A host shift of diamondback moth from crucifers to peas: Life history traits and genetic mechanisms

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ABSTRACT

The diamondback moth (DBM) is a well-known specialist on cruciferous plants. However, in 2001, a host shift to commercially-grown sugar pea was reported from Kenya. To study this phenomenon further, we compared five laboratory strains, including the pea-adapted strain from Kenya (DBM-P). In feeding trials on cabbage and pea, DBM-P larvae performed comparably well on the two hosts, whereas larvae of the other strains could not survive on pea. The genetic diversity within and between 10-12 individuals of the five strains was investigated using microsatellites and AFLPs. Statistical analyses of the AFLP data revealed high genetic diversity between (Fst = 0.297) and within the strains. To study the inheritance of “pea-feeding”, we conducted single pair matings between DBM-P strain and a cabbage-adapted strain followed by a backcross to either the cabbage (BC I) or the pea-adapted (BC II) strain or by intercrossing the F1 to produce an F2. The results suggest recessive genes to be responsible for the trait “feeding ability on pea”.

INTRODUCTION

Most insect species are phytophagous specialists, using only a limited subset of host plants as food resources, mating locations and oviposition sites (Bernays and Chapman 1994, Schoonhoven et al. 2005). The diamondback moth (DBM), Platella xylostella (L.) (Lepidoptera: Plutellidae), is known to be a specialist restricted to cruciferous plants and in recent years has become the most destructive insect of crucifers throughout the world (Talekar and Shelton 1993). However, in 1999, P. xylostella was first collected from a heavily infested sugar snap pea (Pisum sativum, L.) field in Naivasha in central Kenya and in the following year, P. xylostella also invaded neighbouring mangetout pea fields (Löhr 2001). This would mean that a novel phylogenetically distant plant was incorporated to the host range of the specialist insect P. xylostella.

The host shift of P. xylostella to peas has just been reported (Löhr 2001). So far, research on this host shift has been carried out only by Löhr and Gathu (2002), who studied the feeding performance of the pea-adapted population and selected a cabbage-adapted strain for survival on peas; and by Löhr and Rossbach (2004) who analyzed the impact of the host shift on the parasitoids of diamondback moth.

The underlying mechanisms of this host shift are so far not understood. This study was a first attempt to get initial information on the genetic mechanism of the trait “feeding ability on pea”. Previous studies investigating host plant preferences in phytophagous insects used crosses to determine the mode of inheritance (Tang et al. 2006, Fox et al. 2004). Furthermore, a backcross design has already been used to study other
traits in DBM, such as Bt resistance (Heckel et al. 1999). A first assumption on the underlying pattern of inheritance might be a single dominant or recessive gene being responsible for the trait. However, more complex mechanisms might also be involved. Studies on host preference in butterflies report a strong sex-linked component of host-plant preference (Janz 1998). Fox and Savalli (2000) discovered that maternal effects mediate host expansion in a seed-feeding beetle. Maternal effects or sex-linkage might also play a role in the host shift of diamondback moth to peas, and therefore could be first hypotheses to be tested in a study on the host shift.

**MATERIALS AND METHODS**

**Insects**

Colonies of NO-QA and Waite were maintained as continuous cultures in the laboratory under controlled photoperiod (L16: D8) and temperature (21 ± 1°C). Adults were supplied with a 5% solution of honey in water. Larvae of both strains were reared on kale. Colonies of DBM-C and DBM-P were maintained under controlled conditions (L16: D8; 27 ± 1°C), adults were supplied with 5% honey solution and larvae were reared on cabbage (DBM-C) and pea (DBM-P), respectively. The Geneva 88 strain was reared in the department of Genetics and Evolution at the MPI in Jena, where it was kept at 27 ± 1°C and L16: D8 in environmental chambers.

**Plants**

Plants used for rearing of colonies and for the experiments were kale (Brassica napus), cabbage (Brassica oleracea var. Gloria) and pea (Pisum sativum var. Oregon Sugarpod), reared in the greenhouse at 21-23°C, 50%-60% RH, L14: D10 in Klassmann Tonsubstrat.

**Crosses**

Male and female moths from strains Waite and DBM-P were mated to produce F1, F2 and backcross progeny. All matings were performed reciprocally. Thus, for example, F1 offspring were obtained from both Waite male × DBM-P female and DBM-P male × Waite female crosses. For BC I, the Waite parents of these crosses had been reared on kale and the DBM-P parents on pea. For BC I the backcross was performed to the Waite strain and for BC II the F1 was backcrossed to the DBM-P strain. Matings were performed in paper containers covered with gauze. Adults were supplied with 5% honey solution. Containers were checked daily for egg laying. In Backcross (BC) I, eggs of the F1 generation as well as of F2 and backcross generations were distributed evenly on 3 week-old pea and cabbage plants and larval survivorship on the respective host plants was recorded. For BC II (backcross to DBM-P strain) intra-strain crosses (parental generation) were performed prior to the inter-strain crosses. The offspring of the intra-strain crosses as well as F1 offspring from the inter-strain crosses were reared on kale. Only backcross and F2 offspring were reared on pea and cabbage plants, with 1/3 of the eggs transferred to kale and 2/3 to pea. After pupation, backcross and F2 offspring were frozen for molecular analyses. All steps were carried out in a climate chamber at 21 ± 1°C and L16: D8.

**No-choice Feeding Experiment**

Two treatments were compared: P. xylostella feeding on cabbage and P. xylostella feeding on pea. The no-choice feeding experiment was set up in a transparent plastic box (9L volume). 3 week-old cabbage and
pea plants were taken. In total, 50 eggs of each strain were used, with 25 eggs per host plant species and a single egg per plant. The recorded parameter was survivorship on the respective host plant. The experiment was carried out in an environmental chamber under controlled conditions (L16: D8; 21 ± 1°C).

Molecular Analyses

Genomic DNA extraction was performed according to a modified protocol from Reineke et al. (1998), using CTAB protocol and Qiagen TissueLyser.

AFLP analysis for studying genetic diversity between the strains was carried out with 60 individuals of the five diamondback moth strains (12 individuals per strain). AFLP reactions were carried out according to a protocol by Wilding et al. (2001) and run on a LI-COR™ DNA Analyzer 4300.

Data Analysis

AFLP gels were scored for polymorphic loci using Saga™ Version 3.0 (LI-COR™) software. Data obtained were used to compute genetic diversity indices using software AFLP-SURV version 1.0 (Vekemans et al. 2002) and Hickory v1.0 (Holsinger and Lewis 2003). Nei’s genetic distance tree and neighbour-joining tree were constructed using software PHYLIP (Felsenstein 1995).

RESULTS

No-choice Feeding Experiment

The feeding experiment showed that larvae from the pea-adapted strain performed comparably well on both hosts, cabbage and pea, whereas larvae of the three cabbage-adapted strains (Waite, NO-QA and DBM-C) and larvae from the strain reared on artificial diet (Geneva 88) could not survive on pea (Figure 1).

![Figure 1: Survivorship of five DBM strains on cabbage and pea](image)

Genetic Diversity

The genetic diversity within and between the five strains was determined by scoring 162 polymorphic AFLP loci and using 9 primer combinations. Figure 2 shows an unrooted Nei’s genetic distance tree for the
five analysed strains of *P. xylostella*. The tree shows the genetic distance between the five strains. All five strains are quite genetically diverged, with Geneva 88, Waite and NO-QA showing the highest divergence between each other. The two Kenyan strains (DBM-C and DBM-P) are also genetically diverged but compared to the genetic distances among the other strains their common regional origin is represented by a lower genetic distance. As bootstrap values are below 50 they are not presented in the figure. The extent of genetic diversity within the five strains is shown in Figure 3 using the neighbour-joining method by Saitou and Nei (1987). Five clusters were obtained and as expected, each strain forms a cluster containing the individuals of the respective strain. The inhomogeneous branch lengths reflect the genetic heterozygosity within each strain.

![Figure 2](image1.png)

**Figure 2** Unrooted Nei’s genetic distance tree for five diamondback moth strains based on 162 AFLP markers

![Figure 3](image2.png)

**Figure 3** Neighbour-joining tree for 58 individuals from five diamondback moth strains based on 162 AFLP markers

The genetic diversity between the five strains was further determined by using a Bayesian approach. This method calculated an $F_{ST}$ value of 0.29 for the genetic diversity (Figure 4).
Backcross Experiment

In BC I (backcross to cabbage adapted strain), F₁ progeny were tested on cabbage or pea. F₁ progeny descending from a pea-adapted mother had higher survivorship on pea (14%) than did F₁ progeny of cabbage-adapted mothers (2%). Also, the backcross progeny of BC I was tested on the host cabbage and pea (Table 1). Among BC I progeny, survivorship was uniformly low (1%–3%). A single F₂ family showed a high survivorship on pea (38%).

In BC II (backcross to pea-adapted strain) both parental strains and all F₁ progeny were reared on kale to minimize maternal effects. Among backcross progeny of BC II, survivorship on pea was higher (21%–40%) than in BC I (Table 1). Furthermore, offspring from a pea-adapted grandmother showed a higher survival rate on pea than offspring from a pea-adapted grandfather. Survivorship on kale was high without showing an obvious pattern. The F₂ family showed lower survivorship on pea (8%) than would have been expected from BC I, where both F₁ parents had been raised on pea.

Table 1  Results of Backcross I (BC I to cabbage strain (C)) and Backcross II (BC II to pea strain (P)). F₁ parent reared on ‘cabbage or ‘pea

<table>
<thead>
<tr>
<th>Generation</th>
<th>Cross</th>
<th>Host</th>
<th>No. Eggs</th>
<th>No. Pupae</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC I F₁</td>
<td>Gm × Pfm</td>
<td>Cab</td>
<td>382</td>
<td>219</td>
<td>57</td>
</tr>
<tr>
<td>Pm × Cf</td>
<td>Cab</td>
<td>80</td>
<td>40</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Gm × (C)P f</td>
<td>Cab</td>
<td>558</td>
<td>449</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>(C) m × Cf</td>
<td>Cab</td>
<td>201</td>
<td>124</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Gm × (C)P f</td>
<td>Cab</td>
<td>266</td>
<td>246</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>(C) m × Cf</td>
<td>Cab</td>
<td>122</td>
<td>98</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>F₂</td>
<td>F₁ intercross</td>
<td>Cab</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>BC II Backcross</td>
<td>Pm × (C)P f</td>
<td>Kale</td>
<td>115</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Pm × (PC) f</td>
<td>Kale</td>
<td>229</td>
<td>109</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>278</td>
<td>217</td>
<td>78</td>
</tr>
</tbody>
</table>
### DISCUSSION

The results obtained in the no-choice feeding experiment showed that the cabbage-adapted strains are not able to complete their life cycle on the new host pea. Lühr and Gathu (2002) showed in their selection experiment that survival on pea is possible for individuals from a cabbage-adapted strain; however, they started with an initial number of 250 eggs whereas in our case only 25 eggs of each strain were exposed to pea. It was also assumed that the Geneva 88 strain might have a chance to survive on the new host pea, as it is reared on a glucosinolate-free diet and therefore might not rely on these as feeding stimulants and thereby initiate feeding on pea. But contrary to our assumption, this strain could not survive on pea and even showed the lowest survival rate on cabbage. Based on these results, the Waite strain showing the highest survivorship on cabbage among the cabbage feeding strains was chosen as a crossing partner for the pea-feeding strain in the backcross experiment.

The analysis of the genetic diversity revealed a high genetic differentiation within as well as between the strains. For analysing the inter-strain genetic diversity a program called AFLP-SURV was used. An \( F_{ST} \) value of 0.1626 (\( P < 0.001 \)) was calculated, which according to the interpretation of Hartl and Clark (1997), indicates a great genetic differentiation between the strains. This value was supported by a second analysis using the software Hickory, with a Bayesian approach, in which an \( F_{ST} \) value of 0.29 was computed. The heterogeneity within the five strains was unexpected as laboratory strains were analysed and therefore a high degree of inbreeding and thus homogeneity within the strains would have been expected. Including data from wild type individuals of DBM could help to give a more definite statement.

The backcross experiment was carried out to identify the mode of inheritance of the trait “feeding ability on pea”. The fact that survivorship in the \( F_1 \) generation of BC I occurred at all suggested to us that a partially dominant gene might be responsible for survivorship on pea. However, a maternal effect was also evident as survival on pea was highly determined by the type of mother, with offspring coming from a pea-adapted mother having a higher survivorship on pea. Sex-linkage cannot be responsible here as it would predict the opposite effect. The first assumption of a partially dominant gene being responsible for the trait directed our choice of the cabbage-adapted strain as backcross partner in BC I. However, the low numbers of survival on pea in the BC I backcross generation, together with the high survivorship of \( F_2 \) progeny on pea would be consistent with the hypothesis of one or more recessive genes being responsible for adaptation to pea possibly augmented by the strong maternal effect. To test the assumption of one or more recessive genes being responsible for the trait “feeding ability on pea”, in BC II the backcross was performed to the pea-adapted strain. Furthermore, to avoid any maternal effect, both parental strains and all \( F_1 \) progeny were reared on kale. The survivorship on pea among backcross progeny from BC II was higher than in BC I, which might support our hypothesis of recessive genes being responsible for the ability to feed on pea. Unexpectedly there was a strong grandmaternal effect. The survivorship on pea was higher among progeny descending from a pea-adapted
grandmother than from a cabbage-adapted grandmother. We are unaware of any other examples of such a grandmaternal effect.

The mating design of crossing two different strains or two closely related species for studying the heredity of traits in Lepidoptera is well documented (Heckel et al. 1999, Heckel et al. 2004, Dopman et al. 2004, Marcus 2005) and the knowledge about the biphasic nature of lepidopteran genetics facilitates these analyses. This approach has already been used successfully to map and identify genes associated with pesticide resistance (Heckel et al. 1998, Heckel et al. 1999) in *P. xylostella* and other moth species. Molecular analyses of BC II-progeny in order to generate a linkage map for the trait “feeding ability on pea” are currently in progress.

**Acknowledgements**

We would like to thank Bernhard Löhrt for the DBM-P and DBM-C strains, and the Max-Planck-Gesellschaft for financial support.

**REFERENCES**


Vekemans, X. (2002) AFLP-SURV version 1.0. Distributed by the author, Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.