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Allozyme Variation in Reference and Metal-Exposed Natural Populations of *Orchesella cincta* (Insecta: Collembola)

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Key Word Index—*Orchesella cincta*; Entomobryidae; Collembola; allozyme; glutamate–oxaloacetate transaminase; soil pollution; heavy metal; genetic variability

Abstract—Environmental pollution may affect genetic variation in populations inhabiting polluted sites. In this study, allozyme variation was studied in natural populations of the soil-dwelling insect *Orchesella cincta*. The populations originated from eight sites with different histories of metal contamination and natural enrichment in The Netherlands, Belgium and F.R.G. Slight but significant divergence was observed for four polymorphic loci; 18 loci were monomorphic at all sites. The highest mean values for heterozygosity (*H*), degree of polymorphism (*P*) and mean number of alleles per locus (*A*) were 0.090, 0.182 and 1.36, respectively. Geographic distance was not correlated to genetic divergence. Comparison of allozyme data from investigated populations and from a reference population in Italy showed a remarkable genetic homogeneity in this species, the highest value for Nei's genetic distance (*D*) being 0.035. Among NW European populations, a correlation between metal tolerance characteristics and allozyme frequencies was observed for glutamate-oxaloacetate transaminase (EC. 2.6.1.1).

Introduction

Local pollution may affect genetic variation in a population by selection for resistant phenotypes. The genetic variability of a population determines its abilities to adapt to changing environmental conditions. A low genetic variation is not only often related to low reproductive output and individual growth in laboratory populations (Ayala, 1965; Nei, 1987), but it also decreases the potential for responses to future environmental changes (O'Brien *et al.*, 1985).

Directional selection by heavy metals is often severe, and its effects are both stable and permanent (Bradshaw *et al.*, 1990). Genetic effects of heavy metal pollution are most commonly studied by the electrophoretic analysis of allelic variation at enzyme loci, either by observations on allelic variants in a cline of pollution (Lower, 1975; Schneider *et al.*, 1984), by comparison of geographically isolated populations (Verkleij *et al.*, 1985; Gillespie and Guttman, 1989), or by comparison of effects of exposure on certain genotypes (Lavie and Nevo, 1982; 1986; Hawkins *et al.*, 1989; Benton and Guttman, 1990).

In this paper, attention is focused on the collembolan *Orchesella cincta* (L.) This species inhabits the organic layer of forest soils, a site of heavy metal accumulation (Jones *et al.*, 1988). Metal-tolerant populations of soil animal species have been found at several anthropogenically polluted sites (for a review, see Hopkin, 1989), but relationships between metal tolerance and genetic variation have not been demonstrated.

Genetically determined differences have been shown in tolerant populations of soil isopods (Donker and Bogert, 1991) and in *Orchesella cincta*, by comparisons of clean-cultured first-generation laboratory animals from sites with different histories of metal contamination, i.e. sites with different combinations and concentrations of metals, and duration of exposure. In *O. cincta*, metal-tolerant populations exhibit no growth

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reduction during exposure (Posthuma, 1990), which is associated with an increased immobilization and excretion of metals via the midgut (Posthuma *et al.*, 1992).

In view of the adaptation observed, genetic variation in several reference and polluted *O. cincta* populations was studied by means of enzyme electrophoresis. In order to investigate the effects of laboratory culturing on allozyme frequencies, as indicated above, intergeneration comparisons were made (see Baird *et al.*, 1988). Electrophoresis has proved to be an effective tool in the taxonomic investigation of soil organisms, including Collembola (Hart and Allamong, 1979; Frati *et al.*, 1989). Hence, the species was chosen because of its wide distribution and its role in litter decomposition, its capacity to develop metal tolerance and its suitability for electrophoretic studies.

The present study was designed to evaluate: (1) the effect of culturing on genetic patterns, by comparing allelic frequencies of field and third laboratory generation (F3) individuals; (2) allozymic variability within populations, and the amount of genetic differentiation among natural populations with different histories of metal contamination; (3) the role of geographic distance in genetic divergence; and (4) the relative importance of the forces determining the observed variation, and the possible use of genetic parameters in estimating the impact of heavy metal pollution.

Materials and Methods

The genus *Orchesella* is widely distributed throughout the holarctic region. It is differentiated into many morphologically similar species. Pigmentation and chaetotaxy are the most useful characters for taxonomic diagnosis. Allozymes have been used to assess systematic relationships at both intra- and interspecific level. In a relatively small area a large number of *Orchesella* species were distinguished, which appeared to be genetically distinct (Frati *et al.*, in preparation). The pattern of divergence was interpreted as an indication for both a long history of colonization, and the effects of local selection forces and geographic isolation.

Animals were collected from randomly selected litter samples at three reference sites and five contaminated sites in The Netherlands, Belgium and F.R.G., hereafter referred to as (continental) NW Europe (Fig. 1 and Table 1 for names and locations), and, for further comparisons, at an uncontaminated site in S Europe, NE of Siena (central Italy). At least 600 individuals were captured at each site. According to the experimental design, the animals were either frozen in liquid nitrogen and stored at −80°C, or kept in mass cultures on clean food. Location of sites, details of site characteristics, sampling and culturing techniques have been described by Van Straalen *et al.* (1987) and Posthuma (1990) (NW European sites). Previously studied populations are numbered according to increasing litter cadmium concentrations following Posthuma *et al.* (1992). Two newly incorporated sites, Wekerom and Siena, are both pine forests with background level metal contamination; these sites will be referred to as We and Si. Site characteristics are summarized in Table 1. Characteristics determined for the *O. cincta* populations inhabiting these sites are summarized in Table 2. These data are based on observations on first-generation laboratory animals: the Index of Growth Reduction (IGR) and the excretion efficiency (EE) express tolerance, the former as the ratio of growth reduction determined at two cadmium exposure levels (large negative values indicate strong growth reduction upon exposure to a high cadmium concentration in comparison with the growth reduction of individuals exposed to a low concentration; values close to zero indicate absence of growth response attributable to cadmium), the latter as the relative amount of cadmium excreted following a moult (for further information, see Posthuma, 1990 and Posthuma *et al.*, 1992).

Individuals of the field and F3 generations were homogenized in distilled water, absorbed with a filter paper and loaded on to an 11% starch gel (horizontal starch gel electrophoresis; for complete technical information see Murphy *et al.*, 1990). Buffers for preparing the gels were chosen according to the enzyme system targeted, and analyses were run with technical and staining procedures according to Ayala *et al.* (1972, 1974) and Brewer and Singh (1970). Buffer systems were: (A) bridge buffer: 0.687 M Tris, 0.157 M citric acid; gel buffer: bridge buffer diluted 1:30; (B) bridge buffer: 300 mM boric acid, 60 mM NaOH; gel buffer: 76 mM Tris, 5 mM citric acid; (C) bridge buffer: 135 mM Tris, 39 mM citric acid, 1 mM EDTA; gel buffer: 9 mM Tris, 20 mM citric acid, 1 mM EDTA; (D) bridge buffer: 0.10 M Tris, 0.10 M maleic acid, 0.01 M EDTA, 0.01 M $MgCl_2 \times 6H_2O$; gel buffer: bridge buffer diluted 1:9. Twenty-two loci showed a readily interpretable banding pattern; only one locus per enzyme system was found, and all variants within systems were designated as allozymes. These were (with enzyme commission numbers and buffer systems in parentheses): aconitase (EC. 4.2.1.3, B), adenylate kinase (EC. 2.7.4.3, C), aldehyde oxidase (EC. 1.2.3.1, B), fructose-1-6-diphosphate dehydrogenase (EC. 3.1.3.11, B), fumarase (EC. 4.2.1.2., B), glucose dehydrogenase (EC. 1.1.1.47, B), glucose-6-phosphate dehydrogenase (EC. 1.1.1.49, B), glutamate-oxaloacetate transaminase (Got; EC. 2.6.1.1, B), glyceraldehyde-3-phosphate dehydrogenase (EC. 1.2.1.12, C), α-glycerophosphate dehydrogenase (EC. 1.1.1.8, D), hexokinase (Hk; EC. 2.7.1.1, A),

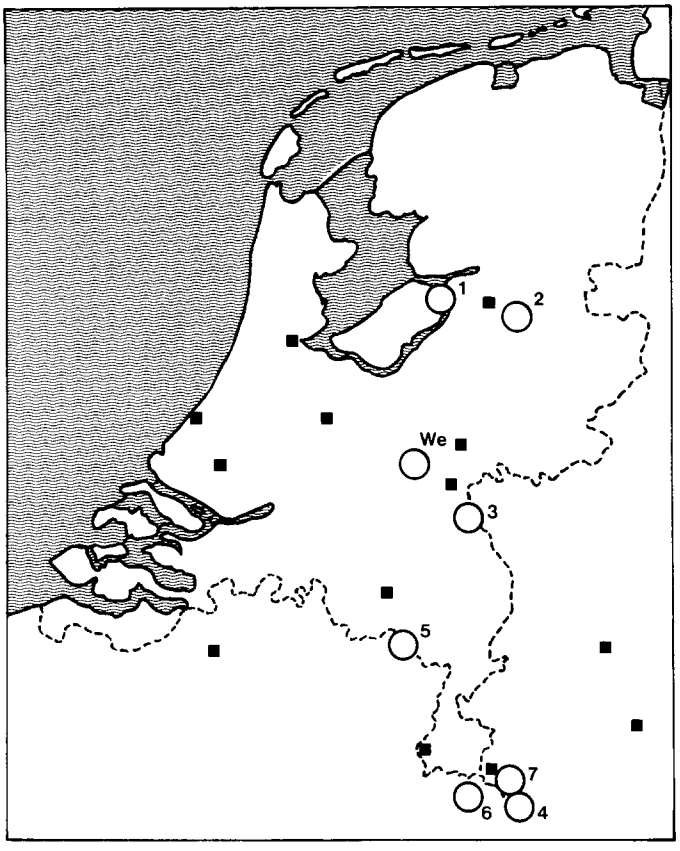

FIG. 1. MAP OF SAMPLING LOCATIONS FOR *ORCHESELLA CINCTA* IN NORTHWESTERN EUROPE. For site codes, see Table 1.

hydroxybutyrate dehydrogenase (EC. 1.1.1.30, D), isocitrate dehydrogenase (EC. 1.1.1.42, C), lactate dehydrogenase (EC. 1.1.1.27, D), malic dehydrogenase (EC. 1.1.1.37, B), malic enzyme (EC. 1.1.1.40, B), mannose phosphate isomerase (EC. 5.3.1.8, A), phosphoglucomutase (Pgm; EC. 2.7.5.1, D), phosphohexoso-isomerase (Phi; EC. 5.3.1.9, A), 6-phosphogluconate dehydrogenase (EC. 1.1.1.44, D), superoxide dismutase (EC. 1.15.1.1, A) and xanthine dehydrogenase (EC. 1.2.3.2, B). Controlled crosses to document the inheritance of the allozyme patterns were not performed. Alleles were designated alphabetically.

Parameters for intrapopulation variability and interpopulation divergence were calculated from observed genotypic frequencies with the computer program BIOSYS-1 (Swofford and Selander, 1981) run on a PC. These included: observed (H_o) and expected (H_e) heterozygosity under Hardy–Weinberg equilibrium; percentage of polymorphic loci (P); average number of alleles per locus (A); genetic distance (D) and identity (I) matrices according to Nei (1972); proportion of interpopulational differentiation (F_{st}: Nei, 1977); UPGMA dendrogram of phenetic relationships (Sneath and Sokal, 1973). Correlations between allozyme and environmental characteristics were calculated with two methods, as a relatively small number of sampling locations was used. Pearson correlations were calculated for all variables, concentration and frequency data were transformed logarithmically and angularly, respectively. Spearman rank-correlations were calculated with untransformed data. Correlations were considered biologically meaningful, if the correlation was supported by both methods with $P \leq 0.01$. A correction method was also applied to avoid interpretation of correlations arising by chance. At a 0.05 level of significance, one out of 20 tests may be significant by chance. A correction was applied by division of the significance level through the number of independent tests (four loci, 10 environmental variables; alleles within loci are dependent), giving a significance level of 0.0013. Correlation calculations were executed with the integrated SPSS statistical program.

Results

Pattern of variation

Effect of culturing. For three populations (1, 4 and 7) individuals from both the field and the F3 generation were analysed. The number of observations and the results for

TABLE 1. SAMPLING SITES AND CHARACTERISTICS OF ACTUAL SAMPLING LOCATIONS WITHIN SITES*

			Total metal concentrations (μmol g^{-1})										Litter pH†
			Litter†‡					Algae†		Field animals†			
Site code	Location	Site characterization	Cd	Zn	Pb	Cu	Ca	Cd	Zn	Cd	Zn	Cu	
1	Roggebotzand (NL)	Reference	0.004	0.5	0.3	0.1	248	0.005	0.5	0.002	0.5	0.7	4.81
2	Dalfsen (NL)	Reference	0.005	1.2	0.5	0.5	38	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
We	Wekerom (NL)	Reference	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Si	Siena-Brolio (I)	Reference	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3	Mook (NL)	Galvanizing plant	0.010	5.8	0.5	0.5	103	0.009	4.5	0.004	0.5	0.8	4.78
4	Breinigerberg (G)	Abandoned mine	0.030	2.6	3.9	1.0	162	0.011	2.4	0.006	0.8	0.5	4.29
5	Budel (NL)	Zinc smelter	0.045	14.5	1.5	0.7	46	0.034	13.9	0.011	1.9	1.1	4.09
6	Plombières (B)	Abandoned mine	0.244	75.2	54.9	2.7	421	0.025	6.2	0.005	1.7	0.6	5.83
7	Stolberg (G)	Lead smelter	0.557	23.9	41.1	20.2	286	0.046	5.1	0.017	1.6	0.4	5.02

*Concentrations are expressed on a dry wt basis. Population coding is based on increasing levels of litter cadmium contamination, following Posthuma *et al.* (1991).

†Data from Posthuma (1990).

‡Data from Van Straalen *et al.* (1987).

NL, The Netherlands; B, Belgium; G, Germany; I, Italy; N.D., not determined.

TABLE 2. CHARACTERISTICS OF *ORCHESELLA CINCTA* POPULATIONS (first generation laboratory animals) FROM REFERENCE AND METAL-CONTAMINATED SITES*

Site code	Blank growth rate† (μg week^{-1}, age four to six weeks) Females	Males	Index of Growth Reduction‡ Females	Cadmium Excretion Efficiency† (%)
1	138	112	−0.834	37.8
2	N.D.	N.D.	N.D.	30.0
We	N.D.	N.D.	N.D.	N.D.
Si	N.D.	N.D.	N.D.	N.D.
3	168	127	−0.819	N.D.
4	168	124	−0.718	N.D.
5	164	105	−1.256	34.9
6	165	141	−0.113	47.3
7	145	136	−0.295	44.7

*For site codes: see Table 1.
†Data from Posthuma *et al.* (1991).
‡Data from Posthuma (1990).
N.D., not determined.

four polymorphic loci are summarized in Table 3; the 18 other loci were monomorphic. Contingency table analyses of allelic frequencies, run for each locus in these populations, showed that the differences between the generations were not significant, except for the Phi-locus in population 1 and the Got-locus in population 4. Thus, as inter-generation differences were not similar for all populations, it is concluded that allelic frequency shifts have been caused by stochastic effects of small observation numbers, rather than by selection during culturing. Therefore, data from field and F3 animals were pooled and analysed together.

TABLE 3. OBSERVED ALLELE FREQUENCY VARIATION IN *ORCHESELLA CINCTA* AT FOUR POLYMORPHIC LOCI IN FIELD-CAPTURED ANIMALS AND CLEAN-CULTURED THIRD-GENERATION LABORATORY ANIMALS (F_3) FROM THREE SAMPLING SITES*

		Population 1		4		7	
Locus		Field	F_3	Field	F_3	Field	F_3
Got	(*N*)	(67)	(48)	(7)	(48)	(29)	(48)
	A	0.56	0.50	0.36	0.56	0.69	0.73
	B	0.40	0.46	0.50	0.42	0.28	0.27
	C	0.04	0.04	0.14	0.02	0.03	0.00
χ^2, *df*, *P*	0.802, 2, 0.670$^{n.s.}$		6.162, 2, 0.046†		3.393, 2, 0.184$^{n.s.}$	
Hk	(*N*)	(27)	(89)	(12)	(56)	(57)	(32)
	A	0.15	0.23	0.67	0.56	0.32	0.31
	B	0.85	0.77	0.33	0.44	0.68	0.69
χ^2, *df*, *P*	1.680, 1, 0.195$^{n.s.}$		0.881, 1, 0.348$^{n.s.}$		0.027, 1, 0.869$^{n.s.}$	
Pgm	(*N*)	(58)	(84)	(16)	(58)	(69)	(35)
	A	0.20	0.15	0.13	0.13	0.20	0.27
	B	0.34	0.33	0.59	0.60	0.53	0.44
	C	0.47	0.52	0.28	0.27	0.26	0.29
	D	0.00	0.01	0.00	0.00	0.01	0.00
χ^2, *df*, *P*	2.079, 3, 0.556$^{n.s.}$		0.026, 2, 0.987$^{n.s.}$		2.268, 3, 0.519$^{n.s.}$	
Phi	(*N*)	(49)	(57)	(17)	(59)	(49)	(33)
	A	0.12	0.10	0.00	0.05	0.12	0.09
	B	0.82	0.89	0.88	0.70	0.82	0.74
	C	0.06	0.01	0.12	0.25	0.06	0.17
χ^2, *df*, *P*	11.785, 2, 0.003‡		5.189, 2, 0.075$^{n.s.}$		4.861, 2, 0.088$^{n.s.}$	

*For site codes: see Table 1. Contingency table analysis of frequency differences between field captured animals and F_3 are given for each allele within populations.
n.s., $P > 0.05$; †$0.01 < P \leq 0.005$; ‡$0.001 < P \leq 0.01$. *N* = number of individuals.

Within-population variation. Table 4 summarizes the overall measures of allelic variability. From the 22 loci observed in F3 animals, or in field and F3 animals together, 18 loci were monomorphic for the same allele in all populations, including site Siena–Brolio (Si). The mean proportion of polymorphic loci was therefore similar in all populations ($P = 0.182$). Four loci (*Got, Hk, Pgm* and *Phi*) were polymorphic. None of the populations contained a unique allele for the four polymorphic loci. Expected mean heterozygosities were low and constant (min. 0.043 for site Si, max 0.090 for site 4). Small deviations from Hardy–Weinberg equilibrium were observed only for the locus *Got* in populations 1 and 2, and for *Phi* in population 4. The mean number of alleles per locus was low ($1.18 \leq A \leq 1.36$). It is concluded that overall measures of allelic variation within *Orchesella cincta* populations are relatively low compared with other arthropod species (Nevo *et al.*, 1984).

TABLE 4. ALLELE FREQUENCY VARIATION IN NINE POPULATIONS OF *ORCHESELLA CINCTA* FROM NW EUROPE AND ITALY*

Locus		Population 1	2	We	Si	3	4	5	6	7
Got	(*N*)	(115)	(26)	(51)	(20)	(51)	(55)	(35)	(55)	(77)
	A	0.535	0.558	0.461	0.725	0.373	0.536	0.343	0.673	0.714
	B	0.426	0.423	0.539	0.275	0.569	0.427	0.600	0.318	0.272
	C	0.039	0.019	0.000	0.000	0.059	0.036	0.057	0.009	0.013
	He	0.531	0.510	0.497	0.399	0.534	0.528	0.519	0.446	0.415
	Ho	0.478	0.577	0.451	0.350	0.667	0.527	0.571	0.509	0.506
	H–W	‡	‡	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hk	(*N*)	(116)	(64)	(29)	(33)	(33)	(68)	(42)	(44)	(89)
	A	0.211	0.281	0.190	0.030	0.318	0.581	0.167	0.227	0.320
	B	0.789	0.719	0.810	0.970	0.682	0.419	0.833	0.773	0.680
	He	0.333	0.404	0.307	0.059	0.434	0.487	0.278	0.351	0.435
	Ho	0.371	0.406	0.310	0.061	0.333	0.456	0.238	0.455	0.461
	H–W	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pgm	(*N*)	(142)	(71)	(46)	(36)	(46)	(74)	(40)	(46)	(104)
	A	0.169	0.310	0.228	0.000	0.130	0.128	0.363	0.152	0.226
	B	0.331	0.423	0.337	0.000	0.522	0.601	0.188	0.348	0.500
	C	0.496	0.268	0.435	0.681	0.348	0.270	0.450	0.500	0.269
	D	0.004	0.000	0.000	0.319	0.000	0.000	0.000	0.005	0.005
	He	0.615	0.653	0.645	0.435	0.590	0.549	0.631	0.606	0.626
	Ho	0.570	0.549	0.630	0.528	0.543	0.581	0.550	0.587	0.529
	H–W	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Phi	(*N*)	(106)	(56)	(46)	(23)	(44)	(76)	(39)	(43)	(82)
	A	0.075	0.009	0.022	0.022	0.023	0.039	0.013	0.012	0.110
	B	0.873	0.929	0.902	0.978	0.795	0.737	0.962	0.837	0.787
	C	0.052	0.063	0.076	0.000	0.182	0.224	0.026	0.151	0.104
	He	0.230	0.134	0.180	0.043	0.334	0.405	0.075	0.276	0.358
	Ho	0.236	0.107	0.196	0.043	0.409	0.316	0.077	0.326	0.366
	H–W	n.s.	n.s.	n.s.	n.s.	n.s.	‡	n.s.	n.s.	n.s.
Mean (22 loci)	He	0.078	0.078	0.075	0.043	0.087	0.090	0.069	0.077	0.084
	Ho	0.075	0.075	0.072	0.045	0.089	0.085	0.065	0.085	0.085
P (22 loci)		0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182
A (22 loci)		1.36	1.32	1.27	1.18	1.32	1.32	1.32	1.36	1.36

*For site codes, see Table 1. The four loci shown were polymorphic; 18 other loci were monomorphic.

†Field and F_3 data pooled; Si; field-captured animals; other populations; F_3 animals.

N, number of individuals; He, estimated mean heterozygosity; Ho, observed mean heterozygosity; H–W, deviations from Hardy–Weinberg proportions: n.s., $P > 0.05$; ‡$0.01 < P \leq 0.05$. *P*, proportion of polymorphic loci; *A*, mean number of alleles per locus.

Among-population variation. Contingency table analyses of allelic distributions showed that a significant interpopulation heterogeneity was present for all polymorphic loci, with and without the data from Si (all $P \leq 0.001$). The proportion of interpopulation differentiation was determined with *F*-statistics. The proportion of interpopulation variation varied between 5.1 and 10.5% (Table 5); about 8.4% of the observed total variation in the species is due to interpopulation genetic differences. Genetic identity (*I*) and genetic distance values (*D*) between populations are shown in Table 6. Distance values were very low: the highest value of *D* was 0.035, between Si and site 4, and is remarkably low considering the geographic distance existing between them. The distance values are in the lower range of those observed among local populations in other species (Ayala, 1975).

Genetic distance matrices were summarized following the UPGMA algorithm and Fig. 2 shows the resulting pattern of divergence. Based on allele frequencies of the four polymorphic loci, the populations from Si and site 4 diverge most from the other populations, which are mainly grouped together. The branch lengths, in this case, do not necessarily represent the time of divergence, as metal selection may have changed the rate of evolutionary change in some populations. It is concluded that overall genetic divergence of *Orchesella cincta* populations is low, but that interpopulation divergence exists for all polymorphic loci.

Geographic arrangement

The geographic distance varied from 3 km between sites 4 and 7, to more than 1200 km for the distance between the NW European sites and the Italian site. The Pearson correlation coefficient between genetic and geographic distances (log-transformed) was determined for all combinations of populations, and for the NW European

TABLE 5. PROPORTIONS OF INTERPOPULATION DIFFERENTIATION AT FOUR POLYMORPHIC LOCI AMONG NINE POPULATIONS OF *ORCHESELLA CINCTA*

Locus	F_{st}
Got	0.060
Hk	0.105
Pgm	0.102
Phi	0.051
Mean (four loci)	0.084

F_{st}, proportion of interpopulational differentiation.

TABLE 6. GENETIC IDENTITY (above diagonal) AND GENETIC DISTANCE (below diagonal) BETWEEN NINE POPULATIONS OF *ORCHESELLA CINCTA**

	1	2	We	Si	3	4	5	6	7
1	—	0.998	0.999	0.990	0.996	0.989	0.996	0.999	0.996
2	0.002	—	0.998	0.983	0.997	0.992	0.995	0.997	0.998
We	0.001	0.002	—	0.987	0.997	0.988	0.998	0.997	0.994
Si	0.010	0.018	0.013	—	0.977	0.966	0.985	0.990	0.981
3	0.004	0.003	0.003	0.023	—	0.955	0.993	0.994	0.994
4	0.011	0.008	0.012	0.035	0.005	—	0.981	0.990	0.994
5	0.004	0.005	0.002	0.015	0.007	0.019	—	0.993	0.998
6	0.001	0.003	0.003	0.010	0.006	0.010	0.007	—	0.997
7	0.004	0.002	0.006	0.019	0.006	0.006	0.012	0.003	—

*For site codes, see Table 1.

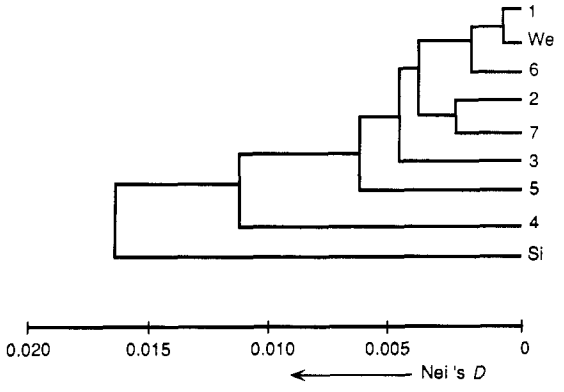

FIG. 2. DENDROGRAM OF THE GENETIC DIVERGENCE OF NINE POPULATIONS OF *ORCHESELLA CINCTA* FROM NW AND S EUROPE, WITH DIFFERENT HISTORIES OF METAL CONTAMINATION. For site codes, see Table 1.

populations separately. The former correlation was significant ($r = 0.484$, $df = 35$, $P \leq 0.001$), but among the NW European populations geographic and genetic distances were not correlated ($r = -0.026$, $df = 27$, $P > 0.05$). It is concluded that factors related to geographic distance have been important in determining genetic divergence on a large geographic scale (>1200 km), but that other factors determined the small-scale divergence on a small geographic scale (3–250 km).

Allozyme frequencies, environmental characteristics and metal tolerance

Correlations between allozyme frequencies, tolerance characteristics and environmental characteristics are summarized in Table 7 for the NW European populations. The Italian site was not incorporated in the calculations, as geographic distance influenced allozyme variation. Allele *Pgm D* was not incorporated as it was present in only two of the NW European sites. The growth tolerance of males (IGR) was not incorporated due to low numbers of individuals and consequently high variability (Posthuma, 1990).

Zinc concentration of the litter and the cadmium or zinc concentration in field algae or field-captured animals were not correlated to any of the alleles; overall measures of allelic variability (*P, A, H*) were not correlated to any of the environmental or tolerance characteristics (data not shown). Most alleles from the polymorphic loci were correlated with at least one other variable, either by Pearson, Spearman, or both methods. Correlations supported by both methods, also after application of the correction factor for number of tests, were found only for *Got* alleles: the frequency of the *Got A* allele was negatively correlated with the concentration of copper found in field animals; it was positively correlated with the blank growth rate of males (determined between four and six weeks after hatching) and with the IGR determined for females. Consequently, the frequencies of the alleles *B* and *C* of *Got* showed the reverse response, but, due to the low number of locations, correlations may be spurious. Correlations of *Got* alleles with cadmium EE were not significant, as this correlation was based on only five locations; however, the signs and values of these correlations were as expected, as IGR and EE are positively associated (Posthuma *et al.*, 1992). The consistent associations between the frequencies of *Got* alleles and copper in field animals and IGR are summarized in Fig. 3.

It is concluded that natural metal-exposed populations of *Orchesella cincta* did not exhibit a lower overall allozyme variation compared to populations from reference sites, but that the frequency of *Got* alleles is correlated with metal tolerance.

TABLE 7. CORRELATION MATRIX OF POLYMORPHIC LOCI WITH CONTAMINATION (top) AND POPULATION (bottom) CHARACTERISTICS IN NW EUROPEAN POPULATIONS OF *ORCHESELLA CINCTA**

		Total metal concentration in litter								Metal concentrations in field animals		Litter pH	
		Cd		Pb		Cu		Ca		Cu			
Locus		r	r_s	r	r_s	r	r_s	r	r_s	r	r_s	r	r_s
Got	A	0.739†	0.464	0.764†	0.595	0.702†	0.631	0.672†	0.571	−0.871†	−0.943‡	0.694	0.771†
	B	−0.750†	−0.357	−0.767†	−0.451	−0.714†	−0.487	−0.700†	−0.607	0.880‡	0.829†	−0.680	−0.886‡
	C	−0.674†	−0.500	−0.761†	−0.685†	−0.626	−0.649	−0.491	−0.571	0.751†	0.771†	−0.786†	−0.714
Hk	A	−0.052	0.214	0.030	0.306	0.085	0.414	0.065	0.143	−0.566	−0.771†	−0.238	0.143
	B	0.024	−0.214	−0.065	−0.306	−0.109	−0.414	−0.128	−0.143	0.566	0.771†	0.238	−0.143
Pgm	A	−0.003	0.071	−0.184	−0.144	0.013	−0.072	−0.687†	−0.393	0.519	0.257	−0.418	−0.029
	B	0.134	0.071	0.116	0.180	0.251	0.252	0.244	0.036	−0.722	−0.543	0.057	0.029
	C	−0.164	0.036	0.018	0.090	−0.330	−0.090	0.295	0.500	0.572	0.486	0.322	0.257
Phi	A	0.532	0.143	0.243	−0.018	0.549	0.162	0.558	0.464	−0.630	−0.543	0.004	0.086
	B	−0.335	−0.321	−0.395	−0.414	−0.384	−0.487	−0.660	−0.500	0.825†	0.771†	−0.287	−0.143
	C	0.102	0.179	0.315	0.378	0.158	0.342	0.437	0.286	−0.570	−0.429	0.265	0.086
n		7		7		7		7		6		6	

		Blank growth rate				Index of Growth Reduction		Cadmium Excretion Efficiency	
		Females		Males		Females			
Locus		r	r_s	r	r_s	r	r_s	r	r_s
Got	A	−0.418	−0.145	0.740†	0.771	0.905‡	0.886‡	0.668	0.600
	B	0.436	0.406	−0.732†	−0.714	−0.897‡	−0.771†	−0.689	−0.600
	C	0.244	0.174	−0.788†	−0.657	−0.937‡	−0.829‡	−0.561	−0.700
Hk	A	0.319	0.493	0.289	0.486	0.157	0.600	0.000	0.200
	B	−0.319	−0.493	−0.289	−0.486	−0.157	−0.600	−0.182	−0.200
Phi	A	−0.823†	−0.464	0.109	−0.086	0.236	−0.029	0.395	0.400
	B	−0.081	−0.493	−0.689	−0.486	−0.578	0.600	−0.814†	−0.800
	C	0.510	0.811†	0.669	0.486	0.479	0.543	0.780	0.700
n		6		6		6		5	

*Characteristics not significantly correlated with allele frequencies and the rare allele *Pgm-D* were omitted.

r, Pearsonian correlation (allele frequencies and excretion efficiency: angular transformation; concentrations; logarithmic transformation); r_s, Spearman rank correlation; n, number of locations incorporated in correlation coefficient; n.s. $P > 0.05$; †$0.01 < P \leq 0.05$; ‡$0.001 < P \leq 0.01$.

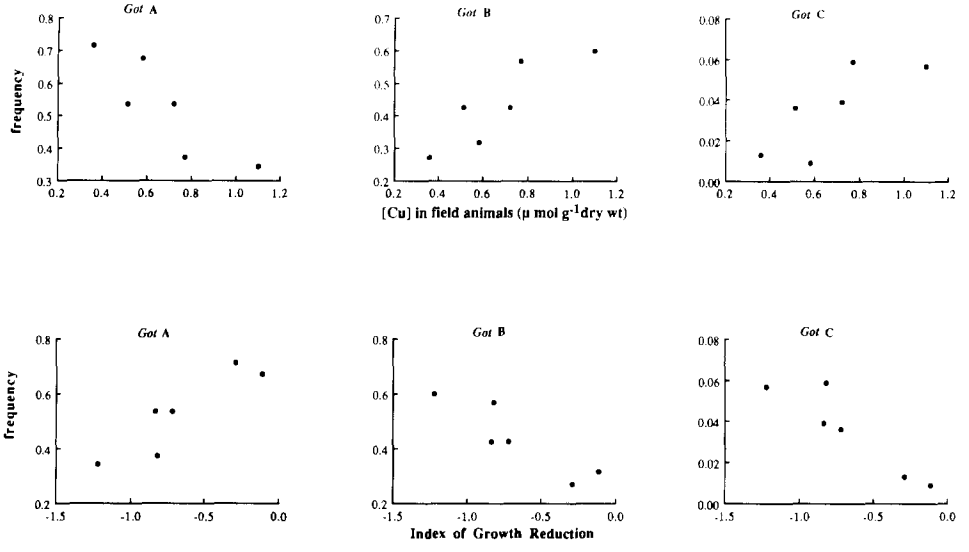

FIG. 3. SCATTERGRAMS OF SIGNIFICANT CORRELATIONS BETWEEN POPULATION CHARACTERISTICS AND ALLELIC FREQUENCIES IN *ORCHESELLA CINCTA*. Top: scattergrams of *Got* alleles (A–C) and copper concentrations in field-captured specimens. Bottom: scattergrams of *Got* alleles (A–C) and Index of Growth Reduction (large negative values indicate strong growth reduction upon cadmium exposure; values close to zero indicate absence of growth response; for further explanation see Posthuma *et al.* (1992).

Discussion

The current study showed that *Orchesella cincta* has to be regarded as a very homogeneous species throughout the NW and S European sampling sites. Metal contamination of the sites did not significantly diminish overall allozymic variation measured in 22 loci and did not induce unique marker alleles. Significant divergence between populations was observed for all polymorphic loci. The correlation between *Got* alleles and metal tolerance characteristics may indicate a response to selection by heavy metals at this locus, or at a genetically correlated locus.

The patterns of variation and population divergence observed in *O. cincta* can be compared to other reports with the summarizing parameter genetic distance (*D*). The values of *D* incorporate both the significant divergences observed at the level of the polymorphic loci, as well as the large number of monomorphic loci in *O. cincta.* The highest value of *D* found in this study was 0.035, between the populations from site 4 and Si; NW European populations with different histories of metal contamination showed lower values of *D.* This genetic homogeneity is remarkable from both a systematic and ecotoxicological point of view. Collembola are considered to be relatively immobile species. Individual *O. cincta* shows movements in response to environmental stimuli in the order of magnitude of metres, e.g. by climbing trees, which may result in air dispersal (Bowden *et al.*, 1976). Furthermore, they show aggregative behaviour, resulting in a relatively high degree of inbreeding within aggregates (Hedlund *et al.*, 1990). At a larger scale (hectares) the species does not show an observable pattern of migration in litter (Joosse, 1969), but air currents may cause passive dispersion of collembolans along large distances (Johnson, 1969). Man can also influence the distribution of species, especially in those, like *O. cincta*, closely related to urban settlement. No information is available concerning actual numbers of viable individuals and distances of dispersion, but we assume, on the basis of the presented observations, that dispersion is not a main cause of genetic homogeneity, especially between the most distant populations. Using electrophoretic analyses, Frati

et al. (in preparation) found a value of genetic distance of 0.027 between two *O. cincta* populations in Tuscany (central Italy); values above 1.543 were found between *O. cincta* and the congeneric species *O. villosa, O. chiantica* and *O. ranzii* in the same area. Other collembolan species showed larger divergence between geographically distant populations ($D \geq 0.224$, Fanciulli *et al.*, 1986a,b; 1991). Within the genus *Orchesella, O. cincta* is considered to be a relatively old species, the allozyme frequencies of which, indicated by the present results, have not been seriously affected by geographic isolation, or by local stochastic and systematic factors.

In this study, previous observations in Italian *O. cincta* populations (Frati *et al.*, in preparation) are confirmed by overall measures of genetic variability: overall genetic divergence was only correlated with large-scale geographic distance, whereas other correlations were not significant. The pattern of (slight) divergence observed among NW European populations should be attributed to factors other than geographic distance. The divergence of population 4 may have been particularly influenced by isolation. The capture location at site 4 is located near natural ore outcrops at a mining site, which was in operation from the Roman ages up to the second half of the 19th century (Vorbrüggen, 1981). It is located in an area (probably including site 7) where lead and zinc ores naturally occur at the soil surface (Schwickerath, 1954), and is only 3 km separated from site 7. Several sampling observations suggest that the *O. cincta* population in site 4 is small and isolated; it inhabits patches of pine litter, at the sharp borderline of the mine and a *Picea* forest (Simon, 1975) in which *O. cincta* was never found; field individuals contain relatively low metal concentrations (Table 1) and do not exhibit increased cadmium tolerance (Posthuma, 1990). Therefore, the divergence of population 4 may have resulted from the stochastic effects of small population size, probably based upon a founder effect, long-term isolation and weak metal exposure. The effects of small population size and isolation of this kind seems to be less important for the populations in the intermediate and highly polluted sites 5, 7 and probably 6; inventory sampling showed that *O. cincta* occurred in forest habitats along gradients of 10–16 km (sites 5 and 7) and at a site next to the mine (site 6, Van Straalen *et al.*, 1987). The divergence of these populations from the other large populations (1, 2, We, Si, and 3) must be attributed to factors other than isolations. The calcium concentration of the litter has been suggested to be an important factor in the relatively low cadmium excretion efficiency of population 5 (Posthuma *et al.*, 1992); this hypothesis is supported by the correlation pattern, which tends to show a direct or indirect association of *Got* alleles with the calcium status of the litter.

Notwithstanding the overall genetic homogeneity of the species, populations of *O. cincta* exposed to high concentrations of heavy metals for a long time exhibit significantly higher levels of metal tolerance than populations exposed to lower concentrations for shorter periods (Posthuma, 1990; Posthuma *et al.*, 1992). Thus metal contamination has affected continuously variable characteristics without affecting overall measures of genetic variability. Similar results in a series of reference and metal-exposed populations of *Silene cucubalus* were found by Verkleij *et al.* (1985; 1987), who suggested that this effect may have resulted from a lack of loci controlling metal tolerance in the sample of loci scored. Contrary to *O. cincta*, this plant species showed a high degree of within-population variability. If the sample of loci is representative of the genetic variability of the genome, then it is remarkable that *O. cincta* has developed a genetically based tolerance with so little genetic variation. On the other hand, if metal tolerance involves many loci, each with a small effect, then quantitative genetic studies should be done to obtain information on the amount of genetic variation involved.

Contrary to the absence of correlations with overall measures of variability, systematic changes of allelic frequencies were found for specific loci, especially for the *Got* locus. Many authors have found *post hoc* correlations between environmental

variables and the frequencies of specific alleles. Five out of 10 isozyme loci in *Drosophila melanogaster* showed gene frequency changes associated with metal contamination along an 11-km gradient from a metal smelter, within seven years after the first emissions (Lower, 1975). Similar results were obtained for desert landsnails (69% of the loci correlated with an environmental variable; Nevo *et al.*, 1981), and marine organisms (correlations with metal contamination: Nevo *et al.*, 1983, and references therein; Gillespie and Guttman, 1989). Such correlations may indicate the loci which are specifically influenced by the selective agent, but do not directly show the ecological relevance of the observed changes (Koehn, 1978). Stronger evidence for associations between pollution-mediated selection and allozyme frequencies has been derived from experimentally exposed animals in which genotype-dependent survival was determined (Lavie and Nevo, 1982; 1986; Chagnon and Guttman, 1989; Gillespie and Guttman, 1989; Hawkins *et al.*, 1989; Benton and Guttman, 1990). In *O. cincta* the most consistent correlations were observed between *Got* alleles and the tolerance characteristics IGR (significant) and cadmium excretion efficiency (spurious). Correlations of similar sign and magnitude were also observed with the litter concentrations of the xenobiotic metals cadmium and lead, and with the micronutrient copper. Copper concentrations in field animals, however, showed correlations of opposite sign, but this correlation may be influenced by active physiological regulation (Table 1, Van Straalen *et al.*, 1987), comparable to the stability of zinc concentrations in exposed animals (Posthuma *et al.*, 1992). These data support the interpretation that metal selection affects specific loci rather than overall variability. Metals are known to influence the activity of many enzymes (Bowen, 1966; Webb, 1966). The enzyme Got is involved in both amino acid metabolism and the tricarboxylic acid cycle, and a weak effect of zinc deficiency on Got-mediated breakdown of amino acids has been reported in rats (Macapinlac *et al.*, 1966). However, decisive questions on the causes of the patterns observed (environmental effects or both environmental and genetic effects; Bergmann *et al.*, 1987), the role of Got in metal tolerance (Koehn, 1978), and its possible use as a marker of the development of metal tolerance in *O. cincta* remain to be answered.

The present results show that metal selection may have influenced specific allozymes, which may be associated, directly or indirectly, with metal tolerance. This observation draws attention to specific rather than general effects of metals on genetic variability. Research focusing on the specific effects of heavy metals on the genetic variability of a quantitative tolerance characteristic (i.e. the inheritability of excretion efficiency) is in progress and will be reported elsewhere.

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