Published in Journal of Chemical Ecology 19, issue 3, 395-410, 1993 1
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PHYSIOLOGICAL SOURCES OF VARIATION IN CHEMICAL DEFENSE OF *Oreina gloriosa* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract—The defensive secretion of the alpine chrysomelid *Oreina gloriosa* is a complex mixture of mainly cardenolides and tyrosine betaine. Individually sampled secretions of adult laboratory-reared and field-collected beetles were analyzed by reverse-phase HPLC; 16 secretion components were quantified. Quantities and concentrations of different components were significantly affected by the age, sex, and reproductive status of individual beetles. Aging was correlated with marked increases (up to 4.4-fold) and decreases (up to 2.7-fold) of quantities and concentrations of several components. Differences between the sexes were smaller, but quantities of all components and concentrations of several components were larger in laboratory-reared females than in males. There was less of one component of the secretion in mated than unmated females, but the concentrations of four secretion components were higher (up to 1.6-fold) in mated females.

Key Words—*Oreina gloriosa*, Coleoptera, Chrysomelidae, chemical defense, cardenolides, quantitative variation, aging, HPLC.

INTRODUCTION

Chemical defense of adult *Oreina gloriosa* is based mainly on the exocrine secretion of a variety of de novo synthesized cardenolides, tyrosine betaine, and ethanolamine at the surface of pronotum and elytra (Van Oycke et al., 1988; Eggenberger et al., 1992). As is typical of arthropod defensive secretions (Blum, 1981), the constituents of the secretion of *O. gloriosa* display quantitative variation among populations and, to a smaller extent, within populations (Eggenberger and Rowell-Rahier, 1991).

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Quantitative variation in the composition of exocrine secretions is probably based on variation in the rate of enzymatic reactions involved in biosynthesis and degradation and may be affected by genetic, physiological, and environmental factors.

Genetic sources of variation were found in the pheromonal secretion of the butterfly *Colias eurytheme* (Sappington and Taylor, 1990). In the defensive secretion of the leaf beetle *O. gloriosa*, genetic differences explained a considerable part of the total variation (Eggenberger and Rowell-Rahier, 1992).

Physiological sources of variation in secretion composition have been frequently described (refs. in Blum, 1981). Jackson and Bartelt (1986) found a dramatic change in quantity and composition of cuticular hydrocarbons of adult *Drosophila virilis* between eclosion and day 8. Quantitative changes in hydrocarbons with aging were also observed in the milkweed bug *Oncopeltus fasciatus* (Jackson, 1983) and in the spruce sawfly *Pikonema alaskensis* (Bartelt et al., 1984). Aphid sex pheromones were reported to increase in quantity until day 6 while changing in composition (Hardie et al., 1990). Kuwahara (1979) found an increasing ratio of neral to geraniol in the courtship pheromone of aging male *Pieris melete* (Lepidoptera).

Seasonal variation in the composition of the defensive secretion of *Oreina gloriosa* was reported by Eggenberger et al. (1992). Such variation could reflect seasonal variation in environmental factors (e.g., predation pressure or nutritional quality) or could be due to a seasonally changing age structure of the population, provided that the composition of the secretion is affected by the age of the individual beetle.

We recently reported on the genetic sources of variation in the concentration of 16 secretion components of *O. gloriosa* (Eggenberger and Rowell-Rahier, 1992). The average genetic variance component proved to account for 31% of total variation, leaving 69% of unexplained variation that could potentially be accounted for by physiological factors. In this paper we examine the effects of age, sex, and mating on the defensive secretion of *O. gloriosa* using both field-collected and laboratory-reared specimens.

METHODS AND MATERIALS

Field-collected beetles were all derived from the same locality in Saas Grund (Wallis, Swiss Alps) at 1860 m above sea level. To prevent premature secretion release, beetles were transported from the site of capture in cooled containers. In the laboratory, the beetles were maintained individually in separate plastic containers randomly distributed in cooled incubators at constant temperature (17°C) and a variable photoperiod matching seasonal change under natural conditions. The beetles were regularly provided with fresh leaves of their

food plant *Peucedanum ostruthium* (Apiaceae), which were shipped weekly from the original locality. Release of the defensive secretion was induced by mechanical irritation of each beetle held with forceps under the binocular microscope. Pronotal secretions were taken up individually in capillary tubes and quantified. The collected secretions were each dissolved in 50 μl acetonitrile–water 1 : 10, plus 2 μg ouabain (Merck) as internal standard, filtered (0.2 μm pore size) and stored individually in microtubes (Treff) at $-70°C$.

The effects of age and sex on secretion composition were studied in both field-collected and laboratory-reared beetles. Rearing of beetles was started in July 1989 with the offspring of 48 field-collected females, which had already mated in the field with unknown males. The temperature of incubators was 17°C in summer and 2°C in winter. The development of 553 adult offspring was completed in June 1990. Details of the rearing methods may be found in Eggenberger and Rowell-Rahier (1992). The secretions of a total of 236 offspring belonging to 27 families were sampled either two weeks after emergence or 10 weeks after emergence. Adult offspring were sexed on the basis of body size (female *O. gloriosa* are larger than the males). They were maintained individually, except for 24 females, which, for the purpose of investigating the effects of mating, were maintained in pairs with males collected in the field. We used males that had overwintered in the field as adults, since males do not mate in their first summer (personal observation). Pairing was started in August, six weeks after emergence of laboratory-reared beetles. Females are referred to as ''mated'' when the male was seen in mating position on top of the female. In order to investigate the effect of mating on secretion regeneration, the pronotal glands of 18 females were depleted before pairing.

Definitive sex determination of reared beetles was accomplished by dissection in winter 1990–1991. The second experiment to investigate the effects of age and sex on secretion composition was carried out with beetles collected in August 1991 in the field. They were aged approximately on the basis of cuticle hardness the day after capture. Individual secretions were collected from 20 newly emerged beetles, which are characterized by a soft cuticle until about one week after eclosion. Another 40 newly emerged beetles, which had not been provoked to release secretion during age classification, were maintained separately in incubators at 17°C. The secretions of 20 of them were collected individually after one week and of the remainder after two weeks. After removal of the secretion, the beetles were killed by freezing and sexed by dissection.

Separation and quantification of the constituents of the secretion were performed by reverse-phase HPLC. A 20-μl sample of each secretion was manually injected and analyzed using a Waters HPLC system (Waters 510 pumps; Waters 994 photodiode array detector; Maxima 820 data analysis system). Solvents were acetonitrile (Baker) and water (Merck). Separation was performed using a

Macherey-Nagel cartridge system (C-18; 3 μm particle size; 4 × 130 mm; 0.45 ml/min; 15–42% acetonitrile in 36 min). The separated components were quantified by measuring UV absorbance at 220 nm. The quantity of the components (microgram ouabain equivalents) was twice the respective peak area divided by the area of the internal standard peak (ouabain). Concentration (microgram oua-

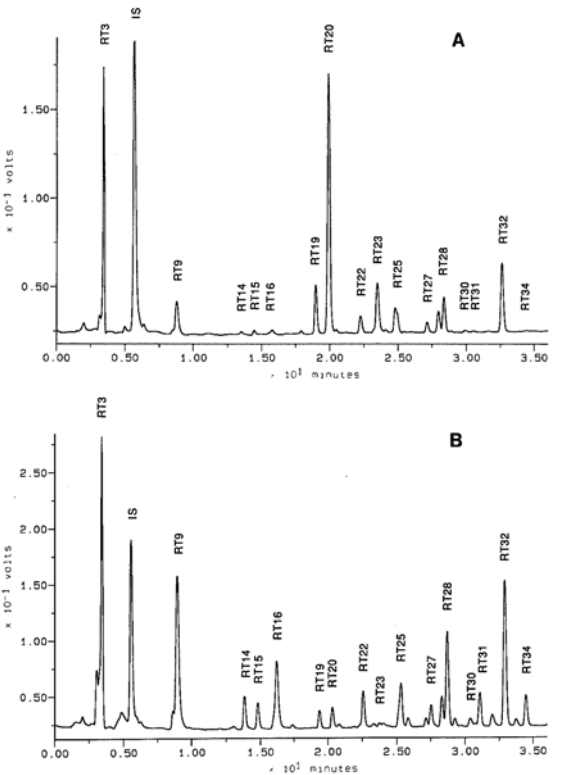

FIG. 1. HPLC trace of the defensive secretion emitted by an individual beetle at age 1 week (A) and 3 weeks (B).

TABLE 1. CHEMICAL STRUCTURE OF COMPOUNDS IN DEFENSIVE SECRETION OF *Oreina gloriosa*

RT3	*N*,*N*,*N*-trimethyltyrosine (tyrosine betaine)
RT9	sarmentogenin-3-*O*-β-D-allopyranoside
RT14	monoacetyl derivative of RT9
RT15	cardenolide
RT16	sarmentogenin-3-*O*-6′-*O*-acetyl-β-D-allopyranoside
RT19	periplogenin-3-*O*-[β-D-xylopyranosyl-(1 → 4)-β-D-allopyranoside]
RT20	periplogenin-3-*O*-β-D-allopyranoside
RT22	digitoxigenin-3-*O*-[β-D-xylopyranosyl-(1 → 4)-β-D-allopyranoside]
RT25	mono- or diacetyl derivative of RT22
RT28	mono- or diacetyl derivatives of RT22 and RT20
RT23	digitoxigenin-3-*O*-β-D-allopyranoside
RT27	mono- or diacetyl derivative of RT23
RT30	cardenolide
RT32	digitoxigenin-3-*O*-[β-D-xylopyranosyl (1 → 4)-2′, 3′-di-*O*-acetyl-β-D-allopyranoside]
RT31	cardenolide
RT34	cardenolide

bain equivalents per microliter secretion) of secretion components was calculated by dividing the respective quantity by the secretion volume. Quantified components are named RT3 to RT34 according to their retention time (Figure 1). The chemical structure of the compounds identified to date are given in Table 1 (see Eggenberger et al., 1992, for identification).

Data analysis was performed using SAS (SAS Institute Inc., 1990) on a VAX 8840. Multivariate (MANOVA) and univariate (ANOVA) two-way factorial analyses of variance were carried out with PROC GLM using type III sums of squares. Multiple comparisons of means were performed by Tukey's range tests. Data were square-root transformed prior to analyses of variance for the reasons detailed in Eggenberger and Rowell-Rahier (1992).

RESULTS

In the experiments on laboratory-reared beetles data were obtained from 138 2-week-old and 92 10-week-old offspring of 27 female *O. gloriosa*. Quantities and concentrations of 16 secretion components were tested for differences between age groups and sexes by two-way analyses of variance (Tables 2 and 3). Total quantity was significantly larger in females than in males and significantly smaller in 2-week-old beetles than in 10-week-old ones. The effect of the interaction between age and sex on total quantity was significant since the increase of total quantity with age was more distinct in females than in males.

TABLE 2. EFFECTS OF AGE AND SEX ON COMPOSITION OF SECRETION OF LABORATORY-REARED BEETLES[a]

	μg per female		μg per male		Effect[b]		
	Age 2 (N = 78)	Age 10 (N = 38)	Age 2 (N = 51)	Age 10 (N = 45)	Age	Sex	Age * Sex
RT3	3.39 ± 0.16	5.74 ± 0.28	2.75 ± 0.14	3.55 ± 0.24	***	***	**
RT9	2.79 ± 0.16	3.52 ± 0.39	1.92 ± 0.13	1.97 ± 0.17	NS	***	NS
RT14	0.26 ± 0.02	0.94 ± 0.06	0.19 ± 0.01	0.54 ± 0.03	***	***	***
RT15	0.20 ± 0.01	0.48 ± 0.03	0.16 ± 0.01	0.28 ± 0.02	***	***	**
RT16	0.72 ± 0.05	3.57 ± 0.26	0.51 ± 0.04	1.96 ± 0.13	***	***	***
RT19	0.28 ± 0.02	0.19 ± 0.02	0.21 ± 0.02	0.08 ± 0.01	***	***	*
RT20	0.53 ± 0.05	0.28 ± 0.04	0.38 ± 0.03	0.16 ± 0.02	***	**	NS
RT22	0.46 ± 0.02	0.63 ± 0.05	0.37 ± 0.03	0.41 ± 0.03	**	***	NS
RT23	0.39 ± 0.03	0.43 ± 0.04	0.31 ± 0.02	0.27 ± 0.02	NS	***	NS
RT25	0.78 ± 0.04	0.87 ± 0.07	0.62 ± 0.03	0.50 ± 0.03	NS	***	*
RT27	0.37 ± 0.03	0.43 ± 0.04	0.32 ± 0.03	0.28 ± 0.02	NS	**	NS
RT28	1.26 ± 0.07	2.02 ± 0.14	0.94 ± 0.05	1.22 ± 0.08	***	***	*
RT30	0.15 ± 0.01	0.27 ± 0.03	0.09 ± 0.01	0.18 ± 0.02	***	***	NS
RT31	0.28 ± 0.02	0.70 ± 0.07	0.18 ± 0.02	0.48 ± 0.04	***	***	NS
RT32	1.38 ± 0.07	2.45 ± 0.15	0.88 ± 0.07	1.89 ± 0.11	***	***	NS
RT34	0.31 ± 0.02	0.46 ± 0.04	0.22 ± 0.02	0.32 ± 0.03	***	***	NS
Sum	13.55 ± 0.55	22.96 ± 1.14	10.07 ± 0.46	14.09 ± 0.67	***	***	**
MANOVA					***	***	***

[a]Mean quantity (±SE) of secretion components of 2-week-old (age 2) and 10-week-old (age 10) beetles.
[b]***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

TABLE 3. EFFECTS OF AGE AND SEX ON COMPOSITION OF SECRETION OF LABORATORY-REARED BEETLES[a]

	μg/μl per female		μg/μl per male		Effect[b]		
	Age 2 (N = 72)	Age 10 (N = 37)	Age 2 (N = 50)	Age 10 (N = 44)	Age	Sex	Age * sex
RT3	53.91 ± 1.64	70.81 ± 3.40	50.18 ± 1.87	61.53 ± 3.07	***	**	NS
RT9	43.54 ± 2.03	40.61 ± 3.41	33.75 ± 1.73	34.74 ± 2.66	NS	**	NS
RT14	4.23 ± 0.28	11.43 ± 0.71	3.54 ± 0.22	9.66 ± 0.50	***	**	NS
RT15	3.30 ± 0.25	5.83 ± 0.43	3.08 ± 0.24	5.08 ± 0.30	***	NS	NS
RT16	12.13 ± 0.88	43.29 ± 2.91	9.66 ± 0.69	34.69 ± 2.09	***	**	NS
RT19	4.91 ± 0.54	2.24 ± 0.23	4.15 ± 0.44	1.37 ± 0.20	***	*	NS
RT20	8.43 ± 0.81	3.08 ± 0.33	6.90 ± 0.55	2.77 ± 0.32	***	NS	NS
RT22	7.46 ± 0.39	7.60 ± 0.53	6.88 ± 0.44	7.19 ± 0.64	NS	NS	NS
RT23	5.96 ± 0.36	5.14 ± 0.43	5.71 ± 0.43	4.93 ± 0.50	*	NS	NS
RT25	12.86 ± 0.70	10.46 ± 0.74	11.68 ± 0.70	8.72 ± 0.55	***	*	NS
RT27	5.72 ± 0.33	5.16 ± 0.43	5.77 ± 0.49	5.16 ± 0.49	NS	NS	NS
RT28	21.02 ± 1.20	24.51 ± 1.82	18.06 ± 1.11	21.29 ± 1.38	*	*	NS
RT30	2.40 ± 0.15	3.22 ± 0.31	1.69 ± 0.13	3.36 ± 0.32	***	NS	*
RT31	4.48 ± 0.37	8.74 ± 0.86	3.42 ± 0.33	8.52 ± 0.79	***	NS	NS
RT32	23.05 ± 1.20	29.90 ± 1.79	15.86 ± 1.03	33.69 ± 1.87	***	NS	***
RT34	5.11 ± 0.40	5.71 ± 0.56	3.98 ± 0.36	5.82 ± 0.54	*	NS	NS
Sum	218.5 ± 6.7	277.7 ± 10.4	184.3 ± 6.3	248.5 ± 9.6	***	***	NS
MANOVA					***	***	***

[a]Mean concentration (±SE) of secretion components of 2-week-old (age 2) and 10-week-old (age 10) beetles.
[b]***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

The overall effects of age, sex, and of the interaction between age and sex on the quantities of 16 secretion components were significant using MANOVA. Quantities of all components of the secretion were significantly larger in females than in males. There was significantly less of 10 components (RT3, RT14, RT15, RT16, RT22, RT28, RT30, RT31, RT32, and RT34) and significantly more of two others (RT19 and RT20) in 2-week-old beetles than in 10-week-old ones. The change in mean quantity with age was most pronounced in components RT16 (4.4-fold increase), RT14 (3.3-fold increase), RT31 (2.5-fold increase), RT20 (2.1-fold decrease), and RT19 (1.9-fold decrease). The effect of the interaction between age and sex was significant for seven components (RT3, RT14, RT15, RT16, RT19, RT25, and RT28), attributable to a different change in quantity with age in female and male *O. gloriosa*. Total concentration was significantly higher in females than in males and significantly lower in 2-week-old beetles than in 10-week-old ones. The overall effects of age, sex, and of the interaction between age and sex on the concentrations of 16 components of the secretion were significant. Concentrations of seven components (RT3, RT9, RT14, RT16, RT19, RT25, and RT28) were significantly higher in females than in males. Concentrations of nine components (RT3, RT14, RT15, RT16, RT28, RT30, RT31, RT32, and RT34) were significantly lower in 2-week-old beetles than in 10-week-old ones, whereas the concentrations of four other components (RT19, RT20, RT23, and RT25) were significantly higher in 2-week-old beetles. The change in mean concentration with age (Figure 2)

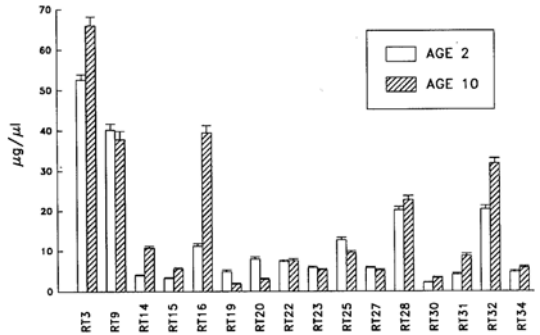

FIG. 2. Effects of age on the composition of the secretion of laboratory-reared beetles. Mean concentration (±SE) of secretion components of 2-week-old (age 2) and 10-week-old (age 10) beetles (see Table 3 for statistics).

was most pronounced for RT16 (3.5-fold increase), RT14 (2.7-fold increase), RT31 (2.1-fold increase), RT20 (2.7-fold increase), and RT19 (2.7-fold decrease). The effect of the interaction between age and sex was significant for two components (RT30 and RT32), where the increase of concentration with age was more distinct in female *O. gloriosa* than in males.

The effects of age and sex on quantity and concentration of 16 components in the secretion of field-collected beetles were investigated using 19 approximately 1-week-old individuals, 18 approximately 2-week-old beetles, and 18 approximately 3-week-old beetles. Differences between age groups and sexes were tested by two-way analyses of variance followed by multiple comparisons of age-group means (Table 4). The total quantity of secretion was significantly larger in females (7.40 ± 0.78 μg) than in males (5.96 ± 0.58 μg) and significantly different among age groups. The overall effect of age on quantity of 16 secretion components was significant, whereas overall effects of sex and of the interaction between age and sex were not significant. The quantities of 13 components (RT3, RT9, RT14, RT15, RT16, RT22, RT25, RT27, RT28, RT30, RT31, RT32, and RT34) increased significantly with age, whereas the quantity of one component (RT20) was significantly larger in 1-week-old beetles than in either 2- or 3-week-old beetles. Total concentration was significantly different among age groups, but not significantly different between the sexes. The overall effect of age on the concentrations of 16 components of the secretion was significant, but the overall effects of sex and of the interaction between age and sex were not significant. Concentrations of 12 components (RT3, RT9, RT14, RT15, RT16, RT22, RT27, RT28, RT30, RT31, RT32, and RT34) increased significantly with age, whereas the concentrations of two components (RT19 and RT20) were significantly higher in 1-week-old beetles than in 3-week-old ones (Figure 3).

The effects of mating and of the interaction between mating and secretion regeneration on quantity and concentration of 16 secretion components were tested by two-way analyses of variance on data of mated and unmated laboratory-reared 10-week-old females (Table 5). There was no overall significant effect of the interaction between mating and secretion regeneration on either quantity or concentration, showing that regeneration of secretion does not affect mating-related changes in composition of the secretion. The overall effect of mating on quantity was significant since the quantity of one component (RT9) was significantly smaller in mated than in unmated females. Total concentration was significantly higher in mated female *O. gloriosa* than in unmated ones. The overall effect of mating on concentration was significant. Concentrations of four components (RT3, RT22, RT25, and RT28) were significantly higher in mated females than in unmated ones. The mating-related change of mean concentration (Figure 4) was most pronounced in RT28 (1.6-fold).

TABLE 4. EFFECTS OF AGE ON COMPOSITION OF SECRETION OF FIELD-COLLECTED BEETLES[a]

	Quantity (μg)			Concentration (μg/μl)		
	Age 1 (N = 19)	Age 2 (N = 18)	Age 3 (N = 18)	Age 1 (N = 19)	Age 2 (N = 18)	Age 3 (N = 18)
RT3	1.17 ± 0.10a	1.77 ± 0.19b	3.03 ± 0.31c	30.3 ± 1.7a	43.7 ± 3.8b	50.8 ± 2.5b
RT9	0.46 ± 0.06a	1.12 ± 0.12b	1.68 ± 0.15c	11.3 ± 1.3a	27.9 ± 2.7b	29.5 ± 2.4b
RT14	0.03 ± 0.01a	0.11 ± 0.02b	0.29 ± 0.04c	0.7 ± 0.1a	2.5 ± 0.4b	5.1 ± 0.5c
RT15	0.04 ± 0.01a	0.13 ± 0.03b	0.25 ± 0.02c	0.9 ± 0.2a	3.0 ± 0.6b	4.6 ± 0.6c
RT16	0.06 ± 0.02a	0.25 ± 0.05b	0.83 ± 0.13c	1.5 ± 0.3a	5.6 ± 1.1b	14.1 ± 1.5c
RT19	0.10 ± 0.02a	0.07 ± 0.01a	0.06 ± 0.01a	2.4 ± 0.4a	2.2 ± 0.5ab	1.1 ± 0.2b
RT20	0.45 ± 0.09a	0.17 ± 0.03b	0.16 ± 0.02b	11.1 ± 2.3a	4.4 ± 0.7b	3.1 ± 0.5b
RT22	0.13 ± 0.02a	0.18 ± 0.02a	0.29 ± 0.03b	3.1 ± 0.4a	4.4 ± 0.5ab	5.0 ± 0.4b
RT23	0.23 ± 0.03a	0.20 ± 0.03a	0.20 ± 0.02a	5.7 ± 0.7a	4.7 ± 0.9a	3.9 ± 0.5a
RT25	0.27 ± 0.03a	0.33 ± 0.04a	0.46 ± 0.03b	6.7 ± 0.7a	8.6 ± 1.2a	8.6 ± 0.8a
RT27	0.12 ± 0.02a	0.21 ± 0.03b	0.33 ± 0.04c	3.0 ± 0.4a	5.1 ± 0.6b	5.9 ± 0.7b
RT28	0.35 ± 0.06a	0.43 ± 0.05a	0.86 + 0.08b	8.5 ± 1.2a	10.9 ± 1.5a	15.4 ± 1.3b
RT30	0.02 ± 0.01a	0.06 ± 0.01b	0.14 ± 0.02c	0.6 ± 0.1a	1.4 ± 0.2b	2.5 ± 0.3c
RT31	0.02 ± 0.01a	0.09 ± 0.02b	0.21 ± 0.03c	0.6 ± 0.2a	2.0 ± 0.3b	3.5 ± 0.4c
RT32	0.43 ± 0.08a	0.52 ± 0.06a	1.17 ± 0.10 b	10.2 ± 1.5a	13.6 ± 1.8a	20.7 ± 2.3b
RT34	0.04 ± 0.01a	0.11 ± 0.02b	0.23 ± 0.03c	0.9 ± 0.2a	2.7 ± 0.5b	3.9 ± 0.5b
Sum	3.92 ± 0.42a	5.72 ± 0.55b	10.19 ± 0.64c	97.5 ± 6.8a	142.7 ± 12.5b	177.6 ± 8.5c
		P = 0.0001 (MANOVA)			P = 0.0001 (MANOVA)	

[a]Means that do not share a letter in common are significantly ($P < 0.05$) different.

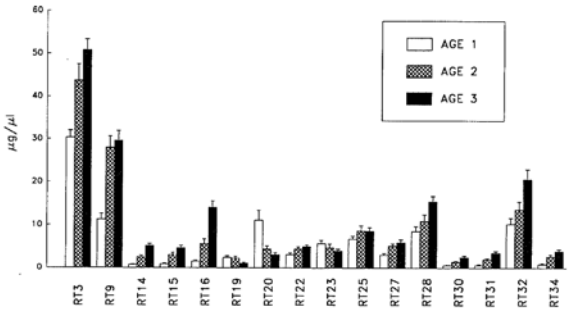

FIG. 3. Effects of age on the composition of the secretion of field-collected beetles. Mean concentration (±SE) of secretion components of 1-week-old (age 1), 2-week-old (age 2), and 3-week-old (age 3) beetles (see Table 4 for statistics).

DISCUSSION

The biological significance of the complexity of most defensive secretions is still unclear. Pasteels et al. (1983) suggested that the complexity of defensive blends may reduce counteradaptation by predators, analogous to the situation in plants, or could be due to the presence of precursors of the active compounds in the secretion, suggesting the composition of secretion to be the inevitable consequence of biosynthesis rather than of significance for chemical defense. Synergism between different components of defensive secretions has been demonstrated by Dettner and Grümmer (1986), who showed the ratio of components to correlate with penetration rate through the integument of predatory arthropods. In pheromones, the composition of secretory mixtures has been proven to be adaptive (refs. in Baker, 1989). Pheromonal activity of secretory compounds consequently may be an additional source of the complexity of defensive secretion blends. The use of defensive secretions as pheromones has been suggested to be highly adaptive, because no additional metabolic pathway would be required for pheromone biosynthesis and receptor proteins would be already present in the form of enzymes of the biosynthetic pathway (Blum, 1981). The pheromonal function of defensive compounds stored in glands has been suggested to be based on the slow leakage of gland contents, assuming defensive glands are not hermetically sealed (Attygalle et al., 1991).

In an attempt to clarify the biological significance of the secretory mixture

TABLE 5. EFFECTS OF MATING ON COMPOSITION OF SECRETION OF LABORATORY-REARED FEMALES[a]

	Quantity (μg)			Concentration (μg/μl)		
	Mated (N = 24)	Unmated (N = 94)	Effect[b] of mating	Mated (N = 23)	Unmated (N = 93)	Effect[b] of mating
RT3	5.31 ± 0.56	5.90 ± 0.19	NS	90.66 ± 7.58	73.42 ± 1.96	*
RT9	1.82 ± 0.25	2.86 ± 0.22	*	30.57 ± 4.27	33.20 ± 2.02	NS
RT14	0.63 ± 0.06	0.76 ± 0.04	NS	10.80 ± 1.06	9.56 ± 0.44	NS
RT15	0.33 ± 0.04	0.40 ± 0.02	NS	5.77 ± 0.70	5.07 ± 0.27	NS
RT16	2.28 ± 0.24	2.72 ± 0.15	NS	39.19 ± 4.16	34.14 ± 1.80	NS
RT19	0.12 ± 0.04	0.12 ± 0.01	NS	1.83 ± 0.51	1.51 ± 0.18	NS
RT20	0.13 ± 0.03	0.20 ± 0.02	NS	2.08 ± 0.39	2.28 ± 0.22	NS
RT22	0.40 ± 0.06	0.46 ± 0.03	NS	6.90 ± 0.96	5.74 ± 0.35	*
RT23	0.29 ± 0.03	0.38 ± 0.02	NS	4.87 ± 0.66	4.69 ± 0.27	NS
RT25	0.56 ± 0.08	0.64 ± 0.04	NS	10.01 ± 1.30	7.79 ± 0.50	*
RT27	0.36 ± 0.03	0.44 ± 0.03	NS	6.30 ± 0.55	5.37 ± 0.28	NS
RT28	1.60 ± 0.18	1.43 ± 0.09	NS	28.23 ± 3.03	17.59 ± 1.14	***
RT30	0.20 ± 0.02	0.24 ± 0.02	NS	3.39 ± 0.33	2.94 ± 0.18	NS
RT31	0.35 ± 0.06	0.45 ± 0.04	NS	6.46 ± 1.31	5.67 ± 0.49	NS
RT32	1.63 ± 0.14	1.99 ± 0.09	NS	28.72 ± 2.39	24.95 ± 1.13	NS
RT34	0.25 ± 0.05	0.31 ± 0.03	NS	4.65 ± 0.98	3.93 ± 0.33	NS
Sum	16.25 ± 1.25	19.29 ± 0.67	NS	280.4 ± 17.9	237.8 ± 6.6	**
	P = 0.0104 (MANOVA)			P = 0.0003 (MANOVA)		

[a]Mean quantity (±SE) and mean concentration (±SE) of secretion components.
[b]***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

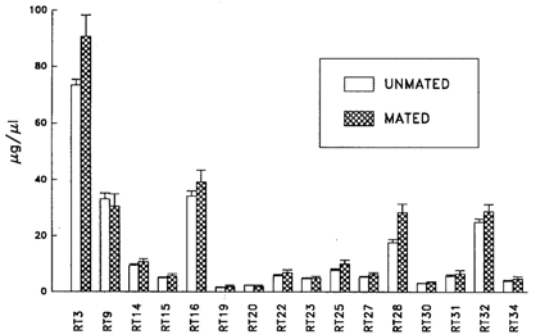

FIG. 4. Effects of mating on the composition of the secretion of laboratory-reared beetles. Mean concentration (±SE) of secretion components of mated and unmated females (see Table 5 for statistics).

of *Oreina gloriosa*, we assessed the effects of age, sex, and mating on quantity and concentration of 16 secretion components. Seasonal effects on the same components were reported in Eggenberger et al. (1992). Comparing both studies, both the season of capture of beetles in the field and the age of laboratory-reared beetles mainly affect the same components of the secretion, producing the most pronounced changes in both quantity and concentration in the same five components (RT14, RT16, RT19, RT20, and RT31). Regarding season, however, the changes go in opposite directions in field-collected and laboratory-reared beetles. This may be explained by the fact that field-collected beetles were sampled in June, just after overwintering, and in August, at the end of the season, when the population consisted of both overwintered and newly emerged beetles, resulting in a distinctly higher mean age in June than in August. Laboratory-reared beetles, on the other hand, were 2 weeks old when secretions were sampled in July and 10 weeks old in September, showing increasing mean age over the course of the season. Seasonal differences of components of the secretion are therefore thought to be for the most part due to seasonal differences in the age structure of the population, rather than seasonal variation in environmental factors such as predation pressure or nutritional quality.

Newly emerged beetles have less and more dilute secretions and consequently are less well protected chemically against predators than older ones.

This appears not to be ecologically beneficial, since newly emerged beetles are already more vulnerable to predation than older ones by virtue of their softer cuticle. The increase of total quantity and total concentration in both sexes may therefore reflect the time necessary to synthesize defensive compounds from dietary input rather than having adaptive significance.

The quantities of all components of the secretion, as well as the concentrations of several of them, are larger in laboratory-reared females than in males. However, in the secretion of field-collected beetles, the differences between the sexes are not as distinct as in laboratory-reared beetles (Eggenberger et al., 1992). This inconsistency may reflect the fact that several factors that affect the defensive secretion are under the investigator's control in the laboratory, but not in the field. Sexual differences between field-collected beetles may consequently be blurred by interactions with such factors such as regeneration of the secretion (Eggenberger and Rowell-Rahier, in preparation), reproductive status, and age.

Considering the effects of age and sex and of the interaction between them on the constituents of the secretion (Figure 5), it seems conceivable that beetles of different sexes and ages are distinguishable on the basis of their secretion. The ecological implications that follow from this may prove interesting, since it would enable a beetle to get information on the sex and age of another beetle, provided that an adequate sensory apparatus exists. Considering the life history of *O. gloriosa*, this would be beneficial. Development from larva to adult takes

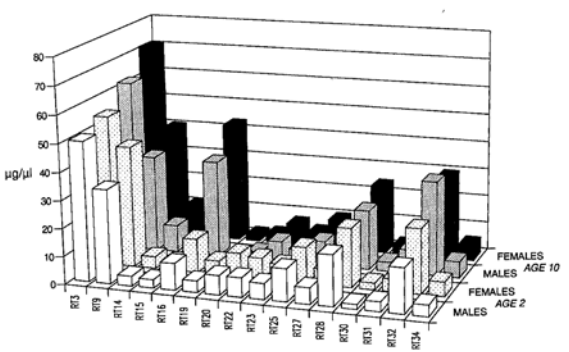

FIG. 5. Effects of age and sex on the composition of the secretion of laboratory-reared beetles. Mean concentration of secretion components of 2-week-old (age 2) and 10-week-old (age 10) beetles (see Table 3 for statistics).

about one year. Overwintered male and female beetles appear in June and decrease during August when the new generation appears, resulting in overlapping generations of overwintered and newly emerged beetles. Newly emerged females mate a few days after eclosion (Rowell-Rahier, personal observation), producing larvae after overwintering. Although adult *O. gloriosa* can live as long as three years, most beetles are likely to die in the second summer. Mating with newly emerged females would consequently be more advantageous than mating with overwintered females, suggesting that a mechanism by which male *O. gloriosa* can distinguish between newly emerged and overwintered females may exist.

With one exception only, the quantities of individual constituents were not affected by mating. On the other hand, total concentration as well as the concentrations of four components are higher in the secretions of mated females than in those of unmated ones. Although mated females may be heavier than unmated ones, the effects of mating are not thought to be based merely on the dependence of secretion concentration on body weight (Eggenberger et al., 1992), since secretion quantity, which also is dependent on body weight, should then also be larger in mated than in unmated females. This is not the case. In view of the higher concentration of the components, mated females seem to be better protected against predators than unmated ones. This would be advantageous. However, it is not clear whether the concentration of the components is more important than the quantity for chemical defense. Regeneration of the secretion following gland depletion does not affect mating-related changes in secretion composition. This specificity of secretion composition in relation to the reproductive status of the individual beetle may again indicate a potential role of secretion components in intraspecific communication.

In summary, the age of the individual beetle is shown to be an important factor affecting the composition of the secretion, and it may also account for most of the seasonal differences observed. Although the quantitative differences in the components of the secretion of males and females as well as mated and unmated females seem to be rather small, a pheromonal function of the secretion cannot be excluded.

Acknowledgments—We thank H.F Rowell, B. Hägele, and an anonymous reviewer for constructive comments on the manuscript; J.M. Pasteels for helpful advice; J. Bonato-Schmidli for help with the beetles; H. and G. Bumann for kindly shipping plants to Basel; Ch. and W. Kempel for help with PC equipment; and the Swiss National Science Foundation for financial support (grant 31-26263.89).

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