 0030-0950/80/0002-0091 $1.50/0

SPECIES AND POPULATION VARIABILITY OF *OSMORHIZA LONGISTYLIS* AND *OSMORHIZA CLAYTONII*[1]

CAROL P. OSTERTAG and RICHARD J. JENSEN[2], Department of Biological Sciences, Wright State University, Dayton, OH 45435

Abstract. Four populations of *Osmorhiza*, containing either or both *O. longistylis* and *O. claytonii* (Sweet Cicely), were sampled to determine interspecific and intraspecific patterns of morphological variation. Discriminant analysis permitted easy separation of the species when presence or absence of anise scent was used as a criterion variable. The same method permitted separation of 2 populations of *O. longistylis* yet did not completely separate 2 populations of *O. claytonii*. Significant differences were found for a variety of characters between the 2 populations of *O. claytonii* as well as between the 2 *O. longistylis* populations and between the species. Style length and anise scent were recognized as reliable species indicators whereas pubescence characters were unreliable. Population differences are presumed to be the result of habitat differences, while species differences are apparently genetic.

OHIO J. SCI. 80(2): 91, 1980

Osmorhiza longistylis (Torr.) DC. and *Osmorhiza claytonii* (Michx.) C. B. Clarke (Sweet Cicely) are closely related species of the genus *Osmorhiza*. Both species are abundant in the eastern United States and extend into Canada. Within this range, *O. claytonii* is more prevalent to the north and east and *O. longistylis* is predominant to the south and west (Constance and Shan 1948). Individuals of both species commonly grow in moist, wood habitats.

These species show such a high degree of morphological similarity that they were once treated as a single species (Clarke 1871 as cited in Constance and Shan 1948). Although modern taxonomic treatments differentiate the two, there remains considerable disagreement as to the basis for this differentiation. Only one character, style length, is acknowledged as consistently reliable in separating the species. Styles of *O. longistylis* range from 2 to 4 mm while those of *O. claytonii* never exceed 1.5 mm (Fernald 1950, Gleason 1963, Blackwell 1975, Weishaupt 1971, Constance and Shan 1948, Small 1971). Several, but not all, descriptions mention that individuals of *O. longistylis* can be characterized by an anise scent which is absent from individuals of *O. claytonii* (Fernald 1950, Blackwell 1975, Weishaupt 1971, Wharton and Barbour 1971). A third character that is sometimes used to distinguish the species, and is perhaps the most variable, is degree of pubescence. According to Blackwell (1975), stems of *O. claytonii* are highly pubescent whereas *O. longistylis* stems are essentially glabrous, except at the nodes. Wharton and Barbour (1971) state that *O. claytonii* differs from *O. longistylis* by being softly hairy throughout. Pubescence of leaf blades is also used as a discriminating character (Small 1972), the leaf blade rachis of *O. claytonii* being described as having villous hairs while that of *O. longistylis* is glabrous or has shorter hairs.

There has also been some question as to whether or not these 2 species grow in mixed populations. According to Fernald (1950), they are rarely, if ever, found growing together. Constance and Shan (1948) report frequently collecting specimens of both species at the same time and place. They also claim that there was no difficulty, within mixed populations, in labeling a plant as belonging to

[1]Manuscript received 7 December 1978 and in revised form 20 June 1979 (#78-71).

[2]Present address: Dept. of Biology, Saint Mary's College, Notre Dame, IN 46556.

one species or the other and that there must be a genetic barrier allowing the species to maintain their distinctness.

Our study was prompted by the discovery of two mixed populations of *Osmorhiza*. We decided to determine the reliability of various characters for separating the 2 taxa and to see if their overlap in space and flowering time gave any indication of natural hybridization, as evidenced by the degree of interpopulation variation.

MATERIALS AND METHODS

Twenty plants were collected from each of 4 areas spatially separated so that they could be considered distinct natural populations. Two of these populations were located in densely wooded, moist habitats in the biology preserve on the campus of Wright State University, and the third population was at another site on the same campus in a drier, more open habitat. A fourth population was located in a relatively open, sunny area along the banks of Massey's Creek near Wilberforce, Ohio. Each population was sampled during one week, 2–9 May 1977, with plants collected randomly by marking points and then selecting the plant closest to each point in any direction.

For each plant, data for 17 phenotypic characters were recorded (table 1). The presence or absence of anise scent and style length were determined and recorded prior to pressing the specimens. The other characters were recorded after each collection had been pressed, dried, and properly filed. All quantitative characters were expressed as counts or mm (1–14 in table 1 and 2), and qualitative characters (15–17 in table 1 and 2) were divided into numerically coded classes so that data could be recorded quantitatively.

Leaf blade and petiole lengths were recorded from the lowest stem leaf. Leaflet length and width were taken from the first lateral leaflet of the same leaf, and leaf blade and leaflet lengths were measured from the point at which the leaflets branched to the blade tips. Number and length of bracts, both involucral and involucel, were averages of either 2 or 3 values depending on availability of data. The same was true for ray length, peduncle length, and number of rays. Whenever possible, the values to be averaged were taken from 3 different flowers or inflorescences. Measurements were consistently taken from those flowers still possessing petals and stamens. Stem pubescence characters were recorded from a point midway along the stem between the base and point of departure of the upper stem leaves. The length was an average of 3 trichomes. Four categories of pubescence density were decided upon and a typical plant chosen as an example of each, and placement of plants into categories was based upon visual comparison with these examples.

Discriminant analysis with an SPSS computer package was utilized to compare the two spe-

TABLE 1

Characters employed and their F-ratios and discriminant function coefficients for populations of Osmorhiza longistylis and Osmorhiza claytonii.

Character	Discriminant Analysis					
	Between Species		Between Populations *O. longistylis*		Between Populations *O. claytonii*	
	F-ratio	Coef.	F-ratio	Coef.	F-ratio	Coef.
1. Trichome Length†	—	—	—	—	—	—
2. Leaf Blade Length	5.07*	−0.68	8.07**	−1.04	11.40**	0.23
3. Leaflet Length	2.96	0.56	6.01*	−0.01	12.70**	−1.07
4. Leaflet Width	0.34	−0.14	5.59	0.25	8.18**	0.12
5. Petiole Length†	—	—	—	—	—	—
6. Peduncle Length	25.70**	0.41	0.03	0.05	16.03**	0.83
7. Number of Involucral Bracts	0.10	−0.18	0.65	−0.51	0.02	0.25
8. Length of Involucral Bracts	7.75**	−0.49	0.40	0.50	15.75**	0.29
9. Number of Involucel Bracts	68.53**	0.23	21.50**	−0.69	1.65	0.31
10. Length of Involucel Bracts	12.74**	0.25	0.83	0.07	16.75**	0.11
11. Number of Rays	149.00**	0.44	2.61	−0.02	1.35	0.14
12. Length of Rays	7.19**	−0.08	0.35	−0.04	36.08**	−1.51
13. Pedicel Length	31.19**	0.00	0.15	0.88	4.95	−0.53
14. Style Length	450.40**	0.78	74.10**	−1.00	4.59*	0.63
15. Stem Pubescence	4.17*	−0.12	5.85*	−0.24	2.94	−0.30
16. Leaflet Pubescence	34.02**	−0.34	1.98	−0.11	0.76	−0.38
17. Anise Scent†	—	—	—	—	—	—

†Data for characters 1, 5, 17 not included in this table.
*F-ratio significant at 0.05 level.
**F-ratio significant at 0.01 level.

cies (Nie *et al* 1975). The criterion variable of anise scent was used to assign individuals to groups *a priori*. Two other discriminant analyses were prepared to allow comparison of separate populations of both taxa. In each case, a population code was used as the variable for assigning individuals to the proper population. One analysis involved the 2 pure populations of *O. longistylis*, and the other compared individuals of *O. claytonii* from 2 populations that, although containing both species, were dominated by the latter. Species and population character means were evaluated by standard t-tests for cases in which the variances were equal and by modified t-tests for cases in which the variances were unequal (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

The histogram (fig. 1A) demonstrates that the discriminant function generated by the analysis allows separation of the 2 species with no overlap or misidentified individuals, *i.e.*, the use of presence or absence of anise scent permits the recognition of 2 statistically different groups corresponding to the 2 species. The midpoint of the axis (denoted by 0) represents the boundary to determine if any individuals have been misidentified. Since all *O. claytonii* individuals are to the left of this point and all *O. longistylis* individuals are to the right of this point, no misclassification has occurred. Eleven characters differed significantly between the 2 species when the variances were compared (table 1). As expected, style length is considerably different between the 2 species and had the highest discriminant function coefficient. *O. longistylis* had longer leaf blades and peduncles, shorter involucral bracts, more and longer involucel bracts, more and longer rays, longer pedicels, and less pubescent stems and leaves. The 2 species were clearly distinct morphologically and, in our sample, there was no evidence of hybridization as indicated by the clear separation shown in figure 1A. If hybrids were present, we would expect to see a continuum between the two centroids as shown by Ledig *et al* (1969) in a discriminant analysis of 2 oak species.

In comparing separate populations of the same species, we found that in one case (fig. 1B, *O. longistylis*) there were no misclassified individuals, while in the other (fig. 1C, *O. claytonii*) there were 2 misclassified individuals. In both cases, there was almost a continuum between the group centroids. The differences between these populations were believed to be a result of a simple environmental parameter since one population was in a dense woods and the other in a relatively open habitat. It would seem that the situation is analogous to that of sun leaves versus shade leaves commonly observed for some plants, especially oaks. Shade leaves are often longer and broader and, if lobed, have shallower sinuses than sun leaves.

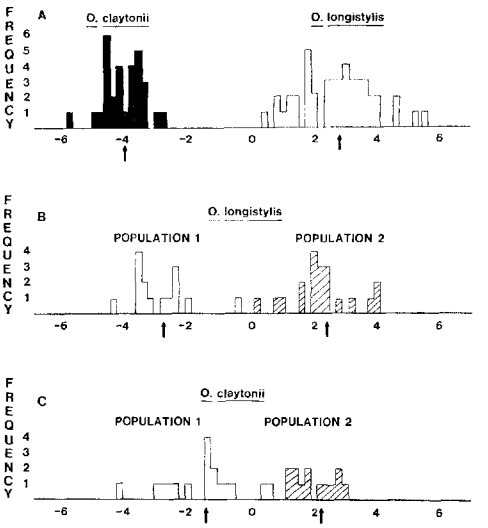

FIGURE 1. Histograms Based on Discriminant Analyses of Species and Population Groups. A. *Osmorhiza longistylis* and *Osmorhiza claytonii;* B. Two populations of *O. longistylis;* C. Two populations of *O. claytonii*. Arrows indicate group centroids.

Our comparison of the 2 species demonstrated that, besides the commonly cited differences in style length and anise scent, the 2 species differed in *O. longistylis* having shorter trichomes (when present), shorter petioles, longer peduncles, more and longer involucel bracts and rays, longer pedicels, and a reduced degree of leaflet pubescence (table 2). It should be noted that the lack of a marked difference in stem pubescence between these 2 taxa suggests that this character should not be used for recognition and classification.

Our values were quite similar to those of Lowry and Jones (1979) for style length, number of rays, and number of

involucel bracts. Comparison of Lowry and Jones' values with those presented in table 2 showed that the difference (calculated as: (larger value–smaller value)/smaller value) never exceeded 7%. This finding suggests a high degree of constancy for these characters since the values reported by Lowry and Jones were achieved by examination of several hundred specimens throughout the range of each of the 2 species.

Based on the results of the discriminant analyses there were several characters that showed significant differences between populations of the same species. These characters (table 2) coincided to a significant variation between populations. Constance and Shan (1948), in discussing the status of varieties and formae in *Osmorhiza*, prefer not to recognize such one character entities because they "usually show little community of characters and do not form discrete populations." We are not advocating the recognition of formal taxonomic entities based on variations in the characters we have analyzed, but we do feel that our analyses suggest that within both taxa there are groups of characters that permit the recognition of distinct natural populations.

It is interesting to speculate on the mechanisms that permit these 2 species

TABLE 2

Comparisons of characters between species and between populations of the same species.†

Char.#	*O. longistylis*	*O. claytonii*	Between Populations *O. longistylis* Population 1	Between Populations *O. longistylis* Population 2	Between Populations *O. claytonii* Population 1	Between Populations *O. claytonii* Population 2
1. (mm)	0.8± 0.3**	1.5± 0.2	0.6± 0.1	1.1± 0.2	1.4± 0.3**	1.6± 0.2
2. (mm)	206.9±47.6	184.7±31.8	196.6±49.5**	239.9±36.6	165.5±27.2**	199.3±27.2
3. (mm)	165.3±40.5	150.9±26.9	156.7±44.5**	189.1±28.9	133.8±21.3	163.8±23.8
4. (mm)	99.9±24.9	96.9±18.9	94.1±28.9**	114.1±18.9	86.8±17.5**	104.6±16.4
5. (mm)	111.0±52.5**	154.9±56.5	117.9±59.2	134.0±43.7	172.8±55.0**	140.3±56.9
6. (mm)	49.4±16.6**	28.8±17.2	52.9±15.2	53.1±17.3	13.9± 6.4**	37.7±16.1
7. (No.)	1.9± 0.5	1.9± 0.4	1.8± 0.6	1.9± 0.5	1.9± 0.5	1.9± 0.3
8. (mm)	6.0± 1.4	7.2± 2.5	5.9± 1.6	6.2± 1.1	5.5± 1.1**	8.5± 2.5
9. (No.)	5.1± 0.5**	3.9± 0.7	4.8± 0.5	5.4± 0.2	3.8± 0.7**	4.1± 0.6
10. (mm)	4.3± 0.9**	3.6± 0.8	4.2± 1.0	4.6± 0.8	3.0± 0.5**	3.9± 0.7
11. (No.)	5.5± 0.7**	3.7± 0.4	5.5± 0.7	5.8± 0.8	3.6± 0.4**	3.7± 0.4
12. (mm)	18.1± 5.7*	14.0± 7.0	19.7± 5.7	18.2± 6.4	8.1± 2.9**	18.5± 5.7
13. (mm)	4.7± 0.6**	3.9± 0.5	4.8± 0.7	4.7± 0.6	3.7± 0.6**	4.1± 0.4
14. (mm)	2.6± 0.4**	1.1± 0.1	2.3± 0.2**	2.9± 0.3	1.0± 0.1**	1.1± 0.1
15.	2.0± 1.3	2.5± 0.8	1.4± 0.9**	2.4± 1.3	2.7± 0.9	2.2± 0.4
16.	2.3± 0.6**	3.1± 0.5	2.2± 0.5	2.5± 0.6	3.0± 0.6*	3.2± 0.5
17.	2.0± 0.0	1.0± 0.0	2.0± 0.0	2.0± 0.0	1.0± 0.0	1.0± 0.0

†Characters numbered and arranged in the same sequence as in table 1.
#Data are presented as mean±SD.
*Difference in means significant at 0.05 level.
**Difference in means significant at 0.01 level.

large extent with those shown to have significant F-ratios in table 1. These differences are of particular interest in view of Constance and Shan's (1948) comments concerning intraspecific variation in *O. claytonii*, which they reported as remarkably uniform and without significant variations. It is not clear to us what they meant by significant variations, but our data suggested that, at the level of the local population, *O. claytonii* was more variable than *O. longistylis*, *i.e.*, it had more characters that illustrate to remain as distinct as they appear to be. Our limited observations suggest that spatial, environmental, and temporal isolating mechanisms are not involved. These 2 species grow in mixed populations and flower at about the same time. While pollinator specificity may be possible, the Umbelliferae in general are promiscuous plants (Bell 1971, Grant 1949), and we have observed a wide variety of potential pollinators on both species. There does not appear to be a cytological barrier because both species

have a haploid chromosome number of 11 (Bell and Constance 1957). Although at this time we can offer no direct proof, we suggest that the most probable species barrier would seem to be a combination of incompatibility factors operating at any or all stages between gamete production and the immature hybrid invidivual.

Acknowledgments. We wish to thank the Department of Biological Sciences and College of Science and Engineering, Wright State University, for providing financial support for this project. Computer facilities were provided by the Research and Instruction Computation Center at Wright State University; figure 1 was prepared by Mr. Bruce Adams of the Wright State Instructional Graphics Center.

LITERATURE CITED

Bell, C. R. 1971 Breeding systems and floral biology in the Umbelliferae. *In:* The biology and chemistry of the Umbelliferae (V. H. Heywood, Ed.), Academic Press, London. pp. 93–107.

——— and L. Constance 1957 Chromosome numbers in Umbelliferae. Amer. J. Bot. 44: 565–572.

Blackwell, W. H., Jr. 1975 Guide to the spring flora of the tristate area. Kendall/Hunt Publ. Co., Dubuque. 79 pp.

Constance, L. and R. H. Shan 1948 The genus *Osmorhiza* (Umbelliferae): a study in geographic affinities. Univ. Calif. Pub. Bot. 23: 111–156.

Fernald, M. L. 1950 Gray's manual of botany. American Book Co., New York. 1632 pp.

Gleason, H. A. 1963 The new Britton and Brown illustrated flora of the north-eastern United States and adjacent Canada. Hafner Publ. Co., New York. Vol. 2.

Grant, V. 1949 Pollination systems as isolating mechanisms in Angiosperms. Evolution 3: 82–97.

Ledig, F. T., R. W. Wilson, J. W. Duffield and G. Maxwell 1969 A discriminant analysis of introgression between *Quercus prinus* L. and *Quercus alba* L. Bull. Torrey Bot. Club 96: 156–163.

Lowry, P. P., II and A. G. Jones 1979 Biosystematic investigations and taxonomy of *Osmorhiza* Rafinesque section *Osmorhiza* (Apiaceae) in North America. Amer. Midl. Nat. 101: 21–27.

Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner and D. H. Bent 1975 Statistical package for the social sciences. McGraw-Hill Book Co., New York. 675 pp.

Small, J. K. 1972 Manual of the southeastern flora. Hafner Publ. Co., New York. 1554 pp.

Sokal, R. R. and F. J. Rohlf 1969 Biometry. W. H. Freeman, San Francisco. 776 pp.

Weishaupt, C. G. 1971 Vascular plants of Ohio. Kendall/Hunt Publ. Co., Dubuque. 292 pp.

Wharton, M. E. and R. W. Barbour 1971 The wildflowers and ferns of Kentucky. Univ. Press of Kentucky, Lexington. 344 pp.