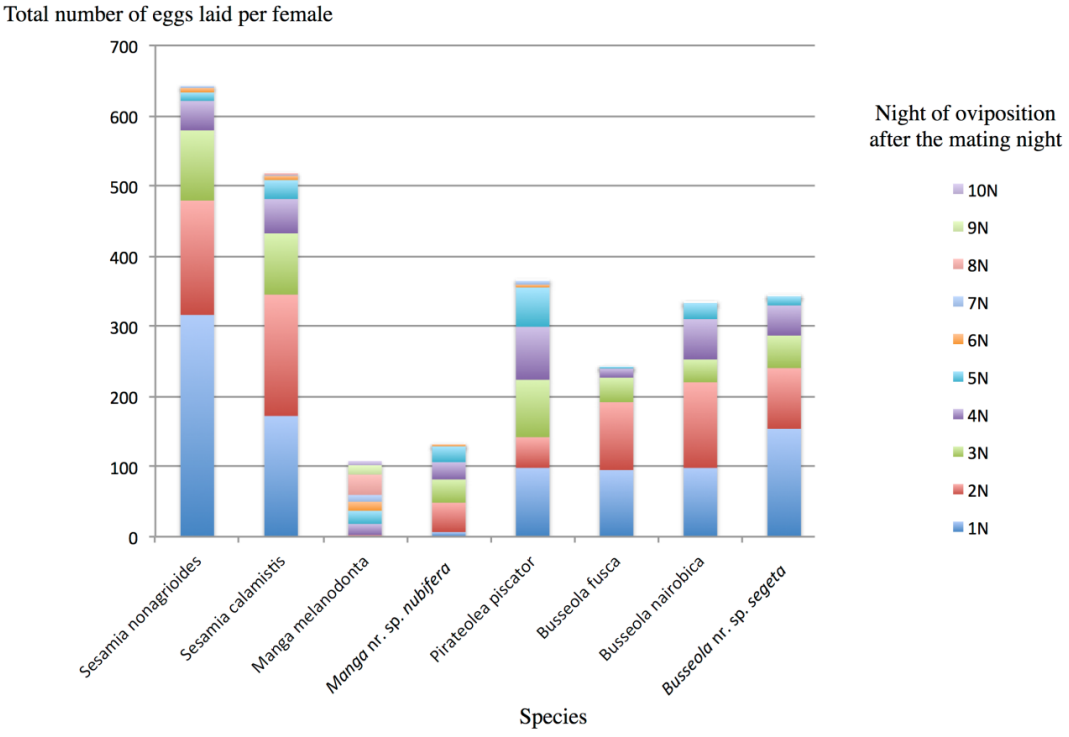

Figure 3. Mean total number of eggs laid per female (n = 10–20) of different Lepidoptera species during several nights (1N, 2N … 10N: first night, second night … tenth night) after the night of mating.

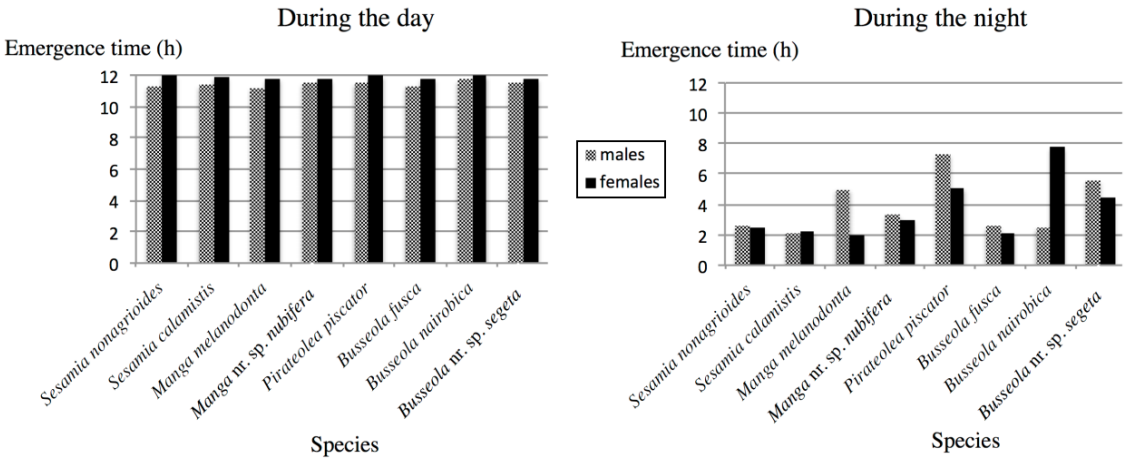

Figure 4. Mean emergence times (n = 20–70) of males and females during the day (left) and during the night (right) of different Lepidoptera species.

By contrast, the parameters linked to the oviposition (timing and egg load per night) were significantly correlated with phylogeny. Oviposition in the species used in this study occurred on the night after the mating night. No information is available on the place where moths mate in the wild, but the oviposition delay can have physiological origins and it gives the insect time to find a suitable host. These aspects might be species specific and are correlated with the phylogeny. Similar to other noctuid species such as *Heliothis zea* (Boddie) and *Spodoptera ornithogalli* Guenée[42] and already reported for *B. fusca*,[17] oviposition peaked the first three nights after the mating night and then rapidly decreased. This was particularly true for *Sesamia* spp. but less for species in the *Manga* genus, corroborating the significant correlation of this parameter with phylogeny.

Table 3. Mean ratios (in %, n = 3) of pheromone components identified in the pheromone glands of the different Lepidoptera species used in this study.

PHEROMONE COMPONENTS	*Busseola fusca*	*Busseola nairobica*	*Busseola* sp. nr. *segeta*	*Manga melanodonta*	*Manga* sp. nr. *nubifera*	*Pirateolea picator*	*Sesamia calamistis*	*Sesamia nonagrioides*
Tetradecanol (TDol)						2		
Z9-tetradecenol (Z9 TDol)						21		
Z11-tetradecenol (Z11 TDol)		5	10					
Tetradecenyl acetate (TDA)				8		6	6	
Z9-tetradecenyl acetate (Z9-TDA)	5	5	5	69	85	65	19	
E9 tetradecenyl acetate (E9-TDA)				19.8	9			
Z11-tetradecenyl acetate (Z11-TDA)	67	61	61		0.7			
E11-tetradecenyl acetate (E11-TDA)	16	20	15					
Z11-hexadecenol (Z11 HDol)						0.58	5	
Hexadecanyl acetate (HDA)							5	
Z9-hexadecenyl acetate (Z9-HDA)						5.42		
Z11-hexadecenyl acetate (Z11-HDA)	11	10	10	3.2	3.1		65	63.3
Z11-hexadecenal (Z11-Hdal)								36.7

The results on sex pheromone components indicated that all the noctuid species exhibited C14 and C16 acetate components in their sex pheromones. The presence of both C16 and C14 components shows that these species share the same biosynthesis pathway, namely, oxidizing (ie, double bond appearance by loss of hydrogen) C16 compounds into C14 compounds. Among the 13 components identified, only four, namely, Z9-TDA, Z11-TDA, E11-TDA, and Z11-HDA, were mostly correlated with the phylogeny of the stem borer moth species studied. These pheromone components were shown to be present in a large number of Lepidoptera species, including several families such as Crambidae, Gelechiidae, Noctuidae, Pieridae, Pyralidae, Tortricidae, and Yponomeutidae.[43] However, some differences in their abundances occur between Lepidoptera families. For example, E11-TDA is more frequently found in Tortricidae and Z9-TDA is more represented in Noctuidae whereas E11-TDA is more rare (B. Frérot, personal communication).

Conclusion

Some traits measured in this study contribute to premating isolation and reinforcement of reproductive isolation in stem borer noctuids. Accelerated evolution of those traits can occur for host races that differ in their host specialization as larvae, but that share a habitat as adults. Host races would pay a postmating fitness cost in the absence of premating reproductive barrier.[44] Pheromone components and mating time are generally good candidates for premating selected traits in host race formation. Other traits such as emergence time are more constrained by environmental adaptation. They are more likely to be under conservative selection and exhibit phylogenetic inertia. Our study indicated that most pheromone components (nine out of 13) as well as mating time do not exhibit any phylogenetic signal. This might be due to a rapid evolution of these traits. As discussed, traits linked to oviposition showed phylogenetic signal, suggesting that inertia can be due to conservative selection. Alternatively, the absence of a phylogenetic signal can be due to the little power of the test when little variation is present on the trait. This is the case for emergence time characterizing the noctuid family and for the majority of pheromone components (particularly true when the component was detected in a single or in two insect species only). Therefore, although it is difficult to conclude on the validation of the hypothesis that all traits evaluated in this study are under diversifying selection related to speciation reinforcement, some of them—such as oviposition time, egg loads per night, and four pheromone components (Z9-TDA, Z11-TDA, E11-TDA, and Z11-HDA)—are better candidates to contribute to such diversifying selection.

Acknowledgment

The authors thank Fritz Schulthess for his critical review of the manuscript and English corrections.

Author Contributions

Conceived and designed the experiments: P-AC. Analyzed the pheromone components: BF and GG. Conducted the experiments: P-AC and PA. Conducted the molecular analyses and the phylogeny: CC-D. Analyzed the data: SD. Wrote the first draft of the manuscript: P-AC. Contributed to the writing of the manuscript: P-AC, SD, BF, CC-D, and BLR. All authors reviewed and approved of the final manuscript.

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