Duplicate tubes contain 0.1 ml of sperm suspension were placed in boiling water for 1 min to stop their metabolism. 0.5 ml of buffer (glycylglycine-semicarbazide 0.1 M, pH 10) containing 0.05 mg NAD and 0.10 mg lactic dehydrogenase (from rabbit muscle) was added to the solution. Then it was incubated at 37 °C for 90 min. Finally, 4.4 ml of NaOH (with EDTA) was added. The fluorescence of the sample was read in an Aminco Bowman Spectrophotometer with excitatory light at 340 nm and set to pass emitted light at 460 nm. In all cases, parallel samples containing pure lactic acid were read.

Results and discussion. The histogram shows the motility of both control and anaerobic cells. Irrespective of the method employed, concordant results were obtained from a total of 13 experiments. The histogram shows the results of 8 experiments using capillary tube method. No differences between the 2 batches were observed when the data mentioned above were studied comparatively.

The production of small, but measurable amounts of lactic acid by anaerobic spermatozoa, demonstrated the existence of an active glycolytic pathway (table 1).

The anaerobic motility of washed spermatozoa and the presence of lactic acid in anoxia would demonstrate that the energy for motility in anaerobiosis, depends neither upon anaerobic oxidative processes nor upon the utilization of extracellular glycolyzable substrates, but probably upon the anaerobic utilization of endogenous substrates, such as glycogen. Anderson and Personne[13] located this polysaccharide in the residual cytoplasm of amphibian spermatozoa and, more recently, the presence of glycogen in the nucleus acrosome and middle piece of *R. clamitants* was cytochemically and morphologically demonstrated by Poirier[14].

In aerobiosis, the oxygen consumption varies between 2.6 and 4.2 µl $O_2/10^8$ cells/h at 30 °C (table 2). From results obtained in current experiments, it could be observed that the Z_{O_2} of *B. arenarum* spermatozoa are substantially changed when cells are incubated with substrates from Krebs' cycle such as succinate, or metabolites which alter oxygen consumption e.g. rotenone, dinitrophenol, oligomycin, antimycin, etc.[5]. These values were found to be below those reported for other species. Furthermore, a Z_{O_2} of 6.0 has been reported for *Echinus esculentus* at 15 °C, while for bull, cock, rabbit and ram Z_{O_2} values of 21, 7, 11 and 22, respectively, have been reported. Spermatozoa removed from *Loligo pealli* spermatophores exhibited a Z_{O_2} of 10 at 20–25 °C. The oxygen consumption of *B. arenarum* is higher than *Balanus balanus* spermatozoa, which was reported as only 0.18 µl $O_2/10^8$ cells/h at 10 °C[16].

According to the results of this first approach to the study of *B. arenarum* spermatozoa, it can be concluded that there are, in the cell, operative oxidative-glycolytic pathways which utilize endogenous substrate. Peterson and Freund[17] have demonstrated that the oxidative metabolism in human spermatozoa is less effective than glycolysis in maintaining ATP cellular levels or in supporting sperm motility. In the absence of glucose, ATP level declines concomitantly with a motility loss, while in the presence of glucose, ATP level remains constant for several hours and the decline in motility is low. These cellular levels of ATP did not maintain motility either when incubated with succinate, which is utilized at high rates, or with pyruvate, which is utilized at lower rates.

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Comparison of sterols from a phytophagous and predacious species of the family Coccinellidae

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Summary. The sterols of a phytophagous and a predacious species of the family Coccinellidae were found to be quite different from each other, indicating that adaptation to different diet regimens is reflected in the utilization and metabolism of dietary sterols.

The Mexican bean beetle, *Epilachna varivestis* Mulsant, which is one of a limited number of plant feeding insects in the otherwise predominantly predacious family Coccinellidae, was recently found to have unique pathways and final products of sterol utilization and metabolism[2,3]. This insect reduces most of its dietary C_{28}- and C_{29}-Δ^5-plant sterols to stanols, which are then dealkylated to the C_{27}-stanol cholestanol. Significant quantities of Δ^7-cholestenol (lathosterol) are then formed, and little, if any, of the plant sterol is metabolized to cholesterol. Saturated sterols (stanols) comprise 50–75% of the total sterols of this insect, but Δ^5-sterols, including cholesterol, are only minor sterol components in all stages of the Mexican bean beetle. To determine whether this unique pattern of sterol utilization and metabolism is characteristic of coccinellids in general or is peculiar to this particular phytophagous species, we compared the sterols of the Mexican bean beetle with those of a predacious coccinellid *Coccinella septempunctata* (L.), that feeds on phytophagous insects (aphids).

Mexican bean beetle adults were obtained from larvae reared as previously described[2] in 1-gal glass jars on bouquets of leaves of Clark variety soybean, *Glycine max*

(L.) Merr. Adult *C. septempunctata*, that had fed on aphids, were field-collected from a wild population in New Jersey. The sterols from both species were obtained by the same extraction and purification procedures used previously for the Mexican bean beetle studies[4]. The total sterols were purified, after saponification of the crude lipid extracts, by column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland)[5], and the sterol fractions from the columns were subjected to digitonide precipitation for further clean-up. The sterols were acetylated (in pyridine: acetic anhydride, 1:1), and the saturated and unsaturated sterol acetates were separated by chromatography on 3-g columns of 20% $AgNO_3$-impregnated Unisil as described previously[2]. Monitoring aliquots of these column fractions by chromatography on 20% $AgNO_3$-impregnated silica gel H chromatoplates developed in benzene: n-hexane (1:1) revealed the presence of a large amount of saturated sterol acetates from the Mexican bean beetle samples, but only very small quantities of saturated sterol acetates from *C. septempunctata* samples. Most of the sterol acetates from *C. septempunctata* behaved as Δ^5-sterol acetates on the chromatoplates, but a noticeable amount of sterol acetate from this insect was much more polar than the Δ^5-sterol acetates, and migrated similarly to 7-dehydrocholesterol acetate. A UV-spectrum, taken in methanol, of a sample of sterol acetate from *C. septempunctata* was typical for a sterol with a $\Delta^{5,7}$-diene system (shoulder at 264 nm, peaks at 272, 282 and 294 nm), thus providing further evidence for the presence of 7-dehydrocholesterol in this insect.

Also the sterol acetate fractions from argentation column chromatography of samples from both species were analyzed and quantitated by gas liquid chromatography (GLC). The results of the identification of the *C. septempunctata* sterol acetate fractions on 2 GLC systems are listed in table 1. The sterol content of Mexican bean beetle adults was thoroughly discussed in an earlier paper[2].

Table 1. GLC analyses of sterol acetates prepared from sterols of *Coccinella septempunctata*

Compound	RRT* on 1.0% OV-17		RRT on 0.75% SE-30	
	Standard	Insect	Standard	Insect
Cholesterol acetate	3.64	3.64	2.67	2.66
Cholestanol acetate	3.65	3.63	2.72	2.71
7-Dehydrocholesterol acetate	4.22	4.22	2.97	2.96
Campesterol acetate	4.83	4.87	3.49	3.43
Campestanol acetate	4.87	4.83	3.55	3.49
Stigmasterol acetate	5.29	5.28	3.78	3.83
Stigmastanol acetate	6.07	5.96	4.47	4.44
Sitosterol acetate	6.06	6.04	4.35	4.37

* Retention time relative to cholestane.

Table 2. Comparison of sterols of adults of the Mexican bean beetle and *Coccinella septempunctata*

Sterol	Mexican bean beetle	*Coccinella septempunctata*
Cholesterol	4.5*	46.4*
Cholestanol	50.7	1.3
Δ^7-Cholestenol	11.8	–
7-Dehydrocholesterol	–	3.7
Campesterol	T**	12.2
Campestanol	6.0	0.6
Δ^7-Campestenol	2.0	–
Stigmasterol	1.4	5.5
Stigmastanol	20.3	0.8
Sitosterol	2.3	29.5
Δ^7-Stigmastenol	1.0	–
Total saturated sterols	77.0	2.7

* Relative percent of total sterols. ** Detectable trace.

The relative percentages of sterols found in both the Mexican bean beetle and *C. septempunctata* are listed in table 2. The sterols of the latter insect are largely those that would be expected from a predacious insect feeding on its normal diet of phytophagous insects that dealkylate phytosterols: Cholesterol comprised nearly half (46.4%) of the total sterol; sitosterol, campesterol and stigmasterol accounted for 29.5, 12.2 and 5.5%, respectively. However, all the saturated sterols taken together were only 2.7% of the total sterols of this insect. In the case of the Mexican bean beetle, 77% of the sterols consisted of the stanols cholestanol, campestanol and stigmastanol, whereas the combined Δ^5-sterols typically found in insects, such as cholesterol, campesterol, stigmasterol and sitosterol, were only 8.2% of the total sterols. In previous studies[3], the Mexican bean beetle was shown to produce a significant amount of the Δ^7-sterol lathosterol, and this was the first report of this sterol as a major sterol in a phytophagous insect. From the data of the present study, we cannot determine whether the larvae or adults of *C. septempunctata* biosynthesize the 7-dehydrocholesterol found in the adult sterols or whether it might have come from the prey of this insect. In any case, the level of 7-dehydrocholesterol (3.7%) was higher than in most insects (usually it is found only in trace amounts). However, a number of insects are able to incorporate the Δ^7-bond into various sterols[6], and 2 insects have been shown to require a dietary source of a Δ^7-sterol[7,8]. The importance of the Δ^7-bond in insect sterol metabolism arises from the fact that all insect molting hormones isolated to date contain a Δ^7-bond and Δ^7-sterols have been implicated as precursors of these steroid hormones in insects[9].

If, as we had previously postulated[3], phytophagy arose secondarily in an insect such as the Mexican bean beetle, which was originally predacious, as are most lady bug beetles (Coccinellidae), then the sterols found in *C. septempunctata* could well represent the normal sterol pattern for these predacious species. They seem to be largely a reflection of the dietary sterols derived from their prey, with perhaps the exception of 7-dehydrocholesterol, which could quite likely be produced by this predator. Studies of related species with differing feeding habits such as these phytophagous and predacious species of the family Coccinellidae provide an excellent source of information concerning biochemical and physiological adaptation in relation to phylogeny and speciation within related groups of insects.

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