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GENETIC COMPONENT OF VARIATION IN CHEMICAL DEFENSE OF *Oreina gloriosa* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract—Defensive secretions of adult *Oreina gloriosa*, liberated at the surface of the pronotum and elytra, contain a complex mixture of cardenolides, and ethanolamine. Proportions and concentrations of constituents determined by reverse-phase HPLC show considerable variation among individual beetles. Heritabilities of proportions of five main components were estimated by mother–offspring regression providing a validation of the less reliable full-sib correlation estimates. Average heritabilities based on the two methods were 0.51 and 0.58, respectively, estimated by using offspring of two age groups. Regression estimates of 2- and 10-week-old offspring differed significantly for one secretion constituent (RT16). Heritability estimates of concentrations of 16 secretion components were calculated by full-sib correlation analysis. Average heritability was 0.45, indicating a significant genetic component. Estimates did not differ significantly between the two age groups. We also estimated heritabilities of concentrations by a two-way model including data from offspring of both age groups. Heritability estimates based on this model are thought to correspond approximately to estimates based on samples from natural populations. The average of these estimates was lower ($h^2 = 0.31$) than the average heritability of each age group separately ($h^2 = 0.45$), suggesting a developmental effect on variation in chemical defense of *O. gloriosa*.

Key Words—*Oreina gloriosa*, Coleoptera, Chrysomelidae, chemical defense, allomone, cardenolides, quantitative variation, heritability.

INTRODUCTION

The alpine beetle *Oreina gloriosa* belongs to one of the most conspicuous families of Coleoptera, the Chrysomelidae. Adult *O. gloriosa* are slow-moving,

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brightly colored, and they often densely aggregate on their host plant *Peucedanum ostruthium* (Apiaceae), resulting in high visual exposure to predators. Nevertheless this chrysomelid shows a high survival rate (0.73–0.96 per week) (Eggenberger, 1989) and can live as long as three years (Rowell-Rahier, unpublished data), suggesting an effective protection by its chemical defense (Pasteels et al., 1988). The defensive strategy of adult *O. gloriosa* is based on the exocrine secretion of a complex mixture of cardenolides and ethanolamine at the surface of the pronotum and elytra (Van Oycke et al., 1988). Since the host plant of this monophagous beetle is known to contain no cardenolides, *O. gloriosa* produces its defensive chemicals de novo. The ability of leaf beetles to synthesize cardenolides de novo has been confirmed by labeling experiments (Van Oycke et al., 1987).

Chemical defense is an important component of the interaction between prey and predator, which is thought to be under strong selection for efficiency, since production, particularly de novo biosynthesis, of defensive chemicals is costly (Rowell-Rahier and Pasteels, 1986). In order to produce evolutionary change, however, selection requires heritable variation, which is measured and expressed as additive genetic variance. The ratio of additive genetic variance to phenotypic variance is defined as the heritability (h^2), which is estimated from the degree of resemblance between relatives by regression of offspring on parents or correlation between sibs (Falconer, 1989).

Although genetic sources of variation in exocrine secretions were shown to exist in pheromones of butterflies (Sappington and Taylor, 1990) and hypothesized for the kairomones of a prey–predator interaction (Herms et al., 1991), this is the first report dealing with heritabilities of chemical defense characters.

We recently reported on variation in chemical defense between populations of *O. gloriosa* (Eggenberger and Rowell-Rahier, 1991). Interpopulational variation in the constituents of the defensive secretion proved to be significantly correlated with genetic variation of six enzyme loci, suggesting that a considerable part of the total phenotypic variation in chemical defense is caused by genetic differences.

In this study we demonstrate a significant genetic component to the intrapopulational variation in chemical defense of *O. gloriosa*.

METHODS AND MATERIALS

Collection and Culture of Beetles. One hundred fifty adult *O. gloriosa* were collected randomly in July 1989 in Saas (Wallis, Swiss Alps) and brought in cooled boxes to the laboratory. The beetles were maintained individually in separate plastic containers on their food plant *P. ostruthium*, which was shipped weekly from the original locality. The offspring of the 48 collected females,

which had mated in the field with unknown males, were reared in plastic containers at constant temperature (17°C) and a constantly changing light–dark regime corresponding to natural conditions. In September 1989 all larvae buried themselves in the substrate provided: soil covered with regularly moistened spruce bark. Pupation was not completed by November, and the temperature was decreased to 2°C. At the end of June 1990, the temperature was raised to 17°C, triggering pupation and the emergence of 553 adults. The offspring of 27 females were maintained separately under the conditions previously described. The 27 families were selected so that there were at least five offspring per family.

Sample Preparation and Chromatographic Analysis. Pronotal secretion liberated as a result of mechanical irritation was collected in a capillary tube (0.2 mm ID) and quantified. The parental generation was "milked" in July 1989, a few days after arrival in the laboratory. Samples were dissolved in 50 μl acetonitrile–water 1:10 and stored individually at −70°C. The secretions of one group of offspring were sampled in July 1990 (two weeks after emergence) and those of the other group in September 1990 (10 weeks after emergence). Individual samples were dissolved in 50 μl acetonitrile–water 1:10 plus 2 μg ouabain (Merck), as internal standard. After filtration (0.2 μm pore size), 20-μl aliquots were analyzed by HPLC, [two-pump system (Waters 510); detector: photodiode array (Waters 994), 220 nm; column: Macherey-Nagel cartridge, C-18, 3 μm, 4 × 130 mm; eluent: chromatography grade acetonitrile (Baker) and water (Merck), 15–42% acetonitrile linear in 36 min, 0.45 ml/min; data analysis system: Maxima 820 data station] (Figure 1). The proportions of the main secretion components (RT3, RT9, RT16, RT28, and RT32) were calculated by dividing the respective peak area by the total area of main peaks. Concentrations (micrograms ouabain equivalents per microliter secretion) of 16 secretion components were twice their peak area divided by the area of the internal standard peak (ouabain) and the secretion volume. Since samples of the parental generation were run without an internal standard, concentrations could be calculated only for offspring.

Statistical Methods. Data were analyzed using SAS (SAS Institute Inc., 1990) on a VAX 8840. Weighted regression of offspring on mother was carried out using PROC REG. Weighting factors for regression were calculated by the procedure of Falconer (1989). Heritability estimates were twice the slope of the regression with a precision of twice the standard error of the slope. Random effect model analyses of variance were performed using PROC GLM. Prior to analyses of variance, proportions were arcsine transformed (Sokal and Rohlf, 1981). Concentrations were square-root transformed since residual distributions showed a poorer fit to Gaussian distributions (PROC UNIVARIATE) if other commonly applied transformations (Sokal and Rohlf, 1981) were used. Variance components were estimated by the maximum likelihood method (PROC VARCOMP), which agreed better with heritability estimates based on mother–off-

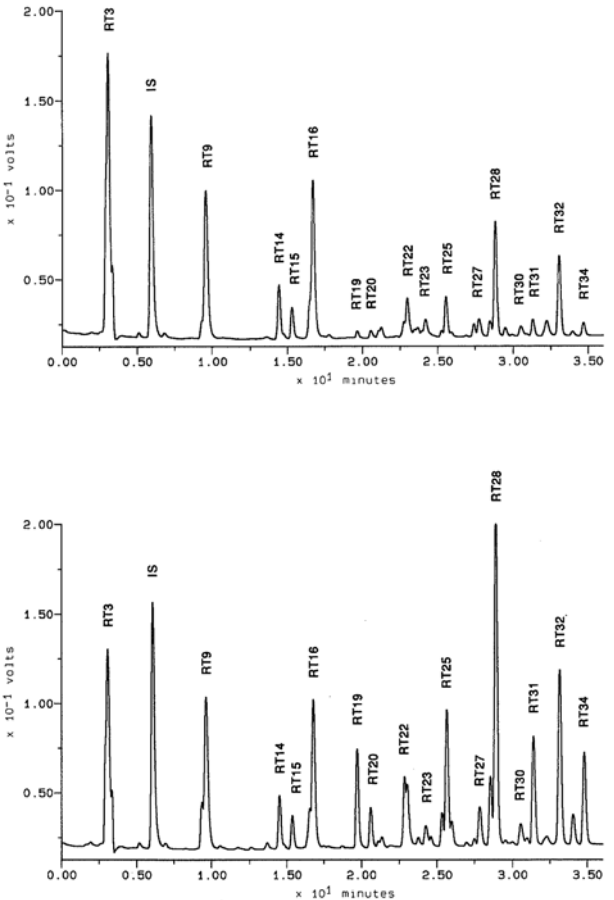


FIG. 1. Chromatograms obtained by reverse-phase HPLC of the defensive secretions emitted by individual beetles.

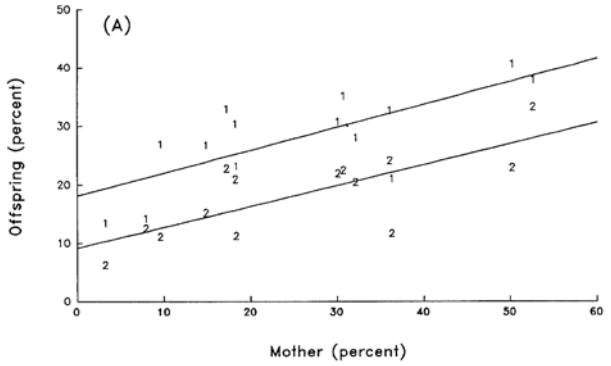
spring regression than either the restricted maximum likelihood method or the type-1 sum-of-squares method. Genetic and developmental effects were designated as random since we were interested in the variation that exists when genotype and age are randomly distributed as in natural populations. Heritability estimates based on full-sib analysis were twice the intraclass correlation coefficient (among-family variance component divided by total variance) with standard errors estimated by the delta technique (Bulmer, 1985).

Heritability estimates of proportions of secretion components were estimated using both parent–offspring regression and full-sib correlation analysis. Parent–offspring regression was carried out with data from 14 females and two different groups of their offspring, which were "milked" two and 10 weeks after emergence, respectively. The proportions of each of the five main components in the secretion of individual offspring were averaged per family and regressed for each age group separately on the proportions measured in their individual female parents. Full-sib correlation analysis was performed using data from the 138 living 2-week-old offspring belonging to 27 families and 92 10-week-old offspring of 25 females, respectively. (The numbers of families used for estimating heritabilities were different for parent–offspring regression and full-sib correlation because secretions of female parents were not always available.) The data for each group were tested for differences among families, which was assumed to be a random factor.

Heritabilities of concentrations of secretion components were estimated using full-sib correlation analysis. (Parent–offspring regression was not used since concentrations were not available for the parental generation.) Full-sib correlation was carried out on the same two age groups as used to estimate heritabilities of proportions. However, since some data on secretion volume were lost, the number of analyzed offspring was different. Data for each age-group were tested for differences among families.

RESULTS

Proportions of Five Main Components. Heritability estimates based on parent–offspring regression were significant ($P < 0.05$) for RT9 and RT16 of both age groups (Figure 2) and for RT3 of 2-week-old offspring (Table 1). Estimates for RT16 differed significantly ($P < 0.05$) between the two age groups. The among-family variance term was significant (Tables 2 and 3) for all secretion components in both age groups, with the single exception of RT3 in 10-week-old offspring. Heritabilities estimated by full-sib correlation analysis were significant for RT9, RT16, and RT28 in both age groups and for RT3 in 2-week-old offspring (Tables 2 and 3). Mother–offspring estimates were slightly lower than full-sib estimates, which are known to be biased by dominance and



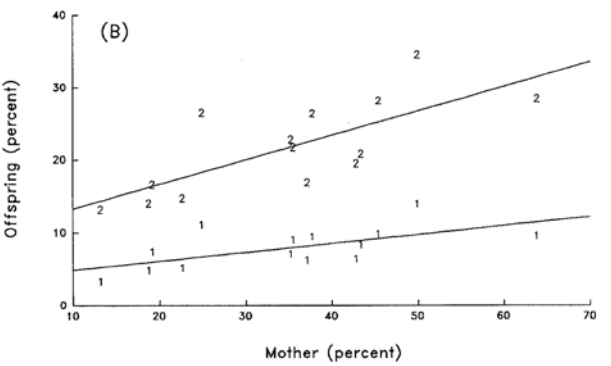


FIG. 2. Proportions of constituents in the secretion of offspring (averaged per family) in relation to those from the secretion of their mothers. Regression were carried out with 2-week-old (1) and 10-week-old (2) offspring. Slopes did not differ significantly for RT9 (A) but were significantly ($P < 0.05$) different for RT16 (B).

TABLE 1. MEAN PERCENTAGES (±SE) OF 5 MAIN SECRETION COMPONENTS OF TWO GROUPS OF OFFSPRING AND THEIR MOTHERS[a]

Secretion component	Mother (N = 14) Percent	Offspring 1 (age 2 weeks, N = 69) Percent	Offspring 1 (age 2 weeks, N = 69) h^2	Offspring 2 (age 10 weeks, N = 56) Percent	Offspring 2 (age 10 weeks, N = 56) h^2
RT3	22.2 ± 3.0	36.2 ± 0.9	0.52 ± 0.17**[b]	31.7 ± 0.9	0.23 ± 0.14 ns
RT9	25.5 ± 4.0	27.6 ± 1.1	0.78 ± 0.21**	19.4 ± 1.2	0.74 ± 0.18**
RT16	35.0 ± 3.7	8.1 ± 0.5	0.24 ± 0.09*	21.0 ± 1.0	0.67 ± 0.18**
RT28	6.1 ± 0.8	14.0 ± 0.5	0.57 ± 0.54 ns	11.3 ± 0.6	0.83 ± 0.49 ns
RT32	11.2 ± 1.2	14.1 ± 0.6	0.21 ± 0.35 ns	16.6 ± 0.6	0.22 ± 0.28 ns
Average			0.47		0.54

[a]Heritabilities (h^2 ± SE) were estimated by mother–offspring regression.
[b]**$P < 0.01$; *$P < 0.05$.

TABLE 2. MEAN PERCENTAGES (±SE) OF 5 MAIN SECRETION COMPONENTS OF 2-WEEK-OLD BEETLES[a]

Secretion component	Offspring 1 (age 2 weeks, N = 138) Percent	Offspring 1 (age 2 weeks, N = 138) h^2	ANOVA (effect of family) df = 26 Type III MS	F value	P value
RT3	37.1 ± 0.6	0.37 ± 0.16*[b]	0.0096	2.213	0.0023
RT9	27.7 ± 0.7	0.97 ± 0.18**	0.0289	5.932	0.0001
RT16	7.7 ± 0.3	0.63 ± 0.19**	0.0109	3.431	0.0001
RT28	13.6 ± 0.4	0.66 ± 0.19**	0.0087	3.660	0.0001
RT32	13.9 ± 0.4	0.26 ± 0.15 ns	0.0073	1.757	0.0234
Average		0.58			

[a]Arcsine transformed data were tested for differences among families by random effect model analysis of variance. Heritabilities (h^2 ± SE) were estimated by full-sib correlation analysis.
[b]**$P < 0.01$; *$P < 0.05$.

environmental effects (Falconer, 1989). However, the heritabilities estimated by the two methods did not differ significantly.

Concentrations of 16 Components. Fifteen of the 16 among-family variance components of 2-week-old offspring were significant, whereas with 10-week-old offspring five among-family variance components were not significant (Tables 4 and 5). Heritability estimates were significant for five secretion components (RT9, RT16, RT27, RT30, and RT34) of both age groups; the estimates of seven other secretion components (RT14, RT15, RT19, RT20, RT23, RT25,

TABLE 3. MEAN PERCENTAGES (±SE) OF 5 MAIN SECRETION COMPONENTS OF 10-WEEK-OLD BEETLES[a]

Secretion component	Offspring 2 (age 10 weeks, $N = 92$) Percent	h^2	ANOVA (effect of family) $df = 24$ Type III MS	F value	P value
RT3	33.6 ± 0.9	0.15 ± 0.17 ns	0.0100	1.328	0.1816
RT9	19.0 ± 0.9	0.80 ± 0.24**[b]	0.0288	3.525	0.0001
RT16	19.9 ± 0.8	1.05 ± 0.22**	0.0204	5.155	0.0001
RT28	11.4 ± 0.4	0.60 ± 0.25*	0.0084	2.781	0.0005
RT32	16.1 ± 0.5	0.25 ± 0.26 ns	0.0063	1.998	0.0140
Average		0.57			

[a] Arcsine transformed data were tested for differences among families by random effect model analysis of variance. Heritabilities (h^2 ± SE) were estimated by full-sib correlation analysis.
[b] **$P < 0.01$; $P < 0.05$.

TABLE 4. MEAN CONCENTRATIONS (±SE) OF 16 SECRETION COMPONENTS OF 2-WEEK-OLD BEETLES[a]

Secretion component	Offspring 1 (age 2 weeks, $N = 131$) Amount (μg/μl)	h^2	ANOVA (effect of family) $df = 26$ Type III MS	F value	P value
RT3	52.7 ± 1.3	0.22 ± 0.15 ns	1.4345	1.624	0.0454
RT9	40.2 ± 1.5	0.47 ± 0.19*[b]	3.4884	2.497	0.0006
RT14	4.0 ± 0.2	0.31 ± 0.16 ns	0.3878	1.876	0.0138
RT15	3.2 ± 0.2	0.30 ± 0.17 ns	0.4401	1.892	0.0128
RT16	11.2 ± 0.6	0.41 ± 0.18*	1.5020	2.291	0.0017
RT19	4.8 ± 0.4	0.94 ± 0.20**	1.8060	5.033	0.0001
RT20	7.9 ± 0.5	0.75 ± 0.20**	2.0203	3.903	0.0001
RT22	7.4 ± 0.3	0.32 ± 0.18 ns	0.6219	2.015	0.0069
RT23	5.9 ± 0.3	0.30 ± 0.18 ns	0.5525	1.905	0.0119
RT25	12.7 ± 0.5	0.68 ± 0.20**	1.5402	3.568	0.0001
RT27	5.8 ± 0.3	0.56 ± 0.19**	0.7618	2.904	0.0001
RT28	20.1 ± 9.8	0.35 ± 0.18 ns	1.8225	2.122	0.0040
RT30	2.1 ± 0.1	0.43 ± 0.19*	0.3045	2.385	0.0010
RT31	4.2 ± 0.3	0.36 ± 0.18 ns	0.8610	2.118	0.0041
RT32	20.4 ± 0.9	0.13 ± 0.15 ns	1.5275	1.388	0.1255
RT34	4.7 ± 0.3	0.45 ± 0.19*	0.9557	2.472	0.0007
Average		0.44			

[a] Square-root transformed data were tested for differences among families by random effect model analysis of variance. Heritabilities (h^2 ± SE) were estimated by full-sib correlation analysis.
[b] **$P < 0.01$; *$P < 0.05$.

TABLE 5. MEAN CONCENTRATIONS (±SE) OF 16 SECRETION COMPONENTS OF 10-WEEK-OLD BEETLES[a]

Secretion component	Offspring 2 (age 10 weeks, $N = 90$) Amount ($\mu g/\mu l$)	h^2	ANOVA (effect of family) $df = 24$ Type III MS	F value	P value
RT3	66.0 ± 2.2	0.19 ± 0.20 ns	2.0143	1.481	0.1076
RT9	37.8 ± 2.0	0.59 ± 0.24*[b]	5.0083	2.621	0.0011
RT14	10.7 ± 0.5	0.60 ± 0.25*	0.7510	2.670	0.0009
RT15	5.6 ± 0.3	0.66 ± 0.23**	0.4719	2.858	0.0004
RT16	39.5 ± 1.8	0.58 ± 0.25*	3.4536	2.580	0.0013
RT19	1.8 ± 0.1	0.14 ± 0.17 ns	0.2932	1.155	0.3153
RT20	2.9 ± 0.2	0.41 ± 0.21 ns	0.5139	1.856	0.0257
RT22	7.5 ± 0.4	0.29 ± 0.19 ns	0.5766	1.561	0.0803
RT23	5.2 ± 0.3	0.60 ± 0.22*	0.7469	2.671	0.0009
RT25	9.6 ± 0.4	0.21 ± 0.18 ns	0.5660	1.344	0.1736
RT27	5.2 ± 0.3	0.57 ± 0.23*	0.5553	2.430	0.0025
RT28	22.7 ± 1.0	0.30 ± 0.25 ns	1.6703	1.704	0.0466
RT30	3.3 ± 0.2	0.74 ± 0.23**	0.4929	3.152	0.0001
RT31	8.7 ± 0.5	0.64 ± 0.26*	1.6849	2.858	0.0004
RT32	31.8 ± 1.2	0.15 ± 0.23 ns	1.3938	1.544	0.0855
RT34	5.8 ± 0.4	0.74 ± 0.26	1.1570	3.220	0.0001
Average		0.46			

[a] Square-root transformed data were tested for differences among families by random effect model analysis of variance. Heritabilities ($h^2 \pm$ SE) were estimated by full-sib correlation analysis.
[b] $^{**}P < 0.01$; $^{*}P < 0.05$.

and RT31) were significant for one age group only (Tables 4 and 5). Although average heritabilities of the two age groups were almost identical, some estimates showed apparent differences that were not significant when tested using Fisher's z-transformation (Zar, 1984). Heritabilities for both age groups together were estimated by a two-way model designating family, age, and the interaction term as random effects. P values for among-family variance components are listed in Table 6, showing 13 significant among-family variance components of which nine were also significant for each age group separately (Tables 4 and 5). Heritability estimates were significant for seven secretion components (RT9, RT15, RT20, RT22, RT28, RT31, and RT34). However, only two estimates (RT9 and RT34) were also significant for each age group when considered separately. Average heritability was lower (0.31) than the estimates of each age group considered separately (0.44 and 0.46), because variance associated with age increased the total variance and because some genetic variance was accounted for by the interaction term.

TABLE 6. MEAN CONCENTRATIONS (±SE) OF 16 SECRETION COMPONENTS OF 2- AND 10-WEEK-OLD BEETLES[a]

Secretion component	Offspring 1 and 2 (age 2 and 10 weeks, $N = 221$) Amount ($\mu g/\mu l$)	h^2	ANOVA (effect of family) $df = 26$ Type III MS	F value	P value
RT3	58.1 ± 1.2	0.11 ± 0.11 ns	2.0056	1.409	0.2000
RT9	39.2 ± 1.2	0.57 ± 0.16**[b]	7.0131	5.411	0.0001
RT14	6.7 ± 0.3	0.20 ± 0.14 ns	0.8824	3.491	0.0014
RT15	4.2 ± 0.2	0.38 ± 0.16*	0.7023	5.477	0.0001
RT16	22.7 ± 1.2	0.19 ± 0.14 ns	3.8835	3.384	0.0018
RT19	3.6 ± 0.2	0.43 ± 0.21 ns	1.2864	2.827	0.0062
RT20	5.9 ± 0.4	0.44 ± 0.20*	1.7442	4.142	0.0004
RT22	7.4 ± 0.2	0.36 ± 0.13*	0.9279	3.937	0.0006
RT23	5.6 ± 0.2	0.05 ± 0.16 ns	0.6769	1.142	0.3733
RT25	11.4 ± 0.4	0.30 ± 0.16 ns	1.2827	2.140	0.0321
RT27	5.5 ± 0.2	0.32 ± 0.18 ns	0.9163	2.343	0.0195
RT28	21.2 ± 0.7	0.39 ± 0.14*	2.4607	3.360	0.0018
RT30	2.6 ± 0.1	0.13 ± 0.18 ns	0.4507	1.355	0.2281
RT31	6.0 ± 0.3	0.38 ± 0.15*	1.9829	2.971	0.0044
RT32	25.0 ± 0.8	0.20 ± 0.11 ns	2.2194	2.975	0.0043
RT34	5.2 ± 0.2	0.53 ± 0.15**	1.6891	3.692	0.0009
Average		0.31			

[a] Square-root transformed data were tested for differences among families by random effect two-way model analysis of variance. Heritabilities ($h^2 \pm$ SE) were estimated by full-sib correlation analysis using a random effect two-way model.
[b] $^{**}P < 0.01$; $^{*}P < 0.05$.

DISCUSSION

Heritable variation is required for evolutionary adaptation. Chemical defense is thought to be the result of evolutionary adaptation. In this study we found significant heritabilities for quantitative traits of chemical defense as estimated by mother–offspring regression and full-sib correlation analysis. Hence, predators may be directing the evolution of the chemical defense of *O. gloriosa*.

Since heritability estimates based on full-sib correlation are likely to be inflated by variance due to common environment (Falconer, 1989), we tried to minimize the environmental variance component by experimental design: all adult beetles were kept randomly distributed under the same controlled environmental conditions. Full-sib estimates are further biased by dominance variance (Falconer, 1989), a part of nonadditive genetic variance due to dominance (heterozygote not intermediate between homozygotes) or overdominance (hetero-

zygote more extreme than either homozygote). Mother–offspring regression estimates are known to be less biased and therefore more reliable than full-sib estimates (Falconer, 1989). Because data on secretion concentrations in the parental generation were not available, mother–offspring estimates could be calculated only for proportions. However, full-sib estimates were not significantly different from mother–offspring estimates, providing a validation of the less reliable full-sib estimates.

Standard errors of heritability estimates based on full-sib correlation are rather large, due to the relatively low power of maximum likelihood estimates (Scheiner and Lyman, 1989). This is obvious, since many heritability estimates are not significantly different from zero, even though the ANOVAs show significant among-family variance components.

We have suggested previously that quantitative variation in chemical defense between populations of *Oreina gloriosa* is partly genetically determined (Eggenberger and Rowell-Rahier, 1991). The present study shows the average heritability of proportions to be 54% of the intrapopulational variation, confirming this hypothesis.

There were no significant differences between the full-sib estimates of 2-week-old and 10-week-old offspring, not even for RT16, which shows significantly different mother–offspring regression estimates (Figure 2). Since heritability is the ratio of additive genetic and phenotypic variance, heritability change during development is a consequence of drift of this ratio, which can be due to change in both genetic and environmental variance. Change in genetic variance is thought to be the result of different gene expression. Genetic control of physiological response is reviewed by Humphreys (1991). Change in phenotypic variance may be caused by environmental or interaction effects. The pattern of change in the relative magnitude of phenotypic and additive genetic variance during growth can be rather complex (e.g., Henrich, 1989).

We also present heritabilities of concentrations based on a two-way model including data from both age groups. These estimates are thought to correspond more closely to estimates of populations with natural age structure than do estimates based on data from each age group separately. However, it should be emphasized that heritabilities depend on the magnitude of all variance components and therefore are valid only for the population for which they are estimated.

The biological significance of the complexity of exocrine secretion mixtures is obscure. It is thought that different components of the secretion may exert different effects or that the proportions of different components may confer specificity (Chapman, 1982). In a three-component allomone system investigated by Dettner and Grümmer (1986), the ratio of constituents was shown to correlate with the maximal penetration rate through the integument of a predatory arthropod. The secretion of *O. gloriosa* may be even more complex than pre-

sented here, since only major components detectable at UV 220 nm were quantified. All constituents are highly polar and, with the exception of RT3, show UV spectra typical of cardenolides. Because of the similarity of the constituents, the complexity of the mixture may be the inevitable consequence of its biosynthesis rather than of significance for chemical defense (or both). The exocrine secretion of *O. gloriosa* may also act as a pheromone (e.g., sex pheromone) and the mixture of constituents may therefore be a component of reproductive fitness. However, to our knowledge, there is no evidence supporting pheromonal function of the secretion.

In summary, heritability estimates show a considerable part of the variation in chemical defense of *Oreina gloriosa* to be genetically determined. Physiological and environmental sources of this variation will be discussed in following papers.

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