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ANALYSES OF MOLLUSCAN SHELLS BY THE DERIVATOGRAPHIC FINGERPRINT METHOD

(Figs. 1–19)

Abstract: Recent and fossil molluscan shells have been analysed using the comparative derivatographic method. It has become manifest that a record taken with an appropriate programme and sensitivity can reproducibly reflect the shell properties carrying taxonomic specificity. Due to the heterogeneity of the organic and inorganic components of the shell structures, the thermoanalytical part-processes are difficult to interpret separately, however, taken all together, they can yield a charactetistic „fingerprint". The analysis of „derivatographic fingerprints" is effectuated by the comparison of DTG and TG relations in a co-ordinate system. Such an analysis of molluscan shells may yield possibilities for the evolutional phylogenetic evaluation of recent species, of the determination of the fragmentary material of fossil species.

Резюме: Автор сравнительным дериватографическим методом анализировал современные и ископаемые *Mollusca*-раковины. Стало ясным, что приготовленный соответствующей программой и чувствительностью снимок воспроизводимо отражает свойства, обозначающие системную специфичность раковин. Из гетерогенности органических и неорганических компонентов, образующих структуру раковин, следует, что термоаналитические частные процессы могут трудно объясняться отдельно, но они в совокупности дают характерный отпечаток (фингерпринт). Анализу «дериватографического отпечатка (фингерпринта)» служит сравнение ДТГ-, ТГ отношений в системе координат. Таким анализом *Mollusca* раковин, может быть, дается возможность для эволюционно-филогенетической оценки современных видов или для определения обломков ископаемых видов.

1. Presentation of the problem

The soft parts of Pelecypods and Gastropods (apart from a few specific groups) are covered by a hard shell made up of inorganic and organic substances. Out of this organic and inorganic complex of substances let us select one protein component of the shell: conchioline.

The rapid development of molecular-biological, comparative biochemical research in the last decades has led to the final elucidation of this biochemical product. J. Roche et al. (1951), S. Tanaka and H. Hatano (1953), S. Tanaka et al. (1960), C. Gregoire et al. (1955), A. Beedham (1958), K. A. Piez (1961), P. E. Hare (1962, 1963, 1965), P. E. Hare and P. H. Abelson (1964, 1965, 1967), P. E. Hare and R. M. Mitterer (1966), E. T. Degens and S. Love (1965), E. T. Degens and R. H. Parker (1965) have cleared up on a varied range of samples the structure of conchioline incorporated in the solid material of the shell. The method applied by these workers is based on the qualitative and quantitative comparison of amino acids, constituents of protein molecules. The method, the so-called amino acid spectrum analysis has supplied information about substances, different from the so

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far known shell proteins, and carrying specificity within the taxonomic unit. This indication of taxonomic specifity has also been proved by other procedures. Thus, for example, the conchioline isolates investigated through X-ray diffraction analysis by K. M. Wilbur an N. Watable (1963) showed particular properties characteristic of species. C. Gregoire (1957, 1958, 1959, 1960) in electron microscopic studies on decalcified conchioline, revealed its submicroscopic tissue-struture, and reffered to the latter as giving characteristic patterns for each particular taxonomic unit.

Parallel with these were performed general physiological investigations of molluscan shells. R. F. Sognnaes (1960, 1964) in comprehensive studies describes the calcification processes of biological systems, i. e. shell formation and regeneration.

Thus, a sequence of assumptions has been drawn up concerning the shell: shell protein is a genetically programmed unit, as a templete, which its free acid and basic groups provides the bonding positions for inorganic anions and cations, thus creating the inumerable varieties of shell-building structures, determining the visible morphology of the whole shell. Very naturally, in our days more and more efforts are being made to approach evolutional systematic problems, to make decisions on particular developmental relations not by way of morphological analysis, but through primary investigations on conchioline. E. T. Degens and H. Schmidt (1966), E. T. Degens, D. W. Spencer and R. H. Parker (1967), M. T. Ghiselin et al. (1967) have succeeded in many cases to clear up or even to revise sequences of evolution by the latest method of shell amino acid spectrum analysis by computerized factorial analysis.

Like any kind of neo-systematic knowledge on evolution which is rooted in, and derived from paleosystematics, studies of molecular evolution can also be essentially supported by paleobiochemical data. Parallel with the previous ideas was raised the claim to extend investigations to fossile sample material. Pioneering paleobiochemical investigations on conchioline are linked with the name of P. H. Abelson (1964a, b, 1955, 1956, 1957a, b, 1959, 1962), who was first to prove that the decomposition products of proteins, oligopeptides, peptides, amino acids can be traced in non-recrystallized, non-dissolved fossil shells, not having undergone thermal or bacterial decomposition. The above-listed workers, nearly all of them, parallel with recent samples have assessed the possibilities on fossil samples as well. Pondering the complex character of the methods applied by them, the laboriousness of the particular tests, there arose the necessity of selecting and elaborating a procedure capable of giving possibility to simplify the process of determination.

As is known, the thermoanalytical examination of a heterogenous system consisting of organic and inorganic components can give a realistic view of the physical and chemical properties of the system. A very good example to prove this claim was given by M. Berényi's work (1967), in which the derivatographic analysis of urinary calculuses as heterogeneous biogenic systems was elaborated. When studying the results obtained by F. Paulik and L. Erdey (1958, 1960, 1968) with home instrumentation, on the basis of the derivatographic method through uniting the methods of thermogravimetry (TG), derivato-thermogravimetry (DTG), differential thermoanalysis (DTA) and thermodilatometry (DT), the author was led to the basic assumption that a derivatogram of the molluscan shell taken with an appropriate programme and sensitivity, will display, in the aggregate, characteristic DTA, DTG, TG, and TD relations, due to the complex, specific-heterogeneous character of the shell even if the part-processes are should not be separated.

II. *Elaboration of the method, investigation of recent and fossil molluscan species*

II. 1. Selection of sample material

The sample material was selected from the phylum *Mollusca*, class *Lamellibranchiata*, although a few analyses have been carried out on recent gastropod shells, too. This decision is supported by the following reasons:

a) lower number of species among pelecypods
b) greater mass, so better preservation of shell material
c) simpler structural properties

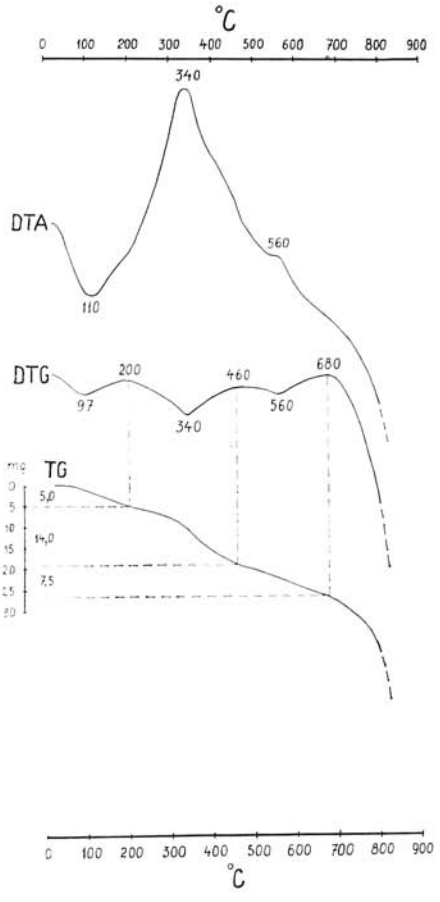
and the fact that the microscopic structural analyses of the shells have been summed up by several authors (O. B. Bøggild 1930; J. J. Oberling 1964; I. D. Taylor et al., 1960). During the investigations 26 recent and 34 fossil species were studied, the number of investigations was raised to 250 by repetition and by parallels from average samples.

Corresponding to the reconnaissance character and phylogenetic aims of the investigations, species of various toxonomic positions, from various ages and localities were selected. Denomination of the species under study, age- and derivational data are given in the figure captions.

II. 2. Description of the method

When examining fossile samples, nothing but well-preserved, non-recrystallized ones, not permeated by ferric or silica solutions were analysed. To reach this aim and to detect the structural constitution, the transversal cross section of the shells was microscopically examined, the mineralogical composition was analysed by IR spectroscopy (Gy. Szöör 1969). The organic-chemical structure was analysed by aminoacid spectra (Gy. Szöör 1967), the regularities of trace element content by quartz spectrography (Gy. Szöör 1971a).

Preparation of the shells for derivatography was performed with a view to spectrographic purity. Sediments attached to the shell and the periostracal layer were removed by soaking in 6n NaOH, and washing with 2n HCl, then rinsing with excess amount of distilled water. Removal of the periostracal layer is highly essential. Informational derivatograms indicated that the periostracal layer left on the shells makes the congruent derivatographic images of more than one individual of one species fairly uncertain. The thickness and chemical state of the periostracal layer is different for the individuals of a living species. It depends on individual age and the mechanical, physico-chemical effect of the biotope. In the case of a recent shell, starting on the way of fossilization, the influence of the environment is amplified by an absolutely uncalculable variety of effects and, it is only chance that determines the state of the periostracal layer of an individual cast ashore and included in the sample material of the museum. This fact and the circumstance that it was only the rarest case that a periostracal layer was found on fossils from older series — recent species only served as reference material — may well account for the complete removal of the layer. Washing with distilled water was followed by drying in air current at 60 °C and then in an exsiccator. The material was ground to a grain size range below 0,06 mm in diameter in an agate mill (Type Fritsch, temperature rise was 1°/hour). For each derivatogram the whole shell material was worked up, and an averaged sample was



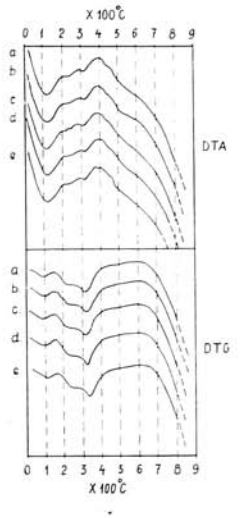


Fig. 2. Derivatograms of individuals of *Cardium edule* (L.) from five different places. a — Varna, b — Galata, c — Burgas, d — Rijeka, e — Dubrovnik.

Fig. 1. The DTA-curve (galvanometer deflection) changes at various sensitivities. Dotted line = Al_2O_3 in the sample holder, Continuous line = shell of *Tridacna elongata* (L.) in the sample holder.

used. Trying a number of possible programmes and sensitivity relations. the derivatograms of the samples were taken with the following programme:

Weighing: The measured weight of the samples varied between 1.5—2.0 g, and in the majority of cases was about a mean value of 1.85 g. The measured weight of inert Al_2O_3 was 1.85 g. (The basic courve of the instrument was also taken with this amount, using Al_2O_3 in both sample holders.)

During measurements the volume and compaction of inert Al_2O_3 were identical with those of the material under study. Crucible: platinum crucible No II. Sensitivities: T = 900 °C (measured in sample), TG = 1/2 sensitivity, DTA = 1/2 sensitivity.

Heating: in furnace No 1. Starting voltage: 90 Volts. Spindle diameter: 15 mm, large disc. Position of nails: third row. Position of drum and heating gearbox: 100'. Oven atmosphere: air, under quartz cup, without exhaustion. Heating rate: 10 °C/min. The T °C scale was calibrated by $\alpha\, SiO_2 \leftrightarrow \beta\, SiO_2$ conversion.

All through the investigations special care was taken that the preparation and derivatographic treatment of each sample be identical to the minutest detail. Thus, identical were the grain sizes, compaction, the crucible, the oven, the application of the

quartz cup, the programme and the sensitivity conditions, temperature of the laboratory. So, it was discovered that, corresponding to its high sensitivity, there was a considerable bend in the DTA base curve of the instrument. On the other hand, choosing 1/2 DTA sensitivity, the hidden thermoanalytical processes, too, manifested themselves clearly. This phenomenon is demonstrated in Fig. 1. Here the changes in the DTA base curve of the instrument are compared with the run of the DTA curve of the shell of the recent species *Tridacna elongata* (1) as a function of varying DTA sensitivities. Well visible is the base-line deformation due to the asymmetric conditions of the oven. In the case of the shell, on the other hand, using the generally applied 1/20, 1/15 and 1/10 DTA sensitivities, it does not appear. At higher sensitivities, however, there is at 450 °C an endothermic minimum.

When establishing the method, it had to be primarily cleared up whether or not there are differences between the ground shell material of one species taken from different localities. Fig. 2 represents derivatograms of individuals of the recent pelecypod species *Cardium edule* (L) from five different places. Sample *a* comes from Varna, sample *b* from Galata, *c* from the district of Burgas on the Bulgarian coast, sample *d* from Rijeka and *e* from the district of Dubrovnik on the Adriatic coast. The samples were collected at different times, thus they displayed different ages and fossilization histories. This is further supported by the differences in thickness of the periostracal layers and the different sizes of the valves. In spite of all, these the derivatograms of shells previously deprived of the periostracal layer, were approximately identical. (Similar comparisons were made with individuals of *Anodonta cygnea*, *Unio pictorum*, *Mytilus edulis*, with near-identical results in each case.) Analysing the derivatograms, there was a ± 5 °C deviation between the minima and maxima of the DTA, DTG curves.

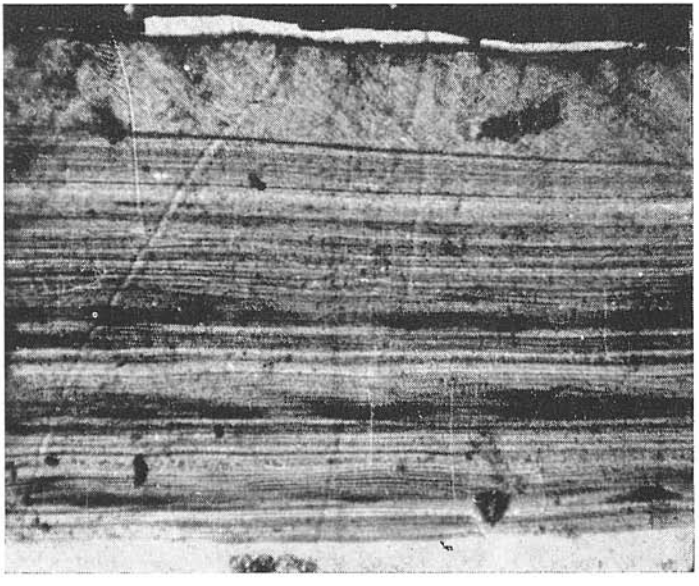


Fig. 3. A microscopic picture of periostracal-, homogeneous calcite-, homogeneous aragonite layer of *Mytilus edulis* (L.) in transmitted light, parallel nicols, 25 ×.

In the following I have proved that the units of the shell-building structure can be identified not only by optical (O. B. Bøggild 1930; J. J. Oberling 1964; I. D. Taylor et al., 1969) or aminoacid spectra (K. A. Piez 1961; P. E. Hare 1963), but by the derivatographic procedure, too. In the course of the experiments, as sample material the shells of those genera were used in which the separate layers were not entangled and could be separated mechanically by splitting (*Unio, Anodonta, Meleagrina, Mytilus*). As an example, the shell of *Mytilus edulis* (L), is built up of the periostracal-, a homogeneous calcite- and a homogeneous aragonite layer (Fig. 3). These differences due to dissimilar physico-chemical structures are well demonstrated by derivatograms taken of the whole shell (Fig. 4) and of the separated structural units (Figs. 5 and 6).

After this procedure, when comparing derivatograms of the ground shell material of a number of recent mollusc species, the assumption was made that the records

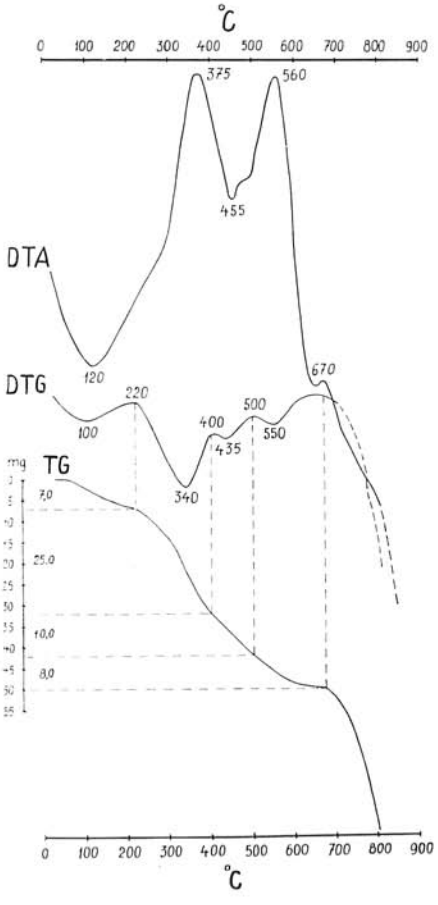


Fig. 4. Derivatographic fingerprint of whole shell of *Mytilus edulis* (L.).

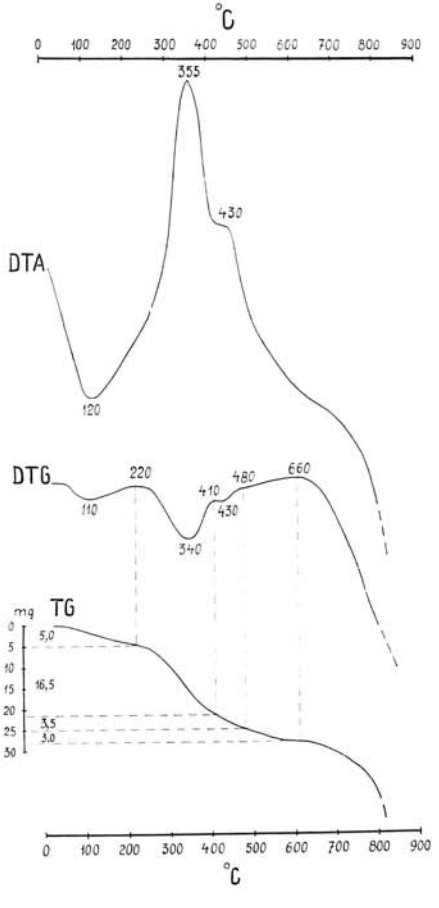


Fig. 5. Derivatographic fingerprint of separated homogeneous calcite layer of *Mytilus edulis* (L.).

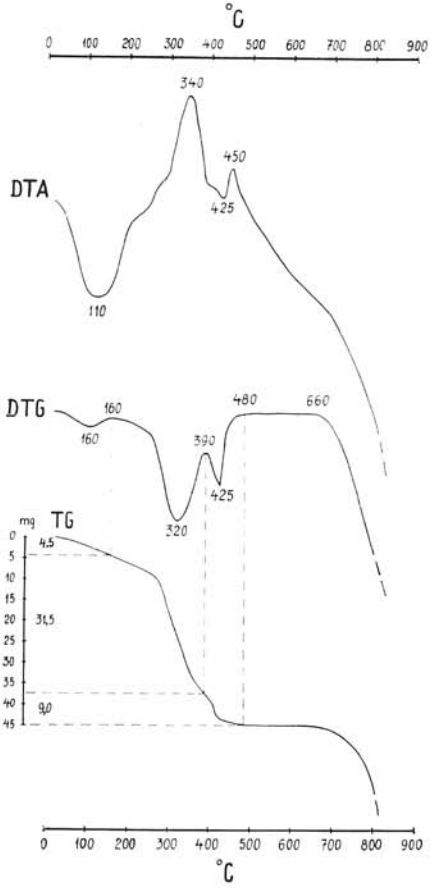


Fig. 6. Derivatographic fingerprint of the separated homogeneous aragonite layer.

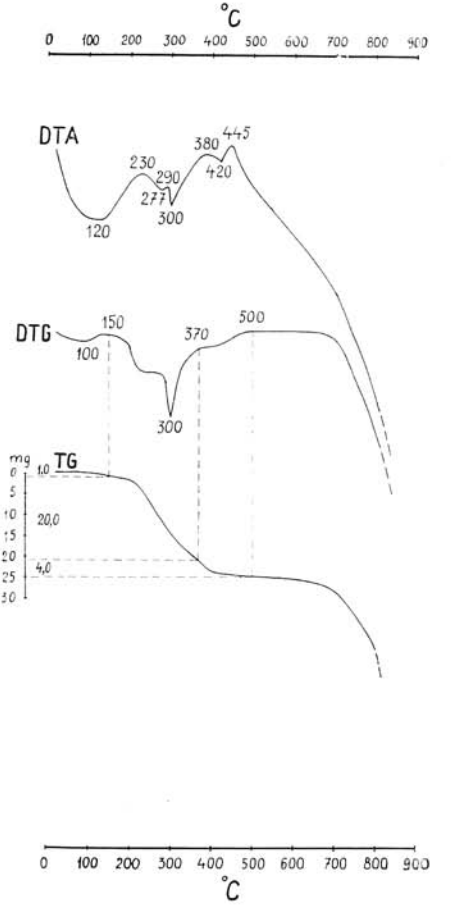


Fig. 7. Derivatographic fingerprint of *Glycymeris pilosus* (L.).

can well represent taxonomic specificity down to species level, or if there is a way for the separation and separate analyses of structural units, to subspecies level (Gy. Szöör 1969).

There is no possibility here to present the derivatograms of all studied recent species, however, the statement can be illustrated well by several examples. Figs. 7, 8, 9 show the derivatograms of species belonging to the order of *Taxodonta* and the family of *Arcidae*. Figs. 10 and 11 give the derivatograms of species of the order *Anisomyaria* and family *Pectinidae*, Fig. 12 that of a species representing the family *Ostreidae* of the same order. Fig. 13 shows the image of a *Murex* species. All species came from the sandy deposit of the Yugoslavian coast and belong to the cenosis of the shelf. Not going here into a detailed analysis of thermoanalytical relations, when comparing the separate figures, it is obvious that all species have different derivatograms, the thermoanalytical character of species of identical genera is similar, but greatly different from

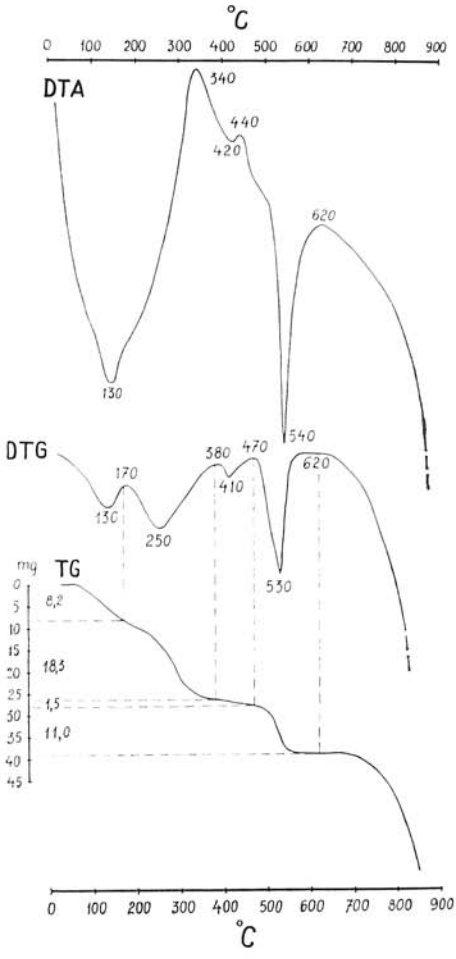


Fig. 8. Derivatographic fingerprint of *Arca noae* (L.).

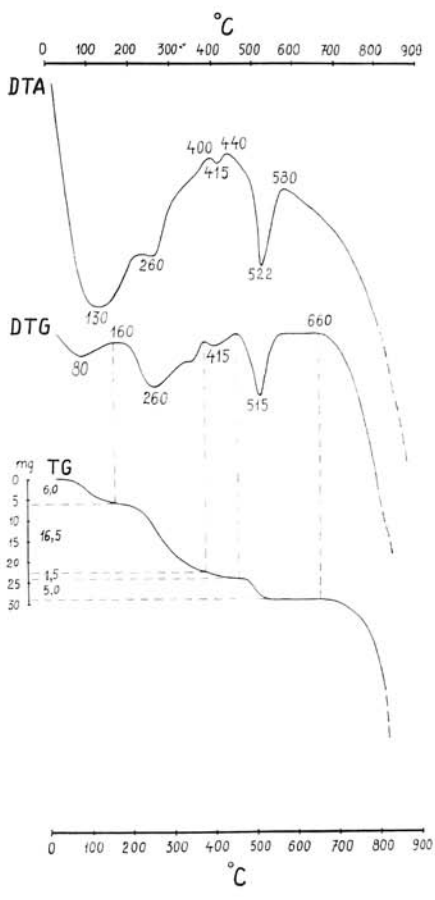


Fig. 9. Derivatographic fingerprint of *Arca barbata* (L.).

that of species representing other genera. This similarity and difference is more markedly manifested surveying higher taxonomic units. Let us compare the records of *Ostrea* and *Pecten* species, both built up mainly of calcite and displaying similar base structures. This example not only illustrates such diagnostical possibilities of the shells, but calls attention to the fact that differences may be due not only to the variety of inorganic structures but to the differences of the organic material.

II. 3. Evaluation of thermoanalytical processes

The thermoanalytical reactions taking place in the course of decomposition are very complex, the particular processes are difficult to differentiate. This is primarily due to the complex heterogeneous inorganic-organic composition of the shells, and can

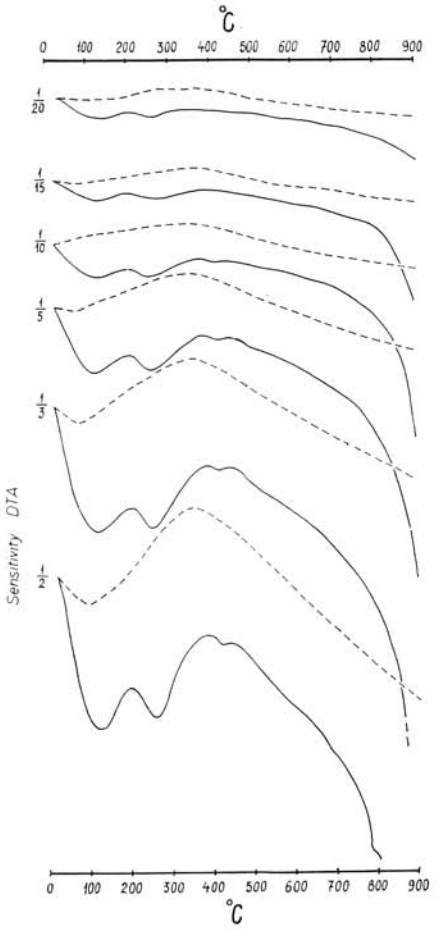


Fig. 10. Derivatographic fingerprint of *Pecten jacobeus* (L.).

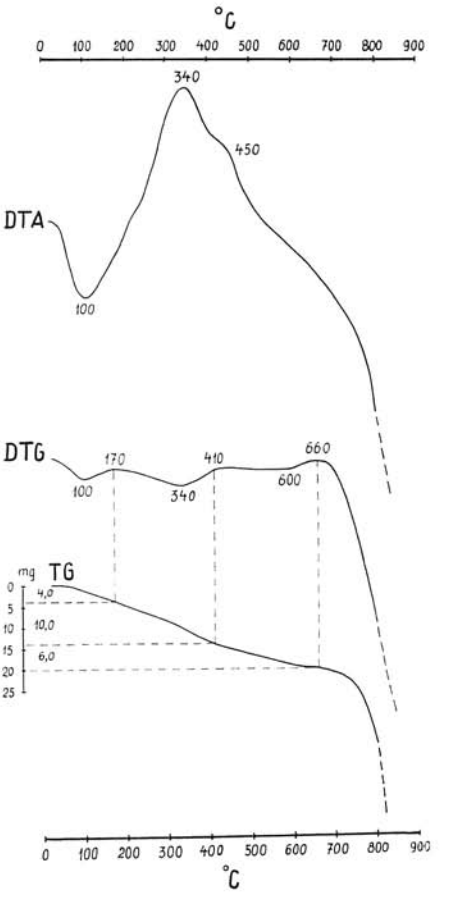


Fig. 11. Derivatographic fingerprint of *Pecten irradians* (L a m.).

be deduced from the following methodological consideratins. Heating of the samples was carried out in platinum crucibles at high compaction: in such circumstances organic material does not burn so fast. a kind of cracking takes place.

The samples examined in our experiments were tested by M. B e r é n y i (1969). Heating them in platnum crucibles n a different apparatus. he obtained identical result (thus proving the reproducibility of the procedure for DTA. DTG relations). However. using a platelet-type sample-holder. the DTA relations got markedly simplified and a great number of slighter effects did not manifest. Current measurements with corundum crucibles seem to prove that with platinum crucibles one has to reckon with the catalytic effect of the platinum sample holder. It is clear from the foregoing that the actual interpretation of changes taking place during pyrolysis can only be effected in the course of a longer series of experiments. destined

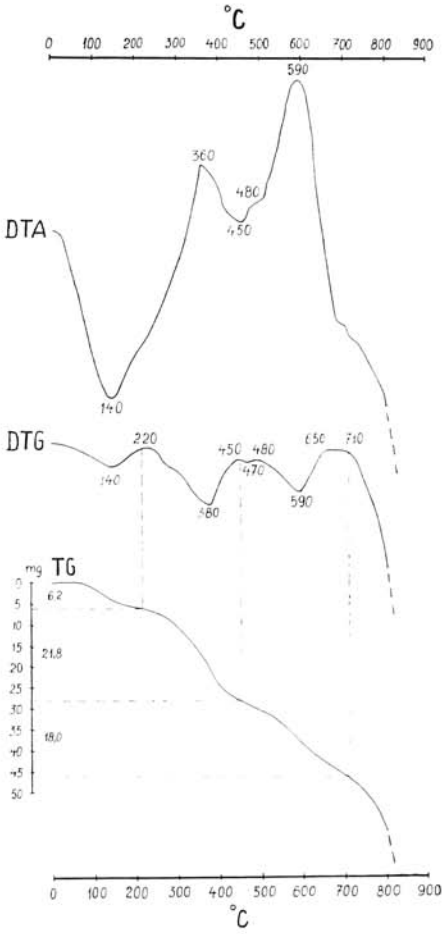


Fig. 12. Derivatographic fingerprint of *Ostrea edulis* (L.).

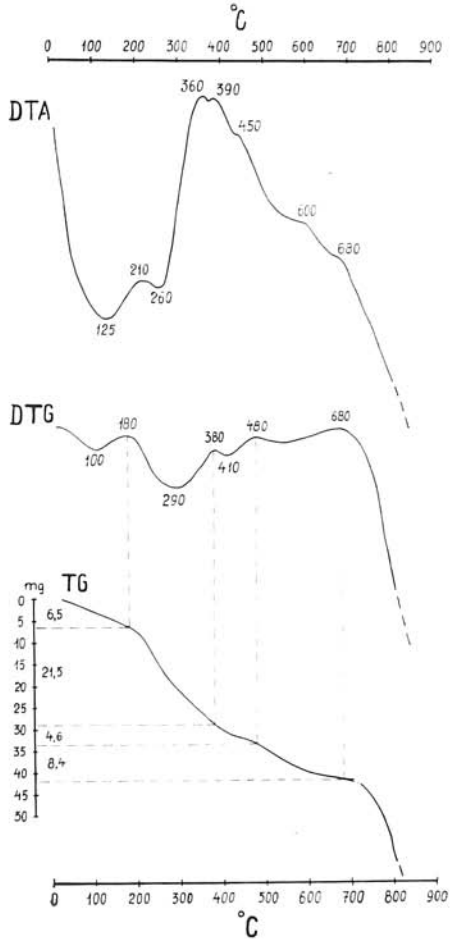


Fig. 13. Derivatographic fingerprint of *Murex* sp.

to solve this very problem. However, it seems necessary to make public the assumptions so far reached. Analysing the derivatographic fingerprints of several recent and fossil molluscan species, under the given experimental conditions, the following thermoanalytical processes can be taken into account (G y. S z ö ő r 1969).

a) *Endothermic reactions involving loss of weight*

1. Loss of adsorptive water in the temperature range of 20 °C—200 °C, which can be observed in all the samples under study.

2. Loss of water bound colloidally on the surface of organic macromolecules. According to M. B e r é n y i (1969) it can be assumed that it is in connection with the carboxylation and deamination of „free" amino acids having decomposition points within this range (glutamic acid = 177 °C, aspartic acid = 212 °C, glycine = 185 °C,

etc.) which can be observed with the families *Arcidae*, *Cardiidae*, *Veneridae* and some species of *Gastropoda*.

3. Loss of water trapped between the aragonite lamellae (J. D. Hudson 1967) within wide temperature limits, from 300—500 °C. It can be observed for all aragonite-containing Lamellibranchiates. This process in all cases coincides with the endothermic minima denoting the irreversible conversion of aragonite → calcite.

4. Unknown endothermic changes involving loss of weight in the temperature range of 500—600 °C. It is markedly apparent within the genus *Arca*.

b) *Exothermic processes involving loss of weight*

1. Slow burning process in the temperature range of 340—375 °C, due to the decomposition of organic substances.

2. Exothermic changes in the temperature range of 500—700 °C in some cases abrupt in other cases gradual according to the amount of organic material (*Ostreidae*, *Pinnidae*, *Mytilidae* families).

3. Hardly noticeable, but reproducible exothermic changes, the cause of which has not been cleared up, possibly denoting the burn-out of built-in organic pigment materials of the shell. (Noticed with species of *Murex*, *Mytilus*, *Pecten*.)

c) *Structural change*

According to G. T. Faust (1950), M. Subba Rao and S. R. Yoganarassimhau (1965) the irreversible aragonite → calcite conversion takes place between 400 °C—500 °C, depending on the presence of contaminating cations. With all the investigated molluscan species the aragonite → calcite conversion took place in this temperature range.

d) From 700 °C upwards the $CaCO_3$ content of the shell is decomposing with the release of CO_2.

The correlative nature of these changes, the presence or absence, strength or hidden character of the separate processes account for the differences in the derivatograms of the particular species. The derivatograms, since the part-processes can only be approximatively interpreted, but in their allover aspect are reproducible and characteristic of the shell material, may be called „derivatographic fingerprints".

II. 4. Comparison of DTG — TG data in system of co-ordinates

An analysis of DTG and TG values, based on the following considerations is suitable to verify the statement so far made and to allow group comparisons of taxonomic character. The thermal decomposition was performed in the 20 °C—900 °C temperature range. The most specific signals from this range were received from the 200 °C—700 °C interval, since from 20 °C to 200 °C the adsorbed water content (marked by Va) is released, which is different for each group according to the environment and antecedents.

Above 700 °C $CaCO_3$ decomposes into CaO and CO_2. Within the range of 200 °C to 700 °C takes place the decomposition of specific proteins and the specific structural elements determined by the former. The mg data of the TG curve were recalculated to weight per cent values and arranged by being assigned to temperature ranges determined by the minimum values of the DTG curve (Gy. Szőr 1969). These intervals are: 200 °C—300 °C, makred as C_1 weight per cent, 300 °C—400 °C, marked as C_2 weight per cent, 400 °C—500 °C, denoted by O_1 weight per cent, 500 °C—700 °C marked O_2 weight per cent. Thus the $C_1 + C_2 + O_1 + O_2 = \Sigma$A-Va weight per cent value denotes all the bound-material loss characteristic of the 200 °C-700 °C temperature range.

us take as an the analysis of the presented derivatograms made with such considetions, as given in Table 1.) The weight per cent loss of material in the part processes were compared by plotting, as a binary function, the $O_1 + O_2$ weight per cent values on the ordinate, the $C_1 + C_2$ weight per cent values on the abscissa. In fact, material losses at low and high temperatures, loss of water, cracking and burning were compared. Drawing perpendicular lines on the ordinate and the abscissa, connecting the intersection points of the resultants, the arrangement of points in the co-ordinate system displayed a kind of regularity. Fig. 14 represents the position of 23 recent pelecypod species in the co-ordinate system. The co-ordinate points of the species representing the individual families fall near each other and, according to the numerical boundaries of the studied samples, well circumscribed fields are separated. (Recent results not incorporated in the present paper completely confirm this aspect.)

With this method the taxonomic specificity of the family can be well observed, at

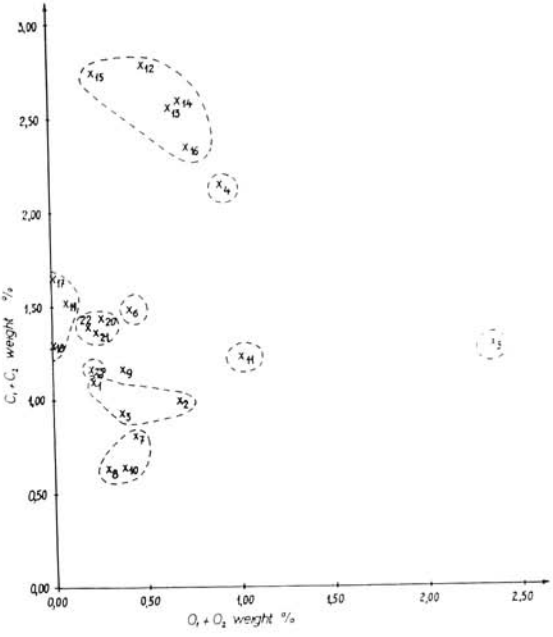


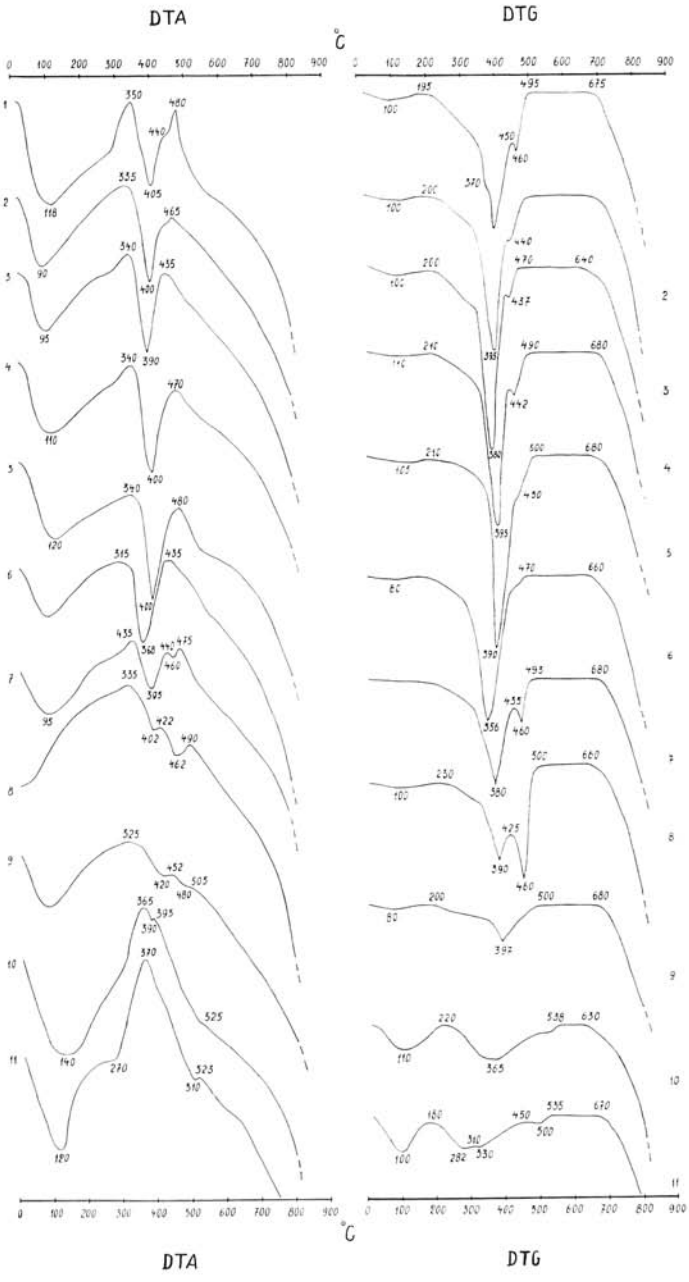
Fig. 14. A comparison of Recent Pelecypod species in C_1+C_2/O_1+O_2 weight per cent co-ordinate system. Samples were collected from freshwaters of Hungary (H) and from the Artic Ocean (A), the Black Sea (B), the Mediterranean Sea (M), the Red Sea (R). Points in co-ordinate system: 1 — *Glycymeris pilosus* (L.), (M), 2 — *Arca noae* (L.), (M), 3 — *Arca barbata* (L.), (M.), 4 — *Meleagrina* (=*Pteria* = *Pinctada*) *margaritifera* (L.), (R), 5 — *Pinna nobilis* (L.), (M), 6 — *Mytilus edulis* (L.), (B), 7 — *Pecten jacobeus* (L.), (M), 8 — *Pecten maximus* (L.), (A), 9 — *Chlamys opercularis* (L.), (A), 10 — *Pecten irradians* (Lam.), (M), 11 — *Ostrea edulis* (L.) (M), 12 — *Unio pictorum* (L.), (H), 13 — *Unio tumidus* (Retz.), (H), 14 — *Anodonta cygnea* (L.), (H), 15 — *Pseudanodonta* (= *Anodonta*) *complatana* (Rossm.), (H), 16 — *Margaritana* (= *Margaritifera*) *margaritifera* (L.), (H), 17 — *Cardium tuberculatum* (L.), (M), 18 — *Cardium edule* (L.), (M), 19 — *Tridacna elongata* (L.), (R), 20 — *Venus cerrucosa* (L.), (B), 21 — *Venus gallina* (L.), (M), 22 — *Donax anatium* (Lam.), (M) 23 — *Tellina tenuis* (L.), (M).

Table 1. DTG-TG fingerprints of Recent Pelecypods

Ordo	Familia	Specimen	V_a weight %	$DTG_{min.}$ °C	C_1 weight %	$DTG_{min.}$ °C	C_2 weight %	$DTG_{min.}$ °C	O_1 weight %	$DTG_{min.}$ °C	O_2 weight %	$DTG_{min.}$ °C	Σ A weight %	Σ A-V_a weight %
TAXODONTA	*Arcidae*	1	0,054	100	1,094	300	—	—	0,218	420	—	—	1,366	1,312
		2	0,443	130	0,990	250	—	—	0,081	410	0,595	530	2,109	1,666
		3	0,345	90	0,938	260	—	—	0,086	415	0,287	515	1,656	1,311
ANISOMYARIA	*Pectenidae*	7	0,287	97	—	—	0,805	340	—	—	0,431	560	1,523	1,236
		10	0,254	98	—	—	0,636	340	—	—	0,382	600	1,272	1,018
	Ostreidae	11	0,348	120	—	—	1,224	380	ny	470	1,011	590	2,583	2,235

Explications: 1 — *Glycymeris pilosus* (L.), 2 — *Arca noae* (L.), 3 — *Arca barbata* (L.), 7 — *Pecten jacobeus* (L.), 10 — *Pecten irradians* (L a m.) — *Ostrea edulis* (L.)

the same time, good possibilities for comparison are available as to the properties and quantity of organic matter included in the shells of the species, as well as to the proportion of water content trapped in structures bound to organic matter.



III. *Investigations of fossil molluscan species*

After it had been proved that by derivatographic fingerprints taxonomic specifity can be traced, the investigations were extended to fossil samples. Now the main aspect was detection of taxonomic specificity, however changes occurring during fossilization were also set as a task of a pilot study.

The investigated fossil species were in good state of preservation. The shell structures were not dissolved or recrystallized during fossilization. To allow a better survey, the derivatograms of fossil species have been classified into groups.

Surveying Fig. 15 and Table 2 both representing fingerprints of mostly Pliocene (only one Pleistocene) fossiles, it is obvious how varied the DTA, DTG and TG relations are. Samples 1—8 are *Unio* species, 9—10 species of *Congeria*, sample 11 represents the shell of a *Pectenculus* species. The thermodynamic processes characteristic of the genus *Unio* can be summed up as follows:

From 20 °C to 200 °C loss of mainly adsorbed wet content (Va); from 300 °C to 400 °C release of water trapped in the aragonite nacreous structure (C_2); 400 °C—500 °C aragonite → calcite conversion and burn-out of organic material (O_1). Of TG relations the dominance of weight loss adjoining the C_2 phase was characteristic.

The DTA-DTG relations of *Congeria* species differ from those of the previous group. Although the DTA curve type of *Congeria ungula caprae* is similar to that of the *Unio* species of samples 7 and 8, in this case; too, the DTG — TG relations are completely different. In the case of *Congeria* species, the loss of adsorbed water content (Va) and a loss of material marked by the temperature values 325 °C an 265 °C can be observed (C_2).

The derivatographic fingerprint of the *Pectenculus* species (sample 11) differs from that of both *Unio* and *Congeria* species. This difference is mainly demonstrated by material losses in the C_2 range. Comparison in the co-ordinate system C_1+C_2/O_1+O_2 is restricted to a comparison C_2/O_1 in the case of Pliocene samples (Fig. 16). The co-ordinate points of the *Unio* species come near one another (with the exception of samples 7 and 8) separated by the points of the two species of *Congeria* and *Pectenculus*.

In Fig. 17 and Table 3 an analysis of the derivatographic fingerprints of Miocene shells is given. Samples 1 and 2 represent different *Venus* species collected at the same place of occurence; samples 3 and 4 show fingerprints of 2 individuals of *Lucina incrassata* found at different localities. Regardless of the conditions of the localities, the two species of the genus *Venus* are similar to each other, and differ from the print of *Lucina incrassata*. In spite of the different places of occurrence, the DTA relations of the two *Lucina incrassata* individuals are almost campletely identical. The different species marked by sample numbers 1, 2, 3 come from the same locality, however, their deriva-

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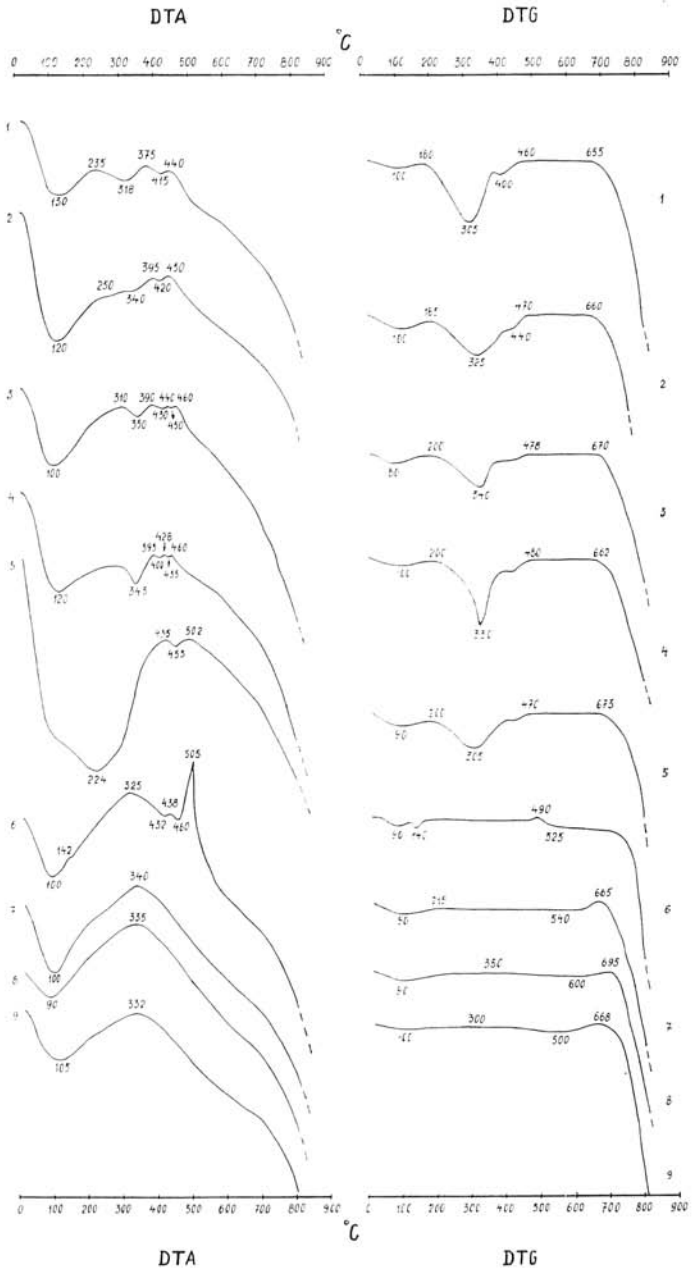
Fig. 15. DTA-, DTG-fingerprints of Pleistocene and Pliocene Pelecypod species.
1 — *Unio sp.*, Pleistocene, Mezözombor (Hungary), 2 — *Unio haevneri* (P e n k), Levantian, Malina (Yugoslavia), 3 — *Unio thalassimus* (Brus.), Levantian, Malino (Yugoslavia), 4 — *Unio pavlovichi* (P a r t s c h), Levantian, Malino (Yugoslavia), 5 — *Unio michalovichi* B r u s.), Lower Pannonian, Radmaneşti (Rumania), 6 — *Unio* sp., Lower Pannonian, Vörendorf (Austria), 7 — *Unio wetzleri* (D u n k l.), Upper Pannonian, Kisbér (Hungary), 8 — *Unio atavus* (P a r t s c h), Upper Pannonian, Kötese (Hungary), 9 — *Congeria ungula caprae* (P a r t s c h), Upper Pannonian, Tihany (Hungary), 10 — *Congeria subglobosa* P a r t s c h), Lower Pannonian, Vörendorf (Austria), 11 — *Pectunculus* (=*Glycymeris*) *bimaculata* (P o l i), Levantian, Torino (Italy).

tographic fingerprints are different. The same is valid for the studied samples of *Lucina incrassata* (sample number 4) and *Arca diluvii* (sample number 5) collected at the same locality from the same facies. Samples 7 and 8 and 9 represent species with calcite



frame from different places of occurrence and different stratigraphic levels. In this case no comparison in the former sense can be effectuated. Table 3 demonstrates that the samples can be classified into 2 groups. For the families *Veneridae*, *Lucinidae*, *Arcidae*

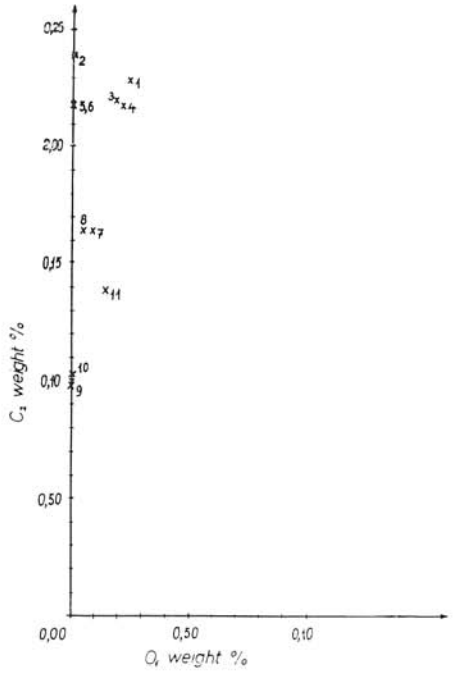


Fig. 16. A comparison of Pleistocene and Pliocene Pelecypod species in C_2/O_1 weight per cent co-ordinate systems. (Numbers as on Fig. 15.)

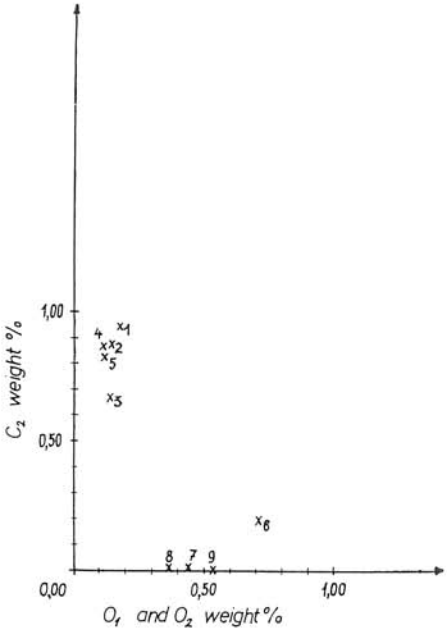


Fig. 18. A comparison of Miocene Pelecypod species in C_2/O_1 an C_2/O_2 weight per cent co-ordinate systems. (Numbers as on Fig. 17.)

the C_2 and O_1 thermoanalytical processes are characteristic. In the case of the families *Ostreidae* and *Pectinidae* on other material losses than C_2 and O_2 can be observed. (On this basis, comparison in the $C_1 + C_2/O_1 + O_2$ co-ordinate system was modified to a C_2/O_1 and O_2 system of comparison.)

In the co-ordinate system seen in Fig. 18 two groups are separated. One of the fields comprises samples 1—5 the other the 6—9. Comparing the co-ordinate system which

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Fig. 17. DTA-, DTG-fingerprints of Miocene Pelecypod species. 1 — *Venus multilamella* (L a m.), Tortonian, Lapugy (Rumania), 2 — *Venus dethrata* (D u b o i s), Tortonian, Lapugy (Rumania), 3 — *Lucina incrassata* (D u b o i s), Tortonian, Lapugy (Rumania), 4 — *Lucina incrassata* (D u b o i s), Tortonian, Bujtur (Rumania), 5 — *Arca diluvii* (L a m.), Tortonian, Bujtur (Rumania), 6 — *Ostrea crassissima* (LAM.), Tortonian, Várpalota (Hungary), 7 — *Pecten praebenedictus* (H o u r n.), Tortonian, Devínska Nová Ves (Slovakia), 8 — *Aequipecten* (= *Chlamys*) *praescabriusculus*, Burdigalian, Mogyoród (Hungary), 9 — *Ostrea* sp., Burdigalian, Budafok (Hungary).

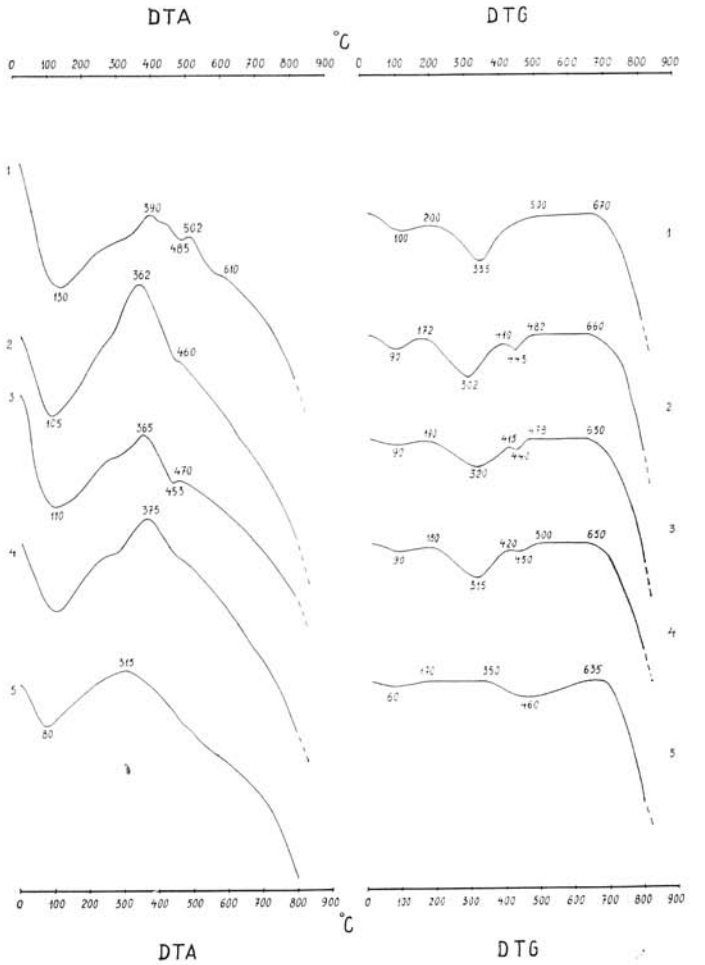

Fig. 19. DTA-, DTG-fingerprints of Paleogene and Jurassic Pelecypod species. 1 — *Cardium cingulatum* (G o l d f.), Oligocene, Eger (Hungary) 2 — *Cardinata planicosta* (L a m.), Eocene, Grigon (France), 3 — *Cytherea semisulcata* (L a m.), Eocene, Grigon (France), 4 — Axinea *pulvinata* (D e p h.), Eocene, Grigon (France), 5 — *Cryphea obliqua* (G o l d f.), Jurassic, Pécs (Hungary).

analyses the Miocene species with the ones analysing the recent or Pliocene species (Figs. 14 and 16), the following conclusions can be drawn. In proportion to the degree of fossilization and time, there is a growing density of projection points. In the case of recent and Pliocene samples, the families are well separated, which is hardly the case with the Miocene ones. The representatives of the families *Veneridae*, *Lucinidae* and *Arcidae* are located close to one another, the families *Ostreidae* and *Pectinidae* in another group. With Miocene shells, by this mode of comparison only the differences due to great basic structure can be detected. In detail, the *Ostrea* and *Pecten* species

Table 2. DTG-TG fingerprints of Pleistocene and Pliocene Pelecypods

Ordo	Familia	Specimen	V_a weight %	DTGmin. °C	C_2 weight %	DTGmin. °C	O_1 weight %	DTGmin. °C	Σ A weight %	Σ A-V_a weight %	Age	
SCHIZODONTA	*Najadidae*	1	0,182	100	2,282	395	0,255	460	2,719	2,537	Neolithic	PLEISTOCENE
		2	0,137	100	2,396	390	ny	~	2,533	2,396		
		3	0,183	90	2,207	389	0,183	437	2,573	2,390		
		4	0,098	110	2,157	390	0,210	442	2,473	2,385		
		5	0,110	105	2,189	390	ny	~	2,290	2,299		
		6	0,133	80	2,174	355	ny	~	2,307	2,174		
		7	0,983		1,627	380	0,813	460	2,440	2,523		
		8	0,157	100	1,635	390	0,408	460	2,190	2,043	Pannonian	PLIOCENE
ANISOMYARIA	*Mytilidae*	9	0,117	80	0,868	397	—	—	0,985	0,868		
		10	0,467	100	1,098	365	ny	~	1,565	1,098		
TAXODONTA	*Arcidae*	11			a/0,693	282					Levantian	
					b/0,776 +	330						
			0,636	100	1,379		0,139	500	2,154	1,518		

Explications: 1 — *Unio* sp., 2 — *Unio haevneri* (Penk), 3 — *Unio thalassimus* (Brus.), 4 — *Unio pavlovichi* (Partsch), 5 — *Unio michalovichi* (Brus.), 6 — *Unio* sp., 7 — *Unio wetzleri* (Dunkl.) 8 — *Unio atavus* (Partsch), 9 — *Congeria ungula caprae* (Partsch), 10 — *Congeria subglobosa* (Partsch), 11 — *Pectunculus (Glycymeris) bimaculata* (Poli).

Table 3. DTG-TG fingerprints of Miocene Pelecypods

Ordo	Familia	Specimen	V_a weight %	$DTG_{min.}$ °C	C_2 weight %	$DTG_{min.}$ °C	O_1 weight %	$DTG_{min.}$ °C	O_2 weight %	$DTG_{min.}$ °C	Σ A weight %	Σ A-V_a weight %	Age	
HETERODONTA	*Veneridae*	1	0,184	100	0,938	305	0,172	400	—	—	1,294	1,110	Tortonian	MIOCENE
		2	0,217	100	0,869	325	0,144	440	—	—	1,230	1,013		
	Lucinidae	3	0,229	80	0,660	340	0,143	440	—	—	1,032	1,803		
		4	0,152	100	0,857	330	0,118	440	—	—	1,127	0,975		
TAXODONTA	*Arcidae*	5	0,247	90	0,820	305	0,112	440	—	—	1,179	0,932		
ANISOMYARIA	*Ostreidae*	6	0,084 0,056 + 0,140	90 140	0,190	375	—	—	0,712	~	1,042	0,902		
	Pectenidae	7	0,299	90	—	—	—	—	0,447	540	0,746	0,447	Burdigalian	
		8	0,369	90	—	—	—	—	0,369	600	0,738	0,369		
	Ostreidae	9	0,160	130	—	—	—	—	0,530	500	0,690	0,530		

Explications: 1 — *Venus multilamella* (Lam.), 2 — *Venus dethrata* (Dubois) 3 — *Lucina incrassata* (Dubois), 4 — *Lucina incrassata* (Dubois), 5 — *Arca diluvii* (Lam.), 6 — *Ostrea crassissima (Lam.)*, 7 — *Pecten praebenedictus* (Tourn), 8 — *Aequipecten (=Chlamys) praescabriusculus*, 9 — *Ostrea* sp.

with calcite frame are characterized by the presence of enriched organic material and by the decrease of water content, the shells of the species with aragonite structure (*Venus, Lucina, Arca*) by great water content and a modest quantity of organic matter.

IV. *Discussion of results. General conclusions*

Studies on recent molluscan shells by the derivatographic fingerprint method, as a novel procedure od investigation, can be made capable of tracing the taxonomic variability brought about by phylogenetic evolution. It has been proved through thermoanalytical analyses that the derivatographic fingerprint, different according to species, appears reproducibly when applied to individuals, too. The taxonomically specific marks are consequences of different biochemical, structural constitution, developed in the course of the evolution of the shells. It has been verified by plotting in the $C_1 + C_2/O_1 + O_2$ system of co-ordinates that according to the present morphological-systematic classification, the phylogenetic relationship is reflected by the chemical, physico-chemical structures of the shell material. The investigation of recent sample material, as a model, can basically secure such an analysis of the taxonomic identification of fossil samples in the cases when the shell material did not undergo far-reaching changes during fossilization. To prove this claim, direct examples are given through the analysis of fossil sample material. Material from the different stratigraphic levels and lithofacies horizons of the Pliocene are similar as to their C_2/O_1 relation, but they sharply differ from the thermoanalytical relations of other genera of *Lamellibranchiata* collected from the corresponding age and lithofacies. This fact can be applied in identifying the found fragmentary material, in the case of Pliocene molluscan samples, to genus-taxon level. (In a developed the method may be suitable to determine the taxonomic specificity of species, too.)

Investigations on the samples of *Lamellibranchiata* from the sandy littoral facies of the Miocene Tortonian stage have given promising results. It is significant that the derivatographic fingerprints of individuals of *Lucina incrassata* from various localities can be regarded identical. This fact suggests the possibility of identification to the species-taxon level, which is supported by the previous statement (Gy. Szőr 1968) that the trace element spectra of the samples are identical, too. Within an identical facies, similarly to the Pliocene samples, identification to the genus-taxon level has been proved in this case, too. This is justified by the study of a previously analysed material of *Gastropoda*, not discussed here (Gy. Szőr 1971b) which, at the same time, calls attention to the fact that taxonomic identification can be exclusively done on the sample material of the same lithofacies, applying derivatography jointly with amino acid spectrum and trace element spectrum analysis.

In the course of the experiments a number of Paleogene samples have been studied (Fig. 19). In spite of being seemingly in a good state of preservation, the shells contained but traces of, or no organic material. Contrary to this, in the shells of Miocene species amino acids can still be detected in quantities enough to give spectra (Gy. Szőr 1967). Derivatographic anlysis gave the surprising result that the thermoanalytical properties of species of different taxonomical groups, collected at identical localities, were identical. This is well demonstrated by the nearly identical DTA-DTG fingerprints of *Cardita planicosta*, *Cytherea semisulcata*, and *Axinea pulvinata*, collected from identic horizon and lithofacies. In the case of Paleogene species no taxonomic identification by the derivatographic fingerprint method can be achieved.

Thus, it can be assumed that identification by the derivatographic method, diagnosis

of fragmentary material can only be performed if the amino acids of the shells are present in sufficient quantities to give spectra, i. e. in the Holocene, Pleistocene, Pliocene and the younger stages of the Miocene. The basis for detecting and deciding on phylogenetic relations is the necessity that the conchioline protein substance be present in relatively unimpaired and undecomposed condition. The amino acid or protein essentially plays the role of an indicator which, by its presence, can prove the favourable fossilizational state of the structures.

When interpreting the results for fossil sample material it must be emphasized that the data given here only suggest the possibility of taxonomic identification. Derivatographic analytics serving the taxonomic identification of fossil fragmentary material may be developed by a longer series of subsequent careful investigations.

As a first step we must endeavour to perform extended studies on comparative sample material and to elucidate the basic thermoanalytical processes.

As a second step the fossil material of type facies must be mapped, proceeding from age to age. The material thus obtained must be compared, considering in every case the diagenetic history of the embedding medium and checking the results by the already elaborated biochemical and optical routine methods.

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