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DEFENSIVE SECRETION PRODUCTION IN THE TENEBRIONID BEETLE, *Zophobas atratus* Effects of Age, Sex, and Milking Frequency

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Abstract—Beetles were milked of their abdominal defensive secretion at three different frequencies over the first 36 days of adult life. Secretion production decreased greatly with age from a maximum of 94 nmol/day at 4–6 days to a basal level of about 25 nmol/day at 30–40 days. Ethyl- and methylquinone comprised the bulk of the secretion and were most strongly affected by age. Benzoquinone was most strongly affected by milking frequency. An average adult produced an estimated 4445 nmol of total secretion in its 5-month life-span but had reservoirs large enough to store 11,000 nmol. Males produced more secretion than females because of their larger size and inherent sexual differences. As they aged, mated females showed a more rapid decrease in production that virgin females. The defensive system appears to be programed to charge glands rapidly with secretion early in adult life and fall of to a low recharge rate little affected by discharge.

Key Words—Chemical defense, *Zophobas atratus*, Coleoptera, Tenebrionidae, defensive secretion, gland recharge, defensive quinone, quinone production.

INTRODUCTION

Two important characteristics of a chemical defensive system are how quickly it is initially charged with secretion and how quickly a depleted system can be recharged with additional secretion. Although much work has been done on arthropod chemical defenses (for reviews see: Roth and Eisner, 1962; Eisner and Meinwald, 1966; Jacobson, 1966; Schildknecht, 1971; Meinwald et al. 1978; Rockstein, 1978; Blum, 1981), little is known about these two characteristics.

Eisner (1958) reported high recharge rates for the carabid beetle, *Brachinus*

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ballistarius LeConte, and Tschinkel (1975) found most tenebrionid species "milked" to apparent depletion can release some secretion again in a day or two, but either of these may be the result of physical effects such as the secretion of water into the defensive reservoirs rather than de novo synthesis (Tschinkel, 1975). The dytiscid beetle, *Agabus obtusatus*, recharges its prothoracic glands in three weeks, while *A. seriatus* requires four weeks to replace 61% of a prothoracic component (Fescemeyer and Mumma, 1983). Carrel (1984) found adult millipedes recharge glands at a constant rate for 100 days, implying a depleted individual needs four months to reload its glands.

Recent studies indicate that age, sex, and size may influence defensive secretion production in beetles. Newly eclosed adults have little secretion, while older adults have much more (Kaneshisa, 1978; Dettner and Schwinger, 1982; Classen and Dettner, 1983). Secretion composition and titer have also been found to vary with age and season (Miller and Mumma, 1974; Dettner, 1979; Newhart and Mumma, 1979a,b; Dettner and Schwinger, 1982; Classen and Dettner, 1983), and some of this variation is likely due to variation in population structure (Dettner, 1979; Classen and Dettner, 1983). Gland size and sex may also have important effects (Kaneshisa, 1978; Dettner and Schwinger, 1982).

In this study, we examined the effect of adult age, sex, and discharge frequency on the defensive secretion production of the abdominal glands in the tenebrionid beetle, *Zophobas atratus*.

MATERIALS AND METHODS

Zophobas atratus is a large tenebrionid beetle from Zamorano, Honduras, laboratory-reared since 1978. Like *Z. rugipes*, it has two pairs of defensive glands. The prothoracic pair exudes the phenolic (phenol, *m*-cresol, and *m*-ethylphenol) secretion, and the abdominal pair, a quinonic mixture (1,4-benzoquinone, 2-methyl-1,4-benzoquinone, and 2-ethyl-1,4-benzoquinone; hereafter referred to as benzoquinone, methylquinone, and ethylquinone, respectively) in equilibrium with an aqueous phase (Tschinkel, 1969). Because the prothoracic glands are difficult to milk, they were not examined in this study.

Zophobas atratus was selected for this study because (1) it is easily reared in the laboratory, (2) the abdominal defensive glands are everted so all secretion can be collected in each milking, and (3) the defensive secretion is simple (see above).

Experimental adults were individually maintained at 30°C in plastic boxes with screened tops and provided bran, cricket feed, and water ad libitum.

Collection and Analysis of Secretion. Secretion was collected by roughly handling a beetle until its glands were everted and then wiping these free of secretion with a small piece of filter paper. The paper was immediately extracted in carbon disulfide and stored at −20°C.

Samples were analyzed within a few days of collection as a single injection into a Varian Aerograph 1400 gas chromatograph with a FID detector (carrier gas: nitrogen, 30 ml/min; column, $\frac{1}{8}$ in. × 5 ft aluminum, 10% OV-101 on HP Chromosorb W (AW DMSW, 80–100 mesh), oven: 150°C). The peak areas were converted to mass by a Shimadzu C-RIA Chromopac recording data processor calibrated with external standards, and the mass was converted to nanomoles for analysis of variance (ANOVA).

Egg Production. Pairs of beetles were maintained in screen-topped plastic boxes at 30°C. The bottom of each plastic box contained a 2-cm layer of Wondra flour covered with a wire screen. Females laid eggs through the screen into the flour. On top of the screen, beetles were fed a chunky baked mixture (unsuitable for oviposition) of bran, cricket feed, Wondra flour, and water. Using a No. 40 U.S. Standard Testing sieve, eggs were sifted from the flour every seven days and counted.

Data were log transformed to normalize and analyzed by analysis of variance (ANOVA) and Duncan's multiple-range test.

RESULTS

Experiment I: Effect of Age, Sex, and Milking Frequency. Beetles were sexed and weighed as pupae and assigned to one of three treatment groups: (1) milked every three days; (2) milked every six days; or (3) milked every 12 days. Each group contained 15 males and 15 females, which all eclosed within 24 hr. Pupal weights were used to eliminate any effects of body size; average pupal weight of males was 0.774 ± 0.089 g and females 0.687 ± 0.073 g. Statistical comparisons were made on the total nanomoles of secretion per gram pupal weight collected at the end of three consecutive 12-day periods. For example, at 12 days, we compared the sum of the first four milkings of the three-day group, the first two milkings of the six-day group, and the first of the 12-day group. Separate analyses were performed for total quinone content and each component quinone.

Age of adult beetles had a tremendous negative effect on their defensive secretion production (Figure 1). Between day 12 and 36, total quinone production fell 64% from 1410 nmol to 509 nmol quinone/g pupal weight (ANOVA, $P < 0.0001$), while methyl- and ethylquinone decreased 72% and 68%, respectively ($P < 0.0001$, Figure 1). Age accounted for 53% of the total variance of these three measures or 80% of the explained variance. No other factor or interaction accounted for more than 4% of the total variation in these measures.

Production of benzoquinone, on the other hand, decreased only 28% over the same period ($P < 0.001$, Figure 1) with age accounting for 7% of the total variance. As a result of the differential decrease in production of each quinone,

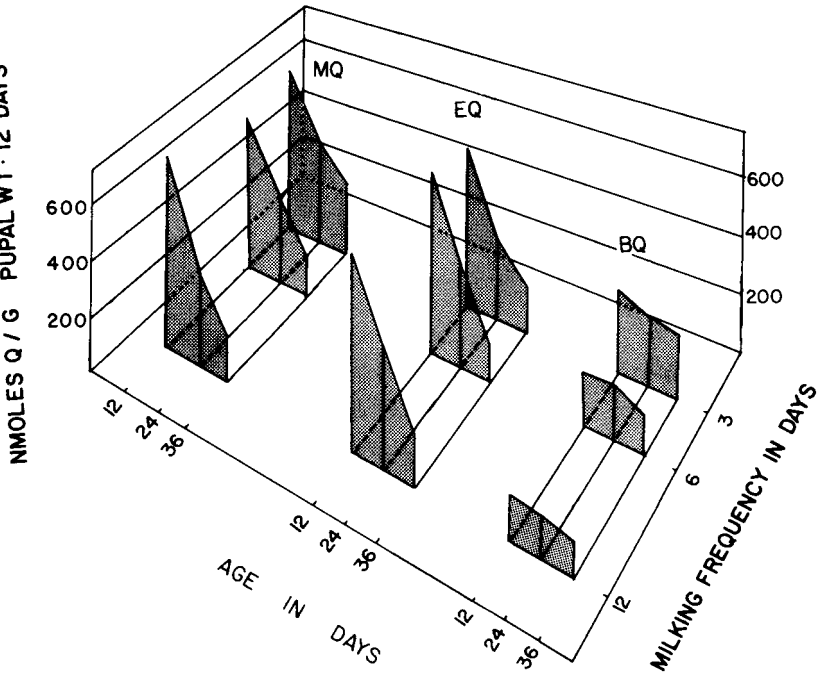

FIG. 1. Production of individual quinones in relation to age and milking frequency (in days). From day 12 to day 36, MQ decreased 72%, EQ, 68%, and BQ, 28%. More frequent milking stimulated production of MQ and BQ. For both MQ and BQ, production decreased more slowly in the three-day group than in the six- or 12-day groups. MQ = methylquinone, EQ = ethylquinone, BQ = benzoquinone, Q = quinone, PUPAL WT = pupal weight.

the composition of the newly produced secretion changed from an initial ratio of 3:3:1 to a final ratio of 1:1:1 of methyl-, ethyl-, and benzoquinone.

Milking stimulated benzoquinone production, causing a two-fold increase of the most frequently milked group over the least ($P < 0.05$; Figure 1). Milking frequency accounted for 22% of total benzoquinone variance. More frequent milking stimulated a small but significant ($P < 0.001$) increase in total and methylquinone (Figure 1) production and accounted for 3% and 4%, respectively, of their total variance.

Males not only produced 16% more total quinone per gram pupal weight than did females, but more of each quinone as well: 16% more methylquinone, 3% more ethylquinone, and 48% more benzoquinone. Total quinone and alkylquinone (methyl- and ethyl-) production was initially greater in females but decreased more rapidly than it did in males ($P < 0.001$, Figure 2). Although

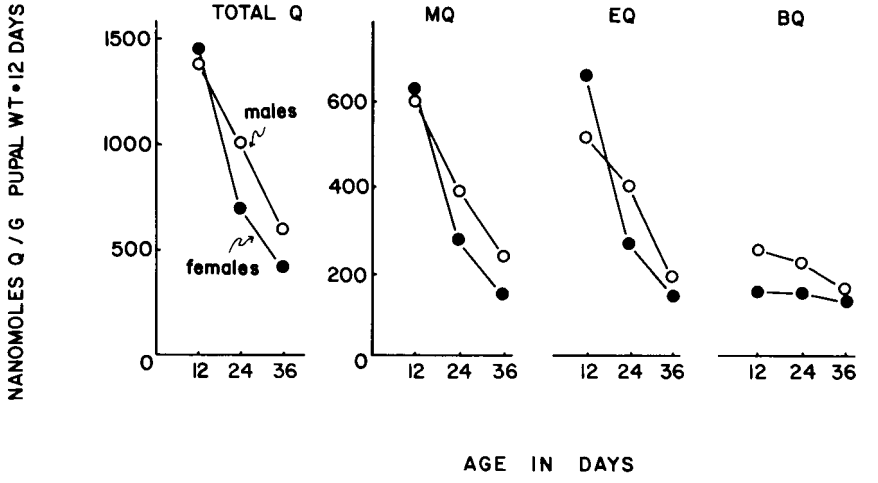

FIG. 2. Interaction of age with sex for total quinones and each component quinone. Males produced more of each measure than females. Production of total Q, MQ, and EQ decreased more quickly in females than in males. TOTAL Q = total quinones, MQ = methylquinone, EQ = ethylquinone, BQ = benzoquinone.

male benzoquinone production decreased 36% during the 36-day period, it always remained higher than female benzoquinone production. Sex accounted for 13% of the total variation in benzoquinone.

It is interesting that methyl- and ethylquinone showed nearly identical responses to all experimental factors (Figures 1 and 2). Duffy and Blum (1976) (in Blum, 1981) established that these alkylquinones are biosynthesized by a pathway independent of that for benzoquinone in *Zophobas rugipes*. If the total amount of quinones reflects defensive potency, then males are 13% more defensively potent per gram pupal weight as shown in Figure 2 or 31% more potent per beetle than females.

Cumulative secretion approached an upper limit with increasing age, indicating that biosynthesis decreased to some basal level (Figure 3). Alkylquinone and total quinone production decreased similarly, while benzoquinone production, which was not strongly affected by age, continued at nearly the same rate.

The average daily production per beetle (including data from experiment II) plotted against age (Figure 4), reveals a rapid decrease from a maximum rate of 94 nmol/beetle/day at 4–6 days to a basal level of about 25 at 30–40 days.

Thus, we found in the order of importance of effect: (1) age was the most significant factor, having its greatest effect on total and alkylquinone production; total quinone production was greatest at 4–6 days and decreased to a basal level at 54 days; (2) males produced more secretion than females because of and in-

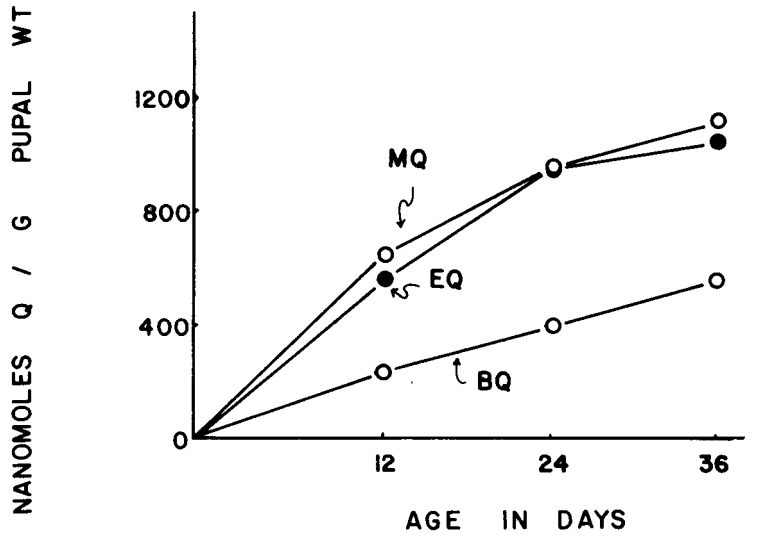

FIG. 3. Cumulative production of each quinone versus beetle age. This shows the approximate gland content in the absence of periodic milking. MQ = methylquinone, EQ = ethylquinone, BQ = benzoquinone, Q = quinone.

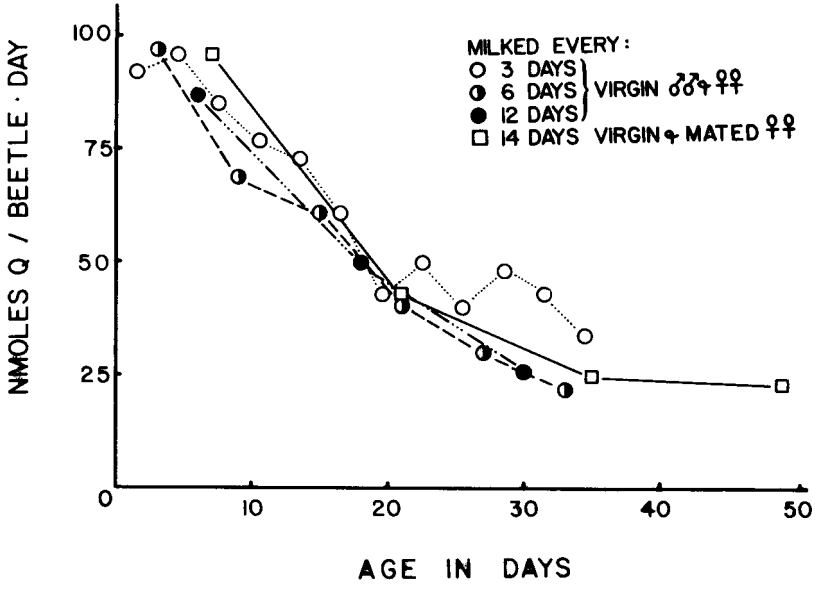

FIG. 4. Average daily secretion production per beetle. Each point is the average for its collection period and is plotted in the center of that period.

dependent of size differences; (3) milking frequency primarily stimulated benzoquinone production; and (4) the alkylquinones occurred in nearly equal quantities, comprised the bulk of the secretion, and were more strongly and almost equally affected by treatment factors.

Experiment II: Effect of Egg Production. Because the decrease in production between day 12 and day 24 (Figure 3) coincides with the onset of egg production in females, we tested the hypothesis that egg production had a negative effect on secretion production (perhaps by causing material to be shunted away from the defensive system to the reproductive system). This experiment differed from experiment I as follows: (1) beetles were placed into one of two treatment groups: virgin (isolated females) or mated (one male and one female with ad libitum, but unknown mating); (2) eggs were collected every seven days; (3) females were milked every 14 days; (4) the males were never milked; and (5) the experiment lasted 56 days. Quinone data were again converted to nanomoles, pupal weight-adjusted, log transformed, and analyzed by ANOVA.

As in experiment I, age accounted for the largest portion of the total variance of each measure: total quinones, 69%; methylquinone, 64%; ethylquinone, 69%; and benzoquinone, 17%.

Although mated females laid nearly twice as many eggs as virgin females ($P < 0.05$), mating did not significantly affect secretion production but did cause total and individual quinone production to decline more rapidly with age. This interaction explained 2% of the total variance of each measure (age by treatment interaction; $P < 0.05$). Egg production, however, was not significantly related to secretion production, perhaps because the beetle does not channel very much energy into defensive secretion production. A larger trade-off between secretion and egg production would be likely in species investing more energy in secretion.

These results suggest that the virgin females in the previous experiment were also reasonably representative of mated females with respect to secretion production.

DISCUSSION

The rapid decline in quinone production suggests that the defensive system in *Z. atratus* is programed to charge glands rapidly early in adult life and drop off to a low recharge rate. Kaneshisa (1978) found defensive titers of newly eclosed tenebrionid beetles reached a maximum about one month after emergence, which agrees with our study. Carrel (1984) found millipedes recharge at a low constant rate for 100 days. Since this millipede passes its secretion from one molt to the next (Carrel, 1984), a high recharge rate immediately following a molt would be unnecessary and might explain the observed recharge rate. The high recharge rates in dytiscid beetles reported by Fescemeyer and Mumma

(1983) may reflect a different defensive strategy, i.e., discharge and loss of secretion, and the greater recharge this demands.

The older a *Z. atratus* is when it loses its defensive secretion, the longer its defenses are reduced. For example, by 12 days an average beetle produces 1035 nmol of secretion. At 24 and 36 days a beetle would require an additional 42 and 50 days, respectively, to produce another 1035 nmol. If all secretion were lost after 42 days (25% of an adult's life-span), it would never be completely replaced. However, if secretion were allowed to accumulate, older beetles would have greater defensive stores, assuming quinones are stable for this period. Calculations of reservoir volume indicate that a beetle could store up to 11,000 nmol, but an average beetle, which lives five months in the lab, could only produce 4445 nmol of secretion. Thus the reservoirs are never more than $\frac{1}{3}$ full of secretion, which probably adheres as a film on the densely folded walls.

Beetles probably retain secretion for most of their lives (4–12 months in the lab) because: (1) they use their secretion very efficiently—no secretion is lost by spraying or exuding and the densely folded reservoir walls help retain the secretion—so it is very unlikely all secretion would be lost in an encounter with a predator; (2) beetles probably encounter predators at a much lower frequency than they were milked in this experiment; and (3) they also have a pair of thoracic defensive glands which exude a phenolic secretion as a supplementary defense. The thoracic secretion is only released when the applied stimulus is relatively severe, while the abdominal glands are probably the first line of defense in encounters with predators. Animals pinched and poked with forceps while standing, readily everted their abdominal glands but did not release their thoracic secretion. When lifted off the substrate, 43% released their thoracic secretion, while 100% everted their abdominal glands ($N = 23$) (personal observation). The thoracic secretion not only flows over the beetle's cuticle and may present a greater threat to the beetle's own well-being, but it is more likely to be lost than the abdominal secretion.

The decrease in quinone production parallels decreasing reproductive potential with increasing age in insects. Older individuals have lower reproductive potential (Engelmann, 1970) and therefore expend less energy on survival, i.e., defensive secretion production.

Because production of alkylquinones is so much greater than that of benzoquinone, in the absence of reservoir depletion and secretion degradation, the quinone ratio changes very little with increasing age. An unusual quinone ratio could only occur if an individual lost most of its secretion after 36 days and was therefore recharging when production of the three quinones is nearly equal.

Males produced 31% more secretion per beetle than females. Perhaps males seek out females and thus are exposed to more predation, which in turn favors those which produce more secretion. This is likely the case in *Glomeris mar-*

ginata, where males produce almost 1.6 times as much defensive secretion per gram body weight as females (Carrel, 1984).

Finally, our study indicates that quantitative and qualitative studies of defensive secretion cannot be effectively investigated without considering the effects of age, sex, or previous discharge. We believe that the low recharge rate or basal rate is a reflection of this beetle's life history as well as the efficiency with which the secretion is used.

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