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Genetics of resistance against defences of the host plant *Barbarea vulgaris* in a Danish flea beetle population

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One essential aspect of the study of the evolution of host-plant use by insects is (variation in) its genetic basis. The genetic basis of the ability of a flea beetle (*Phyllotreta nemorum*) to use the crucifer *Barbarea vulgaris* ssp. *arcuata* (G type) as a host plant was studied in a Danish population (Kværkeby) occurring naturally on this atypical host plant. Evidence was found that this ability was determined by a single, major, autosomal gene, although the presence of genes at additional loci at lower frequencies could not be excluded. No evidence was found for sex-linked inheritance, which was common in a second population in Denmark (Ejby) using *Barbarea* as a host plant. All beetles in the Kværkeby sample were homozygous 'resistant' to *Barbarea* defence. After crossing resistant F_1 offspring from pairs consisting of a field-collected beetle and a susceptible one amongst each other, genotyping the F_2 (reared on radish) showed a 1:2:1 ratio of homozygous resistant, heterozygous and susceptible beetles. No evidence was found for a reduction in the viability of beetles that were homozygous resistant at the autosomal locus, in contrast to what had been found earlier for two backcrossed lines founded by beetles from Ejby. The results show that there is variation in the genetic basis of host-plant use across local populations and imply that population structure should form part of the study of the interaction between *P. nemorum* and its host plants.

Keywords: major gene; host-plant use; population structure; maternal effects; coadapted gene complex; coevolution

1. INTRODUCTION

Over the past decades there has been an increasing emphasis on the role of spatial structure in evolutionary and ecological processes, including interactions between species (Mopper & Strauss 1998; Hanski 1999; Nuismer *et al*. 1999; Thompson 1999*a*–*c*). Populations typically consist of genetically differentiated subpopulations and interactions between species are likely to vary in different parts of their geographical range due to local differences in the physical and biological environment and due to local differences in the genetic architecture of interacting populations (Thompson 1999*a*). Host-plant use by phytophagous insects provides an example of interspecific interactions. Many insects are closely adapted to their host plants and their specialized feeding habits are assumed to have evolved by a series of changes in host-plant use in evolutionary time. These changes may have involved host shifts as well as a narrowing and broadening of the host-plant range. Changes in host-plant range may require genetic variation in preference as well as performance of phytophagous insects on different host plants (e.g. Gould 1983; Mitter & Futuyma 1983; Futuyma & Peterson 1985; Thompson 1988; Via 1990). Evolutionary changes in host use are therefore dependent on the genetic architecture of interacting species, but it is still unknown how the outcome of evolutionary processes is influenced by differences in this genetic architecture (Thompson 1999*a*).

Adaptation to new host plants may involve genes with small or large effects and any intermediates between these extremes (Orr & Coyne 1992; Janz 1998; Jones 1998; Sezer & Butlin 1998). Interactions between the flea beetle *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae: Alticinae) and one of its potential natural host plants *Barbarea vulgaris* ssp. *arcuata* (Opiz.) Simkovics provide an example of where major genes have an important influence on host-plant use (Nielsen 1992, 1996, 1997*a*,*b*). Moreover, there are local differences in the genetic architecture of the insect since (i) insect populations differ in their ability to live on the plant, and (ii) different modes of inheritance (autosomal and sex linked) predominate in different populations using *B. vulgaris* ssp. *arcuata* as a natural host plant (de Jong & Nielsen 1999). The interaction between *P. nemorum* and *B. vulgaris* ssp. *arcuata* may therefore provide insight into the ways in which differences in genetic architecture influence evolutionary interactions.

P. nemorum is a common pest of particular cruciferous vegetables, such as radish and turnip (Alford 1999) and also attacks naturally occurring crucifers. The larvae are leafminers and the adult beetles feed on the same plants. Two types of *Barbarea* have been distinguished (Nielsen 1997*a*), one of which, the 'G type' (in the remainder of this paper referred to as '*Barbarea*'), is unsuitable as a host plant for the majority of *P. nemorum* during spring and summer in the field and is always unsuitable when reared under constant laboratory conditions. However, three populations have been discovered in east Denmark where

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P. nemorum uses this type of *Barbarea* as its major host plant. Studies on beetles from one of these populations (Ejby) have revealed that the ability of the beetles to use *Barbarea* as a host plant is determined by major genes (which have been called 'resistance' or R genes) (see de Jong & Nielsen 1999), of which the most common ones show sex-linked inheritance (in *P. nemorum* males are the heterogametic sex) (Smith & Virkki 1978; Segarra & Petitpierre 1990). Autosomal inheritance was also detected, but at a low frequency (Nielsen 1997*b*). The alleles conferring the resistance to *Barbarea* defence are dominant (Nielsen 1997*b*). Experiments with near-isogenic lines with different R genes have shown that (i) the R genes appear to be specific for the defences of *Barbarea* (Nielsen 1999; J. K. Nielsen, P. W. de Jong and H. O. Frandsen, unpublished data), (ii) they do not show pleiotropic effects across host plants (Nielsen 1999; J. K. Nielsen, P. W. de Jong and H. O. Frandsen, unpublished data), and (iii) beetles which are homozygous resistant at autosomal loci from Ejby have a low viability (de Jong & Nielsen 2000). Nevertheless, in east Denmark R genes occur at a very high frequency in populations using *Barbarea* as a host plant, but are relatively rare in populations using other host plants (de Jong & Nielsen 1999). It is likely that local selection interacts with the distribution of host plants resulting in the observed distribution of resistant beetles. However, we need more information about the genetic basis of resistance in different localities before we can study what processes determine the geographical distribution of resistance in *P. nemorum*.

A survey of populations of *P. nemorum* in east Denmark showed that, in one of the two other populations where *Barbarea* is used as a natural host plant (Kværkeby), autosomal inheritance of R genes seemed to be most common and sex linkage, such as found in Ejby, rare (de Jong & Nielsen 1999). Crosses between field-collected beetles and Susceptible ones from a line originating from Taastrup (ST line) (Nielsen 1997*a*) yielded a high larval survival on *Barbarea*, suggesting that many individuals from this population were either homozygous resistant at one autosomal locus or had R genes at more loci (de Jong & Nielsen 1999). The distinction between these two possibilities is particularly relevant if the reduction in viability of homozygous resistant beetles, such as found in the Ejby lines, were a general phenomenon. In that case, the field-collected beetles may be heterozygous at a number of loci rather than homozygous resistant at one (de Jong & Nielsen 2000). To distinguish between these possibilities, more detailed crossing experiments are needed. The present paper describes the genetics of resistance of *P. nemorum* to defences in *Barbarea* in Kværkeby in more detail in order to answer the following questions.

(i) What is the predominant mode of inheritance of R genes in Kværkeby and are there any differences to that in Ejby?
(ii) Is there any evidence for the presence of R genes at more than one autosomal locus within the Kværkeby population?
(iii) Is there any evidence for a reduction in viability of individuals that are homozygous resistant at one autosomal locus, such as found for the lines derived from the Ejby population?

2. MATERIAL AND METHODS

(a) *Plants*

Radish seeds (*Raphanus sativus* L. cv. Københavns Torve) were commercially obtained (Dæhnfeldt, Odense). Seeds of *B. vulgaris* ssp. *arcuata* (G type) were collected in Herlev in 1994 (accession no. 3) (Nielsen 1997*a*). Seeds were sown in a peat–vermiculite (grade 3) mixture (*ca.* 5:1). Plants were grown at 20 ± 2 °C and a photoperiod of 18 L:6 D (400 W HPI/T lamps). Leaves from plants in the vegetative stage were used for bioassays (see § 2(d)).

(b) *Insect samples*

Larvae of *P. nemorum* L. were collected at a natural growth site of *Barbarea* in Kværkeby (site 9) (de Jong & Nielsen 1999) by picking leaves in which larvae had mined. Sampling took place in June 1998 at the time of year when the G type of *Barbarea* is unsuitable for susceptible larvae. Larvae that were nearly fully grown were collected from many different plants in order to avoid family bias in the samples. The leaves containing the larvae were put into plastic vials (500 ml) with a layer (*ca.* 5 cm) of moist vermiculite. Fully grown larvae left their mines (a few days after the collection date) and burrowed into the vermiculite where they pupated. After approximately two weeks beetles emerged. They were sexed using ventral abdominal and antennal characters (see de Jong & Nielsen 1999). Since the beetles were not used immediately, diapause was induced as described in de Jong & Nielsen (1999). They were kept in diapause for approximately four months until the start of the experiments.

(c) *Maintenance of insects*

In order to determine the genotypes of beetles, survival on *Barbarea* of neonate larval offspring from particular pairs of beetles was tested in bioassays (see § 2(d)). Pairs were kept in plastic vials (158 ml) with a moist charcoal–gypsum bottom layer and closed with a plastic lid with a hole which was plugged with cotton wool (Nielsen 1978). They were kept at 24 ± 2 °C and 18 L:6 D and were fed three times per week with radish cotyledons. Eggs were laid in crevices in the bottom layer and hatched after four to five days. Pairs of beetles were then transferred to new vials. Neonate larvae were used in the bioassays before they had had access to food. Adult offspring were obtained by rearing larvae through to adults using the pots with vermiculite as described in § 2(b). If they were reared on *Barbarea* (after the bioassays), larvae were reared on detached leaves all the way through (see § 2(d)). However, in cases where larvae were reared on radish, intact growing plants were used until the final larval instar, when leaves were picked and put into the pots with vermiculite.

(d) *Determination of insect genotype*

The genotypes of beetles were determined by crossing them with the fully susceptible ST line which does not contain any R genes and is unable to survive on *Barbarea* (Nielsen 1997*a*; de Jong & Nielsen 2000). Survival of their larval offspring on *Barbarea* was examined in bioassays and compared with their expected survival with different modes of inheritance. For example, a cross between a beetle which is homozygous resistant at one autosomal locus (AA genotype, i.e. an allele conferring resistance is dominant) and a beetle from the ST line (aa genotype) is expected to lead to 100% survival of their larval offspring (Aa genotype) on *Barbarea*. A heterozygous (Aa genotype) beetle

crossed with an ST beetle leads to 50% survival as does a cross involving a beetle with one sex-linked resistance gene (see Nielsen 1997*b*; de Jong & Nielsen 1999, 2000) and so forth. If more loci are involved, different classes of larval survival are expected. Bioassays were performed by putting detached rosette leaves of *Barbarea* individually into small plastic vials (25 ml) containing a moist strip of filter paper. Five larvae were transferred to each vial with a moist paint brush. Fifty to one hundred larvae per pair of beetles were put into bioassays at a rate of *ca.* 15–25 per day. The vials were kept at 24 ± 2 °C and 18 L:6 D. After three to four days, larval survival was examined using a stereomicroscope (× 60 magnification). Surviving, resistant larvae had produced a mine and moved when touched with a pair of forceps. Susceptible larvae had not grown and had died in a tiny mine or on the leaf surface. Leaves containing the surviving larvae were put into 500 ml vials with moist vermiculite and fresh laboratory-reared *Barbarea* leaves were regularly added in order to allow the larvae to complete their development. The sex ratios of emerging beetles were determined in order to distinguish between autosomal and sex-linked inheritance of the R genes (Nielsen 1997*b*; de Jong & Nielsen 1999). The sex ratio in this species is extremely close to 50% when not under selection (de Jong & Nielsen 2000). Thus, if the sex ratio was not significantly different from 50% (binomial test), autosomal inheritance was assumed, whereas a biased sex ratio indicated sex linkage of the R genes. Control bioassays using radish leaves were performed with offspring from some pairs (P crosses, see §2(e)) in order to assess mortality due to handling the larvae. As was found before (de Jong & Nielsen 1999, 2000) this background mortality (in three- to four-day larval bioassays) was *ca.* 10–20% ($n = 5$ pairs, median survival = 90.0% and range = 83.3–93.2%). In the analysis of larval survival on *Barbarea*, pairs producing fewer than 30 larvae (e.g. when one parent died) were excluded from the analysis throughout this study. We assumed that pairs producing at least 30 larvae formed an unbiased sample from those that were originally started, since failure to produce sufficiently many larvae was equally often due to early death of a resistant, as of an ST beetle (see de Jong & Nielsen (2000) where the same procedure was followed). If a resistant beetle was crossed with more than one ST beetle (because of early death of the first ST beetle), larval survival data were pooled.

(e) *Experimental design*

A crossing schedule (figure 1) was designed based on earlier results (Nielsen 1997*b*; de Jong & Nielsen 1999, 2000) where evidence was found for the presence of major R genes. Field-collected beetles (eight males and eight females) were initially crossed with the ST line (P crosses) (figure 1). Some of the resulting F_1 larvae were reared on *Barbarea* and some on radish. Of the adult F_1 reared on *Barbarea*, one or two males descending from each original P cross were backcrossed with the ST line (F_1 backcross) in order to allow segregation of major R genes. Larval backcross progeny were tested and resistant larvae reared through on *Barbarea* in order to determine the sex ratio. The remaining F_1 adults reared on *Barbarea* constituted four groups: males and females descending from a field-collected male and a field-collected female, respectively. Eleven crosses were made between F_1 beetles descending from eight different P crosses (inter-F_1 crosses) so that both types of females were mated with both types of males (four combinations with at least two pairs per combination) in order to (i) investigate whether major (autosomal) R genes occurred at different loci, and

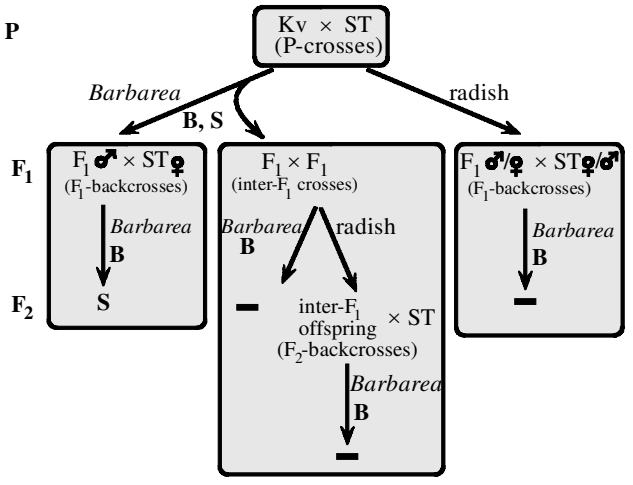

Figure 1. Schematic representation of the experimental design. The plant on which larvae were reared is indicated next to the arrows. A 'B' indicates where bioassays were performed, an 'S' where the sex ratio was determined and a dash that larvae were discarded after the bioassays.

(ii) examine whether there was any maternal effect on the differential survival of different genotypes. Larval offspring from these inter-F_1 crosses (F_2) were tested on *Barbarea* and the remaining larvae from eight of these crosses, involving beetles descending from seven field-collected individuals, were reared on radish in order to eliminate any host-plant effects on the differential survival of genotypes. Resulting F_2 adults (both males and females) were crossed with ST beetles (F_2 backcrosses) and larval offspring tested on *Barbarea* in order to genotype the F_2.

The second group of F_1 beetles, those reared on radish (offspring from three different P crosses), were backcrossed with ST beetles (F_1 backcrosses) in order to examine whether any of the F_1 beetles were non-resistant. The presence of such cases would imply that the field-collected parent was not homozygous resistant at any R locus, but heterozygous at different loci.

3. RESULTS

(a) F_1 beetles

The progeny of all 16 field-collected beetles crossed with ST beetles showed a high proportion of survival on *Barbarea* (figure 2*a*) ($n = 16$, median = 87.40% and range = 70.2–91.9%). Control bioassays on radish, which is a suitable host plant for resistant as well as susceptible beetles, showed that larval survival on radish is not significantly different from that on *Barbarea* (Wilcoxon matched-pairs, signed-ranks test, $n = 5$, $z = 0.14$ and $p = 0.89$). This indicates that all F_1 larvae are resistant. Crosses amongst field-collected beetles from the same cohort produced offspring that, when tested on intact *Barbarea* plants, also had survival rates similar to that on radish (P. W. de Jong, P. Meijer and J. K. Nielsen, unpublished results). The phenotype of the F_1 larvae in the present study is therefore similar to that in the Kværkeby population, suggesting that the resistance to *Barbarea* defence is determined by major, dominant genes at one or more loci. The sex ratio of the F_1 beetles was not significantly different from 50% for any pair (two-tailed, binomial tests overall, percentage females = 50.0, $n = 291$ and $p = 1.0$).

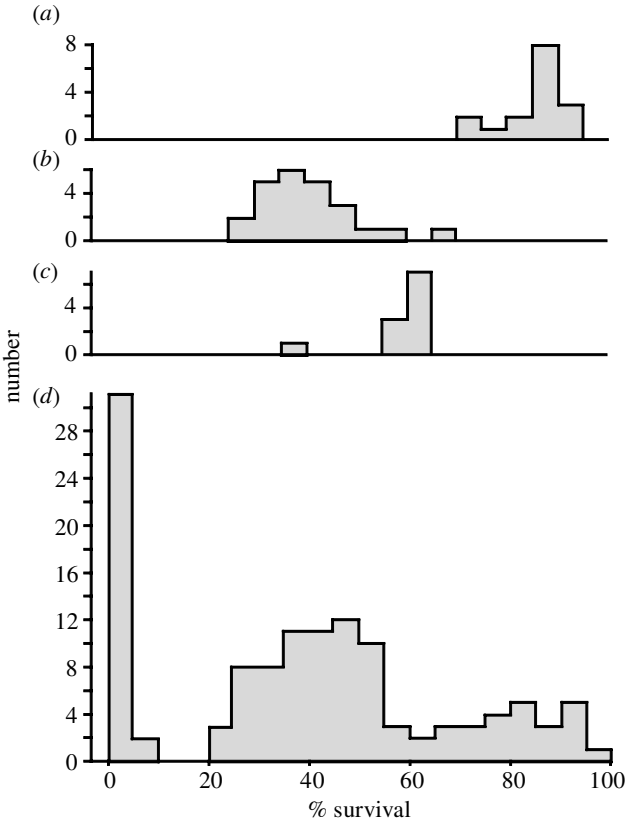

Figure 2. Larval survival determined in bioassays from crosses between (*a*) Kværkeby males and females and ST beetles (P crosses), (*b*) male F_1 offspring from (*a*) × ST females (F_1-backcross progeny), (*c*) male and female offspring from (*a*) (inter-F_1 crosses) and (d) male and female offspring from (*c*) × ST beetles (F_2 backcrosses).

This is consistent with autosomal inheritance of R genes or, if no autosomal R genes are present, location of R genes on both sex chromosomes, such as found earlier in Ejby (Nielsen 1997*b*). If there is no sex linkage of the R genes, the field-collected beetles must be either homozygous resistant at at least one autosomal locus or more loci are involved.

(b) *Progeny of F_1 backcrosses*

The larval survival in all but one family of backcross progeny from F_1 males reared on *Barbarea* was close to 50% (figure 2*b*) ($n = 24$, median = 39.2% and range = 28.0–66.0%) and very similar in range to findings in earlier papers (Nielsen 1997*b*; de Jong & Nielsen 1999, 2000) where evidence was found that beetles were heterozygous at one major R locus. The sex ratios did not differ significantly from 50% males and females for any of the individual families, nor for the pooled families (binomial tests overall, percentage females = 52.6, $n = 500$ and $p = 0.26$) and were not different among crosses involving offspring from a field-collected male (158 females and 146 males) and female (105 females and 91 males), respectively. This is consistent with the R gene being present at an autosomal locus. Hence, no evidence was found for sex linkage of R genes. If more than one major locus were involved in the resistance to *Barbarea* defence in Kværkeby, each showing complete dominance of the R allele and without epistatic interactions and linkage, larval survival in the backcross progeny would be expected to include classes larger than 50% (with a multiple heterozygous father). Since this was only found for one possible case out of 24 (66% survival, which was significantly higher than 50%) (one-tailed, binomial test, $n = 50$ and $p = 0.016$), the results are fully consistent with the Kværkeby beetles in our sample being homozygous resistant at one autosomal locus and the possible presence at a low frequency of an R gene at an additional locus. The data do not support the possibility that all beetles in Kværkeby were heterozygous at more than one locus and homozygous resistant at none (see de Jong & Nielsen 2000). In that case at least one-third of the backcross-progeny families should have shown a survival on *Barbarea* higher than 50% (e.g. with two unlinked loci, one-third of the survivors on *Barbarea* are expected to be double heterozygotes, leading to 75% survival in the backcross progeny).

This result (fixation at one autosomal R locus in the Kværkeby population) was corroborated by examining survival of the backcross-progeny larvae from F_1 parents that had been reared on radish (so that all genotypes could survive) on *Barbarea*. The backcross progeny showed a uniform larval survival close to 50% and no cases were found where none of the backcross-progeny larvae survived on *Barbarea* ($n = 14$ pairs, median = 37.35% and range = 20.0–51.0%). This is further evidence that the field-collected beetles were homozygous resistant at one autosomal locus (AA) and that all F_1 beetles were heterozygous (Aa). If more loci had been involved and beetles were not homozygous resistant at any locus, backcross progeny would have shown classes of survival larger than 50% and of 0% survival, respectively.

(c) *Inter-F_1 crosses*

If one autosomal locus is involved and all resistant genotypes survive equally well, the expected survival of progeny from inter-F_1 crosses on *Barbarea* (Aa × Aa) is 75%. The observed survival was slightly lower (figure 2*c*) ($n = 11$ pairs, median = 61.3% and range = 38.9–64.8%), which is consistent with expectations when taking 10–20% background mortality into account. A lower survival (38.9%) was observed in one family which also produced the smallest number of larvae (36). This may have influenced the accuracy of the estimate of survival for this family; the results of P crosses, F_1 backcrosses, inter-F_1 crosses and F_2 backcrosses based on beetles derived from the same field-collected individuals did not show any outliers. Offspring from inter-F_1 crosses were also reared on radish. Larval survival from crosses between resulting F_2 adults and ST beetles (F_2 backcrosses) is shown in figure 3. The frequency distributions of the survival rates are similar for the eight families and tend to show three classes: one with no, one with intermediate (up to 50%) and one with high (*ca.* 70% and higher) larval survival. The pooled results clearly show these three distinct classes (figure 2*d*). Again, this is in agreement with the segregation of alleles at one autosomal locus. If more unlinked loci had been involved, a more complex mixture of genotypes would have resulted, leading to intermediate classes of survival rates of between 50 and 100% survival and fewer cases with 0% survival. The three peaks in larval survival were very

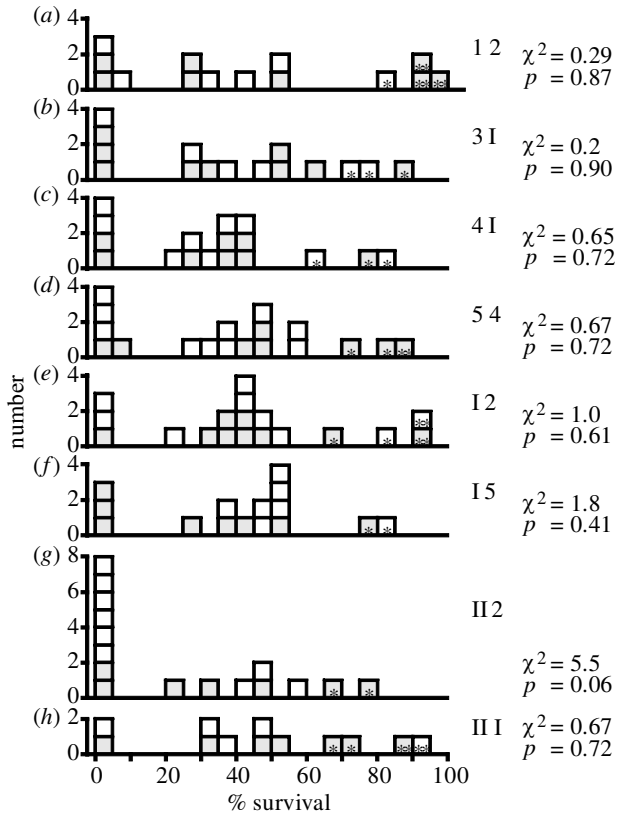

Figure 3. Survival on *Barbarea* of larval F_2-backcross progeny of male (open blocks) and female (shaded blocks) offspring of the eight inter-F_1 crosses. The eight crosses are labelled with two numbers representing the F_1 female and male, respectively. The numbers indicate the original field-collected parents of the F_1 beetles. Arabic numbers refer to field-collected males and Roman numbers to field-collected females. For example, two different male F_1 offspring from field-collected female I (see (*b*) and (*c*)) were crossed respectively with a female offspring from field-collected (*b*) male 3 and (*c*) male 4. (*a–d*) Inter-F_1 crosses with a female offspring of a field-collected male. (*e–h*) Inter-F_1 crosses with a female offspring of a field-collected female. An asterisk indicates a percentage survival which is significantly higher than 50% and a double asterisk a percentage survival which is significantly higher than 75% (binomial tests). The χ^2-values are of χ^2 goodness-of-fit tests in which the observed frequency distributions were compared with a 1:2:1 ratio of 0% survival, survival which was not significantly higher than 50% and survival higher than 50%.

similar to those found in an earlier study (de Jong & Nielsen 2000) with lines of beetles carrying an R gene at one autosomal locus: 0, *ca.* 40 and *ca.* 80% survival. These survival rates correspond to the expected 0, 50 and 100% survival if R alleles segregate at one autosomal locus when taking some background mortality into account. Thus, the beetles which were originally collected in Kværkeby were homozygous resistant at the same autosomal locus. Even the (unlikely) case of the beetles in the Kværkeby sample being homozygous resistant at single but different loci can be excluded with our data. In this case, the maximum survival rate in the F_2 backcrosses would have been 75% (from the double heterozygote × ST). A number of cases show survival rates which are significantly higher than 75% (figure 3) (binomial tests), consistent with the one-locus situation.

(d) *Survival of different genotypes in F_2 beetles*

Assuming the presence of an R gene at one autosomal locus in the F_1 beetles which were crossed between each other, the survival rates of aa (susceptible), Aa and AA genotypes on radish can be examined by classifying the observed survival rates on *Barbarea* in the F_2 backcrosses into three classes and comparing the frequency of beetles falling within each class with the expected 1:2:1 ratio. The aa genotype is readily identified since it results in 0% larval survival. The Aa and AA genotypes were distinguished by applying a binomial test to the larval survival data with an expected survival of 50% (see figure 3). Crosses that produced a larval survival significantly higher than 50% were assumed to involve beetles of the AA genotype. In none of the eight frequency distributions (figure 3) was there a significant deviation from a 1:2:1 ratio of aa:Aa:AA genotypes (χ^2 goodness-of-fit tests) (see figure 3).

The overall ratio of the three genotypes (aa:Aa:AA) is 33:67:25 which is not significantly different from expectations if all genotypes survive equally well on radish ($\chi^2_2 = 1.67$, $n = 125$ and $p = 0.43$). Thus, there was no evidence for reduced survival of the AA genotype. The results were the same for subsets of the data in which the genotyped beetles were offspring from inter-F_1 crosses in which the female descended from a field-collected male (figure 3*a–d*) and female (figure 3*e–h*), respectively (ratios of genotypes 17:34:13 and 16:33:12) ($\chi^2_2 = 0.013$ and $p = 0.99$) and also for the two sexes of the genotyped beetles (ratios of genotypes, females 16:32:16 and males 17:35:9) ($\chi^2_2 = 2.05$ and $p = 0.36$). Thus, there was no evidence for any maternal effects influencing the survival rates of the different genotypes.

4. DISCUSSION

Our results demonstrate that the ability of the Kværkeby population of *P. nemorum* to use *Barbarea* as a host plant is controlled by dominant alleles of major autosomal R genes. Furthermore, the data support the hypothesis that the majority of beetles from this population are homozygous resistant at a single autosomal locus. The possibility of the presence of R genes at additional loci cannot be excluded, but the data suggest that such genes, if present, are relatively rare. The hypothesis that the majority of beetles in Kværkeby are heterozygous at different, unlinked, autosomal loci (see de Jong & Nielsen 2000) is not supported by the data. Under this hypothesis, a larger genotypic variation is expected in the F_1 and F_2 beetles and at least some F_1 beetles would be susceptible. No such variation was observed and the segregation patterns in F_1, F_2 and backcrosses support the conclusion of homozygosity for resistance at a single, major R locus. This finding may help elucidate some apparent discrepancies between the distribution of R genes in the field and results from previous laboratory assays.

(i) Beetles which are homozygous for R genes or which contain several R genes have been found in two Danish populations living on *Barbarea* (Ejby and Kværkeby).
(ii) R genes are present at low frequencies in populations living on other plants, but always in heterozygous condition (de Jong & Nielsen 1999).

(iii) Beetles which are homozygous resistant for an autosomal gene (from Ejby) suffer a severe fitness disadvantage compared to other genotypes (de Jong & Nielsen 2000).

These observations may indicate that beetles which are heterozygous at several loci experience a fitness advantage in comparison with beetles which are homozygous resistant at a single locus. This situation has been described in Ejby, where sex-linked genes (both X and Y linked) were reported to be predominant (Nielsen 1997*b*), although later investigations (de Jong & Nielsen 2000) have also found evidence for the occurrence of autosomal genes in this population. The genetic structure in Kværkeby is different from the one in Ejby, since beetles which are homozygous resistant for an autosomal gene predominate in the population. This result demonstrates that homozygous-resistant beetles do not necessarily suffer from a high fitness disadvantage in natural populations relative to heterozygous-resistant individuals and we were unable to detect any fitness reduction in beetles which were homozygous for the R gene from Kværkeby.

The homozygous-resistant beetles for which a fitness disadvantage was reported were produced in the following way. Initially, a line was produced which was isogenic with the ST line except for the presence of an autosomal R gene in heterozygous condition. The heterozygous lines were produced by repeated backcrossing (four to five generations) of males from the lines with females from the ST line. When these heterozygous beetles were crossed among each other, there was a significant reduction in the proportion of the AA genotype (homozygous resistant at the autosomal locus) in comparison with expectations (de Jong & Nielsen 2000).

There are at least three possible ways of explaining the apparent discrepancy between the low survival of homozygous-resistant beetles produced in the laboratory and their common occurrence in the field. The first one is that R genes may exist at different autosomal loci, some of which result in a low survival of homozygous-resistant beetles and some with no such effect. If this is true, the gene at the former locus must be rare or absent in Kværkeby, whereas in Ejby it may be present at a higher frequency. However, the founders of both lines with an autosomal R gene from Ejby appeared to be homozygous resistant (J. K. Nielsen, unpublished results), whereas after repeated backcrossing with the ST line, homozygous-resistant individuals showed a highly reduced viability (de Jong & Nielsen 2000). Although it provides no conclusive evidence of whether the autosomal genes in Ejby and Kværkeby are identical, this suggests that the reduced survival of homozygous-resistant beetles in the Ejby lines was caused by the backcrossing procedure.

Since the backcrossing always involved the pairing of resistant males with ST females (see above), a second possibility is that maternal effects influence the survival of homozygous-resistant beetles in the field. However, for the Kværkeby sample no difference was found between subsets of the data in which a field-collected male or female was initially crossed with an ST beetle. Hence, no evidence was found for maternal effects influencing the fitness of the AA genotype.

The third hypothesis, which is consistent with the data for the Ejby population but which may apply more generally, is that the R gene at an autosomal locus has a deleterious effect, but that in populations on *Barbarea* in the field there is strong selection in favour of modifier genes which counteract the negative fitness consequences of the R gene. The resistance in the field to *Barbarea* defence is then refined by a coadapted gene complex which is broken up by the process of backcrossing to susceptible beetles to produce the near-isogenic lines. A similar mechanism is known to influence traits in other systems. One example is that of polymorphic Batesian mimicry in *Papilio* (Clarke & Sheppard 1963). The mimetic patterns are governed by major genes, but 'fine-tuned' by modifier genes. Hybridization between races containing and not containing a particular mimetic pattern breaks up the close resemblance of the mimics. In addition, in insect–host plant interactions the operation of modifier genes has been suggested to play a role after an initial mutation of large effect (Sezer & Butlin 1998). Other examples include those of resistance against insecticides. In the Australian sheep blowfly *Lucilia cuprina*, the fitness of genotypes which are resistant to diazinon is reduced when the genetic background of resistant genotypes is disrupted, which is explained by the selection in favour of modifiers with exposure to the insecticide (McKenzie *et al.* 1982). More work is needed in order to determine whether disruption of coadapted gene complexes is indeed the mechanism responsible for the high mortality in homozygous-resistant *Phyllotreta* from backcrossed lines and investigate the generality of this mechanism across different populations (including Kværkeby).

One way of pursuing this possibility further is directly related to the consequences of this mechanism for the distribution of R genes in field populations. In east Denmark, beetles which are collected on *Barbarea* are all resistant to its defences, but, of those that are collected on other host plants, such as *Sinapis arvensis* (even in close proximity to *Barbarea*), a low proportion usually show resistance to *Barbarea* defences (de Jong & Nielsen 1999). One possible explanation for the rarity of R genes on host plants other than *Barbarea* are trade-offs across host plants which are associated with the presence of R genes. A trade-off would result from a cost of resistance in terms of efficiency in using the alternative host plant. This can be measured as a negative genetic correlation in performance characters across different host plants. Whereas trade-offs have been sought repeatedly, they have rarely been found (see Via (1990) and references therein) and *P. nemorum* appears to be no exception (Nielsen 1996, 1999; P. W. de Jong, P. Meijer and J. K. Nielsen, unpublished data; J. K. Nielsen, P. W. de Jong and H. O. Frandsen, unpublished data). Other potential costs associated with the presence of R genes and the use of *Barbarea* include, among others, susceptibility to parasitoids and pathogens. Another factor that may influence the distribution of R genes is the amount of gene flow between local populations of *P. nemorum*. However, a mere limitation to gene flow in the absence of any other mechanism is unlikely to prevent the spread of R genes to alternative host plants. However, if the disruption of coadapted gene complexes is responsible for the mortality

of beetles which are homozygous for an autosomal R gene in our backcrossed lines, this may also play a role in the field. Thus, there is selection in favour of beetles carrying R genes on *Barbarea*, leading to coadapted gene complexes if the R genes confer a fitness disadvantage in homozygous condition. On other host plants, beetles without such R genes can survive and reproduce and are expected to be initially common. Immigrants in such populations originating from *Barbarea* are expected to mate predominantly with beetles lacking R genes. This may continue for several generations, disrupting any coadapted gene complexes in the migrants. Hence, this mechanism prevents the R genes from becoming fixed in populations on host plants other than *Barbarea*, given a certain limitation to gene flow. Crossing beetles collected on host plants other than *Barbarea* but which are heterozygous for an autosomal resistance gene is thus expected to lead to an increased mortality of homozygous-resistant individuals, whereas those collected on *Barbarea* produce offspring of which those homozygous for the R gene survive.

The present data and those from Nielsen (1997*b*) both provide evidence for major genes with large effect at a few loci determining the ability of *P. nemorum* to use *B. vulgaris* ssp. *arcuata* (G type) as a host plant. This is consistent with one of the possible models of the genetic basis of adaptive traits (see Jones 1998; Sezer & Butlin 1998): a few genetic factors with large effect, with the possible presence of modifiers. Jones (1998) reached the tentative conclusion that such an 'oligogenic' basis of natural adaptations may be the norm. Depending on the level of gene flow, genes of large effect may be favoured relative to a slow accumulation of smaller changes when the latter would lead to a reduction in fitness on the 'old' host plant without conferring the ability of colonization of the new host. Mutations of large effect may lead to initial establishment on the new host, followed by selection of modifier genes (Sezer & Butlin 1998). However, in the flea beetle system the mode of inheritance of R genes varies dramatically across populations. Whereas in Ejby, autosomal inheritance was relatively rare (Nielsen 1997*b*), all beetles in the Kværkeby sample showed autosomal inheritance. This suggests that gene flow between the Kværkeby and Ejby populations, which are separated by 44 km, is indeed limited, which is also supported by allozyme studies (P. W. de Jong, H. de Vos and J. K. Nielsen, unpublished data). It also raises the question of how resistance to *Barbarea* defence has evolved in the different populations. R genes at different loci may have originated as independent mutations or as one mutation at one locus and subsequent translocation or non-random segregation of the chromosomes on which the R gene is located (Nielsen 1997*b*, 1999) in some populations, but not in others.

Irrespective of the origin of the R genes at different loci, the results from this study highlight the necessity of studying host-plant adaptation of *P. nemorum* in a geographical context (Thompson 1994, 1999*a*–*c*; see also Sword & Dopman 1999). Although the host plants of *P. nemorum*, including *Barbarea*, are very common, they occur in local patches which may impose a geographical structure on *P. nemorum* populations. This geographical structure is likely to limit the spread of R genes and may lead to locally adapted populations. There are certainly geographical differences in the genetic basis of host-plant use in *P. nemorum*, which in turn will influence the evolution of host-plant use in this species.

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