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MATE CHOICE AND TOXICITY IN TWO SPECIES OF LEAF BEETLES WITH DIFFERENT TYPES OF CHEMICAL DEFENSE

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Abstract—Evidence for the use of defensive compounds for sexual purposes is scarce, even though sexual selection might have some importance for the evolution of defensive traits. This study investigates the effect of defense-related traits and body size on mating success in two sister species of leaf beetle differing in their type of chemical defense. *Oreina gloriosa* produces autogenous cardenolides, whereas *O. cacaliae* sequesters pyrrolizidine alkaloids from its food plant. Larger *O. gloriosa* males with more toxin or higher toxin concentration had a mating advantage, likely due to direct or indirect female choice. In the laboratory, particular pairings recurred repeatedly in this species, indicating mate fidelity. *O. gloriosa* females were also subject to sexual selection, possibly by male choice, because larger females and those with higher toxin concentration mated more readily and more often. In *O. cacaliae*, in contrast, sexual selection for toxicity and body size was not detected, or at best was much weaker. Because toxicity is heritable in *O. gloriosa* but environment-dependent in *O. cacaliae*, individuals of the former species could be choosing well-defended partners with "good genes." Our study suggests that sexual selection may contribute to the maintenance of heritable defensive traits.

Key Words—Sexual selection, chemical defense, cardiac glycosides, pyrrolizidine alkaloids, sequestration vs. *de novo* synthesis, assortative mating, *Oreina*, Chrysomelidae.

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INTRODUCTION

A wide variety of insect species exploit chemical defenses to protect themselves against natural enemies (Euw et al., 1967; Brower, 1984; Bowers, 1992). Defensive traits such as toxic compounds most likely evolve in response to predation pressure, and may be constrained by various physiological parameters. An additional mechanism selecting for toxicity may arise if individuals benefit from mating with a well-defended partner. Some authors have proposed that defensive secretions may have a direct pheromonal effect, provided that glands are not sealed and volatile compounds can diffuse (Attygalle et al., 1991; Eggenberger and Rowell-Rahier, 1993a). In other species, defensive compounds have been shown to be precursors of sexual pheromones (Trigo and Brown, 1990; Dussourd et al., 1991; Amano et al., 1999). Toxicity may be a reliable trait for assessing overall mate quality, as it is costly (Zahavi, 1975; Pasteels et al., 1990; Bowers, 1992; Andersson and Iwasa, 1996). However, evidence for the use of defensive compounds for sexual purposes in insects in most cases concerns plant-derived compounds (Nishida and Fukami, 1990; Trigo and Brown, 1990; Dussourd et al., 1991; Amano et al., 1999). Sexual selection for defensive compounds may occur when individuals of the choosing sex benefit from this choice. This is the case with the moth *Utetheisa ornatrix* (Dussourd et al., 1991), where females choose males advertising a toxin-rich spermatophore, a nuptial gift that females use to defend their offspring. In turnip sawflies, more distasteful females (i.e., containing greater amounts of diterpenes) have greater mating success (Amano et al., 1999), which may indicate male choice for toxic females. Clearly, more evidence is needed to evaluate the role of sexual selection in the evolution of chemical defenses.

Two fundamentally different means of chemical defense occur within the alpine leaf beetle genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae). Some species, like *O. gloriosa*, defend themselves by synthesizing cardenolides (autogenous defense: Van Oycke et al., 1987; Eggenberger and Rowell-Rahier, 1993a,b). Other species, like *O. cacaliae*, are defended by pyrrolizidine alkaloids (PAs) acquired from their food-plant, which they store in defensive glands (toxin sequestration: Rowell-Rahier et al., 1991; Pasteels et al., 1992). *Oreina cacaliae* is, thus, dependent on its food-plant for defense against predators. In neither species are defensive compounds provided to offspring by their mother (Eggenberger and Rowell-Rahier, 1992; Dobler and Rowell-Rahier, 1994). However, in *O. gloriosa*, the concentration of 8 out of 16 cardenolides in the defensive secretion is heritable ($h^2 =$ ca.0.5, Eggenberger and Rowell-Rahier, 1992), and these components account for 31% of the total intrapopulation variation. Defensive capability is, therefore, at least partly genetically determined. Thus, in this species, there is the potential for selection for "good genes" to operate (Andersson, 1994), and toxicity may be the basis for mate choice if detectable by the partner. Indeed, an individual that chooses a well-defended partner would be favored by natural selection

because this enhances the survival of its offspring through higher resistance to predators. In contrast, this would be less likely in sequestering species such as *O. cacaliae*, where toxicity is primarily related to host plant content of PAs (Isman, 1977; Isman et al., 1977; Brower et al., 1984; Bowers, 1992). Toxicity will also be influenced by foraging or physiological conversion capacity, so may serve as an indicator of "good genes," but nothing is known about the heritability of these traits.

Here, we investigate sexual selection in these two leaf beetle species in the laboratory and the field, using modern standardized methods for quantifying sexual selection (Lande and Arnold, 1983; Arnold and Wade, 1984a,b; Brodie et al., 1995). In addition to the defensive traits of toxin volume and concentration (which are physiological traits rarely assessed in the sexual selection context; Kingsolver et al., 2001), body size was also analyzed because it has been shown to affect mate choice in many species (Andersson, 1994; Bonduriansky, 2001). Although supposedly rare and poorly documented (Andersson, 1994), male choice for females may occur when males are in some way limited in their number of matings, or if the quality of their mates strongly influences their fitness. This may be the case in toxic insects, so we specifically investigate this possibility. Lastly, we provide a general description of the mating behavior of both species.

MATERIALS AND METHODS

Collection of Beetles. Laboratory Experiment. This experiment was designed to record the mating pattern for each individual *O. gloriosa* and *O. cacaliae*. For both species, we collected 60 adults of each sex. *O. cacaliae* was sampled near La Fouly in the Val Ferret (Valais, Swiss Alps, 45.56 N, 7.05 E, alt. 1500 m) in early May 2000, when individuals emerge from the ground, dig out from the snow, and start feeding on *Petasites paradoxus* (Asteraceae). Individuals of this species were sexed using their sexually dimorphic tarsi (Lohse and Luche, 1994). *O. gloriosa* was sampled in Saas Grund (Valais, Swiss Alps, 46.08 N, 7.57 E, alt. 1800 m) at the beginning of June 2000, when they start feeding on *Peucedanum ostruthium* (Apiaceae). As sexual dimorphism of tarsi does not occur in this species, beetles were sexed using body weight. Previous studies have documented the weight distribution of both sexes, females being heavier than males (mean weight and standard deviation of females and males, respectively, 0.094 ± 0.013 g and 0.067 ± 0.006 g). We avoided using individuals weighing between 0.077 and 0.091 g. Beetles lighter than 0.077 g were taken as males, and those heavier than 0.091g as females. In another experiment in which sex could be checked because females larviposited, this method produced only a 2% error rate. To minimize disturbance during transportation, beetles along with leaves of their food-plant were placed in plaster-bottomed boxes with high humidity.

Field Sampling. An instant sampling was performed in order to document the mating pattern of both species in the field. Adult beetles were collected at the same sites and dates as for the laboratory experiment. Two categories of beetles were collected for each species: mating couples ($N = 31$ in *O. gloriosa*, $N = 25$ in *O. cacaliae*), and beetles that were not mating at the time (31 beetles of each sex in *O. gloriosa*, 25 in *O. cacaliae*). The sampling dates correspond to their peak mating time in the field. We collected beetles that were active (feeding and mating) on the leaves of their food plants, which should represent a random sample of the individuals in the population active at the time, as inspection on the ground under the food plants revealed the presence of few beetles and no mating. The sex ratio in the field is typically male-biased, with up to 84% of individuals being males (Kalberer et al., unpublished data). Each mating pair and single beetle was carefully placed into a separate vial to minimize disturbance while transporting them to the laboratory. All vials were plaster-bottomed to ensure high humidity, and provided with a fresh piece of the food-plant. Field-sampled beetles were milked for their defensive secretion within 24 hr after arrival in the laboratory (see below). All beetles were released at their respective field sites at the end of the experiment.

Experimental Procedure. The laboratory experiment lasted from 1 to 30 June, 2000, for *O. gloriosa*, and from 3 to 30 May, 2000, for *O. cacaliae*. These dates correspond to the mating period in the field, as indicated by field observations from the previous 4 years (Knoll, Kalberer and Nessi, unpublished data). For each species, we placed 30 males and 30 females in each of two 30- $\times$50-cm trays. This approximately reflects the natural density on plant patches in the field. Beetles were individually marked with correction fluid (Tipp-Ex) and bee labels on the elytrae. Defensive secretions and weights of all individuals were taken within 2 days at the end of the experiment. The holding trays had wet filter paper at the bottom to ensure humidity, and were placed in the laboratory at room temperature (range: 19–25°C), away from direct sunlight. Fresh food-plants were provided every day for food and shelter.

We recorded every mating by inspection every 4 hr (point sampling) over the entire period of the experiment. As preliminary observations indicated that matings last on average 6 hr in both species, we were confident that nearly all copulations were observed. After six nights of observation, we confirmed that nocturnal copulations are rare (only one mating was observed); hence, no survey was done between 11 p.m. and 6 a.m. Each mating pair was carefully removed from the tray and kept in a vial until the partners separated. Afterwards, beetles were released into the trays in order to keep insect density approximately constant in both trays. At the end of the experiment, each beetle that survived (62% of *O. gloriosa* and 48% of *O. cacaliae*) was weighed and milked for its defensive secretions as described below. As every copulation was recorded, chronological order of mating and the total number of copulations could be determined for all

individuals, allowing us to correlate these mating attributes to the measured traits. To estimate the duration of the different phases of mating, 12 mating pairs of *O. gloriosa* and 13 of *O. cacaliae* were observed every hour for the duration of the mating.

Measurement of Defensive Secretions. Defensive secretions were collected by holding the beetle under the microscope and gently tapping its pronotum and elytrae with fine forceps until drops of secretion appeared from the gland openings. The stimulus was applied as long as defensive liquid could be emitted. Drops were collected with a calibrated glass capillary, and the volume of the secretion (as represented by the height of the liquid in the capillary tube) was measured with a graduated lens on the microscope. Each secretion was stored individually in 150 μl of methanol in the freezer. Beetles were weighed to the nearest 10^{-4}g.

Sample Preparation and Chromatographic Analysis of Cardenolides. A preliminary trial revealed that the spectrophotometric method described by Dobler and Rowell-Rahier (1994) is not sensitive enough to analyze individual secretions. Thus, the samples were prepared and the concentration of total cardenolides in the secretions of *O. gloriosa* determined using reverse-phase high pressure liquid chromatography (HPLC) as described by Eggenberger and Rowell-Rahier (1993b), using a Varian Star chromatography workstation system with automated injection. We used ouabain (Sigma) as an internal standard, and concentrations are expressed as μg equiv. ouabain/μl. The minimum detected value was 0.95 $\mu g/\mu$l of secretion, and the standard deviation equalled 0.7% of the mean.

Sample Preparation and Chromatographic Analysis of PAs. Samples were prepared, and the concentration of total PAs in the secretions of *O. cacaliae* determined, using capillary gas chromatography (GC) as described by Rowell-Rahier et al. (1991), using a HP1-MS 30-m $\times$ 0.025-mm $\times$ 0.25-μm column, and senecionin as external standard. Concentrations are expressed as μg equiv. senecionin/μl. The minimum detected value was 0.01 μg/μl, and the standard deviation equalled 6.4% of the mean. Gas chromatography coupled with mass spectrometry (GC/MS) was used to confirm that the peaks corresponded to PAs.

Statistical Analysis. Sexual selection was quantified by calculating uni- and multivariate linear and quadratic selection coefficients (or gradients) using regression, following Lande and Arnold (1983) and Arnold and Wade (1984a,b). For each sample, we produced standardized z-scores for all measured traits (body weight, toxin concentration, and secretion volume) by subtracting the sample mean from each value and dividing the difference by the standard deviation: $z_i = (x_i - \bar{x})/SD_x$. For the field data, we estimated the effect of all traits on pairing success (yes or no) and on assortative mating given pairing. Relative pairing success (i.e., relative fitness $w_i = W_i/\bar{w}$) of males and females was calculated as absolute pairing success (1 or 0) divided by an estimate of the operational sex ratio [(i.e., mean fitness: Brodie and Janzen (1996)]. Because of the complex structure of habitat, the operational sex ratio could not be reliably estimated in the field

at the time of sampling. We, therefore, obtained an estimate of the proportions of paired and unpaired males and females from the laboratory experiment. This estimate was calculated as the average proportions determined at 9 point samples in time corresponding to the first 9 days of the experiment. We used the univariate models of relative fitness on standardized body size $w = c + \beta_1 z$ to estimate the linear (β_1) and $w = c + \beta_1' z + (\gamma_1/2)z^2$ to estimate the quadratic (γ_1) coefficients. The resulting coefficients β_1 and γ_1 are the linear and nonlinear (quadratic) selection differentials, reflecting the combined effects of direct and indirect selection on body size (Endler, 1986; Brodie et al., 1995). We used the corresponding trivariate models $w = c + \beta_{2,\mathrm{bw}} z_{\mathrm{bw}} + \beta_{2,\mathrm{tc}} z_{\mathrm{tc}}$ for the linear ($\beta_{2,j}$) and $w = c + \beta'_{2,\mathrm{bw}} z_{\mathrm{bw}} + (\gamma_{2,\mathrm{bw}}/2) z^2_{\mathrm{bw}} + \beta'_{2,\mathrm{tv}} z_t + (\gamma_{2,\mathrm{tv}}/2) z^2_{\mathrm{tc}} + \gamma_{2,\mathrm{tc}} z^2_{\mathrm{tc}}$ for the quadratic ($\gamma_{2,j}$) coefficients, where the subscripts bw, tv, and tc refer to body weight, secretion volume, and toxin concentration, respectively. These coefficients are the multivariate linear and nonlinear selection gradients (Brodie et al., 1995). The difference of the regression coefficients from a slope of zero (the null hypothesis of no selection) was tested for each species, whereby for the binary field data, least-squares regression was used to derive the estimate, but logistic regression was used to test for significance (Brodie et al., 1995; Blanckenhorn et al., 1999). Assortative mating (homogamy) with regard to all traits was assessed by analogously regressing the relative trait value in one sex on the z-standardized trait value in the other. Since fecundity is typically positively correlated with body size in insects (Honek, 1993), female size estimates male reproductive success. Analogous models were used in the laboratory experiment, using two measures of mating success reflecting different selection episodes, separately for each sex. Relative fitness $w_i = W_i/\overline{w}$ was computed as (1) the total number of matings of an individual divided by the mean number of matings (which were the same for both sexes because the sex ratio in the laboratory cages was unity); and (2) the rank order of the first mating of an individual, reflecting time to first mating, divided by the mean rank of all matings that occurred during the entire experiment (reflecting multiple matings of many individuals). Assortative mating was not assessed in the laboratory, as most beetles that mate also multiply. Although there were two replicate population cages for each species, all selection coefficients were calculated for both data sets combined because the estimates did not differ significantly. All measured traits were square-root–transformed to fit model assumptions.

Mate Fidelity and Random Mating Simulation. From the results, we suspected that matings were not random in *O. gloriosa*, because certain combinations of partners were observed several times. In order to see if this pattern might have arisen under random mating, we performed a simulation using the S-PLUS 2000 software. The program displayed two lists of 182 randomly chosen numbers (because there were an average of 182 matings per tray) ranging from 1 to 30 (because each tray contained 30 individuals of each sex). Each list represented beetles of one sex, and mating events were represented by the two numbers on the same line.

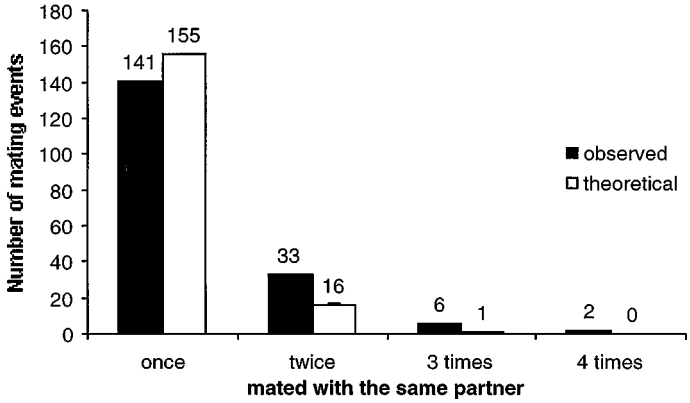

FIG. 1. Observed and calculated numbers of occurrences of repeated mating with a particular partner one to four times. For *O. gloriosa*, calculated numbers were obtained by a simulation assuming random mating. Note that standard errors of theoretical means are too small to be visualized on the figure.

The program then scored the number of "pairings" (i.e., combinations) occurring once, twice, or more often. We repeated this simulation 300 times, and recorded the mean numbers of expected matings in each category, and their standard errors (Figure 1). A goodness-of-fit Chi-square test was performed to see if the differences between observed and calculated values were significant. For this test, we used only two categories ("once the same partner" and "more than once") because the expected values were too small in the other categories.

RESULTS

Field Study. Our field sample consisted of 31 and 25 mating pairs, and 31 and 25 single beetles of each sex for *O. gloriosa* and *O. cacaliae*, respectively. Because of technical problems, there are some missing values for the concentration measurements (Table 1). Toxin volume and concentration were negatively correlated in *O. gloriosa* ($R = -0.30$, $P < 0.05$), whereas all other traits were uncorrelated. In *O. cacaliae*, weight and toxin concentration were positively correlated ($R = 0.33$, $P < 0.01$), and volume and concentration negatively ($R = -0.58$, $P < 0.001$).

In *O. gloriosa*, large males and those with large toxin volumes had a mating advantage, whereas toxin concentration did not influence pairing success (Table 1). These effects were independent, as uni- and multivariate selection differentials were congruent. Furthermore, males and females paired assortatively with regard to body weight and toxin concentration, but not toxin volume (Table 1). Therefore,

TABLE 1. MEAN (±SE) TRAIT VALUES OF PAIRED AND UNPAIRED INDIVIDUALS, UNIVARIATE (β_1) AND MULTIVARIATE (β_2 LINEAR SEXUAL SELECTION COEFFICIENTS FOR MALE AND FEMALE PAIRING SUCCESS AND ASSORTATIVE MATING IN THE FIELD FOR TWO SPECIES OF LEAF BEETLE

Trait	Mean ± SD paired (N)	Mean ± SD unpaired (N)	Pairing success		Assortative mating, Univariate β_1
			Univariate β_1	Multivariate β_2	
O. gloriosa females					
Body weight (g)	0.101 ± 0.01 (31)	0.100 ± 0.011 (31)	+0.055 ± 0.241	+0.139 ± 0.470	+0.063 ± 0.016*
Toxin volume (μl)	0.112 ± 0.047 (31)	0.123 ± 0.089 (31)	−0.102 ± 0.195	−0.664 ± 0.303*	−0.107 ± 0.076
Toxin concentration (μg/μl)	18.68 ± 10.88 (14)	23.18 ± 8.95 (12)	−0.733 ± 0.547	−0.878 ± 0.548	+0.392 ± 0.163*
O. gloriosa males					
Body weight (g)	0.070 ± 0.006 (31)	0.064 ± 0.005 (31)	+1.035 ± 0.232*	+1.072 ± 0.318*	+0.048 ± 0.012*
Toxin volume (μl)	0.138 ± 0.058 (31)	0.094 ± 0.065 (31)	+0.623 ± 0.220*	+0.719 ± 0.276*	−0.107 ± 0.076
Toxin concentration (μg/μl)	22.33 ± 7.64 (12)	26.82 ± 8.36 (15)	−0.461 ± 0.319	−0.253 ± 0.230	+0.199 ± 0.083*
O. cacaliae females					
Body weight (g)	0.092 ± 0.008 (24)	0.090 ± 0.011 (24)	+0.128 ± 0.245	−0.579 ± 0.366	+0.008 ± 0.019
Toxin volume (μl)	0.048 ± 0.039 (24)	0.073 ± 0.038 (24)	−0.606 ± 0.279*	−0.637 ± 0.572	+0.111 ± 0.172
Toxin concentration (μg/μl)	0.075 ± 0.089 (19)	0.147 ± 0.161 (8)	−0.657 ± 0.434	−0.337 ± 0.681	−0.214 ± 0.286
O. cacaliae males					
Body weight (g)	0.066 ± 0.009 (24)	0.061 ± 0.007 (25)	+0.544 ± 0.304	+0.800 ± 0.694	+0.013 ± 0.031
Toxin volume (μl)	0.078 ± 0.045 (24)	0.060 ± 0.033 (25)	+0.499 ± 0.313	+0.768 ± 0.857	+0.078 ± 0.121
Toxin concentration (μg/μl)	0.118 ± 0.142 (19)	0.161 ± 0.131 (17)	−0.367 ± 0.359	−0.524 ± 0.345	−0.214 ± 0.286

* $P < 0.05$.

all three traits positively affected male mating success in *O. gloriosa*. Aside from the assortative mating, which is reciprocal (however, male and female estimates of course vary quantitatively), and a negative multivariate linear selection gradient for toxin volume, female mating success appeared random with regard to all traits (Table 1).

In *O. cacaliae* males, sexual selection differentials for pairing success were qualitatively similar to those in *O. gloriosa*, but lower and nonsignificant, perhaps because of the lower sample size (i.e., there tended to be a positive effect of body weight and toxin volume on mating success; Table 1). There was no assortative mating. Toxin volume negatively affected the mating success of *O. cacaliae* females, but this was not significant in the multivariate analysis (Table 1). So again, sexual selection on females with regard to the three traits was largely absent, whereas sexual selection on male body weight and toxin volume may be present. Non-linear selection differentials were largely non-significant and are not presented in Table 1.

Laboratory Study. General Mating Behavior. Mean pairing duration was similar in both species (6.1 ± 2.8 hr and 6.7 ± 2.4 hr in *O. gloriosa* and *O. cacaliae*, respectively). We could distinguish two phases: true copulation, during which the male copulatory organ was fully inserted into the female's reproductive tract, and "guarding," during which the male clings onto the female's back but no intromission occurs. The duration of these two pairing phases was different in the two species: in *O. gloriosa* true copulation usually lasted less than 1 hr (less than 10 min on average), whereas in *O. cacaliae* all mating time consisted of true copulation. Although the switch between copulation and guarding was observed only once out of the 12 matings observed, we think that the guarding phase occurred after true copulation. Neither of the two species showed any apparent courtship. In both species, males often tried to mate with females that were running away. Most of the time these unwilling females were successful in escaping. Direct contest for access to mates was not evident in males or females. Although in some cases potential male competitors approached a mating pair and even climbed onto the back of the male already in place, this never caused the current pairing to end. All this suggests a mating system of male scramble competition for access to females, combined with some sort of (direct or indirect) female choice (Andersson, 1994; Rowe et al., 1994; Wiley and Poston, 1996).

Sexual Selection. We observed a total of 384 matings in *O. gloriosa* and 77 in *O. cacaliae* during the laboratory experiment. Again, the toxin concentration sample size was reduced because of technical problems. In *O. gloriosa*, all males mated and only 3% of the females did not, whereas in *O. cacaliae*, 28% of males and 59% of females remained unmated. The estimated average proportion of beetles mating at any point in time was 9% for *O. gloriosa* and 6% for *O. cacaliae*. This suggests that *O. cacaliae* might be less active overall (at least in the laboratory) than *O. gloriosa*.

There were no significant phenotypic correlations among the three traits in the laboratory experiment. In contrast to the field data, body weight and toxin volume had no effect on the number or order of mating in *O. gloriosa* males (Table 2). Only toxin concentration positively affected mating order, i.e., males with high concentration mated first (Table 2). Furthermore, females with higher toxin concentrations mated more often, and heavier females mated sooner (Table 2).

In *O. cacaliae*, there were no significant effects of any of the three traits on the number and order of mating in the laboratory (Table 2). Nonlinear selection coefficients were nonsignificant and are not presented.

Mate Fidelity and Random Mating Simulation. Remating with the same partner was never observed in *O. cacaliae*. In *O. gloriosa*, repeated pairings of the same two partners made up 21% (82 of 384) of all pairings observed (Figure. 1). There were 12 mates that remated three times, and 4 mates that remated four times. (Note that in the figure these numbers were divided by 2 to represent the situation in only one of the two trays.) Our simulation showed that we would expect fewer rematings under random mating ($\chi^2 = 32.01$, $df = 1$, $P < 0.001$; Figure. 1).

DISCUSSION

Our study suggests that defensive toxins and body size play a role in sexual selection in *O. gloriosa*, a species that autogenously produces toxins (Eggenberger and Rowell-Rahier, 1992). Sexual selection on physiological traits in general, and on toxic defensive compounds in particular, is rarely documented in the literature (e.g., Kingsolver et al., 2001). Although field and lab results are not entirely congruent, sexual selection apparently acts on male *O. gloriosa*: larger males with either greater secretion volume (field) or higher toxin concentration (laboratory) have a mating advantage. This is possibly due to direct (active) or indirect (passive) female choice (Rowe et al., 1994; cf. Wiley and Poston, 1996).

Moreover, there are indications that *O. gloriosa* females are subject to sexual selection as well, perhaps by male choice, as larger females mated more readily and those with higher toxin concentration more often. Males might compete for the largest receptive females, which typically have higher fecundity in many insects (Honek, 1993). Additionally, since toxicity is heritable in this species (Eggenberger and Rowell-Rahier, 1992), individuals could increase the chances of survival of their offspring by choosing well-defended partners with "good genes." Although poorly documented, male choice for females has been shown to operate in numerous species (Waring-Wilde, 1996; Cunningham and Birkhead, 1998; Amano et al., 1999; Gwynne and Bailey, 1999; Bonduriansky, 2001). Although male choice is in theory more likely to operate when the OSR is balanced or even female-biased (as is rare in nature but was the case in our lab experiment), the OSR does not in any straightforward way predict observed differences in choosiness (Bonduriansky,

TABLE 2. MEAN (±SE) TRAIT VALUES, UNIVARIATE (β_1) AND MULTIVARIATE (β_2) LINEAR SEXUAL SELECTION COEFFICIENTS FOR THE NUMBER OF MALE AND FEMALE MATING PARTNERS AND THE SPEED OF THE FIRST MATING (MATING ORDER) IN THE LABORATORY FOR TWO SPECIES OF LEAF BEETLE

		Number of matings		Mating order	
Trait	Mean ±SD, paired (N)	Univariate β_1	Multivariate β_2	Univariate β_1	Multivariate β_2
O. gloriosa females					
Body weight (g)	0.099 ± 0.009 (28)	$+0.169 \pm 0.093$	$+0.176 \pm 0.091$	$+0.278 \pm 0.132^*$	$+0.263 \pm 0.127^*$
Toxin volume (μl)	0.049 ± 0.029 (28)	-0.010 ± 0.099	-0.098 ± 0.094	$+0.094 \pm 0.152$	-0.013 ± 0.152
Toxin concentration (μg/μl)	22.93 ± 14.64 (28)	$+0.179 \pm 0.092$	$+0.189 \pm 0.092^*$	$+0.206 \pm 0.147$	$+0.183 \pm 0.149$
*O. gloriosa*males					
Body weight (g)	0.072 ± 0.006 (31)	-0.040 ± 0.089	-0.066 ± 0.095	$+0.105 \pm 0.119$	-0.035 ± 0.116
Toxin volume (μl)	0.040 ± 0.023 (30)	$+0.076 \pm 0.091$	$+0.085 \pm 0.095$	$+0.158 \pm 0.124$	$+0.118 \pm 0.120$
Toxin concentration (μg/μl)	16.63 ± 12.74 (31)	$+0.051 \pm 0.089$	$+0.053 \pm 0.093$	$+0.301 \pm 0.119^*$	$+0.281 \pm 0.126^*$
O. cacaliae females					
Body weight (g)	0.092 ± 0.010 (16)	$+0.142 \pm 0.412$	-0.344 ± 1.174	-0.276 ± 0.479	$+0.116 \pm 0.506$
Toxin volume (μl)	0.074 ± 0.053 (16)	$+0.132 \pm 0.413$	-0.143 ± 0.966	$+0.234 \pm 0.416$	-0.040 ± 0.422
Toxin concentration (μg/μl)	0.109 ± 0.111 (7)	$+0.882 \pm 0.599$	$+0.818 \pm 0.928$	$+0.593 \pm 0.259$	$+0.646 \pm 0.408$
O. cacaliae males					
Body weight (g)	0.075 ± 0.007 (25)	-0.045 ± 0.192	$+0.216 \pm 0.252$	-0.288 ± 0.156	-0.233 ± 0.210
Toxin volume (μl)	0.125 ± 0.072 (25)	-0.178 ± 0.189	-0.253 ± 0.370	$+0.290 \pm 0.267$	$+0.260 \pm 0.291$
Toxin concentration (μg/μl)	0.156 ± 0.110 (15)	$+0.154 \pm 0.226$	$+0.079 \pm 0.249$	$+0.104 \pm 0.186$	$+0.181 \pm 0.199$

$^*P < 0.05$.

2001). However, reports of male mate preferences are primarily from species and situations with balanced or female-biased sex ratios, especially when males are limited in the number of copulations they can perform, when males do not have to invest a lot in searching for females (as occurs in gregarious insects like *Oreina*) and there is ample choice, and when there is large variation in female quality (which is the case in *O. gloriosa*). Furthermore, in *Oreina* spp., toxins are costly to produce/sequester (for both sexes), and mating might be costly (e.g., in terms of predation) even for males. All these conditions together may limit the number of female partners a male can typically obtain in a season, potentially selecting for male (in addition to female) choice based on toxicity. Additionally, in the laboratory experiment, particular pairings recurred repeatedly in *O. gloriosa*, clearly indicating nonrandom mate fidelity. Contrary to the laboratory experiment, sexual selection on females was largely absent in the field sample, where more pronounced male–male scramble competition may have primarily favored large, fit males and rendered mate choice for toxicity secondary. However, the evidence for male choice in the lab experiment remains weak, and there are alternative interpretations possible, including a lab artifact.

In *O. cacaliae*, in contrast, which sequester their toxins from their food plants (Rowell-Rahier et al., 1991; Pasteels et al., 1992), such selection is absent or at least much weaker. The smaller samples for this species limit the interpretation of the data, as the field estimates of sexual selection in *O. cacaliae* were roughly similar to those of *O. gloriosa,* albeit a bit lower (Table 1). Results for *O. cacaliae* should be regarded as preliminary. However, sexual selection for "good genes" would not necessarily be expected in *O. cacaliae*, in which toxin sequestration is highly dependent on the PA content of their host plants (cf. Isman, 1977; Isman et al., 1977; Brower et al., 1984; Bowers, 1992). First, because toxin sequestration is often thought to be less costly than *de novo* synthesis (even though this is difficult to show: Pasteels et al., 1990; Bowers, 1992), mate choice for toxicity may be less likely to evolve in *O. cacaliae* than *O. gloriosa*. Second, even though physiological sequestering efficiency or food-plant choice might also be heritable, environmental variation should be large in comparison. Indeed, in *O. cacaliae*, toxicity was not consistent over seven samplings in a 6-week experiment (Labeyrie, unpublished data). Nevertheless, most other examples of sexual selection in relation to chemical defense concern sequestering species (Nishida and Fukami, 1990; Trigo and Brown, 1990; Dussourd et al., 1991; Amano et al., 1999). In these studies, this could be associated with spermatophores offered by males to females, which do not occur in our leaf beetles.

Mean pairing duration was long (ca. 6 hr) and approximately similar in both species. However, in *O. gloriosa*, actual intromission (as opposed to guarding) duration was much shorter (< 1 hr) than in *O. cacaliae*, where it lasts for the whole time. We also found that *O. cacaliae* mated much less frequently than *O. gloriosa*, although we have no data on differential activity rates of males or

females for either species. It is possible that these differences in copulation frequency and duration between the two species are related, suggesting more sexual conflict in *O. cacaliae* (cf. Rowe et al., 1994). Copulation duration is typically correlated with insemination and fertilization rates and/or spermatophore size (He and Tsubaki, 1992; Birkhead and Møller, 1998; Micholitsch et al., 2000), although genital contact does not always imply that sperm are being transferred (Rubenstein, 1989). Longer copulations are also often indicative of greater sperm competition and should lead to faster sperm depletion. Everything else being equal, this should allow *O. gloriosa* males to mate more often than males of *O. cacaliae*.

The field and laboratory observations revealed that females often successfully avoid matings by running away. As there appears to be no courtship, and females show no other rejection behavior (such as shaking; e.g., Rowe et al., 1994), this would be their primary expression of mate choice. However, the use of aerial or contact pheromones for sexual attraction is not inconceivable in leaf beetles (Edwards and Seabrook, 1997; Shu et al., 1999; Ruther et al., 2000). For toxicity to serve in mate choice, some such cue is necessary that individuals may use for assessing toxicity of potential mates. As far as we could observe, the beetles only emit their defensive liquid when disturbed by a predator, and not when they mate. This makes it unlikely that toxin concentration is directly assessed by mates before or during mating. However, as defense glands are under neural regulation (Schooneveld et al., 1992), it is possible that small amounts of secretions are released from the glands in a sexual context as a (contact) pheromone (Trigo and Brown, 1990; Attygalle et al., 1991; Dussourd et al., 1991; Eggenberger and Rowell-Rahier, 1993; Amano et al., 1999). We have no direct evidence for this process in *Oreina* leaf beetles.

In this study, the mean secretion volume differed between the field sample and the lab experiment. This is most likely due to different physical conditions, including air humidity, water availability, and temperature. Indeed, the lab air humidity (ca. 60%) was considerably lower than in the field. Beetles may have been limited by the quantity of liquid available for producing large volumes of secretion and consequently may have reduced the volume produced to a threshold (40–43% of the volume in the field) necessary for effective defense. This could explain why no effect of secretion volume was detected in the laboratory.

In conclusion, reciprocal male and female choice for well-defended, in addition to large, partners can to some degree explain the mating patterns observed in *O. gloriosa*, but probably not in *O. cacaliae*. Even though the exact mechanism and signals involved in sexual selection are currently unclear, the study suggests that sexual selection may contribute to the maintenance, although not necessarily the origin, of defensive traits in the leaf beetle *O. gloriosa* and probably other species as well.

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