Belg. J. Zool. - Volume 128 (1998) - issue 2 - pages 119-134 - Brussels 1998

Received: 18 February 1998

GENETIC COMPARISON OF TWO COLOUR MORPHS OF *PHYLLOTRETA TETRASTIGMA* (COLEOPTERA: CHRISOMELIDAE, ALTICINAE)

PETER VERDYCK ([1,2]), HANS DE WOLF ([2]), KONJEV DESENDER ([1]),
JAN HULSELMANS ([2]) & PATRICK GROOTAERT ([1])

([1]) Department of Entomology, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussel

([2]) Evolutionary Biology Group, Department of Biology, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen,
e-mail: verdyck@kbinirsnb.be

Abstract. In the flea beetle species *Phyllotreta tetrastigma* two colour morphs (2B and 4S) exist in western Europe. We test whether these forms are genetically differentiated using two techniques: Polyacrylamide gel electrophoresis (PAGE) of allozyme loci and Isoelectric Focusing (IEF) of general proteins. PAGE reveals that both forms are not genetically isolated and IEF did not show any differences in banding patterns between them. Both forms are considered to interbreed and to belong to the same gene pool.

Key words: color forms, *Phyllotreta*, Chrysomelidae, genetics, allozymes, Isoelectric Focusing.

INTRODUCTION

Colour polymorphism is known in many different groups of animals (*e.g.* BOOTH, 1990; CORDERO, 1990; BACKELJAU *et al.* 1992; HOLLOWAY, 1993). Variation in colour can be due to host plant choice, season, temperature during development and/or can be genetically determined. Undoubtedly, the best studied cases are of species of Lepidoptera (*e.g.* COOK *et al.*, 1990; KINGSOLVER & WIERNASZ, 1991; SMITH *et al.*, 1993; PAULSEN, 1994) and these have aroused much interest concerning the adaptive value, heritability and phenotypic plasticity of their different colorations.

Although more limited in number, several genetic studies on beetle species considered colour polymorphism. LIEBHERR (1983) showed that in the ground beetle *Agonum decorum* Say, 1823 the red-green colour polymorphism and hirsute-glabrous setational polymorphism are determined by unlinked, autosomal diallelic loci. HANTULA *et al.* (1987) failed to detect genetic differences associated with colour polymorphism in the weevil *Diaprepes abbreviatus* (L., 1764).

In chrysomelid beetles the amount of colour variation is often very large, but the number of studies on the phenomenon remains rather limited. In chrysomelid systematics colour characteristics have been used to distinguish between different species (BROWN,

1956; MOHR, 1966; DOGUET, 1986, 1994), but in many cases (and often in the very same studies), the systematic value of colour characteristics is questioned (BROWN, 1956; DOGUET, 1986) as a considerable amount of variation in colour pattern is found within a single species, and sometimes even within a single population. In some cases ecological differences are detected which can be helpfull in making systematic decisions (BROWN, 1956), but in most cases they do not allow authors to conclude whether different colour forms are genetically isolated, and thus represent biological species, or not. In several instances a more profound study revealed that systematic differences based on coloration were incorrect.

Probably the first study on the genetics of colour polymorphism in chrysomelids was the work of THOMAS (1964) on the cassid beetles of the genus *Aspidomorpha* Hope, 1840. He discovered that three former species (*A. adhaerens* (Weber,1801), *A. testudinaria* (Montrouzier, 1855) and *A. phyllis* (Boheman, 1862)) were in fact colour morphs of a single species. FUJIYAMA & ARIMOTO (1988) found random mating between two colour forms of *Chrysolina aurichalcea* (Mannerheim). VASCONCELLOS-NETO (1988) studied the genetics of *Chelymorpha cribraria* (Fabricius, 1775) and found that the elytral colour is expressed by three different alleles of a single gene, whereas pronotum colour is a quantitative character under polygenic control. BOITEAU *et al.* (1994) studied the genetics of a beige elytral mutant of the Colorado potato beetle *Leptinotarsa decemlineata* Say, 1824 and found that inheritance was controlled by two dominant genes. BOITEAU (1994) discusses the genetics of several mutations (white body, pearleye, black body and beige elytra) of *L. decemlineata* and discovered that the beige mutant has lower fitness. LU & LOGAN (1994) showed that larval colour variation in *L. decemlineata* is controlled by two epistatic loci. VERDYCK *et al.* (1996) did not find genetic isolation between two colour forms of *Phyllotreta cruciferae* (Goeze, 1777).

Here we study *Phyllotreta tetrastigma* (Comolli, 1837), one of the larger species within the genus *Phyllotreta* (Coleoptera: Chrysomelidae), widely distributed throughout Europe and Western Asia. The species is closely related to *P. dilatata* Thomson, 1866 and *P. flexuosa* (Illiger, 1794), from which it is distinguished by minor differences in the elytral colour pattern (DOGUET, 1986, 1994). *P. tetrastigma* is a monophagous species (NIELSEN, 1978b) feeding only on *Cardamine sp.* It is almost exclusively found on large bittercress, *Cardamine amara* (NIELSEN, 1978a; NIELSEN, 1978b), though we found one population feeding on *Cardamine flexuosa.* This species is monophagous in the field, but in laboratory conditions it will also feed on other cruciferous plants (NIELSEN, 1978b).

Within *P. tetrastigma*, two types of elytral colour pattern can be distinguished. Type 2B has a yellow band (which is narrowed in the middle) on each elytron. Type 4S has two yellow spots on each elytron. Variation within each type is considerable and few specimens are intermediate. In most places both forms can be found, which means that they are sympatric, even the series of syntypes contains specimens of both forms (VERDYCK *et al.*, 1995). VERDYCK *et al.* (in press) did not find morphological differences between them. This study aims to test for genetic isolation between the two colour forms of *P. tetrastigma*, and to examine several aspects of population genetics in the species.

MATERIAL AND METHODS

Populations studied

Six populations of *P. tetrastigma,* in which both forms were present were sampled in Western Europe in the period 1990 to 1994 (Table 1). All animals were collected from the leaves of their host plant using an aspirator. In all cases the host plant was *Cardamine amara*, except in the Udenhout (NL) population where the animals fed on *Cardamine flexuosa*. All habitats sampled were characterised as wet and shady places in woods. Animals were transported alive to the laboratory or immediately frozen in liquid nitrogen. They were stored at -80°C until sample preparation.

TABLE 1

Populations and numbers of P. tetrastigma *studied, numeric contribution of both forms (4S-2B) in each population*

Locality	*Country*	*Lon, Lat*	*No of specimens*	*4S*	*2B*	*% 4S*	*% 2B*
Celles sur Plaine	France	48/57'N, 6/57'E	127	20	107	15.7	84.3
Chimay	Belgium	50/3'N, 4/19'E	119	14	105	11.8	88.2
Geisenfeld	Germany	48/41'N, 11/37'E	84	6	78	7.1	92.9
Stenholts Vang	Denmark	55/57'N, 12/21'E	64	14	50	21.9	78.1
Udenhout	Netherlands	51/37'N, 5/9'E	77	13	64	16.9	83.1
Zoersel	Belgium	51/16'N, 4/42'E	220	72	148	32.7	67.3
Total			691	139	552	20.1	79.9

Electrophoretic analysis

For sample preparation the abdomens of the animals were removed and single abdomens were homogenized in 25µl sucrose solution (20% w/v). Crude homogenates were centrifuged at 4°C for 45 minutes at 15,000 rpm (27.200 g) and were stored at -80°C until electrophoresis.

Nine enzyme systems coding for ten different loci were screened: aconitase (*ACON*, 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 (*MP-1* and *MP-2*, 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.). Staining recipes were adapted from HARRIS & HOPKINSON (1976). The buffer systems used were a Tris/Citric Acid (TC) system pH 8.0 (0.1M) [for *ACON, AMY, GPD, ICD, MP-1, MP-2* and *PGM*] and a Tris/Boric Acid/EDTA (TBE) system pH 8.9 (0.1M) [for *AAT, MDH* and *PEP*]. For each sample 5µl of supernatant was applied to 6% polyacrylamide gels. Vertical polyacrylamide gel electrophoresis (PAGE) was performed with Hoefer Mighty Small System II, running gels for 15 minutes at 25 Volts, then 15 minutes at 50 Volts and finally for 1 or 3 hours at 150 Volts (for TC and

TBE buffer respectively). Alleles were designated alphabetically according to decreasing mobility, the fastest allele (the most anodal one) being A.

Hardy-Weinberg equilibrium

The six geographic populations (4S and 2B combined) were tested for deviations from Hardy-Weinberg (HW) equilibrium using exact probabilities (SWOFFORD & SELANDER, 1989), (corrected for multiple comparisons using sequentially rejective Bonferroni test). The same was done for the 4S and 2B form within each locality.

Population differentiation

Hierarchical F-statistics (WRIGHT, 1965, 1978) were used to analyse genetic differentiation at two different levels. In our hierarchy F refers to form, L to locality and T to total. In this study we have six populations, and within each population we have two forms: 4S and 2B. In this way differentiation among forms within localities is described by F_{FL}, and so on. We also performed contingency table analyses of heterogeneity among forms (for each population) and among populations, and calculated fixation indices F and coefficients of heterozygote deviation D.

Genetic distances, Clustering and Multivariate Analysis

There has been much discussion on which clustering method is the best, and in his overview BUTH (1984) concluded that for closely related species there are difficulties in obtaining a correct topology with most methods. Here the two most predominantly used genetic distances, (NEI (1978) unbiased genetic distances and Modified Rogers distances (WRIGHT, 1978)) were calculated between populations. Both were used for the construction of UPGMA dendrograms, but only Rogers distance could be used for construction of a Wagner tree with midpoint rooting. A multivariate analysis of the allele frequencies was performed using the correspondence analysis (CA option) of the program CANOCO (V3.2) (TER BRAAK, 1988), and the population scores on the two first canonical axes are plotted.

Isoelectric Focusing

A second evaluation of genetic differentiation was performed using Isoelectric Focusing (IEF) of general proteins. This was done with PhastSystem from Pharmacia LKB. Using a 8/1 sample applicator, 1 :1 of sample was applied to an IEF gel with pH range 4-6.5. Samples of both forms were chosen randomly from specimens of the six populations. The program running conditions are as in VERDYCK *et al.* (1992). After a run of 500 Vh the proteins have moved to their isoelectric point and are visualized by means of silver staining according to the PhastSystem IEF silver staining program performed with PhastSystem developing unit. Gels were air dried and maintained in standard 4x4 cm slides for storage and further manipulation.

Gels were projected on a slide viewer for visual interpretation. As only adjacent lanes were compared (the same lane not being used twice), each gel (8 lanes) gave 4 comparisons. Counts were made for the number of bands visible in both lanes and for the number of bands unique for lane one and lane two respectively. Lanes of bad quality were excluded from analysis and parts of lanes that were not clearly interpretable were not used.

The mean number of bands for the two forms was compared using a Mann-Whitney U test. We calculated three different similarity measures. The similarity S_F as defined by FERGUSON (1980) and used in similar studies of BACKELJAU (1985) and VERDYCK *et al.* (1992, 1996) is defined as the number of bands of common mobility divided by the maximum number of bands for an individual. The Jaccard (S_J) and Dice (S_D) indices, as defined in SNEATH & SOKAL (1973), take into account both the specimens sampled (thus avoiding strong influences of individuals with an extremely high number of bands). Bands in common in both individuals are given more weight in S_D.

Three groups of similarities (between two 4S forms (4S4S), between two 2B forms (2B2B) and between 4S and 2B forms (4S2B) are calculated (4S4S and 2B2B = intraform similarities, 4S2B = interform similarities)). Fifteen comparisons were used for each group. The three different similarities were compared using ANOVA. To avoid interdependance of the comparisons, individuals were never used twice.

RESULTS

Populations studied

In total we screened 691 animals (139 of the 4S form and 552 of the 2B form) (Table 1). In all six populations studied the 4S form is less abundant compared to the 2B form. The proportion of the 4S form varies from 7.1 to 32.7 % (mean ± st.dev. : 17.7 ± 8.9), the proportion of the 2B form varies from 67.3 to 92.9 % (mean ± st.dev. : 81.5 ± 10.6). In many studies coloration types are known to vary geographically (*e.g.* ABBAS, 1988; BURKE, 1989; SILFVERBERG, 1991, 1994). Here we always find dominance of the same form. Of course a study on geographic variation should include many more populations. As *P. tetrastigma* has only one generation each year, and the adults are only active during a short period in which their host plants are abundant, seasonal variation can be excluded.

Electrophoretic analysis

All loci screened, with in total 25 alleles, are polymorphic (Table 2). Three loci (*AAT, MP-2* and *PGM*) are polymorphic at all localities, *aco* is polymorphic at four, *MP-1* and *AMY* at three and *ICD* at two localities. The remaining loci (*MDH, PEP* and *GPD*) are only polymorphic in one locality. None of the populations is polymorphic for all 10 loci. The highest percentage of loci polymorphic is found in Chimay (80%), the lowest in Celles sur Plaine and Stenholts Vang (both 40 %). An example of an allozyme profile is shown in Fig. 1.

No diagnostic loci for the colour forms were found. Five alleles (*MDH*-B, *PEP*-B, *GPD*-B, *MP-1*-A and *MP-2*-A) were only found in the 2B form, one allele (*AAT*-D) was

only found in the 4S form. But all of these alleles had very low frequencies (<0.005, except for *MP-1*-A with 0.012), and can be considered rare alleles.

TABLE 2

Allele frequencies for 10 allozyme loci in six populations of P. tetrastigma

Locus	*Allele*	*Celles sur Plaine*	*Chimay*	*Geisenfeld*	*Stenholts Vang*	*Udenhout*	*Zoersel*
AAT	n	61	46	40	39	40	81
	A	0.516	0.467	0.563	0.397	0.675	0.543
	B	0.451	0.500	0.425	0.577	0.287	0.457
	C	0.025	0.033	0.013	0.026	0.038	0.000
	D	0.008	0.000	0.000	0.000	0.000	0.000
MDH	n	63	46	40	39	40	88
	A	1.000	1.000	1.000	1.000	1.000	0.994
	B	0.000	0.000	0.000	0.000	0.000	0.006
PEP	n	47	41	40	24	14	56
	A	1.000	0.988	1.000	1.000	1.000	1.000
	B	0.000	0.012	0.000	0.000	0.000	0.000
ACON	n	41	50	40	38	44	90
	A	0.098	0.110	0.025	0.000	0.000	0.006
	B	0.902	0.890	0.975	1.000	1.000	0.994
GPD	n	46	46	33	34	23	46
	A	1.000	1.000	1.000	1.000	1.000	0.989
	B	0.000	0.000	0.000	0.000	0.000	0.011
MP-1	n	52	55	35	26	35	54
	A	0.000	0.036	0.014	0.000	0.014	0.000
	B	1.000	0.964	0.986	1.000	0.986	1.000
MP-2	n	45	51	43	25	27	48
	A	0.000	0.010	0.000	0.000	0.000	0.000
	B	0.878	0.990	0.965	0.840	0.889	0.865
	C	0.122	0.000	0.035	0.160	0.111	0.135
ICD	n	50	48	39	24	46	100
	A	1.000	0990	1.000	0.979	1.000	1.000
	B	0.000	0.010	0.000	0.021	0.000	0.000
AMY	n	44	55	36	11	41	83
	A	1.000	0.991	1.000	1.000	0.976	0.988
	B	0.000	0.009	0.000	0.000	0.024	0.012
PGM	n	59	49	36	18	37	93
	A	0.025	0.051	0.056	0.000	0.176	0.059
	B	0.593	0.724	0.625	0.694	0.257	0.667
	C	0.356	0.214	0.278	0.306	0.446	0.247
	D	0.025	0.010	0.042	0.000	0.122	0.027
	H_{exp}	0.145	0.132	0.119	0.127	0.144	0.130
	H_{obs}	0.121	0.114	0.103	0.124	0.104	0.099

H_{exp} : expected heterozygosity assuming Hardy-Weinberg equilibrium; H_{obs} : observed heterozygosity

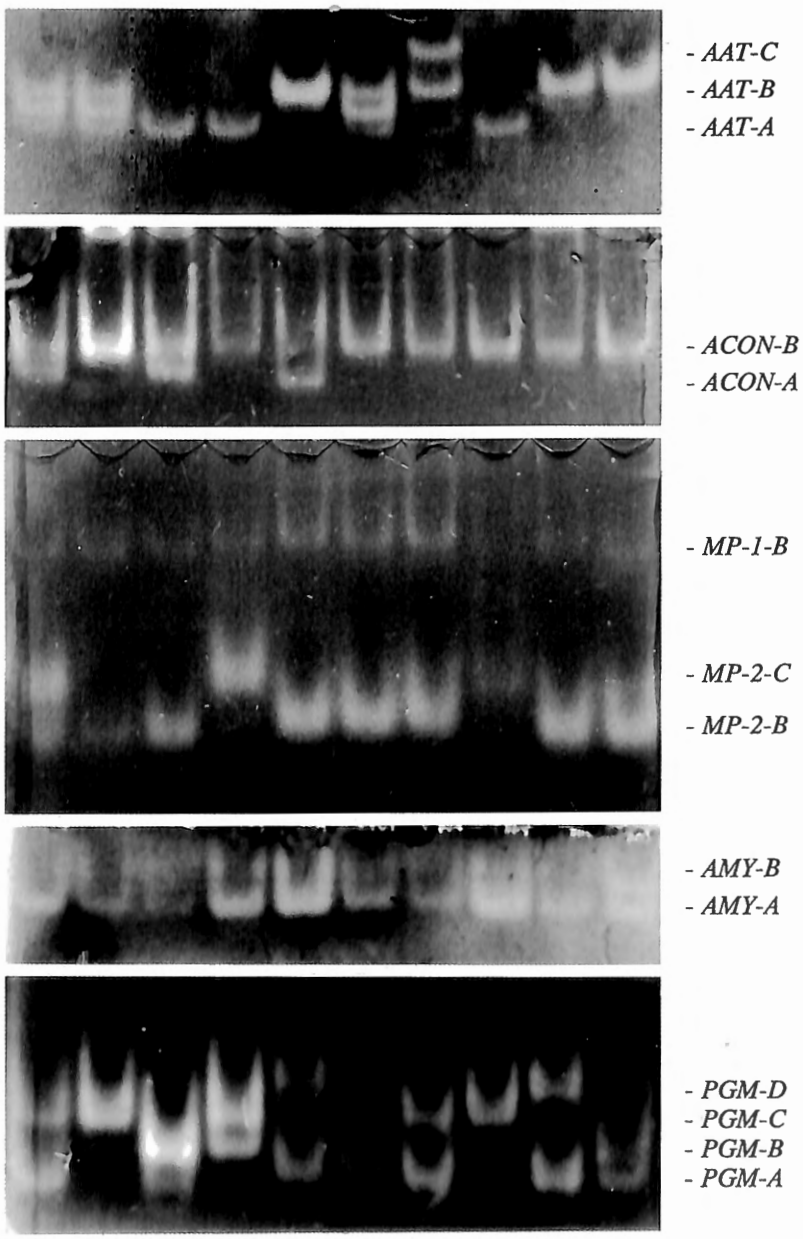

Fig. 1. – Example of allelic variation in the most variable loci : from top to bottom : *AAT, ACON, MP-1 and MP-2, AMY, PGM* (alleles are indicated)

Hardy-Weinberg equilibrium

The analysis for the six localities (the two forms mixed) shows only two deviations from Hardy-Weinberg equilibrium: *MP-2* for Celles sur Plaine and Zoersel (exact probabilities, sequential Bonferroni corrected, $p < 0.05$) (Table 3). Both significant deviations from Hardy-Weinberg equilibrium are caused by heterozygote deficiencies (Table 3).

TABLE 3

Significance test using exact probabilities (sequential Bonferroni corrected), fixation index F and coefficient for heterozygote deficiency D of allozyme loci

Locus	*Celles sur Plaine*	*Chimay*	*Geisenfeld*	*Stenholts Vang*	*Udenhout*	*Zoersel*
AAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F=-0.208	F=0.180	F=0.055	F=-0.210	F=0.022	F=0.055
	D=0.198	D=-0.189	D=-0.167	D=0.195	D=-0.035	D=-0.061
MDH	-	-	-	-	-	n.s.
	-	-	-	-	-	F=-0.006
	-	-	-	-	-	D=0.000
PEP	-	n.s.	-	-	-	-
	-	F=-0.012	-	-	-	-
	-	D=0.000	-	-	-	-
ACON	n.s.	n.s.	n.s.	-	-	n.s.
	F=0.446	F=0.285	F=1.000	-	-	F=-0.006
	D=-0.453	D=-0.292	D=-1.000	-	-	D=0.000
GPD	-	-	-	-	-	n.s.
	-	-	-	-	-	F=-0.011
	-	-	-	-	-	D=0.000
MP-1	-	n.s.	n.s.	-	n.s.	-
	-	F=-0.038	F=-0.014	-	F=-0.014	-
	-	D=0.028	D= 0.000	-	D=0.000	-
MP-2	p<0.05	n.s.	n.s.	n.s.	n.s.	p<0.05
	F=0.689	F=-0.010	F=0.655	F=0.702	F=0.625	F=0.733
	D=-0.693	D=0.000	D=-0.659	D=-0.708	D=-0.632	D=-0.736
ICD	-	n.s.	-	n.s.	-	-
	-	F=-0.011	-	F=-0.021	-	-
	-	D=0.000	-	D=0.000	-	-
AMY	-	n.s.	-	-	n.s.	n.s.
	-	F=-0.009	-	-	F=1.000	F=-0.012
	-	D=0.000	-	-	D=-1.000	D=0.006
PGM	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F=0.218	F=0.043	F=0.052	F=-0.178	F=0.294	F=0.210
	D=-0.225	D=-0.053	D=-0.065	D=0.145	D=-0.304	D=-0.215

The analysis for all localities divided in two forms reveals similar results. We find significant deviations from Hardy-Weinberg equilibrium for *MP-2* at the same localities. Both deviations are found in the 2B form (in Celles sur Plaine the 4S form is not polymorphic).

Population differentiation

Table 4 shows the variance components and hierarchical F-statistics combined across loci for the different levels. For this multilocus estimate the variance was smaller among forms within localities than among localities within the total population, and F_{FL} is smaller than F_{LT}.

TABLE 4

Variance components and F-statistics combined across allozyme loci

X	Y	variance component	F_{XY}
form (F)	locality (L)	0.01410	0.011
form (F)	total (T)	0.03295	0.026
locality (L)	total (T)	0.01885	0.015

Contingency chi-square analysis of allele frequency differences (sequential Bonferroni corrected) between populations do not show significant heterogeneity in any of the localities (not for any locus, nor for all loci combined) between both forms (4S-2B) ($p=0.05$).

Chi-square analysis (for both forms combined) gives significant differentiation between localities at 5 loci (*ACON, MP-2* and *PGM*) ($p<0.05$). Three out of 7 other loci (*MDH, PEP* and *GPD*) are only polymorphic in one locality (table not shown).

Genetic distances, Clustering and Multivariate Analysis

NEI (1978) unbiased genetic distance and Modified Rogers distance (WRIGHT, 1978) were calculated (table 5). The first one varied between 0.000 and 0.024 (mean = 0.007), the second one between 0.036 and 0.148 (mean = 0.078). Three dendrograms were constructed. The population of Udenhout always branched off first, while the position of the other populations changed in the different dendrograms. Only the Wagner tree based on Rogers modified distance is shown in Fig. 2.

TABLE 5

Matrix of genetic distances of allozyme data: above diagonal: NEI (1978) unbiased genetic distance, below diagonal Rogers modified distance (WRIGHT, 1978)

Population	*1.*	*2.*	*3.*	*4.*	*5.*	*6.*
1. Celles sur Plaine	-	0.003	0.000	0.002	0.011	0.001
2. Chimay	0.061	-	0.001	0.003	0.024	0.003
3. Geisenfeld	0.043	0.049	-	0.003	0.011	0.000
4. Stenholts Vang	0.058	0.070	0.069	-	0.022	0.001
5. Udenhout	0.106	0.148	0.107	0.146	-	0.015
6. Zoersel	0.043	0.060	0.036	0.049	0.118	-

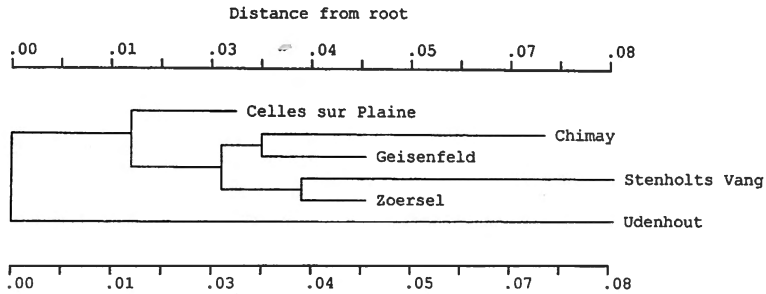

Fig. 2. – Wagner tree based on Modified Rogers distance (WRIGHT, 1978), Cophenetic correlation = .981, rooted at midpoint of longest path

In the correspondence analysis, the cumulative percentage of the variance explained by the canonical axes is 52.7 for CA1 and 79.1 for CA2. The populations scores plotted for CA1 and CA2 (Fig. 3) show a clear separation of the Udenhout population from the other populations according to the first axis. This axis is strongly correlated with the allele frequencies of *PGM-B, PGM-D, AAT-B, PGM-A, GOT-A* and *PGM-C*. According to the second axis the Chimay population is more or less clearly separated from the other populations, although differences here are less marked. This axis is strongly correlated with the alleles *MP2-B, MP2-C, MP1-A* and *MP1-B*.

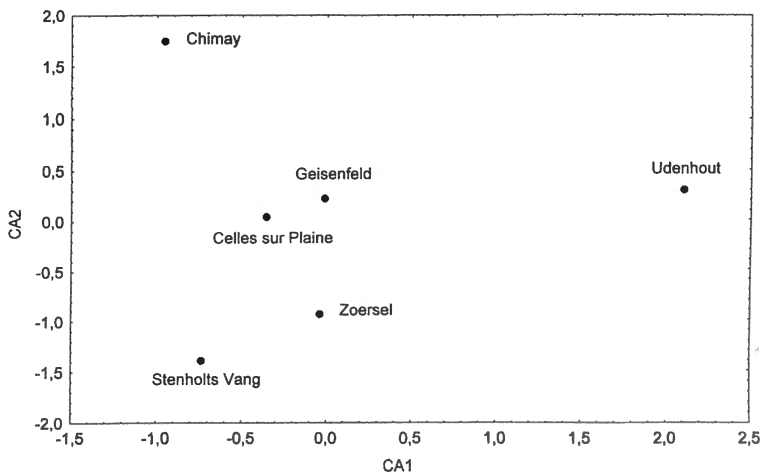

Fig. 3. – Biplot of the population scores on the first two canonical axes of the CA analysis

Isoelectric Focusing

An example of a gel is shown in Fig. 4. The average number of bands for both forms was calculated (4S : mean ± st. dev. : 47.69 ± 6.06 ; 2B : mean ± st. dev. : 45.13 ± 6.02).

Between both forms no significant difference in the number of bands was detected (Mann-Whitney U test ; $p < 0.05$).

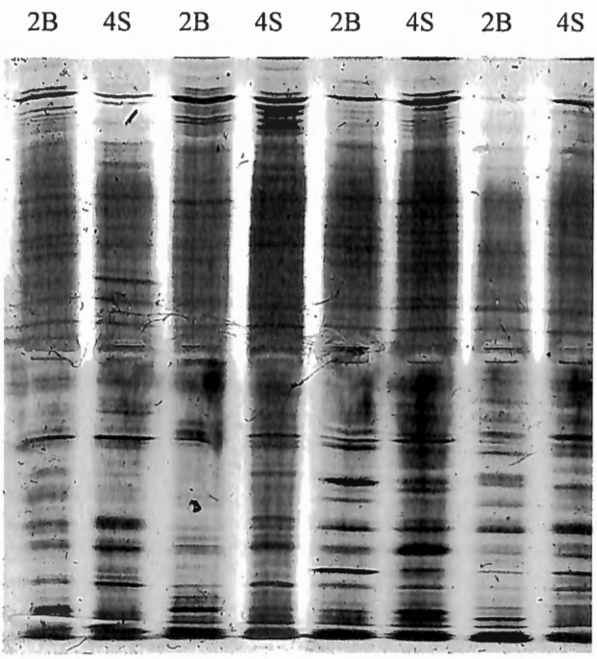

Fig. 4. – IEF pattern : forms from left to right : 2B,4S,2B,4S,2B,4S,2B,4S

Similarities S_F, S_J and S_D are given in table 6. Differences between the three types of comparisons (DD, DL and LL) were not significant (ANOVA, df = 2,42 ; $p>0.1$).

TABLE 6

Basic statistical data on IEF protein polymorphisms for similarities S_F, S_J, S_D for the three types of comparisons

comp.	simil.	Mean	st. dev.	# comparisons
4S4S	S_F	0.879	0.030	15
	S_J	0.822	0.050	15
	S_D	0.902	0.030	15
4S2B	S_F	0.870	0.037	15
	S_J	0.806	0.054	15
	S_D	0.892	0.033	15
2B2B	S_F	0.871	0.030	15
	S_J	0.818	0.047	15
	S_D	0.899	0.028	15

DISCUSSION

P. tetrastigma is a genetically very variable species showing high heterozygosity values and being polymorphic at all ten loci studied. VERDYCK *et al.* (1996) studied another *Phyllotreta* species (*P. cruciferae)* and found that it was only variable at five of these loci (*AAT, MDH, GPD, AMY* and *PGM*) of which only the *AAT* locus showed variation in all populations.

Genetic population structuring has been studied in several other chrysomelid species and KNOLL *et al.* (1996) give an overview of several studies with special emphasis on Alpine populations. In hierarchical studies F_{ST} values have been compared at different micro-and macrogeographic scales (between trees, localities a few kilometers apart and localities sometimes 500 km apart). In *Plagiodera versicolora* F_{ST} values between 0.006 and 0.098 were found between trees within localities (MCCAULEY *et al.*, 1988), while in *Chrysomela aeneicollis* , large F_{ST} values were found (for several loci) at the same level (RANK, 1992). Our F_{FL} value of 0.011 fits in this spectrum. Between localities F_{LT} values varied from 0.003 to 0.057 in *P. versicolora* (MCCAULEY *et al.*, 1988), from 0.010 to 0.135 in *Chrysomela aeneicollis* (RANK 1992), from 0.011 and 0.053 in *Phratora vitellinae* (Linnaeus, 1758) and from 0.066 and 0.094 in *Oreina cacaliae* (Schrank, 1785) (KNOLL *et al.,* 1996). The F_{LT} value of 0.015 for *P. tetrastigma* is (although not exceptional) rather low for populations at such geographic scale. Apparently there is an important amount of gene flow between these geographically sometimes very distant populations, leading to little genetic differentiation at a large geographic scale. Although the resolving power of band sharing with IEF is limited and the allozymes tested provide only a limited sample of the genome, we suspect that important genetic differentiation would have been detected using these techniques. A promising future strategy to further explore this question would be assessment of variability in microsatellite DNA. In that case it would be interesting to obtain more populations from a large geographic range.

The clear separation of the Udenhout population in the Wagner tree is possibly due to the fact that this was the only population feeding on *Cardamine flexuosa*. This result indicates that the formation of host plant races can lead to genetic separation and speciation, an evolutionary pathway finding support in several other insect groups. The best studied case is probably that of the sibling species in the *Rhagoletis* fruit flies (FEDER *et al.,* 1988, 1989, 1990a, 1990b; MCPHERON *et al.,* 1988; BERLOCHER *et al.,* 1993). GOYER *et al.* (1995) also demonstrated host-associated genetic differentiation in the fruittree leafroller *Archips argyrospila*, suggesting formation of sibling species by means of different hosts and GULDEMOND (1990a, 1990b) discussed host plant shift, host race formation and speciation in the aphid genus *Cryptomyzus*.

The IEF results demonstrated that both forms do not differ significantly in the number of bands, and inter- and intraform similarities do not show any significant differences, indicating lack of general protein pattern differences in both forms.

VERDYCK *et al.* (1996) did not find evidence for genetic isolation between the colour forms in *P. cruciferae*. For *P. tetrastigma* results of allozymes and IEF lead to a similar conclusion. As neither allozyme nor IEF results indicate genetic differentiation between the 4S and 2B form of *P. tetrastigma,* both forms are considered interbreeding and belong-

ing to the same populations. Colour differences in *P. tetrastigma* are without systematic value, and again confirm the conclusion of VERDYCK *et al.* (1996) that a profound study of colour variation in chrysomelid species is necessary before systematic conclusions can be drawn.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. J.K. Nielsen (Royal Veterinary and Agricultural University, Frederiksberg, Denmark) for providing the *P. tetrastigma* material from Stenholts Vang (Denmark), M. Döberl for sending the material from Geisenfeld, S. Simokovic for helping in the collection of material from Chimay and Udenhout and J. Scheirs for helping collecting material in Zoersel. Also sincere thanks to Dr. T. Backeljau for reviewing the manucript.

P.V. and H.D.W. are Postdoctoral Fellows of the Fund for Scientific Research - Flanders (Belgium) (F.W.O.).

REFERENCES

ABBAS, I., K. NAKAMURA, H. KATAKURA & H. SASAJI (1988) – Geographical variation of elytral spot patterns in the phytophagous ladybird, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae) in the province of Sumatra Barat, Indonesia. *Res. Popul. Ecol.*, **30**: 43-56.

BACKELJAU, T. (1985) – Estimation of genic similarity within and between *Arion hortensis s.l.* and *A. intermedius* by means of isoelectric focused esterase patterns in hepatopancreas homogenates (Mollusca, Pulmonata: Arionidae). *Z. zool. Syst. Evolut.-forsch.*, **23**(1): 38-49.

BACKELJAU, T., C.P. DE BRITO, R.M. TRISTAO DA CUNHA & A.M. FRIAS MARTINS (1992) – Colour polymorphism and genetic strains in *Arion intermedius* from Flores, Azores (Mollusca: Pulmonata). *Biol. J. Linn. Soc.*, **46**: 131-143.

BERLOCHER, S.H., B.A. MCPHERON, J.L. FEDER & G.L. BUSH (1993) – Genetic differentiation at allozyme loci in the *Rhagoletis pomonella* (Diptera: Tephritidae) species complex. *Ann. ent. Soc. Am.*, **86**(6): 716-727.

BOITEAU, G., G.C.C.TAI & M.E. DREW (1994) – Genetics and biological fitness of a beige elytral mutant of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Can. J. Zool.*, **72**: 312-318.

BOITEAU, G. (1994) – Genetics of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In: *Novel aspects of the Biology of Chrysomelidae.* JOLIVET, P.H., COX M.L. & E. PETITPIERRE, E. (Eds.). Kluwer Academic Publishers, Dordrecht: 231-236.

BOOTH, C.L. (1990) – Evolutionary significance of ontogenetic colour change in animals. *Biol. J. Linn. Soc.*, **40**: 125-163.

BROWN, W.J. (1956) – Sibling species in the Chrysomelidae. In: *Proceedings Tenth International Congress of Entomology.* E.C. BECKER (Ed). Mortimer Limited, Ottawa, Canada: 103-109.

BURKE, D.P.T. (1989) – Variation in body colour in western Irish populations of *Cepaea nemoralis* (L.). *Biol. J. Linn. Soc.*, **36**: 55-63.

BUTH, D.G. (1984) – The application of electrophoretic data in systematic studies. *Ann. Rev. Ecol. Syst.*, **15**: 501-522.

COOK, L.M., K.D. RIGBY & M.R.D. SEAWARD (1990) – Melanic moths and changes in epiphytic vegetation in north-west England and north Wales. *Biol. J. Linn. Soc.*, **39 (4)**: 343-354.

CORDERO, A. (1990) – The inheritance of female polymorphism in the damselfly *Ischnura graellsi* (Rambur) (Odonata: Coenagrionidae). *Heredity,* **64**: 341-346.

DOGUET, S. (1986) – Mise B jour du catalogue des Alticinae de la faune de France: le genre *Phyllotreta* Chevrolat (Col. Chrysomelidae). *l'Entomologiste,* **42** (3): 145-149.

DOGUET, S. (1994) – Coléoptéres Chrysomelidae volume 2: Alticinae. *Faune de France,* Fédération Française des Sociétés de Sciences Naturelles, Paris, France. **80**: 694 pp.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1988) – Genetic differentiation between sympatric host races of *Rhagoletis pomonella*. *Nature,* **336**: 61-64.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1989) – Are the apple maggot (*Rhagoletis pomonella*) and the blueberry maggot (*R. mendax*) distinct species? Implications for sympatric speciation. *Entomol. exp. appl.,* **51**: 113-123.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1990a) – The geographic pattern of genetic differentiation between host-associated populations of *Rhagoletis pomonella* (Diptera: Tephritidae) in the eastern United States. *Evolution,* **44**: 570-594.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1990b) – Regional, local, and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in Western Michigan. *Evolution,* **44**: 596-608.

FERGUSON, A. (1980) – Biochemical systematics and evolution. Blackie & Son., Glasgow.

FUJIYAMA, S. & K. ARIMOTO (1988) – Genetics of the two colour forms of *Chrysolina aurichalcea* (Mannerheim) (Coleoptera: Chrysomelidae) and their gene frequencies in two mountainous areas of central Honshu, Japan. In: *Biology of Chrysomelidae*. P. JOLIVET, E. PETITPIERRE & T.H. HSIAO (Eds.). Kluwer Academic Publishers, Dordrecht: 205-215.

GOYER, R.A., T.D. PAINE, D.P. PASHLEY, G.J. LENHARD, J.R. MEEKER & C.C. HANLON (1995) – Geographic and host-associated differentiation in the fruittree leafroller (Lepidoptera: Tortricidae). *Ann. ent. Soc. Am.*, **88** (4): 391-396.

GULDEMOND, J.A. (1990a) – Host plant shift, host race formation and speciation in *Cryptomyzus* (Homoptera, Aphididae). *Acta Phytopathol. Ent. Hungarica,* **25**: 89-96.

GULDEMOND, J.A. (1990b) – Evolutionary genetics of the aphid *Cryptomyzus*, with a preliminary analysis of the inheritance of host plant preference, reproductive performance and host-alteration. *Entomol. exp. appl.,* **57**: 65-76.

HANTULA, J., A. SAURA, J. LOKKI & N. VIRKKI (1987) – Genic and color polymorphism in Puerto Rican phyllobiine weevils *Diaprepes abbreviatus* (L.) and *Compsus maricao* Wolcott. *J. Agric. Univ. Puerto Rico,* **4**: 391-397.

HARRIS, H. & D.A. HOPKINSON (1976) – Handbook of enzyme electrophoresis in human genetics. North Holland publishing Co., Amsterdam.

HOLLOWAY, G.J. (1993) – Phenotypic variation in colour pattern and seasonal plasticity in *Eristalis* hoverflies (Diptera: Syrphidae). *Ecol. Entomol.,* **18**: 209-217.

KINGSOLVER, J.G. & D.C. WIERNASZ (1991) – Development, function, and the quantitative genetics of wing melanin pattern in *Pieris* butterflies. *Evolution,* **45** (6): 1480-1492.

KNOLL, S., M. ROWELL-RAHIER, P. MARDULYN & J.M. PASTEELS (1996) Spatial genetic structure of leaf beetle species with special emphasis on Alpine populations. In: *Chrysomelidae Biology, vol. 1: The classification, phylogeny and genetics*. P.H.A. JOLIVET & M.L. COX (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands: 379-388.

LIEBHERR, J.K. (1983) – Genetic basis for polymorphism in the ground beetle, *Agonum decorum* (Say) (Coleoptera: Carabidae). *Ann. ent. Soc. Am.*, **76** (3): 349-358.

LU, W. & P. LOGAN (1994) – Inheritance of larval body color in *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae). *Ann. ent. Soc. Am.* **87** (4) : 454-459.

MCCAULEY, D.E., M.J. WADE, F.J. REDEN & M. WÖHLTMAN (1988) – Spatial and temporal variation in group relatedness : evidence from the important willow leaf beetle. *Evolution,* **42** : 184-192.

MCPHERON, B.A., D.C. SMITH & S.H. BERLOCHER (1988). – Genetic differences between host races of the apple magot fly. *Nature,* **336** : 64-66.

MOHR, K.H. (1966) – Chrysomelidae. In : *Die Käfer Mitteleuropas*. 9, H. FREUDE, K.W. HARDE & G.A. LOHSE (Eds.). Goecke & Evers, Krefeld : 95-297.

NEI, M. (1978) – Estimation of average heteroygosity and genetic distance from a small number of individuals. *Genetics,* **89** : 583-590.

NIELSEN, J.K. (1978a) – Host plant discrimination within cruciferae : feeding responses of four leaf beetles (Coleoptera : Chrysomelidae) to glucosinolates, cucurbitans and cardenolides. *Entomol. exp. appl.,* **24** : 41-54.

NIELSEN, J.K. (1978b) – Host plant selection of monophagous and oligophagous flea beetles feeding on crucifers. *Entomol. exp. appl.,* **24** : 362-369.

PAULSEN, S.M. (1994) – Quantitative genetics of butterfly wing color patterns. *Developmental genetics,* **15** : 79-91.

RANK, N.E. (1992) – A hierarchical analysis of genetic differentiation in a montane leaf beetle *Chrysomela aeneicollis* (Coleoptera : Chrysomelidae). *Evolution,* **46** : 1097-1111.

SILFVERBERG, H. (1991) – Colour variation in Finnish Chrysomelidae (Coleoptera). 1. Cryptocephalinae. *Elytron suppl.,* **5** (1) : 149-157.

SILFVERBERG, H. (1994) – Colour variation in Finnish Chrysomelidae (Coleoptera). 2 The genus *Gonioctena.* In : *Proceedings of the Third International Symposium of the Chrysomelidae Beijing, 1992.* FURTH, D.G. (Ed.). Backhuys Publishers, Leiden : 31-37.

SMITH, D.A.S., D.F. OWEN, I.J. GORDON & A.M. OWINY (1993) – Polymorphism and evolution in the butterfly *Danaus chrysippus* (L.) (Lepidoptera : Danainae). *Heredity,* **71** : 242-251.

SNEATH, P.H.A. & R.R. SOKAL (1973) – Numerical Taxonomy. W.H. Freeman and company, San Francisco : 573 pp.

SWOFFORD, D.L. & R.B. SELANDER (1989) – BIOSYS-1 : A computer program for the analysis of allelelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Illinois.

TER BRAAK, C.J.F. (1988) – CANOCO : a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1 and update notes version 3.1). Technical Report, Agriculture Mathematics Group, Wageningen.

THOMAS, S. (1964) – Some aspects of life history, genetics, distribution and taxonomy of *Aspidomorpha adhaerens* (Weber, 1801) (Cassidinae, Coleoptera). Ph. D. thesis, University of Amsterdam : 264 pp.

VASCONCELLOS-NETO, J. (1988) – Genetics of *Chelymorpha cribraria*, Cassidinae colour patterns and their ecological meanings. In : *Biology of Chrysomelidae*.P. JOLIVET, E. PETITPIERRE & T.H. HSIAO (Eds.). Kluwer Academic Publishers, Dordrecht : 217-232.

VERDYCK, P., T. BACKELJAU, K. BREUGELMANS & J. HULSELMANS (1992) – The use of isoelectric focusing to solve systematic problems in the genus *Phyllotreta* (Coleoptera, Chrysomelidae). *Belg. J. Bot.,* **125** (2) : 310-314.

VERDYCK, P., L. DE BRUYN, J. HULSELMANS & J. SCHEIRS (1995) – Lectotype designation and discussion on the type material of three closely related Chrysomelid species : *Phyllotreta dilatata*

Thomson, 1866, *P. flexuosa* (Illiger, 1794) and *P. tetrastigma* (Comolli, 1837) (Col., Chrysomelidae). *Bull. Soc. ent. France,* **100** (3) : 298.

VERDYCK, P., H. DE WOLF, T. BACKELJAU & J. HULSELMANS (1996) – A genetic study of two colour forms of *Phyllotreta cruciferae* (Chrysomelidae : Alticinae). In : *Chrysomelidae Biology, vol. 1 : The classification, phylogeny and genetics.* P.H.A. JOLIVET & M.L. COX (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands : 389-397.

VERDYCK, P., L. DE BRUYN & J. HULSELMANS (in press.) – A morphological study of *Phyllotreta tetrastigma* (Coleoptera : Chrysomelidae : Alticinae) including two colour forms. *J. Nat. Hist.*

WRIGHT, S. (1965) – The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution,* **19** : 395-420.

WRIGHT, S. (1978) – Evolution and genetics of populations, vol. **4** Variability within and among natural populations. University of Chicago Press, Chicago : 580 pp.