The digestive enzymes of some psammophilous tenebrionid beetles from South West Africa

by

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CONTENTS

1 INTRODUCTION

This investigation evaluates the role of terrestrial invertebrates in the process of degradation and secondary humification of litter. This process is known particularly in many temperate, humid and moderately humid environments but little is known about desert species. We have assessed the action of the digestive enzymes of some Namib Desert tenebrionid beetles. (*Onymacris plana* Per., *O. laeviceps* Gebien, *O. regatipennis Haag* and *Physosterna g. globosa* Haag), feeding on dead vegetation.

The natural history of these species is summarised below·

1.1 Onymacris plana

Lives on sand dunes and about perennial grasses and the Narras plant, *Acanthosicyos horrida* in the Namib Desert, south and west of the Kuiseb River (Koch, 1961, 1962; Holm and Edney, 1973). It is a common tenebrionid of dunes and less common in the flat interdune valleys and is sometimes found on the dune crests. It is mainly a summer species, though it is present all through the year. Strictly diurnal, it runs rapidly on the hot sand. It is omnivorous and feeds mainly on vegetal debris, but also on green plants and dead animals. In the summer it is present during intervals when it can maintain body temperatures below 44°C.

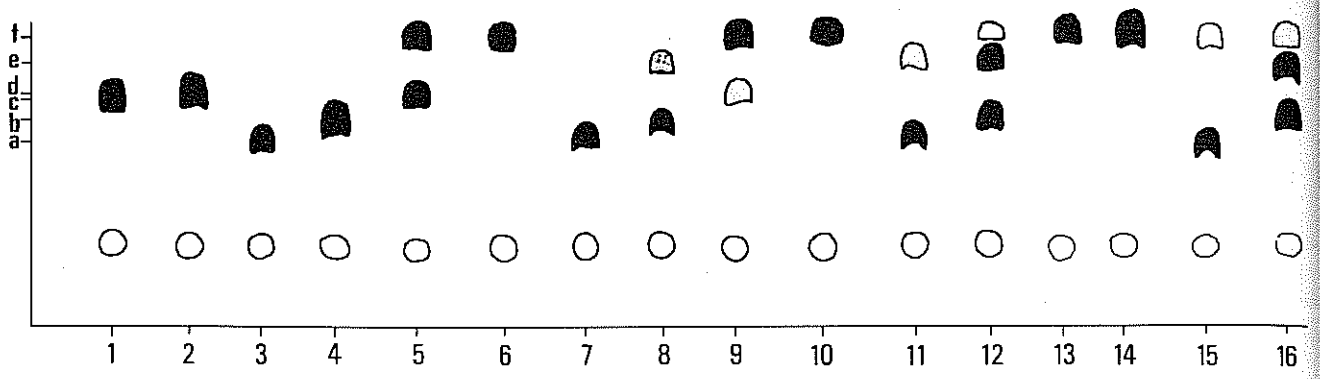
1.2 Onymacris laeviceps

Is also an inhabitant of sand dunes. It is diurnal to crepuscular and also mainly a summer species. Its habitat and food habits are similar to those of *O. plana*, although on the average this species spends more time foraging at the base of dune slipfaces. This insect occasionally climbs into the thorny grass *Stipagrostis sabulicola* and feeds on its seeds.

1.3 Physosterna globosa

This is a typical example of river inhabiting beetles on the margins of the Namib dunes. It is common along river-beds, floodplains and occasionally occurs on the gravel plains. In this respect it differs sharply from *Onymacris plana* which exclusively inhabits niches of the dunes. In some habitats *Physosterna globosa* and *Onymacris rugatipennis* are sympatric, e.g., the Kuiseb riverbed and the reddish sand, are consolidated and more vegetated marginal dunes at the eastern fringes of the central Namib. These are the typical habitats for *O. rugatipennis* and the closest habitats of *P. globosa* to the true Namib dune. The feeding habits of *P. globosa* have adjusted to the available organic material as have those of the dune species. All of them apparently feed on any debris available, from drifted and dry plant debris to dead animal materials. As the habitats of *P. globosa* and *O. rugatipennis* are richer in all these materials, food is more plentiful and the selection wider. Both *P. globosa* and *O. rugatipennis* are abundant in summer and strictly diurnal with an activity cycle similar to that of *O. plana*, which is not usually found in the river bed.

Figure 1: Thin-layer chromatography of reduced sugars



1-blank of cellobiose; 2-blank of maltose; 3-blank of melibiose; 4-blank of lactose; 5-*Onymacris plana* + cellobiose; 6-*Onymacris plana* + maltose; 7-*Onymacris plana* + melibiose; 8-*Onymacris plana* + lactose; 9-*Physosterna g. globosa* + cellobiose; 10-*P. g. globosa* + maltose; 11-*P. g. globosa* + melibiose; 12-*P. g. globosa* + lactose; 13-*Onymacris laeviceps* + cellobiose; 14-*O. laeviceps* + maltose; 15-*O. laeviceps* + melibiose; 16-*O. laeviceps* + lactose
a — melibiose; b — lactose; c — cellobiose; d — maltose; e — galactose; f — glucose.

Separation on KIESELGEL 60 buffered with 0.1 N boric acid. Solvent: butanol — acetone H_2O (40 — 50 — 10). Indicator: 20% sulphuric acid + 0.2% ethanolic solution of naphtoresorcinol (v/v) heated to 100—105°C for 5—10 minutes.

2 METHODS

For the extraction of digestive enzymes, the whole digestive tract was used. It was ground in a crucible with quartz sand and distilled in water at 4°C. We thus obtained both exo- and endoenzymes, contained in about 10 mg of fresh tissue per 1 cm^3 of water, which represents a high concentration of standard enzyme units.

In order to demonstrate the enzymatic action on oligo- and polysaccharides, Fehling's reagent was used for all non-reducing saccharides, whereas chromatography was employed for the reducing substances (Figure 1). Here we used ascending thin-layer chromatography, on plates of Kieselgel 60, buffered with 0.1 N boric acid and activated at high temperature. The solvent was butanol/acetone/water (40/50/10). Staining was accomplished by means of a solution of 20% sulphuric acid and 0.2% alcoholic solution of 1:3. dihydroxynaphthalene (naphtho-resorcinol) (v/v) and heated to 100—105°C for 5—10 minutes. For the determination of N acetyl-glucosamine, the product from splitting of chitin, the method described by Aminoff (1952) was used. For quantitative detection of the amino acids, obtained by the enzymatic attack from proteins, we used a method by which formaldehyde reacts with the amino groups so that the existing carboxylic groups can be titrated. For oils, beeswax and tributyrrin we used bromothymol-blue as an indicator. When enzymatic attack occurs the colour change shows an increase in acidity of the solution, owing to the formation of fatty acids. For the different kinds of cork (common cork, or *Quercus suber* cork, birch cork and maple cork) the production of suberic acid was taken into consideration, which gives the solution an intense dark colouration.

All the determinations were made by incubating the intestinal tract and the substrate for 24 hours at 37°C. For oligo- and polysaccharides a pH equal to that of the digestive extract of the different beetles was maintained (ranging from 5.2 to 5.8), since our previous research has shown that the optimal pH for the digestion of carbohydrates is slightly acid. In contrast, for proteases and esterases the determinations were done at pH 7.5.

3 RESULTS AND DISCUSSION

The results are presented in tables 1, 2 and 3.

As far as oligo-saccharides are concerned, both *Onymacris* and *Physosterna* show a remarkable digestive activity in comparison to all the assayed black beetles so far, some of which come from arid environments, such as Israel, Arizona, Sicily etc. (cfr. Marcuzzi and Turchetto 1975: Marcuzzi and Turchetto, in press). They seem therefore, to possess α- and β-glucosidases, fructosidases and α- and β- galactosidases, with the exception of *O. plana* and *O. rugatipennis*, which appear not to possess an α- galactosidase, since they do not split melibiose, while *O. rugatipennis* digestion does not even attack raffinose. These results are consistent with our previous observations on different tenebrionids, which are easily able to break down di- and trisaccharides.

The polysaccharides as a whole seem to be less well digested, except glycogen which is first broken down by an amylase and then by an α- glucosidase into glucose, as we have shown. Among the species considered, only *O. laeviceps* is able to utilise a broader alimentary system, compared with the other species, since it demonstrated an enzyme activity on all the polysaccharides we assayed. This species is able — within limits — to digest cotton, rayon and filter paper, which suggests the existence of an holocellulase. The ability of the animal to split several kinds of holocellulose in the presence of bacteriostatic substances, such as toluene and thymol, which were always included in the substrate, (already used with success by Zinkler 1971; Nielsen 1962; Parkin 1940; Ripper 1930, etc.) can be attributed to the presence of a true animal cellulase. The phenomenon has so far never been demonstrated in tenebrionids, and is, moreover, very rare in the animal kingdom (cfr — also Monsour & Monsour—Bek, 1934).

Among the proteins we assayed, only gelatin, salmin and albumine were consistently broken down by our species: *O. laeviceps* is able to break down elastine and sericine as well.

A remarkable digestive ability in *O. laeviceps* is also shown as far as oils, vegetal esters, beeswax and tributyrrin are con-

cerned. Only *O. rugatipennis* seems to possess very few esterases in which it differs from the other species.

We are not able at the present stage of research to say whether this feature can be correlated with ecology of the various species. Further research both in the laboratory and in the field should prove to be very rewarding.

TABLE 1: Enzymatic action on oligo- and polysaccharides

OLIGO- AND POLYSACCHARIDES

	Onymacris plana	Onymacris rugatipennis	Onymacris laeviceps	Physosterna g. globosa
TREHALOSE	++	+	+++	+++
SUCROSE	+++	+++	+++	+++
CELLOBIOSE	++	++	++	+
MALTOSE	++	±	++	++
MELIBIOSE	–	–	±	+
LACTOSE	±	+	++	++
ARBUTIN	++	+++	+++	+++
SALICIN	++	+++	++	++
MELLEZITOSE	+++	+++	+++	+++
RAFFINOSE	+	–	++	++
STARCH	–	–	±	+
GLYCOGEN	+++	+++	++	+++
COTTON WOOL	–	–	±	–
RAYON	–	–	±	–
GAUZE	+	–	+	+
CAROB	+	–	±	+
IVORY NUT	+	+	±	±
CHITOSAN	–	–	±	–
CHITIN	+	±	+++	–

Di- and trisaccharides, arbutin, salicin, starch and glycogen are pure substances; cotton wool is pure holocellulose; rayon is regenerated cellulose; gauze is hydrocellulose treated with H_3PO_4, (not present in nature); carob is a galactomannane extracted from carob seeds (*Ceratonia siliqua*); ivory nut is a mannane of *Phytelephas macrocarpa* seeds, consisting of mannane A (97.6%) and mannane B; chitin and chitosan were extracted from the beetles' elytra.

TABLE 2: Enzymatic action on proteins

PROTEINS

	Onymacris plana	Onymacris rugatipennis	Onymacris laeviceps	Physosterna g. globosa
GELATIN	+++	+	+	+
CASEIN	–	–	–	–
SALMIN	+	+	++	+
ELASTIN	–	–	±	–
FIBROIN	–	–	–	–
SERICIN	–	–	±	–
ALBUMIN	++	++	+++	++

All chemically pure substances were used with the exception of elastine (prepared and purified by the Institute of Anatomy of this University), fibroin and sericin (obtained from silk worm cocoons by means of a prolonged ebullition). The gelatin we used is that employed in photogrnphic plates: these are exposed to digestion and then developed to reveal the degree of enzymatic attack on the gelatine.

TABLE 3: Enzymatic action on oils and esters

OILS AND ESTERS

	Onymacris plana	Onymacris rugatipennis	Onymacris laeviceps	Physosterna g. globosa
OLIVE OIL	+	±	+++	+
GRAPE SEED OIL	+	±	+++	+
CORN OIL	±	–	+	–
PEANUT OIL	±	–	–	±
SUNFLOWER OIL	+	–	++	±
SOY BEAN OIL	±	–	++	±
LINSEED OIL	±	–	±	±
CASTOR OIL	–	–	±	–
OAK CORK	±	–	±	±
BIRCH CORK	±	–	–	–
MAPLE CORK	±	–	±	–
BEES WAX	±	–	±	±
TRIBUTYRRIN	+++	+++	+++	+++

We used all pure, refined oils; the corks are raw, natural substances; the beeswax is crude i.e. a complex mixture of cerotic, melissic and palmitic acids, linked with melissic and cetilic alcohols.

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