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### ► To cite this version:

Marion Orsucci, Philippe Audiot, Alexandra Pommier, Christophe Raynaud, Beatrice Ramora, et al.. Host specialization involving attraction, avoidance and performance, in two phytophagous moth species. *Journal of Evolutionary Biology*, 2016, 29 (1), pp.114-125. 10.1111/jeb.12766 . hal-02631296

**HAL Id: hal-02631296**

**<https://hal.science/hal-02631296>**

Submitted on 2 Sep 2020

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# Host specialization involving attraction, avoidance and performance, in two phytophagous moth species

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## Keywords:

attraction;  
avoidance;  
host specialization;  
performance;  
phytophagous insect;  
preference.

## Abstract

Host specialization plays a key role in the extreme diversification of phytophagous insects. Whereas proximate mechanisms of specialization have been studied extensively, their consequences for species divergence remain unclear. Preference for, and performance on hosts are thought to be a major source of divergence in phytophagous insects. We assessed these major components of specialization in two moth species, the European corn borer (ECB) and the Adzuki bean borer (ABB), by testing their oviposition behaviour in different conditions (choice or no-choice set-ups) and their performances, by reciprocal transplant at the larval stage on the usual host and an alternative host plant. We demonstrated that both ABB and ECB have a strong preference for their host plants for oviposition, but that relative larval performances on the usual host and an alternative host differed according to the experiment and the trait considered (weight or survival). Finally, we show for the first time that the preference for maize in ECB conceals a strong avoidance of mugwort. The differences in performance, attraction and avoidance between ECB and ABB are discussed in the light of the underlying mechanisms and divergence process.

## Introduction

Ecological specialization is the process by which an organism adapts to a restricted subset of possible environments. One of the most striking features of phytophagous insects is their general tendency to specialize on a small range of host plants (Jaenike, 1990; Bernays & Chapman, 1994; Rasmann & Agrawal, 2011). By facilitating or even triggering speciation, this specialization may have played a significant role in the extreme diversification of phytophagous insects observed today (Futuyma & Moreno, 1988; Via & Hawthorne, 2002; Forister *et al.*, 2012). The proximate mechanisms of specialization, such as divergent selection, habitat choice

and habitat-specific performances, have been the subject of extensive empirical (Jaenike, 1990; Agrawal, 2000; Via & Hawthorne, 2002) and theoretical (De Meeûs *et al.*, 1993; Kirkpatrick & Ravigné, 2002; Ravigné *et al.*, 2009) studies. The ultimate mechanisms linking host specialization and population (and species) divergence are more poorly documented (but see Matsuhashi *et al.*, 2010 for a review and the textbook model of *Rhagoletis pomonella*, Feder *et al.*, 1994).

When insects specialize on one or a few host plants, they optimize their fitness in a restricted ecological niche through changes in behaviour or physiological traits (Bernays, 2001). According to the maxim 'Jack of all trades, master of none', the performance trade-offs associated with specialization enable specialists to outperform generalists on a subset of host plants (Caley & Munday, 2003; Fry, 2009). Conversely, specialists cannot maintain such high levels of performance over a broader range of host plants. Thus, specialized phytophagous insects tend to develop a greater capacity to digest and detoxify specific plant compounds (Agrawal,

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2000) and to recognize specific host plants as suitable (Gripenberg *et al.*, 2010) than generalist species, but they often lose the ability to exploit a large range of host plants (Futuyma & Moreno, 1988; Jaenike, 1990). Consequently, among phytophagous insects, specialized species are prone to stronger trade-offs than generalists, resulting in better performances on their usual host plants than on other plants (Futuyma & Moreno, 1988).

Two key life history traits make a major contribution to host plant specialization: *performance* (measured as the fitness of the larvae feeding on a given host plant) and *preference* (measured as host plant choice under conditions of equal host abundance and availability). During specialization, selection is expected to favour females laying eggs on host plants improving their fitness by increasing the survival and reproductive success of their offspring (the 'mother knows best' hypothesis; Valladares & Lawton, 1991). As an illustration of the above expectation, several studies on phytophagous species have indeed reported a positive correlation between larval performance and female egg-laying preferences (Singer *et al.*, 1988; Thompson, 1988; Craig & Ohgushi, 2002; in Gripenberg *et al.*, 2010). However, other studies (Valladares & Lawton, 1991; Keeler & Chew, 2008) have shown that larvae perform as well on other plants as on those on which females lay their eggs, suggesting that specialization can, in some cases, be driven solely by adult female preference (Thompson, 1988). *Pieris*-ovipositing females even lay eggs on *Thlipsis arvense*, a crucifer species lethal for their offspring, highlighting the complexity of the balance between adult and larval selection, which involves a large number of factors, including the availability of various host plants or the time lag between the introduction of an exotic plant and its identification by the insect as a suitable or unsuitable host (Chew, 1977). Finally, some authors have shown that adult host preference be shaped not only by offspring performance, but also by the optimization of adult performance, accounting for the apparent conflicts between adult oviposition choice and offspring development (Scheirs *et al.*, 2000).

The adult's choice of host for oviposition results from a complex process involving host attraction and/or host avoidance. In empirical studies, the preference for a host may cover the attraction for a suitable host, or the avoidance of another unsuitable host, or a combination of the two. These two components of preference may sometimes be difficult to disentangle, particularly in experimental choice experiments, in which two or more hosts are provided (Linn *et al.*, 2005; Feder & Forbes, 2007; Uesugi, 2008; Feder *et al.*, 2012). Both avoidance and attraction are components of the preference for a particular habitat, but they have different consequences for speciation, particularly as concerns the fitness of hybrids between parental taxa. If a hybrid is heterozygous for one or several loci involved in host

attraction, it will tend to have equal probabilities of occupying the niches of the two parental taxa (Feder & Forbes, 2007). In such cases, the hybrids maintain gene flow between the parental taxa, delaying divergence. By contrast, a hybrid between taxa avoiding particular host plants may suffer chemical conflicts, due to the antagonistic effects of plant volatile compounds, for example resulting in a tendency not to recognize any of the parental host plants (Linn *et al.*, 2005; Feder & Forbes, 2007). This would interrupt gene flow between the parental taxa and accelerate divergence. Like host attraction, host avoidance can promote and reinforce prezygotic barriers if the mixture of native and repulsive hosts interferes with the meeting and mating of sexual partners. The attraction–avoidance conflict occurs only in hybrids, resulting in their simultaneous attraction to *and* repulsion by a given plant. These conflicts should lead to a disruption of gene flow, *via* hybrid maladaptation and the isolation of specialized parental populations.

*Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), the European corn borer (ECB), was until recently considered to be a polyphagous moth and a major pest of maize crops. In the last decade, two genetically differentiated host races of ECB have been identified in Western Europe, one feeding mostly on maize (*Zea mays* L.), and the other feeding on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.; Bontemps *et al.*, 2004; Leniaud *et al.*, 2006; Malausa *et al.*, 2007a). After reviewing the available phenotypic, behavioural and genetic data, Frolov *et al.* (2007) suggested that these two taxa should be promoted to separate species, with populations feeding on maize corresponding to *O. nubilalis* (ECB), whereas those feeding on various dicotyledons (notably hop and mugwort) belong to *Ostrinia scapularis* (ABB for adzuki bean borer). The common names ECB and ABB refer to the main cultivated hosts of these insects. The wild and native host is not known for ECB, and this host may be mugwort or wild hop for ABB (Malausa *et al.*, 2007b; Alexandre *et al.*, 2013). These two species display strong, but not complete, reproductive isolation. The proportion of hybrids has been evaluated directly (by behavioural or biochemical analyses of mating events) or indirectly (through population genetics), in both semi-natural (Bethenod *et al.*, 2005; Malausa *et al.*, 2008) and natural (Malausa *et al.*, 2005, 2007a) conditions. This proportion is very low, probably < 1%, suggesting a high level of assortative mating (> 95%; Malausa *et al.*, 2005). Various potential sources of disruptive selection have been investigated and may have contributed to specialization: temporal isolation (Thomas *et al.*, 2003), parasitism (Thomas *et al.*, 2003; Bethenod *et al.*, 2005; Péliissié *et al.*, 2010) and pheromonal communication (Pélozuelo *et al.*, 2004). Moreover, the specialization of *O. nubilalis* on maize and of *O. scapularis* on mugwort has been characterized by reciprocal larval transplant experiments, with

larvae from French populations (Calcagno *et al.*, 2007). Adult females preferentially lay their eggs on their usual hosts, this preference being more pronounced for *O. nubilalis* than for *O. scapularis* (Bethenod *et al.*, 2005; Malausa *et al.*, 2005, 2008). These studies suggest that larval performance is positively correlated with female host preference. However, this correlation has not been formally tested, and experimental differences (e.g. source of the moth populations, host plants, experimental conditions) might affect the results obtained. Of particular importance, host avoidance has never been assessed in *Ostrinia* spp., despite the potentially major effects of this phenomenon, together with strong attraction, on assortative mating and species divergence.

In this study, we carried out two experiments to investigate the major components of the insect–host relationship: (i) a reciprocal transplant (RT) experiment with larvae reared on their usual host and on alternative host plants, to evaluate larval performance, (ii) an oviposition experiment (OV), focusing on the host preference of gravid females in mixed stands of the usual host and alternative host plants, with the quantification of host avoidance by gravid females in no-choice tests with stands of the usual host or alternative host plants. We then discuss the role of the patterns of performance, attraction and avoidance observed in these experiments, in the divergence between ECB and ABB.

## Materials and methods

### Sampling and rearing of moths

We sampled 116 ECB and 133 ABB diapausing larvae from maize and mugwort stands, respectively, near Versailles (48°48'19"N, 2°08'06"E, Ile-de-France region, see Table S1) in February 2013. The larvae were kept in the dark, at 4 °C, to allow them to complete diapause. Diapause was terminated by a gradual daily increase in temperature until the stable rearing conditions were achieved: 24 °C, 70% relative humidity (RH) and a 16 : 8 h light : dark (L : D) photoperiod. After pupation and emergence, we placed about 10 male and 10 female adults in home-made cages for several days, for mating and egg laying. The eggs were then transferred to plastic rearing boxes containing an artificial diet (from Poitout *et al.*, 1972) for production of the next generation.

### Plant rearing

Maize plants (maize line B73) were produced from organic seeds at the DIASCOPE experimental research station (INRA, Mauguio, France, 43°36'37"N, 3°58'35"E), in 6-L plastic pots filled with soil. Mugwort rhizomes were sampled near Versailles in March 2013 and transplanted to 12.5-L plastic pots at the DIASCOPE

station for further growth and experiments. Maize and mugwort were grown in a heated, pesticide-free greenhouse (25 °C, 60% RH and 16 : 8 h L : D). Plants were treated with Marseille soap 15 days before the experiments to remove aphids and were rinsed the day before the start of the experiment.

### Experimental conditions

The OV and RT experiments described below were conducted in outdoor conditions at the DIASCOPE INRA research station. Eight 2-m-high cages covering a surface area of 9 m<sup>2</sup> (3 m × 3 m) were built with iron frames covered with an insect-proof netting (150 µm) to prevent contamination and escapes. Automatic misting was used to keep humidity levels constant within the cages.

### Oviposition experiment

The OV experiment involved three different set-ups: *choice*, *usual host* and *alternative host*. In the choice experiment, each cage contained 10 maize and five mugwort plants (the numbers of maize and mugwort plants were adjusted to ensure that equivalent amounts of biomass were available for the two species) arranged in two patches (one patch of maize and one patch of mugwort). These two patches were located at opposite corners of the cage. For the usual- and alternative-host set-ups, we used either 20 maize or 10 mugwort plants. The plants were arranged in two equal patches (2 × 10 maize or 2 × 5 mugwort plants) located at opposite corners of the cage. We released 21–25 virgin ECB or ABB females reared in the laboratory and 15 virgin males of the same strain per cage, and left them together for three nights (72 h).

For each moth species, the three experimental set-ups were used on different dates, to prevent the mixing of volatile plant compounds. Each set-up was used twice (i.e. two replicates per set-up).

At the end of the third night, all the adults were recovered from each cage, and the leaves and stalks of all maize and/or mugwort plants and the netting covering the cage were carefully screened for the presence of egg masses. All egg masses were counted and for each cage, we noted the plant species from which the egg mass was collected. For analyses, these data were transformed into the following variables (see Table 1):

- 1 One component of the fertility of each moth species, expressed as the total number of egg masses produced by females of the species concerned in a given cage (i.e. the total egg output per female, laid either on plants or on the cage netting).
- 2 The percentage of egg masses laid on one particular site (one given plant species or the cage netting) was also determined.

**Table 1** Statistical analysis for the oviposition experiment (a) and for the reciprocal transplant experiment (b).

	Model	Δd.f.	Statistic	P
(a) Oviposition experiment (OV)				
Models for the fertility component (F)				
1. <b>F = Species + Set-up + Species:Set-up</b>	LM (N)			
2. F = Species + Set-up		2	8.58	0.0177
Models for the percentage of egg masses (EM) collected on the different laying sites in choice condition				
1. <b>EM = Laying site + Species + Laying site:Species</b>	GLM (B)			
2. EM = Laying site + Species		2	13.549	< 0.0001
Models for the adult capture sites (ACS)				
1. ACS = Species + Site + Set-up + Species:Site + Species:Set-up + Site:Set-up	GLM (B)			
2. <b>ACS = Species + Site + Set-up + Species:Site + Species:Set-up</b>		2	2.929	0.0535
3. ACS = Species + Site + Set-up + Species:Set-up		2	7.4231	0.0006
Models for the number of egg masses laid on the cage netting (EMcg)				
1. <b>EMcg = Set-up + Species + Set-up:Species</b>	GLM (B)			
2. EMcg = Set-up + Species		2	57.888	< 0.0001
(b) Reciprocal transplant experiment (RT)				
Models for the larval survival (LS)				
1. <b>LS = Species + Plant + Species:Plant + (Replicate)</b>	GLMER (B)			
2. LS = Species + Plant + (Replicate)		1	5.2482	0.022
Models for the larval weight (W) at the fourth instar				
1. W = Species + Plant + Sexe + Species:Plant + Plant:Sexe + Species:Sexe + (Replicate)	GLMER (P)			
2. <b>W = Species + Plant + Sexe + Species:Plant + (Replicate)</b>		2	3.403	0.1824
3. W = Species + Plant + Sexe + (Replicate)		1	20.662	< 0.0001

For each variable is indicated the model formula with factors, interactions (:) and random effects in parenthesis, the statistical model and the error distribution (B = Binomial, N = Normal, P = Poisson), the differences in degree of freedom between the simplified model and the previous model, the chi-squared test (for ratio data) or *F* (for number data) statistics, and the *P*-value of likelihood ratio test. Only, the best model (in bold) and the closest ones are presented, but all interactions were tested.

**3** The adult survival rate (calculated as the number of adults still alive after 3 days over the number of adults released into the cage) and the adult resting site, corresponding to the site (plants, cage netting or ground) from which the adults were recovered 3 days after their release.

The OV experiment was carried out over a period of 1 month, with the first adult moths released on July 17th, and the last released on 16 August 2013.

### Reciprocal transplant experiment

The RT experiment involved controlled infestations of maize and mugwort plants with fertile egg masses of the two moth species reared as described above. The RT experiment was conducted in the same cages and conditions as the OV experiment, with four different set-ups: (i) maize plants infested with ECB; (ii) mugwort plants infested with ECB; (iii) maize plants infested with ABB and (iv) mugwort plants infested with ABB. Each cage contained two replicates of the same set-up. Thus, four cages each contained 48 maize plants (24 per replicate) and the other four cages each contained 20 mugwort plants (10 per replicate).

We began these infestations in the second week of May 2013. We deposited fertile egg masses on the plants between May 13th and 16th for ECB and May 14th to

23rd for ABB, for all set-ups. Egg masses were obtained from individual mating pairs. Each pair was placed in an individual cylindrical plastic box (8.5 cm in diameter, 11 cm deep, containing a wad of moist cotton and a 15 × 1.5 cm strip of paper for resting and oviposition) incubated at 25 °C, under a 16 : 8 h (L : D) photoperiod, in a growth chamber. Most mating pairs (29 and 33, for ECB and ABB, respectively) produced about 10 egg masses, each of which contained about 20 eggs (personal observation). The total number of egg masses per mating pair was split into batches for the infestation of different set-ups and replicates, so that the different cages were infested with batches of similar pools of sibs. In total, we deposited about 400 eggs for the mugwort replicates and 480 eggs for the maize replicates, that is one mature egg mass per maize plant and two per mugwort plant, as mugwort had a greater biomass per plant.

Two to three weeks after egg mass deposition, we assessed how far larval development had advanced by opening a few maize and mugwort stalks specifically for this purpose. Larval stage was determined by the width of the head capsule (Tseng, 1985). As this parameter may vary with environment and species, we calibrated this parameter for the two moth species before carrying out the full-scale experiments, by measuring head capsule size range per larval stage on dedicated samples, observed over the entire larval cycle (five instars). We

opened the remaining stalks to collect the larvae when the fourth instar predominated, generally about 1 month after deposition on maize and 1 month and a half after deposition on mugwort, regardless of the moth species considered.

We measured two variables (Table 1):

- 1 Larval survival, corresponding to the number of larvae alive at the end of the experiment over the initial number of larvae.
- 2 The weight of each larva.

As female larvae were expected to be heavier than males, on average (Calcagno *et al.*, 2007), we determined the sex of each larva. As it is impossible to determine the sex of the larva on the basis of external features at this stage, we used a molecular sex testing procedure, as follows. Genomic DNA was extracted from each L4 larva with the AllPrep DNA/RNA 96 Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. We then determined the sex of each larva by the multiplex PCR amplification of two microsatellite markers (ONZ1 and ONW1) on the Z and W heterochromosomes, respectively (Coates & Hellmich, 2003). If only ONZ1 was amplified, the larva was considered to be male (homogametic sex), whereas the amplification of both loci (ONZ1 and ONW1) indicated that the larva was female (heterogametic sex). We observed no departure from a sex ratio of 1 : 1 for any of the ECB or ABB samples.

### Statistical analyses

Statistical analyses were performed with the 'lme4' package (Bates *et al.*, 2014) of R software (Version R.3.0.3; R Development Core Team 2008). We used different linear models (generalized and/or mixed), depending on the distribution of the residuals (Table 1). Various candidate models, corresponding to different biological hypotheses and combinations of variables/factors, were fitted to the data by maximum-likelihood methods (see details in Table 1). Model selection was performed as follows: the significance of the different terms was assessed, beginning with higher-order terms using likelihood ratio tests (LRT). Nonsignificant terms ( $P > 0.05$ ) were removed (Crawley, 2007a,b). The factor levels for qualitative

variables not significantly different from each other were grouped together (LRT, Crawley, 2007b).

### Results

The best linear models (with significant factors and interactions) for all variables of the OV and RT experiments are summarized in Table 1.

#### Oviposition experiment: egg output and laying

Female fertility, measured as the total number of egg masses produced by females in a given cage, was explained by species, set-up and their interaction ( $P = 0.028$ ; Table 1). On average, ECB females laid twice as many egg masses in the presence of their usual host plant (*choice* and *usual host* set-ups) as they did in the presence of the alternative host (*alternative host* set-up;  $P = 0.002$ ; Table 2). For the ABB, fertility was higher in the presence of the usual host only (*usual host* set-up) than in the presence of the alternative host only or both plants (*choice* and *alternative host* set-ups;  $P = 0.002$ ; Table 2).

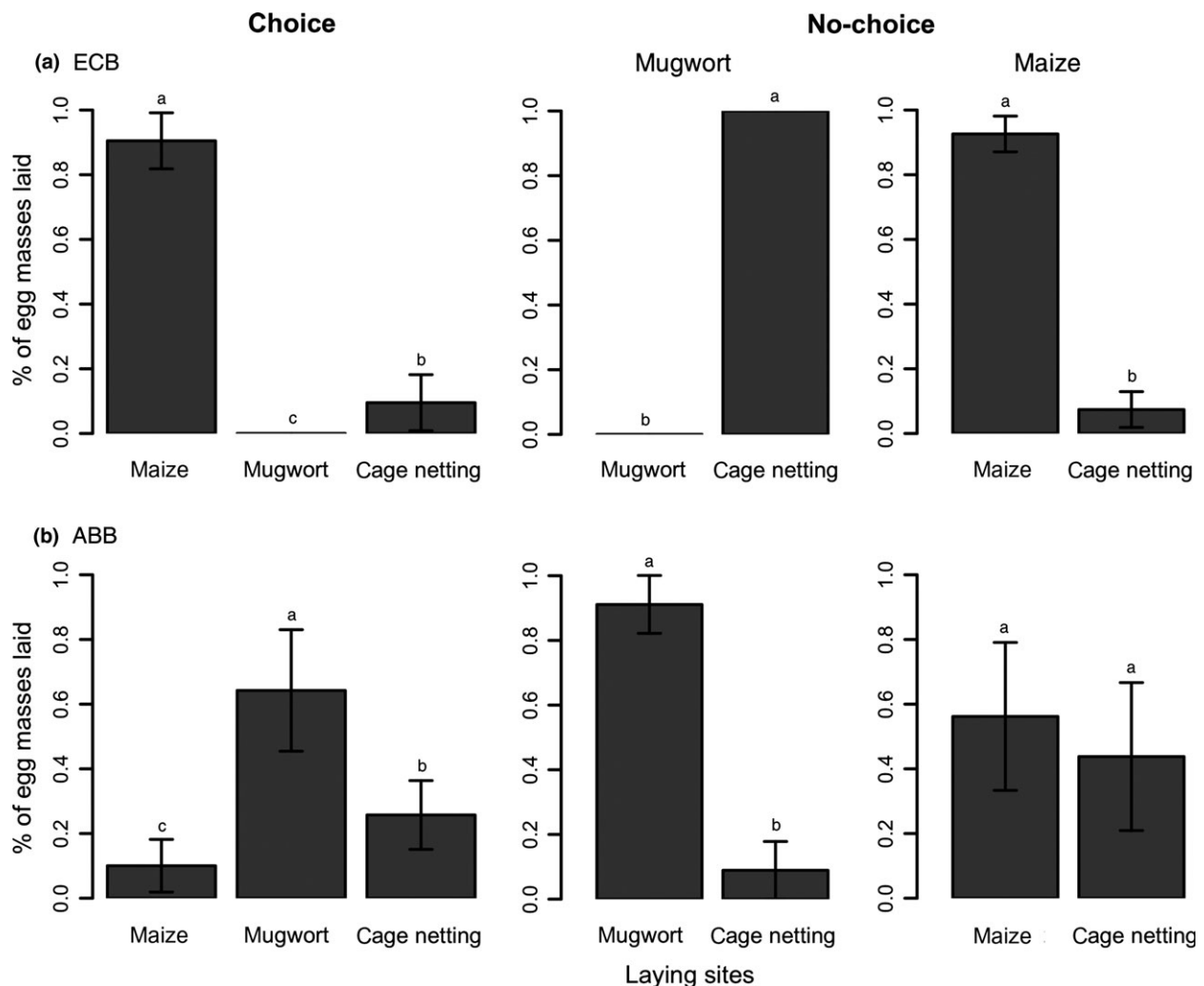
The percentage of egg masses laid on different substrates by female moths in the experimental cages was explained by species, laying site and their interaction ( $P < 0.0001$ , Table 1). In the choice set-up, ECB females laid significantly more egg masses on maize than on mugwort and cage netting, whereas ABB females laid significantly more egg masses on mugwort than on maize and cage netting (Fig. 1a for ECB and Fig. 1b for ABB). More than 90% of ECB egg masses were found on the usual host, and none were found on mugwort (Fig. 1a). For ABB, 64%, 25% and 11% of egg masses were found on mugwort, cage netting and maize, respectively.

The percentage of egg masses laid on the cage netting was explained by species, experimental set-up and their interaction ( $P < 0.0001$ , Table 1). In the absence of maize (i.e. in the alternative-host set-up), ECB females laid 100% of their egg masses on the cage netting (Fig. 1a). Indeed, in cages containing only mugwort, none of the 45 and 60 egg masses found in the two replicates was recovered from the mugwort plants. Conversely, when maize plants were present in the

**Table 2** Raw data for adult survival and fertility component for the two moth species in three set-ups of oviposition experiment.

Species	Set-ups	$N_{\text{rep}}$	$N_{\text{f deposit}}$ (SD)	$N_{\text{m deposit}}$ (SD)	Adult survival			Fertility component $F$ (SD)
					$N_{\text{f sampled}}$ (SD)	$N_{\text{m sampled}}$ (SD)	AS (SD)	
ECB	Usual host	2	25 (0)	15 (0)	20.5 (0.71)	12.5 (0.71)	0.825 (0.38)a	111.5 (6.36)a
	Alternative host	2	25 (0)	15 (0)	15 (4.95)	8.5 (2.83)	0.588 (0.49)a	52.5 (10.61)b
	Choice	2	25 (0)	15 (0)	21 (1.41)	14 (1.41)	0.875 (0.34)a	91.5 (36.06)a
ABB	Usual host	2	25 (0)	15 (0)	18.5 (2.12)	8.5 (0.71)	0.675 (0.47)a	85.5 (21.92)a
	Alternative host	2	23 (2.83)	15 (0)	17 (4.24)	9 (5.66)	0.684 (0.46)a	36.5 (9.19)b
	Choice	2	24 (1.14)	15 (0)	16.5 (2.12)	11.5 (2.12)	0.718 (0.45)a	43 (14.14)b

ABB, Adzuki bean borer; ECB, European corn borer. Different letters for AS and F indicate significant differences.



**Fig. 1** Percentage of egg masses laid on mugwort, maize or cage netting by European corn borer (ECB) (a) and Adzuki bean borer (ABB) (b) in three experimental set-ups: cages with both maize and mugwort (choice), cages with mugwort only and cages with maize only (no-choice). Different letters above bars indicate significant differences in the number of egg masses observed per plant/cage netting ( $P < 0.05$ ).

cage (i.e. choice and alternative-host set-ups), this percentage fell to  $< 10\%$  (Fig. 1a). ABB females also laid a percentage of their egg masses on the cage netting, but this percentage was significantly lower when mugwort was the only host plant present in the cage (17% in the *usual-host* set-up) than in the presence of maize (30% in the *choice* and 43% in *alternative-host* set-ups;  $P = 0.006$ ; Fig. 1b). It is noteworthy that, in both species, the proportion of egg masses laid on the cage netting was not significantly different between the two set-ups containing maize ( $P = 0.271$  for ECB and  $P = 0.216$  for ABB).

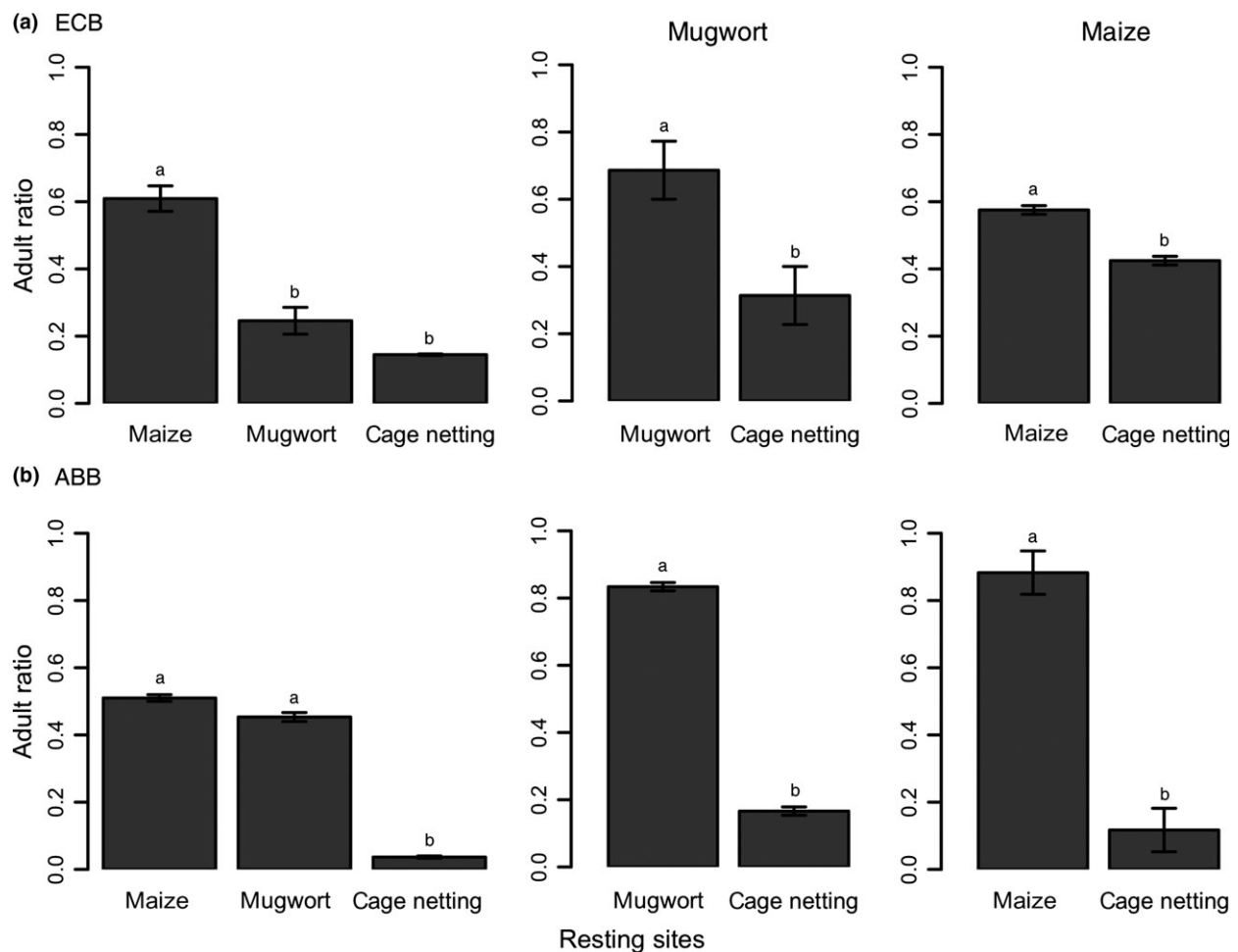
#### Oviposition experiment: survival and resting site

Adult survival was similar between ECB and ABB ( $P = 0.495$ ) and between set-ups ( $P = 0.377$ ). ECB

survival was lower in cages containing only mugwort (58% in the alternative set-up) than in cages containing maize (82% and 87% in the *usual-host* and *choice* set-ups, respectively), but this trend was not significant ( $P = 0.381$  and  $P = 0.546$  for comparisons of survival rates between the *usual-host* and *alternative-host* set-ups and between the *alternative-host* and *choice* set-ups, respectively; Table 2).

The adults of both species were more frequently recovered from plants than from the cage netting (Fig. 2). Capture site varied with experimental set-up, as follows:

**1** In the *choice* set-up, adult resting site was explained by species, site and their interaction ( $P < 0.0001$ , Table 1). ABB adults were more frequently recovered from host plants than from the cage netting ( $P < 0.001$ , Table 1 and Fig. 2). However, they were



**Fig. 2** Ratio of adults sampled on mugwort, maize or cage netting by European corn borer (ECB) (a) and Adzuki bean borer (ABB) (b) in three experimental set-ups: cages with both maize and mugwort (choice), cages with mugwort only and cages with maize only (no-choice). Different letters above the bars indicate significant differences in the number of egg masses observed per plant/cage netting ( $P < 0.05$ ).

not found preferentially on mugwort rather than maize ( $P = 0.735$ ). By contrast, ECB adults were recovered much more frequently from maize plants than from other sites ( $P < 0.0001$ ). The few ECB not found on maize were present on mugwort plants or on the cage netting, with no clear preference between the two ( $P = 0.215$ ).

- 2 In the *usual-host* set-up, adult resting site was explained by species, site and their interaction ( $P = 0.009$ , Table 1). ABB adults were recovered more frequently from mugwort than from the cage netting ( $P < 0.001$ ). ECB was also recovered more frequently from its usual host plant, but the difference in the proportion of adults on maize and on the cage netting was not significant ( $P = 0.314$ ).
- 3 Finally, in the *alternative-host* set-up, adult resting site was explained by species and site, and their interaction, with marginal significance ( $P = 0.055$ , Table 1).

Adults of both ABB and ECB were recovered more frequently from their alternative hosts than from the cage netting ( $P = 0.040$  and  $P < 0.0001$  for ECB and ABB, respectively).

### Reciprocal transplant experiment: larval performance

Larval survival was explained by species and host plant, and their interaction ( $P = 0.022$ , Table 1). The survival of ECB larvae was similar (about 12%, similar to previous results for a field experiment reported by Calcagno *et al.*, 2007) on maize and mugwort ( $P = 0.627$ , Fig. 3a), whereas ABB larvae survival rates were higher for mugwort (usual host) than for maize (23% vs. 11%,  $P = 0.005$ , Fig. 3b).

Larval weight at the fourth instar was explained by species, host plant and sex, with a significant interaction

between species and host plant ( $P < 0.0001$ , Table 1). The females were heavier than the males ( $P < 0.0001$ ).

For both species, larvae feeding on maize were heavier than those feeding on mugwort ( $P < 0.0001$  for both species). On mugwort, ECB larvae were significantly heavier than ABB larvae (15 vs. 8 mg,  $P < 0.0001$ ). On maize, larval weight did not differ significantly between the two moth species ( $P = 0.276$ ).

## Discussion

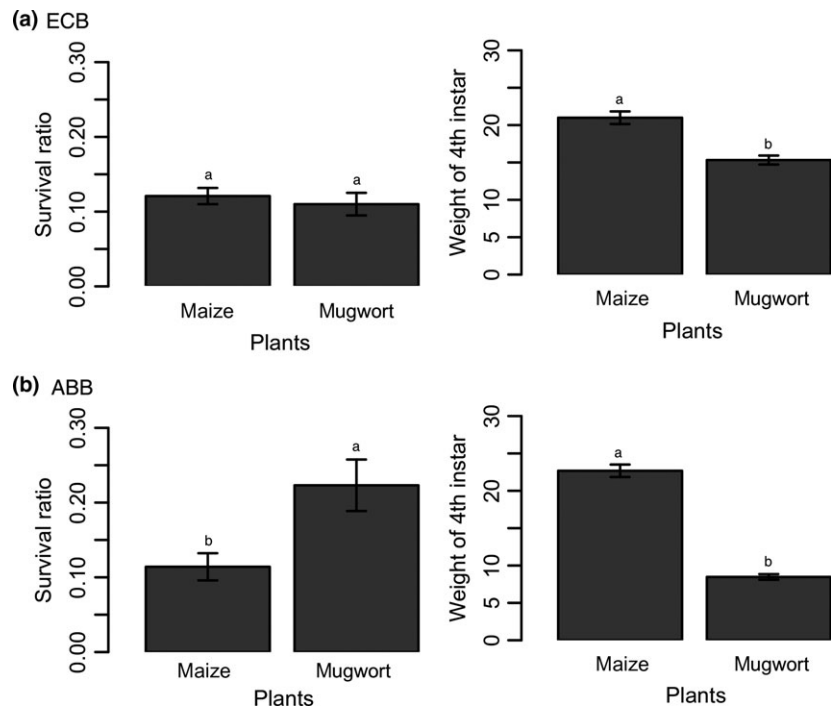
### Variability of the pattern of specialization

Natural selection would be expected to favour insects laying their eggs on the most suitable hosts for the development and survival of their offspring, at least in the absence of other sources of selection (such as parasitoids or predators). In systems in which the larvae display little dispersal, adult ovipositing choice is even more crucial, because the larvae are unlikely to move far from their birth place, even if it is unsuitable.

In ECB and ABB, host preference at the adult stage was strong and apparent, through the choice of oviposition, the resting sites and the fertility rates (Figs 1 and 2, Table 2). By contrast, differences in larval performance were less marked and were detected through various trends towards similar or contrasting patterns in ECB and ABB (Table 3). First, in the OV experiment, the females laid more egg masses in the presence of their usual host plant (whether alone or in the presence of the alternative host) than in its absence

(Fig. 1). This greater fertility on the usual host may result in a larger population in the next generation than would be observed on an alternative host and may therefore be considered to indicate higher performance *sensu lato*. Second, in the RT experiment, larvae had higher survival rates (for ABB) and were heavier (for ECB) on their usual host plant than on the alternative host plant (Table 3). Larval weight may be considered an indirect fitness component, as it is related to post-diapause survival rate, for example heavier larvae are more likely to survive diapause, Tauber *et al.* (1986).

In terms of weight gain, maize appeared to be the best food source not only for the ECB (as expected), but also for the ABB (unexpected). Maize may be a richer resource of primary metabolites for larval growth (ECB and ABB). However, some of its compounds, such as DIMBOA (Campos *et al.*, 1989), may be toxic to the ABB, potentially accounting for the lower survival of ABB larvae on this plant than on mugwort. The ECB may have developed a capacity to detoxify this toxic compound, enabling its larvae to survive equally well on maize and on mugwort. However, in a previous RT experiment with ECB and ABB, Calcagno *et al.* (2007) found that late fifth instars of ABB were heavier and survived better on mugwort than on maize, contrasting with our observations for the fourth instar. We focused on the fourth instar, because we wanted to investigate the effects of host plant on larvae (e.g. for digestion and detoxification) rather than on diapause features. The diapause, which would probably be more conspicu-



**Fig. 3** Larval survival rate and weight at the 4th larval instar for European corn borer (ECB) (a) and Adzuki bean borer (ABB) (b) reared on maize or mugwort. Different letters above the bars indicate significant differences in the survival rates ( $P < 0.05$ ).

**Table 3** Summary of results obtained in this study.

Stage	LHT	ECB	ABB
Adult	Fertility	Higher on maize and choice cages than on mugwort cage	Higher on mugwort cage than on maize and choice cages
	Oviposition	Strong preference for maize	Slight preference for mugwort
	Capture site	Evidence for avoidance (never lay egg masses on mugwort)	No evidence for avoidance
Larvae		Slightly more often on plants than on cage netting	More often on plants than on cage netting
	Survival	More often on maize than on mugwort	Same proportion on maize than on mugwort
	Weight at 4th instar	Similar on maize and mugwort	Much higher on mugwort
		Higher on maize than on mugwort	Higher on maize than on mugwort

ABB, Adzuki bean borer; ECB, European corn borer.

ous in the fifth instar, affects larval survival, weight and developmental time and could interfere with assessments of the effect of plant species. In addition, weight is sensitive to larval density (Agrawal, 2004; Calcagno *et al.*, 2007). Hence, the differences between the results obtained by Calcagno *et al.* (2007) and those reported here may therefore reflect differences in the experimental conditions and biological response.

Overall, for ECB, survival was similar on maize and mugwort, but growth was greater on maize. This lends some support to the ‘mother knows best’ hypothesis in terms of growth, but is inconclusive in terms of survival. For ABB, females preferred mugwort, but larval growth was actually greater on maize. Thus, two different dimensions of larval performance (growth and survival) responded differently, indicating an ambiguous relationship between female preference and larval performance and only weak support for the ‘mother knows best hypothesis’. In ECB and ABB, specialization thus appears to be driven mostly by a strong host preference, as repeatedly observed in different studies and environmental conditions.

We also obtained the first evidence that the preferences of the ECB involve a strong avoidance of mugwort. Previous studies on ECB have already revealed a pattern of host preference in adult females (Bethenod *et al.*, 2005; Malausa *et al.*, 2008), but attraction for maize was not distinguished from avoidance of an alternative host. Such a distinction was made possible in this study by the use of a ‘no-choice’ experimental design, and the comparison of egg-laying behaviour in the presence and absence of mugwort. Indeed, while in host-choice conditions or in the presence of maize only, ECB females laid < 10% of their eggs on the cage netting, this percentage reached 100% in cases in which ECB females were provided only with mugwort. This behaviour strongly suggests that ECB females avoid laying their eggs on mugwort (Fig. 1a). Further support for this hypothesis is provided by the very different behaviour of ABB females, which, in these no-choice conditions, laid their eggs equally frequently on maize and the cage netting (Fig. 1b). Oviposition choices may not necessarily result from dispersal or resting behaviour, because ECB adult females were also recovered

from mugwort and the cage netting (Fig. 2, assuming that the capture sites reflect resting preferences). Interestingly, however, in no-choice mugwort conditions, ECB adult survival tended to be lower than in maize or choice conditions (although this result was not significant in our experiment). No such trend was observed for ABB in the presence of maize. Further investigation of these patterns of resting and survival behaviour is required, to identify putative repellent or toxic effects of some volatile or leaf-surface components of mugwort. The difference between ECB and ABB for avoidance raises questions about the underlying mechanisms, the role of this difference in the specialization of ECB in terms of other traits (e.g. preference) and the process of divergence between ECB and ABB.

### Candidate mechanisms for host avoidance

Feder *et al.* (2012) suggested that the physiological basis for habitat avoidance is probably based on a few genes with strong effects on attraction–repulsion behaviour, because the probability of selecting a successful allelic combination for attraction or avoidance would otherwise be low and, as a consequence, making the divergence of specialized host races unlikely. The most studied candidate mechanisms in this attraction/repulsion system involve the components of the chemosensory system (Smadja & Butlin, 2009): the olfactory receptor neurons (ORNs), the odorant-binding proteins and the transmembrane odorant receptors (ORs). In *Drosophila*, Semmelhack & Wang (2009) showed that the activation or inactivation of individual glomeruli (ORN-OR complexes) could account for attraction or aversion to apple cider vinegar. In *Caenorhabditis elegans*, Tsunozaki *et al.* (2008) found that mutations in a single olfactory neuron could lead to switching between attraction and avoidance behaviour. This result was unexpected, because other neurons in *C. elegans* are known to direct attraction or repulsion, but not both. In flight tunnel assays, Leppik & Frérot (2012) demonstrated the role of volatile organic compounds (VOCs) emitted by maize, hop and mugwort in host choice by *Ostrinia* females. The experiments involved choices between two putative host plants, but not no-choice

trials with a single plant. These authors thus assessed attraction, but not avoidance. A chemical analysis of maize, hop and mugwort VOCs further suggested that *Ostrinia* spp. host fidelity is driven by plant-specific ratios of ubiquitous VOCs (Leppik & Frérot, 2012).

### Role of host attraction and avoidance as mating barriers

Models of population or species divergence in phytophagous insects highlight the key role for host plant-associated mating as a premating isolation mechanism. There is no clear evidence to suggest that the reported premating barriers between the ABB and ECB are due to such a 'magic trait', simultaneously ensuring reproductive isolation and adaptation to the host plant (Gavrilets, 2004; Servedio *et al.*, 2011), as reported for the host fidelity (mating and development on the same plants) of *Rhagoletis pomonella* (Feder *et al.*, 1994) and *Zeiraphera diniana* (Emelianov *et al.*, 2001).

Indeed, ECBs can cover distances of several hundred metres in flight (Qureshi *et al.*, 2005) and do not mate exclusively on their host plants. They also mate in dense foxtail grass and patches of other herbaceous, nonhost plants (Showers *et al.*, 1976). ECB may not preferentially mate close to maize, but they avoid mugwort, for both oviposition and resting, as demonstrated here. This behaviour may contribute to assortative mating by establishing a previously unsuspected local micro-allopatry. This micro-allopatry may be reinforced by the interference of host attraction/avoidance with pheromone cues. Chemosensory mechanisms of pheromonal communication in *Ostrinia* spp. are known to account at least partly for reproductive isolation (Pélozuelo *et al.*, 2007). In some parts of their geographical ranges, ABB and ECB populations share the same 'E' and 'Z' pheromone blends (characterized by inverse ratios of the E and Z isomers of  $\Delta 11$ -tetradecenyl acetate), whereas, in northern France, ABB females release, and males preferentially respond to the 'E' blend, whereas ECB females and males use a 'Z' blend (Pélozuelo *et al.*, 2004; Bethenod *et al.*, 2005). The potential interactions between these pheromone cues and plant odours have yet to be studied in ABB and ECB, but at least two studies in other moth species have documented stronger male attraction when plant odours and sexual pheromones are mixed than in the presence of only one compound (Emelianov *et al.*, 2003; Trona *et al.*, 2013). In ECB, the putative synergic effects of the E blend and mugwort volatile chemicals may contribute significantly to isolation from ABB and merit further study.

Finally, if prezygotic mating barriers are considered to predominate over post-zygotic barriers in ECB and ABB, as suggested by the strong homogamy observed in natural populations (Malausa *et al.*, 2005) and the

survival to the adult stage of F1 hybrids (Bourguet *et al.*, 2014), then the strong pattern of mugwort avoidance in ECB suggests that hybrid fitness should be reconsidered, not in terms of survival, but in terms of *behaviour*. Indeed, as suggested by Feder & Forbes (2007), host avoidance and attraction have different evolutionary implications, because insects that are heterozygous for avoidance alleles to alternate host plants (as e.g. a ABB  $\times$  ECB hybrid) may display behavioural conflicts and have difficulty finding any suitable host for feeding, oviposition or mating. Feder & Forbes (2007) developed an epistatic model involving attraction, avoidance and performance genes, and showed that the role of avoidance in species divergence depends on the strength of host discrimination and performance trade-offs. Studies of these components of the genetic architecture should be highly relevant in this context, because ABB and ECB display contrasting specialization patterns: strong avoidance and attraction and moderate larval trade-off for the ECB and moderate preference, absence of avoidance and significant larval trade-off for ABB.

### Acknowledgments

We thank S. Ponsard and B. Facon for constructive discussions and comments on the manuscript. We thank M. Vabre for the mounting and maintenance of the experimental cages. This work received supports from ANR Adapt-Ome (ANR-13-BSV7-0012-01) to RS, DB and AZ, and from the University of Montpellier for MO PhD studentship.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Summary of sampling localities.

Data deposited at Dryad: doi: 10.5061/dryad.6b1h8

Received 22 May 2015; revised 11 September 2015; accepted 18 September 2015