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Mono- and oligophagous *Phyllotreta* (Coleoptera: Chrysomelidae) species: the relation between host plant range and genetic diversity

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ABSTRACT. The niche-width variation hypothesis predicts a positive correlation between niche width and genetic variability. Here we evaluated this hypothesis by comparing genetic variability in four monophagous (narrow niche) and six oligophagous (broad niche) species of the beetle genus *Phyllotreta* Chevrolat, 1837 (Coleoptera: Chrysomelidae: Alticinae). The results, obtained over a similar geographic range, demonstrate that for all genetic variability measures used (average number of alleles per locus, percentage of loci polymorphic, observed heterozygosity and expected heterozygosity under Hardy-Weinberg conditions) the monophagous species showed a significantly higher genetic variability compared to the oligophagous species. This result is at variance with the niche-width variation hypothesis.

KEY WORDS : Niche-width variation hypothesis, allozymes, genetic variability, phytophagous insects.

INTRODUCTION

In 1965 VAN VALEN postulated the "niche-width variation hypothesis" stating that "wider niches would permit greater phenotypic variation if this variation is controlled to a significant extent by the adaptive diversity of the niche". Although in his original definition Van Valen does not talk about genetic variation, in later studies the hypothesis was mainly interpreted in a genetic context (SOMERO & SOULÉ, 1974; STEINER, 1977; LACY, 1982; LAVIE & NEVO, 1986; NOY et al., 1987) predicting a positive correlation between niche breadth and genetic variability. Several studies testing the hypothesis have been performed and almost all of them confirm it (e.g. BABBEL & SELANDER, 1974; STEINER, 1977; LAVIE & NEVO, 1981; 1986). The most crucial factor in testing the hypothesis is of course the definition of the niche, and the ability to find a group of closely related organisms in which one subgroup clearly has a broader niche than the other. Although niche components such as geographic (e.g. BABBEL & SELANDER, 1974; LAVIE et al., 1993) or physiological range (NOY et al. 1987) are often used in studies of this kind, it is difficult to really quantify them as niche parameters. In the same way NEVO et al. (1984) compared genetic variation between habitat specialists and generalists over 669 species (literature data) and found more genetic variation in generalists for vertebrates, *Drosophila* and molluscs, and (depending on the combination of categories used to define generalists) also for insects. Several studies testing the hypothesis compared genetic variability between organisms belonging to different genera (e.g. SOMERO & SOULÉ, 1974; MITTER & FUTUYMA, 1979; LACY, 1992; LAVIE et al., 1993). As the amount of genetic variation can differ greatly between animal groups it is preferable only to compare closely related organisms, differing mainly in clearly quantifiable niche components. LAVIE et al. (1993) stated that although hitherto the niche-width variation hypothesis has been supported by many studies, additional critical tests are imperative.

A group of animals that seems to be ideally suited for testing the hypothesis is found in the phytophagous insects. The niche of a phytophagous insect is mainly determined by the number of host plant species it is able to live on. The range of host plants of an insect species will depend on both chemical and morphological characteristics of the plants concerned. Co-evolution, chemical adaptation, fitness, predation and parasitism rates all may have their influence on the effective host plant spectrum (MATSUDA, 1988; SOETENS et al., 1991; JERMY, 1994; METCALF, 1994). Host-associations are the results of complex interactions between the plants' defensive systems and the insects' possibility to survive and reproduce on them (HSIAO, 1969; SIEMENS & JOHNSON, 1990), reaching a climax of adaptation in those insects that are even capable of sequestration of plants' defensive chemicals for use as their own defensive secretions (PASTEELS et al., 1988a, 1988b 1994; ROWELL-RAHIER et al., 1991; PASTEELS, 1993).

Within the phytophagous insects, some genera contain only generalist species, while in others specialisation (on often unrelated plant species) seems to be the rule. However, within certain groups of closely related species, both specialists and generalists are found. These groups provide an excellent opportunity to study the above hypothesis. To our knowledge only two studies testing the hypothesis (and showing contradicting results) on phytophagous insects (MITTER & FUTUYMA, 1979; LACY, 1982) have been performed, unfortunately both using comparisons between species of different genera.

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Within the beetle family Chrysomelidae, the genus *Phyllotreta* (also known as the cruciferous flea beetles) contains both mono- and oligophagous species, all feeding within the plant family of the Brassicaceae or the related families Resedaceae and Caparidaceae (Mohr, 1966; Nielsen, 1978; Doguet, 1995). The only exception in this genus is *Phyllotreta vittula* (Redtenbacher, 1849), which, besides accepting various Brassicaceae, also feeds on grasses and cereals (Kostromitin, 1973; Vig, 1998a, 1998b). Ecology and host plant relationships of several *Phyllotreta* species have been studied extensively (Nielsen, 1978; 1988; Nielsen et al., 1979a 1979b; Lamb & Palaniswamy, 1990; Palaniswamy & Lamb, 1993; Palaniswamy & Bodnaryk, 1994; Milbrath et al., 1995). Both larvae and adults live on the same plants, indicating a close relation between host and parasite. Larvae feed externally on the roots (e.g. *P. cruciferae* (Goeze, 1777), *P. consobrina* (Curtis, 1837)) or mine the leaves (e.g. *P. tetrastigma* (Comolli, 1859), *P. flexuosa* (Illiger, 1794). Cruciferous plants contain several defensive chemicals to protect them from insects, and even inhibit growth of other plants (Bodnaryk, 1991; Dimock, et al. 1991; Ghaout et al., 1991; Bodnaryk, 1992; Yamane et al., 1992). Acceptability of a plant for a *Phyllotreta* species may depend on the presence and/or absence of certain chemicals, which can act as attractants or deterrents (Feeny *et al.,* 1970; Hicks, 1974; Nielsen, 1988; Bodnaryk, 1991,1992), and certain morphological characteristics such as pubescence of the leaves, stems and pods (Lamb, 1980; Palaniswamy & Lamb, 1993; Palaniswamy & Bodnaryk, 1994). This association of closely related insects with closely related host plant species makes *Phyllotreta* a suitable genus for evaluation of the niche-width variation hypothesis.

MATERIAL AND METHODS

We obtained material from 36 *Phyllotreta* populations from six countries (Table 1). All animals were collected from their host plants using an aspirator or a sweepnet. In total about 5000 specimens comprising 10 different species were used for a study of genetic variation using allozyme electrophoresis. Due to the small size of the animals, only three to five loci could be evaluated per specimen. The species were divided into two groups: monophagous (11 populations, 4 species: *P. astrachanica* Lopatin, 1977, *P. dilatata* Thomson, 1866, *P. flexuosa* and *P. tetrastigma*) and oligophagous (25 populations, 6 species: *P. aerea* Allard, 1895, *P. atra* (Fabricius, 1775), *P. consobrina*, *P. cruciferae*, *P. nigripes* (Fabricius, 1775) and *P. ochripes* (Curtis, 1837)). A species was considered monophagous if it only lives on one host plant species or on host plant species belonging to the same genus; it was considered oligophagous if it lives on host plants of several genera (Bernays & Chapman, 1994). Living on a host plant means that in outdoor conditions the species are known to feed and reproduce on the plants concerned. Although for some monophagous species it is known that they survive on other non-host plants in the laboratory (Nielsen, 1978), we do not take this into account. The separation into these two groups does not reflect any phylogenetic relationships (unpublished data). The average distances between two populations of a monophagous species (mean ± st. dev.: 421 ± 241km) and between two populations of an oligophagous species (mean ± st. dev.: 466 ± 264 km) were not significantly different (Mann-Whitney U test, $p > 0.05$).

Vertical polyacrylamide gel electrophoresis (PAGE) was performed for 10 loci: aconitase (ACO, E.C. 4.2.1.3), α-amylase (AMY, E.C. 3.2.1.1), α-glycerophosphate dehydrogenase (GPD, E.C. 1.1.1.8), aspartate aminotransferase (AAT, E.C. 2.6.1.1), isocitric dehydrogenase (ICD, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), mannose phosphate isomerase (MP1 and MP2, E.C. 5.3.1.8 [2 loci]), peptidase (Leu-Ala) (PEP, E.C. 3.4.-.-) and phosphoglucomutase (PGM, E.C., 5.4.2.2.). Sample preparation, storage, electrophoresis running conditions and buffer systems used are as in Verdyck et al. (1996). As low sample numbers could give rise to aberrations of the variability measures, only populations for which the mean number of specimens per locus was larger than 10 were included in this study.

Genetic variability was studied using four different criteria: the average number of alleles per locus, the percentage of loci polymorphic, the observed heterozygosity (Hobs.) and the expected heterozygosity under Hardy-Weinberg conditions (Hexp.). All calculations were performed using BIOSYS-1 (Swofford & Selander, 1989). For each of the measures a Mann-Whitney U test was used to check for significant differences between monophagous and/or oligophagous species.

Populations were tested for deviation from Hardy-Weinberg equilibrium using exact probabilities, corrected for multiple comparisons using sequential Bonferroni correction. Fixation index (F) and coefficients of heterozygote deviation ($D= H_o-H_e/H_e$) were calculated per locus for each population. To compare between groups, the coefficients were averaged over all polymorphic loci (for which significant deviation from Hardy-Weinberg equilibrium was found) for each of the populations. These averages were compared using a Mann-Whitney U test.

RESULTS

Table 1 gives an overview of all genetic variability measures calculated for all 36 populations. Table 2 gives the minimum, maximum and mean values with standard deviation for each variability measure for the mono- and the oligophagous species. The mean sample size did not differ significantly between the mono- and oligophagous species (Mann-Whitney U test, $p>0.05$). For all the measures, the monophagous species showed a significantly higher genetic variability compared to the oligophagous species (Mann-Whitney U test, $p<0.05$).

The results of the exact probability tests (after sequential Bonferroni correction) showed only significant deviation from Hardy Weinberg equilibrium in the populations *P. aerea* [loc.: Breisach] for MP1, *P. aerea* [loc.: Fulda] for ACO and MP1, *P. aerea* [loc.: Ludwigsberg] for ACO and *P. tetrastigma* [loc.: Zoersel] for MP2. Thirty two out of 36 populations (nine out of ten monophagous and 23 out of 26 oligophagous) showed no deviations from Hardy-Weinberg equilibrium for any of their variable loci. For the mono- and oligophagous species, respectively, one out of 56 and four out of 68 cases of polymorphism were significantly different from Hardy-Weinberg equilibrium (exact probabilities, $p<0.05$).

TABLE 1

Genetic variability (four measures: number of alleles per locus, percentage of polymorphic loci, observed (Hobs) and expected heterozygosity (Hexp)) at 10 loci in all populations for the different *Phyllotreta* species; M= monophagous; O= oligophagous

Species	Population (country)	M/O	Mean sample size/locus	Mean No of alleles/locus	% of loci polym. (no.crit.)	% of loci polym. (95%)	Hobs.	Hexp.
P.aerea	Breisach am Rhein (D)	O	30.5	1.7	40	30	0.058	0.144
P.aerea	Fulda (D)	O	36.9	1.5	30	30	0.066	0.138
P.aerea	Gembloux (B)	O	29.7	1.4	30	30	0.079	0.109
P.aerea	Ludwigsburg (D)	O	29.7	1.6	40	30	0.067	0.139
P.aerea	Wimereux (F)	O	29.5	1.5	30	30	0.083	0.151
P.astrachanica	St.-Aignan-Grandlieu (F)	M	16.4	1.3	30	10	0.059	0.060
P.atra	Celles sur Plaine (F)	O	24.2	1.5	30	20	0.076	0.074
P.atra	Fulda (D)	O	20.6	1.4	40	10	0.065	0.065
P.atra	Stansted (UK)	O	31.3	1.4	30	30	0.050	0.053
P.consobrina	Berchem (B)	O	42.2	1.4	30	20	0.058	0.084
P.consobrina	Gembloux (B)	O	35.4	1.3	20	20	0.066	0.079
P.consobrina	Wimereux (F)	O	28.0	1.3	20	20	0.070	0.083
P.cruciferae	Berchem (B)	O	64.9	1.2	20	10	0.027	0.024
P.cruciferae	Breisach am Rhein (D)	O	37.5	1.4	20	10	0.028	0.028
P.cruciferae	Frederiksberg (DK)	O	43.4	1.3	20	10	0.020	0.019
P.cruciferae	Gembloux (B)	O	83.0	1.3	30	10	0.012	0.014
P.cruciferae	Strasbourg (D)	O	43.8	1.4	40	10	0.027	0.026
P.cruciferae	Wimereux (F)	O	40.8	1.3	20	10	0.023	0.021
P.dilatata	Deurne (B)	M	44.0	1.7	50	30	0.076	0.092
P.dilatata	St.-Aignan-Grandlieu (F)	M	30.0	1.7	60	40	0.062	0.099
P.dilatata	St.-Philbert-de-Grand-Lieu (F)	M	30.1	1.6	50	20	0.059	0.085
P.flexuosa	Udenhout (NL)	M	10.5	1.7	40	20	0.150	0.124
P.nigripes	Berchem (B)	O	35.4	1.2	20	0	0.006	0.012
P.nigripes	Gembloux (B)	O	25.7	1.1	10	10	0.015	0.014
P.nigripes	Fulda (D)	O	39.0	1.5	50	10	0.027	0.029
P.nigripes	Taastrup (DK)	O	16.6	1.1	10	10	0.013	0.012
P.nigripes	Wimereux (F)	O	16.6	1.3	20	10	0.023	0.043
P.ochripes	Deurne (B)	O	35.6	1.1	10	0	0.000	0.008
P.ochripes	Oisterwijk (NL)	O	34.9	1.4	30	10	0.008	0.025
P.ochripes	St.-Philbert-de-Grand-Lieu (F)	O	43.3	1.6	50	0	0.014	0.018
P.tetrastigma	Celles sur Plaine (F)	M	50.8	1.7	40	40	0.119	0.141
P.tetrastigma	Chimay (F)	M	48.7	2.1	80	40	0.114	0.138
P.tetrastigma	Geisenfeld (D)	M	38.2	1.8	50	20	0.103	0.119
P.tetrastigma	Stenholts Vang (DK)	M	27.8	1.5	40	30	0.124	0.127
P.tetrastigma	Udenhout (NL)	M	34.7	1.8	50	30	0.104	0.144
P.tetrastigma	Zoersel (B)	M	73.9	1.9	70	30	0.099	0.130

TABLE 2

Genetic variability in mono- and oligophagous species (four measures: number of alleles per locus, percentage of polymorphic loci, observed (Hobs) and expected heterozygosity (Hexp))

	Mean		St.Dev		Minimum		Maximum	
	M	O	M	O	M	O	M	O
Sample Size	36.827	35.940	17.471	14.086	10.500	16.600	73.900	83.000
No Alleles/locus	1.709	1.368	0.207	0.157	1.300	1.100	2.100	1.700
% loci polym.)	50.909	27.600	14.460	11.284	30.000	10.000	80.000	50.000
Hobs.	0.097	0.039	0.030	0.027	0.059	0.000	0.150	0.083
Hexp.	0.114	0.056	0.027	0.047	0.060	0.008	0.144	0.151

In most populations the expected heterozygosity (Hexp.) was somewhat larger than the observed heterozygosity (Hobs.). The coefficient of heterozygote deviation (D) (Koehn et al. 1976) was negative in all five cases where deviations were found; the fixation index (F) was always positive (Table 3). These results show that, in these few cases, there were fewer heterozygotes than expected under Hardy-Weinberg conditions.

TABLE 3

Values for D (coefficient of heterozygote deviation) and F (fixation index) for loci not in Hardy-Weinberg equilibrium

Population	ACO	MP1	MP2
P. aerea (Breisach)	-	F= 0.755 D= -0.759	-
P. aerea (Fulda)	F= 0.707 D= -0.720	F= 0.908 D= -0.909	
P. aerea (Ludwigsberg)	F= 0.757 D= -0.761	-	-
P. tetrastigma (Zoersel)	-	-	F= 0.733 D= -0.736

DISCUSSION

Hsiao (1989) compared genetic variability studies for 30 Coleopteran species belonging to five families and found a mean heterozygosity (Hexp) of 0.168, amongst the highest recorded in insects. Within the Chrysomelidae he found mean heterozygosities between 0.081 and 0.206. In more recent studies on chrysomelids the following ranges are found: 0.061 to 0.238 in *Diabrotica* species (recalculated from Krysan et al., 1989), 0.003 to 0.195 in *Ophraella* species (recalculated from Futuyma & McCafferty, 1990), 0.077 to 0.624 in *Oreina* species (recalculated from Rowell-Rahier (1992) and Rowell-Rahier & Pasteels (1994)). The heterozygosity values in *Phyllotreta* species seem to be slightly lower than in most other chrysomelid groups (Hexp between 0.060 and 0.144 in monophagous and between 0.008 and 0.151 in oligophagous species). For the monophagous species the mean heterozygosity value (0.114) does not differ very much from the average heterozygosity value in other Coleoptera (0.168) (Hsiao, 1989). For the oligophagous species the mean value (0.056), however, is amongst the lowest found in Coleoptera.

The results clearly indicate that the specialized monophagous *Phyllotreta* species are genetically more variable than generalist oligophagous species. This is at variance with the niche-width variation hypothesis. Until now most of the studies testing this hypothesis confirmed it (e.g. Avise & Selander, 1972; Lacy, 1982; Nevo et al., 1984 (analysing literature data for 669 species); Lavie & Nevo, 1986; Noy et al., 1987; Lavie et al., 1993; Pavliek & Nevo, 1994). However, some studies (Sabath, 1974; Somero & Soulé, 1974; Mitter & Futuyma 1979) did not support the hypothesis.

Although in this study we only tested 10 different loci, representing only a small part of the genome (which makes it impossible to extend our conclusions towards the genetic variability of the entire genome), the finding of strongly significant differences for all genetic variability measures allows us to reject the hypothesis for the *Phyllotreta* species studied.

How can the low genetic variability in the oligophagous species be explained? The monophagous species have always been found in natural habitats, whereas most of the oligophagous populations were found in agricultural or at least cultivated habitats (such as botanical gardens) (except *P. atra* from Celles sur Plaine and the three populations of *P. ochripes*). Populations in agricultural and cultivated habitats may be more subjected to drastic changes in numbers (insecticide use) and more affected by selective pressures (crop rotation), and may be extinguished and recolonized more often than populations in more stable natural habitats. Therefore founder events might be more important in these oligophagous animals, resulting in lower genetic variability. If this were true for many of the populations, we would expect different loci to be variable in different localities. However, our results show that within a species, the variable loci are often the same within different localities (although this may be an indication for selection). Moreover, we did not find higher genetic variability in the four oligophagous populations collected in natural habitats, where founder effects are supposed to be less important.

An alternative explanation for this phenomenon may be found in the host plant use by the different species. The monophagous *Phyllotreta* species studied feed on plants such as *Cardamine* sp. or *Rorippa* sp. These plants are very abundant during a short period of the year, as after flowering they disappear completely. Animals feeding on them have only limited time to complete their cycle. As for the *Phyllotreta* species both larvae and adults live on the same plants, and correct timing is crucial. When the plants will be there is related to weather conditions changing from year to year. This system may cause an evolutionary stress for the monophagous species and, if timing and development are genetically controlled, we can assume an advantage in variable populations, maintaining polymorphism in these species. For the oligophagous species there is always an assortment of cruciferous plants to be found, from early spring to early winter, and timing is supposedly less important, resulting in less polymorphism.

Here we find higher genetic variability for both observed and expected heterozygosity values in the specialist *Phyllotreta* species. Relationships between heterozygosity and several fitness traits have been studied extensively in several organisms, showing that highly heterozygous individuals usually have superior growth rates, more buffered developmental processes and lower morphological variation (Mitton & Grant, 1984; Zouros, 1987; Houle, 1989). In bivalves multiple-locus heterozygosity is positively correlated with growth and viability (Zouros & Foltz, 1984; Holley & Foltz, 1987; Zouros, 1987; Gaffney et al., 1990). Although it has never been studied in chrysomelid beetles, it is possible that also in these animals, under stressful conditions, heterozygous genotypes perform better than more homozygous genotypes.

In conclusion we can state that all alternative explanations for the refutation can be valid. The data are straightforward, and our paper represents a strong case for the rejection of the niche-width variation hypothesis. Although this does not mean that it can not be valid for some organisms, the hypothesis should not be taken for granted in general.

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