

When Do *Paederus riparius* Rove Beetles (Coleoptera : Staphylinidae) Biosynthesize Their Unique Hemolymph Toxin Pederin?

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Pederin analyses of single *Paederus riparius* specimens reared from the egg and kept for prolonged periods of time show that only the females are able to biosynthesize the substance. Preimaginal stages efficiently store pederin transferred by the females into their eggs and the males' pederin content decreases slowly over time. Only males with access to eggs containing the substance moderately increase their pederin load. The females begin to accumulate the toxin a few weeks after imaginal eclosion and build up reserves for the egg laying period within 60 days. This is discussed with regard to the life cycle of *P. riparius*. During reproduction, when the females lose pederin due to maternal transfer, they must replenish their pederin reserves. This biosynthesis is not reflected in the amount found per individual.

Introduction

Paederus rove beetles possess a toxic amide, which causes blistering several hours after their hemolymph comes into contact with human skin. As recently reported from Nigeria (Okiwelu *et al.*, 1996), outbreaks of such lesions occur in the tropics and subtropics after one or more *Paederus* species increase in abundance during the rains, and therefore an extensive literature is devoted to this phenomenon (Frank and Kanamitsu, 1987).

Studying *P. fuscipes* in Italy, Pavan and Bo (1953) obtained crystals of the toxin which they named pederin. Millions of beetles were extracted (Pavan, 1975) to determine the molecular structure of the amide (Fig. 1) which took two rivaling groups several years for a final solution (Cardani *et al.*, 1965; Matsumoto *et al.*, 1968). Related substances are hitherto known only from marine

sponges, from which several compounds with the same substructure as pederin have been isolated (Perry *et al.*, 1988; Sakemi *et al.*, 1988; Fusetani *et al.*, 1992; Matsunaga *et al.*, 1992; Kobayashi *et al.*, 1993). This substructure, especially the centrally located α -hydroxyamidoacetal functionality, probably causes the biological activities which the substances have in common, for example their cytotoxic activity (Thompson *et al.*, 1992).

For the chemist pederin synthesis is a real challenge (Kociński *et al.*, 1991). How the beetles get the substance is known only to a rather limited extent. Pederin biosynthesis in *P. fuscipes* is outlined as a kind of polyketide synthesis (Cardani *et al.*, 1973) but the details are unknown. An investigation of that issue should take into account that the beetles might not biosynthesize the substance at all times, as indicated by low incorporation of radioactive precursors during late season (Cardani *et al.*, 1973).

A TLC-method developed by Kellner and Dettner (1995) enables us to study the pederin content of single specimens. Analyzing *P. fuscipes* and *P. riparius*, it was found that females are able to accumulate pederin over time when fed with a wholly artificial diet, which suggests biosynthetic capabilities that are independent of special precursors. The developmental stages could not be shown to biosynthesize the substance but unexpectedly lost about 50% of the amount received maternally after the second larval stage. The males

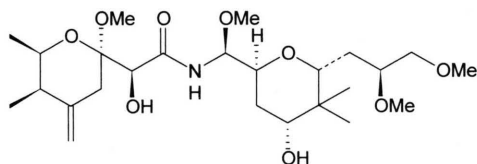


Fig. 1. Structural formula of pederin (Kociński *et al.*, 1991).

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also didn't accumulate pederin but sequestered the amide if fed with eggs containing the substance. Regarding the limited sample size for imagines, it was concluded preliminarily that only females might have biosynthetic capabilities for pederin.

In addition, the females of both species studied could be classified into two distinct types termed (+)-females and (–)-females respectively. Whereas the (+)-females transfer appreciable amounts of pederin into every egg (*P. riparius*: 0.4–2.1 µg per egg), the (–)-females obviously lack the biosynthetic pathway leading to pederin, are thus unable to accumulate the toxin, and lay eggs without pederin. If fed with the substance (–)-females also lay eggs with pederin. When the egg-laying period is already terminated, the (–)-females like males store pederin that they have ingested over prolonged periods of time.

As pederin is obviously a valuable substance for *Paederus*, which can be determined months after ingestion, biosynthetic capabilities for pederin should be reflected in changes in individual pederin content. This study investigates offspring of *P. riparius* (+)-females kept under controlled laboratory conditions to determine the times of increase and decrease of pederin content which reflects biosynthetic activity or lack of it.

Materials and Methods

Beetles and breeding conditions

Imagines of *Paederus riparius* (Linné 1758) were collected from several sites in northeastern Bavaria, Germany. Pairs were kept separately as de-

scribed by Kellner and Dettner (1995), and the eggs were gathered three times per week. Larvae were reared on moist absorbent paper in 24-cell wells (1.7 cm diameter of the wells). The feeding regime used earlier was modified in order to increase breeding success: As before, larvae were fed twice a day with frozen *Drosophila melanogaster* (strain vg). Additionally specimens were fed once during each larval stage with a piece of either a *Tenebrio molitor* larva or a *Calliphora* pupa. Using this method, considerably more imagines were obtained and fewer larvae and pupae died (Fig. 2). From each of four arbitrarily chosen females, second larval stages (larvae 2), prepupae, pupae, and freshly emerged imagines were preserved at –25 °C for pederin analysis.

Reared imagines were transferred to petri dishes (9 cm diameter) with moist absorbent paper where they were fed with living *Drosophila* flies. Within three months at constant conditions (20 °C, 15 L : 9 D) samples of beetles were frozen after some fixed time period had elapsed in order to represent different age classes. The remaining beetles were hibernated artificially by placing them 2–4 months in a climate chamber at 12 °C with 9 hr of photophase. Pairs were founded using this filial generation. Beetles of the same sex were not mixed to be able to recognize their individual ages. From time to time pairs were fed with pieces of *Tenebrio molitor* larvae or *Calliphora* pupae. Their offspring was reared as described above and a few second generation pairs were started.

Since reproductive females lay eggs daily, the females that ceased to lay eggs for at least one week

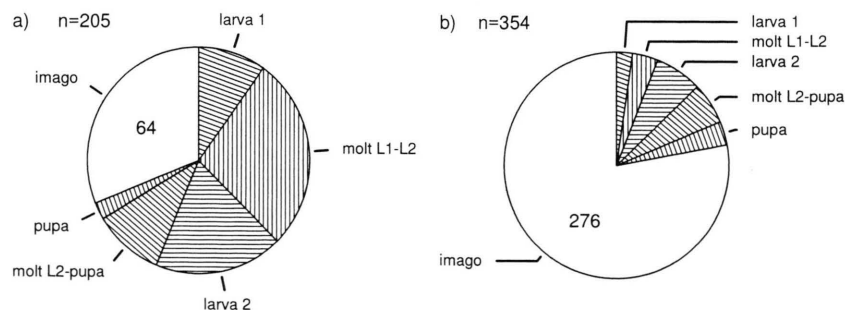


Fig. 2. Outcome of *Paederus riparius* rearing experiments under two different feeding regimes: a) Larvae fed twice a day with frozen *Drosophila melanogaster* (strain vg) as described by Kellner and Dettner (1995), b) as in (a), but one feeding in each larval stage consisted of a piece of either *Tenebrio molitor* larvae or *Calliphora* pupae. Specimens developed successfully (open sector) or died during different developmental stages (hatched sectors).

were frozen together with their mates. Pairs without reproduction (no eggs found within 1–2 months) were again hibernated artificially or they were ultimately frozen as those specimens that had died only recently. Subsequently age classes of beetles older than three months were chosen arbitrarily from the known ages of individuals because the dates of preservation resulted in a continuum of ages.

Measurements

A number of beetles were weighed prior to preservation (Sartorius Supermicro 4504 MP 8). Weight of other specimens was estimated from measurements taken after preservation using an ocular micrometer at a dissecting microscope. Body length and head width, measured across the eyes, are both correlated with fresh weight (body length: $r = 0.85$, head width: $r = 0.58$). Multiple regression results in an equation ($F(2, 83) = 185.83$, $p < 0.001$) from which weight can be approximated ($-17.28 + 1.61 \cdot \text{body length} + 9.69 \cdot \text{head width}$; $r = 0.90$. For all correlations: $n = 86$, $df = 84$, $p < 0.001$). This correlation is obvious as feeding state influences body length especially by the extensible abdomen and head width, which is fixed after imaginal eclosion, is a measure of stoutness.

Pederin analysis

Individual pederin content was determined according to the method described in detail by Kellner and Dettner (1995). Extraction of specimens ground in 30 μl demineralized H_2O was accomplished by three successive volumes of 100 μl ethyl acetate. Extracts were fractionated using 50 μl H_2O and 100 μl hexane. The watery phase was dried, (diluted), and applied to HPTLC plates (0.2 mm silica gel 60, Merck), which were developed in ethyl acetate and stained in anisaldehyde – sulfuric acid – acetic acid (1 : 2 : 100 v/v/v, 2 min at 90 °C). Plates were documented on color slides, and black-and-white video images were analyzed with a computer program (BASys 1D, Biotec Fischer GmbH, Reiskirchen). Pederin spots at $R_f = 0.22$ could be quantified by means of known amounts of authentic pederin which were treated like the samples and applied to the same plates.

Statistical analysis

Data were analyzed using CSS (StatSoft Inc., Tulsa, Oklahoma, release 2.1). Oneway ANOVA was performed to test for significant differences of pederin content among groups of individuals. In case of significant results, planned comparisons were carried out between successive age classes.

Results

Lack of pederin loss during development

A decrease of pederin content in pupae and young adults, which was observed in *Paederus riparius* by Kellner and Dettner (1995), is not confirmed here (Table I). Indeed, the amount of pederin transferred by the females to their eggs is preserved efficiently through all developmental stages. Variation between females in the amount transferred is thus represented in the data but there is clearly no difference among developmental stages (Table I).

Pederin content of females

Females are able to accumulate pederin over time. Values as high as 20 times the amount received maternally can be attained. The amount found per individual is, however, not correlated with its fresh weight ($r = 0.05$, $n = 54$, $df = 52$. Only females containing more than 2 μg pederin are included, i.e. those that had biosynthesized at least some small amount). Therefore, pederin amounts are given in the following without calculating con-

Table I. Pederin in descendants of four *Paederus riparius* (+)-females during part of their development and ANOVAs of the data. Imagines were preserved within two days after eclosion. n. s.: not significant, ***: $p < 0.001$.

Stage	<i>n</i>	Mean [μg]	SEM
Larva 2	7	1.23	0.104
Prepupa	6	1.21	0.036
Pupa	8	1.22	0.054
Imago	7	1.18	0.102

Source of variation	<i>df</i>	<i>MS</i>	<i>F</i>	
Stages	3	0.00386	0.085	n. s.
Within	12	0.04568		
Females	3	0.22511	12.492	***
Within	12	0.01802		

centrations as these would only obscure changes in pederin load.

Pederin increase proceeds early in the rather long-lived imaginal stage of *P. riparius* females (Fig. 3). Planned comparisons of successive age class means show that only between the second (20–31 days) and third (60–84 days) age class is a significant difference found ($t = 2.698$, $p = 0.01$, $df = 34$). Although individual females can accumulate an appreciable amount of pederin within 3–4 weeks (as indicated by the maximum of 8.4 μg pederin in the second age class, which was determined in a 21-day-old female, Fig. 3) the females on the whole do not accumulate sufficient amounts within this short period of time to differ from the freshly emerged females (0–7 days). On

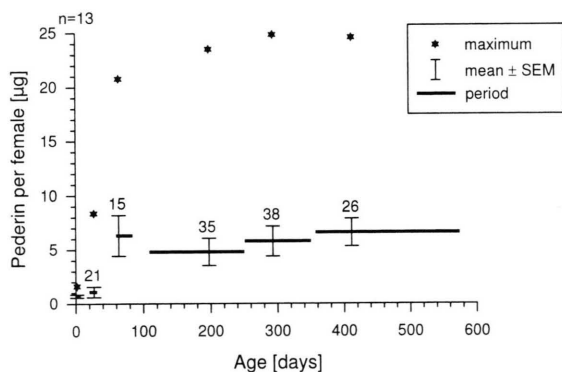


Fig. 3. Pederin content of *Paederus riparius* females in several age classes (n is given above the error bars, $F(5, 142) = 2.89$, $p < 0.05$). Pederin mean and maximum are plotted versus mean age of the specimens in the respective age class whose range is also shown.

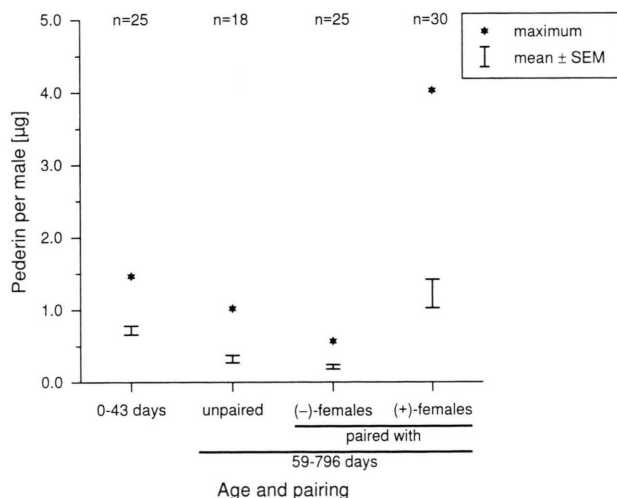


Fig. 4. Pederin content of *Paederus riparius* males classified according to age and pairing.

the other hand, 60-day-old females already possess pederin amounts equivalent to those of much older females. As the youngest female to start with laying eggs was aged 127 days (runners-up were 132 and 133 days old, respectively), pederin biosynthesis occurs well before the egg laying period begins.

Pederin content of males

Contrary to the females, males of *P. riparius* are not able to accumulate pederin when kept separately. Only males paired with (+)-females may reach pederin amounts higher than the quantity transferred maternally (Fig. 4). Mates of (+)-females thus differ from mates of (-)-females (1% confidence intervals of the means do not overlap).

Restricting the analysis to males that were either unpaired or paired with a (-)-female, the males show a steady loss of pederin over time (Fig. 5). Age class means differ significantly between the first (0–43 days) and second (59–193 days) class ($t = 4.972$, $p < 0.001$, $df = 41$) and nearly so between the second and third class ($t = 1.868$, $p = 0.066$, $df = 41$). As in females, the most prominent change in pederin content is located about 40–60 days after imaginal eclosion.

Discussion

The absence of dwindling pederin amounts during larval development can probably be attributed to the improved feeding regime. As earlier results (Kellner and Dettner, 1995) were based on speci-

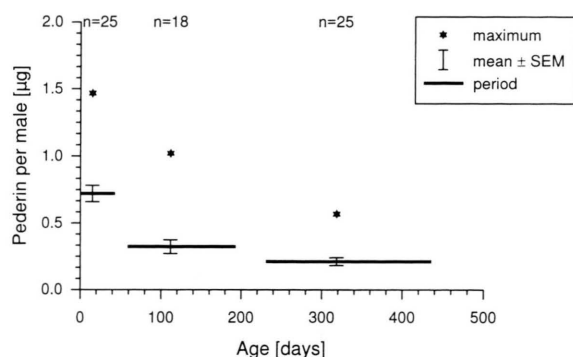


Fig. 5. Pederin content of *Paederus riparius* males in several age classes (graphic presentation as for females in Fig. 3, $F(2, 65) = 31.78$, $p < 0.001$). The males included in the analysis were not paired with (+)-females, i.e. had no access to extraneous pederin sources.

mens coming from rearing experiments with high mortality of developmental stages, the determination of pederin content of different *P. riparius* stages might be flawed due to extraction of moribund specimens. Such individuals could have lost pederin by decomposition. Although pederin appears to be stable in living as well as dried specimens (Pavlovsky and Stein, 1936), it is unstable towards acid (Kociński *et al.*, 1991). Beetles might prevent such decomposition by compartmentalization or proteins. By feeding larvae with a more diverse diet which resulted in most larvae reaching imaginal stage, it can be assumed that the specimens preserved for pederin analysis would otherwise have been living much longer and therefore were not in a physiologically unnatural state.

The females grow heavier after imaginal eclosion but they do not concurrently accumulate pederin. Pederin biosynthesis seems to be an independent process which is not directly related to adequate food supply. As pederin is needed for transfer into the eggs, females accumulate the substance before they begin to lay eggs. There is, however, a noticeable delay after pederin accumulation until eggs are produced. This might be caused by a natural life cycle which consists of imagines reproducing in spring, larvae developing in summer, and young adults adapted for awaiting the forthcoming winter (Boháč, 1985, own observations). That is why it was not possible to induce the filial generations to reproduce in the laboratory without an artificial hibernation. Under natural cir-

cumstances pederin biosynthesis may thus occur already in autumn and does not depend on reproduction.

For the females it is not sufficient to accumulate pederin reserves before reproduction. Biosynthesis has also to replenish pederin amounts transferred into the eggs during reproduction. Otherwise their pederin resources would be exhausted within a few days (for a period of several weeks females lay one to five eggs per day containing 0.4–2.1 µg pederin, Kellner and Dettner, 1995). After termination of egg laying, which is most probably determined endogenously, pederin content of the females is not lower as compared to others. This indicates a rather efficient pederin replenishment.

As the males lose pederin over time, they are apparently unable to biosynthesize the substance. The loss could be attributed to decomposition or pederin discharge through the faeces, which was reported by Pavan (1975). More likely, however, the substance is discharged by an abdominal exocrine gland, where small quantities of pederin can be found in the reservoir (unpublished data).

If fed with eggs containing pederin males sequester amounts of the substance much greater than that received maternally (Kellner and Dettner, 1995). As the males paired with (+)-females possess an elevated pederin content, they most probably have also ingested the substance. The eggs were accessible for some time after deposition and the males could have preyed on them. In view of the number of eggs laid by the females and the moderate pederin amounts found in the males (up to 4 µg), this increase is nevertheless rather slight. The males may not seek eggs very energetically but eat them by chance.

The confinement of pederin biosynthesis to the (+)-females of *Paederus* is unique in comparison with other defensive hemolymph compounds described in different beetle taxa. Adaline, for example, is produced by female and male ladybird beetles (Coccinellidae, de Jong *et al.*, 1991). In oedemerid beetles, cantharidin is biosynthesized by both sexes as well as the larvae (Holz *et al.*, 1994) whereas in most meloid beetles the males undertake the duty of cantharidin biosynthesis and the females get this substance during copulation to transfer it into their eggs (Sierra *et al.*, 1976; Carrel *et al.*, 1993; Dettner, 1997).

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