A BIOSYSTEMATIC STUDY OF THE RELATIONSHIP OF *NAMA HISPIDUM* AND *NAMA STEVENSII*

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A BIOSYSTEMATIC STUDY OF THE RELATIONSHIP

OF NAMA HISPIDUM AND

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PREFACE

This study is concerned with the reproductive biology and morphological relationship between Nama hispidum and Nama stevensii. In order to better understand this relationship, a more intense study was needed.

Special thanks is given to my major adviser, Dr. Ronald J. Tyrl, for his guidance and assistance throughout this study and to the members of my committee, Dr. Paul E. Richardson and Dr. L. Herbert Bruneau.

The author wishes to express his appreciation to Dr. Charles D. Michener, at the University of Kansas; and to Dr. Jerome G. Rozen, Jr., at the American Museum of Natural History for their time and expertise in the identification of the insects collected during this study.

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CHAPTER I

INTRODUCTION

Nama is a small genus of the Hydrophyllaceae comprising 15 annual and 17 perennial species. Distributed in drier habitats thirty-one species occur in the southwestern United States and Northern Mexico, two in the Carribean, five in South America and one in Hawaii (Hitchcock, 1933).

Plants vary from prostrate to erect, and from herbaceous to suffrutescent. The leaves are mostly alternate and entire. The flowers are borne singly in the leaf axils or in reduced laterial or terminal clusters. The calyx is divided nearly to the base, the lobes are linear-lanceolate to spatulate and the corolla is tubular. The stamens are mostly subequal to unequal. The filament bases vary, although usually somewhat dilated, while the adnate portion may have or lack free margins. There are two styles which are usually free, but are sometimes partially to completely united. The seeds are numerous and variously pitted.

In southwestern Oklahoma and adjacent Texas, only two species of Nama occur (Hitchcock, 1933; Waterfall, 1969; Correll and Johnston, 1970). One is N. hispidum, described by Asa Gray in 1861. A widespread and variable taxon, it is found growing in sandy or gravelly soils with populations occurring from southern California and northern Mexico to Oklahoma (Figure 1).

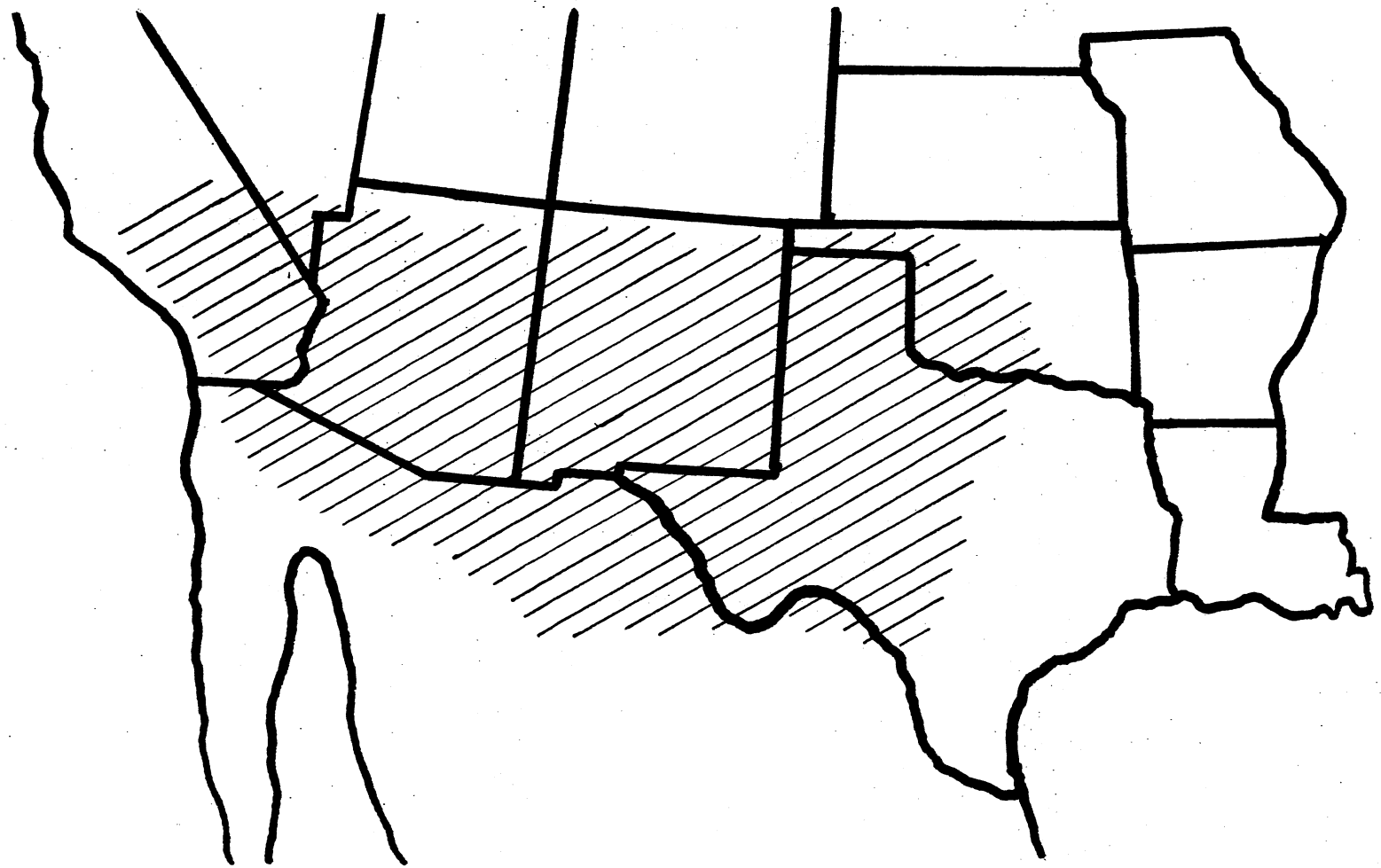

Figure 1. Distribution of Nama hispidum in the United States and Mexico.

The second species is N. stevensii. Collected near Alva, Woods County, Oklahoma by George Stevens in 1913 and described by C. L. Hitchcock in 1933, N. stevensii is found growing only on the gypsum soils of Oklahoma and Texas (Figure 2).

These two taxa appear to be closely related. Although they are apparently highly restricted as to soil type, they differ consistently in only one morphological character--the nature of the pubescence. N. hispidum, as the specific epithet implies, is characterized by hispid hairs. N. stevensii, on the other hand, is characterized by appressed hairs. This pubescence differences has been emphasized in keys by Hitchcock (1933), Waterfall (1969), and Correll and Johnston (1970).

In addition to the pubescence, the two taxa exhibit less conspicuous differences in leaf shape and size, mode of branching, and level of stamen filament insertion and degree of fusion with the corolla (cf. Table I) (Correll and Johnston, 1970; Benenati, 1974). The leaves of N. hispidum vary from linear oblong to obovate and are much more variable than those of N. stevensii. They range from 10-15 mm long and 1-5 mm wide. The leaves of N. stevensii, on the other hand, are linear-lanceolate and are usually 10-13 mm long and 1-3 mm wide.

In general, N. hispidum exhibits sparse branching above the stem base with the leaves not densely crowded (Figure 3a). Therefore N. hispidum has an erect, narrow appearance. N. stevensii, on the other hand, is branched from the base with the upper branches and leaves crowded thus giving a dense rounded appearance (Figure 3b).

With respect to the nature of the stamen filament, the adnate portion of the filament of N. hispidum is not much wider than the free

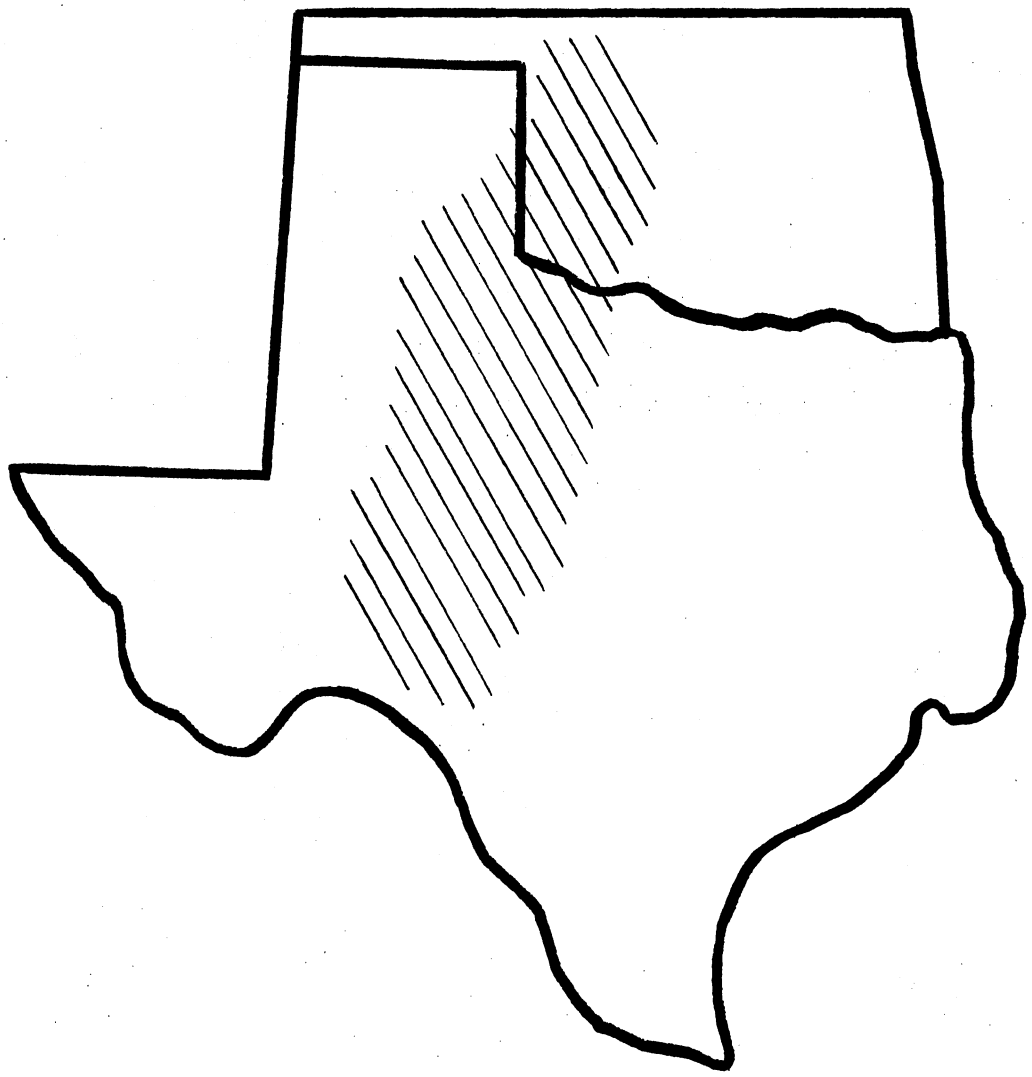

Figure 2. Distribution of Nama stevensii in Oklahoma and Texas.

TABLE I

CHARACTER SYNOPSIS OF *N. HISPIDUM* AND *N. STEVENSII*

NAMA HISPIDUM	*NAMA STEVENSII*
Sandy soil	Gypsum soil
Strigose- hispid to hispid	Appressed strigose
Simple at base to branching	Branching at base
Sparingly branched above	Few branched
Slender, erect	Erect
10-40 cm tall	5-25 cm tall
Leaves linear to oblong-spathulate 1-5 cm long 1-5 mm wide tapering gradually to base slightly revolute hispid	Leaves linear-lanceolate 1-3 cm long 1-3 mm wide clasping, sessile revolute strigose-hispid
Flowers single or in cymes	Flowers single, sessile
Calyx lobes linear-lanceolate	Calyx lobes linear-lanceolate
Corolla tubular-campanulate 8-15 mm long	Corolla tubular 8 mm long
Stamens ½ corolla length filaments thick, terete unequal insertion 1-4 mm from corolla base adnate portion same as free margins not free	Stamens not more than ½ filaments terete very unequal insertion 1-3 mm from corolla base adnate portion wider margins free
Styles 2-4 mm long	Styles 4 mm long
Capsules with 20-100 seeds	Capsules with 40-50 seeds
Seeds 0.5 mm long alveolate-reticulate	Seeds 0.3 mm long alveolate, yellow

Figure 3. General Morphology of (A) Nama hispidum and (B) Nama stevensii.

portion; whereas, in N. stevensii, the filament base is dilated to widened into narrowly free-margined scales about equaling the free portion (Hitchcock, 1933; Correll and Johnston, 1970).

Despite their general acceptance as two species by Hitchcock (1933); Waterfall (1969); and Correll and Johnston (1970), there is some question as to whether these two taxa are distinct. Correll and Johnston suggest that N. stevensii may be only a gypsophilous phase of N. hispidum and indeed a gypsophilic variety of N. hispidum, var. gypsicola, has been described from Nuevo Leon, Mexico by I. M. Johnston in 1941.

Initial observations of these taxa suggested a considerable amount of variability and overlap in the characters generally used to distinguish the two taxa. Further, a large mixed population of plants identifiable as both N. stevensii and N. hispidum was found in Harmon county along the bluffs of the Red River. In this area, a gypsum bluff abutted the sandy floor plain. In the sand-gyp rubble at the base, plants of the two taxa were growing sympatrically with some individuals exhibiting intermediate morphology.

Additionally, Benenati (1974) suggested that these two taxa exhibited phenotypic plasticity. She reported that wide leaves and spreading leaf pubescence were characteristic of both taxa on soils low-in or lacking gypsum. As the gypsum content increased, leaf width apparently narrowed, the leaf hairs were more closely appressed, and the difference between the adnate and free portions of the stamen filament became more distinct.

Thus the objective of this study has been to investigate the relationship of N. hispidum and N. stevensii in order to determine

if they are two distinct species or merely two genetic phases of one species. The work has involved detailed studies of the reproductive biology, an analysis of the patterns of morphological variation, a characterization of the flavonoid patterns by chromatography and intra- and interspecific hybridizations.

CHAPTER II

STUDY SITES

Nama hispidum and *N. stevensii* were studied and collected at fifteen study sites in western and southwestern Oklahoma (Table II, Figure 4). The two taxa were growing together in only one population, located in Harmon County.

Plants were studied in the field and selected individuals were transplanted into pots and subsequently transported to the University of Oklahoma Biological Station on Lake Taxoma for further study and manipulation. Other individuals were collected, pressed, and dried for later study of morphology. Voucher specimens were deposited in the Oklahoma State University Herbarium (OKLA). Insects were deposited in the Oklahoma State University Entomological Collections.

TABLE II

N. HISPIDUM AND *N. STEVENSII*
STUDY SITES

Accession	County	Range	Township	Section	Soil Association	Notes
Grummer 159	Custer	R15W	T12N	13	WCQ	*N. stevensii*. 2.0 mi. N. and 1.2 mi. W. on OK Hwy 54 from the Weatherford Cemetery; S. of road on gyp outcrop.
Grummer 160	Custer	R15W	T12N	13	WCQ	*N. stevensii*. 1.8 mi. W. of OK Hwy 54 and Weatherford Cemetery. N. of road on gyp outcrop.
Grummer 161	Washita	R14W	T11N	14	WCQ	*N. hispidum*. 20 m N. and 17 m. W. of boatlaunch area of Crowder Lake shore. sandy loam.
Grummer 162	Caddo	R10W	T10N	4	CQ	*N. hispidum*. 0.8 mi. W. of Salyer Lake on OK Hwy 152. S. side of road. sandy loam.
Grummer 163	Beckham	R25W	T8N	7	NBM	*N. stevensii*. 9.2 mi. S. of OK Hwy 30 from I-40. Roadside gyp outcrop.

TABLE II (continued)

Accession	County	Range	Township	Section	Soil Association	Notes
Tyrl 748	Blaine	R12W	T19N	27	VQ	N. stevensii. 8.3 mi. W. of Okeene. Main street intersection with OK Hwy. 51. 300 yds. N. of Hwy on section line road. Top of gyp ridge.
Tyrl 749	Blaine	R12W	T17N	12	VQ	N. stevensii. Gyp outcrop behind Roman Nose State Park Stable. very rare.
Tyrl 750	Custer	R15W	T12N	13	WCQ	N. stevensii. 1.3 mi. W. of OK Hwy 54 on service road along I-40. Entrance to farm. Gyp hummock. rare.
Tyrl 760 761 762 Grummer 165	Harmon	R26W	T1N	5	ETY	N. stevensii. 0.25 mi. N.E. of bridge over Red River of road between Hollis and Goodlet, Texas. along ditch bank and fence row, adjacent to field.

TABLE II (continued)

Accession	County	Range	Township	Section	Soil Association	Notes
Tyrl 764 Grummer 164	Jackson	R26W	T1S	6	ETY	N. hispidum. 0.5 mi. N. of OK Hwy 34-44 bridge across Red River. Stabilized sand dunes. E. side of hwy next to abandoned RR buildings and track.
Tyrl 765	Wilbarger Texas	R20W	T2S	6	ETY	N. hispidum. 0.3 mi. S. of Red River Bridge on US Hwy 283. Stabilized sand dunes of river deep sand soil. abundant.
Tyrl 766	Jackson	R23W	T1S	15	FHT	N. stevensii. 2 mi. E. of Eldorado on OK Hwy 5 and 500 yds S. of road on small gyp outcrop.
Tyrl 768	Comanche	R13W	T3N	11	GMTs.	N. hispidum. 0.5 mi. NW of entrance to Mt. Scott campground 3.4 Mi. E. of jct. of OK Hwys 49 and 115. Granite hillside. slopes of Mt. Scott.

TABLE II (continued)

Accession	County	Range	Township	Section	Soil Association	Notes
Tyrl 769	Caddo	R10W	T8N	4	CQ	N. hispidum. 400 m. N. of Gracemont main street. Red sandy soil; fence separation of highway right of way from grazed pasture.
Tyrl 839	Caddo	R10W	T10N	24	CQ	N. hispidum. 5.1 mi. W. of OK Hwy 152. 300 M. W. of Salyer Lake Club House. Eroded banks at base of sandstone bluffs. numerous.
Tyrl 845	Caddo	R10W	T6N	35	CQ	N. stevensii. Western of Cement Oil Field adjacent to OK Hwy 8, ca. 5.0 mi. W. of Cement OK post office. exposed gyp ridge scattered plants.

Soil Association Types: WCQ= Woodward-Carey-Quinlan; CQ= Cobb-Quinlan; NBM- Nobscot-Brownfield-Miles; VQ= Vernon-Quinlan; ETY= Enterprise-Tipton-Yahola; GMTs= Granitic Mountains; FHT= Foard-Hollister-Tillman (Oklahoma Water Resrouces Board, 1972).

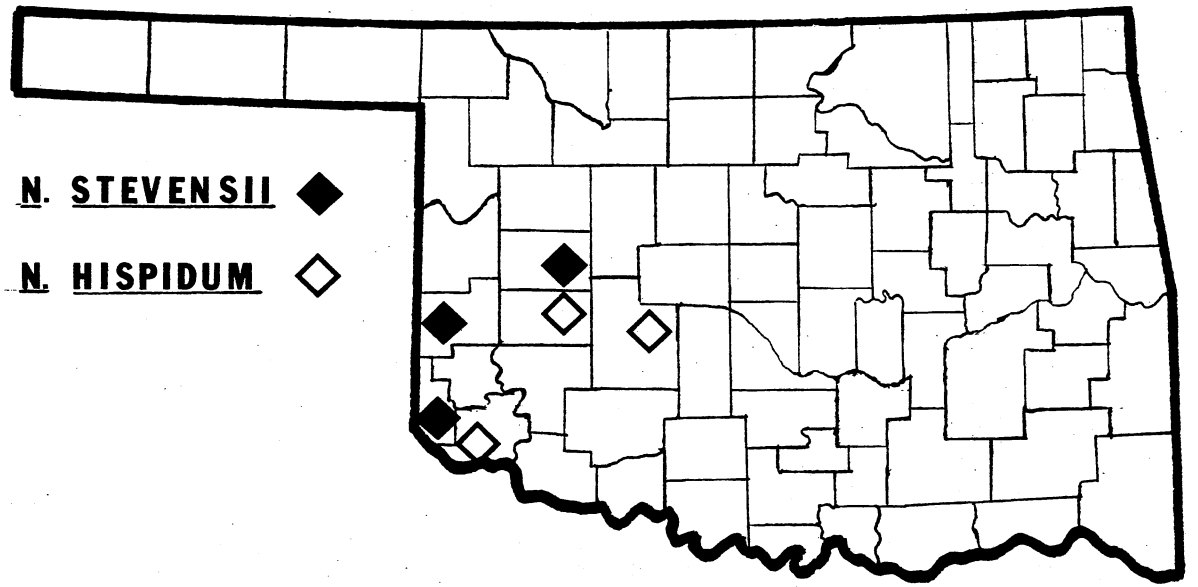

Figure 4. Collection Sites of Nama used in Crossing Experiments.

CHAPTER III

ANALYSIS OF MORPHOLOGICAL VARIATION

Initial work involved an analysis of the patterns of morphological variation within and between the two taxa. Preliminary observations had indicated that the majority of characters exhibited continuous variation. As mentioned previosly, only two features are discontinuous and can be used to consistently separate N. hispidum and N. stevensii. They are soil type (sand versus gypsum) and pubesence (erect hispid hairs versus appressed). Thus it was my intention to (1) determine the range of variation exhibited by each taxon and (2) to see if characters other than soil type and pubesence were sufficient to delimit the two taxa.

A variety of methods to study variation patterns have been developed and include the construction of morphological indices, scatter diagrams, polygonal graphs, phenograms, and cluster analysis. A morphological index (hybrid index sensu Anderson, 1949) was formulated to study the range of variation exhibited by each taxon. Fourteen characters of 303 plants from 11 populations were measured; the range of variation of each determined; size or character classes established; and index values assigned (Table III).

Histograms were then constructed to graphically show the distribution of index values (Figure 5). As is apparent, two taxa may be distinguished with Nama stevensii appearing to be more homogenous

TABLE III

MORPHOLOGICAL INDEX OF NAMA CHARACTERS

Character	Index Value 1	Index Value 3	Index Value 5
Plant Height	2.0 - 6.5	6.6 - 15.0	15.1 - 22.0 cm
Branching	0.0 - 0.2		0.2 - 11.0 cm
Internode Length	0.0 - 0.1	0.1 - 9.0	9.1 - 30.0 mm
Leaf Width	1.0 - 2.0		2.1 - 5.0 mm
Leaf Length	0.5 - 0.9	1.0 - 1.8	1.9 - 3.0 mm
Pedicel Length	0.0 - 0.1	1.0 - 1.5	2.0 - 8.0 mm
Calyx Lobe Length	3.0 - 4.5	4.6 - 5.5	6.0 - 7.5 mm
Corolla Tube Length	3.0 - 4.5	5.0 - 6.0	6.5 - 9.0 mm
Style Length	1.0 - 1.5	2.0	2.5 - 3.0 mm
Free Filament Length	1.0	1.5	2.0 - 2.5 mm
Hair Appression	Appressed		Erect
Hair Base	Non Bulbous		Bulbous
Filament Adnation	Wider		Equal
Filament Margins	Free		Not Equal

"Typical" N. stevensii have a value of 14, and "Typical" N. hispidum have a value of 70.

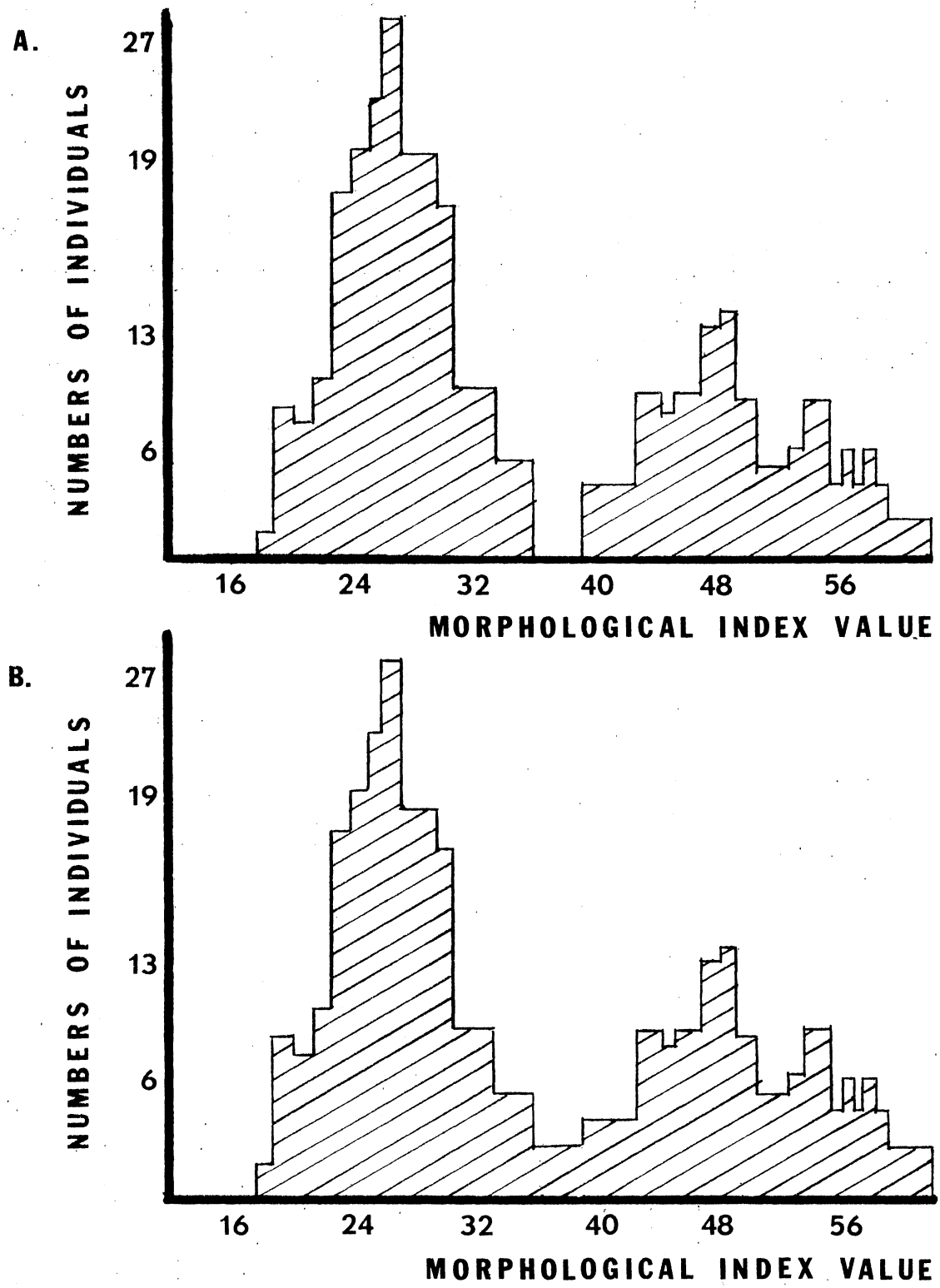

Figure 5. Morphological Index Values of Nama hispidum and Nama stevensii. A. Without plants of Hollis population (760,761,762) B. Including plants of Hollis population (760,761,762).

than N. hispidum.

The histogram patterns change slightly when plants from near Hollis in Harmon county (accession #760, 761, 762) are included (Figure 5b). This locality is characterized by a soil mixture of gypsum and sand. Intermediate plants were present; therefore, it appears that the soil possibly influences the morphological characteristics of the two taxa.

Another approach employed to study morphological variation is the formation of a discriminat function. This was an approach developed by Sir Ronald Fisher in the 1930's when the taxonomist Edgar Anderson presented him with the problem of how best to discriminate between species of Iris (Burnaby, 1966).

This approach is especially valuable in the classification of very similar plant taxa separated by slight quantitative differences as appears to be the case in Nama. The discriminant function is essentially a weighted average of a set of measurements. The measurements are multiplied by statistical factors to give maximum separation of pairs of groups when the products are added together (Fisher, 1936; Burnaby, 1966). Following the establishment of this discriminant function between the two groups, individual plants can be positioned in one group or the other. This positioning is then compared with the position determined by the taxonomist. The discriminant function employed here is based on the 11 quantitative characters previously used in the construction of the morphological indices. The qualitative characters of soil type and pubescence were not used in order to determine if N. hispidum and N. stevensii could be differentiated on the basis of these other highly variable characters. The Statistical Analysis

System (SAS) (Davis, 1971; Barr and Goodnight, 1972) was employed to produce the discriminant function.

Analysis via the discriminant function of N. hispidum populations indicated that they were uniform with one exception (Table IV). There were no changes in identification. The exceptional plant was from the gypsum-sand site near Hollis. However, analysis via the discriminant function of the N. stevensii populations (Table V), indicates that a large number of plants, on the basis of these 11 quantitative characters, could be classified as N. hispidum, if soil type and pubescence were ignored.

Three of the N. stevensii populations were found to be very variable with numerous plants being reclassified (Tables VI, VII, VIII). All plants were readily identified as N. stevensii and possessed hairs. In the Okeene population 38 of the 47 were reclassified as N. hispidum.

The same situation is observed in a population from a gypsum outcrop near Weatherford (Table VII). Of the 62 plants originally identified as N. stevensii, 51 are reclassified as N. hispidum.

The third population is from the gypsum-sand site near Hollis (Table VIII). Of the 11 plants initially identified as N. stevensii, nine were reclassified.

An analysis of variance for each character of these three heterogenous populations was calculated to determine if a single character was responsible for the separation of the two groups. The analysis revealed that there was no single character responsible for the separation of these two groups at the traditionally significant levels. However, many of the characters did show a tendency toward separation with probabilities of mean differences at the 50, 60 and 70% levels

TABLE IV

DISCRIMINANT FUNCTION ANALYSIS
OF *NAMA HISPIDUM* POPULATIONS

	Taxon Taxonomic	Identification Discriminant
N. stevensii	0	1
N. hispidum	63	62

TABLE V

DISCRIMINANT FUNCTION ANALYSIS
OF *NAMA STEVENSII* POPULATIONS

	Taxon Taxonomic	Identification Discriminant
N. stevensii	134	36
N. hispidum	0	98

TABLE VI

DISCRIMINANT FUNCTION ANALYSIS
OKEENE GYPSUM POPULATION

	Taxon Taxonomic	Identification Discriminant
N. stevensii	47	9
N. hispidum	0	38

TABLE VII

DISCRIMINANT FUNCTION ANALYSIS OF
WEATHERFORD GYPSUM POPULATION

	Taxon Taxonomic	Identification Discriminant
N. stevensii	62	11
N. hispidum	0	51

TABLE VIII

DISCRIMINANT FUNCTION ANALYSIS OF
HOLLIS SAND-GYPSUM POPULATION

	Taxon Taxonomic	Identification Discriminant
N. stevensii	11	2
N. hispidum	0	9

(Table IX). The discriminant function is indeed indicating that all characters together result in morphological separation, thus permitting taxonomic recognition.

TABLE IX

ANALYSIS OF VARIANCE OF
MEASURED CHARACTERS

Character	Population 748 (percent)	Population 750 (percent)	Populations 760-761-762 (percent)
Plant Height	61	53	53
Branching	54	2	71
Internode Length	54	20	1
Leaf Width	71	25	5
Leaf Length	24	54	82
Pedicel Length	1	54	35
Calyx Lobe Length	9	72	65
Corolla Tube Length	14	27	32
Style Length	53	14	35
Free Filament	60	85	1
Filament Insertion	68	91	1

CHAPTER IV

PHENOLOGICAL PATTERNS

In late March and early April seed germination occurs. Growth is rapid and by early to mid May, Nama reaches a height of 6 to 12 cm. At this time, the reproductive phase of growth is initiated. Buds appear and when 2 to 4 mm long have a pale white corolla (color 14A1 or 14A2 according to the 1961 classification of Kornerup). The second day after appearance the buds have doubled in size with the corolla becoming purple (14A4). At sunrise on the third day after the buds are visible, the corollas of N. hispidum appear dark purplish to lavender in color (14B6, 14B7) and have reached their full length of 8 to 15 mm. The corollas of N. stevensii, however, are light purplish in color (14A6, 14A7) and range from 8 to 10 mm. The flowers of both taxa are borne singly or in 3-5 flowered terminal cymes and the corollas are funnelform to campanulate.

At approximately 10:30 hours C.D.T., the color of the corolla deepens (14A8, 14B8) as the lobes open. Between 11:00 and 11:30, the flowers open completely exposing the two stigmas and five anthers in the throat. At this point, however, there is a slight difference between the two taxa. The flowers of N. hispidum typically open 10-15 minutes earlier than those of N. stevensii. This was the only difference in flowering sequence noted between the two taxa.

The spatial positioning of the anthers in the throat of the

corolla is such that three are typically below the stigmas and two above. The anthers are extrorse; the anther dehiscing outward and away from the stigmas. Thus unless the flower is entered or probed by an insect, pollen is not normally deposited on the stigmas.

Stigma receptivity was determined using the procedure of Dionne and Spicer (1957). Twenty-five flowers of each taxon were tagged prior to anthesis. Utilizing standard procedures, hand pollinations were made at the beginning of anthesis and at five hour intervals until the termination of anthesis. Pollen from the same plant as well as pollen from other plants were utilized. One and one-half to two hours after each pollination, the style was removed and fixed in chloroform: ethyl alcohol: acetic acid (6:3:1, v/v) for one hour. The style was then stained in a safranin 0 - aniline blue stain for 10-15 minutes, mounted, and examined with a compound microscope. Stigma receptivity was determined by the germination of pollen tubes on the stigmatic surface. The stigmas were found to be receptive throughout anthesis.

Approximately 30 minutes after the beginning of anthesis, the anther sacs split open longitudinally releasing pollen. The beginning of dehiscence is characterized by the splitting of the epidermis followed by full exposure of the pollen. The entire process lasts from 30 to 45 seconds. However, all five of the anthers do not dehisce simultaneously. Typically the lower anthers dehisce first followed by the upper ones.

Pollen grain morphology of six populations of N. stevensii and six populations of N. hispidum was studied and compared. Two hundred pollen grains per population were measured (Figure 6). The pollen grains of N. hispidum and N. stevensii are identical morphologically, being

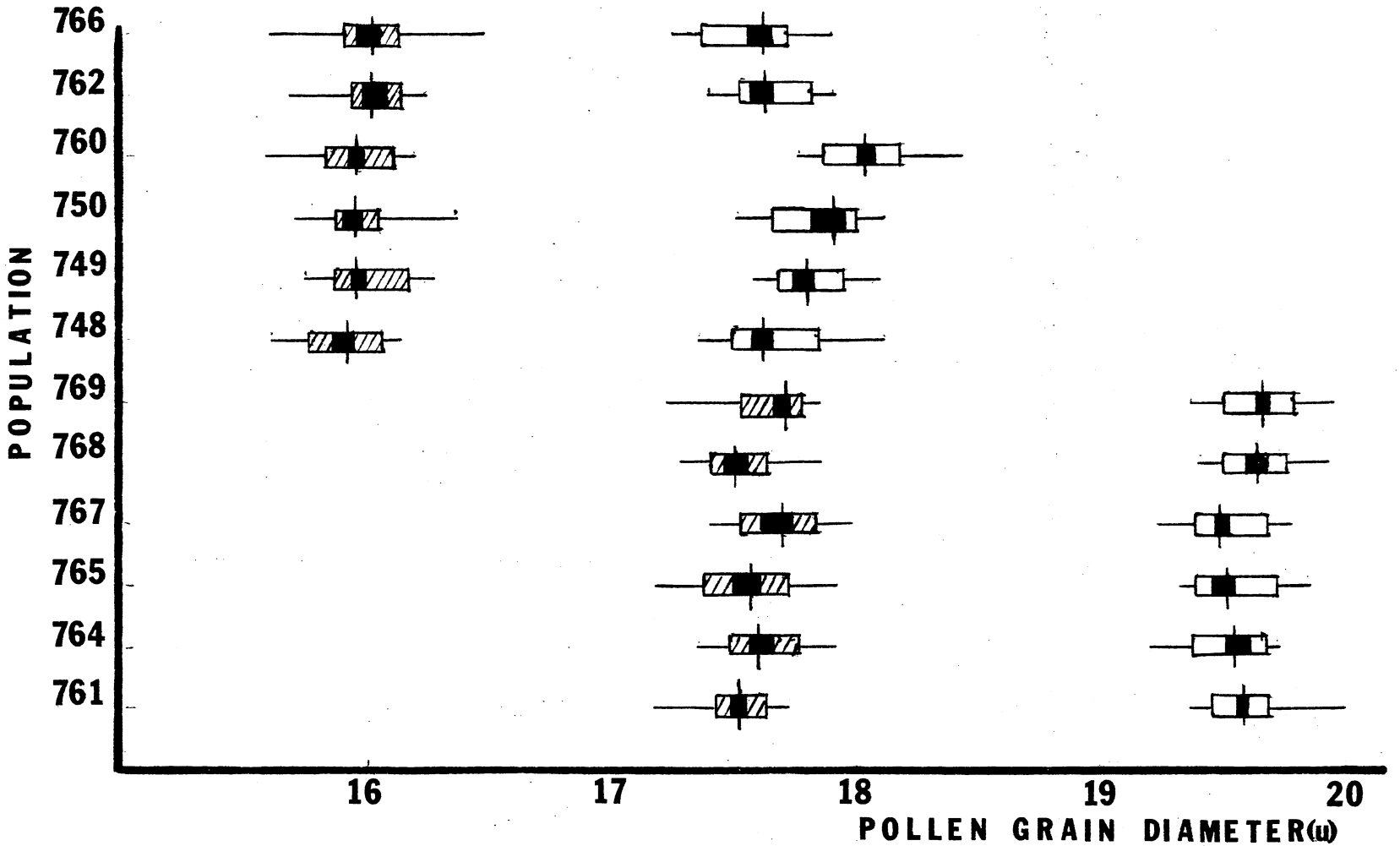

Figure 6. Measurements of Nama 100 pollen grain diameters per population. Mean, standard deviation, and standard error of mean.

= Polar view. = Equatorial view.

tricolporate (Erdtman, 1952). Pollen grain size differs between the two taxa. The pollen grains of N. hispidum are typically 1.77 u larger than those of N. stevensii.

On the day following flower opening, or approximately three days after bud appearance, the corolla falls off carrying with it the five stamens and leaving behind the one pistil and five sepals. Within seconds, the five sepals tightly enclose the pistil. Fruiting occurs and approximately 2-2½ weeks after flowering the capsule reaches maturity. The mature capsule is from 3-4 mm long and is light green (28A5) in color. At the end of 4-6 weeks, the capsule turns brown in color and dehisces.

There are 20-100 seeds per N. hispidum capsule and 40-50 seeds per N. stevensii capsule. The seeds are 0.3-0.5 mm long and are light yellow in color.

CHAPTER V

POLLINATION ECOLOGY

Observations of insects visiting Nama were made during the summer months of 1976 and 1977. Their behavior was recorded and then they were captured, pinned, labeled and identified. Each insect collected was examined for Nama pollen. Pollen was removed from the putative pollinators and transferred to microscope slides; the pollen then stained using safranin O-aniline blue (Dionne and Spicer, 1957).

Nama pollinators begin foraging at the beginning of flower anthesis. As noted earlier, this occurs between 11:00 and 11:30 hours C.D.T. Activity lessens at approximately 14:00 hours C.D.T. when the sun's heat is more intense. Pollinator activity as well as flower anthesis is delayed if the sky is overcast.

Specodosoma pratti Crawford, (Halictidae-Halictinae) is believed to be the major pollinator of N. hispidum in southwestern Oklahoma. Rozen and McGinley (1976) report that Conanthalictus dicksoni and C. conanthi pollinate N. hispidum in New Mexico. These two bees are closely related to Specodosoma and their activity is similar to that of S. pratti.

A previously undescribed species of bee was discovered to be the major pollinator of N. stevensii. It is Nomadopsis beamerorum, also a member of the family Halictinae, but in the subfamily Arenidae (J. G. Rozen, personal communication). These bees, as S. pratti,

are small, slender bees ranging from 4-6 mm in length.

The pollination behavior of both species is similar. Distinct approach patterns were not observed. Females briefly circle the plant and then apparently randomly land on a newly opened flower. Older flowers were not visited. The bee lands on the lip of the corolla, moving head first into the tube. Pollen is collected from all five anthers as the bee moves around the corolla lip. The pollen is collected with the front legs and packed among the ventral hairs of the hind legs. Occasionally the bee enters the corolla tube completely. Pollination is effected when the front or rear legs come in contact with the stigmas. As discussed below, Nama is an obligate outcrosser. The bee withdraws from the corolla tube, takes off, and moves to another flower in the same infloresence or moves to the flowers of the nearest plant repeating its movements.

The males of these two species carried small pollen loads, and exhibited a different behavior pattern; flying in a rapid zigzag fashion around the plant before landing on the flower. The males land primarily on newly opened flowers, but also on older ones, unlike the females. Nesting sites were found 30-90 cm away from the plants. Mating was also observed in the vicinity of the plants. According to Rozen and McGinley, this rapid zigzag fashion is apparently a part of their mating ritual.

At the sand-gypsum area near Hollis, S. pratti and Nomadopsis beamerorum both occur. Infrequent visits of Nomadopsis were observed on N. hispidum as well as S. pratti on N. stevensii. Although these visits were brief, active pollination did occur. Therefore, it is believed that cross pollination is possible between N. hispidum and

N. stevensii.

Another bee was observed visiting separate populations of both taxa on two occasions. One bee was collected on N. hispidum (#764) while four were collected on N. stevensii (#160). Although the behavior of this bee was very similar to S. pratti and Nomadopsis, it is not believed to be one of the major pollinators of Nama.

CHAPTER VI

GENETIC RELATIONSHIPS

As the data presented in chapter III indicate, morphology is of limited value in determining whether N. stevensii and N. hispidum are distinct species or merely ecotypes or varieties of a single species. The analysis of data from experimental hybridizations within and between each taxon can give an understanding of genetic continuity. Elucidation of gene flow patterns within each taxon and between the two taxa were examined utilizing standard hybridization techniques (Radford et al, 1974) (Appendix). Hybridizations were performed on established potted plants located at the University of Oklahoma Biological Station during the summer of 1976. All plants were kept within insect exclusion cages during the crossing experiments to prevent insect visits. The results of these hybridizations are summarized in Table X and Table XI.

As can be seen, fruit and seed set in nature is high. However, it is apparent that the activity of Specodosoma and Nomadopsis are crucial. In addition to the absence of self pollination due to the spatial relations of anthers and stigmas, there is an internal isolating barrier to self-fertilization. Nama stevensii and N. hispidum are self-incompatible due to a failure of pollen tube growth. Sequential sectioning of the style (Ramming et al, 1973) revealed that the pollen tubes grew only some 0.7 to 1.0 mm through the style after

TABLE X

RESULTS OF HYBRIDIZATION EXPERIMENTS TO ELUCIDATE GENETIC RELATIONSHIPS IN NAMA

Crosses	Number of Crosses Made	Capsule Development (Percent)	Seed Development (Percent)
Controls			
N. hispidum		96	96
N. stevensii		92	97
Wind Pollination			
N. hispidum		0	0
N. stevensii		0	0
Apomixis			
N. hispidum		0	0
N. stevensii		0	0
Self Compatibility			
N. hispidum		0	0
N. stevensii		0	0
Intrapopulational			
N. hispidum	10	90	91
N. stevensii	36	97	90
Intraspecific			
N. hispidum	21	100	71
N. stevensii	63	92	84
Interspecific			
N. hispidum X *N. stevensii*	132	90	28
N. stevensii X *N. hispidum*	110	91	25

germination. The style of Nama is approximately 2.2 mm in length.

Although the tubular corolla and inserted essential organs indicate entomophily, the possibility of wind pollination was examined. Observation of 25 flowers from various populations of each taxon revealed that wind pollination does not play a role in the reproductive biology of Nama. Pollen was not deposited on the stigmas, as expected.

Emasculation and bagging of 25 flowers of each taxon revealed that apomictic activity does not occur. There was no capsule or seed development. Experimentation to exclude pseudogamy was not performed.

For each taxon, crosses within populations and between populations were highly successful.

Two hundred-forty-two reciprocal crosses were made between plants of N. stevensii and N. hispidum. Two hundred-twenty-one capsules developed. All capsules appeared normal with respect to size and time of development. However, upon dissection it was discovered that seed set was greatly reduced. Seeds ranged from very small, black and shrivelled to full size and light yellow in color. Seed development was determined to be 26.73%.

CHAPTER VII

CHROMATOGRAPHY

Working in conjunction with Wayland Ezell at St. Cloud State University in Minnesota, chromatograms of flavonoid constituents were developed. Dried leaves were ground to a powder using mortar and pestle. A concentrated extract was obtained from the powdered material using petroleum ether and absolute methanol. This extract was then spotted on Whatman No. 1 filter paper and developed in two dimensions using BAW (n-butanol: acetic acid: water; 1:1:1 v/v) and 2% aqueous acetic acid. Developed chromatograms were viewed under UV light and the visible colors recorded.

Nama hispidum has a flavonoid array of five pigments (Figure 7a). N. stevensii has the same five pigments but also exhibits four additional ones (Figure 7b). Plants from the gyp-sand rubble site near Hollis in Harmon county exhibited either one array or the other; the arrays correlating with the morphology features of pubesence and degree of branching.

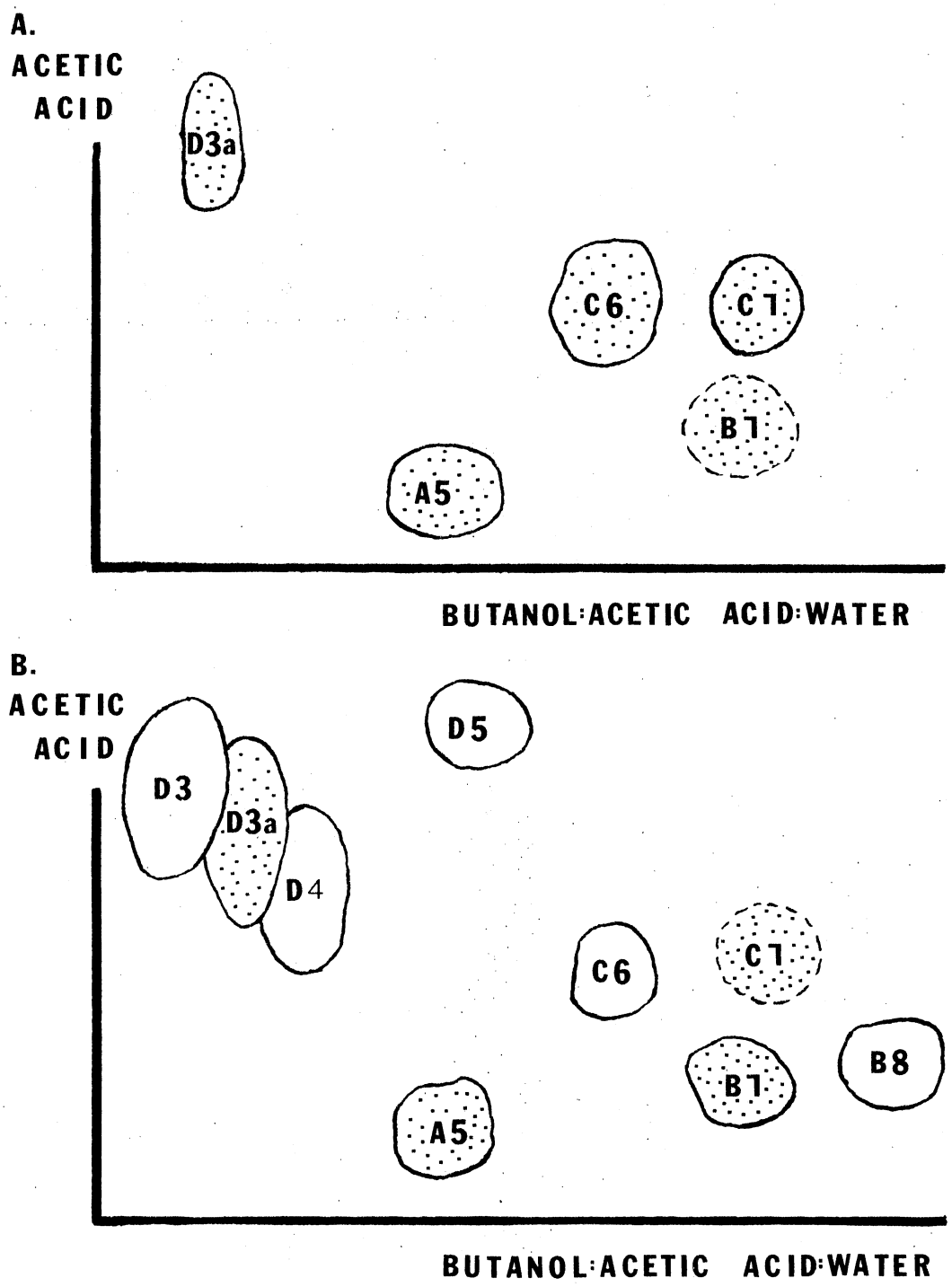

Figure 7. Chromatograms of the flavonoid constituents of Nama hispidum and N. stevensii. A. Five pigment array of N. hispidum (Populations 764,769,839). B. Nine pigment array of N. stevensii (Populations 748,750,845). Stippuled spot indicates constitutent common to both taxa; dashed line indicates trace occurence.

CHAPTER VIII

CONCLUSIONS

The relationship of Nama stevensii and Nama hispidum presents a challenging problem for the taxonomist. He is confronted with marked similarities and yet distinctive differences between the two taxa. The two differ consistently in only two phenotypic characters--soil type and the nature of their pubescence. Both have similar phenological and genetic patterns. In contrast, each taxon has different major insect pollinators albeit exhibiting similar behavior patterns. The chomatographic data indicate distinct differences. Gene flow between the two taxa is greatly reduced. Finally, they are highly restricted as to soil type.

On the basis of the information collected to date, one might hypothesize that the widely distributed and variable Nama hispidum has given rise to the gypsophilic Nama stevensii. The description of the gypsophilic variety of hispidum in Mexico (Johnston, 1941) suggests that this phenomenon has occured more than once. An examination of the morphology of this taxon and comparison with N. stevensii is needed.

The marked similarities in morphology, insect behavior, and flowering sequence are indicative of a relatively recent origin.

At this time it is felt that continued recognition of these two taxa as species best reflects their biological relationship. They are ecologically and genetically distinct.

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APPENDIX

PROCEDURES EMPLOYED IN CROSSING METHODS

Controls: Flowers were tagged in the field but otherwise undisturbed through flowering and fruiting. The mature capsules were dissected and the number of seeds counted in order to determine the natural fruit and seed set.

Self-Compatibility: One hundred-fifty flowers of each taxon were emasculated at anthesis. The stigmas of each were thoroughly covered with the pollen from the excised anthers. Insect exclusion cages were placed over the plants. Approximately three weeks later the capsules were collected and scored as developed or not developed, dissected open and the developed seeds counted.

Wind Pollination: Twenty-five flowers of each taxon were selected and marked for observation prior to opening. Insect exclusion cages were placed over the plants. Approximately three weeks later the capsules were collected and scored as developed or not developed, dissected open and the developed seeds counted.

Apomixis: Twenty-five flowers of each taxon were emasculated prior to anthesis. Insect exclusion cages were placed over the plants and the flowers subsequently examined for capsule development and seed set.

Intrapopulational: Thirty-six flowers of N. stevensii and ten of N. hispidum were emasculated prior to anthesis. The stigmas of each were

thoroughly hand pollinated with pollen from another plant of the same population. Insect exclusion cages were placed over the plants. Approximately three weeks later the capsules were collected and scored as developed or not developed, dissected open and the developed seeds counted.

Interpopulational: Sixty-three flowers of N. stevensii and three of N. hispidum were emasculated prior to anthesis. The stigmas of each were thoroughly hand pollinated with pollen from plants of a different population. Insect exclusion cages were placed over the plants. At the end of approximately three weeks the capsules were collected and scored as developed or not developed, dissected open and the developed seeds counted.

Intertaxon: Two-hundred-forty-two flowers of N. stevensii and N. hispidum were emasculated prior to anthesis. The stigmas of each were thoroughly hand pollinated with pollen from a plant of the other taxon. Reciprocal crosses involving all populations were made. Insect exclusion cages were placed over the plants. Approximately three weeks later the capsules were collected and scored as developed or not developed, dissected open and the developed seeds counted.

TABLE XI

RESULTS OF EXPERIMENTAL HYBRIDIZATIONS

Cross	Number of Crosses	Fruit Developed	Seed/Ovules
Intrapopulational			
165-01 X 165-12	8	8	410/429
165-12 X 165-35	5	5	222/222
165-12 X 165-02	2	2	92/92
165-15 X 165-22	5	5	159/163
165-15 X 165-12	4	4	100/105
165-17 X 165-22	3	3	127/133
165-17 X 165-12	2	1	0/9
165-22 X 165-12	7	7	112/200
164-01 X 164-21	2	1	32/32
164-01 X 164-19	3	3	85/90
164-10 X 164-16	1	1	77/83
164-19 X 164-10	3	3	101/101
164-21 X 164-17	1	1	37/60
Interpopulational			
160-01 X 163-01	3	3	81/81
160-02 X 165-17	17	14	18/32
160-03 X 165-12	6	6	250/250
162-01 X 164-12	1	1	0/32
162-13 X 164-9	8	3	49/55
163-01 X 160-01	4	4	159/159

TABLE XI (continued)

Cross	Number of Crosses	Fruit Developed	Seed/Ovules
163-01 X 165-31	2	2	99/99
164-02 X 161-11	4	4	180/225
164-16 X 161-02	2	2	86/90
164-18 X 162-12	6	6	59/88
165-01 X 160-01	2	2	0/97
165-04 X 160-01	3	3	229/229
164-12 X 160-02	5	5	22/30
165-12 X 160-01	2	2	93/93
165-17 X 163-01	3	3	30/91
165-26 X 160-02	8	8	33/70
165-30 X 160-01	3	1	5/7
165-31 X 163-01	2	2	112/112
165-31 X 160-02	3	3	21/22
Intertaxon			
160-01 X 161-02	9	8	35/241
160-01 X 164-01	2	2	15/59
160-02 X 164-04	7	4	2/24
160-02 X 164-12	1	0	0/38
160-03 X 164-16	7	7	69/267
161-02 X 165-12	4	4	83/115
161-02 X 165-01	6	5	127/148
161-02 X 159-02	2	2	12/74
161-03 X 160-01	2	2	5/62

TABLE XI (continued)

Cross	Number of Crosses	Fruit Developed	Seed/Ovules
162-01 X 165-14	2	2	0/65
162-01 X 165-17	3	3	98/103
163-01 X 164-32	1	1	5/37
164-01 X 159-01	2	1	33/33
164-01 X 163-01	7	7	156/293
164-02 X 163-01	8	8	0/248
164-02 X 160-01	6	6	25/126
164-10 X 165-22	4	2	0/59
164-10 X 160-02	5	3	2/98
164-10 X 165-12	8	8	35/324
164-12 X 165-31	1	1	3/31
164-12 X 165-13	4	4	7/123
164-12 X 165-12	9	7	17/231
164-12 X 165-15	4	4	1/110
164-13 X 163-01	2	2	5/43
164-13 X 165-12	2	2	18/66
164-16 X 160-02	6	6	9/109
164-16 X 165-17	6	6	13/183
164-17 X 165-12	3	3	0/106
164-17 X 163-01	13	7	13/239
164-17 X 165-14	4	3	9/87
164-19 X 159-01	1	1	6/16
164-19 X 160-01	5	5	3/84

TABLE XI (continued)

Cross	Number of Crosses	Fruit Developed	Seed/Ovules
164-21 X 165-13	3	3	14/60
164-21 X 165-15	2	2	10/45
164-22 X 165-31	2	2	0/54
164-22 X 165-22	2	2	2/106
164-30 X 160-02	3	3	0/14
164-30 X 165-31	2	2	5/39
164-31 X 165-17	2	2	47/52
165-01 X 164-16	6	6	181/283
165-01 X 164-29	2	2	81/81
165-02 X 164-17	1	1	93/93
165-02 X 161-02	7	6	28/294
165-04 X 164-16	8	8	75/369
165-12 X 164-12	6	6	42/161
165-13 X 164-09	3	3	46/86
165-14 X 164-16	8	5	7/105
165-14 X 164-16	4	4	56/145
165-15 X 162-01	6	6	116/203
165-15 X 164-12	4	4	74/155
165-17 X 164-19	2	2	17/51
165-21 X 164-01	2	2	19/93
165-22 X 162-01	3	3	49/116
165-26 X 164-01	4	4	52/141
165-30 X 164-19	3	3	14/107

TABLE XI (continued)

Cross	Number of Crosses	Fruit Developed	Seed/Ovules
165-31 X 164-21	2	2	26/70
165-34 X 164-12	1	1	36/36
165-35 X 164-16	2	2	31/80
165-35 X 164-12	9	9	153/395

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