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Chemical Defence and Genetic Variation

Interpopulational Study of *Oreina gloriosa* (Coleoptera: Chrysomelidae)

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The conspicuous color and aggregated distribution of many chrysomelids is associated with defensive mechanisms against predators [1]. The defensive strategy of adults of the alpine species *Oreina gloriosa* is based on the exocrine secretion of a complex mixture of de novo synthesized cardenolides and ethanolamine at the surface of the pronotum and elytra [2]. The efficiency of the defensive strategy of *O. gloriosa* is strongly supported by its high survival rate (0.73 to 0.96 per week) estimated by Jolly's capture-recapture method [3]. Recapture of marked adults also shows that these can live as long as 3 years [4].

Variation of quantitative characteristics is ubiquitous; it is thought to be subjected to much adaptive evolution and to provide the substrate for selective improvement. However, variation in chemical defence, between both individuals and populations, has not been given much attention so far, although chemical defence is an important component of the interaction between prey and predator. Normally, within a population, the standard deviation of phenotypic values does not exceed 10% of the mean [5]. In contrast, we show below that the main constituents of the defensive secretions of *O. gloriosa* display a clearly higher quantitative variation among populations and to a smaller extent also within populations (see Table 1). The phenotypic variation of the defensive secretion could be caused by environmental variation. Clear differences between geographically separated (and thus probably reproductively isolated) populations, however, suggest that the phenotypic variation is at least partly due to genetic variation.

The hypothesis that the quantitative variation of the defensive secretions could be associated with genetic varia-

Table 1. Variation in chemical defence of *O. gloriosa*. Mean percentages (±SEM) of main components determined by liquid chromatography. Transformed (arcsine) proportions were tested for differences among populations by analysis of variance. Three main components (RT3, RT9, and RT16) are significantly ($p < 0.05$) different between populations

Population	*N*	RT3	RT9	RT16	RT28	RT32
Saas	8	43.6±6.0	23.7±2.5	15.8±3.3	8.7±0.7	8.2±1.0
Gruben	6	28.9±3.1	27.9±3.2	22.6±3.0	9.3±1.5	11.3±1.6
Zinal	12	29.5±3.5	30.7±2.7	13.0±2.6	12.6±1.7	14.2±1.9
Grimentz	16	27.2±2.2	34.1±3.3	13.4±3.2	12.0±1.4	13.4±1.0
Ferpècle	6	23.9±4.9	49.9±6.1	1.3±1.0	11.2±2.3	13.7±2.5
ANOVA (1-way)						
F-value		3.53	5.49	3.57	0.98	2.10
p		0.014	0.001	0.013	0.427	0.100

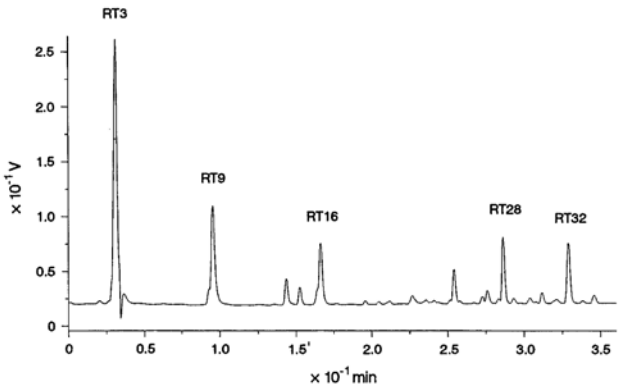

Fig. 1. Chromatogram obtained from liquid chromatography (RP-HPLC; ACN/H_2O-gradient; 254 nm) of the defensive compounds emitted by a single beetle

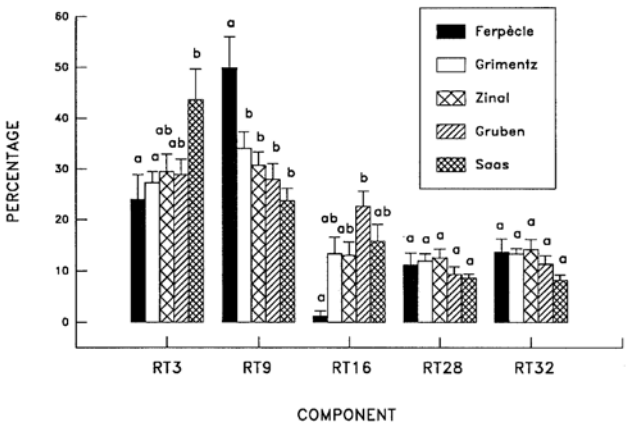

Fig. 2. Mean percentages (±SEM) of five main components in defensive secretions determined by liquid chromatography. Numbers of individuals are listed in Table 1. Means which do not share a letter in common are significantly ($p < 0.05$) different (analysis of variance; Tukey-Kramer test for multiple comparison)

tion was tested by correlating phenotypic variation of the defensive secretion with molecular variation of six enzymes. The individuals which were collected in the field for this purpose were derived from five populations located in four of five neighboring tributory valleys of the Rhone (Swiss Alps). These are successively the Val d'Hérens (Ferpècle), Val d'Anniviers (two populations: Grimentz and Zinal), Turtmanntal (Gruben) and, skipping one valley, Saastal (Saas Grund). Although distances between the locations are small, ranging from 7 to 30 km, the studied populations are separated by high-altitude ridges. Genetic differentiation may be further promoted by the distribution of the host plant (*Peucedanum ostruthium;* Apiaceae) which occurs in distinct patches and the low dispersion rate of *O. gloriosa* (0.5 m/day) [6] which flies very rarely or not at all.

The quantitative variation in chemical defence was determined from secretions of 16 individuals of each population. In the laboratory each individual was irritated mechanically and the liberated secretion was taken up with a small piece of filter paper. Each sample was dissolved in 30 µl acetonitrile/water 1:10 and stored in a freezer at −70 °C. The analytical separation was performed with RP-HPLC (solvent A: water, B: acetonitrile, 15–42 % B linear in 36 min, 0.45 ml/min; column: RP18, 3 µm, 4 × 130 mm; detection: 254 nm). Figure 1 shows a resulting chromatogram. Since the sensitivity of the analytical system was too low for some samples, only 48 analyses of a potential 80 could be statistically evaluated. The proportions of the main peaks (RT3, RT9, RT16, RT28, and RT32) were calculated by dividing the respective area by the total area of main peaks. These proportions, arcsine-transformed, were separately tested for differences among the five populations by analysis of variance (SAS; Proc GLM). The components RT3, RT9, and RT16 are significantly ($p < 0.05$) different among populations (Table 1, Fig. 2). Comparisons of means (Tukey-Kramer) are significant ($p < 0.05$) for differences between the Saas and Grimentz populations and between those of Saas and Ferpècle (RT3), between that of Ferpècle and all other populations (RT9) and between the populations of Gruben and Ferpècle (RT16).

Genetic variation of *O. gloriosa* among populations was studied by examining with starch gel electrophoresis the enzyme products of six genetic loci (ACON, PGM, MDH, FUM, ICDH, and α-GPDH). For this analysis a total of 363 adult beetles was collected in the field and stored in liquid nitrogen, 148 individuals were derived from Saas, 38 from Zinal and 59 from Gruben, Grimentz and Ferpècle, respectively. The thoracic muscles of each beetle were homogenized and absorbed on two filter-paper wicks. Each sample was placed in a vertical slit made in the gels, which were prepared by the method in [7]. Two buffer systems were used: Tris-citrate buffer pH 8 [8] (FUM and ICDH) and Tris-citrate buffer pH 6.7 [9] (α-GPDH, ACON, PGM, and MDH). The gels were run at 50 mA in a

refrigerated chamber and stained after run times of 4 h (MDH, FUM, and ICDH) and 5 h, respectively (ACON, PGM, and MDH).

Three loci (FUM, ICDH, and α-GPDH) are monomorphic in all populations. The three-banded heterozygotes of MDH indicate a dimeric enzyme. Heterozygotes of PGM and ACON show a two-banded pattern typical for monomeric enzymes. Allele frequencies of the polymorphic loci are listed in Table 2. The mean heterozygosity of the population in Saas ($H = 0.12$) is similar to the mean heterozygosity of *Oreina speciosissima* in the Swiss Alps ($H = 0.16$) [4] and also to the mean heterozygosity of other insect species ($H = 0.15$) [10]. The low mean heterozygosities of the populations in Gruben ($H = 0.06$), Zinal ($H = 0.02$), Grimentz ($H = 0.01$), and Ferpècle ($H = 0.01$) suggest small effective population sizes of these populations compared to the population in Saas. This is supported by data on population density [6] which is clearly higher in Saas (13 adults/m²) than in Gruben (7 adults/m²), Zinal (6 adults/m²) and Grimentz (5 adults/m²). Genetic Nei-distances [11] between populations are listed in Table 3. According to these values, the two populations (Zinal and Grimentz), which are situated in the same valley (Val d'Anniviers), are genetically identical. There are also no measurable genetic differences between the populations of the Val d'Anniviers and the population in the adjoining Val d'Hérens (Ferpècle). However, the Nei-distances between the populations, which are farther apart spatially, are similar to the average Nei-distance ($D = 0.05$) between three populations of *Oreina cacaliae* in the Alps [4] and between 11 populations of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) in the USA and Europe ($D = 0.022$) [12].

Phenotypic distances in chemical defence between the populations were calculated by clustering the percentages of the main components (Table 1). Standardized mean percentages of each population were subjected to UPGMA cluster analysis (SAS; Proc Cluster). The resulting average distances between clusters are listed as phenotypic distances in Table 3.

Rank correlation (SAS; Proc Corr) between genetic (Nei) and geographic distances shows a significantly positive relationship (Kendall's $r = 0.787$; $p = 0.002$). Geographical separation seems to reduce gene flow between separated populations and may thereby promote genetic divergence. The dependence of both genetic and phenotypic distance on geographic distance is shown in Fig. 3. With increasing distance between locations both genetic and phenotypic distances between populations increase. This conformity of genetic and phenotypic distances suggest that phenotypic differences in chemical defence are at least in part attributable to genetic differences. According to the rank correlation (SAS; Proc Corr) between genetic and phenotypic distance (Kendall's $r = 0.641$; $p = 0.02$), 41 % of variation in chemical defence could be explained by genetic differences. In addition to genetic divergence, environmental differences between locations, e.g., in climate,

Table 2. Genetic variation in five populations of *O. gloriosa*. Allel frequencies of polymorphic loci investigated by starch gel electrophoresis

Allel		Saas	Gruben	Zinal	Grimentz	Ferpècle
ACON	88	0.000	0.009	0.000	0.000	0.009
	100	0.934	0.941	0.974	0.971	0.975
	114	0.066	0.051	0.026	0.029	0.017
	N	129	59	38	51	59
PGM	80	0.004	0.033	0.013	0.000	0.000
	92	0.049	0.067	0.013	0.000	0.000
	100	0.578	0.867	0.974	1.000	1.000
	108	0.283	0.033	0.000	0.000	0.000
	116	0.086	0.000	0.000	0.000	0.000
	N	122	60	38	59	59
MDH	76	0.010	0.000	0.000	0.000	0.000
	100	0.990	1.000	1.000	1.000	1.000
	N	148	59	38	51	59

Table 3. Phenotypic, genetic, and geographic distances between populations of *O. gloriosa* in the Swiss Alps. Phenotypic distances are equivalent to average distances between clusters (determined by UPGMA cluster analysis) of mean populational proportions of main defensive components (investigated by liquid chromatography). Genetic Nei-distances were determined by starch gel electrophoresis of six enzymes. Kendall's rank correlations between all distances are significant ($p < 0.05$)

Populations	Distance between populations		
	phenotypic	genetic	geographic [km]
Saas-Ferpècle	1.227	0.023	30
Saas-Grimentz	1.227	0.023	28
Saas-Zinal	1.227	0.021	23
Saas-Gruben	1.227	0.014	19
Gruben-Ferpècle	0.959	0.002	19
Grimentz-Ferpècle	0.959	0.000	13
Gruben-Zinal	0.765	0.001	12
Gruben-Grimentz	0.765	0.002	11
Zinal-Grimentz	0.212	0.000	10
Zinal-Ferpècle	0.959	0.000	7
Rank correlations			
Distance between locations [km]	$r = 0.714$**	$r = 0.787$**	
Genetic distance	$r = 0.641$*		

** $p < 0.01$
* $p < 0.05$

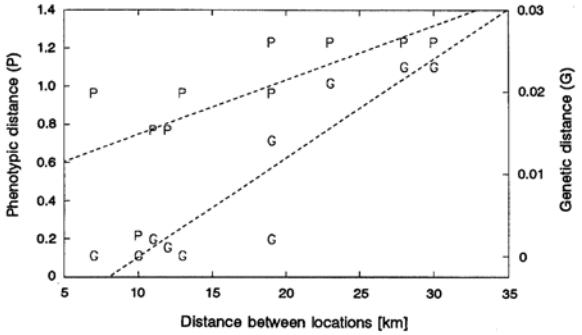

Fig. 3. Comparison of phenotypic, genetic, and geographic distances between populations of *O. gloriosa* in the Swiss Alps. Phenotypic distances reflecting quantitative differences of defensive secretions among populations. Genetic Nei-distances were determined by starch gel electrophoresis of six enzymes

food quality, or predation pressure, as well as physiological differences, e.g., in age and reproductive status, may also contribute to the quantitative variation in chemical defence among populations of *Oreina gloriosa*.

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