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Polymorphism in a flea beetle for the ability to use an atypical host plant

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The flea beetle *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae: Alticinae) is polymorphic for its ability to use *Barbarea vulgaris* R. Br. (Brassicaceae) as a host plant. The genetic factors influencing this ability show both sex-linked and autosomal inheritance. Evidence was found for the presence of major genes such as those found in earlier studies, but also of genes with a smaller effect which have not previously been found. Although the ability to survive on *B. vulgaris* exists in most populations in eastern Denmark, it is usually at a low frequency. Beetles collected on *B. vulgaris*, however, usually produced larvae that survived on this plant. The inheritance and the abundance of the ability to use *B. vulgaris* are discussed in the context of the evolution of the interaction between *P. nemorum* and its atypical host plant.

Keywords: major genes; *Phyllotreta*; sex linkage; polymorphism

1. INTRODUCTION

The interactions between phytophagous insects and their host plants provide ideal systems to study evolutionary processes. Many plant species are chemically defended against herbivory, and the successful utilization of a host plant by a phytophagous insect requires adaptations on the physiological and behavioural levels (Via 1990). To understand the evolution of host-plant use, the genetics of these adaptations need to be studied (Thompson 1994). Much research has been devoted to the study of interactions between phytophagous insects and their host plants, but detailed knowledge about the genetic background of those interactions has only been obtained for a few cases (Desroches *et al.* 1997; Grenier *et al.* 1997; Hare & Kennedy 1986; Hatchett & Gallun 1970; Hatchett *et al.* 1993; Lu & Logan 1994*a*,*b*; Nielsen 1997*a*,*b*; Puterka & Peters 1989; Via 1990). The colonization of *Barbarea vulgaris* by the flea beetle *Phyllotreta nemorum*, an event which appears to have occurred recently (Nielsen 1997*b*), provides an opportunity to study *Barbarea*–flea interactions at different levels, including the genetic aspects.

The larvae of *P. nemorum* are leafminers on a limited number of Cruciferae, while the adult beetles feed on the same plants. In Denmark, some plants from the genus *Barbarea* are suitable host plants for *P. nemorum* (Nielsen 1996). However, *P. nemorum* cannot complete its larval development on plants from the majority of populations of *B. vulgaris* ssp. *arcuata*, although plant suitability for larval development varies throughout the season and also between leaves of the same plant (Nielsen 1997*a*). A population has been found in Denmark (Ejby) where large numbers of *P. nemorum* were feeding on the normally unsuitable *B. vulgaris* (Nielsen 1997*a*). Laboratory studies have shown that beetles originating from the population on *B. vulgaris* produced larvae that were able to complete development on this plant, while the majority of larvae from another population were not. Results from crosses between beetles from which larvae were and were not able to survive on *B. vulgaris*, respectively, are consistent with the presence of major genes, the dominant alleles of which conferred larval survival. These genes have been called 'resistance' (*R*) genes in earlier studies (Nielsen 1997*b*), referring to the resistance of carriers of these genes to plant defences (for another example of this terminology see Jones (1998)). In this study we will adopt the same terminology, and the terms 'resistant' and 'susceptible' in this paper refer to the beetles (not to the plants). The percentage of progeny of a resistant–susceptible cross surviving on *B. vulgaris* yielded information about whether the resistant beetle was heterozygous for the *R*-gene, or whether this beetle was homozygous or carried more than one resistance gene. Based on the sex ratio of survivors on *B. vulgaris*, it was concluded that at least two sex-linked genes are involved (on the X- and Y-chromosome, respectively) as well as one or more autosomal genes (Nielsen 1997*b*).

To understand the evolution of the interaction between *P. nemorum* and its host plants, information is required about the presence of *R*-genes in different flea beetle populations. This paper describes the abundance of genetic factors with sex-linked and autosomal inheritance in *P. nemorum* populations in eastern Denmark, where evidence was sought for the presence of major genes similar to those described above. The aim was to discover to what extent

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Figure 1. Map of the localities in eastern Denmark where collections were made. 1: Gniben; 2: Lynæs; 3: Solbjerg; 4: Skuldelev; 5: Bjergsted; 6: Assentorp; 7: Taastrup; 8: Amager; 9: Kværkeby; 10: Myrup; 11: Maglebrænde; E: Ejby.

the populations previously studied (Taastrup and Ejby; Nielsen 1997*b*) are representative for the presence of *R*-genes in other flea beetle populations in eastern Denmark.

2. MATERIAL AND METHODS

On various sampling occasions between 1992 and 1997 adult *P. nemorum* beetles and/or larvae were collected on several host plants at 11 sites on the islands Zealand and Falster in eastern Denmark (figure 1). The plants on which sampling took place included *Sinapis arvensis*, *Rorippa islandica*, *Armoracia lapathifolia*, *Barbarea vulgaris* ssp. *arcuata* (G-type and P-type; see Huang *et al.* 1994; Nielsen 1997*a*; the G-type can be morphologically and chemically distinguished from the P-type, and is unsuitable for larval development of *P. nemorum*), *Raphanus raphanistrum* and *Cardaria draba*. Adult beetles were collected using an aspirator, larvae by picking the leaves in which they had mined. All sampling took place in May–August (usually in July–August), which is the period that the G-type of *B. vulgaris* is least suitable for susceptible *P. nemorum* (Nielsen 1997*a*).

In the laboratory, adult beetles were sexed under a stereo microscope (25×), using ventral abdominal characters (females have a narrow, median groove on the caudal end of the abdomen, while in males this groove is much more pronounced, forming a cone-shaped depression) and the antennae (in males the third and fourth segment of the antennae are clearly thicker than the other segments). The leaves containing larvae were put in plastic vials (500 ml), closed with a plastic lid with a central hole (diameter 1.5 cm), which was closed with cotton wool. The vials contained a layer (*ca.* 5 cm) of moist vermiculite (medium grain) or a mixture of moist peat and vermiculite. Fully grown larvae dug themselves into the vermiculite/peat, in which they pupated. Emerging beetles (after about two weeks) were sexed and the different sexes were kept separate. Beetles were either used in crosses immediately (see below), or diapause was induced by feeding them for 2–4 weeks (1–2× per week) with radish leaves (plants were grown at 20 °C and a L18:D6 photoperiod) and keeping them under low light conditions at about 20 °C. After about two weeks beetles thus kept stopped feeding and were transferred to small plastic vials (12.5 ml) containing a folded piece of tissue paper and covered with a piece of nylon netting kept in place by a plastic lid with a central hole (maximum ten beetles per vial). The small vials were kept in a 500 ml vial with a moist piece of filter paper on the bottom and sealed with a lid, which was put in a refrigerator (4 °C). Diapausing beetles could thus be kept for up to one year, and they were used for crosses after a period of minimal two months in the refrigerator.

Crosses were set up between males originating from field populations and females from the same population or virgin females from a line of susceptible beetles (unable to survive in *B. vulgaris*: ST-line; see Nielsen 1996, 1997*b*). Pairs were kept in plastic vials (158 ml) with a moist gypsum/charcoal bottom layer (Nielsen 1978) closed with a lid with a cotton wool stopper. They were kept at 24 °C and a photoperiod of L18:D6. Beetles were fed three times per week with radish cotyledons or young radish leaves. Eggs were laid in crevices in the bottom layer and beetles were transferred to new vials every 5 d or when eggs hatched (5–6 d).

Neonate larvae emerging in the vials were used in bioassays to test their ability to survive on *B. vulgaris*. *B. vulgaris* ssp. *arcuata* (G-type) seeds were collected at Herlev in 1994 (accession no. 3; Nielsen 1997*a*). They were sown in a peat/vermiculite mixture and plants were grown at 20 °C, L18:D6. Detached rosette leaves from these plants were individually put into small, plastic vials (25 ml) together with a moist strip of filter paper. Larvae were picked up with a moist paintbrush (size 000) and five larvae were transferred to each leaf. Vials were sealed with a plastic lid and labelled. About 50–100 larvae of each cross were thus used in bioassays. Survival of the larvae was measured after 3–4 d at 22±2 °C and L18:D6 using a stereo microscope (×6). Surviving, resistant larvae were at that time close to the first moult and were substantially larger than susceptible larvae that had died in very small mines or were found on the surfaces of leaves without any marked growth. Leaves with surviving larvae were transferred to plastic vials (500 ml) with a layer of moist vermiculite (see above). Fresh *Barbarea* leaves were regularly added, and old leaves were removed as larvae entered the new ones and eventually pupated in the vermiculite. The sex ratio of emerging beetles was determined. Larval survival was evaluated using the range of survival in previous studies (Nielsen 1997*b*; P. W. de Jong and J. K. Nielsen, unpublished data) to define classes of survival supporting the presence of major genes similar to those found earlier. Beetles were assumed to possess major *R*-genes of which the dominant alleles conferred resistance, similar to those found previously (Nielsen 1997*b*; P. W. de Jong and J. K. Nielsen, unpublished data) if survival of progeny from crosses with the ST line exceeded 25% on the G-type of *B. vulgaris* ssp. *arcuata*. Beetles were assumed to be homozygous or to contain several major genes if survival rates exceeded 56%, taking some background mortality into

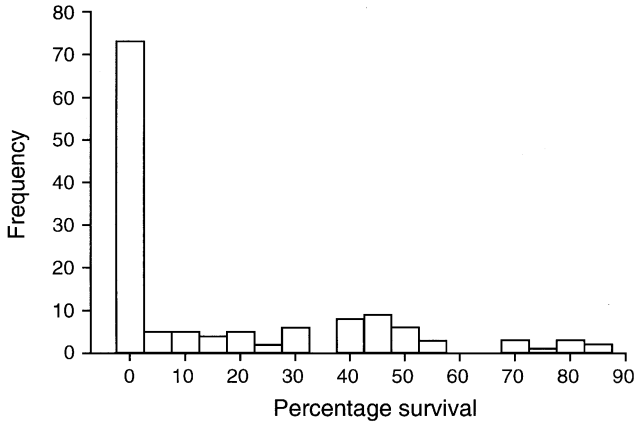

Figure 2. Frequency distribution of the percentage survival of larvae from the pooled crosses with ST-beetles in Appendix A. If one male was crossed with more than one ST-female, data were pooled. Family-sizes smaller than 30 were excluded from the data set.

account (ideally survival would be 75–100%). Beetles were assumed to be heterozygous at one locus if larval survival on *B. vulgaris* ssp. *arcuata* was 26–55%. Survival lower than 25% could not be explained by the presence of major genes similar to those previously found, but could be due to genes with a smaller effect. Such genes are labelled 'unclassified', and so are apparently major genes (26–55% larval survival on *B. vulgaris* ssp. *arcuata*) from crosses in which the number of adult offspring produced was insufficient (<6) for determination of sex ratios (see below). Location of genes on different chromosomes was determined from crosses between heterozygous males and ST-females. X-linkage is assumed if only female progeny from the cross survives on the G-type of *B. vulgaris* ssp. *arcuata* (males are heterogametic; Segarra & Petitpierre 1990), Y-linkage if only male progeny survives, and autosomal inheritance if the sex ratio does not deviate significantly from 50% of each sex (binomial test; note that if *P. nemorum* is reared on the suitable radish, sex ratios are very close to 50% (P. W. de Jong and J. K. Nielsen, unpublished data), indicating that the expected sex ratio in this beetle is 50% when not under selection; see also Nielsen 1996, 1997*b*). Genes are classified as either autosomal or X-linked (A/X) if they are carried by a female (sex ratios do not deviate from 50% in either case). Characterization of major genes from apparently homozygous beetles was done by examining their heterozygous progeny. Male offspring from crosses between homozygous males and ST-females were mated with ST-females and their progeny was investigated as described above. Control bioassays were performed to estimate mortality of larvae due to handling, using suitable radish leaves instead of *Barbarea*.

3. RESULTS

(a) *Polymorphism*

Most eastern Danish flea beetle populations are polymorphic for the ability to survive on *B. vulgaris* ssp. *arcuata* (G-type). Figure 2 shows a frequency histogram of the percentage survival of all crosses with ST-beetles from Appendix A producing $\geqslant 30$ larvae. The distribution is non-normal (Kolmogorov–Smirnov test, $n=135$, $p<0.01$) and tends to show three peaks, at 0%, 40–50% and 80% survival, coinciding with the expected proportions of survival if single, major genes segregate, as found for Ejby (taking background mortality into account). Genes conferring the ability to survive in the G-type of *Barbarea vulgaris* ssp. *arcuata* (*R*-genes) are rare in Denmark. From 144 males investigated in the present study (i.e. Ejby not included), only nine (6.3%) produced a larval survival larger than 56% when crossed with an ST female (homozygous or more than one *R*-gene), while 19 (13.2%) produced a survival between 26% and 55% (heterozygous; table 1). All the males producing a high larval survival were collected on the G-type of *Barbarea vulgaris* ssp. *arcuata* (Kværkeby), while those producing a lower larval survival were collected on other plants as well. These results are consistent with the presence at low frequencies of *R*-genes similar or identical to those identified previously in Ejby. Relatively low survival rates (5–25%) found in progeny from some crosses (e.g. Assentorp, Taastrup, Myrup, Maglebrænde) do not directly support the presence of the same type of *R*-genes in these populations, since similarly low survival rates have not been found before. The effects of these genes on survival rates on *Barbarea vulgaris* ssp. *arcuata* require further investigation. Some crosses had a very low larval survival in the three-day bioassays (usually $<5\%$), but higher than 0. If the survival to the adult stage was 0, these beetles were adjudged not to contain any *R*-genes, but the results do not exclude the possibility that genes conferring low larval survival are present. Two populations (Amager and Solbjerg) did not show any evidence for the occurrence of *R*-genes. Taastrup is the origin of the susceptible ST-line which is unable to survive on leaves of the G-type of *Barbarea vulgaris* ssp. *arcuata* (0% survival during the period when this study was performed; data not shown), but even in this population *R*-genes occurred at low frequencies (table 1; Appendix A).

The survival data and the sex ratios of survivors were combined to enable an interpretation in terms of sex linkage of *R*-genes (table 2). Autosomal inheritance seemed to be the most abundant; evidence was found in at least four populations. Two crosses (from Assentorp and Bjergsted, respectively) produced only female progeny when a male from the field was mated with an ST-female, which is an indication of X-linkage of the *R*-genes. In other cases, it was impossible to distinguish between X-linked or autosomal inheritance either because the female contained the *R*-gene(s) or too few ($\leqslant 6$) adults emerged to determine whether sex ratios were skewed (table 2; Appendix A).

(b) *Distribution*

Since the sample sizes are small, it is not possible to draw strict conclusions about gene frequencies and geographical distribution. The genes were found, albeit in low frequencies, in isolated, coastal populations (Gniben, Lynæs) where *B. vulgaris* was not growing, as well as in inland localities where the plant is common (figure 1). On the other hand, the genes have not been found on Amager, although the G-type of *Barbarea vulgaris* ssp. *arcuata* is very common there.

The control bioassays on radish leaves carried out for some of the crosses (data not shown) yielded a larval survival ranging from 83.0–100% (overall survival: 93.0%, $n=1383$), showing that handling of the larvae or other factors leads only to extremely low background mortality.

Table 1. *Classification of field collected males according to survival rates (0–5%, 6–25%, 26–50%, >56%) of their progeny on the G-type of* B v. *ssp.* arcuata *in three-day bioassays*

(Host plants of larvae are given; more mobile adults may have originated from other plants or even localities.)

			no. of males in different classes[b]			
locality	host plant	stage[a] collected	0–5%	6–25%	26–55%	>56%
1 Gniben	*Cardaria draba*	l,a	12	1	0	0
2 Lynæs	*Cardaria draba*	l	9	1	1	0
3 Solbjerg	*Sinapis arvensis*	l	9	0	0	0
4 Skuldelev	*Sinapis arvensis*	l	9	2	0	0
5 Bjergsted	*R. raphanistrum*	l,a	11	0	3	0
6 Assentorp	*B.v.* ssp. *arcuata*[c]	l	9	2	1	0
7 Taastrup	*Raphanus sativus*	a	18	4	1	0
8 Amager	*Sinapis arvensis*	l,a	11	0	0	0
9a Kværkeby	*Sinapis arvensis*	l	1	0	1	0
9b Kværkeby (1992)	*B.v.* ssp. *arcuata*[d]	l,a	0	1	6	3
9c Kværkeby (1997)	*B.v.* ssp. *arcuata*[d]	l	0	0	2	6
10 Myrup	*Sinapis arvensis*	l	5	2	3	0
11 Maglebrænde	*Sinapis arvensis*	l	6	3	1	0
E Ejby (1990)[e]	*B.v.* ssp. *arcuata*[d]	l	0	0	0	10
total			100	16	19	19

[a] l, larvae; a, adults.
[b] If a male was mated with several females, only data from crosses with females from the ST-line (combined) are included; for the full data set, see Appendix A.
[c] P-type of *B.v.* ssp. *arcuata* which is suitable for all *P. nemorum* larvae.
[d] G-type of *B.v.* ssp. *arcuata* which is unsuitable for susceptible *P. nemorum* larvae.
[e] Data from Nielsen (1997*b*).

Table 2. *Occurrence of beetles possessing different R-genes conferring ability to survive on the G-type of* Barbarea vulgaris *ssp.* arcuata *in eastern Denmark*

(A, autosomal inheritance; X, X-linked inheritance; Y, Y-linked inheritance; A/X, autosomal or X-linked inheritance (distinction not possible); U, unclassifiable gene of small effect or low survival until adulthood preventing accurate sex-ratio determination; for host plants in the different sites see table 1.)

		no. of beetles (in parentheses) with evidence for different	frequency (%)	
site	sample size	types of genes	all genes	major genes
1 Gniben	24	A/X (1); U (1)	8	4
2 Lynæs	15	A/X (1)	7	7
3 Solbjerg	9	—	0	0
4 Skuldelev	11	U (2)	18	0
5 Bjergsted	25	A (2); X (1)	12	12
6 Assentorp	24	A (2); X (1); A/X (2)	21	17
7 Taastrup	23	U (5)	22	4
8 Amager	13	—	0	0
9a Kværkeby	2	A (1)	—	—
9b Kværkeby	10	A (4); U (6)	100	90
9c Kværkeby	8	A (6); Y (1); U (1)	100	100
10 Myrup	10	A/X (1); U (4)	50	10
11 Maglebrænde	10	A (1); U (3)	40	10
E Ejby (1990)	20	A, X and Y	100	100

4. DISCUSSION

(a) *Polymorphism and abundance of R-genes with different modes of inheritance*

The results from this study clearly show that *P. nemorum* is polymorphic for the ability to survive on the atypical host plant *B. vulgaris* ssp. *arcuata* (G-type). In 9 out of 11 studied populations at least one of the classes of genes (autosomal, X-linked, Y-linked and unidentified) were present. Beetles that were homozygous for major *R*-genes or carried several *R*-genes (high larval survival) were found only in populations living on the G-type of *B. vulgaris* ssp. *arcuata* (Kværkeby and Ejby). These two populations are also the only sites where evidence for Y-linkage has been found, while X-linked and autosomal inheritance seem to have a wider distribution in eastern Denmark.

Y-linked genes are carried only by males and it is unlikely that a population can persist on defended types of *B. vulgaris* unless autosomal or X-linked genes are also present to enable female survival. Beetles which met the criteria for being heterozygous were found in several populations, but always at low frequencies (<20%, table 2), and estimated gene frequencies are therefore low (<0.1). The presence of low frequencies of homozygous beetles (<1%) is in agreement with the expectation if the population with a low gene frequency is in Hardy–Weinberg equilibrium. Although gene frequencies cannot be estimated very accurately in the present study, it is evident that *R*-genes are more abundant in populations living on the G-type of *B. vulgaris* ssp. *arcuata* compared to populations using other plant species. It was not possible to demonstrate any differences in gene frequencies between populations living on other host plants. These observations support the idea that evolution of insect–plant interactions is to be viewed in a geographical context and the outcome may depend on local differences in the physical environment, the available host plants, as well as in the local differences in genetic structures of populations (Thompson 1994).

(b) *Evidence for genes with small effect*

Our data provide evidence for the presence of major *R*-genes such as previously identified (Nielsen 1997*b*; P. W. de Jong and J. K. Nielsen, unpublished data; many of our survival data tend to fall into similar classes; see figure 2). In some of the crosses, however, larval survival in the bioassays was below 25% (e.g. Taastrup, Myrup, Skuldelev, Maglebrænde; see Appendix A). This result is an indication that there may be different genes within each class (Y-linked, X-linked and autosomal), some conferring 100% survival to their carrier, but others with a much smaller effect. The action of such genes may combine, influencing the survival time of larvae on *B. vulgaris* in a polygenic fashion, or else the survival of larvae may represent a threshold trait (Falconer & Mackay 1996; Hartl & Clark 1997). Examples are known from the literature where traits that are influenced by major loci, such as insecticide resistance, are also affected by polygenic modifiers (Via (1990), and references therein). Jones (1998) suggests that resistance in *Drosophila sechellia* to a host plant's toxin has an 'oligogenic basis', and found factors influencing the resistance on different chromosomes, including the X-chromosome. He suggests that natural adaptations may typically have an oligogenic basis. Sezer & Butlin (1998) propose that a sympatric host shift is most likely initiated by a mutation of large effect, possibly followed by the evolution of modifier genes. The possibility that the variability in larval survival is caused by variation in toxicity of *Barbarea* leaves (Nielsen, 1997*b*) is extremely unlikely in our experiments, since the plants were grown under standardized conditions and similar leaves were used for all bioassays.

(c) *Rarity of R-genes*

There are several possible explanations for the rarity of *R*-genes. If *B. vulgaris* (G-type) is only rarely used as a host plant, and the *R*-genes confer no other advantage than survival on this plant, selection pressures causing the spread of *R*-genes are low. These selection pressures, and hence the rarity of *R*-genes, are thus influenced by the distribution of potential host plants of *P. nemorum* and the mobility of the beetles. In general, all of our studied populations are in close proximity to sites where *B. vulgaris* ssp. *arcuata* (G-type) has been found, with the exception of Gniben (1) and Maglebrænde (11, see figure 1), but at present nothing is known about the mobility of the flea beetles, so that gene flow cannot be assessed. Even if *B. vulgaris* is used as a host plant, selection on the spread of *R*-genes may not always be strong, since there is variation in the suitability of *B. vulgaris* for feeding by *P. nemorum*, both seasonal and between different parts of the plants (Nielsen 1997*a*). On the other hand, the variation in suitability may facilitate the colonization of *Barbarea* (Nielsen 1997*a*) and provide the conditions for development of resistance to any compounds rendering (parts of) the plants unsuitable for feeding if alternative food gets scarce. The association we found in this study between the abundance of beetles carrying *R*-genes and the use of *B. vulgaris* as a host plant suggests that there is selection for the development of resistance in the beetles on this host plant. The rarity of the *R*-genes may also be explained by a recent origin of the ability of *P. nemorum* to use this plant as a host plant (Nielsen 1997*a*). A third testable possibility concerns trade-offs associated with the presence of these genes (Rausher 1984). Although Nielsen (1996) did not find any evidence for a negative effect of *R*-genes on performance on alternative host plants, recent observations on an autosomal line suggest that beetles that are homozygous for this gene have a strongly reduced fitness, both on *B. vulgaris* ssp. *arcuata* (G-type) and radish (P. W. de Jong and J. K. Nielsen, unpublished data).

(d) *Mode of inheritance*

The results from this and previous studies (Nielsen 1997*b*) show that the ability to survive on *B. vulgaris* ssp. *arcuata* (G-type) is both sex linked and autosomally inherited. Sex-linked inheritance of various traits, including those related to host-plant use, has been found in a number of insects (see Nielsen 1997*b* and references therein; Jones 1998), and in other cases of insect–plant relations, autosomal genes are involved in host-plant preferences (e.g. Huettel & Bush 1972). However, the involvement of both autosomal inheritance and genetic factors on both sex chromosomes in host-plant use, such as that found in *P. nemorum*, appears to be a very unusual situation. The study of this system provides, therefore, a rare opportunity to study the evolution of host-plant use through different modes of inheritance.

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APPENDIX A

Table A1. *Larval survival and sex ratio of progeny from crosses of beetles from various flea beetle populations in eastern Denmark*

(A binomial test was performed to test for departure from 50% females in surviving offspring: [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$, two-tailed test; [d]offspring (reared on *B.v.* ssp. *arcuata* in the laboratory) of cross with field-collected beetle(s) tested; [e]F_2 beetles used in the cross; [f]the same number refers to the same individual male; some males have been mated with females from the field as well as from the ST line; the addition of a letter to beetles with the same number indicates that these beetles are different, but related individuals. A=autosomal, X=X-linked, Y=Y-linked, A/X=autosomal or X-linked inheritance (distinction not possible); *U*=unclassifiable gene of small effect or low survival until adulthood preventing accurate sex-ratio determination, and hence sex linkage of the gene; for explanation of interpretation in classes of genes, see text.)

		larvae survival		adults		
♂ no.[f]	♀ from	no.	proportion	no.	proportion ♀	interpretation
1. Gniben						
1	ST	44	0	—	—	no *R* genes
2	Gniben	75	0	—	—	no *R* genes
3	Gniben	59	0	—	—	no *R* genes
4	Gniben	100	0.02	0	—	no *R* genes
5	Gniben	50	0	—	—	no *R* genes
6	Gniben	115	0.052	0	—	no *R* genes
7	Gniben	69	0.377	7	0.571 (n.s.)	A/X (in ♀)
7	ST	120	0	—	—	
8	Gniben	95	0	—	—	no *R* genes
9	Gniben	130	0	—	—	no *R* genes
10	Gniben	60	0	—	—	no *R* genes
11	Gniben	100	0.01	0	—	no *R* genes
12	Gniben	60	0	—	—	no *R* genes
13	ST	100	0.15	2	1.0 (n.s.)	U (in ♂)
2. Lynæs						
1	Lynæs	100	0.26	16	0.5 (n.s.)	A/X (in ♂ or ♀)
2	Lynæs	175	0	—	—	no *R* genes
3	Lynæs	150	0	—	—	no *R* genes
4	Lynæs	100	0	—	—	no *R* genes
5	ST	100	0	—	—	no *R* genes
6	ST	80	0	—	—	no *R* genes
7	ST	70	0	—	—	no *R* genes
8	ST	90	0	—	—	no *R* genes
9	ST	100	0	—	—	no *R* genes
10	ST	83	0	—	—	no *R* genes
11	ST	71	0.07	0	—	no *R* genes
3. Solbjerg						
1	ST	60	0	—	—	no *R* genes
2	ST	52	0	—	—	no *R* genes
3	ST	32	0	—	—	no *R* genes
4	ST	34	0	—	—	no *R* genes
5	ST	57	0	—	—	no *R* genes
6	ST	9	0	—	—	no *R* genes
6	ST	53	0	—	—	no *R* genes
7	ST	60	0	—	—	no *R* genes
8	ST	69	0	—	—	no *R* genes
9	ST	53	0	—	—	no *R* genes
4. Skuldelev						
1	ST	49	0	—	—	no *R* genes
2	ST	65	0.062	1	0 (n.s.)	U (in ♂)
2	ST	107	0.028	0	—	
3	ST	63	0	—	—	no *R* genes
4	ST	63	0	—	—	no *R* genes
5	ST	51	0	—	—	no *R* genes
5	ST	50	0	—	—	
6	ST	50	0.08	3	0.67 (n.s.)	U (in ♂)
6	ST	31	0.032	0	—	
6	ST	91	0	—	—	
7	ST	72	0	—	—	no *R* genes
8	ST	62	0	—	—	no *R* genes
9	ST	58	0	—	—	no *R* genes

10	ST	104	0	—	—	no *R* genes
11	ST	42	0	—	—	no *R* genes
11	ST	64	0	—	—	
5. Bjergsted						
1	Bjergsted	48	0	—	—	no *R* genes
2	Bjergsted	53	0	—	—	no *R* genes
3	Bjergsted	90	0	—	—	no *R* genes
4	Bjergsted	130	0	—	—	no *R* genes
5	Bjergsted	107	0.486	35	0.629 (n.s.)	A (in ♂ or ♀)
5a[d]	ST	110	0.382	4	0.25 (n.s.)	
5b[d]	ST	54	0.50	8	0.625 (n.s.)	
6	Bjergsted	75	0.04	0	—	no *R* genes
6	ST	60	0	—	—	
7	Bjergsted	132	0	—	—	no *R* genes
8	Bjergsted	120	0.017	0	—	no *R* genes
8	ST	85	0	—	—	
9	Bjergsted	98	0	—	—	no *R* genes
9	ST	50	0	—	—	
10	Bjergsted	125	0.256	19	1.0[c]	X (in ♂)
10	ST	85	0.494	12	1.0[c]	
11	Bjergsted	145	0.31	21	0.571 (n.s.)	A (in ♂ or ♀)
11[d]	ST	99	0.485	7	0.429 (n.s.)	
12	ST	105	0	—	—	no *R* genes
13	ST	100	0	—	—	no *R* genes
14	ST	100	0	—	—	no *R* genes
6. Assentorp						
1	Assentorp	50	0	—	—	no *R* genes
2	Assentorp	119	0.033	0	—	no *R* genes
2	ST	120	0.027	0	—	
3	Assentorp	171	0.257	4	1.0 (n.s.)	X (in ♂)
3	ST	132	0.182	16	1.0[c]	
4	Assentorp	90	0	—	—	no *R* genes
5	Assentorp	40	0	—	—	no *R* genes
6	Assentorp	100	0	—	—	no *R* genes
7	Assentorp	100	0	—	—	no *R* genes
8	Assentorp	110	0.273	3	0.67 (n.s.)	A/X (in ♀)
8	ST	110	0	—	—	
9	Assentorp	165	0.309	24	0.33 (n.s.)	A (in ♂)
9	ST	108	0.426	8	0.875 (n.s.)	
10	Assentorp	120	0	—	—	no *R* genes
11	Assentorp	130	0.346	15	0.80[a]	A/X (in ♀)
11	ST	108	0	—	—	
12	Assentorp	118	0.322	11	0.455 (n.s.)	A (in ♂)
12	ST	125	0.224	1	1.0 (n.s.)	
7. Taastrup						
1	ST	100	0	—	—	no *R* genes
2	ST	110	0	—	—	no *R* genes
3	ST	100	0	—	—	no *R* genes
4	ST	80	0	—	—	no *R* genes
5	ST	105	0	—	—	no *R* genes
6	ST	50	0	—	—	no *R* genes
7	ST	170	0.082	5	1.0 (n.s.)	U (in ♂)
8	ST	160	0.088	6	0.33 (n.s.)	U (in ♂)
9	ST	114	0	—	—	no *R* genes
10	ST	90	0	—	—	no *R* genes
11	ST	93	0.01	0	—	no *R* genes
12	ST	89	0.157	3	1.0 (n.s.)	U (in ♂)
13	ST	87	0	—	—	no *R* genes
14	ST	100	0.11	0	—	U (in ♂)
15	ST	67	0	—	—	no *R* genes
16	ST	90	0	—	—	no *R* genes
17	ST	105	0	—	—	no *R* genes
18	ST	100	0	—	—	no *R* genes
19	ST	95	0	—	—	no *R* genes
20	ST	100	0	—	—	no *R* genes
21	ST	97	0	—	—	no *R* genes

(*Cont.*)

Appendix Table (*Cont.*)

		larvae survival		adults		
♂ no.[f]	♀ from	no.	proportion	no.	proportion ♀	interpretation
22	ST	100	0	—	—	no *R* genes
23	ST	44	0.318	1	0 (n.s.)	U (in ♂)
8. Amager Fælled						
1	Amager	65	0	—	—	no *R* genes
2	ST	150	0	—	—	no *R* genes
3	ST	51	0	—	—	no *R* genes
4	ST	110	0	—	—	no *R* genes
5	ST	100	0	—	—	no *R* genes
6	Amager	130	0	—	—	no *R* genes
6	ST	105	0	—	—	
7	ST	100	0	—	—	no *R* genes
8	ST	80	0	—	—	no *R* genes
9	ST	100	0	—	—	no *R* genes
10	ST	100	0	—	—	no *R* genes
11	ST	100	0	—	—	no *R* genes
9. Kværkeby a						
1	ST	60	0	—	—	no *R* genes
2	ST	94	0.532	18	0.556 (n.s.)	A (in ♂)
2[e]	ST	189	0.434	15	0.467 (n.s.)	
9. Kværkeby b (1992)						
1[d]	ST	137	0.686	2	0.50 (n.s.)	U (in ♂)
2a[d]	ST	151	0.715	1	1.0 (n.s.)	U (in ♂)
2b[d]	ST	155	0.20	3	0.333 (n.s.)	A (in ♂)
2b[e]	ST	68	0.309	12	0.50 (n.s.)	
2c[d]	ST	155	0.51	11	0.455 (n.s.)	A (in ♂)
2c[e]	ST	216	0.514	22	0.636 (n.s.)	
3[d]	ST	176	0.801	12	0.583 (n.s.)	A(in ♂)
3[e]	ST	178	0.230	9	0.67 (n.s.)	
4	ST	77	0.558	—	—	U (in ♂)
5	ST	118	0.424	3	0.67 (n.s.)	U (in ♂)
6	ST	56	0.089	0	—	U (in ♂)
7	ST	95	0.421	1	0 (n.s.)	A (in ♂)
7[e]	ST	245	0.42	41	0.683[a]	
8	ST	60	0.450	0	—	U (in ♂)
9. Kværkeby c (1997)						
1	ST	90	0.356	16	0.562 (n.s.)	A(in)
1	ST	114	0.491	20	0.50 (n.s.)	
2	ST	72	0.833	22	0.50 (n.s.)	A (in ♂)
2a[d]	ST	124	0.419	10	0.50 (n.s.)	
2b[d]	ST	64	0.422	7	0.429 (n.s.)	
3	ST	108	0.861	27	0.407 (n.s.)	A (in ♂)
3a[d]	ST	113	0.407	24	0.417 (n.s.)	
3b[d]	ST	73	0.315	8	0.625 (n.s.)	
4	ST	56	0.769	20	0.60 (n.s.)	A (in ♂)
4a[d]	ST	79	0.519	21	0.67 (n.s.)	
4a[d]	ST	107	0.551	34	0.50 (n.s.)	
4b[d]	ST	41	0.463	2	0 (n.s.)	
5	ST	111	0.775	17	0.412 (n.s.)	Y (in ♂)
5[d]	ST	11	0.091	1	0 (n.s.)	
5[d]	ST	77	0.481	19	0[c]	
6	ST	91	0.846	8	0.625 (n.s.)	A (in ♂)
6	ST	112	0.804	23	0.565 (n.s.)	
6a[d]	ST	110	0.40	26	0.385 (n.s.)	
6a[d]	ST	2	1.0	6	0.50 (n.s.)	
6b[d]	ST	8	0.25	0	—	
6b[d]	ST	14	0.50	1	0 (n.s.)	
6b[d]	ST	94	0.213	4	0.75 (n.s.)	
6c[d]	ST	100	0.52	43	0.442 (n.s.)	
6d[d]	ST	105	0.457	18	0.67 (n.s.)	
7	ST	37	0.676	8	1.0[b]	U (in ♂)
8	ST	63	0.302	5	0.60 (n.s.)	A (in ♂)

10. Myrup						
1	ST	115	0.043	0	—	no *R* genes
2	ST	98	0.112	2	0.5 (n.s.)	U (in ♂)
3	ST	124	0.177	1	1.0 (n.s.)	U (in ♂)
4	ST	68	0.294	0	—	U (in ♂)
5	ST	95	0	—	—	no *R* genes
6	ST	95	0	—	—	no *R* genes
7	ST	105	0	—	—	no *R* genes
8	ST	195	0.436	6	0.33 (n.s.)	A/X (in ♂)
9	ST	93	0	—	—	no *R* genes
10	ST	160	0.294	3	0.33 (n.s.)	U (in ♂)
11. Maglebrænde						
1	ST	61	0	—	—	no *R* genes
2	ST	78	0	—	—	no *R* genes
2	ST	14	0	—	—	
3	ST	114	0.465	44	0.50 (n.s.)	A (in ♂)
4	ST	107	0	—	—	no *R* genes
5	ST	53	0	—	—	no *R* genes
6	ST	104	0.163	14	0.50 (n.s.)	U (in ♂)
7	ST	52	0.135	—	—	U (in ♂)
8	ST	64	0	—	—	no *R* genes
8	ST	80	0	—	—	
9	ST	46	0	—	—	no *R* genes
10	ST	62	0.194	0	—	U (in ♂)

REFERENCES

Desroches, P., Mandon, N., Baehr, J. C. & Huignard, J. 1997 Mediation of host-plant use by a glucoside in *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *J. Ins. Physiol.* **43**, 439–446.

Falconer, D. S. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*, 4th edn. Harlow: Longman.

Grenier, A. M., Mbaiguinam, M. & Delobel, B. 1997 Genetical analysis of the ability of the rice weevil *Sitophilus oryzae* (Coleoptera, Curculionidae) to breed on split peas. *Heredity* **79**, 15–23.

Hare, J. D. & Kennedy, G. G. 1986 Genetic variation in plant–insect associations: survival of *Leptinotarsa decemlineata* populations on *Solanum carolinense*. *Evolution* **40**, 1031–1043.

Hartl, D. L. & Clark, A. G. 1997 *Principles of population genetics*, 3rd edn. Sunderland, MA: Sinauer Associates.

Hatchett, J. H. & Gallun, R. L. 1970 Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheats having different genes for resistance. *Ann. Ent. Soc. Am.* **63**, 1400–1407.

Hatchett, J. H., Sears, R. G. & Cox, T. S. 1993 Inheritance of resistance to Hessian fly in rye and in wheat–rye translocation lines. *Crop Sci.* **33**, 730–734.

Huang, X., Renwick, J. A. A. & Sachdev-Gupta, K. 1994 Oviposition stimulants in *Barbarea vulgaris* for *Pieris rapae* and *P. napi oleracea*: isolation, identification and differential activity. *J. Chem. Ecol.* **20**, 423–437.

Huettel, M. D. & Bush, G. L. 1972 The genetics of host selection and its bearing on sympatric speciation in *Procecidochares* (Diptera: Tephretidae). *Entomol. Exp. Appl.* **15**, 465–480.

Jones, C. D. 1998 The genetic basis of *Drosophila sechellia*'s resistance to a host plant toxin. *Genetics* **149**, 1899–1908.

Lu, W. & Logan, P. 1994*a* Geographic variation in larval feeding acceptance and performance of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Ann. Ent. Soc. Am.* **87**, 460–469.

Lu, W. & Logan, P. 1994*b* Genetic variation in oviposition between and within populations of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Ann. Ent. Soc. Am.* **87**, 634–640.

Nielsen, J. K. 1978 Host plant discrimination within Cruciferae: feeding responses of four leaf beetles (Coleoptera: Chrysomelidae) to glucosinolates, cucurbitacins and cardenolides. *Entomol. Exp. Appl.* **24**, 41–54.

Nielsen, J. K. 1996 Intraspecific variability in adult flea beetle behaviour and larval performance on an atypical host plant. *Entomol. Exp. Appl.* **80**, 160–162.

Nielsen, J. K. 1997*a* Variation in defences of the plant *Barbarea vulgaris* and in counteradaptations by the flea beetle *Phyllotreta nemorum*. *Entomol. Exp. Appl.* **82**, 25–35.

Nielsen, J. K. 1997*b* Genetics of the ability of *Phyllotreta nemorum* larvae to survive in an atypical host plant, *Barbarea vulgaris* ssp. *arcuata*. *Entomol. Exp. Appl.* **82**, 37–44.

Puterka, G. J. & Peters, D. C. 1989 Inheritance of greenbug, *Schizaphis graminum* (Rondani), virulence to *gb2* and *gb3* resistance genes in wheat. *Genome* **32**, 109–114.

Rausher, M. D. 1984 Trade-offs in performance on different hosts: evidence from within- and between-site variation in the beetle *Deloyala guttata*. *Evolution* **38**, 582–595.

Segarra, C. & Petitpierre, E. 1990 Chromosomal survey in three genera of Alticinae (Coleoptera, Chrysomelidae). *Cytobios* **64**, 169–174.

Sezer, M. & Butlin, R. K. 1998 The genetic basis of host plant adaptation in the brown planthopper (*Nilaparvata lugens*). *Heredity* **80**, 499–508.

Thompson, J. N. 1994 *The coevolutionary process*. University of Chicago Press.

Via, S. 1990 Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *A. Rev. Ent.* **35**, 421–446.

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