The Use of Paper Partition Chromatography for Taxonomic Studies of Land Snails

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Buzzati-Traverso & Rechnitzer (1953) have shown that comparisons of the paper chromatograms obtained from tissue extracts of fishes indicate differences between species, and that within a species the patterns are remarkably constant irrespective of the size or age of the fish. They claim also that comparable results have been obtained in other phyla, and that preliminary results suggest that the technique can be used to distinguish stocks of the same species belonging to geographically isolated populations. In another study Buzzati-Traverso (1953) has found also that paper chromatography of tissues of genetically known strains of *Drosophila melanogaster* and of certain plants gives a constant and distinctive pattern for each strain. This work suggests that chromatographic analysis of tissue extracts may be of value not only as an important tool in taxonomic and population genetics studies, but, as Buzzati-Traverso points out, as an aid to understanding the biochemical basis of individuality.

In order to test the validity of Buzzati-Traverso's procedure when applied to an invertebrate phylum, a rapid method of analysis has been developed based on the use of chromatography on filter-paper disks (Rutter, 1950; Giri, 1953). Several species of land snail were used as experimental material, and the resultant patterns have been studied to determine the effect of size of animal and of variable factors in both natural and artificial environments. The results indicate that the method is likely to be of great value in the taxonomic study of land snails.

EXPERIMENTAL

Methods

Paper chromatography was carried out using circles of Whatman no. 3 paper diameter 12.5 or 15.0 cm. Extracts of tissue cut from living specimens were made by crushing small pieces of the tissue 1.5 cm. from the centre of the paper, the amount of tissue being such that when compressed and rotated with moderate manual pressure using a small glass stopper a spot approximately 0.5–0.75 cm. diameter was produced. Generally four spots were prepared on each circle. The spots were allowed to dry in a current of warm air. If kept dry the prepared papers could be stored for several days before chromatographing without noticeable effects on

the resultant patterns. The solvent finally adopted was butanol:acetic acid:water (100:22:50, v/v). The solvent was contained in a 10 cm. diameter Petri dish, the paper being placed on a glass plate on top of the Petri dish and covered with an inverted pie dish. A filter-paper wick descending through a hole in the plate fed solvent to the centre of the filter-paper disk. All chromatography was carried out in a constant temperature room at $20 \pm 1^\circ$ for 4–6 hr. At the end of the run the disk was dried horizontally in a current of warm air and examined for the ultraviolet fluorescence and absorption pattern using a Wood's glass mercury-vapour discharge lamp. R_F values of the visible fluorescent and absorption bands were measured directly with calipers whilst the pattern was being examined under the mercury-vapour discharge lamp. Photographic records of the pattern were made by printing through the paper disk. Full details of the fluorescent pattern are not reproducible photographically, however, since the filter paper itself fluoresces faintly and obscures coloured bands of low intensity which are clearly visible to the eye.

Materials

The patterns from seven species of land snails are described below. The species were: *Theba pisana* (Müll), *Helix aspersa* (Müll), *Bothriembryon indutus* (Menke), *B. kingii* (Gray), *B. dux* (Pfeifer), *B. leeuwinensis* (Smith), *Austrosuccinia contenta* (Iredale).

The first two species are common European snails first reported in Australia about 60 years ago. The species of the genus *Bothriembryon* and *A. contenta* are native to West Australia.

Specimens of *T. pisana* were collected from four different localities, namely beach dunes at Cottesloe, Albany and Hopetoun and along the river foreshore in Perth. *H. aspersa* was obtained from house gardens in Perth. All the specimens of native snails were topotypes of species listed by Iredale (1939).

The tissue used for preparing extracts was cut from the posterior or lateral edges of the foot. If the animal was withdrawn into the shell or had an epiphragm it was placed in water until the foot was extended. The shells were numbered to facilitate comparisons of the chromatographic patterns from individuals within a species.

In one experiment young specimens of *T. pisana* collected in the field were reared for 4 months in the laboratory on different diets. The animals were kept in glass jars and divided into three groups. Group 1 was fed on lettuce leaves, group 2 on buffalo grass (*Stenotaphrum secundatum*) and group 3 was provided with a diet of filter paper impregnated with a mixture of yeast, cod liver oil and calcium carbonate (cf. Wagge, 1952). The food was changed twice weekly.

RESULTS

Differences between species

Each of the species of snail studied gave characteristic chromatographic patterns which could be distinguished clearly by their ultraviolet absorption and fluorescence patterns. The main characteristics of the various patterns are recorded in Fig. 1. No attempt has been made as yet to identify chemically the various components.

Effect of age and geographical locality

Three different age groups of *H. aspersa* and *T. pisana* were collected in the field. Specimens of either species were feeding on a single plant species in a restricted area. No significant differences

between young, intermediate or mature animals could be detected in the characteristic patterns for either species, even when the R_F values for the four principal fluorescent bands in the pattern of *H. aspersa* and the R_F value of the outer edge of the fluorescing zone in the pattern of *T. pisana* were measured for each specimen (Table 1).

No significant difference was found between mature specimens of *T. pisana* from four widely separated localities (Table 2).

Effect of different diets on *Theba pisana*

A large number of very young specimens of *T. pisana* were collected on the beach dunes at Cottesloe and were reared in glass jars on the three different diets described in the previous section.

Fig. 1. Characteristic fluorescence and absorption patterns obtained by chromatographing fresh tissue squashes of the foot muscle of various land snails.

Table 1. Mean R_F values for the main characteristics in the chromatographic pattern of fresh tissue extracts of snails of different ages

A. <i>Helix aspersa</i> (12 animals/group)				
R_F values for four principal fluorescent lines (mean $\pm$ s.e.)				
Age group	1	2	3	4
Young	0.16 $\pm$ 0.025	0.26 $\pm$ 0.028	0.35 $\pm$ 0.028	0.51 $\pm$ 0.019
Intermediate	0.17 $\pm$ 0.018	0.28 $\pm$ 0.015	0.37 $\pm$ 0.024	0.55 $\pm$ 0.014
Mature	0.18 $\pm$ 0.032	0.28 $\pm$ 0.029	0.36 $\pm$ 0.035	0.54 $\pm$ 0.030
B. <i>Theba pisana</i> (16 animals/group)				
R_F values for edge of fluorescent flare				
Age group	Mean $\pm$ s.e.		Range	
Young	0.43 $\pm$ 0.007		0.40–0.50	
Intermediate	0.41 $\pm$ 0.009		0.34–0.42	
Mature	0.39 $\pm$ 0.007		0.33–0.42	

Table 2. Mean R_F values from the chromatographic pattern of fresh tissue extracts of mature specimens of *Theba pisana* from different geographical localities

Locality	No. of animals	R_F values for edge of fluorescent flare	
		Mean $\pm$ s.e.	Range
Cottesloe	16	0.39 ± 0.007	0.33-0.43
Perth	15	0.38 ± 0.010	0.35-0.39
Albany	16	0.43 ± 0.007	0.38-0.46
Hopetoun	16	0.39 ± 0.005	0.35-0.41

Table 3. Mean R_F values from the chromatographic pattern of fresh tissue extracts of *Theba pisana* reared in the laboratory on different diets

Four animals per group.

Diet	R_F	
	Mean	Range
Buffalo grass	0.38	0.34-0.42
Lettuce	0.37	0.35-0.40
Synthetic	0.35	0.33-0.36

R_F values are for edge of fluorescent flare.

At the end of four months extracts of tissue from a sample of the animals on each of the diets were subjected to chromatography. No obvious differences could be detected between any of the patterns obtained from the animals reared in the laboratory (Table 3), which were identical with those obtained from *T. pisana* of different ages, and from different geographical localities.

DISCUSSION

The method of paper chromatography applied to fresh tissue extracts developed by Buzzati-Traverso & Rechnitzer (1953) and the modification of it described here are both rapid and easy to carry out. The evidence so far available suggests that the patterns of such chromatograms are highly characteristic for a single species, and are uninfluenced by wide variations in environmental conditions or by age. The common European snail *T. pisana* is now widely dispersed along a narrow coastal belt in Western Australia over a distance of 1000 miles and is exposed to considerable differences in climate, soil and vegetation. Specimens taken from localities 300 miles apart showed no significant differences in the chromatographic pattern obtained from extracts

of the foot musculature. Further, attempts to modify the pattern by rearing young snails on three different diets for four months in the laboratory gave rise to no significant differences in the patterns obtained. On the other hand, each of the seven separate species of land snail studied gave distinctively different patterns. On the basis of these preliminary observations, therefore, it seems clear that paper chromatography of simple tissue extracts offers a tool which is likely to be of considerable value to the taxonomist. With the further refinement of this kind of study and the identification or quantitative measurement of some of the components responsible for the patterns it is possible that degrees of relationship between closely related species may be assessed. If this is realized a new approach to the problems of phylogeny will be opened up which may be used in conjunction with the ecological, serological and biometrical methods of analysis now employed in evolutionary studies.

SUMMARY

1. Paper chromatography has been applied to fresh tissue extracts of the foot muscle of seven species of land snails. The characteristic ultraviolet fluorescence and absorption patterns obtained, using butanol:acetic acid:water as the solvent, are described. Each species gives a pattern which can be distinguished clearly from the others.

2. The effect on the patterns of variations in age for *T. pisana* and *H. aspersa* and of geographical locality and of three different diets under laboratory conditions for *T. pisana* has been studied. No significant differences were found in the characteristic pattern under any of the conditions studied.

3. The potential value of chromatography of fresh tissue extracts for systematic and phylogenetic investigations is discussed.

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