of fixing technique probably caused these and thereby apparently closed the matter. However, there are now, including those mentioned in our two papers, sufficient observations from different sources to conclude that kidney tubular alkaline phosphatase may be markedly variable in fish and reptile species. Further work is needed to define the conditions that govern these changes and to determine the consequent functional alterations.

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Application of Paper Chromatography to Taxonomic Studies

The preliminary work of Buzzati-Traverso and Rechnitzer (*1*) suggested that the simple method of squashing fresh tissue on filter paper, followed by one-dimensional chromatographic separation of ninhydrin-positive and ultraviolet-fluorescent substances, could yield results of value in taxonomic and population-genetic studies. These expectations have been amply fulfilled in an extensive investigation of the dipteran family Drosophilidae (*2*).

The method as previously applied has, however, suffered from limitations imposed by the inadequate separation of complex mixtures that is afforded by one-dimensional chromatography. In *Drosophila melanogaster*, for example, differences between males and females with respect to ninhydrin-positive materials are readily demonstrable by means of two-dimensional chromatography (Figs. 1 and 2). The most striking difference is the presence of a peptide in males that is absent in females, but quantitative differences exist as well (*3*). When, however, the same solvents are used separately in the development of one-dimensional chromatograms, the differences either fail to be disclosed (in the case of the butanol, acetic acid, and water mixture) or spurious differences are observed (in the case of 80-percent aqueous phenol).

These misleading observations are the result of a number of factors. In the first place, the spots observed on one-dimensional chromatograms frequently consist

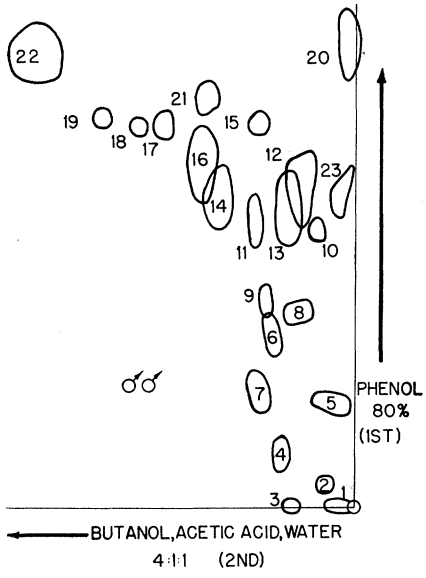

Fig. 1. Two-dimensional chromatogram of free ninhydrin-positive substances in ten decapitated *D. melanogaster* males that were squashed directly on Whatman No. 1 filter paper. First solvent: 80-percent aqueous phenol for 20 hours at 25°C. Second solvent: *n*-butanol, glacial acetic acid, and distilled water (4 to 1 to 1 by volume) for 18 hours at 25°C. Identity of spots: *1*, unknown; *2*, pupine (?); *3*, unknown; *4*, aspartic acid; *5*, cystine; *6*, serine; *7*, glutamic acid; *8*, taurine; *9*, glycine; *10*, lysine; *11*, threonine; *12*, histidine and/or arginine; *13*, glutamine; *14*, α-alanine; *15*, methionine; *16*, β-alanine; *17*, tryptophan; *18*, valine; *19*, norvaline; *20*, front peptide (?); *21*, proline; *22*, leucines; *23*, sex peptide (specific to males).

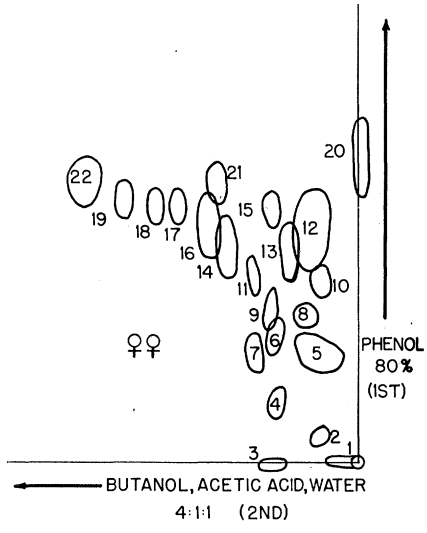

Fig. 2. Two-dimensional chromatogram of free ninhydrin-positive substances in eight decapitated *D. melanogaster* females. Development and identity were the same as in Fig. 1. Note absence of sex peptide, spot *23*.

of two or more substances with similar R_f values, and the differences between the sexes are thus obscured. Further, inspection of Figs. 1 and 2 discloses a systematic depression of R_f values in females in the dimension that is developed with phenol. In one-dimensional chromatograms developed with this solvent, this depression of R_f values results in a compaction of spots and an apparent reduction in the number of ninhydrin-positive substances in females as compared with the number in males.

These observations (*4*) illustrate the obvious advantages of two-dimensional chromatography and suggest its more extensive use in the application of paper chromatography to problems of taxonomy and population genetics. They also suggest that the establishment of the identity of or difference between substances in different species or populations should not depend only on R_f values and such gross observations as color of spots but should also include qualitative identification by means of more extensive chromatographic and chemical procedures whenever possible. Quantitative measurements as well as qualitative identifications would be highly desirable.

Thus, an examination of the distribution of individual, identified substances among species or populations would be preferable to that of over-all chromatographic patterns. As a first approach, hierarchies of chromatographic similarities and differences should be useful in the construction of taxonomic categories, although a more refined approach might be provided by methods of multivariate analysis (*5*). Properly used, chromatographic methods should come to occupy a position in modern taxonomy similar to that occupied by serologic methods.

A more complete account of the methods of chromatography and identification employed in this work, as well as an analysis of R_f values, will be published elsewhere.

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References and Notes

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