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Edward E. Schilling,
Major Professor

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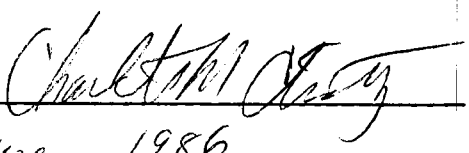
Signature 

Date June 1986

A BIOSYSTEMATIC STUDY OF THE RELATIONSHIPS OF *HIERACIUM GRONOVII* L., *H. VENOSUM* L. AND *H. TRAILLII* GREENE (COMPOSITAE: LACTUCEAE)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Charlotte M. Christy

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ABSTRACT

The relationships of H. gronovii L., H. venosum L. and H. traillii Greene were investigated for two reasons. First, to determine if H. traillii represents a hybrid of H. gronovii and H. venosum and second, to determine methods of identifying natural hybrids between H. gronovii and H. venosum. Data from reproductive behavior, artificial hybridization, cytology, morphology and flavonoid chemistry were used.

Differences between species in reproductive behavior were noted. Although all three taxa are self-fertile, only in H. gronovii will self-pollination occur in isolated heads. In H. venosum and H. traillii, self-pollination does not occur in isolated heads.

Artificial hybrids between H. gronovii and H. venosum were easily produced. These hybrids appeared vigorous, and set viable seed when self-pollinated.

Cytological differences occur between the species. All three taxa are diploid, $2n = 9_{II}$. Irregular chromosome pairing was not observed in any of the taxa or in artificial hybrids between H. gronovii and H. venosum. Meiotic abnormalities observed resulted from division failure and possible paracentric inversions (bridge and fragment figures). The highest frequency of meiotic abnormalities occurred in inter-populational hybrids of H. gronovii, suggesting that its tendency toward selfing has produced genetic differences between populations.

Multivariate analyses of morphology indicate that all three taxa are distinctive. Artificial hybrids between H. gronovii and H. venosum are also distinctive. Although hybrids exhibited a wide range of variability, they combined characters of the parents. They are best identified as possessing the wide ligules (at least 1-1/2 mm) of H. venosum and the hair types and distribution of H. gronovii.

All flavonoids isolated were based on the flavones apigenin and luteolin. A comparison of flavonoid complements suggests that all taxa have distinctive profiles. However, the presence of unidentified compounds, one each in H. gronovii and H. traillii, prevents a definite decision on this. Flavonoid data do not support the interpretation of H. traillii as a hybrid, as it lacks a distinctive compound found in H. gronovii.

The similarities of H. venosum and H. traillii in reproductive behavior, morphology and flavonoid chemistry indicate that these two taxa have a closer relationship to each other than to H. gronovii. Based on these data, the wide range of morphological variability in H. venosum and the small distribution and more specialized ecological preferences of H. traillii (when compared to H. venosum), it is hypothesized that H. traillii represents a schizoendemic species--the endemic result of gradual speciation--which evolved from H. venosum.

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CHAPTER I

INTRODUCTION

The genus Hieracium L. (Compositae - Lactuceae) contains circa 1000 species, of mostly temperate distribution (Willis, 1973). All species are perennial herbs with milky sap, fibrous roots and a base chromosome number of x = 9 (Cronquist, 1980). Hieracium is well known for the taxonomic problems caused by apomixis, polyploidy and hybridization among amphimictic and facultatively apomictic taxa.

Natural hybridization has been a cause of taxonomic confusion in subgenus Stenotheca (Monn.) Torrey & Gray. This predominantly North American subgenus contains only diploid and amphimictic taxa (Vuilleumier, 1973; Guppy, 1978; Cronquist, 1980). Cronquist (1980) lists seven species of subgenus Stenotheca for eastern North America, although a total of twelve named taxa for this area can be found in older literature (e.g. Britton and Brown, 1913; Small, 1933). The nomenclatural confusion resulted from the description of several putative hybrids as species (Figure I-1) and from problems with the recognition or circumscription of taxa. In eastern Tennessee, four species (H. gronovii L., H. scabrum Mich., H. paniculatum L. and H. venosum L.) may be expected to occur. These taxa have been reported to hybridize naturally in five of the six possible combinations (Figure I-1).

Some of the confusion in taxonomic status involves H. traillii Greene. Hieracium traillii has been considered a probable hybrid by Fernald (1950) and the equivalent of H. marianum, a putative hybrid of

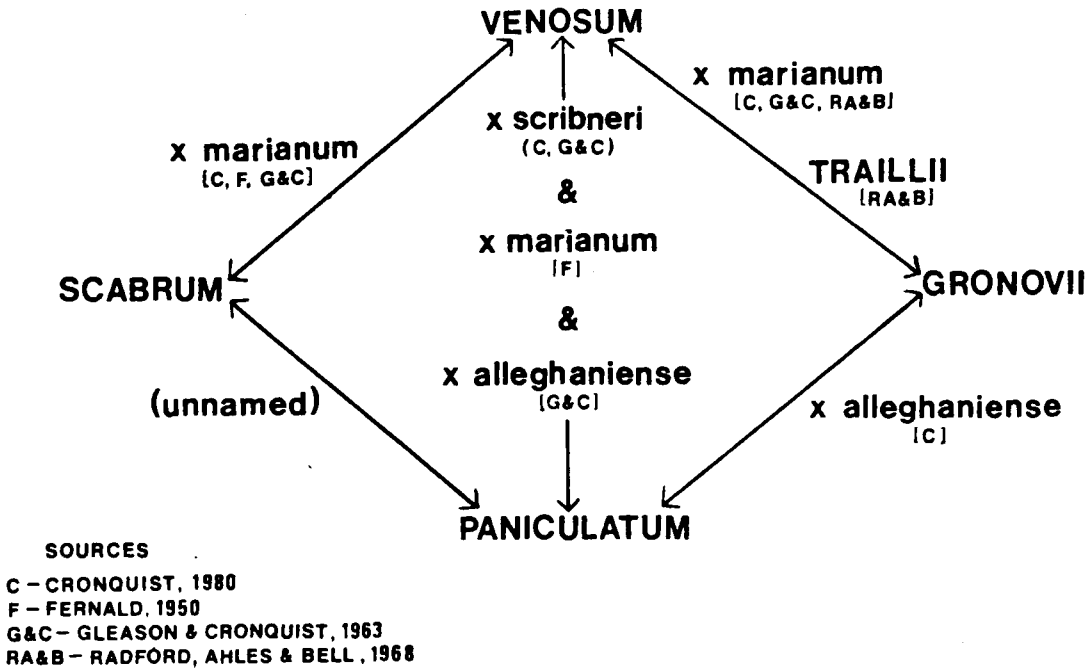

Figure I-1. Reported natural hybridizations and putative parentage of named hybrids in Eastern North American Hieracium. Species names are in capital letters. Arrows indicate combinations in which hybridization has been reported. The names that have been associated with these hybrids are listed along the arrows.

H. gronovii and H. venosum, by Radford, Ahles and Bell (1968). Other authors have considered H. traillii a distinct species (e.g. Johnson, 1977; Cronquist, 1980). Specimens matching the description of H. traillii are found in a restricted geographic area (Figure I-2), commonly in association with shale substrates (Johnson, 1977). The questionable taxonomic status of H. traillii has hampered evaluation of the possibility that it is a shale-barren endemic (Keener, 1983).

A comparison of descriptions (Johnson, 1977; Cronquist, 1980) of H. gronovii, H. traillii and H. venosum (see also page 40) suggests that H. traillii and H. venosum are superficially similar in appearance and that H. gronovii is morphologically distinct from these two taxa. Hieracium gronovii is characterized by the absence of a basal rosette at maturity, leaves and setose hairs distributed on the lower half of the stem, a narrow, cylindrical, corymbose inflorescence with stellate and glandular hairs and fusiform achenes. Hieracium venosum is characterized by the presence of a basal rosette at maturity, leaves with red-purple vein margins, the stem glabrous and scapose or with few leaves, a spreading, corymbose, sparsely hairy inflorescence and truncate achenes. Hieracium traillii is characterized by the presence of a basal rosette at maturity, no red-purple leaf coloration, a scapose stem, spreading, corymbose inflorescence with stout branches, dense stellate and glandular hairs and involucral bracts with setose hairs. While H. traillii has morphological similarities to both H. gronovii and H. venosum, it also appears to have distinctive morphological characters and habitat preferences.

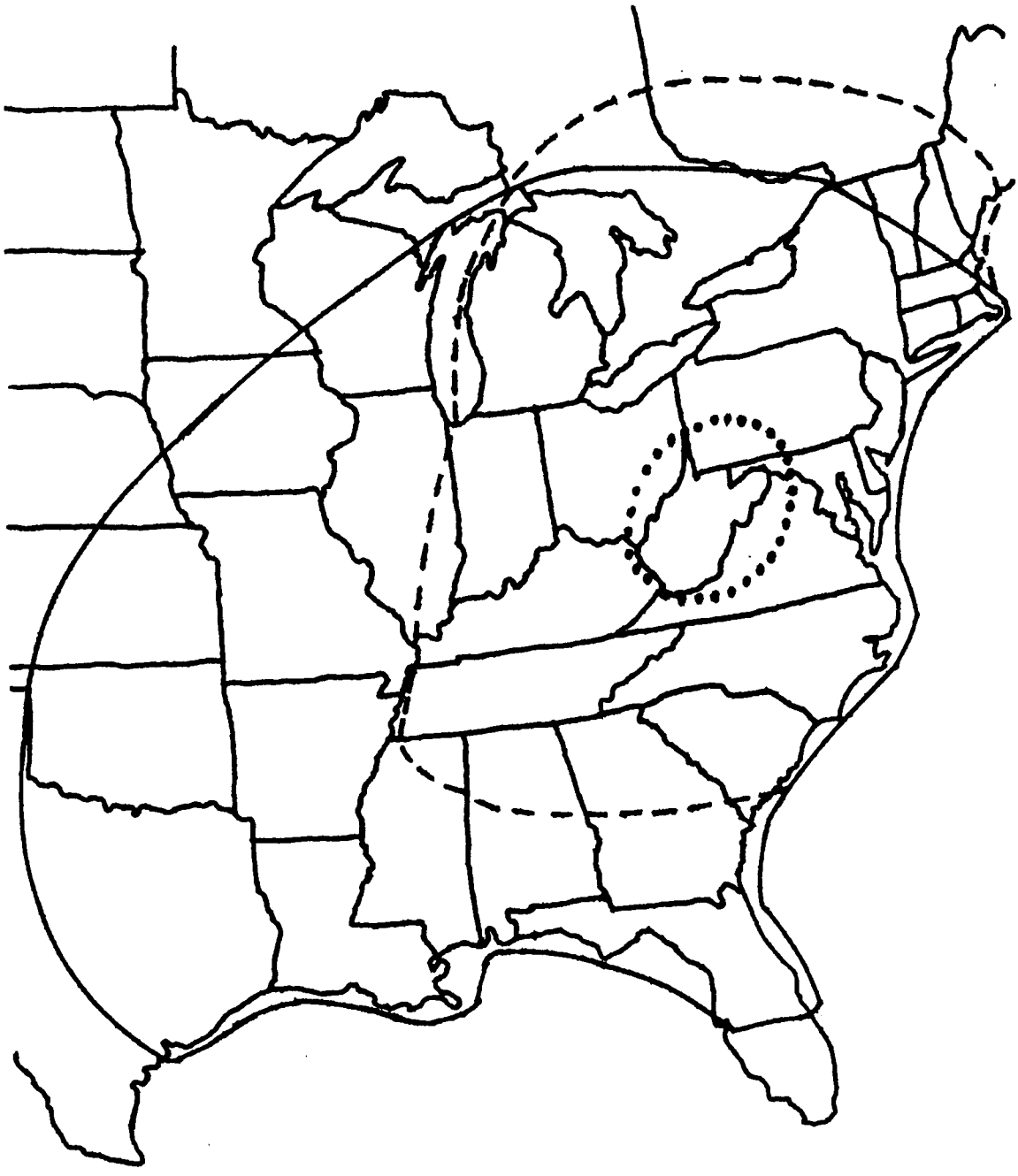

Figure I-2. Distributions of Hieracium gronovii, H. venosum and H. traillii. Distribution limits are indicated by a solid line for H. gronovii, a dashed line for H. venosum and a dotted line for H. traillii.

The purposes of this study are to assess the possibility that H. traillii represents a hybrid of H. venosum and H. gronovii and to discover methods of recognizing natural hybrids between H. venosum and H. gronovii. Data from artificial hybridization, morphology, flavonoid compounds and cytology were used in this study, as they have proven useful in comparisons of other hybridizing taxa in this subgenus (Guppy and Bohm, 1976; Guppy, 1978). Artificial hybrids of H. gronovii and H. venosum were compared to their parental taxa and to H. traillii. Artificial hybrids were also surveyed for unique characters or character combinations that might be useful in the identification of naturally occurring hybrids.

CHAPTER II

HYBRIDIZATION STUDIES

Since the early part of this century, natural hybridization among plants has been increasingly recognized as a relatively common occurrence (Stace, 1975). Biosystematic studies have found both artificial and natural hybrids a useful source of information for understanding and interpreting the evolution, origin and relationships of many species. Data from artificial hybrids is subject to interpretation, as differences between artificial and natural hybrids in appearance or behavior can be caused by genetic variability within a species over space and time.

Previous work with hybridization in Hieracium subgenus Stenotheca includes that of Guppy and Bohm (1976) and Guppy (1978). While investigating the relationships among the species of Hieracium in British Columbia, Guppy found two hybrid complexes. Guppy's artificial hybrids both within and between these complexes were morphologically intermediate to their parents and meiotically normal. Patterns of flavonoid glycosides were species-specific, and the artificial hybrids examined possessed additive flavonoid profiles. These results suggest that a similar approach may be worthwhile in defining the relationship of H. gronovii and H. venosum and the status of H. traillii.

Reciprocal artificial hybridizations were attempted between H. gronovii and H. venosum. Attempts to cause these taxa to self-fertilize were also made, so that any progeny could be compared to

suspected hybrids. The hybrids were compared with H. traillii and surveyed for character patterns possibly useful in recognition of natural hybrids. Hybrid specimens were also surveyed for the presence of meiotic abnormalities or other visible effects of the interactions of two different genomes.

Methods

Specimens of H. gronovii, H. venosum and H. traillii were collected between July 1983 and June 1984 and transplanted into Botany Department gardens located at the University of Tennessee campus in Knoxville. Collection data for specimens used in crossing and selfing attempts is listed in Table II-1. Few crosses involving H. traillii could be attempted due to scarcity of specimens. Self-fertilization of artificial hybrids was also attempted.

Flower heads for crossing were isolated, before the flowers opened, in insect-proof bags of nylon "Nitex" fabric. Hieracium flowers have been found to be too small to allow emasculation without damage to the flowers or breakage of anthers (Guppy, 1978). Emasculation of flowers was not attempted in this study. Heads of the egg parent were washed, by sloshing them in a beaker of fresh water, which removes most pollen. After drying (5 to 10 min.), they were brushed past a similarly isolated but unwashed head to effect cross-pollination. For self-fertilization, two flower heads of one specimen were isolated and brushed past each other without washing. After pollination, heads were tagged and re-isolated.

Table II-1. Collection numbers and locations of specimens of *Hieracium* used in selfing and crossing attempts.

	State	County	Specimens Used (By Collection Number[1])
Hieracium gronovii	Arkansas	Franklin	229
		Pope	148
	Missouri	Howell	247, 250
		Oregon	261
		Shannon	254
		Taney	155
	Oklahoma	Adair	231, 233
	Tennessee	Coffee	83, 84
		Grundy	79
		Johnson	89
		Morgan	50, 51, 55, 57, 60
		Warren	85
Hieracium traillii	Virginia	Bath	f1, f3, f5, f6
Hieracium venosum	Tennessee	Blount	69
		Grundy	80
		Johnson	86, 87, 91, 97
		Monroe	10, 12, 14
		Morgan	42, 46
	Virginia	Augusta	221, 223, 224, 225
		Bath	171, 173, 213, 214
		Highland	112, 113
		Rockbridge	175, 176, 178, 179, 180-193, 195, 197-203, 208, 209, 211
	West Virginia	Summers	106

[1]All collection numbers are of Christy. Vouchers are deposited at TENN.

Preliminary tests of seed germination produced germination rates and percentages that were adequate for the purposes of this study without stratification or specific temperature and light regimens. Therefore, seeds were germinated in petri dishes on dampened qualitative grade filter paper, at normal room conditions of light and temperature. During the germination period seedlings were removed from the petri dishes every one to three days and placed on a "general purpose" soil mix (top soil:peat:sand:perlite, 1:1:1:1) in a greenhouse misting bed. Established seedlings, with a rosette at least 3 cm in diameter, were transplanted into 2-1/4 inch plastic pots, in the same soil mix. After a 2 to 4 day period of recuperation to minimize any effects of simultaneous transplanting and change of environment, they were removed from the misting bed and placed in a laboratory under artificial lighting provided by two high intensity cool white bulbs at a distance of 2 to 2-1/2 ft. The initial daylength of 12 hours was progressively lengthened, to a maximum of 18 hours, after the first two months of growth to simulate seasonal changes.

A weekly application of 50 ppm gibberellic acid (GA_3, one drop/plant) was used in an attempt to increase the rate of maturation. In other rosette plants, notably biennials, GA has been shown to cause early maturation (Jones, 1973). The rates of maturation in Hieracium seedlings were too varied to determine if GA increased the rate of maturation. Use of GA was discontinued when stem elongation began, prior to blooming. Seedlings at this stage were also transplanted

with minimal root disturbance to a larger container to decrease the chance of accidental dessication.

Results and Discussion

Collections. County locations of all collections made during this study are indicated in Figure II-1. Most descriptions of the typical habitat of *H. gronovii* and *H. venosum* are very similar--dry, open woods (Fernald, 1950; Cronquist, 1980). The most common habitat observed during this study was a dry, partly sunny road or trail bank with little dense ground cover, adjacent to a wooded area. Populations were also observed in relatively undisturbed woods and in clearcut areas. Several mixed populations of *H. gronovii* and *H. venosum* were located in recently disturbed areas. The single population of *H. triallii* was on a steep shale road bank that provided little shade or competition.

Several populations of *H. gronovii* west of the range of *H. venosum* (Figure I-2, Page 4 and Figure II-1) appeared dimorphic. In Oklahoma, a population covering several square yards of a clearcut possessed two distinct forms, both later identified as *H. gronovii*. One set of plants was small, less leafy and in flower or fruit when observed in late June. The other set was robust, leafier and not yet in bud. In Missouri, several counties supported populations, frequently in well-shaded areas, with specimens differing widely in leaf and stem coloration--from a normal green to an atypical dark red. Both coloration and the size differences noted above persisted in transplanted specimens. A wide range of variation in leafiness, size

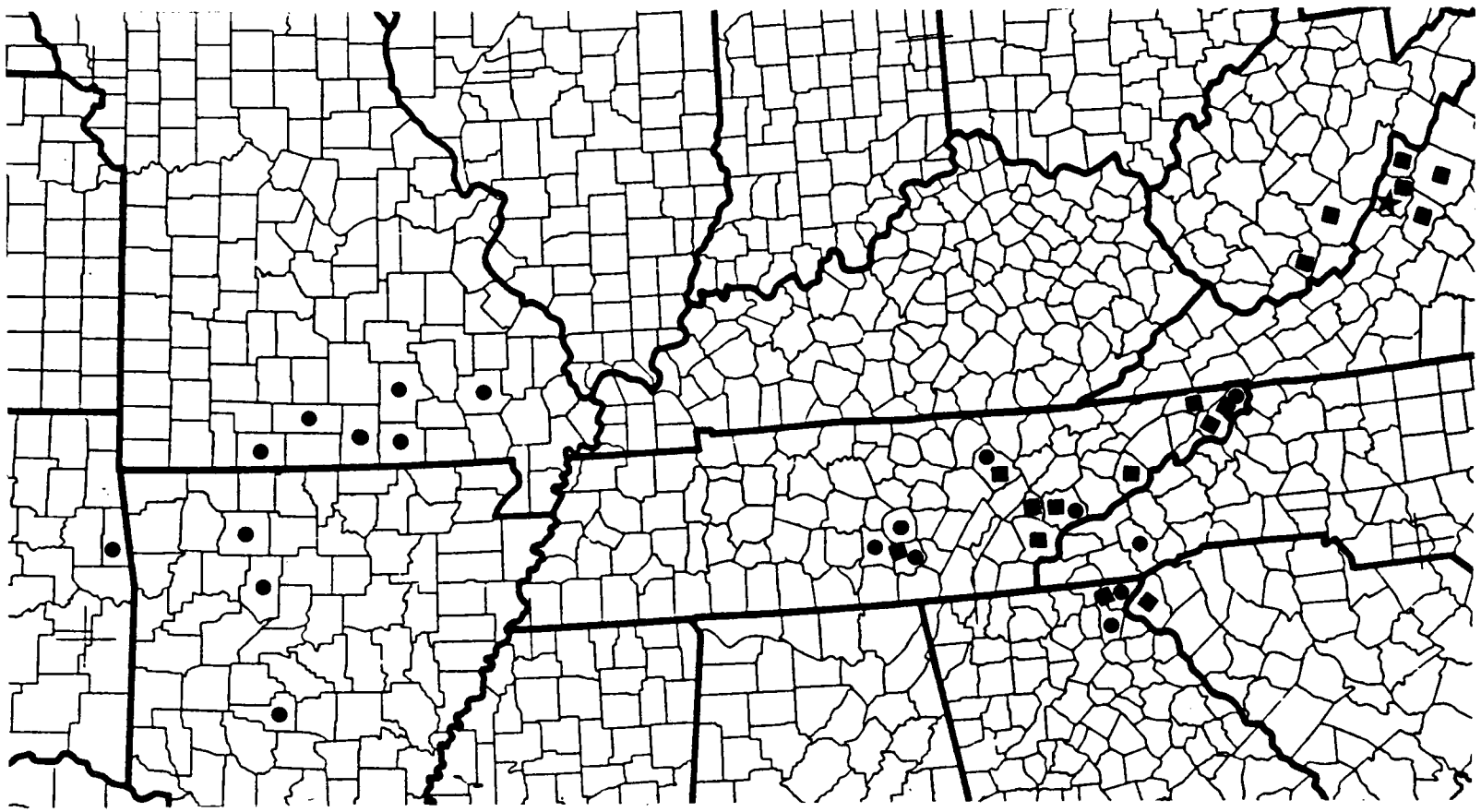

Figure II-1. Map of all Hieracium collections made during this study, by county. Collections of H. gronovii are indicated by a dot, H. venosum by a square and H. traillii by a star.

and inflorescence form in H. gronovii has been noted by Steyermark (1963) as the cause of occasional misidentifications.

Self-fertilization. Both H. gronovii and H. venosum will self-fertilize, but they show marked differences in ability to self-pollinate (Table II-2). In H. gronovii 80% of isolated heads produced seed, and seed set was consistently greater than 50% in both isolated and artificially pollinated heads. In contrast, in H. venosum only 12% of isolated heads set seed, and seed set was less than 20% even when self-pollination was attempted. Artificial hybrids of H. gronovii and H. venosum will set viable seed, but favor H. venosum in their lack of ability to self-pollinate (not shown). Lack of seed set in isolated heads of H. traillii suggests that it, like H. venosum, lacks the ability to self-pollinate in isolated heads, although the sample size is too small to allow firm conclusions.

Hybridization. Results of the attempted crosses are listed in Table II-3. The relative abilities of H. gronovii and H. venosum to self-pollinate are evident both in a comparison of seed set and in the number of failed cross attempts when each was the egg parent. While the direction of the cross affected the number of seeds recovered, it does not appear to affect either seedling survival (approximately 20%) or the frequency of hybrids (approximately 20% of surviving seedlings). Artificial hybrids were identified by morphological comparison to their parents and to progeny of self-fertilzation in both parental taxa. Both the ability of H. gronovii to self-pollinate

Table II-2. Results of isolation and self-pollination attempts in Hieracium gronovii, H. venosum, and H. traillii.

Species	Self-Pollination Results				Frequency of Seed Set[2]	
	Specimen Collection Numbers[1]	Number of Heads Selfed	Number of Flowers Selfed	Number of Filled Seed	Heads Isolated	Heads Self-Pollinated
H. gronovii	50	2	42	7		
	83	4	104	72		
	84	6	135	98		
	57	2	-[3]	34	4/5	24/26
	89	2	-	30		
	155	4	-	37		
	254	2	-	15		
	261	2	-	23		
H. venosum	97	2	43	4		
	195	2	41	21		
	211	2	53	13		
	221	2	52	7		
	223	4	89	16		
	42	8	-	2	2/17	38/78
	86	2	-	4		
	112	2	-	1		
	176	4	-	6		
	192	8	-	5		
	209	2	-	3		
H. traillii	f3	2	-	11	0/2	2/2

[1]All collection numbers are of Christy. Vouchers are deposited at TENN.
[2]Expressed as (number of heads setting seed/total heads tested).
[3]Not determined.

Table II-3. Results of attempted artificial hybridizations in *Hieracium gronovii* and *H. venosum*. The crosses are listed as egg parent x pollen parent.

	Cross Attempt[1]	Number of Replicates	Number of Seeds Recovered	Number of Seeds Germinating	Number of Specimens Recovered	Number of Seedlings Still Immature	Failed Cross Attempts[1]
V x G[2]	171 x 89	1	2	2	0	0	V x G
	173 x 155	1	1	1	0	0	80 x 84
	188 x 57	1	1	1	0	0	97 x 89
	190 x 57	1	5	5	0	1	113 x 84
	195 x 51	1	4	4	2	0	187 x 83
	203 x 50	1	1	1	1	0	195 x 89
	219 x 84	2	18	18	0	2	197 x 79
	221 x 83	2	11	11	1	2	224 x 83
G x V	83 x 202	2	36	35	2	2	G x V
	84 x 80	1	8	7	2	0	84 x 185
	84 x 187	1	7	7	2	1	
	84 x 192	1	14	14	6	1	
	84 x 195	1	13	13	4	6	
	155 x 173	2	58	54	8	0	
	51 x 221	2	22	16	0	0	
Totals							
V x G		10	43	43	4 (1 hybrid)	5	
G x V		10	158	146	24 (5 hybrids)	10 (2 hybrids)	

[1]The parent plants are referred to by collection number (Table II-1).
[2]V = *H. venosum*; G = *H. gronovii*.

and the relative ineffectiveness of human cross-pollination presumably decreased the number of artificial hybrids produced.

Seedling mortality. Although most seedling deaths were without a definite cause the obvious causes included albinism and, prior to production of the first leaf, slug predation. An inability to develop roots and large leaves was noticed in some plants; other, older, seedlings would abruptly cease growth and many of these eventually died, despite changes in GA application and/or frequency of watering.

Conclusions

In conclusion, attempts to produce reciprocal artificial hybrids between H. gronovii and H. venosum were successful. Details of hybrid characters are discussed in subsequent chapters. All three taxa will self-fertilize. This occurs to the greatest degree in H. gronovii, in which self-pollination occurs in isolated heads.

CHAPTER III

CYTOLOGY

Cytology has proven useful in detecting interspecific hybridization because changes in chromosome structure (e.g. inversions, translocations) or number (e.g. polyploidy, aneuploidy) may accompany speciation (Lewis, 1953; Grant, 1981). Hybrids between species that possess such differences often exhibit abnormal meiosis. Meiotic problems not demonstrably due to chromosomal changes may be gene-determined, or perhaps due to some cryptic structural change (Stebbins, 1950).

The genus Hieracium has a base chromosome number of $x = 9$ (Heywood, Harborne and Turner, 1977). In contrast to many other species in this genus, all surveyed species in the subgenus Stenotheca have been found to be diploid and sexually reproducing (Vuilleumier, 1973; Guppy, 1978). Artificial hybrids in several species combinations within subgenus Stenotheca were found by Guppy (1978) to be meiotically normal.

In this study, chromosome numbers were determined for specimens of H. gronovii, H. venosum and H. traillii. Meiosis in these taxa and in hybrids of H. gronovii and H. venosum was surveyed for the presence of any abnormalities potentially useful in either comparing hybrids and parental taxa or in deciding if H. traillii represents a hybrid of H. gronovii and H. venosum.

Methods

Several immature heads from each specimen were fixed in a methanol:chloroform:propionic acid (6:3:2, v/v/v) solution (Cooperrider and Morrison, 1967) and stored under refrigeration. Approximately 20 flowers were removed from a head, teased apart and squashed in a 2% lactic-acetic-orcein stain (Cooperrider and Morrison, 1967). All meiotic stages represented on each slide were surveyed for abnormalities. The frequency of abnormal meiosis for each specimen was determined by scoring 100 meiocytes, in late Telophase II or tetrad stages, when the effects of erratic division could most easily be observed.

Selected specimens were surveyed for viability of the pollen grains that resulted from abnormally-sized microspores. One unopened flower of each specimen was teased apart in a drop of 5% lactophenol cotton blue (Radford, Dickison, Massey and Bell, 1974) and the pollen allowed to stain for 12 to 24 hours. Slides were surveyed to determine if the abnormally sized pollen was darkly stained, which was considered to indicate viability.

Photographs were taken of some meiocytes and pollen using an Olympus PM-10 A 35-mm photographic system with Kodak Panatomic-X, ASA 32, black and white film or Kodak Ektachrome slide film, ASA 64.

Results and Discussion

Cytology. All taxa examined (Table III-1) had a meiotic chromosome number of $2n = 9_{II}$. Previous reports (Federov, 1969; Moore, 1973) of $2n = 18$ for *H. gronovii* and *H. venosum* are confirmed.

Table III-1. Collection numbers and locations of specimens of *Hieracium* examined cytologically.

Species		Voucher[1]	
H. gronovii	Arkansas	Franklin	229
		Newton	150, 151, 153
	Georgia	Habersham	138, 140
		Rabun	133[2], 134[2]
	Missouri	Douglas	244
		Howell	246, 247, 250
		Oregon	265[2]
		Taney	154[2]
		Wayne	156
	North Carolina	Jackson	128
	Oklahoma	Adair	233n, 230n[2]
	Tennessee	Blount	158
		Coffee	84[2]
		Grundy	79
		Johnson	89[2]
		Morgan	51, 57[2]
H. traillii	Virginia	Bath	f2[2], f5[2], f6
H. venosum	Tennessee	Blount	69
		Carter	33[2]
		Cocke	24, 27[2], 28[2]
		Grundy	81[2]
		Johnson	86, 91
		Monroe	14[2]
	Virginia	Augusta	221[2]
		Highland	112[2], 113[2]
		Rockbridge	179, 191[2], 192[2], 196[2], 198[2], 200
	West Virginia	Summers	106[2]

[1]All collection numbers are of Christy. Vouchers are deposited at TENN.
[2]$2n = 9_{II}$.

The count of $2n = 18$ for *H. traillii* (Figure III-1) is reported here for the first time.

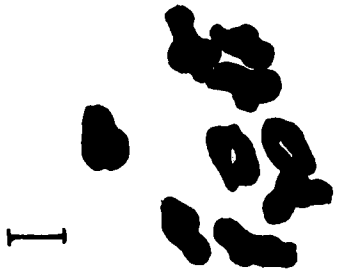

Figure III-1. Diakinesis in *Hieracium traillii* pollen mother cell, showing nine bivalents (9_{II}). Bar = 1 μm.

Meiosis in most parental and many hybrid specimens was normal (Figure III-2). In 50% of all surveyed specimens, at least 98% of meiocytes were normal, and in only 10% of surveyed specimens was the frequency of normal meiocytes less than 90%. No characteristic differences in chromosome pairing or behavior were observed between artificial hybrids and their parental taxa. Meiosis in *H. traillii* was also normal; the available specimens, however, represent only one population.

The observed meiotic abnormalities were of two types--bridge and fragment figures, perhaps resulting from paracentric inversions and non-haploid microspores (Figure III-3). The aberrant microspores apparently result from mis-division or failure of division, perhaps in a manner similar to one of those described in a triploid specimen of *Ceratopteris* (Hickok and Klekowski, 1973). Bridge and fragment

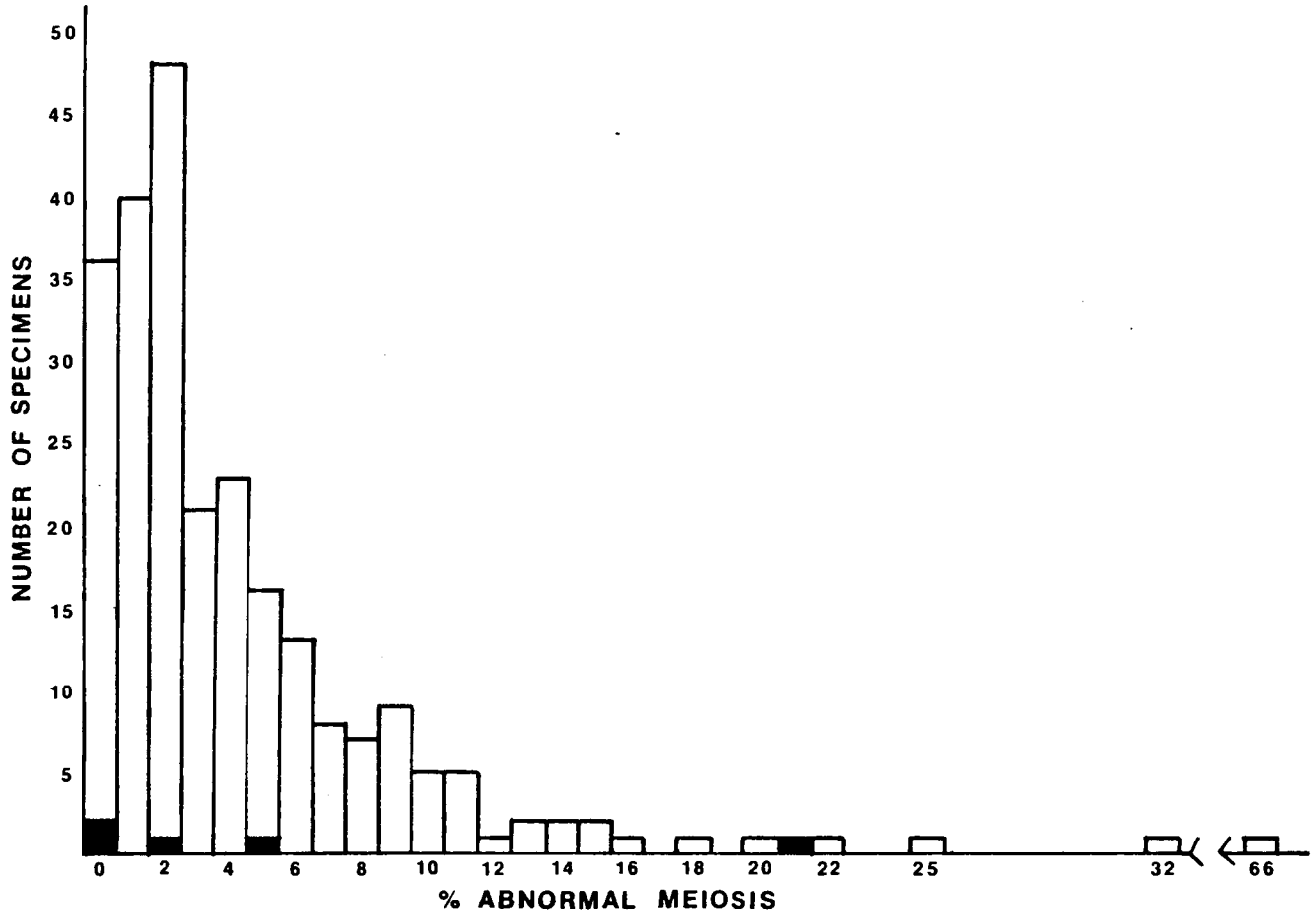

Figure III-2. Histogram of frequency of meiotic abnormalities in 250 specimens of Hieracium. Shading indicates abnormality frequencies of artificial hybrids between H. gronovii and H. venosum.

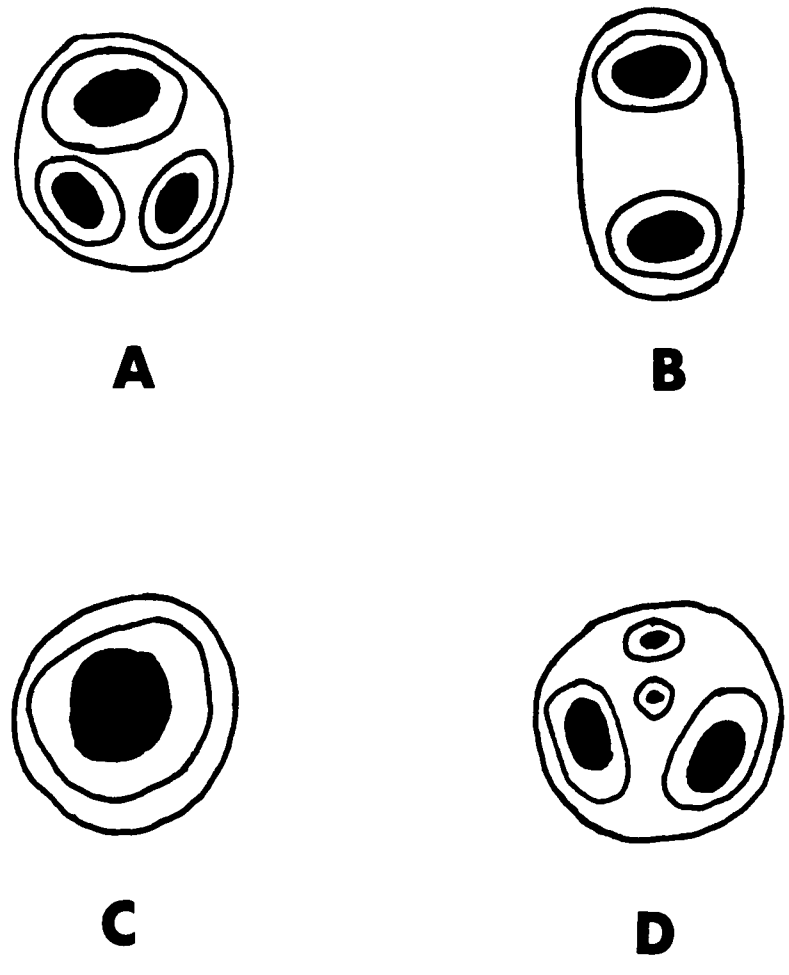

Figure III-3. Diagrams of the four aberrant patterns of microspore production observed in Hieracium. A. triad; B. dyad; C. monad; D. with one or more micronuclei. Relative nuclear size is indicated by solid shading.

figures, while more common in specimens of H. venosum and H. traillii than in H. gronovii or artificial hybrids, were always infrequent. The most commonly observed abnormalities, illustrated in Figure III-3, were divided into four categories:

A. A triad, apparently resulting when one nucleus fails to complete the second meiotic division;

B. A dyad, apparently from complete failure of one of the meiotic divisions;

C. A monad, from various possible causes, including the apparent failure of cytokinesis after divisions of varying degrees of normality;

D. Varying numbers and sizes of microspores, including at least one resulting from a micronucleus.

The above four categories of abnormalities had similar distributions in parental H. gronovii and H. venosum and their hybrid (Table III-2). Hieracium venosum has a higher frequency of micronuclei than H. gronovii; this is probably related to the more frequent observation of bridge and fragment figures in H. venosum.

The hybrid group consists of known artificial hybrids and apparent natural hybrids for which the pollen parent is unknown, but which morphologically and cytologically are within the range of the known hybrids. These hybrids fall between their parents in the upper range of abnormality frequencies recorded for each group, and have similar average values. As a group, the hybrids also have a distribution of abnormalities similar to that of their parents.

Table III-2. Comparison of frequency and distribution of meiotic abnormalities of *Hieracium gronovii*, *H. venosum*, their hybrid and *H. traillii*.

Specimen Group	Number of Specimens	Number of Meiocytes Examined (100/ Specimen)	Range of Total % Abnormal Meiosis[1]	Average % Abnormal Meiosis	Average frequency of abnormality occurrence, by category ((Total observed per category/total meiocytes examined) X 100) A (Triad)	B (Dyad)	C (Monad)	D (Micro-nuclei)
H. gronovii								
Parents	11	1100	0-14	5.5	4.5	0.4	0.1	0.5
Known Selfs	18	1800	0-6 (20)	3.7	3.2	0.2	0.15	0.15
Presumed Intraspecific Crosses	20	2000	1-32 (66)	11.4	6.6	2.0	1.2	1.6
Hybrids of *H. gronovii* and *H. venosum*								
All	15	1500	0-21	4.9	4.1	0.1	0	0.7
Known	5	500	0-5 (21)	5.6	-	-	-	-
H. venosum								
Parents	33	3300	0-13 (25)	5.5	4.0	0.2	0.1	1.2
Known Selfs	6	600	0-6	1.7	1.2	0	0	0.5
Presumed Intraspecific Crosses	48	4800	0-9	2.0	1.4	0.1	0.1	0.4
H. traillii								
Parents	3	300	0-5	3.0	2.3	0	0	0.7

[1]Isolated extremes are indicated in parentheses.

The marked ease with which H. gronovii will self-fertilize has been noted in Chapter II. One possible consequence of frequent selfing is apparent in comparison of the collected specimens of H. gronovii, and their known selfs, to progeny of intraspecific crossing attempts. The parents (#'s 50, 79, 83 and 89) used in intraspecific crosses were from geographically separated populations in Tennessee (Table II-1, page 8). The average frequency of meiotic abnormalities in intraspecific crosses shows an increase of over 100% when compared to the parental group. All four categories of abnormalities occurred at a higher frequency in intraspecific crosses of H. gronovii than in any other group. One possible explanation is that a tendency toward selfing has produced increased homozygosity in individuals and populations and has also increased the probability of "fixing" mutations in a population. Such selfing would contribute to genetic differences between populations, which could cause the observed problems when specimens from different populations are crossed (Stebbins, 1950; Snyder, 1951).

Pollen viability. Only microspores formed by the smallest micronuclei routinely fail to develop into recognizable pollen grains. Conspicuously small pollen grains do not stain in lactophenol cotton blue. Approximately normal to over-sized pollen grains usually appear viable.

Conclusions

In conclusion, there are no characteristic meiotic differences between artificial hybrids of H. gronovii and H. venosum and the

parental taxa. Meiotic characteristics apparently can not be used to discriminate H. traillii and hybrids of H. gronovii and H. venosum. The relationship of H. gronovii and H. venosum fits the "Geum" pattern (perennial, outcrossing herbs with related species intercompatible, chromosomally homologous and isolated by ecological or external factors) of Grant (1981). This pattern also appears in the species of subgenus Stenotheca studied by Guppy (1978). The ability of these taxa to self-fertilize appears to be the only deviation from this pattern.

CHAPTER IV

MORPHOLOGY

The delimitation of plant species and the recognition of their hybrids has traditionally been accomplished with morphological characters (Stace, 1980). Chemical and chromosomal characters are often informative (Lewis, 1953; Alston and Turner, 1963), but morphology remains an efficient method of distinguishing most taxa. Interspecific hybrids frequently are recognized by the possession of characters of both parents. Exceptions to this are common--hybrids can be indistinguishable from one parent or have completely new characteristics (Grant, 1975).

The development of numerical taxonomy was an attempt to remove the subjectivity inherent in the traditional methods of classification. Although these new methods are not completely devoid of subjectivity (Johnson, 1970), their need for precise definitions of the characters chosen has increased the repeatability of classification. These methods have also led to the development of techniques for multivariate analysis that are useful for the comparison of organisms.

Multivariate analyses were used for several purposes in this study. The similarity of artificial hybrids of H. gronovii and H. venosum to each other and to their parental species was evaluated. Artificial hybrids and H. traillii were compared to determine the extent of their similarity and thus evaluate the suggestion that H. traillii may represent a hybrid of H. gronovii and H. venosum. In

addition, artificial hybrids were examined for the presence of any consistent character patterns that might serve to identify naturally occurring hybrids.

Methods

A total of 191 operational taxonomic units (OTU's), the lowest ranking taxon employed in a study (Sokal and Sneath, 1973), was scored for nine characters (Table IV-1). Each OTU consisted of one dried herbarium specimen, derived mostly from collections made during this study and their progeny. The choice of characters and the numbering of character states (0 = typical of H. venosum, 2 = typical of H. gronovii, 1 = intermediate or mixed) allowed the sum of scores for each OTU to serve as a hybrid index value (Anderson, 1949), with raw scores as data for other comparisons. OTU's with identical scores were treated as a single OTU. When specimens of H. traillii were included in an analysis, the modification of some characters and character states was necessary (Table IV-2).

A coefficient of similarity was calculated for each pair of OTU's using the method of Gower (1971). Unweighted pair-group method using arithmetic means (UPGMA) was used for clustering, and principle coordinates analysis (PCA) for ordination. The results of clustering and ordination are complimentary, with cluster analysis best for identifying similar OTU's and ordination best for identifying the relative similarity between groups and dissimilar OTU's. Computations were performed on a DEC-10 computer at the University of Tennessee, using numerical taxonomy programs written by Dr. Edward E. Schilling.

Table IV-1. Characters scored for numerical analyses of *Hieracium gronovii*, *H. venosum* and their hybrids.

1. Presence of stellate hairs on lower stem
   - 0 = absent
   - 1 = infrequent, scattered
   - 2 = common to dense
2. Presence of setose hairs on lower stem
   - 0 = absent
   - 1 = infrequent, scattered
   - 2 = common to dense
3. Leaf distribution
   - 0 = rosette present, not more than one leaf on stem
   - 1 = rosette present, more than one leaf on stem
   - 2 = rosette lacking, several leaves on stem
4. Inflorescence form
   - 0 = spreading, long branches and pedicels (see Figure IV-9)
   - 1 = mixed - usually long branched and spreading with tufts (see Figures IV-10 and IV-11)
   - 2 = cylindrical, short branches and pedicels, "thyrsoid" tufts in some axils (see Figure IV-8)
5. Pedicel length to head length ratio[1]
   - 0 = at least 2.5:1
   - 1 = intermediate or mixed
   - 2 = not more than 2:1
6. Length of Head[1]
   - 0 = at least 8 mm
   - 1 = intermediate or mixed
   - 2 = not more than 7 mm
7. Distribution and frequency of glandular hairs in inflorescence
   - 0 = infrequent to glabrous, on involucre bracts and adjacent pedicel
   - 1 = intermediate
   - 2 = common to dense, extending along branches to rachis
8. Width of ligule
   - 0 = at least 2 mm
   - 1 = intermediate
   - 2 = not more than 1-1/2 mm
9. Presence and extent of red-purple vein margins
   - 0 = present, secondary veins
   - 1 = present or faded, midvein only
   - 2 = absent

[1]Pedicel length = from base of receptacle to first branch;
Head length = from base of receptacle to tip of involucral bracts.

Table IV-2. Characters scored for numerical analyses of *Hieracium gronovii*, *H. venosum*, *H. traillii* and hybrids between *H. gronovii* and *H. venosum*.

1. Leaf distribution
   - 0 = rosette present, not more than one leaf on stem
   - 1 = rosette present, more than one leaf on stem
   - 2 = rosette lacking, several leaves on stem
2. Presence of stellate hairs on lower surface of leaves
   - 0 = present, common
   - 1 = infrequent, scattered
   - 2 = absent
3. Presence of setose hairs on lower stem
   - 0 = present, common
   - 1 = infrequent, scattered
   - 2 = absent
4. Presence and extent of red-purple vein margins
   - 0 = present, on secondary veins
   - 1 = present or faded, on midvein only
   - 2 = absent
5. Inflorescence form
   - 0 = cylindrical, short branches and pedicels, "thyrsoid" tufts in some axils (see Figure IV-8)
   - 1 = intermediate or mixed (see Figures IV-10 and IV-11)
   - 2 = spreading, long branches and pedicels (see Figure IV-9)
   - 3 = spreading, long branches and short stout pedicels (see Figure IV-12)
6. Pedicel length to head length ratio[1]
   - 0 = at least 2.5:1
   - 1 = intermediate or mixed
   - 2 = not more than 2:1
7. Length of head[1]
   - 0 = less than 7 mm
   - 1 = intermediate or mixed
   - 2 = over 8 mm
8. Presence of setose hairs on involucral bracts
   - 0 = present
   - 1 = absent
9. Ligule width
   - 0 = not more than 1-1/2 mm
   - 1 = at least 2 mm
10. Distribution and frequency of glandular hairs in inflorescence
    - 0 = sparse to glabrous, on involucre bracts and adjacent pedicel
    - 1 = intermediate
    - 2 = common to dense, extending along branches to rachis

[1]Pedicel length = from base of receptacle to first branch;
Head length = from base of receptacle to tip of involucral bracts.

Results and Discussion

A histogram of hybrid index values of *H. gronovii*, *H. venosum* and their hybrids (Figure IV-1) illustrates the presence of some variability in parents and in hybrids. The hybrids occupy the middle values, indicating either mixed or intermediate character states.

Hybrids show no consistent pattern of characteristics. The most stable character appears to be possession of ligules over 1-1/2 mm wide and, somewhat less reliably, possession of the types, density and distribution of trichomes typical of *H. gronovii*. The single *H. venosum* X *H. gronovii* hybrid recovered is similar to the *H. gronovii* X *H. venosum* hybrids, suggesting that the direction of the cross does not influence hybrid morphology.

Two specimens collected from natural populations (Cole s.n.; Chester 81-675; both at TENN) that are of probable hybrid origin fall within the phenetic range of the artificial hybrids (Figure IV-2). This may indicate that neither artificial resynthesis of the hybrid nor their growing conditions have markedly affected characteristic morphological features.

Possible explanations for the observed variation in morphology of the artificial hybrids include heterozygosity of parents for form-determining genes and the variation possible within a species. A large number of possible gamete genotypes or the interactions of new and varying gene combinations in the hybrid may account for the diversity observed. *Hieracium venosum* shows potential for a greater degree of heterozygosity than *H. gronovii*, with the progeny of the self-fertilization of one specimen (#195) encompassing most of the

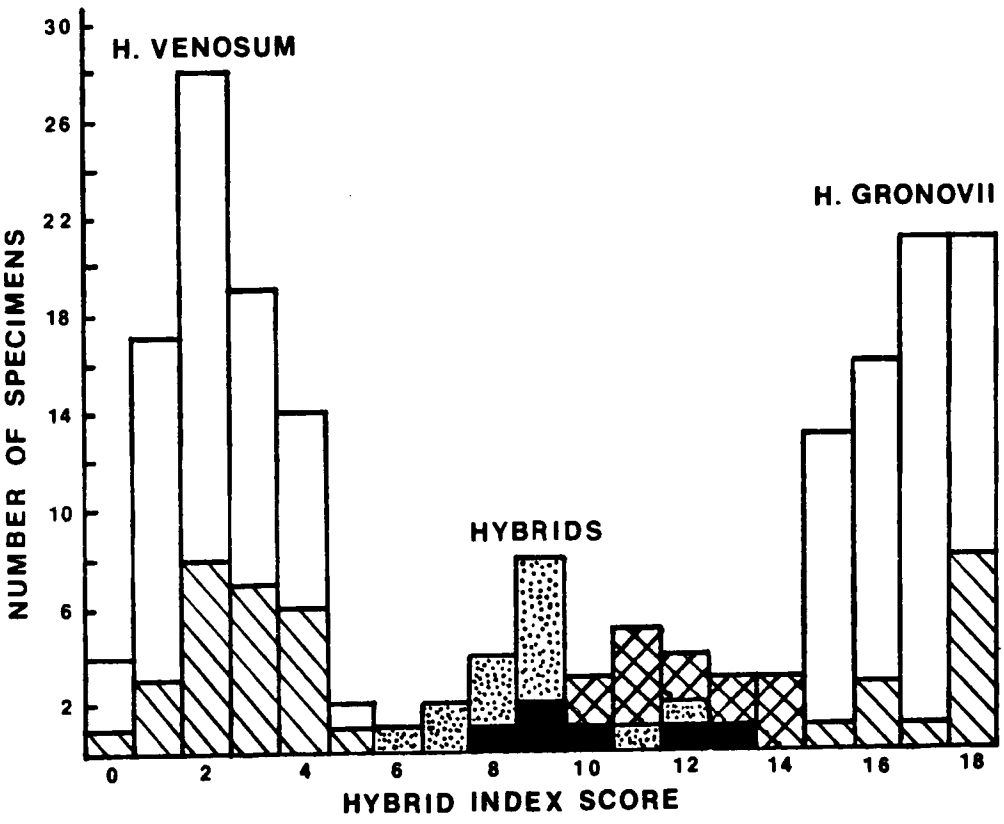

Figure IV-1. Histogram of hybrid index scores of Hieracium gronovii, H. venosum, their artificial hybrid and other intermediate forms using nine characters (Table IV-1). The scores of artificial hybrids are indicated by solid shading, putative hybrids by dots, parental collections by diagonal lines, intermediate forms by cross-hatching and the progeny of selfing and intraspecific crossing by clear areas.

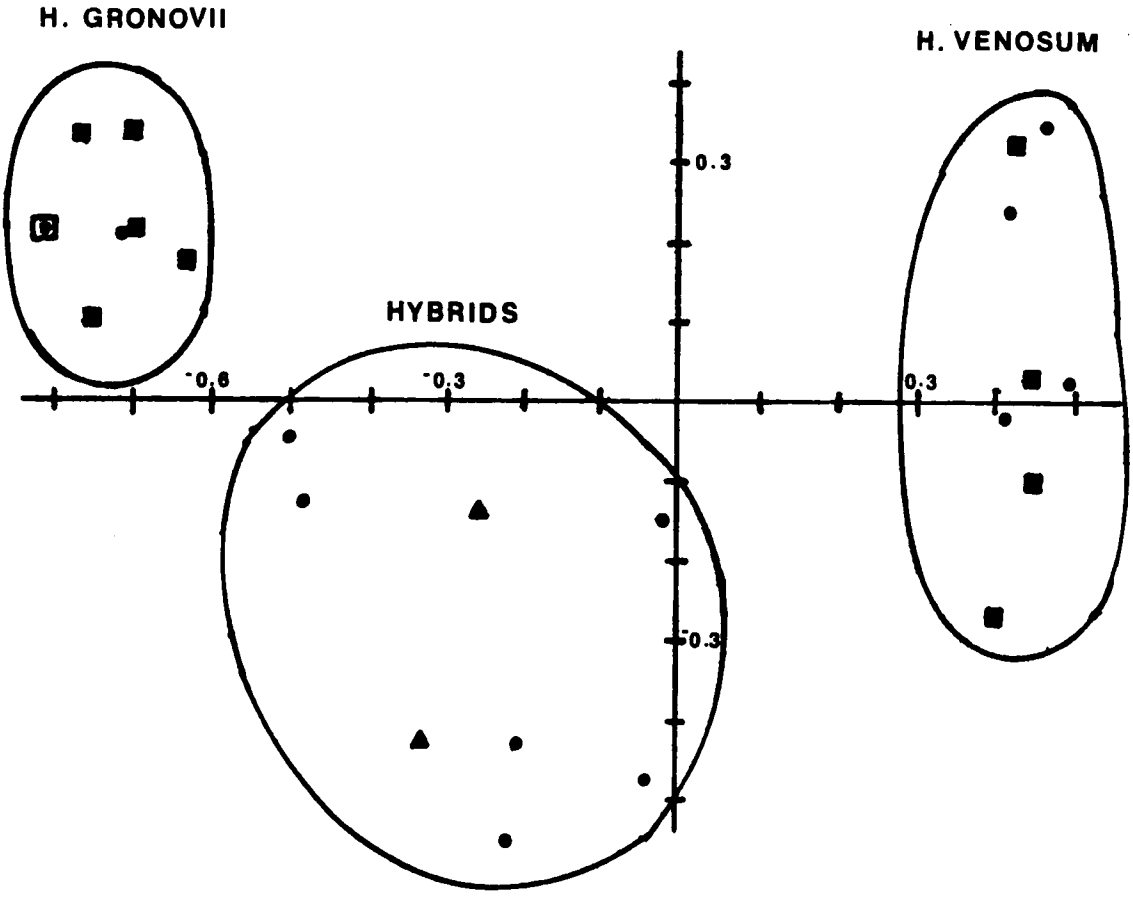

Figure IV-2. Graph of the first two axes determined by Principle Coordinates Analysis of 24 Hieracium OTU's using 9 morphological characters (Table IV-1). Artificial hybrids and their parents are indicated by dots, field-collected hybrids by triangles and the progenies of self-fertilization by squares.

variation exhibited by the other H. venosum OTU's (Figure IV-2). Collections from one population have also shown this same range of variation.

The results of ordination of all OTU's (Figure IV-3) show clear separation between H. venosum and the hybrids. The hybrids and H. gronovii, however, are connected by a number of OTU's. Four of these OTU's represent specimens collected outside the range of H. venosum that do not exhibit segregation for phenetic characters when selfed or crossed. The other five are progenies of H. gronovii, whose pollen parent is unknown. Closer examination of these specimens led to the conclusion that they either represent an extreme in variation for H. gronovii or that the pollen parents were of hybrid origin.

In the phenogram resulting from cluster analysis (Figure IV-4), specimens of H. venosum form a distinct and uniform group, with the exception of a single OTU that clusters with the hybrids. This OTU is separated from the hybrids by ordination (Figure IV-3). OTU's of H. gronovii and of hybrids form less uniform clusters. Several hybrids, and the possible backcrosses discussed above, fall within the H. gronovii cluster, and the stable intermediate forms are split between the hybrid and H. gronovii clusters.

Removal of the two groups of unusual OTU's from the data set produced clear separation of the parental species and their hybrids by ordination (Figure IV-5) and also, except for the H. venosum OTU noted above, in cluster analysis (Figure IV-6). The variability of hybrid morphology is readily apparent in Figure IV-6, with the final clustering of hybrid OTU's occurring at a similarity value of less

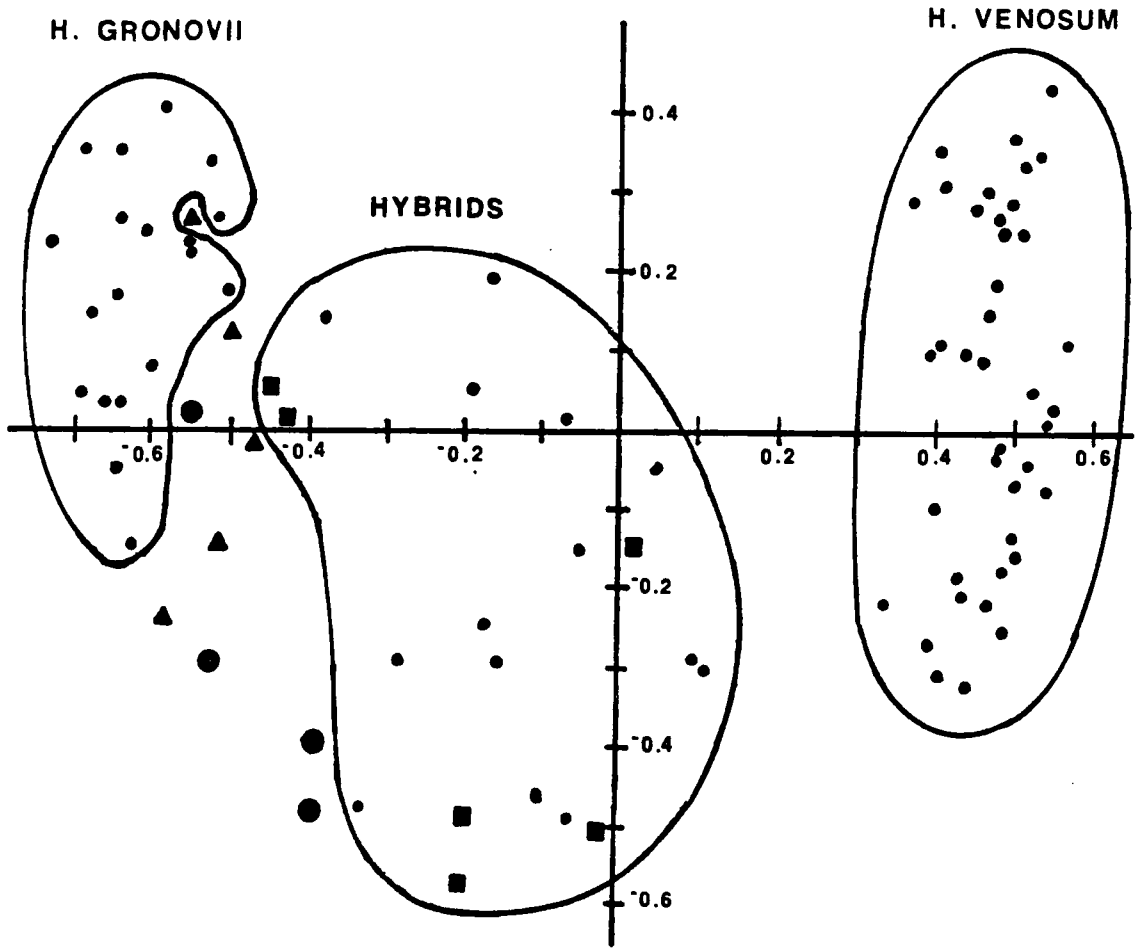

Figure IV-3. Graph of the first two axes determined by Principle Coordinates Analysis of 191 Hieracium OTU's using 9 morphological characters (Table IV-1). Artificial hybrids, within the hybrid group, are indicated by squares. OTU's not assigned to a group include possible backcrosses, indicated by triangles, and stable intermediate forms, indicated by larg dots. Delineation of groups is based on coordinates of the third axis determined by PCA and by similarities between OTU's, as determined by UPGMA Cluster Analysis.

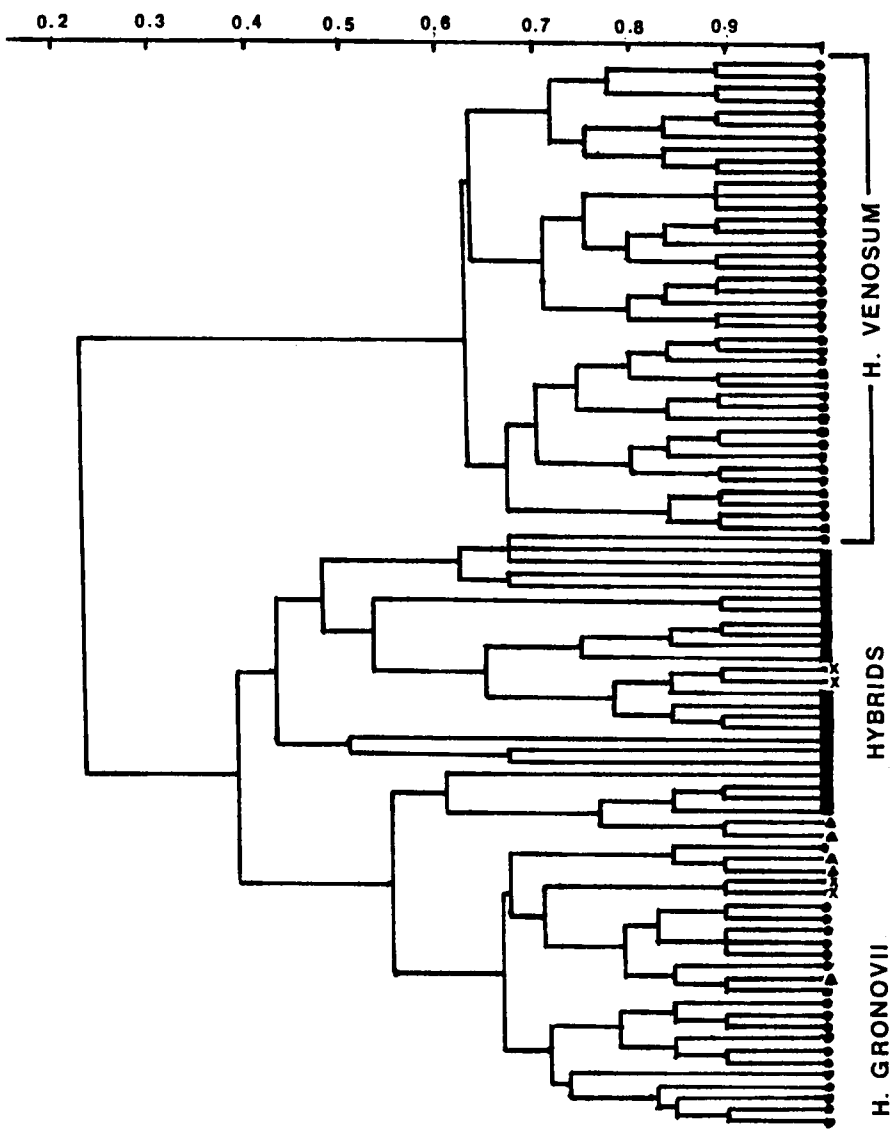

Figure IV-4. Phenogram resulting from UPGMA Cluster Analysis of 191 Hieracium OTU's using 9 morphological characters (Table IV-1). Scale indicates level of similarity based on Gower's Coefficient. Hybrid OTU's are indicated by bars, possible backcrosses by triangles, and stable intermediate forms by "x".

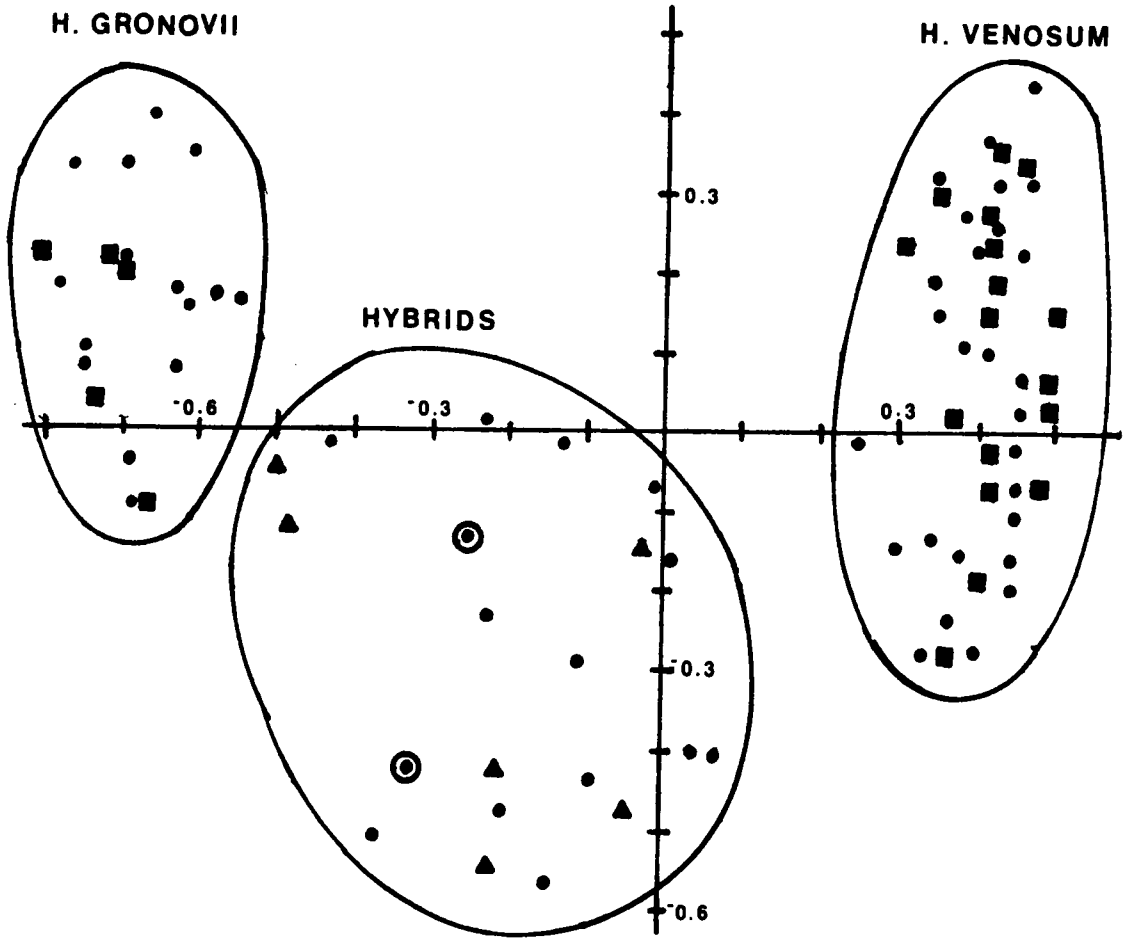

Figure IV-5. Graph of the first two axes determined by Principle Coordinates Analysis of 182 Hieracium OTU's using 9 morphological characters (Table IV-1). Parental collections are indicated by squares, artificial hybrids by triangles and field-collected hybrids by circled dots.

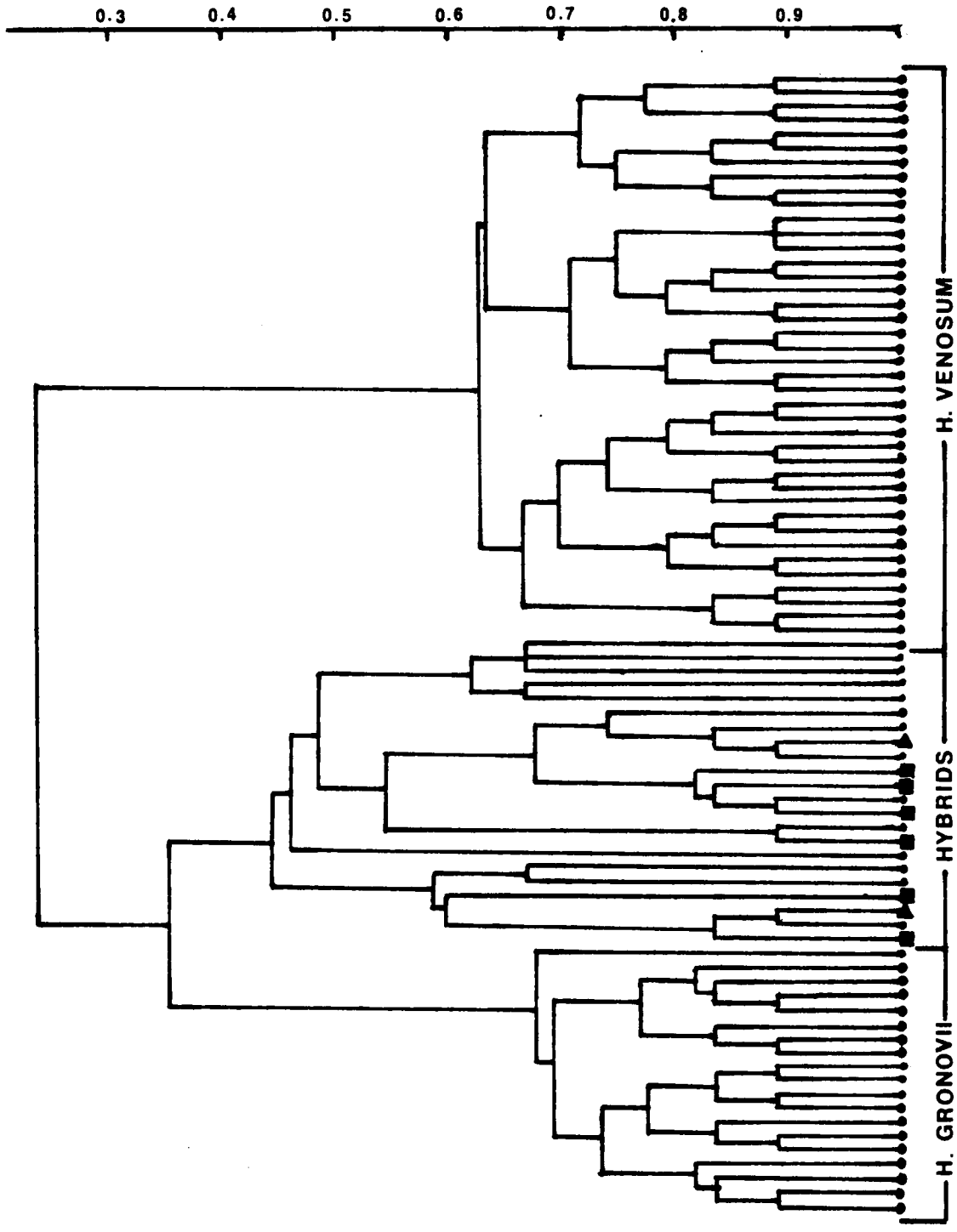

Figure IV-6. Phenogram resulting from UPGMA Cluster Analysis of 182 Hieracium OTU's using 9 morphological characters (Table IV-1). Scale indicates level of similarity based on Gower's Coefficient. Artificial hybrids are indicated by squares and field-collected hybrids by triangles.

than 50%. In contrast, OTU's of H. venosum and H. gronovii, within their respective clusters, have overall similarities of over 60%.

Hieracium traillii is morphologically distinct from artificial hybrids of H. gronovii and H. venosum. The results of ordination (Figure IV-7) illustrate their dissimilarity. Hieracium traillii shares character states (Table IV-3) with both H. gronovii and H. venosum, producing an intermediate position on Axis 1 (Figure IV-7), but has distinctive characters and character states that widely separate it from all other OTU's on Axis 2. A key to separate these taxa is shown below.

Key to Hieracium gronovii, H. venosum, H. traillii and hybrids between H. gronovii and H. venosum

1. Ligules less than 1-1/2 mm wide; inflorescence narrow, cylindrical, corymbose; leaves distributed on lower half of stem . . . . . H. gronovii.
1. Ligules at least 1-1/2 mm wide; inflorescence broad, spreading, corymbose to paniculate; most leaves clustered at base of stem . . . . . 2.
2. Lower stem glabrous; involucral bracts and pedicels sparsely glandular-hairy to glabrous; leaves usually with red-purple coloration on veins . . . . . H. venosum.
2. Lower stem with stellate and/or setose hairs; glandular hairs common in inflorescence; leaves green, occasionally with red-purple coloration at base . . . . . 3.

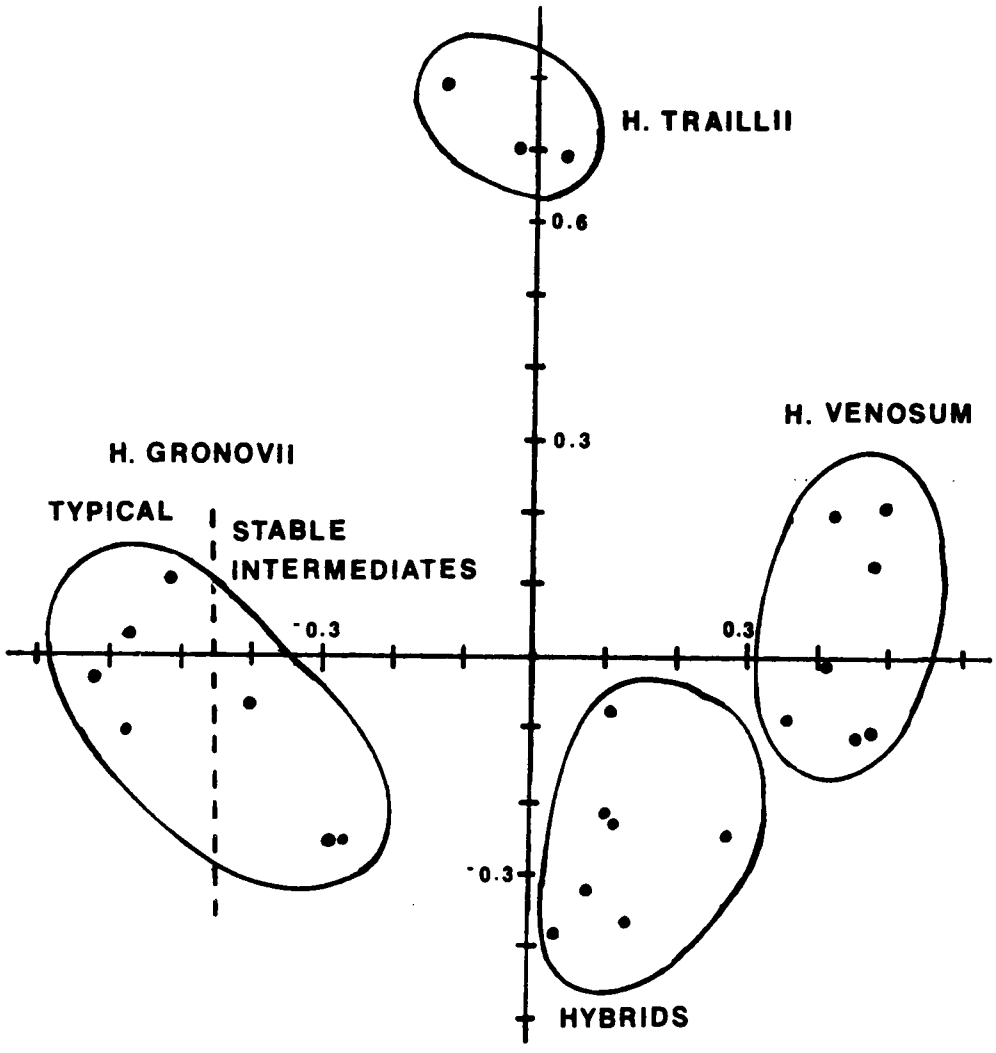

Figure IV-7. Graph of the first two axes determined by Principle Coordinates Analysis of 25 Hieracium OTU's using 10 morphological characters (Table IV-2).

Table IV-3. Comparisons of typical character states of *Hieracium gronovii*, *H. venosum*, their hybrid, and *H. traillii*.

Character	*H. gronovii*	*H. gronovii* x *H. venosum*	*H. venosum*	*H. traillii*
Stem Vestiture	Stellate and setose	Usually stellate and setose	Glabrous	Stellate
Leaf Distribution	Rosette lacking, several leaves on lower half of stem	Mixed, usually a rosette and several stem leaves	Rosette present, scapose or one leaf on stem	Rosette present scapose
Inflorescence Form	Narrow, cylindrical, short branches and pedicels, "thyrsoid" tufts in axils	Mixed, usually with "thyrsoid" tufts and long, spreading branches	Spreading, long branches and pedicels	Spreading, long branches, short stout pedicels
Head Length	Less than 7 mm	Variable	Over 8 mm	Over 8 mm
Inflorescence Vestiture	Obviously stellate and glandular	Usually intermediate to parents	Glabrous or scattered, stellate and glandular	Densely stellate and glandular, heads setose
Ligule Width	Less than 1-1/2 mm	At least 1-1/2 mm	At least 2 mm	At least 2 mm
Vein Margins	Green	Variable	Red-purple	Green

3. Scapose or with 1 reduced leaf on stem; inflorescence with branches at least 1/2 mm wide, densely stellate and glandular-hairy; involucral bracts with setose hairs; lower stem stellate-hairy . . . . . H. traillii.

3. 1 - 2 normal sized leaves on lower stem; inflorescence branches less than 3/8 mm wide, moderately stellate and glandular-hairy; heads lacking setose hairs; lower stem usually with setose hairs . . . . . H. gronovii X H. venosum.

Conclusions

In conclusion, H. gronovii (Figure IV-8) and H. venosum (Figure IV-9) are morphologically distinctive. Their artificial hybrids (Figures IV-10 and IV-11) may be distinguished from them as combining the characters of both, but without a consistent pattern. Hybrids inherit the wider ligules of H. venosum and, typically, the hairiness of H. gronovii. Hieracium traillii (Figure IV-12) shares some characters with both H. gronovii and H. venosum, but is distinct from these species and from their hybrid.

Figure IV-8. Photocopy of Hieracium gronovii (# 84 s'd/3). X 2/3.

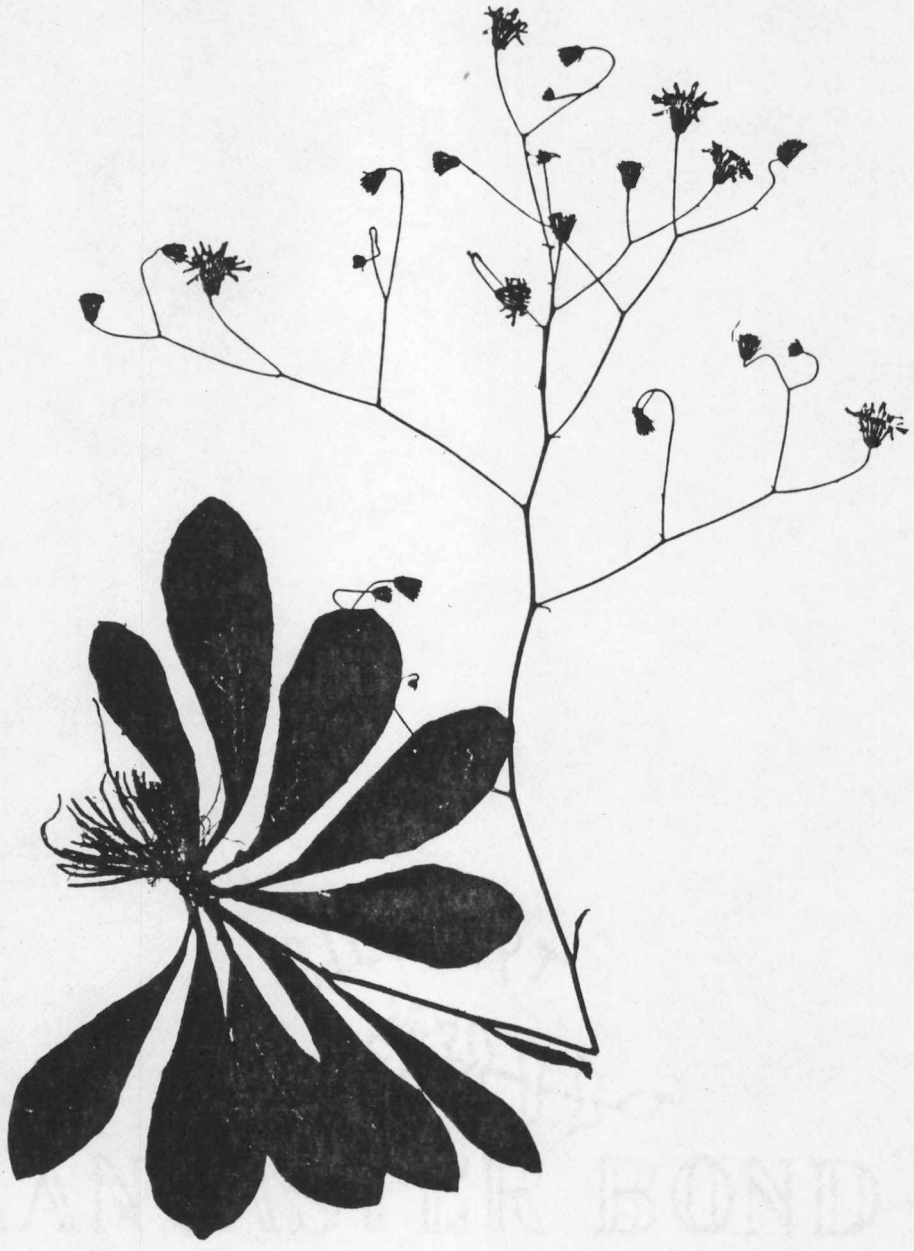

Figure IV-9. Photocopy of Hieracium venosum (# 113 X 112/14). X 1/2.

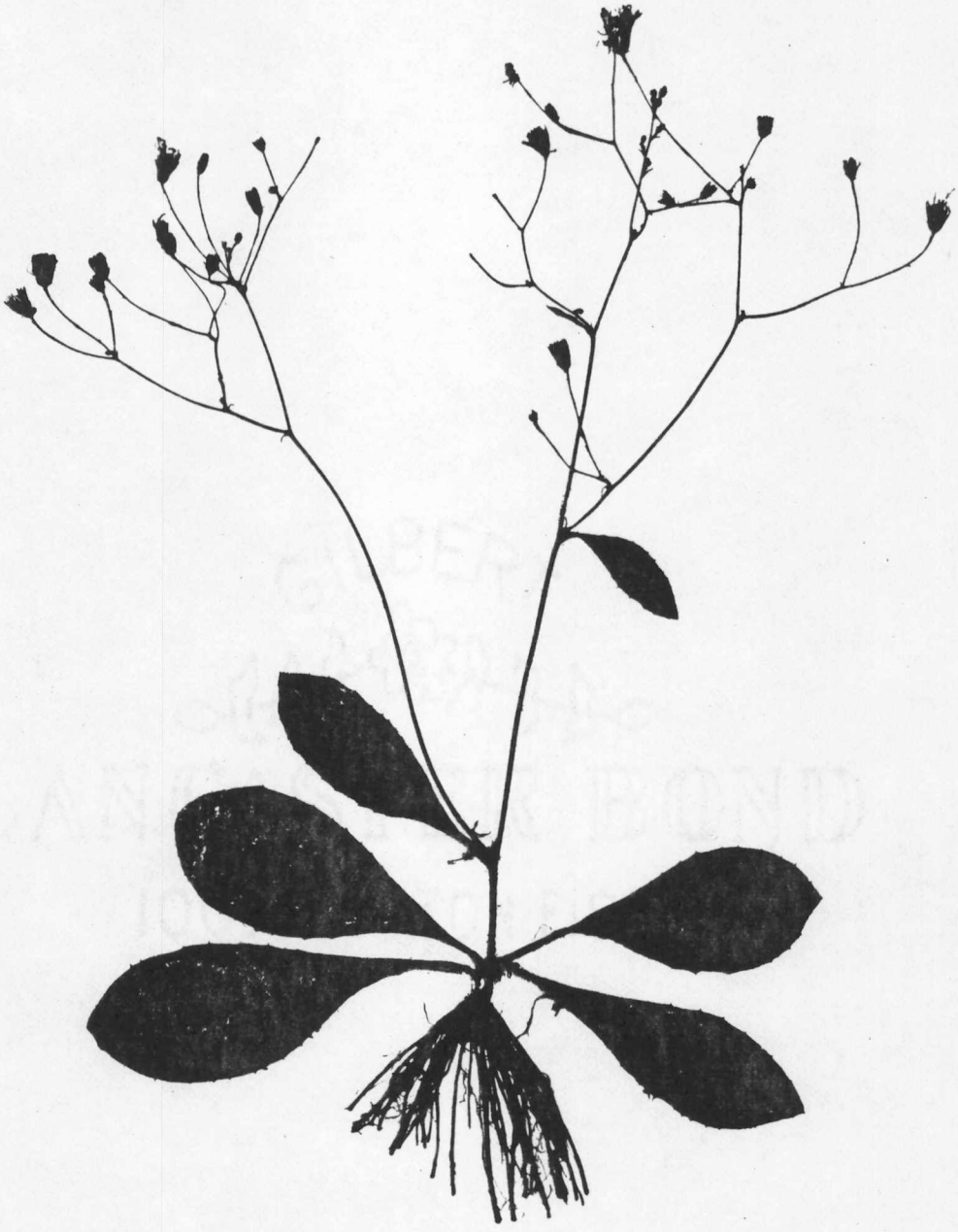

Figure IV-10. Photocopy of artificial Hieracium gronovii X H. venosum hybrid (# 84 X 195/3). X 2/3.

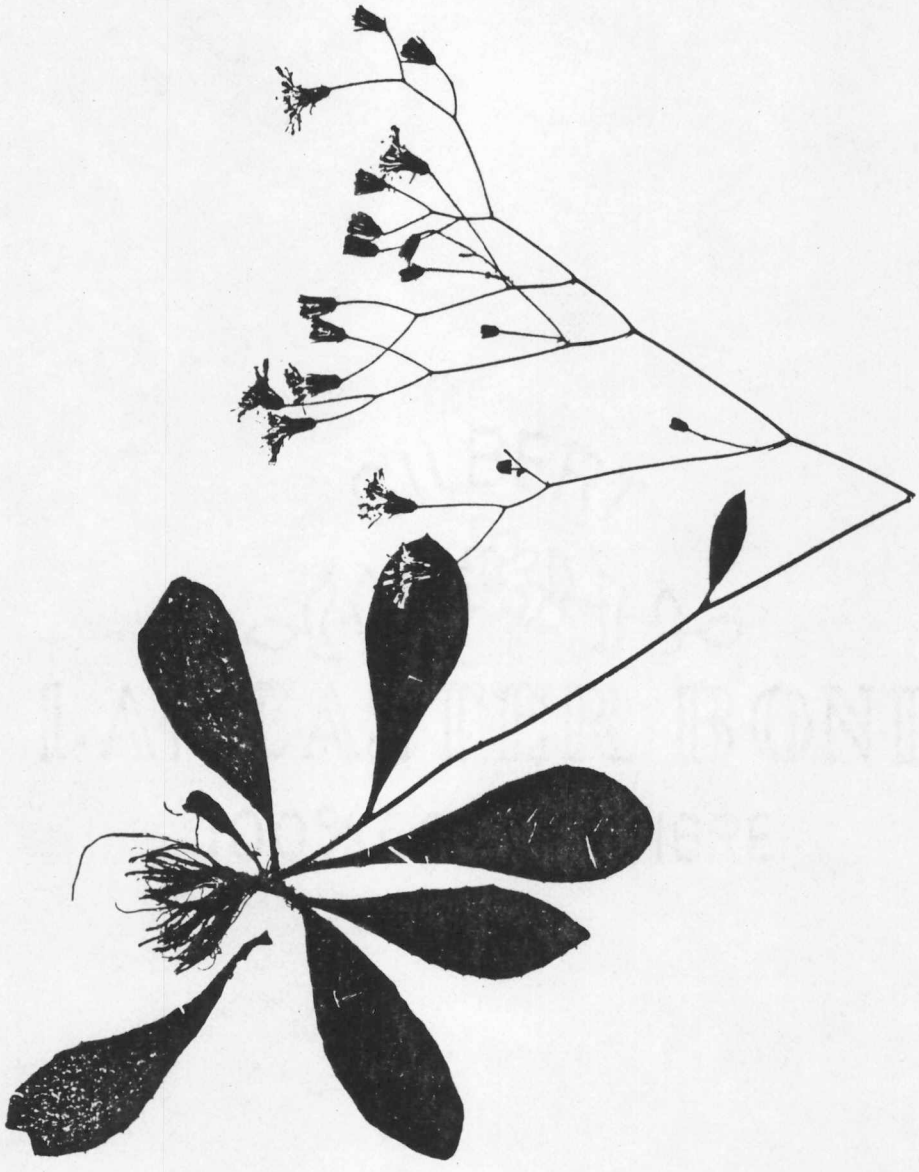

Figure IV-11. Photocopy of artificial Hieracium gronovii X H. venosum hybrid (# 83 X 202/1). X 1/2.

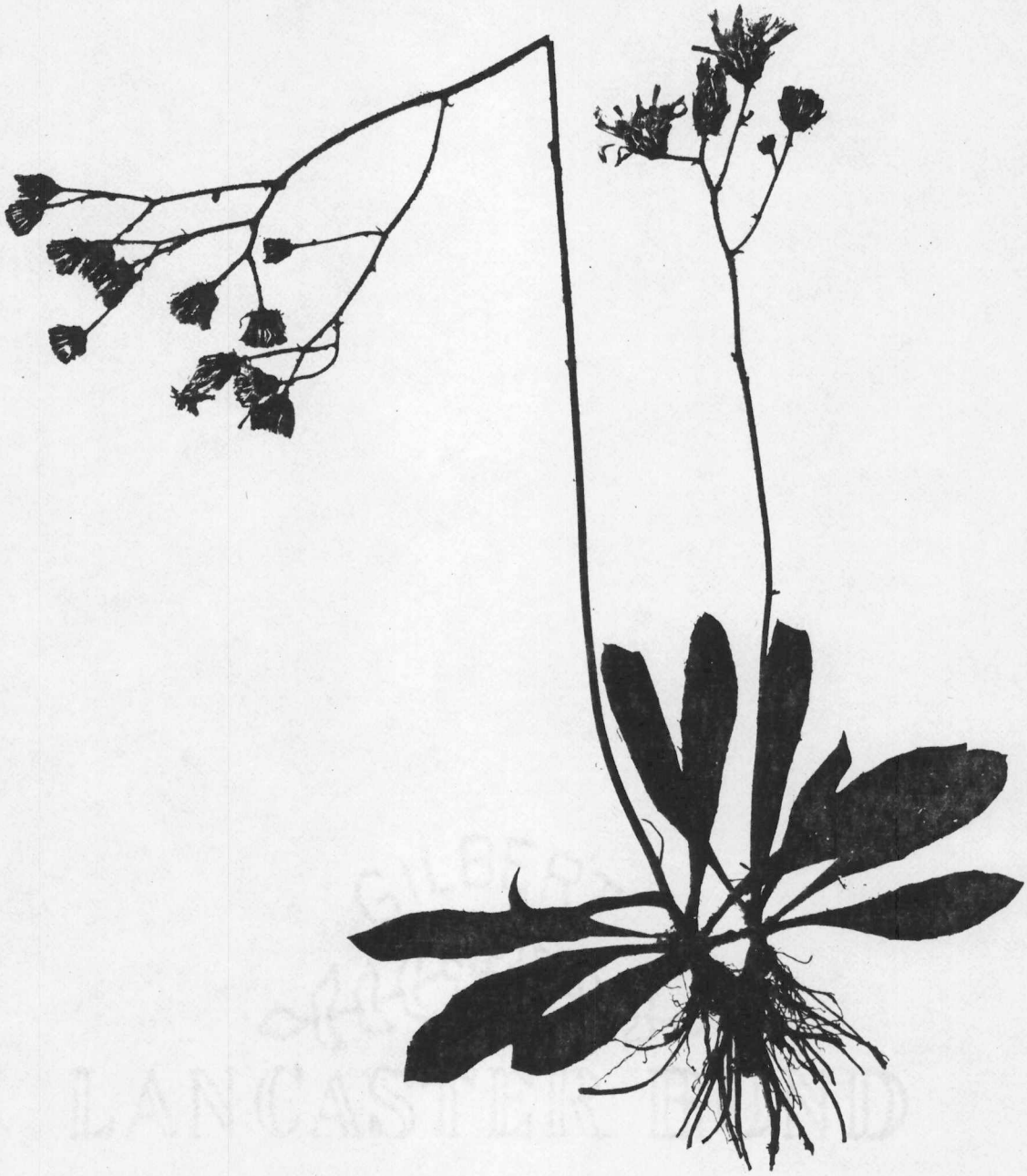

Figure IV-12. Photocopy of Hieracium traillii (# f/2). X 2/3.

CHAPTER V

FLAVONOID CHEMISTRY

The characteristic possession of flavonoid compounds by plants has proven a useful source of taxonomic information (Harborne, Mabry and Mabry, 1975). Flavonoids are a structurally diverse group of phenolic compounds that are easily isolated and identified using standardized methods (Markham, 1982).

Comparisons of flavonoid profiles can be used to detect hybridization. When the parental taxa have species-specific flavonoid complements, the flavonoids of hybrids may exhibit additive inheritance (Alston and Turner, 1963). Unclear results are also possible, including hybrids that produce compounds not found in either parent (Smith and Levin, 1963).

Flavonoid profiles of artificial hybrids in Hieracium subgenus Stenotheca have been found to exhibit additive inheritance (Guppy and Bohm, 1976). The species-specific flavonoid complements of the parental taxa were composed of the relatively common compounds apigenin and luteolin (Figure V-1) and their mono- and di-glycosides. Entire plants were extracted by Guppy and Bohm, without attempting to determine if any of the compounds had specific locations within the plant.

In this study, patterns of flavonoids present in leaves and flowers of H. gronovii, H. venosum and H. traillii were compared for similarity and for evidence of hybrid origin of H. traillii. Different flavonoid compounds may be expected in leaves and flowers,

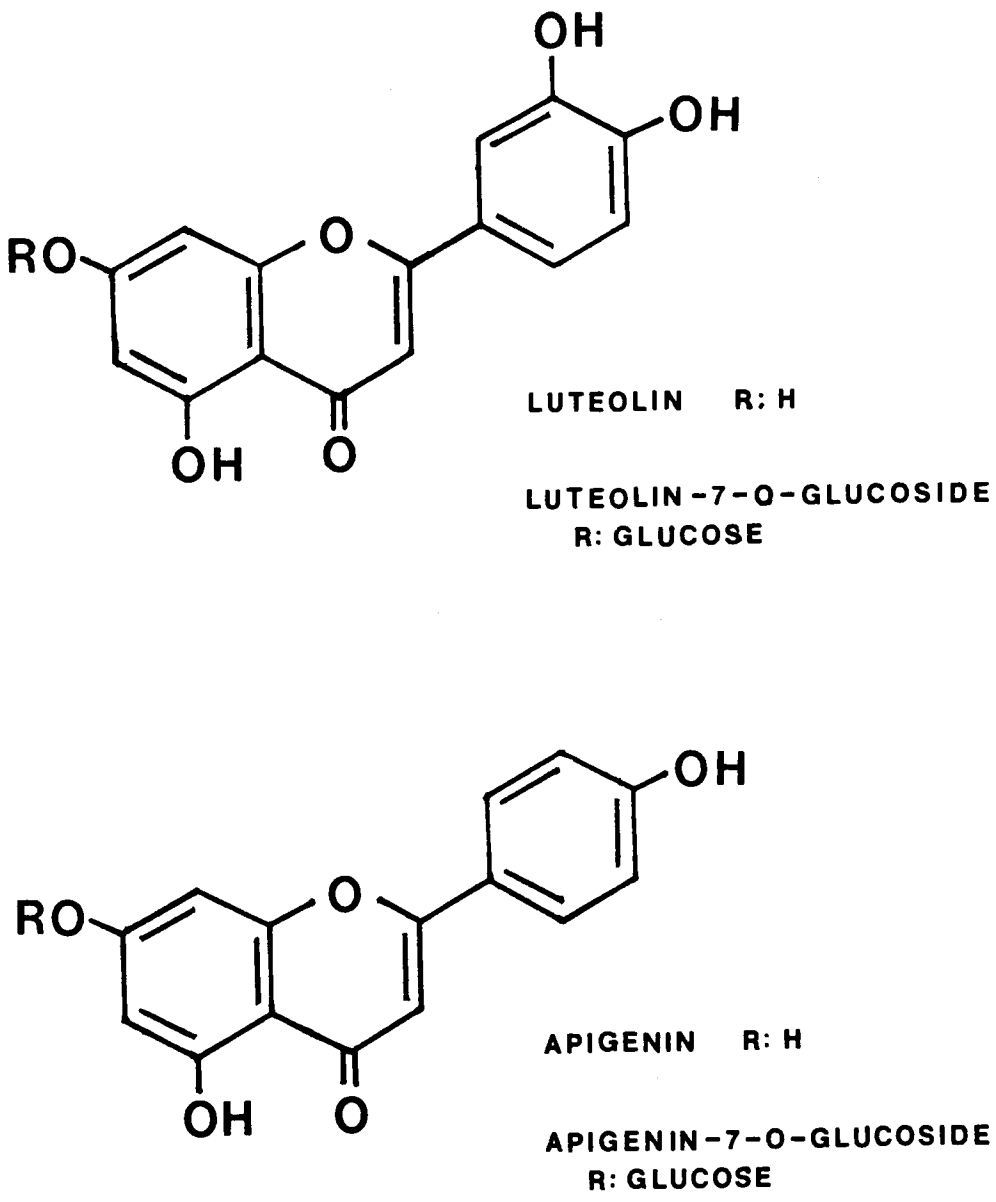

Figure V-1. Molecular structure of flavonoid compounds isolated from Hieracium.

as flavonoids may serve in pollinator attraction or recognition (Harborne and Smith, 1978; Rieseberg and Schilling, 1985). Artificial hybrids of H. gronovii and H. venosum were not included in the comparison because their growth under artificial light appeared to have interfered with flavonoid bio-synthesis (Harborne, 1967).

Methods

Plant material of each species was gathered from transplanted specimens of typical morphology and pooled. Leaves were dried, powdered in a blender and extracted for 24 hours in 85% methanol. Whole flowers, both fresh and dry, were extracted as above. Separation of fresh flowers from the involucre was accomplished by pulling them from the head while squeezing the ovary region with forceps. Compounds were isolated and purified with paper and Sephadex-column chromatography. Identification of compounds was based on Rf values, UV absorption spectra and co-chromatography against standards. The sugars were removed by acid hydrolysis (1N HCl, 40 min. at 100°C) and identified by co-chromatography against standards.

Results and Discussion

When leaf and floral profiles are compared, each of the three taxa appears to have a distinctive flavonoid complement (Table V-1). All three taxa have profiles made of the flavones apigenin and luteolin and their 7-O-glycosides (Figure V-1). Flavonoid profiles based on these simple flavones seem common among the Lactuceae (Guppy and Bohm, 1976; Haywood, Harborne and Turner, 1977; Fusiak and Schilling, 1984).

Table V-1. Distribution of floral and foliar flavonoid compounds in *Hieracium gronovii*, *H. venosum* and *H. traillii*.

Species	Compound Location	Compound				
		Luteolin	Luteolin-7-O-glucoside	Apigenin	Apigenin-7-O-glucoside	Apigenin-7-O-glycoside
H. gronovii	flowers	+	+	+		+
	leaves		+		+	
H. venosum	flowers	+	+	+	+	
	leaves		+		+	
H. traillii	flowers	+	+	+	+	
	leaves		?		+	

Hieracium gronovii and H. traillii each have an incompletely identified compound. In H. gronovii flowers, the apigenin-7-O-glucoside is replaced by a similar compound. This compound has mobility in 40% acetic acid intermediate between that of apigenin and apigenin-7-O-glucoside and does not change color when exposed to NH_3. Ultraviolet spectral data indicate an apigenin-7-O-glycoside and hydrolysis yields only apigenin and glucose. An apparent luteolin-7-O-glycoside from the leaves of H. traillii was visible on two-dimensional chromatograms (developed in TBA, then in 15% acetic acid), but could not be isolated, despite repeated attempts.

The floral and foliar complements of flavonoids show consistent differences (Table V-1). In all three taxa, the aglycones are present in the flowers only. The aglycones are not externally located as they cannot be removed by washing dried flowers in chloroform. It is possible that the aglycones are artifacts produced by enzymatic hydrolysis of glycosides during extraction. If they are not artifacts, their apparent internal location is unusual. According to Wollenweber and Dietz (1981), free aglycones, due to their low solubility in water, are usually located externally or are in association with secretory structures.

A comparison of flavonoid profiles does not provide evidence supporting the interpretation of H. traillii as a hybrid of H. gronovii and H. venosum. If additive inheritance of flavonoids occurs in hybrids of H. gronovii and H. venosum, their flowers should possess both the apigenin-7-O-glucoside of H. venosum and the apigenin-7-O-glycoside of H. gronovii. Hieracium traillii does not have the

distinctive floral compound from H. gronovii and may have a different foliar compound than either of its putative parents.

Conclusions

In conclusion, H. gronovii, H. venosum and H. traillii each appear to have a distinctive flavonoid profile. These profiles are based on simple flavones commonly found both in Hieracium and in the Lactuceae. Evidence from flavonoids does not support an interpretation of H. traillii as a hybrid of H. gronovii and H. venosum.

CHAPTER VI

CONCLUSIONS

The purposes of this study were to evaluate the possibility that H. traillii represents a hybrid of H. gronovii and H. venosum and to determine ways to identify natural hybrids of H. gronovii and H. venosum. Information from reproductive behavior, cytology, morphology and flavonoid chemistry was used to compare artificial hybrids with their parental taxa and H. traillii.

Reproductive behavior. Different reproductive behavior was observed in H. gronovii than in H. venosum and H. traillii. While all three taxa will self-fertilize, isolated heads of H. gronovii readily self-pollinate. Isolated heads of H. venosum and H. traillii rarely self-pollinate. Artificial hybrids between H. venosum and H. gronovii are similar to H. venosum in this respect.

Cytology. Cytological observations also showed H. gronovii to be distinctive. All three taxa have meiotic chromosome numbers of $2n = 9_{II}$, including a first report for H. traillii. Meiotic abnormalities were observed, but severely disturbed meiosis was infrequent. Frequencies of meiotic abnormalities encountered in H. gronovii and H. venosum were similar, although H. venosum had a higher frequency of micronucleus production, apparently associated with a higher frequency of bridge and fragment figures. Meiotic problems in H. traillii appear similar to those of H. venosum. Meiotically, artificial hybrids between H. gronovii and H. venosum are similar to their parents. The most marked meiotic problems occurred in presumed inter-

populational crosses of H. gronovii. Unreduced pollen resulting from failed division may appear viable when stained in lactophenol cotton blue, but its ability to germinate is unknown.

Morphology. All three taxa are morphologically distinctive. Hieracium traillii and H. venosum are superficially similar; given the morphological variability present in H. venosum, the best characters to distinguish these two taxa may be the densely hairy and stout-branched inflorescence of H. traillii. The presence of long-setose hairs on the involucral bracts of H. traillii is usually a distinguishing character, but in exceptional cases can occur in H. venosum (e.g. four specimens of Bowers, Hamilton Co., TN, deposited at TENN). Hieracium gronovii also possesses some morphological variability, which may be related to its wide range and is probably enhanced by its tendency to self-pollinate. Artificial hybrids between H. gronovii and H. venosum have mixed and intermediate characters when compared to their parents. The most consistent features that characterize the hybrids appear to be the wide ligules of H. venosum and the hairiness of H. gronovii.

Flavonoid chemistry. Each species appears to be characterized by a distinctive profile when both leaf and floral flavonoids are considered. Hieracium gronovii and H. traillii each have one unidentified compound. There is a distinctive floral compound in H. gronovii that is not found in H. traillii, further supporting an interpretation of H. traillii as a species, not as a hybrid. Flavonoid profiles of hybrids could not be characterized, apparently due to conditions of lighting during growth.

Hieracium traillii. In reproductive behavior, gross morphology and flavonoid chemistry H. traillii and H. venosum are more similar to each other than to H. gronovii. Interpretation of the data on H. traillii is hampered by the fact that the few specimens available only represent one population. Nevertheless, the data suggest that H. traillii and H. venosum are closely related taxa. In gross morphology H. traillii is similar to the scapose extreme of H. venosum. Reproductive behavior appears identical and flavonoid profiles are similar. Hieracium traillii also occupies a specific habitat within a limited area.

A testable hypothesis that could be drawn from this is that H. traillii evolved on shale barrens, from H. venosum. If this is the case, H. traillii may constitute a schizoendemic--an endemic taxon that originated by gradual speciation (Keener, 1983). Hieracium traillii could also have evolved by quantum speciation (Grant, 1981) associated with adaptation to a shale barren habitat.

Conclusions

In conclusion, data from hybridization, reproductive behavior, cytology, morphology, and flavonoid chemistry indicate that H. gronovii, H. venosum and H. traillii are distinct species. Hieracium traillii is distinct from artificial hybrids between H. gronovii and H. venosum. The similarities between H. traillii and H. venosum that they are closely related species, and the hypothesis is advanced that H. traillii evolved from H. venosum.

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