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Sympatric host races of the European corn borer: adaptation to host plants and hybrid performance

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis*, is a major pest of maize crops. In Europe, two sympatric host races are found: one feeds on maize (*Zea mays*) and the other mainly on mugwort (*Artemisia vulgaris*). The two host races are genetically differentiated, seldom crossing in the laboratory or in the field, and females preferentially lay eggs on their native host species. We conducted two independent experiments, in field and greenhouse conditions, to determine whether the two host races are locally adapted to their host species. The effect of larval density and the performance of hybrids were also investigated. Despite some differences in overall larval feeding performance, both experiments revealed consistent patterns of local adaptation for survival and for larval weight in males. In females the same trend was observed but with weaker statistical support. F1 hybrids did not seem to be disadvantaged compared with the two parental races. Overall, our results showed that both host races are physiologically adapted to their native host. The fitness trade-off between the two host plants provides a potential driving force for ecological speciation in this species.

Introduction

Understanding the origin of species is a major goal of biological research, and speciation, the process by which new species are formed, is one of the most debated fields in evolutionary biology (Gibbons, 1996; Via, 2001; Gavrilets, 2003). Over the past century, the focus has shifted from the allopatry–sympatry debate to identifying the evolutionary forces involved in speciation (Schilthuizen, 2001). The relative contribution of natural selection and of other evolutionary forces is a key issue (Mallet, 2001; Rundle & Nosil, 2005). Specifically, the importance of adaptation to diverging environmental conditions has been highlighted, in the context of ecological speciation (Schluter, 2001). Speciation via host shift is one of the

most plausible modes of ecological speciation (Fry, 2003). This scenario begins with the formation of host races (host-affiliated, genetically differentiated groups within the parental species, Drès & Mallet, 2002). Suppression of gene flow between the two genetic clusters can eventually lead to the formation of two daughter species (Rundle & Nosil, 2005).

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is a polyphagous moth and notorious pest worldwide. Maize (*Zea mays* L.) was colonized after its introduction into Europe, around AD 1500. In France, ECB populations feeding on maize are genetically differentiated from sympatric populations feeding on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.), but these populations with different hosts are morphologically indistinguishable (Bourguet *et al.*, 2000; Martel *et al.*, 2003; Leniaud *et al.*, 2006). The taxonomic status of these two entities remains a matter of debate. They are sometimes considered to be sister species (Frolov *et al.*, 2007), but we shall refer to them as the

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maize race and the mugwort race. These two host races are reproductively isolated, as hybrid matings are very rare in the field (Malausa *et al.* 2005). This cannot result solely from local mating on host plants, as ECBs usually mate in dense foxtail grass and other patches of herbaceous nonhost plants (Showers *et al.*, 1976; but see Dalecky *et al.*, 2006). At least two other factors decrease the frequency of hybridization: the allochronic emergence of the two host races (Thomas *et al.*, 2003, but see Malausa *et al.* 2005) and the assortative mating of adult moths (prezygotic isolation, Bethenod *et al.*, 2005; Malausa *et al.* 2005). Moreover, females of the two host races preferentially lay eggs on their natural host plant (Bethenod *et al.*, 2005; Malausa *et al.*, in press a). Assortative mating results in part from differences in the sex pheromone blend produced by the females and attractive to males (Thomas *et al.*, 2003; Pélozuelo *et al.*, 2004). In France, the ratio of the Z and E isomers of the main pheromone component (11-tetradecenyl acetate) differs between the two races: the maize race pheromone blend contains a 97 : 3 Z/E ratio (Z blend) whereas the mugwort race blend contains an inverse ratio of 1 : 99 Z/E (E blend, Pélozuelo *et al.*, 2004).

The shift to maize occurred some 500 years ago, but molecular studies indicate that the divergence between the two extant host races may predate this host shift (Malausa *et al.*, in press b). The links between host shift and current reproductive isolation, and thus the likelihood of ecological speciation, remain unclear. In particular, the difference in pheromone blend may have been established before, during or after host shift. For ecological adaptation to have played a significant role in the divergence of the two host races, the host species should exert contrasting selection pressures, and there should be a fitness trade-off between them. In this case, antagonistic adaptations could have led to reproductive isolation, either pleiotropically (Caillaud & Via, 2000; Emelianov *et al.*, 2001; Jiggins *et al.*, 2005) or as a result of hybrid counter-selection (Via *et al.*, 2000; Rundle & Nosil, 2005).

Antagonistic adaptations are revealed by patterns of 'local adaptation' (Kawecki & Ebert, 2004). Some authors define local adaptation as better performance of a host race on its native host plant than on the other host plant (the 'home vs. away' definition). Alternatively, some describe local adaptation as better performance of a given host race on its native host plant than of other host races on that plant (the 'local vs. foreign' definition). Both definitions imply that a genotype \times environment (GE) interaction is involved in the determination of fitness (i.e. host races must have different reaction norms). They also require the reaction norms to have opposite slopes (home vs. away) or to cross (local vs. foreign). We will keep both definitions in mind, without adhering strictly to one or the other.

We present here the results of two independent common-garden experiments, in which we investigated

the larval development (survival and weight) of the two host races on their respective host plants – i.e. maize and mugwort. As adult ECBs do not feed and typically live for less than 1 week, larval performance is a major component of fitness in this species. In the Field experiment, we tried to match natural conditions as closely as possible. Maize and mugwort plants were infested with F1 individuals of the pure maize and mugwort ECB host races. Two larval densities were used to generate realistic levels of intraspecific competition. In the Greenhouse experiment, while using only one of the two larval densities, we also investigated the performance of F1 hybrids, to assess the form of the fitness trade-off and the possibility of hybrid counter-selection. We used a full-sib split-brood design, with controlled climate and host biomass, and we increased genetic variability by broader sampling of the parental populations.

Both experiments, despite their highly contrasting settings and environmental conditions, consistently showed that the host races were locally adapted. Local adaptation was particularly marked for survival, and was also observed for the weight of male larvae. For the weight female larvae, the two experiments showed consistent trends but statistical support for local adaptation was weak. Hybrids did not seem to be disadvantaged, but their performance was intermediate. These findings provide an ecological basis for the divergence of the host races, and a potential driving force for ecological speciation in this species.

Materials and methods

Sampling

European corn borer populations were sampled in 2002–2003 for the Field experiment, and in 2004–2005 for the Greenhouse experiment. Larvae of the maize race were extracted from maize stalks in October and November, in several fields around Paris (Ile de France, France, 48°46'N, 2°4'E). Larvae of the mugwort race were extracted from mugwort stands in March, in sites located around Amiens (Picardy, France, 49°90'N, 2°30'E) in 2003, and around Paris in 2005. About 1000 larvae per host race were sampled each year. Larvae were stored at 4 °C for diapause completion. In June they were placed under diapause-breaking conditions (25 °C in 2003 or 23 °C : 19 °C L : D in 2005, with a 16 h : 8 h L : D photoperiod). This is roughly the time at which diapause stops under natural conditions. After 10 days, larvae began to undergo pupation. Pupae were collected daily for subsequent use in the experiments.

Field experiment

In the first week of May 2003, 100 stalks of maize (Novartis hybrid Furio[®], Novartis Seeds, Nérac, France) were sown in a field close to Paris, and 100 stands of

mugwort were collected from one of the sampling sites. After checking for the absence of overwintering ECB larvae, stands were randomly transplanted between the maize seedlings. Each plant was 1.5 m away from its neighbours, and covered with an insect-proof net to prevent infestation by feral ECB populations.

In June 2003, pupae from the two host races were placed in distinct mating cages. A total of 49 maize race moths and about 600 mugwort race moths emerged from these pupae. Moths mated freely within each mating cage. Once all moths had emerged, egg masses were collected on three consecutive days. First instars (L1) hatching from the eggs were placed in plastic boxes at 20 °C, under a 16 : 8 h (L : D) photoperiod, and fed with fragments of the host species they were intended to infest. On 11 July 2003, all larvae belonging to the same host race were pooled, and immediately used for artificial infestation.

Larvae from the maize race and the mugwort race were introduced on the two host species. Two larval densities (number of larvae introduced per plant) were used: 10 and 30. This defined four (two host races × two larval densities) infestation treatments. Each treatment was applied to 25 randomly chosen plants within both host species.

At the end of summer, the number of surviving individuals was recorded for each plant. Larvae were weighed and dissected for sex determination.

Greenhouse experiment

The experiment took place in Montpellier (France, 43°37'N, 3°51'E), within a closed thermo-regulated greenhouse (temperatures limited to 15 °C – minimum – during the night and 25 °C – maximum – during the day). Maize (Novartis hybrid Furio®) was sown in April 2005 and mugwort plants were collected in March 2005 in Lille (France, 50°63'N, 3°7'E), from a site known to be infested by ECB. Plants of both species were grown in individual plastic pots (40 cm high and 25 cm wide) filled with sterilized soil. We checked that they were free of insects before use. We placed 40 plants of each species randomly on a grid in the greenhouse. Each plant was 40 cm away from its nearest neighbours. It was isolated from them by the pot being placed above a 10-cm-deep layer of water, and was completely enclosed in an insect-proof net. This prevented any exchange of larvae between plants. Plants were watered daily by an automatic drip/trickle irrigation device.

In July 2005, virgin moths emerging from pupae were used to form mating pairs. There were four kinds of mating pairs (female × male): Ma × Ma, Mu × Mu, Ma × Mu and Mu × Ma pairs, where Ma and Mu represent moths of the maize and mugwort races respectively. Mating pairs were left in individual plastic boxes with a moistened wad of cotton wool, until oviposition. Both parents were then removed, and egg

masses were left in the box until hatching, yielding a full-sib family of L1. We observed no difference in fecundity or egg viability between intra-race and hybrid crosses, but very strong prezygotic isolation led to only two families being obtained from Ma × Mu pairs.

Each family was split into two groups of 30 L1. The first group was transferred to a randomly chosen maize plant, and the second was transferred to a randomly chosen mugwort plant. If necessary, larvae that hatched early were fed on leaf fragments of the appropriate host species until 30 L1 were available. Infestations took place from 30 July to 15 August 2005. In November, the number of surviving individuals and above-ground dry biomass were recorded for each plant. Larvae were weighed and dissected for sex determination.

Statistical analysis

Statistical analysis was performed with R software (R Development Core Team, 2005; code available upon request). To avoid the need for multiple hypothesis tests, we used an information-theoretical model selection approach (Burnham & Anderson, 2002; Johnson & Omland, 2004; Hobbs & Hilborn, 2006) to detect patterns of local adaptation. A number of candidate models (corresponding to different biological hypotheses) were fitted to the data by maximum-likelihood (ML) methods, and were compared with the second-order expression of the Akaike Information Criterion (AIC), corrected (AICc) quantifies the extent to which the data support a given model (Burnham & Anderson, 2002; Johnson & Omland, 2004). The 'best' model (the one with the lowest AICc) and a confidence set of models (all models less than two AICc units away from the best model) were eventually defined.

The number of surviving individuals per plant was assumed to follow a binomial distribution with parameters N_i , the initial larval density, and s , the probability of survival. In both experiments, we had to compare eight probabilities of survival. For clarity, these probabilities will be coded with letters from a to h in the Results section. For the Greenhouse experiment, s was expressed as a logistic function of host biomass (MASS):

$$s = 1 / (1 + \exp(-\beta - \alpha_{\text{HOST}} * \text{MASS})).$$

Host biomass was centred around its mean value for both host species, so the intercepts β are interpreted naturally as survival probabilities on a host of average weight (\hat{s}), as $\text{logit}(\beta) = \hat{s}$. The slopes α_{HOST} represent the effect of host biomass on survival probability. We allowed a different slope for each host species.

The candidate set of models was obtained by letting the eight s (or the eight β) be either equal to, or different from, each other. This generated 4140 (the Bell number for eight elements) possibilities, ranging from 'all probabilities are equal' to 'all probabilities are different'. For the Greenhouse experiment, we also allowed both, one

or none of the slopes (α_{HOST}) be zero (four possibilities). Some biologically irrelevant models were removed *a priori* (Burnham & Anderson, 2002) to produce the definitive candidate sets.

European corn borer females are heavier than males (Beck, 1989). We therefore analysed the larval weights of females and males separately. Larval weight (quantitative variable W) was described by a linear model, assuming a Gaussian error distribution. The predictors were: (i) the host plant on which the individual developed (factor HOST, two levels); (ii) the type of F1 (factor F1, two and four levels for the Field and Greenhouse experiments respectively); and (iii) the number of larvae initially placed onto the host plant (factor N_i , two levels, for the Field experiment only). We added two covariables: (i) the number of larvae and pupae found on the host plant from which the individual was collected (quantitative variable N_f); and (ii) the biomass of the host plant centred around the mean biomass of the corresponding host species (quantitative variable MASS, for the Greenhouse experiment only). As N_f is the product $N_i \times s$, it incorporates some information from factors N_i , HOST and F1 via survival. Similarly, as mugwort plants are generally smaller than maize plants, MASS incorporates a large part of the information contained in HOST. These covariables were therefore used only in interaction terms: N_f : HOST : F1 : N_i and MASS : HOST respectively. Candidate models were built as all combinations of the predictors detailed above, together with their interactions. The GE interaction indicative of local adaptation was F1 : HOST. Residuals were checked for normality for all models in the confidence set. In the Greenhouse experiment, we also tested for a correlation between survival probabilities on maize and mugwort, across families (Spearman's rank correlation test).

Results

Larval development

We recovered about 500 individuals from the 6000 larvae initially used for infestations (Table 1). Almost all were diapausing L5, but some pupal remains were also found in the Field experiment, on maize only. This indicates that some individuals did not enter diapause and instead developed directly into adults. The proportion of such individuals was significantly higher within the maize race (24.8%, $N = 117$) than within the mugwort race (3.1%, $N = 65$; Fisher's exact test $P < 10^{-4}$). The absence of pupae on mugwort suggests that larvae of the maize race tended to develop faster on maize. No pupae were found in the greenhouse experiment, possibly due to the controlled temperature mimicking conditions at the end of summer.

Survival

Large differences in survival were observed and host races had different reaction norms in all cases (Fig. 1). Simultaneous comparisons of survival probabilities indicated that the mugwort race survived better than the maize race on mugwort, in both experiments and at both larval densities (Table 2 and Fig. 1). On maize, the maize race survived better than the mugwort race in the Field experiment, at both larval densities. In the Greenhouse experiment (density 30), the maize race also survived better than the mugwort race on maize, but the difference was slight: only the sixth best model, with an evidence ratio of 2.64, retained this difference (Table 2). In the Field experiment, at density 10, reaction norms crossed and had opposite slopes, thus satisfying both definitions of local adaptation (Fig. 1).

Table 1 Raw data for survival and weight.

Type of F1	N_i	N_p	N_{tot}	Host species									
				Maize					Mugwort				
				s (%)	♀ mean weight (SD)	n	♂ mean weight (SD)	n	s (%)	♀ mean weight (SD)	n	♂ mean weight (SD)	n
Field experiment													
Ma × Ma	10	25	250	16.4*a	146.6 (15)	13	113.4 (21)	16	6.8b	153.9 (23)	5	103.9 (14)	11
Ma × Ma	30	25	750	10.1*e	152.7 (36)	27	110.6 (14)	19	10.1f	137.3 (18)	46	97.9 (11)	30
Mu × Mu	10	25	250	10.0*c	167.4 (11)	10	121.3 (12)	12	22.0d	150.7 (27)	21	117.6 (14)	34
Mu × Mu	30	25	750	5.2g	163.4 (45)	19	113.0 (22)	17	13.3h	173.0 (22)	56	118.6 (14)	44
Greenhouse experiment													
Ma × Ma	30	11	330	24.5a	113.6 (30)	43	77.8 (17)	38	4.8b	81.2 (28)	12	75.7 (8)	4
Mu × Mu	30	14	420	21.2c	90.7 (26)	39	58.9 (17)	50	9.0d	81.3 (23)	17	62.8 (16)	20
Ma × Mu	30	2	60	10.0e	88.6 (48)	4	92.1 (9)	2	3.3f	60.7 (–)	1	26.2 (–)	1
Mu × Ma	30	11	330	21.5g	92.3 (29)	41	67.4 (20)	30	13.6h	84.6 (33)	23	70.3 (20)	22

N_i is the number of ECB L1 introduced on each plant, N_p is the number of pairs of plants used, and N_{tot} is the total number of larvae introduced on each host species. s is the overall survival probability, including larvae, and in some cases pupae (asterisks). The values of s are the eight survival probabilities to be compared in each experiment, and they are labelled with letters a to h. Mean weight (and standard deviation) of female and male larvae correctly sexed and weighed are expressed in milligrams. n is the number of larvae used for weight estimation.

*Including pupae.

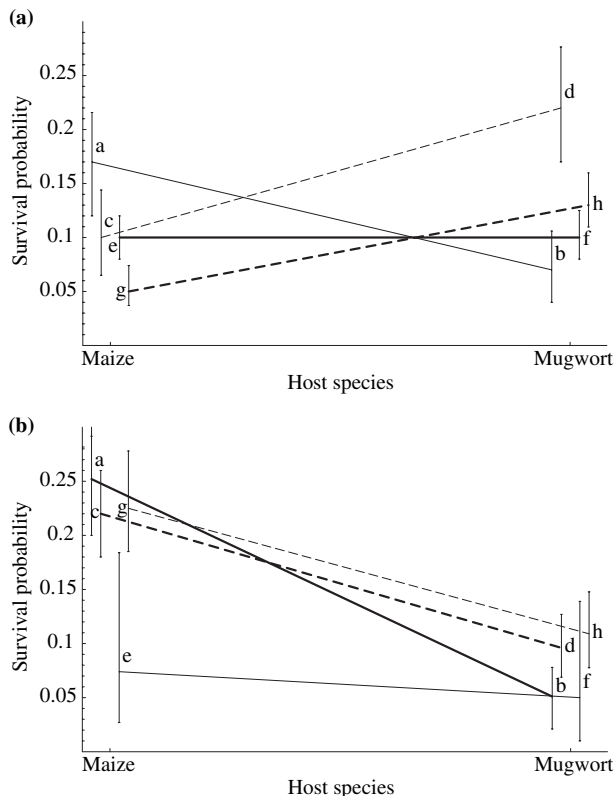


Fig. 1 Local adaptation for survival. To keep as close as possible to the data, we plotted estimates under the full model (i.e. the eight probabilities were allowed to be different). Exact 95% coverage confidence intervals are also plotted. The letter associated with each probability is indicated on the plot (see Table 1), and you can refer to Table 2 to know which are statistically different. (a) Field experiment. Thin lines: $N_i = 10$, bold lines: $N_i = 30$. Solid lines: maize race (Ma \times Ma), dashed lines: mugwort race (Mu \times Mu). (b) Greenhouse experiment. Bold lines: intra-race crosses, with solid lines for Ma \times Ma and dashed lines for Mu \times Mu. Thin lines: hybrid crosses, with solid lines for Ma \times Mu and dashed lines for Mu \times Ma. Survival probabilities are given for host plants of average weight.

Larval density had a strong effect on survival, as did host biomass (Table 2). On maize, shifting from density 10 to density 30 lowered survival probabilities in the Field experiment, as might be expected (Fig. 1). On mugwort, similar results were obtained for the mugwort race, but survival rates were slightly higher at high larval density for the maize race (Fig. 1). In the Greenhouse experiment, maize biomass had a weak negative effect on survival (Fig. 2a). The opposite trend was observed on mugwort, with a strong positive effect of mugwort biomass (Fig. 2b).

In the Greenhouse experiment, survival was furthermore globally lower on mugwort than on maize (Fig. 1). In light of the effect of mugwort biomass, this may result from the large weight difference between maize and mugwort plants that were used in the Greenhouse

experiment (Fig. 2). Mugwort plants grown in pots were smaller than typical mugwort stands grown in the field (VC, personal observation). Comparison with the Field experiment at larval density 30 goes in the same direction: survival was on average smaller in the Greenhouse experiment (Table 1). On the contrary survival on maize was about twice higher in the Greenhouse experiment than in the Field experiment (Table 1). This might be explained by the differing climatic conditions, that were less stressful in the Greenhouse experiment, or from the absence of some natural enemies in the greenhouse.

Hybrids had no obvious survival disadvantage in the Greenhouse experiment (Table 2 and Fig. 1). Mu \times Ma hybrids were systematically pooled with their maternal host race in all models in the confidence set (Table 2). Ma \times Mu hybrids tended to be grouped with their maternal race on mugwort (Fig. 1), although statistical power was too low to rule out their grouping with the other F1 (Table 2). Their survival on maize was lower than that of any of the other F1 (Fig. 1). This finding provided the only evidence of hybrid disadvantage, but as only two families were involved, these results for maize should be interpreted with caution.

Survival probabilities on mugwort and maize were positively correlated across families (Spearman's rank correlation, $\rho = 0.29$, $N = 38$, $P = 0.040$). The correlation was null for maize families ($\rho = 0.07$, $N = 11$, $P = 0.419$), strong for mugwort families ($\rho = 0.55$, $N = 14$, $P = 0.021$) and intermediate for hybrids ($\rho = 0.34$, $N = 13$, $P = 0.123$). These positive correlations indicate variability for general vigour (Fry, 1993), resulting from maternal effects or genetic variability. Although not conclusive, additive behaviour of the correlation across F1 groups supports the genetic variability hypothesis.

Female weight

For the Field experiment, the best model retained the effects of F1 and larval density, but no GE interaction (Table 2). Still, other models were also supported by the data: the F1 : HOST interaction appeared in the second and fifth best supported models, with evidence ratios of 1.42 and 2.46 respectively. Interestingly, the same pattern was observed in the Greenhouse experiment, with F1 : HOST appearing in the second model in the confidence set (Table 2). There is therefore some support for the involvement of GE interaction in determining female weight.

The reaction norms revealed a pattern of local adaptation in both experiments at density 30. In the Field experiment, the slope of the reaction norm was positive for the mugwort race but negative for the maize race (Fig. 3a). In the Greenhouse experiment, the maize race put on much less weight on mugwort than the mugwort race did (Fig. 3b). On the contrary, at density 10 in the

Table 2 Statistical analysis.

Field experiment				
Best models for survival				
1.	a/b/c/d/e = f/g/h	7	793.5	1
Best models for female weight				
1.	W~F1 + Ni + F1:Ni	5	1895.8	1
2.	W~F1 + HOST + Ni + F1 : HOST : Ni	9	1896.5	1.42
3.	W~F1 + Ni	4	1897.2	2.01
4.	W~F1	3	1897.2	2.01
5.	W~F1 + HOST + Ni + F1 : HOST + F1 : Ni	7	1897.6	2.46
Best models for male weight				
1.	W~F1 + HOST + F1 : HOST	5	1546.6	1
2.	W~F1 + HOST + Ni + F1 : HOST	6	1547.5	1.57
Greenhouse experiment				
Best models for survival				
1.	a = c = g/b = e = f/d = h	5	424.58	1
2.	a = c = g/b/d = e = f = h	5	424.88	1.16
3.	a = c = g/b = f/e/d = h	6	425.18	1.35
4.	a = c = g/b/d = h/e = f	6	426.25	2.3
5.	a = c/b/d = e = f = h/g	6	426.25	2.3
6.	a/b/c = g/d = e = f = h	6	426.52	2.64
Best models for female weight				
1.	W~F1 + HOST	6	2564.4	1
2.	W~F1 + HOST + F1 : HOST	9	2566	2.22
Best models for male weight				
1.	W~F1 + HOST + F1 : HOST	9	2219.8	1
2.	W~F1	5	2221.3	2.12

For each model in the confidence sets: formula, number of estimated parameters (K), AICc and evidence ratio (ER). Models are sorted by increasing AICc (best model in bold). Formulas for survival indicate which probabilities are statistically different from one another. Clusters of statistically equal probabilities are separated by slashes (see Table 1 for the letter codes). In the Greenhouse experiment, all models also include two slope parameters for the effect of host biomass, $\alpha_{\text{MAIZE}} = -0.01$ and $\alpha_{\text{MUGWORT}} = 0.06$. They were fitted to the data using a combined simulated annealing and Newton optimization algorithm. Formulae for larval weight use the standard GLM formulation for main effects and interactions. All models for larval weight include a variance parameter for the Gaussian error distribution, not shown in formulas. They were fitted with the R function GLM (IWLS algorithm for minimization of squared errors), and AICc were computed from residual deviances. The full models explained 14.2%/14.5% and 49.1%/47.3% (females/males) of the total deviance, for the Field and Greenhouse experiments respectively.

Field experiment, there was no pattern of local adaptation, as the slopes of the reaction norms had signs opposite to the expectation (Fig. 3a).

The F1 effect was dominant in both experiments. The two host races differed in weight, but, surprisingly, the difference was not the same in the two experiments. In the Field experiment, larvae from the mugwort race were heavier (142 vs. 120 mg), whereas in the Greenhouse experiment, larvae of the maize race were heavier (94 vs. 72 mg, Table 1). Consistent with the survival data, $\text{Mu} \times \text{Ma}$ hybrids had a very similar reaction norm to their maternal race (Fig. 3b).

The effect of larval density (N_i) was strong in the Field experiment (Table 2), but differed according to host race. Females were not always heavier at low than at high larval density (Table 1 and Fig. 3a). For instance, on

mugwort, larvae of the mugwort race tended to be heavier on plants infested with 30 L1 than on those infested with 10 L1 (Table 1).

In the Greenhouse experiment, HOST had a strong effect, females grown on mugwort being on average 17% lighter than those grown on maize (Table 1). As for survival, this effect may be partly accounted for by the difference in size between the two host species (see Fig. 2). The effect of HOST on larval weight may therefore be similar to the strong effect of larval density found in the Field experiment.

Male weight

For the Field experiment, the GE interaction (F1 : HOST) was present in the two best supported models (Table 2).

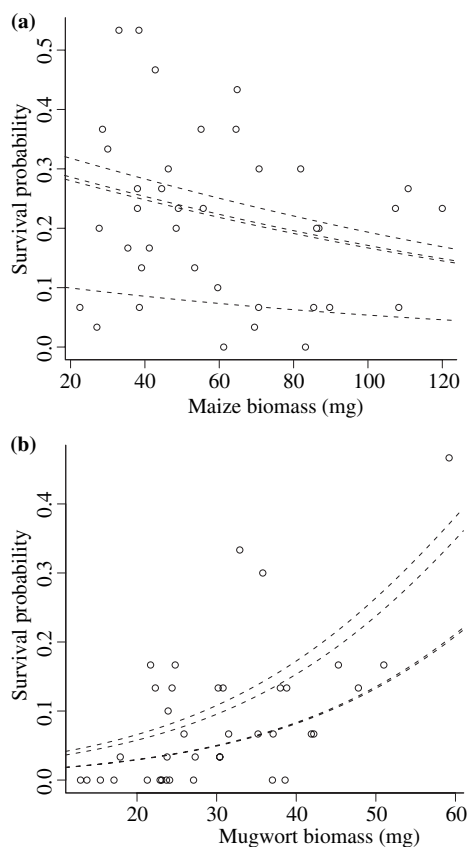


Fig. 2 Effect of plant biomass on survival, for (a) maize and (b) mugwort. Each point represents a pool of 30 L1 introduced on a plant. Dashed curves show logistic regression under the full model, for each F1.

Consistently, the best model for the Greenhouse experiment retained the GE interaction (F1 + HOST + F1 : HOST, Table 2). This indicates very strong support for GE interaction, and the reaction norms revealed a clear pattern of local adaptation (Fig. 3c and d). For instance, at density 30 in the Field experiment, males from the mugwort race were 2.1% heavier than those of the maize race on maize, whereas they were 21.5% heavier on mugwort (Table 1). In both experiments, the relative decrease in weight on mugwort was much smaller for the mugwort race than for the maize race, and at density 30 in the Field experiment, the mugwort race was even heavier on mugwort than on maize (Fig. 3c).

As for females, F1 had a strong effect. Again, the weight difference between host races differed in the two experiments, and the pattern was qualitatively similar to that for females. However, the pattern for hybrids was different, as Mu × Ma male hybrids had intermediate weights (Fig. 3d).

Discussion

Host plant local adaptation

The two host races of the ECB differed considerably in terms of larval development. We showed that they were locally adapted to their host plants for two major fitness components: larval survival and larval weight. The pattern was particularly striking for survival, with two-fold differences in the survival probabilities of the two host races under some conditions. As larval survival is a crucial life-history trait, the resulting differences in fitness may be very important. In both experiments, local adaptation was also observed for male weight. Heavier males, although no more fertile than lighter ones (Royer & McNeil, 1993), may be less likely to die during diapause (Tauber *et al.*, 1986). At the adult stage, they may also have an advantage in male–male competition for access to females (Begon *et al.*, 1996). For female weight, some evidence for local adaptation was obtained in the Greenhouse experiment, even though this hypothesis was weakly supported. Heavier females, like males, may be more likely to survive during diapause, and would also be expected to be more fecund (see, e.g. Iyengar & Eisner, 1999).

Thus, host races have a fitness advantage on their native host plant. In some cases, both criteria for local adaptation – i.e. the local vs. foreign and the home vs. away – were met, as for survival probabilities at larval density 10. In other cases, only one criterion was met. For instance, the local vs. foreign definition held for survival probabilities in the Greenhouse experiment. Local adaptation according to this definition is little documented (e.g. Hawthorne & Via, 2001). By contrast, local adaptation according to the alternative home vs. away definition has repeatedly been reported in Coleoptera (Katakura & Hosogai, 1994), Homoptera (Via *et al.*, 2000), Diptera (Craig *et al.*, 1997) and Hemiptera (Carroll *et al.*, 1997, 1998).

Strict adherence to one of these definitions could have biased our interpretations. Indeed, the failure to detect local adaptation according to the home vs. away criterion probably resulted from intrinsic differences in host quality (e.g. differences in mean host biomass in the Greenhouse experiment), whereas failure to meet the local vs. foreign criterion resulted from intrinsic differences between host races (e.g. differences in mean weight for males in the Field experiment). However, in all cases, fitness was partly determined by a GE interaction, and reaction norms were such that host races had a better response (fitness change) than the other races when moving from their alternative to their native host plant. We argue that this is the most general and operational criterion for local adaptation.

Theoretical models of resource specialization (Levene, 1953; Maynard-Smith, 1966) predict that the fitness advantage required for the sympatric coexistence of host

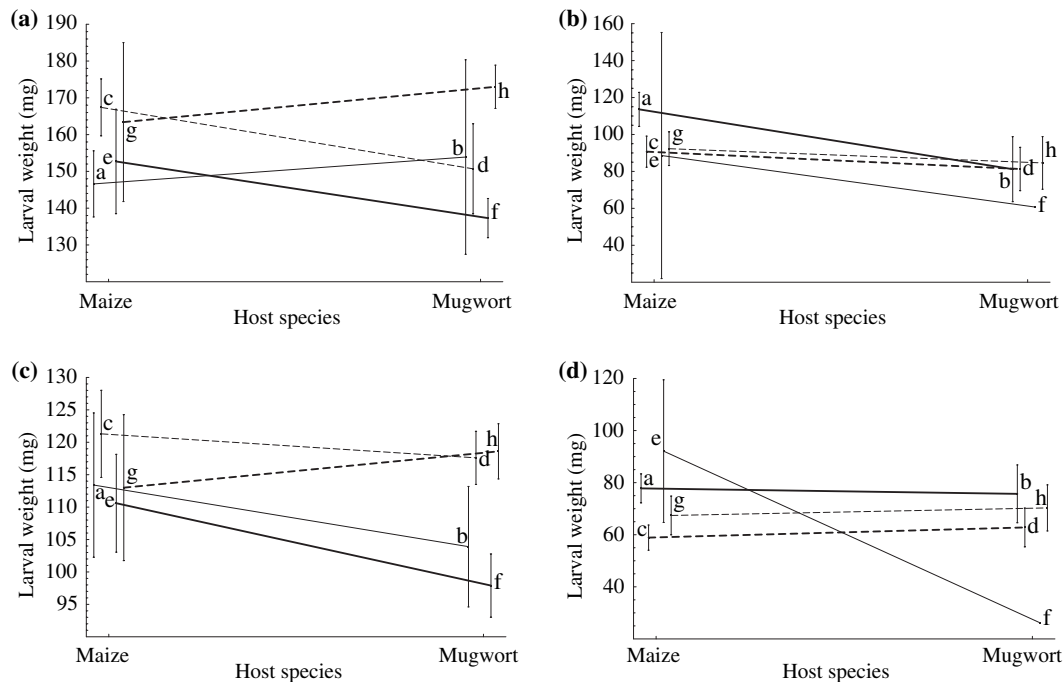


Fig. 3 Local adaptation for larval weight. To keep as close as possible to the data, plotted values are raw values taken from Table 1. You can refer to Table 2 to know which effects are statistically meaningful. Field experiment, for females (a) and males (c). Thin lines: density 10, bold lines: density 30. Solid lines: maize race, dashed lines: mugwort race. Greenhouse experiment, for females (b) and males (d). Bold lines: intra-race crosses, with solid lines for Ma \times Ma and dashed lines for Mu \times Mu. Thin lines: hybrid crosses, with solid lines for Ma \times Mu and dashed lines for Mu \times Ma. The corresponding letter codes for survival probabilities (Table 1 and Fig. 1) are also shown.

racess is smaller on the most abundant resource (Ravigné *et al.*, 2004). Maize is clearly more abundant and sustains larger ECB populations than mugwort, at least in the zone of sympatry (Martel *et al.*, 2003). Our results for survival probability are thus consistent with this prediction: on average, the maize race had a smaller fitness advantage on maize than the mugwort race had on mugwort.

As ECB host races preferentially lay their eggs on their native host plant (Bethenod *et al.*, 2005; Malausa *et al.*, in press a), our results indicate that oviposition preference and subsequent offspring performance are positively correlated. This is a classical expectation from optimal oviposition and host shift speciation theory (Mayhew, 2001; Gavrillets, 2004). Surprisingly, it has seldom been validated – see Via (1986) for Diptera, Hawthorne & Via (2001) for Homoptera and Agrawal (2000) for Acari – indeed, this correlation is often been found to be null or negative (Mayhew, 2001; Bossart, 2003).

Density dependence

There was evidence for density-dependent larval development. We manipulated larval density in the Field experiment, whereas we studied the effect of host plant

biomass at constant larval density in the Greenhouse experiment. These two approaches yielded slightly different results. The main trend was negative density dependence, with poorer performance at high density. This would be expected based on competition for food and/or space (Underwood & Rausher, 2002; Agrawal, 2004). However, previous studies on *O. nubilalis* reported no differences in larval survival rates on maize at different initial densities (from 30 to 120 larvae, Reh & Ohnesorge, 1988). Negative density dependence should result in some local regulation of population size, generating soft selection in theoretical models and favouring the coexistence of host races and host shift speciation (Levene, 1953; Gavrillets, 2004; Ravigné *et al.*, 2004). Some less intuitive results indicating positive density dependence were obtained on maize, in the Field experiment. This suggests that mechanisms other than intraspecific competition may also be operating (Underwood & Rausher, 2002; Rotem & Agrawal, 2003). For instance, maize is known to induce the synthesis of a cysteine proteinase around tissues damaged by insects, and this proteinase is effective against lepidopteran larvae (Pechan *et al.*, 2000). When controlling for host biomass in the Greenhouse experiment, we might have been controlling for host vigour as well as larval density. If more vigorous plants were more able to defend them-

selves against caterpillars, this would have concealed the effect of larval density, possibly inverting the overall trend from negative to positive density dependence.

Hybrid performance

Hybrid counter-selection reduces effective gene flow and can select for assortative mating, thereby favouring host race formation and host shift speciation (Gavrilets, 2004). In the Greenhouse experiment, F1 hybrid performance was mostly similar to that of the maternal host races or intermediate. The only indication of hybrid disadvantage was the poor performance of Ma × Mu hybrids on maize. Interestingly, this is also the hybrid cross for which prezygotic isolation was stronger. However, as only two families were used, further confirmation of this result is required. Overall, our results suggest that hybrids are not strongly disadvantaged and that the fitness trade-off curve is rather concave in shape (Ravigné *et al.*, 2004).

Maternal/cytoplasmic effects may be responsible for this, and more experiments, preferentially involving backcrosses, are required to assess the shape of the trade-off curve robustly. Hybrids may be disadvantaged at the adult stage, particularly during reproduction given that their sexual pheromone blend is altered (Pérezuelo *et al.*, 2004). The reproductive performance of hybrid adults should therefore be investigated to confirm our result.

The absence of strong hybrid counter-selection (post-zygotic isolation), such as hybrid nonviability, is compatible with a recent divergence of the two host races. Indeed, if reproductive isolation had established a very long time ago, well before the host shift, then we would expect the fixation of incompatible mutations to have produced (intrinsic) post-zygotic isolation, as in classical allopatric speciation models (Gavrilets, 2004). Conversely, ecological speciation via host shift does not rely on strong post-zygotic isolation, and several models actually predict ecological speciation and coexistence even when hybrids have intermediate performance (Maynard-Smith, 1966; Gavrilets, 2004).

Conclusion

The ECB seems to be a good candidate for ecological speciation via host shift. Two host races are found in sympatry, and the host shift on maize can be dated to the introduction of this new crop in Europe. Several mechanisms of reproductive isolation have been documented in recent years: allochrony, assortative mating and host choice for oviposition. However, it remained to be demonstrated that ecological adaptation to host plants could have driven the divergence. The two independent experiments reported in this paper, and performed in very different settings, showed that the two host races are locally adapted to their native host species. These findings provide a firm ecological basis for the sympatric

coexistence of the two host races, and a potential driving force for ecological speciation.

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