Differences in oviposition behaviour of two sympatric sibling species of the genus Ostrinia

T. Malausa¹²*, B. Péllié¹, V. Piveteau¹, C. PéliSSier¹, D. Bourguet² and S. Ponsard¹

¹Laboratoire Dynamique de la Biodiversité, Université P. Sabatier – Toulouse III, UMR CNRS 5172, 31 062 Toulouse Cedex 09, France; ²Centre de Biologie et de Gestion des Populations (CBGP), UMR INRA-IRD-SupAgró-CIRAD, Institut National de la Recherche Agronomique, Campus International de Baillarguet, 34 988 Montferrier/Lez, France

Abstract

Changes in host preferences are thought to be a major source of genetic divergence between phytophagous insect taxa. In western Europe, two sympatric taxa, O. nubilalis (the European corn borer) and O. scapulalis, feed mainly on maize and hop or mugwort, respectively. These two species may have diverged without geographic isolation after a host shift of ancestral populations onto maize or another cultivated species (e.g. sorghum). A previous study using inbred laboratory strains revealed that the two species differ in their oviposition choices in maize-mugwort tests. We sampled four natural populations in France (two of each taxon) and tested their oviposition behaviour toward four of their main host plant species: maize, sorghum, mugwort and hop. O. nubilalis females showed a very high preference for laying their eggmasses on maize, whereas O. scapulalis females displayed a more balanced range of preferences. O. nubilalis females were attracted slightly to sorghum, suggesting that this plant is an accidental, rather than a regular and ancestral host plant of O. nubilalis. One important result arising from this study is the significant proportion of eggs laid by both Ostrinia species on hop. This may explain why some stands of hop are sometimes not only infested by O. scapulalis but also by O. nubilalis larvae, a situation preventing assortative mating based on microallopatry. Hence, further studies must be conducted to see whether the host preference in the genus Ostrinia might be linked to assortative mating by a mechanism that is not mediated by the host plant.

Keywords: sympatric speciation, ecological speciation, Ostrinia nubilalis, Ostrinia scapulalis, European corn borer, host race, host preference

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Introduction

Host plant choice profoundly shapes the evolution of phytophagous insect taxa. Indeed, comparisons of plant and insect phylogenies suggest that the diversification of vascular plants played a key role in the unprecedented diversification that resulted in insects, most of which are phytophagous, being by far the most species-rich order of multicellular organisms nowadays (Mitter et al., 1988; Ward et al., 2003). Most phytophagous insects feed on only one or a small number of plant species; and many apparently polyphagous taxa have, upon closer examination, been found to be divided into host races or sibling species, i.e.

*Author for correspondence:
Fax: +33 492 386 401
E-mail: thibaut.malausa@sophia.inra.fr
into sympatric, partially interfertile but genetically differentiated taxa that use different host plants (Drès & Mallet, 2002). The fact that such taxa might represent different steps of ecological and sympatric speciation (Schluter, 2001; Via, 2001; Rundle & Nosil, 2005) has fostered interest for the mechanisms that initiate, maintain or enhance their genetic differentiation (Funk et al., 2002).

Host preference plays a central role in such differentiation by being or becoming associated with traits involved in host performance and/or in assortative mating (Bush, 1975, 1994; Berlocher & Feder, 2002; Drès & Mallet, 2002; Rundle & Nosil, 2005). Association with host performance traits (i.e. traits involved in the ability to survive, grow and mature on a given plant species) favours divergent selection because it ensures that offspring of groups that have undergone different selection pressures are in turn exposed to different selection pressures similar to those experienced by their respective parents. This enables natural selection to steadily act over successive generations, on a consistent set of traits contributing to host adaptation. In addition, host preference can be, or become, associated with the second major ingredient of ecological speciation: reproductive isolation. Such isolation can be the ‘by-product’ of the two lineages adapting to their respective hosts’ spatial distribution or phenology and therefore, mating in different places or different times; or it can be determined by loci not involved in host adaptation, but happening to be genetically linked to host preference loci.

Strong host preferences have been reported in most of the extensively documented host races: Rhagoletis pomonella (Prokopy et al., 1988; Feder et al., 1994), Eurosta solidaginis (Craig et al., 1993, 2001), Zeiraphera diniana (Bovey & Maksymov, 1959; Emelianov et al., 2001, 2003), or Acyrthosiphon pisum (Via & Hawthorne, 2002), all of which have a small (2–4) number of host plants. Most attributes of taxa diverging mainly under ecological selective pressures have also been documented recently in the genus Ostrinia (Lepidoptera: Crambidae). This genus has the interesting property of being extremely polyphagous, e.g. more than 220 host plant species belonging to more than 40 different families for the European corn borer (ECB), Ostrinia nubilalis Hübner (review in Ponsard et al., 2007). Another interesting feature in this genus is the presence of several reproductively isolated taxa infesting maize on the one hand and sorghum on the other (Hu¨bner (review in Ponsard et al., 2007)).

Material and methods

Sampling and plant preparation

Four Ostrinia sp. populations were sampled during the winter 2004–2005, two (Muret and Viabugn) from maize and two (Amiens and Lille) from mugwort (fig. 1). These collection sites are located in France, in areas where O. nubilalis and O. scapulalis occur at different degrees of sympatry. Indeed, whereas populations of these two sibling species co-occur at substantial densities around Paris (e.g. Viabugn and Amiens), populations of O. nubilalis and O. scapulalis are very scarce in northern (around Lille) and southern (around Toulouse) France, respectively. Collecting individuals in the latter areas, in sites where no host plant of the other species was observed over several kilometres, insured that pure populations of each species were collected. Based on the results of Bourguet et al. (2000), Martel et al. (2003), Thomas et al. (2003), Pélouzoelu et al. (2004), Bontemps et al. (2004) and Malausa et al. (2005), we hypothesized that populations collected on mugwort and maize in these areas consisted exclusively of O. nubilalis and O. scapulalis individuals, respectively. Indeed, these authors found that, all over France, Ostrinia females exclusively use the E (when originating from mugwort) or the Z (when originating from maize) sex pheromone blends, suggesting that these two host plants house pure populations of O. nubilalis (on maize) and O. scapulalis (on mugwort). For each population, ~1200 diapausing larvae were collected and maintained at 4°C.
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Two days before its second use. It received ‘blanket’ watering through a hose at least once a day, if not twice, as the weather was extremely hot. This might have reduced the possible influence of putative marking pheromones. As phenology can have a significant impact on host plants attraction (Patch, 1942; Derridj et al., 1989; Lupoli et al., 1990; Spangler & Calvin, 2000), experiments were performed using plants at similar development stages. Maize, sorghum and hop plants were 5, 5–6 and 5–7 weeks old, respectively. The exact age of mugwort plants could not be estimated, but all plants were 70–110 cm high. Finally, host plant combinations were tested in random order over the course of the experiment in order to avoid any relation between time period and host plant species involved in the tests.

For each plant, the total surface available for oviposition was estimated by the following protocol. For each host species, a set of 20–30 plants was randomly chosen. For each plant, all leaves were scanned and their overall surface was estimated by automatically counting the pixels of the resulting pictures using the ImageJ 1.33u software (Abramoff et al., 2004). In addition, the dry weight of the leaf set corresponding to each of a sub-sample of 20–30 plants was recorded for each host species. The XLSTAT 2006.3 program was used to test the fit of linear regression models with leaf dry weight and overall leaf surface as the explanatory and dependant variables, respectively. The regression coefficient of determination ($r^2$) between dry weight and leaf surface were 0.80 (n = 30), 0.47 (n = 29) and 0.89 (n = 28) for maize, hop and sorghum, respectively. Corresponding mean absolute percentage errors (MAPE) of leaf surface estimates were 13.5%, 15.6% and 10.9%, respectively. For these three host plants, we therefore used the regression equation to infer the overall leaf surface of each plant used in the experiment from its dry weight. For mugwort, $r^2$ was lower, 0.33 (n = 23), and the mean absolute percentage error was 34.7%, so that we decided to scan all plants used in the experiments rather than inferring their leaf surfaces from their dry weights. The total plant surface available for oviposition was the sum of the overall leaf surface and the stem surface (circumference at mid-height \times plant height), except for hop, where the stems were very thin and therefore of negligible surface when compared to leaves (<5%). The number of eggmasses on a plant species X after correction with plant surface ratios ($E_{corrX}$) was calculated using equation (1).

$$ E_{corrX} = \frac{S_m}{S_X} \times E_{X} \times \left( \frac{E_1 + E_2}{S_m/S_1 \times E_1 + S_m/S_2 \times E_2} \right) $$

where $S_m$ is the mean surface of both species of plants, $S_1$ and $S_2$ are the surfaces of plants from species 1 and species 2, respectively, and $E_1$ and $E_2$ are the uncorrected number of eggmasses on plant species 1 and 2, respectively.

Data analysis

Proportions of eggmasses laid on each host plant

The proportion of eggmasses, rather than the proportion of eggs, was used as an indicator of host preference. Indeed, both *Ostrinia* species lay their eggs in clusters of ~20 eggs, so that individual eggs cannot be considered as the results of repeated, independent choices. For each host plant combination, we used the GENMOD procedure of SAS (1989) with a
binomial distribution of the residuals and a type 3 analysis to test for the influence of the factors ‘Ostrinia species’ and ‘population (Ostrinia species)’ on the average proportions of eggmasses (corrected or not by the leaf surfaces of the plants) laid on the two host plants available in the cage. A Chi-square test was used to determine whether these proportions were significantly different from 0.5, the proportion expected if females randomly oviposited on the two available host plants.

Size of the eggmasses

Differences between populations and sibling species were further examined by comparing the size of their eggmasses, measured as the number of eggs per eggmass. This analysis was performed on a dataset in which the experimental unit was the host plant individual, i.e. one of the four plants introduced into the cages. We used the MIXED procedure of SAS with repeated measures to test for the influence of the factors ‘Ostrinia species’, ‘population (Ostrinia species)’ and ‘plant species’, as well as the interactions ‘Ostrinia species’ × ‘plant species’ and ‘population (Ostrinia species)’ × ‘plant species’, on the variable ‘number of eggs per eggmass’. ‘Experiment number’ (from 1 to 192) was considered as a random effect in the model. Each eggmass laid by females on a plant was treated as a repeated measure of the number of eggs per eggmass on the corresponding plant. The chosen covariance structure in the REPEATED statement was ‘compound symmetry’ (option ‘/type = CS’). Each plant introduced into a given cage was, thus, considered as subject in the REPEATED statement of the mixed procedure. Comparisons between sibling species and/or populations were performed with the LSMEANS statement and the PDIFF option.

Total number of eggs laid

We examined possible differences in the total number of eggs laid in each host plant combination, i.e. the total number of eggs of all eggmasses collected on the four plants of any given cage. The total number of eggs rather than number of eggmasses was used because we considered that it better estimated the energetic investment of females in oviposition on each plant. Since differences in early female mortality could bias the results by affecting the number of eggs laid over the duration of the experiments, this analysis was carried out on a dataset excluding replicates in which less than two out of three females were alive at the end of the experiment. The GENMOD procedure of SAS, with a normal distribution of the residuals, was used to test for the influence of factors ‘Ostrinia species’, ‘population (Ostrinia species)’, and ‘plant species combination’ on the total number of eggs laid by females. In order to determine whether the plant surface influenced the number of eggs laid, we added ‘plant surface’ as a covariable to the model. The LSMEANS option was used to compare the total number of eggs among all combinations of the three explanatory variables. Bonferroni corrections were applied to all pairwise tests.

Results

Proportions of eggmasses laid on each host plant

In all but one combination of host plants, the proportion of eggmasses laid on each plant was strongly influenced by the factor ‘Ostrinia species’, whether or not this proportion was corrected by the available plant surface (Chi² > 15.64, df = 1, P < 10⁻³ for all tests except for the hop-sorghum combination where Chi² = 4.22, df = 1, P = 0.040 when the proportion was not corrected by plant surface). The only plant combination for which the proportion of eggmasses laid on each host plant was not influenced by the ‘Ostrinia species’ factor was maize–sorghum (Chi² = 0.31, df = 1, P = 0.577 and Chi² = 1.17, df = 1, P = 0.279 with uncorrected and corrected proportions, respectively). A significant influence of the factor ‘population (Ostrinia species)’ was detected in two plant combinations: mugwort–hop (Chi² = 20.83, P = 10⁻³ with both corrected or uncorrected proportions); and mugwort–sorghum (Chi² = 9.92, df = 2, P = 0.007 with corrected proportions but Chi² = 1.01, df = 2, P = 0.604 with uncorrected proportions). LSMEANS pairwise comparisons revealed that the ‘population (Ostrinia species)’ factor only had an influence on the proportion of eggmasses laid on each plant in O. nubilalis.

The proportions, whether or not corrected by the proportion of available plant surfaces, of eggmasses laid by each population on the two types of plants used in the different combinations are given in table 1. When cages contained both maize and mugwort, the two sibling species displayed antagonistic preferences; O. nubilalis females laid >95% of their eggmasses on maize, whereas O. scapulalis females laded 61% (or >70% after correction) of their eggmasses on mugwort. The preference of both Ostrinia species was high enough to significantly reject the null hypothesis that females displayed no oviposition preference, i.e. that they laid 50% of their eggmasses on each plant (P < 10⁻³ for all populations). Although less marked, results for the maize–hop combination displayed the same trend; O. nubilalis females laid between 75 and 85% of their eggmasses on maize, these proportions being significantly (P < 10⁻³) higher than 50% for both populations. Although O. scapulalis females were still less attracted by maize than by hop, the proportions of eggmasses laid on each plant did not significantly differ from 50% (P = 0.359 and P = 0.188 for Lille and Amiens, respectively), unless they were corrected by the relative plant surface available for oviposition (P < 10⁻³ for both populations).

When given the choice between maize and sorghum, all populations strongly, if not exclusively, chose to oviposit on maize, resulting once again in the rejection of the 50% null hypothesis (P < 10⁻³ for all populations for both corrected and uncorrected proportions). In cages containing hop and mugwort, females of both Ostrinia species showed a marked preference for hop; whether corrected or not, the proportion of eggmasses laid on this plant was always significantly higher than 50% (P < 10⁻³) and varied from 75 to 97%. The mugwort–sorghum combination showed that O. scapulalis had a clear and substantial preference for mugwort, as the proportions (corrected or not) were always >75% and significantly different from 50% (P < 10⁻³). Conversely, this combination was the only one not revealing any clear preference in O. nubilalis females. The proportions of eggmasses laid on these two plants were not significantly different from 50% (P > 0.094), except for the Muret population and only after taking available plant surface into consideration (P = 0.049). The last plant combination (hop–sorghum) again revealed very strong preferences. Both O. nubilalis and O. scapulalis preferred to oviposit on hop; the departure from 50% was highly significant for the four Ostrinia populations (P < 10⁻³). This trend was slightly
Table 1. Proportions of eggmasses (corrected or not by the relative amount of available plant surface) and mean numbers of eggs laid during tests involving the various host plant combinations. The 95% confidence intervals (CI) were calculated using the modified Wald method (Agresti & Coull, 1998).

| Host plant combination | Ostrinia species | Population | Proportion of eggmasses laid on host plant #1 | Number of eggs |
|------------------------|-----------------|------------|---------------------------------------------|---------------|
|                        |                 |            | Uncorrected by available plant surface | Corrected by available plant surface |               |
|                        |                 |            | Mean | 95% CI | Mean | 95% CI | Mean | SD |
| Maize Mugwort          | O. scapulalis   | Lille      | 0.39* | 0.32–0.46 | 0.26* | 0.20–0.33 | 590 | 171.3 |
|                        |                 | Amiens     | 0.39* | 0.32–0.46 | 0.27* | 0.21–0.33 | 710 | 310.7 |
|                        | O. nubilalis    | Muret      | 0.98* | 0.96–0.99 | 0.97* | 0.95–0.98 | 1,004 | 293.4 |
|                        |                 | Viabugn    | 0.98* | 0.96–0.99 | 0.95* | 0.92–0.97 | 827 | 211.7 |
| Maize Hop              | O. scapulalis   | Lille      | 0.47  | 0.40–0.54 | 0.36* | 0.30–0.43 | 591 | 137.8 |
|                        |                 | Amiens     | 0.45  | 0.38–0.52 | 0.35* | 0.28–0.43 | 417 | 192.0 |
|                        | O. nubilalis    | Muret      | 0.85* | 0.81–0.89 | 0.80* | 0.76–0.84 | 713 | 204.9 |
|                        |                 | Viabugn    | 0.80* | 0.75–0.83 | 0.75* | 0.70–0.81 | 756 | 193.0 |
| Maize Sorghum          | O. scapulalis   | Lille      | 0.96* | 0.91–0.98 | 0.91* | 0.85–0.94 | 420 | 277.5 |
|                        |                 | Amiens     | 0.96* | 0.91–0.98 | 0.92* | 0.86–0.96 | 652 | 120.2 |
|                        | O. nubilalis    | Muret      | 0.96* | 0.94–0.98 | 0.92* | 0.89–0.95 | 973 | 467.1 |
|                        |                 | Viabugn    | 0.97* | 0.93–0.99 | 0.95* | 0.90–0.98 | 469 | 177.5 |
| Mugwort Hop            | O. scapulalis   | Lille      | 0.18* | 0.14–0.23 | 0.25* | 0.20–0.30 | 451 | 206.6 |
|                        |                 | Amiens     | 0.17* | 0.13–0.23 | 0.21* | 0.16–0.26 | 570 | 356.2 |
|                        | O. nubilalis    | Muret      | 0.03* | 0.02–0.06 | 0.05* | 0.03–0.08 | 686 | 344.5 |
|                        |                 | Viabugn    | 0.13* | 0.10–0.18 | 0.18* | 0.14–0.23 | 474 | 378.0 |
| Mugwort Sorghum        | O. scapulalis   | Lille      | 0.83* | 0.78–0.87 | 0.86* | 0.82–0.90 | 739 | 458.2 |
|                        |                 | Amiens     | 0.79* | 0.71–0.85 | 0.79* | 0.71–0.85 | 329 | 213.8 |
|                        | O. nubilalis    | Muret      | 0.51  | 0.40–0.62 | 0.39* | 0.29–0.50 | 251 | 182.3 |
|                        |                 | Viabugn    | 0.53  | 0.41–0.64 | 0.60  | 0.48–0.71 | 205 | 113.0 |
| Hop Sorghum            | O. scapulalis   | Lille      | 0.93* | 0.87–0.96 | 0.96* | 0.91–0.98 | 406 | 97.6 |
|                        |                 | Amiens     | 0.91* | 0.84–0.96 | 0.93* | 0.85–0.96 | 364 | 199.1 |
|                        | O. nubilalis    | Muret      | 0.90* | 0.85–0.93 | 0.84* | 0.78–0.88 | 528 | 371.0 |
|                        |                 | Viabugn    | 0.84* | 0.78–0.88 | 0.86* | 0.80–0.90 | 585 | 188.6 |

* indicates that the proportion was significantly different from 50%.
stronger in O. scapulalis (with a proportion on hop >90%) than in O. nubilalis (with a proportion on hop varying from 80 to 90%).

**Size of the eggmasses**

Both ‘plant species’ and the ‘plant species × Ostrinia species’ interaction had a significant effect on the number of eggs per eggmass ($F = 15.55$, df = 3, $P < 10^{-3}$ and $F = 6.68$, df = 3, $P < 10^{-3}$, respectively). Conversely, the size of the eggmasses was not influenced by ‘Ostrinia species’, ‘population (Ostrinia species)’, or by the ‘plant species × population (Ostrinia species)’ interaction ($F = 0.12$, df = 1, $P = 0.734$; $F = 1.42$, df = 2, $P = 0.241$; and $F = 0.77$, df = 6, $P = 0.594$, respectively).

The mean sizes of eggmasses laid by each population on the various host plants are given in Table 2. On average, the eggmasses laid by the females of the two Ostrinia species contained ~20 eggs. For O. nubilalis, these eggmasses were significantly smaller when laid on maize and significantly larger when laid on sorghum ($P < 10^{-4}$ and $P = 0.028$, respectively).

**Discussion**

Both O. nubilalis populations showed a high preference for maize (75–98%), while populations of O. scapulalis showed a significant preference (61–83%) for hop and mugwort over maize and sorghum. These results are consistent with those reported by Nagy (1976), Vilkova & Frolov (1978), Manojlovic (1984), Savinelli et al. (1988), Frolov (1991) and Bethenod et al. (2005) on other European populations or strains of Ostrinia. Hence, populations of O. nubilalis and O. scapulalis (sensu Frolov et al., 2007) display consistent host preferences in France and eastern Europe. Overall, results obtained on oviposition in the genus Ostrinia point to a high preference of O. nubilalis for maize and a more balanced range of preferences for O. scapulalis, the latter species being strongly attracted by mugwort, hemp and hop. It is noteworthy that these preferences are of the same order of magnitude (70–100% of eggmasses on native hosts) as those recorded between host races and sibling species of other phytophagous insects: Nilaparvata lugens (Sezer & Butlin, 1998), E. solidaginis (Craig et al., 2001), Galerucella nymphaeae (Pappers et al., 2002) and Z. diniana (Bovey & Maksymov, 1959). The magnitude of these preferences was also similar to those recorded by Prokopy et al. (1988) on R. pomonella in non-choice experiments.

Because sorghum and maize share a very similar morphology and a C4-type photosynthesis and because sorghum has been repeatedly recorded as an ECB host plant in several parts of the world (the former Soviet Union (Frolov et al.,

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**Table 2. Mean sizes (mean number of eggs) and standard deviations (SD) of the eggmasses laid by each population of each Ostrinia sibling species on the various host plants.**

| Host plant | Sibling species | Population | Number of eggs per eggmass |
|------------|----------------|------------|-----------------------------|
|            |                |            | Mean | SD |
| Maize      | O. scapulalis  | Lille      | 23.5 | 1.2 |
|            |                | Amiens     | 21.8 | 1.3 |
|            |                | Muret      | 19.0 | 0.9 |
|            |                | Viabugn    | 18.3 | 0.9 |
| Mugwort    | O. scapulalis  | Lille      | 20.6 | 1.2 |
|            |                | Amiens     | 19.8 | 1.3 |
|            |                | Muret      | 16.5 | 2.0 |
|            |                | Viabugn    | 19.4 | 1.8 |
| Hop        | O. scapulalis  | Lille      | 16.1 | 1.0 |
|            |                | Amiens     | 15.7 | 1.1 |
|            |                | Muret      | 16.4 | 1.0 |
|            |                | Viabugn    | 16.8 | 1.1 |
| Sorghum    | O. scapulalis  | Lille      | 19.3 | 2.1 |
|            |                | Amiens     | 19.6 | 2.3 |
|            |                | Muret      | 21.7 | 1.8 |
|            |                | Viabugn    | 26.3 | 1.9 |
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non-host-plant patches (Showers et al., 1976). In addition, allochrony caused by developmental lags between the different plants seems limited (Thomas et al., 2003) or absent (Malausa et al., 2005), at least in the surroundings of Paris. If further studies were to confirm that the host preference in the genus Ostrinia is linked to assortative mating by a mechanism that is not mediated by the host plant, comparative studies with host races or sibling species, where this appears to be the case, could provide interesting insights.

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