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Elizabeth Rose
University of Tennessee at Chattanooga

Recommended Citation

Rose, Elizabeth, "A study of generic evolution in the plant family Ranunculaceae" (1985). *Honors Theses.*

A STUDY OF GENERIC EVOLUTION IN THE PLANT FAMILY RANUNCULACEAE

by

Elizabeth Rose

Departmental Honors Thesis
University of Tennessee at Chattanooga
Biology
Director: Gene S. Van Horn
March 26, 1985

Examining Committee: [redacted]

Chairman, University Departmental
Honors Committee: [redacted]

ABSTRACT

The eighteen southeastern genera of the Ranunculaceae family yielded eight evolutionary trees when analyzed using the PENNY routine of the PHYLIP program with a data matrix of 19 genera by 41 characters. Each tree required 70 steps, and the trees were the most parsimonious found for the data.

INTRODUCTION

Botany, the study of plants, has gone through several phases of classification. The early botanists, such as Linnaeus (Porter, 1959), were largely concerned with identifying and naming as many different plants as possible. As more plants were identified and named, the focus of research in this area moved to the process of finding the characteristics of particular plants, and using these characteristics to group plants into genera and families. Keys, which are composed of a series of characteristics that can be used to separate and distinguish between different families, were developed to aid in the identification of unidentified plant specimens. These early keys were either based on an artificial system of classification, which separates plants by a few convenient characteristics only, or on a natural system of classification, which separates plants by groups of characters. When the concept of evolution was developed, yet a third type of classification appeared. The phylogenetic, or evolutionary, classification arranged plant families by their presumed evolutionary affinities.

While systems of classification were being developed, it was clear that many species had similar features. Thus, species could be grouped into larger groupings called genera. By the same process, genera could be placed into larger groups called families, and so on. At this time, little has been done with respect to the evolutionary study of genera within a family. One method of finding how these genera are related to each other is to compile a great variety of characters.

In this century research techniques became more sophisticated and yielded more characters for analysis and classification. Biochemistry of plants became an important new source of characters. As new biochemical discoveries were made, many new similarities and dissimilarities were found, causing many changes in plant classification. In addition to the new biochemical information, a large amount of new genetic information was discovered. Many chromosome studies and counts were performed, and this chromosomal information led to classification changes. This great expansion of data was difficult to analyze without another development: the computer.

My project involved a computer-assisted evolutionary study of the southeastern genera of the Ranunculaceae (buttercup) family (Fig. 1). The buttercup family is particularly interesting, for its genera are very diverse. Indeed, there are genera in the Ranunculaceae that botanists feel should not be ranked as separate genera, but instead be placed together in a single genus. Conversely, some genera usually placed in this family may belong in a different family or families. Since evolutionary studies can clarify the relationships between genera, indicating which genera are highly similar to each other and which genera are extremely dissimilar from the others, it is particularly useful to perform such a study for this family.

MATERIALS AND METHODS

The first portion of my research was devoted to gathering the characters, or characteristics, of the different genera and composing a data matrix with these characters (Fig. 2). Each character is composed of two different character states, one of which is considered primitive and

SOUTHEASTERN GENERA OF RANUNCULACEAE

Xanthorhiza
Aquilegia
Delphinium
Aconitum
Hydrastis
Caltha
Enemion
Actaea
Cimicifuga
Clematis
Thalictrum
Myosurus
Ranunculus
Trautvetteria
Hepatica
Anemone
Coptis
Anemonella

Fig. 1

CHARACTER STATE DATA

1. Flowers regular 0
   Flowers irregular 1

2. Plant woody 1
   Plant herbaceous 0

3. Fruit an achene 1
   Fruit not an achene0

4. Fruit a follicle 0
   Fruit not a follicle1

5. Fruit a berry 0
   Fruit not a berry 1

6. Leaves evergreen 1
   Leaves not evergreen0

7. Leaves alternate 0
   Leaves not alternate 1

8. Leaves opposite 1
   Leaves not opposite 0

9. Leaves whorled 1
   Leaves not whorled 0

10. Plant perennial 0
    Plant not perennial 1

11. Nectaries present 1
    Nectaries not present0

12. Flowers dioecious 1
    Flowers perfect 0

13. Rhizomes or stems yellow internally 1
    Rhizomes or stems not yellow internally 0

14. Fruit an utricle 1
    Fruit not an utricle 0

15. Base chromosome number=8 1
    Base chromosome number#8 0

16. Base chromosome number =9 1
    Base chromosome number #9 0

17. Base chromosome number =13 1
    Base chromosome number #13 0

(Fig. 2)

18. Base chromosome number =7 0
Base chromosome number #7 1

19. R-type chromosomes 1
Not R-type 0

20. T-type chromosomes 1
Not T-type 0

21. Seeds wrinkled in follicle 1
Seeds not wrinkled in follicle 0

22. Seeds rounded in follicle 1
Seeds not rounded in follicle 0

23. Seeds ruffled in follicle 1
Seeds not ruffled in follicle 0

24. Seeds in berry rounded 0
Seeds not rounded 1

25. Seeds in berry with scales 1
Seeds without scales 0

26. Petals spurred 1
Petals not spurred 0

27. Sepals spurred 1
Sepals not spurred 0

28. Carpels exactly one 0
Carpels not exactly one 1

29. Sepals small, early decidious 0
Sepals well developed 1

30. Sepals valvate in bud 1
Sepals not valvate in bud 0

31. Sepals imbricate in bud 1
Sepals not imbricate in bud 0

32. Ovules a pair in each ovary 1
Ovules not a pair in each ovary 0

33. Staminodia present 1
Staminodia not present 0

34. Vascular bundle amphicribal 1
Vascular bundle not amphicribal 0

35. Pollen grains furrowed 0
Pollen grains pored 1

(Fig. 2, cont.)

36.	Exine reticulate pitted	1
	Exine otherwise	0
37.	Exine granular	1
	Exine papillate	0
38.	Serologically similar to <u>Xanthorhiza</u>	1
	Serologically dissimilar to <u>Xanthorhiza</u>	0
39.	Serologically similar to <u>Cimicifuga</u>	1
	Serologically dissimilar to <u>Cimicifuga</u>	0
40.	Serologically similar to <u>Aquilegia</u>	1
	Serologically dissimilar to <u>Aquilegia</u>	0
41.	Serologically similar to <u>Delphinium</u>	1
	Serologically dissimilar to <u>Delphinium</u>	0

(Fig.2)

one of which is considered advanced. The primitive character state is assigned a "0", while the advanced character state is assigned a "1". For example, the flowers in certain genera are regular or radially symmetrical, while in other genera the flowers are irregular, or asymmetrical. Regular flowers are considered primitive, and those genera having this type of floral arrangement are given a "0" in the data matrix while those genera with irregular flowers, which are considered advanced, are assigned a "1".

To determine whether a character state is primitive or not, the outgroup criterion is used. An outgroup is a group closely related to the group that is being studied. The family used as an outgroup for this study was the Berberidaceae. Each character is looked at in the outgroup and the character state that is found in the outgroup is considered primitive and assigned a "0" for both the outgroup and the group under study. A hypothetical ancestor, which is composed of all the primitive character states, was constructed to root the evolutionary trees produced from the data. The ancestor exhibited only "0"'s in the data matrix.

In compiling characters, I used published data, herbarium specimens, and seed samples. Data was gathered for each genus. The bulk of my characters were obtained from the published data on the Ranunculaceae. My major source was the Manual of the Vascular Flora of the Carolinas (Radford, Ahles, and Bell, 1968) which describes all of the genera of the buttercup family that are found in the Carolinas. The genera found in the Carolinas are typical of those found in the Southeast, and this book described 16 of the 18 genera I studied. The remaining two genera, Coptis and Anemonella, were described by Gleason (1952). Along with these books I used published chromosome analyses of the Ranunculaceae (Tamura 1964, 1965, 1966; Walter, 1941). The chromosomal information was extremely useful because there is a major distinction among the genera between those with R-type (large) chromosomes and those with T-type (small) chromosomes. Allied with this dichotomy is the fact that most of the R-type genera have a base chromosome number of eight, while the T-type genera have a base chromosome number of seven. A few genera have other general patterns, such as having variably sized chromosomes and base chromosome numbers of nine or thirteen (Fig. 3 and 4). In addition to the major floristic works and chromosome studies, I gathered information from assorted papers on the Ranunculaceae. These papers covered a wide variety of areas, such as pollen types, serological (protein) similarities, and structural information (Jensen and Greven, 1984; Keener, 1977; Smith,1926; Wodehouse, 1936).

The herbarium specimens were used to provide information on bud structure. The buds in the Ranunculaceae are either imbricate or valvate. Imbricate buds are ones in which the parts of the bud are overlapping, while valvate buds are those in which the parts meet, but do not overlap. In order to determine the bud type for each genus, I had to remove a bud from each specimen and observe it using a stereomicroscope, taking the bud apart while noting the arrangement of the parts.

Four types of fruits are found among the 18 genera: achenes, follicles, berries, and utricles. Basically, achenes and utricles are one seeded fruits, while berries and follicles may contain more than one seed. Not only are the fruit types distinguishing characters, but the seeds also

CHROMOSOME SIZE IN THE RANUNCULACEAE

R-TYPE	T-TYPE	OTHERS
Delphinium	Anemonella	Hydrastis
Aconitum	Thalictrum	
Caltha	Coptis	
Actaea	Xanthorhiza	
Cimicifuga	Isopyrum	
Myosurus	Aquilegia	
Ranunculus	Enemion	
Trautvetteria		
Hepatica		
Anemone		

(Fig. 3)

CHROMOSOME NUMBER IN THE RANUNCULACEAE

n=8	n=7	n=9 or 13
Delphinium	Aquilegia	Xanthorhiza
Aconitum	Enemion	Hydrastis
Caltha	Thalictrum	Coptis
Actaea	Anemonella	
Cimicifuga		
Clematis		
Myosurus		
Ranunculus		
Trautvetteria		
Hepatica		
Anemone		

(Fig. 4)

provide useful character information. Using the stereomicroscope I observed seed samples from the genera and was able to obtain many workable characters, such as seeds wrinkled in a follicle vs. unwrinkled in a follicle.

Evolution is thought to be a parsimonious process; i.e. each character has undergone the fewest number of changes possible. This means that ideally each character should have evolved only once among the genera, and such a character is said to be informative. Certain characters, however, are the result of more than one evolutionary change, and these characters are said to be noninformative. In some cases it appears that evolution has occurred more than once for several characters, and these characters were maintained in the data matrix (Fig. 5). Nonetheless, the number of times a character evolves should be a small number, and those characters appearing to have evolved six or more times were discarded (Fig. 6). The process of gathering characters was a continuous one, where I was constantly editing the data, discarding noninformative characters while maintaining informative ones.

Not only did the characters have to be examined and edited, they also had to be viewed in terms of the outgroup, Berberidaceae. A decision had to be made as to which state of the character was found in the outgroup. Often there would be a definitive answer, but in the case of some characters the outgroup contained no character similar to the one I was using, or contained both character states of that particular character. If the outgroup did not have a character analogous to the one I was using, the character state appearing the least number of times in the group under study was given a "1", while the character state appearing the most was assigned a "0". In this case, the ancestor was also assigned a "0". If the

CHARACTERS WITH MORE THAN ONE STEP*

Fruit an achene Fruit not an achene	3 steps
Fruit a follicle Fruit not a follicle	5 steps
Fruit a berry Fruit not a berry	3 steps
Leaves evergreen Leaves not evergreen	2 steps
Nectaries present Nectaries not present	3 steps
Base chromosome number = 7 Base chromosome number ≠7	2 steps
Seeds wrinkled in follicle Seeds not wrinkled in follicle	3 steps
T-type chromosomes Not T-type chromosomes	2 steps
Seeds rounded in follicle Seeds not rounded in follicle	2 steps
sepals spurred sepals not spurred	2 steps
Carpels exactly one Carpels not exactly one	2 steps
Sepals small early decidious Sepals well developed	3 steps
Sepals imbricate in bud Sepals not imbricate in bud	2 steps
Ovules a pair in each ovary Ovules not a pair in each ovary	2 steps
Pollen grains furrowed Pollen grains pored	3 steps
Staminodia present Staminodia not present	4 steps
Exine granular Exine papillate	3 steps

* from data matrix run using the WAGNER program

Fig. (5)

CHARACTERS DELETED

Character	No. of steps
Flowers in panicles, racemes, etc. Flowers otherwise	4
Leaves glabrous Leaves pubescent, pillose, etc.	5
Stamenoids present Stamenoids not present	4
Ovule one in each ovary Ovule more than one in each ovary	4

(Fig. 6)

character states of the character I was using both appeared in the outgroup, a "0" was assigned to the character state which appeared most often in the outgroup.

The dimensions of my final data matrix are 19 genera, including the ancestor, by 41 characters (Fig. 7). The data in this matrix is relatively clean, with only 17 out of the 41 characters using more than one evolutionary step. The first analysis of the final data matrix was accomplished using the WAGNER routine of the PHYLIP program, written by Dr. Joe Felsenstein of the University of Washington, Seattle. This program analyzes the data matrix and composes an evolutionary tree based on the number of characters each genus has in common with the others. Those genera having more characters in common are located near each other on the tree, while those genera having few characters in common with the other genera are located relatively farther away from other genera. The WAGNER routine generates one evolutionary tree, which, unfortunately, may not be the most parsimonious tree. One indication of the weakness of the WAGNER routine is that the resulting tree could be changed merely by changing the order in which the data was given to the computer. Not only would the tree look different, but it would also involve a different number of evolutionary steps. As the goal of this project was to find the most parsimonious trees, or those with the least number of evolutionary steps, the only way to determine if a tree with relatively few steps was generated was to manually shuffle the data as many ways as possible. With a matrix of this size, it would be difficult to present the data in all of its possible permutations, and the author of the program, Dr. Felsenstein, suggested using a total of ten permutations.

DATA MATRIX

Taxa	*Characters*								
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41
Xanthorhiza	01001	00000	10100	10101	01000	00110	11101	00100	0
Coptis	00111	10000	10100	10101	00000	00110	10101	00100	0
Aquilegia	00001	00000	00000	00001	01000	10110	10100	01001	0
Enemion	00001	00000	00100	00001	10000	00110	10000	00000	0
Thalictrum	00111	00000	01000	00001	00000	00100	10001	01001	0
Anemonella	00111	00000	00000	00001	00000	00110	10001	00001	0
Hydrastis	00010	00000	00100	01100	00010	00110	11010	10000	0
Actaea	00010	00000	00001	00110	00001	00000	10000	00010	0
Cimicifuga	00001	00000	00001	00110	10000	00100	10100	00010	0
Caltha	00001	00000	00001	00110	10000	00110	10000	00000	0
Ranunculus	00111	00001	10001	00110	00000	00110	10000	00000	0
Myosurus	00111	00001	10001	00110	00000	01110	10000	00000	0
Trautvetteria	00011	00000	00011	00110	00000	00100	10000	00000	0
Hepatica	00111	10000	00001	00110	00000	00110	10000	00000	0
Anemone	00111	00010	00001	00110	00000	00110	10001	00000	0
Clematis	00111	01100	00001	00110	00000	00111	00000	00000	0
Delphinium	10001	00000	00001	00110	00100	01110	10100	01000	1
Aconitum	10001	00000	10001	00110	00100	01110	10100	01000	1
Ancestor	00000	00000	00000	00000	00000	00000	00000	00000	0

(Fig. 7)

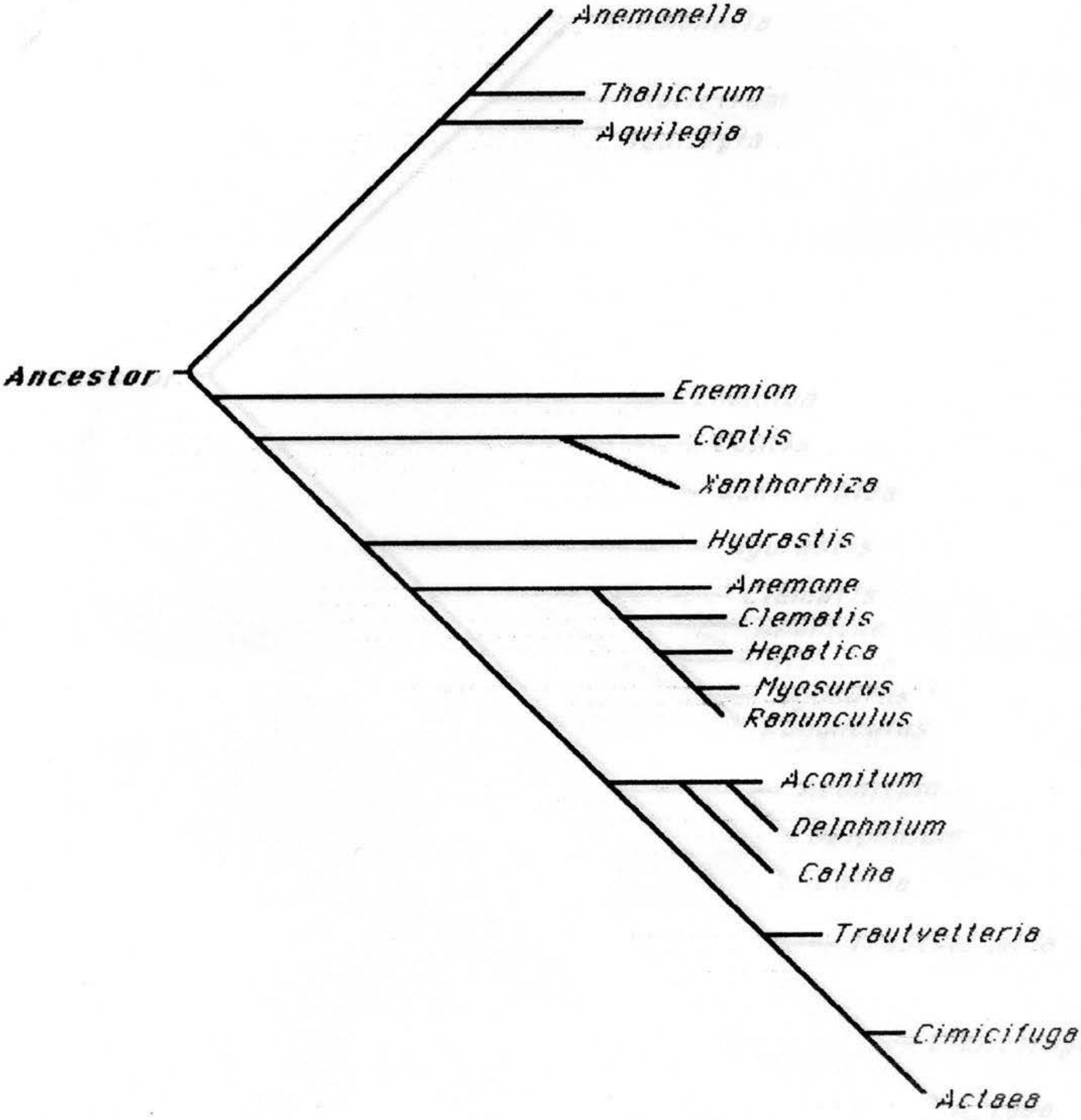
Another routine, PENNY of the PHYLIP program, became available for use on the IBM mainframe computer, which has much more memory capability than the HP3000 used for the WAGNER routine. PENNY, like WAGNER, composes evolutionary trees from the data, but unlike the WAGNER routine, PENNY looks at a large number of trees and searches for the most parsimonious trees that the data will produce.

RESULTS

For the present data, the PENNY routine looked at 300,000 possible trees and found eight trees that were equally parsiminious, each tree having 70 steps, which was a much lower number of steps than was found in any of the trees generated by the WAGNER routine, which had from 72 to 76 steps involved (Fig. 8-14).

As stated earlier, the purpose of undertaking an evolutionary study is to clarify relationaships between families, between genera within families, or between species within a genus. My study of the southeastern genera of the buttercup family produced eight trees that indicate the relationships between the genera as indicated by the data in the data matrix. An examination of the eight trees produced by the PENNY routine reveals that all eight trees are basically the same, with minor differences in the trio of _Clematis_, _Anemone_, and _Hepatica_. Two of the trees generated were congruent.

The major similarity among the trees is the primary branching pattern, which splits the genera into two groups that correlate with their chromosome type and number. Those genera with T-type chromosomes and a base chromosome number of seven, _Aquilegia_, _Anemonella_, and _Thalictrum_, compose one major branch while the other genera, which have R-type chromosomes and base numbers of eight, nine, or thirteen compose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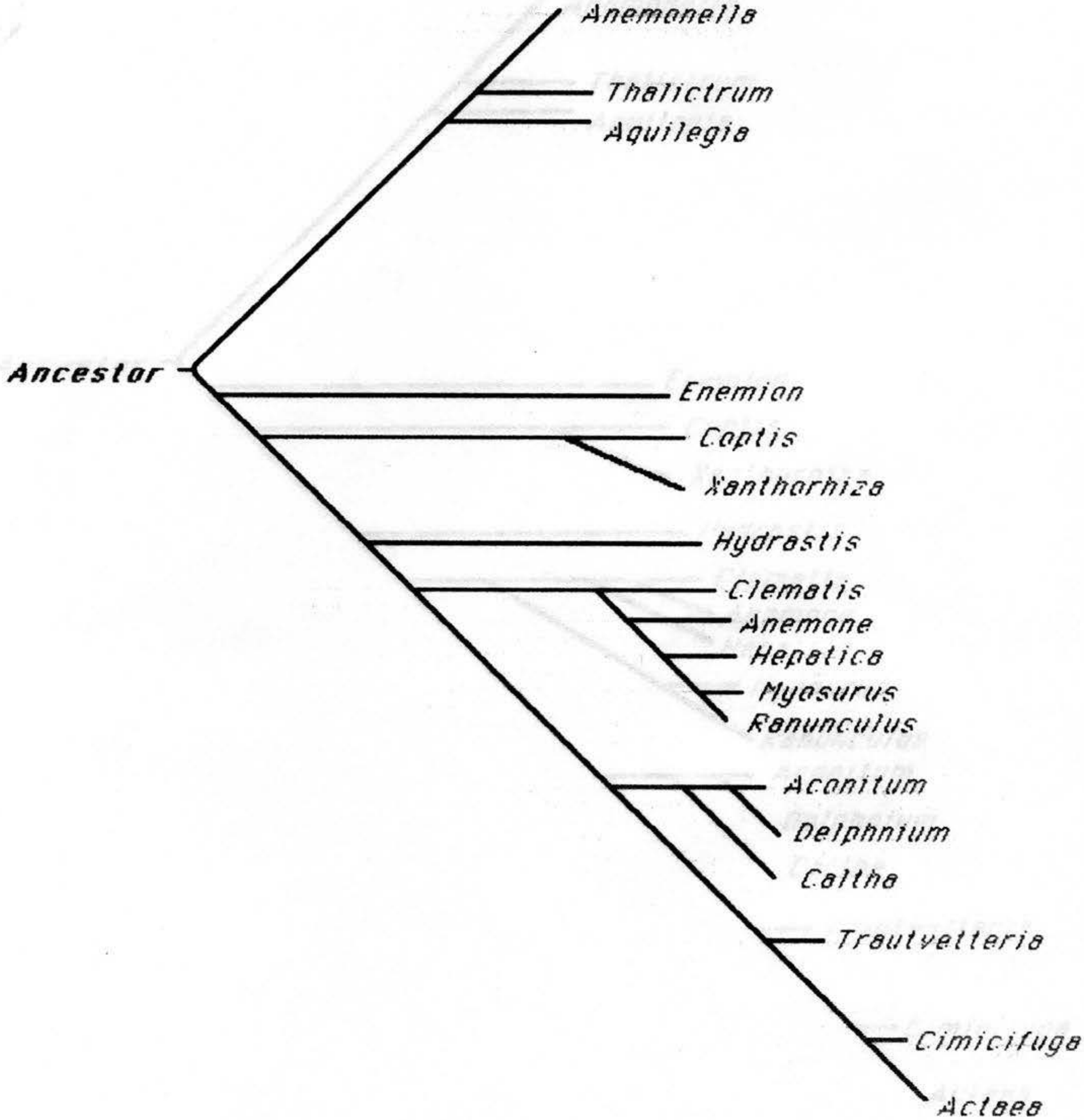
Thalictrum
Aquilegia
Ancestor
Enemion
Coptis
Xanthorhiza
Hydrastis
Anemone
Clematis
Hepatica
Myosurus
Ranunculus
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 8)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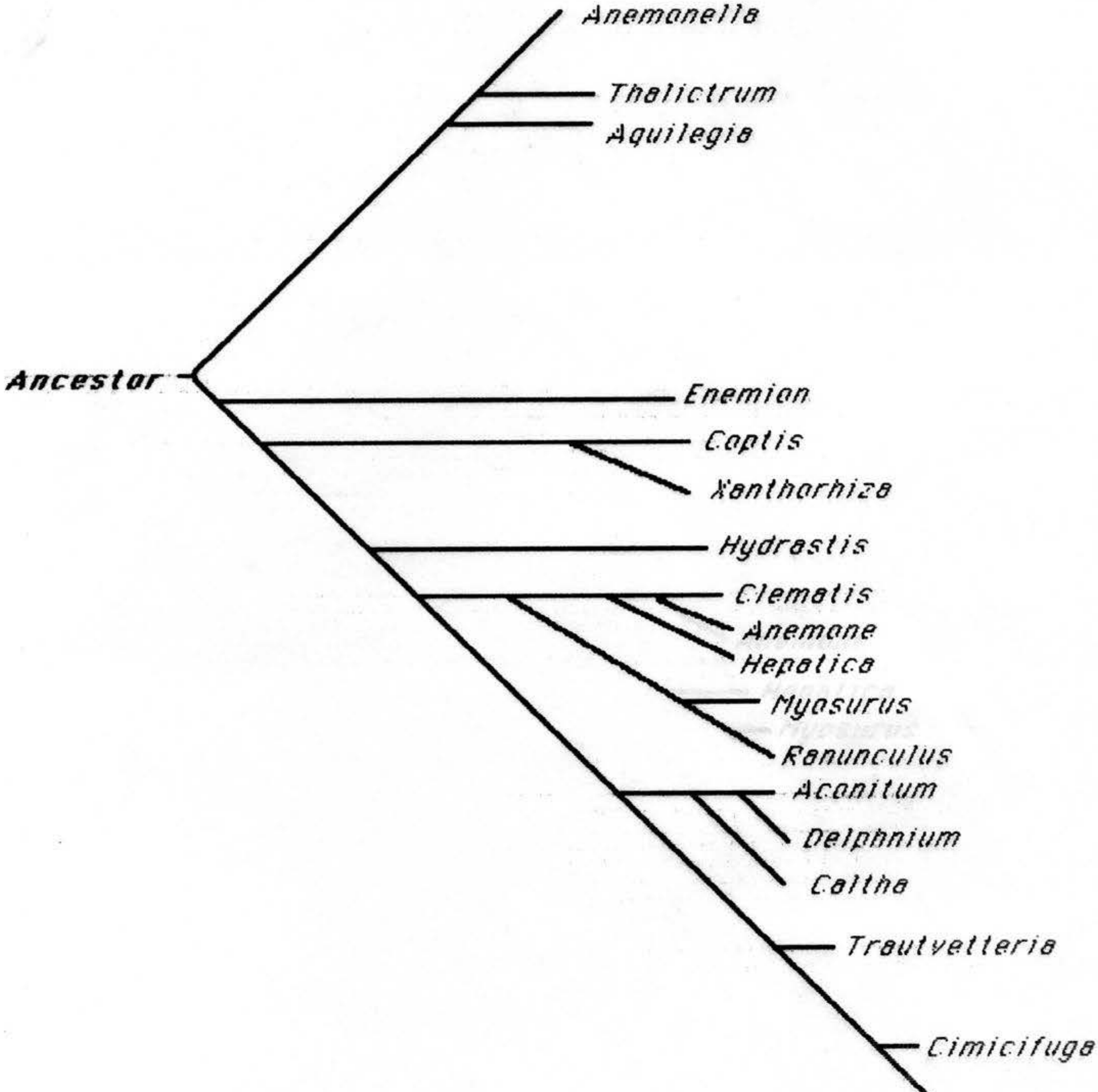
Clematis
Anemone
Hepatica
Myosurus
Ranunculus
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 9)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Clematis
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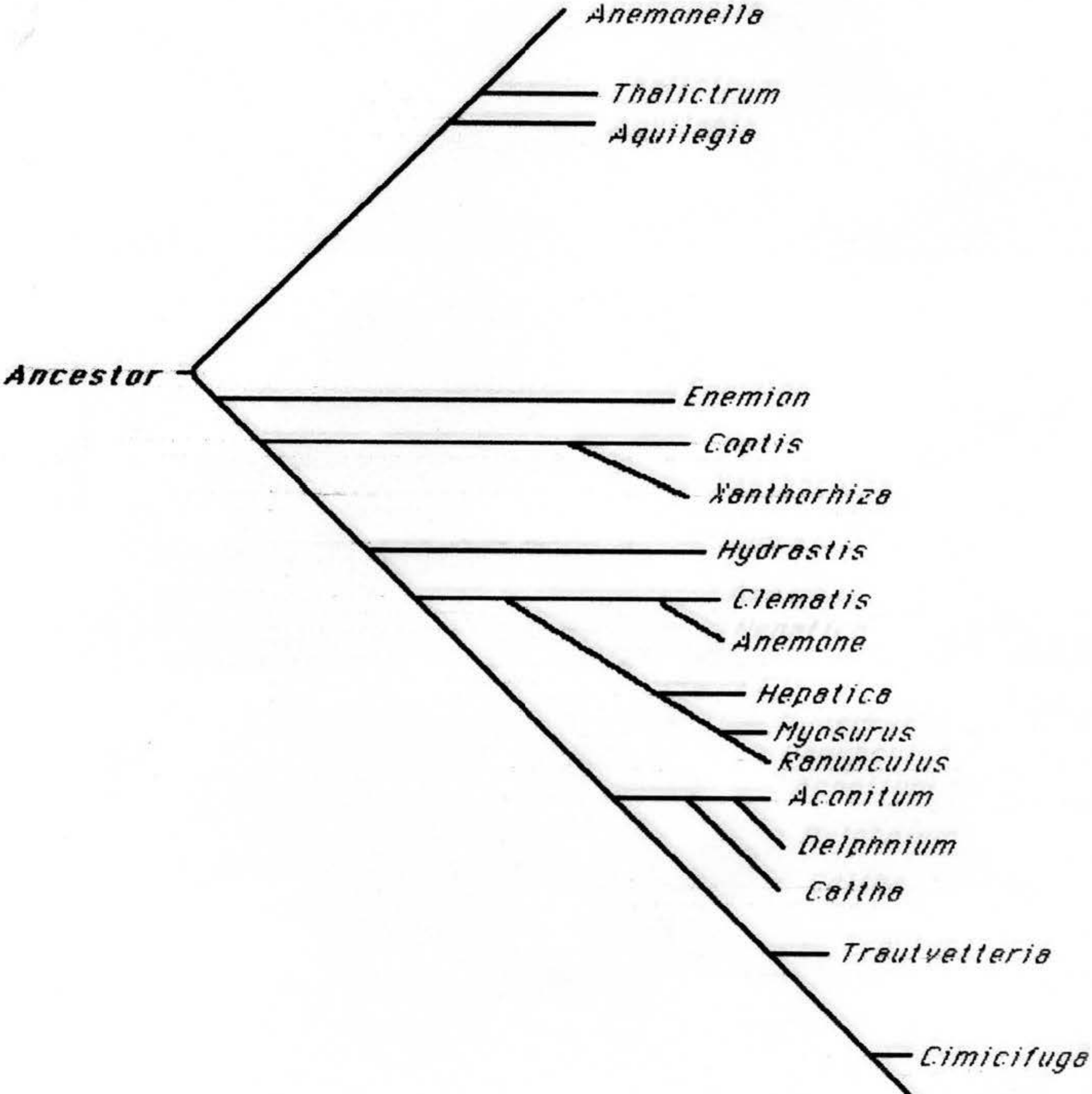
Hepatica
Myosurus
Ranunculus
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 10)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Xanthorhiza
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Clematis
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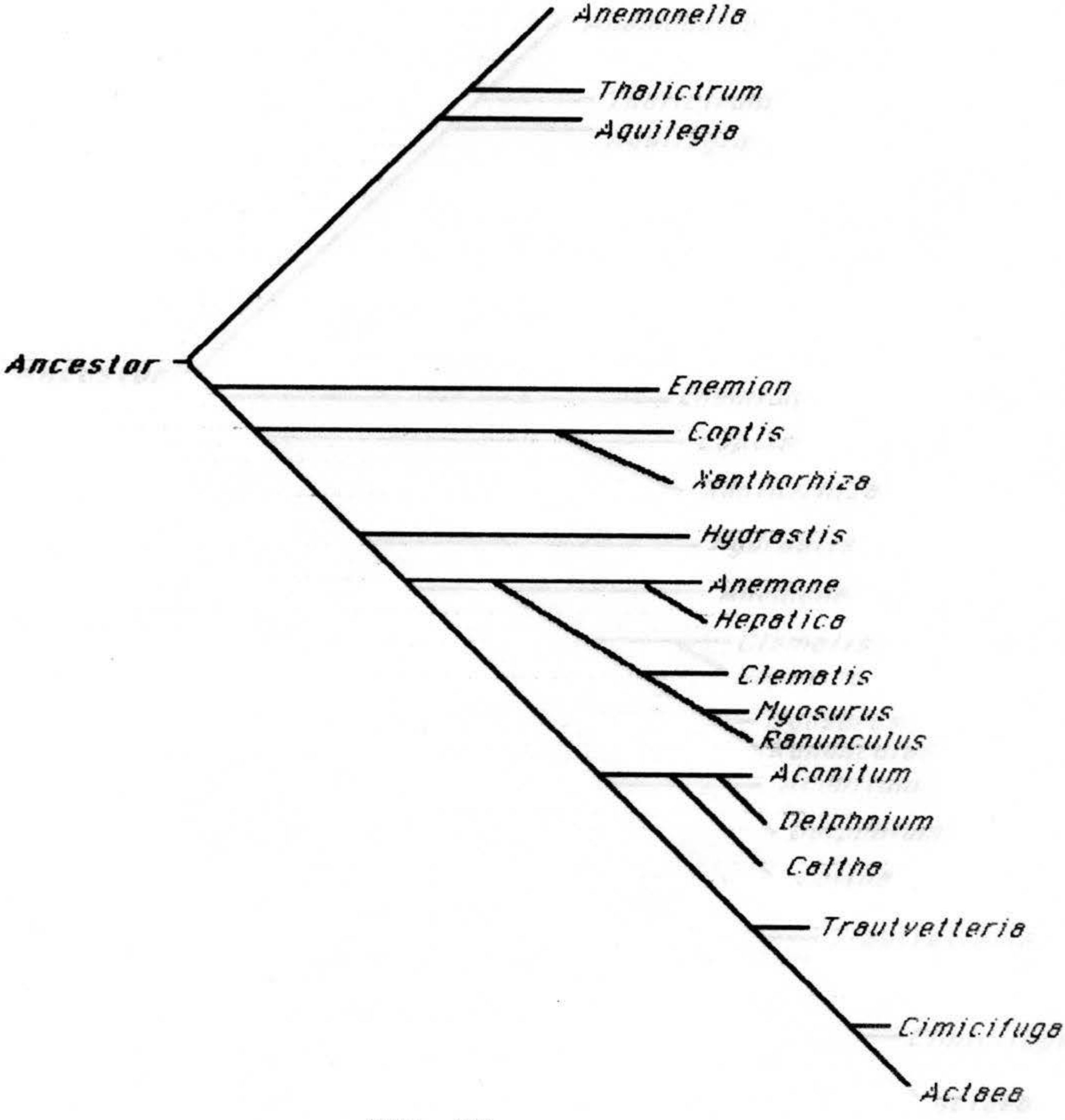
Myosurus
Ranunculus
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 11)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Thalictrum
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Anemone
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Ranunculus
Aconitum
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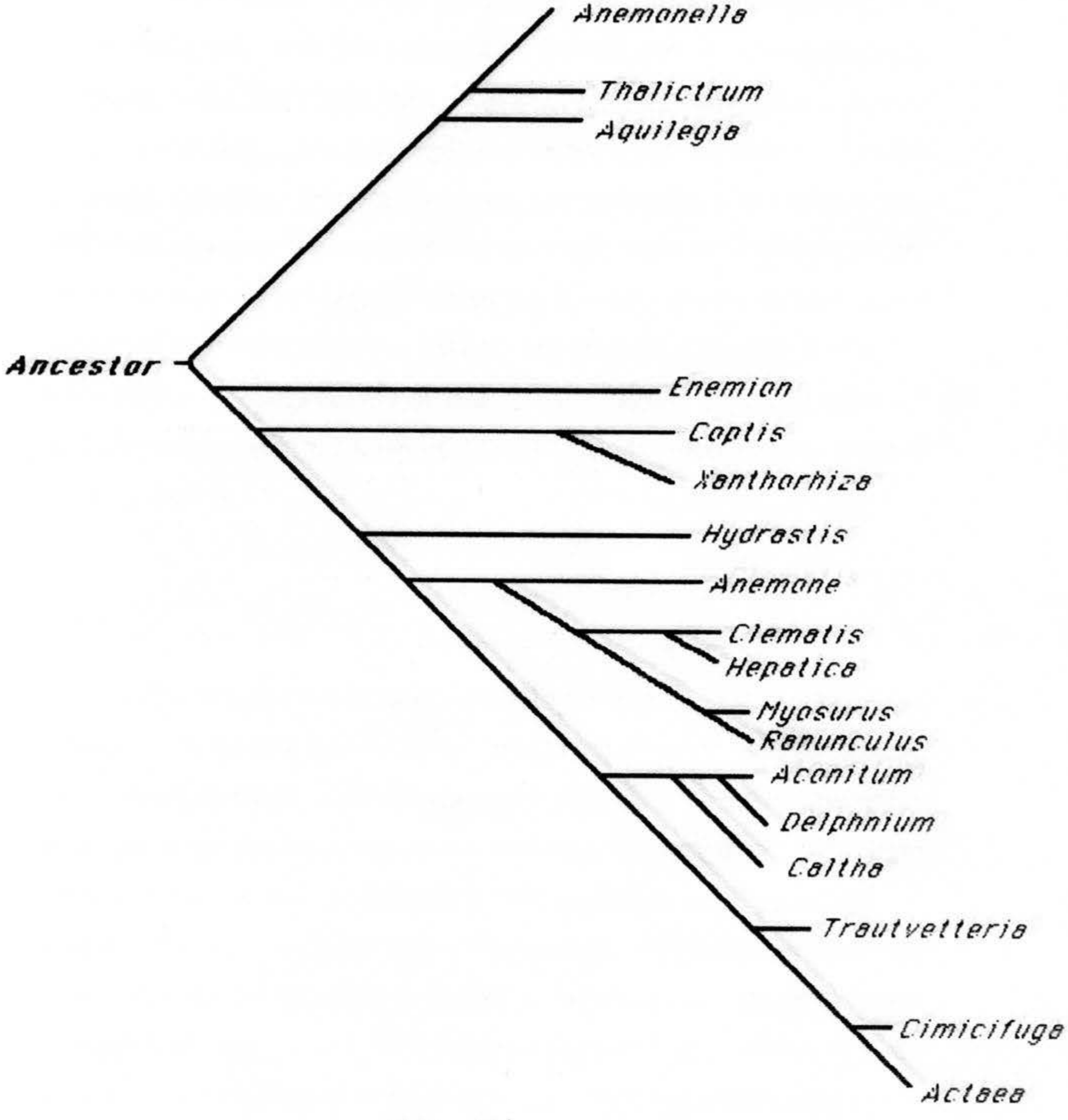
Trautvetteria
Cimicifuga
Actaea

(Fig. 12)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Hydrastis
Anemone
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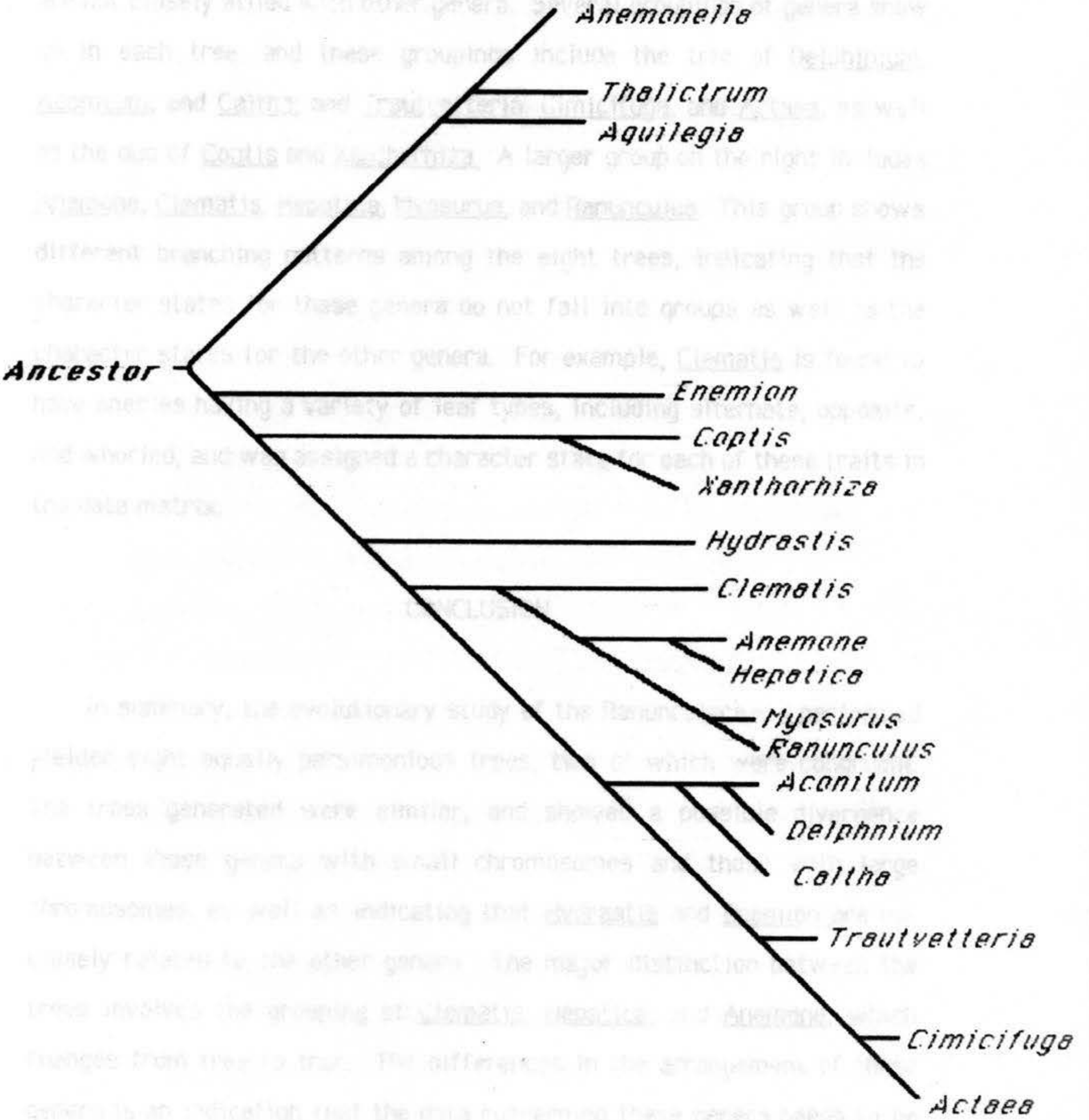
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 13)


Anemonella
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Coptis
Xanthorhiza
Hydrastis
Clematis
Anemone
Hepatica
Myosurus
Ranunculus
Aconitum
Delphnium
Caltha
Trautvetteria
Cimicifuga
Actaea

(Fig. 14)

the other major branch. Among those genera on the right of the ancestor one finds several secondary branching patterns that are consistent among all eight trees. For example, Enemion and Hydrastis branch off singly, and are not closely allied with other genera. Several groupings of genera show up in each tree, and these groupings include the trio of Delphinium, Aconitum, and Caltha; and Trautvetteria, Cimicifuga, and Actaea, as well as the duo of Coptis and Xanthorhiza. A larger group on the right includes Anemone, Clematis, Hepatica, Myosurus, and Ranunculus. This group shows different branching patterns among the eight trees, indicating that the character states for these genera do not fall into groups as well as the character states for the other genera. For example, Clematis is found to have species having a variety of leaf types, including alternate, opposite, and whorled, and was assigned a character state for each of these traits in the data matrix.

CONCLUSION

In summary, the evolutionary study of the Ranunculaceae I performed yielded eight equally parsimonious trees, two of which were congruent. The trees generated were similar, and showed a possible divergence between those genera with small chromosomes and those with large chromosomes, as well as indicating that Hydrastis and Enemion are not closely related to the other genera. The major distinction between the trees involves the grouping of Clematis, Hepatica, and Anemone, which changes from tree to tree. The differences in the arrangement of these genera is an indication that the data concerning these genera needs to be reexamined and further refined.

BIBLIOGRAPHY

Duncan, Wilbur H., and Leonard E. Foote. Wildflowers of the Southeastern United States. Athens: University of Georgia Press, 1975.

Durrell, L.W. and H.D. Harrington. How to Identify Plants. Chicago: The Swallow Press, Inc., 1957.

Gleason, Henry A. New Britton and Brown Illustrated Flora. Vol. 2. New York: Hafner Press, 1952.

Gregory, Walter C. "Phylogenetic and Cytological Studies in the Ranunculaceae." Transactions of the American Philosophical Society, Vol. 31, Part 5 (August, 1941).

Greven, B., and U. Jensen. "Serological Aspects and Phylogenetic Relationships of Magnoliidae." Taxon, Vol. 33. (November, 1984). pp. 567-568.

Keener, Carl S. "Studies in the Ranunculaceae of the Southeastern United States VI. Miscellaneous Genera." SIDA, Vol. 7 (January, 1977). pp. 1-12.

Porter, C.L. Taxonomy of Flowering Plants. San Francisco and London: W. H. Freeman and Co., 1959.

Radford, Albert E., Harry E. Ahles, and C. Ritchie Bell. Manual of the Vascular Flora of the Carolinas. Chapel Hill: The University of North Carolina Press, 1968.

Smith, George Hume. "Vascular Anatomy of Ranalian Flowers." The Botanical Gazette, Vol. 82 (September, 1926) pp. 1-29.

Tamura, Michio. "Morphology, Ecology, and Phylogeny of the Ranunculaceae III." Science Reports, Vol. 13(1), 1964.

Tamura, Michio. "Morphology, Ecology, and Phylogeny of the Ranunculaceae IV." Science Reports, Vol. 14(1), 1965.

Tamura, Michio. "Morphology, Ecology, and Phylogeny of the Ranunculaceae VI." Science Reports, Vol. 15(1), 1966.

Wodehouse, R. P. "Pollen grains in the Identification and Classification of Plants. VII, The Ranunculaceae." Bulletin of the Torrey Botanical Club, Vol. 63 (December, 1936) pp. 495-662.

ACKNOWLEDGEMENTS

I would like to thank Dr. Charles Nelson for his interest in my project and for his help in using the PHYLIP program, as well as the IBM mainframe computer.