

Genetic isolation between two sympatric host plant races of the European corn borer, *Ostrinia nubilalis* Hübner. II: assortative mating and host-plant preferences for oviposition

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The European corn borer, *Ostrinia nubilalis* Hübner, colonized maize (*Zea mays* L.) after its introduction into Europe about 500 years ago and is now considered one of the main pests of this crop. In northern France, two sympatric host races have been described: one feeding on maize and the other on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.). In a previous study, we showed that mating between the two races may be impeded by differences in the timing of moth emergence and in the composition of the sex pheromone produced by the females. In this study, we further investigated the genetic isolation of these two races using strains from the maize (Z strain) and mugwort (E strain) races selected for diagnostic alleles at two allozyme loci. In a

cage containing maize and mugwort plants and located in natural conditions, mating between individuals of the same strain occurred more often than mating between males and females of the E and Z strains. In particular, we obtained no evidence for crosses between Z females and E males. We also found that females of the Z strain laid their eggs almost exclusively on maize, whereas females of the E strain laid their eggs preferentially, but not exclusively, on mugwort. These results suggest that the genetic differentiation between the two host races may also be favored by host-plant preference, one of the first steps toward sympatric speciation. *Heredity* (2005) **94**, 264–270. doi:10.1038/sj.hdy.6800611
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Introduction

For several decades of the last century, speciation was thought to occur mainly, if not exclusively, in allopatric conditions. The importance of sympatric speciation has been reassessed over the last 25 years (Via, 2001), leading to an increase in the number of studies focusing on this particular type of speciation (Mallet, 2001; Via, 2001; Drès and Mallet, 2002). Although the importance of sympatric speciation is still debated (Turelli *et al.*, 2001), various mathematical models have shown that speciation is theoretically possible even in the absence of geographical barrier to gene flow (Kondrashov and Mina, 1986; Kirkpatrick and Ravigné, 2002; Gavrillets, 2003). In addition, the number of biological models known to be undergoing, or to have undergone, sympatric speciation has increased (Drès and Mallet, 2002). Successful host shifts in phytophagous insects have been studied in

particular detail and are now considered one of the best routes for sympatric speciation (see Diehl and Bush, 1984; Via, 2001; Drès and Mallet, 2002). In these insects, the first step toward sympatric speciation involves genetically controlled changes in host preferences and adaptation to the newly colonized host plant. Following these changes, positive assortative mating between individuals adapted to the same host plant may be selected for, eventually leading to genetic differentiation between the two genetic entities and ultimately to speciation. In most studied cases, insects mate on or near their hosts (see Craig *et al.*, 1997 and Prokopy *et al.*, 1971 for striking examples and Drès and Mallet, 2002 for a review on host races) so that the premating isolation of host-plant races is a by-product of the disruptive selection initially induced by host plants.

In this study, we further investigated the genetic isolation of two sympatric host-plant races of the European corn borer (ECB), *Ostrinia nubilalis* (Lepidoptera: Crambidae). This polyphagous moth is native to northern Africa, western Asia and Europe (Hudon *et al.*, 1989), and was accidentally introduced into North America at the beginning of the 20th Century (Baker *et al.*, 1949). *O. nubilalis* feeds on more than 200 weeds and

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cultivated plants (Hodgson, 1928; Ponsard *et al*, 2004) and colonized maize (*Zea mays* L.) after its introduction into Europe about 500 years ago. In France, ECB populations feeding on maize are genetically differentiated from those feeding on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.) (Bourguet *et al*, 2000; Martel *et al*, 2003; Bontemps *et al*, 2004). The exact taxonomic status of these two genetic entities has not yet been defined. In this paper, as in that by Thomas *et al* (2003), we use the terms 'maize race' to indicate a population feeding on maize and 'mugwort race' for a population feeding on either mugwort or hop.

Genetic isolation between sympatric populations of the maize and mugwort ECB host races may result from both differences in the date of moth emergence and differences in the mating communication system (Thomas *et al*, 2003; Pélozuelo *et al*, 2004). In France, at a field site near Paris, mugwort-race moths emerged, on average, 10 days earlier than maize-race moths. This allochrony in the timing of emergence may be a major, but not absolute, cause of isolation between the two races, as the lifetime of ECB adults is estimated at around 10 days (Balachowsky, 1935; Fadamiro and Baker, 1999). At the same field site, females of the two host races produced sex pheromones with different E:Z ratios of the Δ -11-tetradecenyl acetate (Δ 11-14:Ac) isomers. This difference in the mating communication system between the two host-plant races is not restricted to this particular site. All but two females out of 169 collected on mugwort or hop at eight other sites distributed throughout France produced a pheromone blend with a 97:3 ratio (E blend) of the E and Z isomers of Δ 11-14:Ac (Pélozuelo *et al*, 2004). Conversely, all but one of the 176 females extracted from maize stalks collected at six different sites – located in the center and south of France – produced a pheromone blend with a 1:99 E:Z isomeric ratio (Z blend) (Pélozuelo *et al*, 2004). Only three females – one on each type of host plant – of the ~350 analyzed produced a pheromone with a 50:50 E:Z ratio of Δ 11-14:Ac isomers (Pélozuelo *et al*, 2004). These three females were probably hybrid females. Hence, to our knowledge, females producing a Z pheromone blend have never been detected on populations collected on mugwort or hop. Similarly, females producing an E pheromone blend have never been detected in maize fields located in France. We therefore suggest that ECB larvae of the mugwort race can hardly develop on maize and that larvae of the maize race find it difficult to develop on mugwort and hop, and/or that ECB females prefer to lay their eggs on the host plants corresponding to their own race. The small proportion of hybrid females (three in ~350) may be a consequence of strong assortative mating between the host races and/or poor hybrid larval development on maize, hop and mugwort.

We investigated female preferences for oviposition and assortative mating between males and females of the two host races of ECB. We selected two strains, one from the maize race (Z strain) and one from the mugwort race (E strain), for diagnostic alleles at two allozyme loci. Using these strains, we addressed two questions related to host-race formation in *O. nubilalis*. Firstly, we investigated whether mating between the two strains was random in the absence of temporal barriers and in complete sympatry. Secondly, we investigated whether females of the

two strains displayed host-plant preferences for oviposition.

Materials and methods

ECB strains

Origin of the strains: This experiment was performed with two strains, referred to as the E and Z strains. The E strain was established from 195 overwintering larvae collected from hop (*H. lupulus* L.) during the fall of 1999 at Obernai (Alsace, France). These larvae were maintained at 4°C for diapause completion in laboratory conditions. During spring 2000, overwintering larvae were placed under diapause-breaking conditions (25°C under a 16:8 h (L:D) photoperiod, in plastic cups containing a moistened piece of cotton wool). We recovered 135 individuals, which were used to establish the E strain. We analyzed the composition of the pheromones produced by 12 females collected randomly within the batch of this strain. Consistent with previous results (Thomas *et al*, 2003; Pélozuelo *et al*, 2004), these females produced the E pheromone blend. The Z strain was established from eight egg masses (approximately 120 to 160 eggs) collected in maize fields during July 1998 at Les Rues des Vignes (Nord-Pas-de-Calais, France). The composition of the pheromones produced by 24 females of this strain was analyzed. Consistent with previous results (Thomas *et al*, 2003; Pélozuelo *et al*, 2004), these females produced the Z pheromone blend.

Selection for diagnostic allozyme markers: The genetic diversity of each strain was investigated by starch gel allozyme electrophoresis for five unlinked autosomal loci (*Hbdh*, *Pgm*, *Mpi*, *Aat* and *Gpi*) and one sex-linked locus (*Tpi*), as described previously (Bourguet *et al*, 2000). No diagnostic allele was found for any of the loci studied. However, as expected from previous results obtained on populations collected on the two host races (Bourguet *et al*, 2000; Martel *et al*, 2003; Thomas *et al*, 2003; Bontemps *et al*, 2004), the two strains were genetically differentiated at two loci: *Tpi* and *Mpi*. At the *Tpi* locus, the frequency of the *Tpi* 100 allele was significantly higher in the E strain than in the Z strain, whereas the *Tpi* 120 allele was significantly more frequent in the Z strain than in the E strain. At the *Mpi* locus, the *Mpi* 80 and *Mpi* 100 alleles were the main alleles in the Z and E strains, respectively.

Using isofemale lines, we selected both strains for homozygosity at the *Tpi* and *Mpi* loci. The selected E and Z strains were established from the progenies of one and two isofemale lines, respectively. At the *Tpi* locus, the E strain was fixed for the *Tpi* 100 allele, whereas the Z strain was fixed for the *Tpi* 120 allele. At the *Mpi* locus, the E and Z strains were fixed for the *Mpi* 100 and *Mpi* 80 alleles, respectively. Fixation of these diagnostic alleles made it possible to discriminate between the various intra- and interstrain crosses occurring in the experiment described below.

The selection process led to both strains being highly inbred, resulting in a decrease in genetic diversity. Consequently, the mating choices and host-plant preferences for oviposition displayed by the E and Z strains may be less variable than those displayed by E and Z

moths in natural populations. However, there is no *a priori* reason that the process by which we fixed the diagnostic alleles resulted in selection of any of these two traits. Indeed, the reproductive success of ECB females during the rearing was not altered by their possible host-plant preference, as the substrate provided to lay eggs was presumably neutral: it consisted of a sheet of paper (not a particular plant) placed at the top of the breeding cages. Likewise, possible differences in propensity to favor assortative mating among females should not have affected their reproductive success in our experiment. The extent of assortative mating and host-plant preferences for oviposition may have been unintentionally increased – via genetic drift or due to genetic variations being in linkage disequilibrium with the *Mpi* and *Tpi* alleles fixed in the strains – but there is little chance that these traits were not initially present in the natural populations used to create the strains. Hence, although their importance may have been artificially magnified, the mating choices and oviposition preferences observed in this experiment are probably real differences between the maize and mugwort ECB races.

Study site and experimental setting

Study site and cage setting: The experiment took place from spring 2001 to summer 2002 at a field site located at the vicinity of the INRA laboratory at Guyancourt (Ile-de-France, France, 02°04' N, 48°46' E). In this field, we set up a 2-m-high cage covering a ground area of 81 m² (9 × 9 m²), covered by a net. In May 2001, mugwort (*A. vulgaris* L.) was planted and maize (*Z. mays* L.) sown in the cage in the following pattern: three rows of mugwort separated by two strips of three rows of maize. Each row of maize contained about 90 plants and each row of mugwort contained about 15 plants. In total, the cage contained ~540 stalks of maize and ~45 stands of mugwort (each stand of mugwort had around 10 stalks).

Moth releases: From 12 June to 30 July 2001, a total of 127 virgin males of each strain (isolated from females as pupae) were introduced into the cage. During the same period, we released a total of 127 and 123 virgin females of the Z and E strains, respectively. The males of the two strains were the same age and were systematically introduced in identical numbers so that the probabilities of a female mating with a male of her own strain or with a male from the other strain were equal. Crosses, and the individuals obtained from these crosses, were expressed in the form 'female strain × male strain' (either E or Z in each case). For instance, E × Z is a cross between an E female and a Z male.

Egg mass collection and development in laboratory conditions

On six dates (23–27 and 30 July 2001), all maize and mugwort plants in the cage were carefully screened for the presence of egg masses on their leaves and on their stalks. All egg masses encountered during these screenings were immediately collected and brought back to the laboratory. For each egg mass, the plant species from which it was collected, the number of eggs and the percentage hatching were recorded. The offspring of each egg mass were reared in laboratory conditions (25°C under a 16:8 h (L:D) photoperiod) until the adult stage.

Larvae were fed on the maize flour–wheat germ–agar artificial diet described by Gahukar (1975). This diet contained fumidil to control *Nosema* infection and benzoic acid as a preservative. For each egg mass, we recorded the number and sex of emerged moths. These moths were then weighed and frozen at –80°C until electrophoretic analysis of the *Tpi* and *Mpi* loci.

Enzyme electrophoresis

Allozyme electrophoresis was performed on each moth emerged from any of the egg masses collected in the cage. We determine the genotype of individual at the *Mpi* and *Tpi* loci as described previously (Bourguet *et al*, 2000) to identify the strain(s) of its parents. Male genotypes at these two loci allow discrimination between E × E, Z × Z and interstrain parental crosses. Females being hemiploid at the *Tpi* sex-linked locus, female genotypes additionally allow discrimination between Z × E and E × Z parental crosses (Table 1).

Data analysis

We investigated whether individuals mated randomly regardless of strain by testing for a deviation of the observed frequencies of the three types of crosses (ie E × E, Z × Z or interstrain crosses) from those theoretically expected under the hypothesis of random mating (ie genotypic frequencies expected under Hardy–Weinberg equilibrium conditions). For this, we used the corresponding test in the GENEPOP 3.3 software (Raymond and Rousset, 1995).

Results

Mating choice

During the last week of July, 178 egg masses were collected from the two host plants (*A. vulgaris* and *Z. mays*). Of these egg masses, 13 (7.3%) were sterile, six (3.4%) did not give rise to a single adult and nine (5.1%) gave a few moths for which electrophoresis patterns could not be interpreted. We analyzed genotypic composition at the *Tpi* and *Mpi* loci for the progenies of the 150 remaining egg masses. This enabled us to determine the genotype of the female that laid each egg mass and of the male that mated with this female (see Table 1). As females may lay egg masses on several consecutive days, the relative proportions of egg masses on any one day were not independent from those on previous days. The results were therefore analyzed separately for each sampling date. During the week of

Table 1 *Tpi* and *Mpi* genotypes of male and female offspring of the four types of cross between the parental E and Z strains

Crosses	Offspring genotypes			
	<i>Mpi</i>		<i>Tpi</i>	
	Males	Females	Males	Females
E × E	<i>Mpi</i> 100/ <i>Mpi</i> 100	<i>Mpi</i> 100/ <i>Mpi</i> 100	<i>Tpi</i> 100/ <i>Tpi</i> 100	<i>Tpi</i> 100
E × Z	<i>Mpi</i> 80/ <i>Mpi</i> 100	<i>Mpi</i> 80/ <i>Mpi</i> 100	<i>Tpi</i> 100/ <i>Tpi</i> 120	<i>Tpi</i> 120
Z × E	<i>Mpi</i> 80/ <i>Mpi</i> 100	<i>Mpi</i> 80/ <i>Mpi</i> 100	<i>Tpi</i> 100/ <i>Tpi</i> 120	<i>Tpi</i> 100
Z × Z	<i>Mpi</i> 80/ <i>Mpi</i> 80	<i>Mpi</i> 80/ <i>Mpi</i> 80	<i>Tpi</i> 120/ <i>Tpi</i> 120	<i>Tpi</i> 120

The first letter of a cross corresponds to the female.

sampling, the relative proportions of egg masses originating from E × E and Z × Z crosses varied between 43 and 88% and between 0 and 35%, respectively (Table 2). The relative proportion of egg masses originating from interstrain (Z × E and E × Z) crosses ranged between 0 and 26% (Table 2). At five of the six sampling dates, the proportions of hybrid egg masses were significantly lower than expected if random mating occurred between moths of the two strains (Table 2). At these dates, the estimator f of F_{IS} ranged from 0.537 to 1.000. Finally, only one of the two types of hybrid cross, Z females × E males, resulted in the production of fertile egg masses during the week of sampling.

Oviposition on mugwort and maize

Only one of 47 (2%) egg masses laid by Z females was collected on mugwort, all others (98%) were collected on maize (Table 3). The deviation of the relative proportion of egg masses laid by Z females on both plant species from 50:50 was found statistically significant ($P < 0.05$) on four of the six sampling dates (Table 3). A total of 103 egg masses laid by E females were collected: 15 on maize (or 15% overall, varying between 10 and 22% depending on sampling day) and 88 (85%) on mugwort (Table 3). The deviation of the relative proportion of egg masses laid by E females on both plant species from 50:50 was found statistically significant ($P < 0.05$) on five of the six sampling dates.

Discussion

Assortative mating between the selected E and Z strains
In our experiment, the moths introduced into the cages did not mate at random: mating between individuals of the same strain occurred more often than mating between males and females of the E and Z strains. Indeed, on all sampling dates, the proportion of hybrid egg masses in the cage was lower than that expected under Hardy–Weinberg equilibrium. Females of the ECB can lay several egg masses during a night. Consequently, the number of the different types (E, Z or hybrid) of egg mass is not an ideal direct estimate of the numbers of each type of mating. However, laboratory observations showed that E and Z females laid a similar mean number of egg masses per night (Y Thomas, unpublished data). Hence, the number of the different types of egg mass should be roughly proportional to the frequency of each type of mating occurring in the cage. This estimate might be further biased because egg masses for which the offspring were not analyzed – because they were sterile, because all the offspring died before reaching the adult stage or because electrophoresis was not of sufficient quality for reliable interpretation – may have been laid preferentially following a particular type of mating. However, even if all these unanalyzed egg masses were laid by E × Z and/or Z × E crosses, the proportion of hybrid

Table 2 Proportion of egg masses laid following each type of cross, for several sampling dates and for all dates

Date	N total	N fertile	N with adults	N analyzed	Crosses				\hat{f}^a	P-value ^b
					E × E	Z × Z	Z × E	E × Z		
23 July 01	28	25	25	25	0.88	0.08	0.04	0	0.786	0.007
24 July 01	19	18	17	16	0.63	0.25	0.12	0	0.725	0.008
25 July 01	28	24	23	20	0.65	0.35	0.00	0	1.000	<10 ⁻⁵
26 July 01	23	22	22	21	0.43	0.33	0.24	0	0.537	0.025
27 July 01	25	23	22	19	0.74	0.00	0.26	0	-0.125	1.000
30 July 01	55	53	50	49	0.72	0.20	0.08	0	0.783	<10 ⁻⁵
All dates	178	165	159	150	0.69	0.20	0.11	0		

^a F_{IS} estimator of Weir and Cockerham (1984).

^bProbability of deviation from Hardy–Weinberg equilibrium.

N total = number of egg masses collected; N fertile = number of egg masses that produced offspring; N with adults = number of egg masses that produced adult offspring; N analyzed = number of egg masses that produced adults with interpretable electrophoresis patterns.

The first letter of a cross corresponds to the female.

Table 3 Proportion of egg masses laid by each type of female on each of the two host plants for several dates and for all dates

Date	Females Z						Females E					
	N	Maize	Mugwort	χ^2	df	P-value	N	Maize	Mugwort	χ^2	df	P-value
23 July 01	3	1.00	0.00	3.000	1	0.083	22	0.14	0.86	11.636	1	<10 ⁻³
24 July 01	6	1.00	0.00	6.000	1	0.014	10	0.10	0.90	6.400	1	0.011
25 July 01	7	1.00	0.00	7.000	1	0.008	13	0.15	0.85	6.231	1	0.013
26 July 01	12	1.00	0.00	12.000	1	<10 ⁻³	9	0.22	0.78	2.778	1	0.096
27 July 01	5	0.80	0.20	1.800	1	0.180	14	0.14	0.86	7.143	1	0.008
30 July 01	14	1.00	0.00	14.000	1	<10 ⁻³	35	0.14	0.86	17.857	1	<10 ⁻⁴
All dates	47	0.98	0.02				103	0.15	0.85			

Details of the test for difference between the observed proportions of egg masses on each type of host plant compared with an equal proportion on each host plant (ie 50% on maize and 50% on mugwort).

crosses would remain significantly (on July 25 and 30) or almost significantly (on July 23 and 26) lower than expected if the moths had mated randomly. Thus, the low proportion of hybrid egg masses is at least partly, if not entirely, due to positive assortative mating of the two strains.

Mating between the two strains was not symmetric. Indeed, none of the 150 egg masses analyzed during the experiment was laid by females of the E strain after mating with a Z male. All the 17 hybrid egg masses found were laid by Z females after $Z \times E$ crosses. As both $E \times Z$ and $Z \times E$ hybrids can be obtained in the laboratory (Y Thomas unpublished results), the absence of $E \times Z$ egg masses from the analysis is unlikely to be entirely due to postzygotic isolation. The asymmetry in the hybrid crosses may be rather due to the difference in male responses to the pheromone blends. Indeed, in laboratory conditions, Z males do not respond to the E blend, whereas a few E males respond to the Z blend (Glover *et al*, 1990). It is worth noting that, in North American field populations located in the state of New York, the few hybrid females recovered by Glover *et al* (1991) were consistent with hybridization occurring, like in the present study, by E males mating with Z females.

In most studies of phytophagous insects, mating has been shown to take place on or near the host plants (Prokopy *et al*, 1971, 1972; Craig *et al*, 1997; Via, 1999). In these species, assortative mating between host races is thought to evolve as a by-product of the host shift (Rice 1984, 1987). Indeed, for the host races described to date, assortative mating is often weak in the absence of the host plant (eg Craig *et al*, 1993; Feder *et al*, 1994). Our experiment cannot rule out the influence of the host plant as $E \times E$ and $Z \times Z$ mating may have occurred on mugwort and maize, respectively. However, the influence of the host plant on assortative mating is probably limited since the ECB frequently mates on nonhost plants in the field (Showers *et al*, 1976).

Host-plant preferences for oviposition

Preferences for oviposition are a common feature among host races of herbivorous insects (Drès and Mallet, 2002). For example, the larch budmoth has two host races, one feeding on larch and the other on pine (Emelianov *et al*, 2001). Females of the larch race display a strong preference for larch, but are occasionally found on pine, whereas females of the pine race are attracted mainly, but not exclusively, to pine (Emelianov *et al*, 2003). In the experiment described here, females of the two strains displayed striking differences in the relative proportions of egg masses laid on the two host plants. Females of the Z strain laid their egg masses almost exclusively on maize. Conversely, females of the E strain laid their egg masses preferentially, but not exclusively, on mugwort. If we assume that oviposition at random would have resulted in an equal proportion of egg masses laid on the two host plants, then both Z and E females displayed a significant preference for laying their egg masses on maize and mugwort, respectively.

The Z strain was selected from a population collected on maize, whereas the E strain originated from diapausing larvae collected on hop, one of the host plants of the mugwort race (Bourguet *et al*, 2000; Bontemps *et al*, 2004). Our results therefore indicate that both strains

preferred to lay their egg masses on the host plants of their own host races. Each of the host races of *Rhagoletis pomonella* is also most strongly attracted to its own natural host plant. Differences in the attractions of the host races of *R. pomonella* are based primarily on the volatile compounds produced by the host (Linn *et al*, 2003), in addition to visual cues helping the adults to find fruits within the tree canopy (Prokopy and Roitberg, 1984).

Implications for the evolution of the two host races

One of the factors that may reduce the frequency of hybridization between the maize and mugwort races is a difference in the timing of moth emergence (Thomas *et al*, 2003). In this experiment, the mating choices of E and Z moths occurred in the absence of allochronic barriers as E and Z moths were introduced into the cage at the same age. A second factor that may reduce the frequency of hybrid crosses is that moths emerging from maize use the Z pheromone blend, whereas moths emerging from hop and mugwort use the E pheromone blend (Thomas *et al*, 2003; Pélozuelo *et al*, 2004). Female sex pheromones provide long-range cues for mate-finding (Löfstedt, 1993). In the $9 \times 9 \text{ m}^2$ cage into which the E and Z strains were introduced, the influence of female sex pheromone was probably less important than in natural populations. Assortative mating probably resulted from unknown proximate cues in mate choice, although we cannot rule out an effect of the female sex pheromone.

Of the ~ 350 females analyzed by Pélozuelo *et al* (2004), none of those collected on maize produced the E blend and none of those produced on hop and mugwort produced the Z pheromone blend. Three of these females produced an intermediate ratio of E:Z isomers of $\Delta 11-14:\text{Ac}$, characteristic of hybrid females (Pélozuelo *et al*, 2004). The results reported here provide a potential explanation of the relative proportions of E, Z and hybrid moths on maize and mugwort. Indeed, in most regions of France, Z moths emerging from maize are far more numerous than E moths emerging from mugwort and hop – in France maize is grown on 3 million hectares, most of which being significantly infested by the ECB, while mugwort and most of the hop are wild-growing plants. Consequently, even if there were no barrier limiting $Z \times E$ hybrid crosses, such crosses would certainly occur at a much lower frequency than $Z \times Z$ crosses. If Z females lay their eggs exclusively on maize – like the females of the Z strain in our experiment – then hybrid larvae feeding in maize fields are likely to be much rarer than Z larvae, which may account for such larvae being detected in only very small numbers in the data set of Pélozuelo *et al* (2004). However, the difference in the density of moths emerging from maize and mugwort may favor $E \times Z$ hybrid crosses over $E \times E$ crosses. As E females preferred to lay their egg masses on mugwort rather than on maize, substantial quantities of hybrid larvae would be expected to feed on mugwort. The data reported by Thomas *et al* (2003) and Pélozuelo *et al* (2004) showed that this is clearly not the case: overall, in populations collected on mugwort, the proportion of hybrids was $< 1\%$. Our work provides a rational explanation for this very low proportion of hybrids on mugwort, as females of the E strain seem to have mated exclusively with E males.

Conclusions

This study provides new insight into genetic isolation between the two sympatric ECB host races. Thomas *et al* (2003) showed that assortative mating between these races may occur due to differences in the moth emergence timing, with mugwort-race moths emerging, on average, 10 days earlier than maize-race moths. We show here that even in situations of true sympatry and synchrony, the level of hybridization between the maize and mugwort races is lower than expected under a random mating hypothesis. This reduction may be the consequence of the difference in sex pheromone between the two host races or other undetermined cues.

Thomas *et al* (2003) showed that males emerged earlier than females in both host races, favoring one type of hybrid cross (E mugwort females \times Z maize males) over the other (Z maize females \times E mugwort males). If the pattern of mating choice observed in this experiment accurately reflects that occurring in natural conditions, then the hybrid cross (ie Z maize females \times E mugwort males), which is the most likely to occur in sympatric conditions, may be disfavored by differences in the timing of moth emergence between the two host races and between males and females. In other words, one type of hybrid (E mugwort females \times Z maize males) would be mostly prevented by the evolution of specific mating choice, whereas the other (Z maize females \times E mugwort males) would be indirectly disfavored by adaptation to ecological conditions, that is, an indirect consequence of the difference in emergence timing between the two host races.

Our results suggest that the genetic differentiation between these two host races may also be favored by host-plant preference, one of the first steps toward sympatric speciation (Kondrashov and Mina, 1986; Drès and Mallet, 2002). However, the host-plant preference for oviposition of the E and Z strains requires confirmation in moths collected from natural populations. Differences in performance on the host plants (Kondrashov and Mina, 1986; Drès and Mallet, 2002) should also be investigated.

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