Biochemical Lab Activity Supports Evolution Theory

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THIN-LAYER CHROMATOGRAPHY (TLC) is a technique that can be conveniently used in the laboratory to generate evidence supporting the principle that degrees of biochemical similarity reflect degrees of evolutionary relatedness among organisms. When TLC is applied to the analysis of tissue extracts of various organisms, similarities among the extracts can be interpreted as a result of ancestral relationship.

Materials and Methods

The organisms used in this biochemical investigation of evolution are *Erythronium americanum, E. albidum, Trifolium repens, T. pratense,* and *T. arvense.* Plants of these two genera have been used because of their common occurrence and because students can easily relate them morphologically. (Other groups of closely related organisms, of families such as the Hydrophyllaceae, Labiatae, and Fabaceae, could be used.)

The plants are collected when they are in flower; roots are excluded. The plants are washed, superficially dried, placed between layers of newspaper, and allowed to air-dry; or they can be oven-dried, at 45 °C or less.

Plant-tissue extract is obtained by placing approximately 0.4 g of each of the air-dried plants in a drying oven at 45 °C for approximately 12 hours. The oven-dried plant tissues are then pulverized with a glass stirring-rod after being placed in small glass vials. To each of the vials containing the pulverized plant tissue is added 2.5 ml of extracting agent, a methanol-concentrated hydrochloric acid solution (99:1 v/v). The vial is sealed and is placed in the dark at room temperature for 12 hours. The plant-tissue extract may be stored in a refrigerator for several days.

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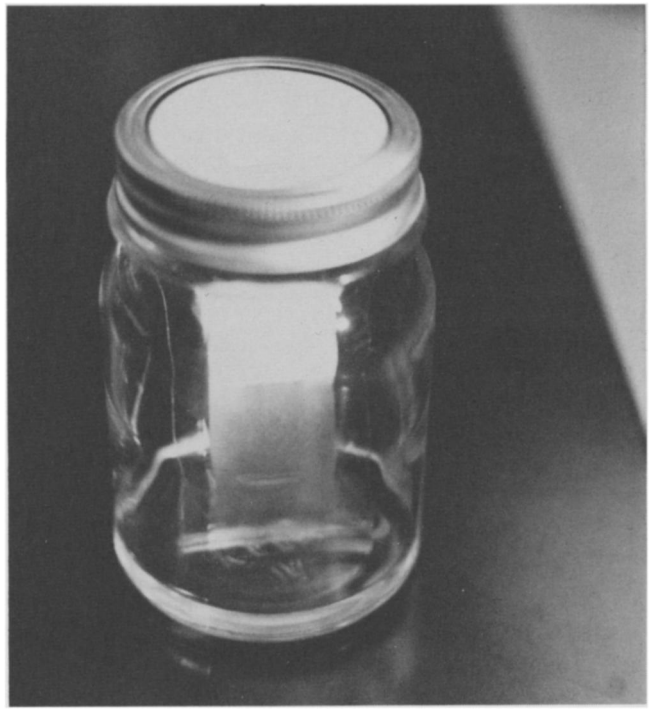

Fig. 1. The chromatographic system is shown with a TLC plate in the process of development. The developing chamber is a canning jar with its lid reversed.

The TLC plates can be prepared any of the following ways: (i) the glass plates may be dipped into the gel–water mixture; (ii) the glass plates may be sprayed with the gel–water mixture; or (iii) the gel–water mixture may be spread over the glass plates. The third method is suggested for this investigation.

The long edges of scrupulously clean 5-by-11-cm glass plates are taped with 1.2-cm moisture-resistant

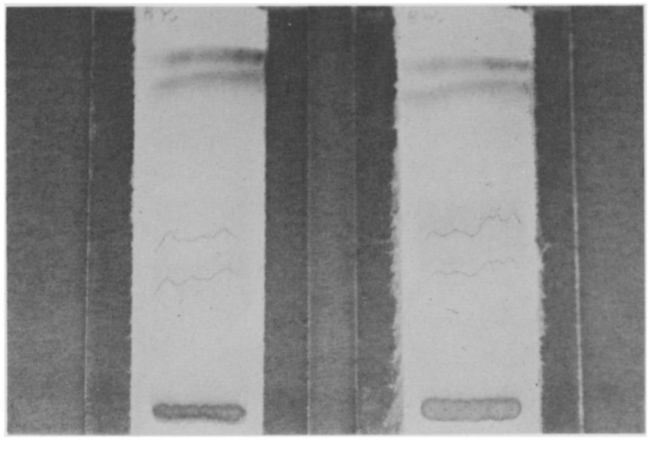

Fig. 2. TLC plates of *E. albidum* (left) and *E. americanum* (right) under ultraviolet light. The TLC plates reveal two similar secondary compound bands having R_f values of 0.51 and 0.41, respectively. The photo has been slightly retouched to bring out the chromatographic bands.

labeling tape. The tape serves as a gauge for limiting the thickness of the gel layer that will be distributed on the surface of the plate. The gel is prepared by placing 14 g of silica gel H for TLC according to Stahl (available from Brinkman Instruments, Des Plaines, Ill. 60016) in a 125-ml Erlenmeyer flask, adding 40 ml of distilled water, and swirling the gel–water mixture for 90 seconds. Small amounts of the gel–water mixture—approximately 2 ml—are quickly poured onto each of the taped glass plates, and the gel is evenly distributed ("struck off") with a 1-cm-diameter glass rod. In distributing the silica gel, a very even layer is desirable. The tape is carefully removed from the plates, and the gel layer is allowed to air-dry. Finally, regardless of the method of coating the glass plates with a silica gel layer, the TLC plates are activated by placing them in a drying oven at 95–100 °C for 30 minutes. The plates should

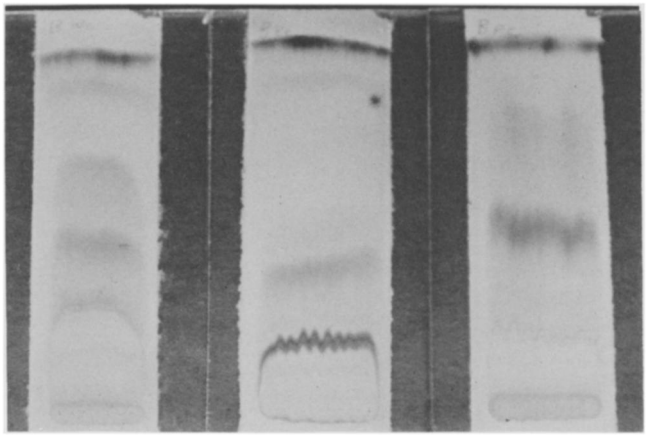

Fig. 3. TLC plates of *T. repens* (left), *T. pratense* (center), and *T. arvense* (right) under ultraviolet light. The TLC plates reveal that *T. repens* and *T. arvense* have a common secondary compound band with an R_f value of 0.50 and that *T. pratense* and *T. arvense* have a common secondary compound band with an R_f value of 0.26. A common secondary compound apparently does not exist for the three *Trifolium* species used in this study.

be cooled to room temperature before being used. The activated plates may be stored for several days if kept in a dry, dust-free environment.

The plant extract can be applied to the TLC plates with a Hamilton microliter syringe or with a microcapillary tube. The latter is made by gently heating the midregion of a 50-μl pipette. As the pipette is heated, the ends are manually drawn apart, to produce a narrow, constricted region. The pipette is then broken in the region of the constriction, to produce two microcapillary tubes, each having an internal diameter of approximately 0.5 mm.

With the use of the microcapillary tube, the extract is spotted as a band across the narrow edge of the silica gel. The band should be approximately 1.5 cm from the bottom edge of the plate. Three applications of the extract is ideal. An insufficient concentration of extract results in a separation that is not readily apparent; an excessive concentration results in an ill-defined separation. The silica gel plates can be labeled at the top by scratching into the gel layer with a needle or a dissection probe.

The extract-spotted silica gel plates are developed in a chromatographic chamber, which may be sophisticated but could be as crude as a 1-pint canning jar. If a canning jar is used, the lid should be reversed so that the rubber sealing ring is up: typical TLC solvents tend to dissolve the rubber.

The recommended chromatographic solvent is a a methanol–chloroform (3:7 v/v) solution. The solvent is added to the TLC developing chamber to a depth of approximately 0.5 cm. Students must be cautioned that the solvent level must not exceed the level of the extract band on the TLC plate: if the solvent level touches the extract band, the extract will dissolve into the solvent, and the TLC plate will be ruined.

The extract-spotted plates are placed in the developing chamber containing the solvent, the chamber is sealed, and the development of the plate is permitted (fig. 1). Development of a TLC plate takes about 20 minutes. The solvent should be allowed to rise to a height of approximately 9–10 cm.

Results

A longwave (ultraviolet) lamp is used to visualize the developed TLC plates. Fig. 2 and 3 show the results that can be expected. The violet bands that appear represent free amino acids. The amino acids are considered to have less significance than the secondary compounds as indicators of evolutionary relatedness.

With regard to both the secondary compounds and the free amino acids, the student can examine the TLC plates for similarities and differences of various bands. Colors and location of the various bands should be taken into account. The degree of ancestral relatedness is reflected by the degree of similarity represented by the separation.

Ratio-to-front (R_f) values for each of the bands

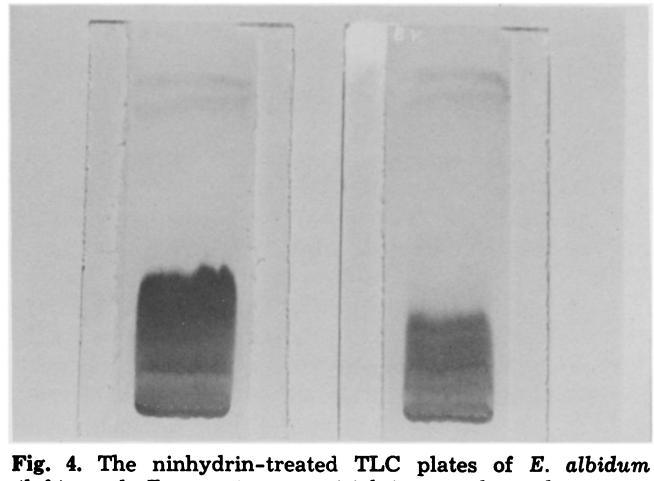

Fig. 4. The ninhydrin-treated TLC plates of *E. albidum* (left) and *E. americanum* (right) reveal at least two common amino acid bands, which have R_f values of 0.27 and 0.11.

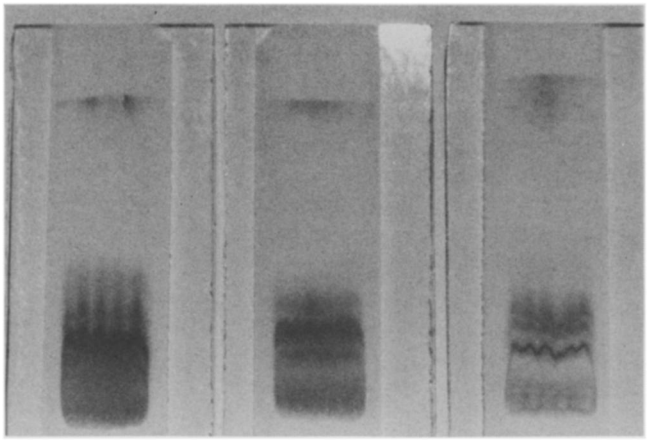

Fig. 5. The ninhydrin-treated TLC plates of *T. repens* (left), *T. pratense* (center), and *T. arvense* (right) reveal at least two common amino acid bands, which have R_f values of 0.18 and 0.29.

can be calculated. The formula used for this calculation is the distance the band traveled, divided by the distance the solvent traveled. R_f values are helpful in comparing location similarities that may exist among the various bands. Similar compounds have similar R_f values.

Conclusions and Recommendations

The results of this investigation indicate that *Erythronium albidum* and *E. americanum* are more closely related to each other than to any of the *Trofolium* species. Similarly, *T. repens, T. pratense,* and *T. arvense* are more closely related to each other than to either of the *Erythronium* species.

An investigative character can be added to this lab activity by including an "unknown" plant extract. The unknown could be any of the five plants used in this lab activity. The students could be asked to associate the unknown with its apparent relative. Or the unknown could be an organism that is not morphologically similar to any of the known organisms. Students could note the obvious differences that exist among the various group representatives and the morphologically different unknown.

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