A note on the taxonomic status of *Euphrasia randii* (Scrophulariaceae)

STEPHEN R. DOWNIE
Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

AND

JOHN MCNEILL
Royal Ontario Museum, 100 Queens Park, Toronto, Ont., Canada M5S 2C6

Received July 11, 1989

DOWNIE, S. R., and MCNEILL, J. 1990. A note on the taxonomic status of *Euphrasia randii* (Scrophulariaceae). Can. J. Bot. **68**: 1531–1535.

A reexamination of the phenetic relationships among the previously recognized infraspecific taxa of *Euphrasia randii* using principal component analyses and four clustering methods indicates that the recognition of these infraspecific taxa is unwarranted. This complex exists as one morphologically variable taxon, *E. randii*.

DOWNIE, S. R., et MCNEILL, J. 1990. A note on hte taxonomic status of *Euphrasia randii* (Scrophulariaceae). Can. J. Bot. **68** : 1531–1535.

À l'aide d'analyses en composantes principales et de quatre méthodes de groupements, les auteurs ont réexaminé les relations phénétiques entre les taxons infraspécifiques antérieurement reconnus du *Euphrasia randii*; l'étude indique que la reconnaissance des taxons infraspécifiques n'est pas justifiée. Ce complexe existe comme un unique taxon, morphologiquement variable, le *E. randii*.

[Traduit par la revue]

Introduction

In 1901 B.L. Robinson described two taxa from Maine: *Euphrasia randii*, and a variety of this species, *E. randii* var. *farlowii*, the latter named for plants possessing smaller leaves and a coarser pubescence. *Euphrasia randii* var. *farlowii* was portrayed as "a puzzling form which more copious material may show to be a distinct species." After examining more material of this species, Fernald and Wiegand (1915) replaced the name *E. randii* by the earlier *E. purpurea* Reeks and divided the species into three varieties, the typical variety and var. *randii* and var. *farlowii*, each of which had two forms based on whether the corolla was white or purple. The varieties were distinguished primarily by the degree of leaf pubescence.

In 1943 Fernald, realizing that *E. purpurea* Reeks was a later homonym of *E. purpurea* Desf., took up the name *E. randii* again and made the necessary new combinations for the varieties and forms that he and Wiegand had previously recognized. The epithet *reeksii* was proposed for what had been the type variety of *E. purpurea* Reeks.

The presence of both white and purple corolla forms within each variety does not suggest that it is a particularly useful taxonomic character, and as Sell and Yeo (1970) stated, names to distinguish these forms are pointless. In the past, the varieties *randii* and *reeksii* were distinguished only according to whether their leaves were sparingly crisp-pubescent or glabrous, respectively (Fernald and Wiegand 1915). Sell and Yeo (1970), in a North American revision of *Euphrasia* based solely upon an examination of herbarium specimens, were of the opinion that this character was inadequate for the separation of these two varieties and consequently upheld only var. *randii* (including var. *reeksii*) and var. *farlowii*. They characterized *E. randii* var. *randii* by its lax habit, long internodes, and obtuse, crenate cauline leaves, and var. *farlowii* by its small size, much-branched habit, small, densely pubescent leaves, and minute flowers. The distributions of their two varieties were wholly sympatric.

The purpose of this study was to ascertain the extent of morphological variation prevalent within and among populations of *E. randii* by way of a multivariate statistical approach and to determine whether any, and if so which, of the previously recognized infraspecific taxa within this species merit taxonomic recognition.

Materials and methods

Specimens from the following herbaria were examined during the course of this investigation: CAN, DAO, GH, MICH, MT, MTMG, and NHA (abbreviations according to Holmgren *et al.* 1981). The recognition and delimitation of *E. randii* from related taxa was made on the basis of previous taxonomic treatments (e.g., Robinson 1901; Fernald and Wiegand 1915; Sell and Yeo 1970) and on inspection of type material. Additional material was obtained during field studies in Maine, New Brunswick, Newfoundland, Nova Scotia, and Quebec. In all, approximately 350 specimens were examined.

The selection of representative herbarium specimens was based on a number of factors. After the removal of duplicates and the rejection of several whose locality data were insufficient for mapping purposes, consideration was given to specimens that consisted of five or more complete individuals (i.e., possessing all diagnostic characters) in an effort to assess intrapopulational variation. Owing to the limited availability of material from P.E.I., two specimens, consisting of two and four complete individuals, respectively, were retained in the dataset. The five individuals selected from a particular locality (whether derived from a single herbarium specimen or local field collection site) constituted a population. Extensive field studies and herbarium specimen observations coupled with our knowledge from previous investigations (Downie *et al.* 1988) suggested that a sample of five individuals is a good representation of the diversity within a population. Of perhaps greater significance, however, is that the maximum number of individuals that could have been chosen from each population was curtailed by material availability. Rarely more than five complete individuals were present on any one herbarium specimen. In this situation, increasing the sample size would have undoubtedly decreased the number of populations that could have been surveyed.

The populations finally selected for use in the following numerical analyses were chosen because they encompassed the geographic range

Printed in Canada / Imprimé au Canada

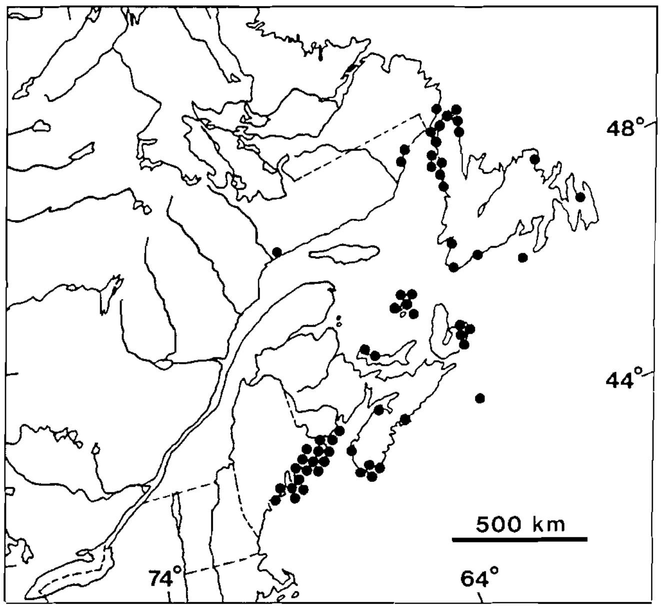

FIG. 1. Distribution of the 59 populations of *E. randii* used in the numerical analyses.

TABLE 1. Morphological characters used in multivariate analyses of *E. randii*

1. Stem height (mm)
2. Stem habit
    1, simple
    2, sparingly branched (usually only 1 pair)
    3, much branched
3. Node at which flowering begins
4. No. of pairs of leaves (or branches)
5. First-flowering bract length (mm)
6. Leaf length (mm)
7. No. of teeth on bracts and (or) leaves
8. Bract and leaf pubescence
    1, glabrous
    2, sparingly pubescent
    3, moderately pubescent
    4, densely pubescent
9. Corolla length (mm)
10. Corolla colour
    1, white with purple veins
    2, purple throughout
11. Calyx tooth length (mm)
12. Calyx tooth apex
    1, acute or rarely obtuse
    2, narrowly acuminate
13. Capsule length (mm)

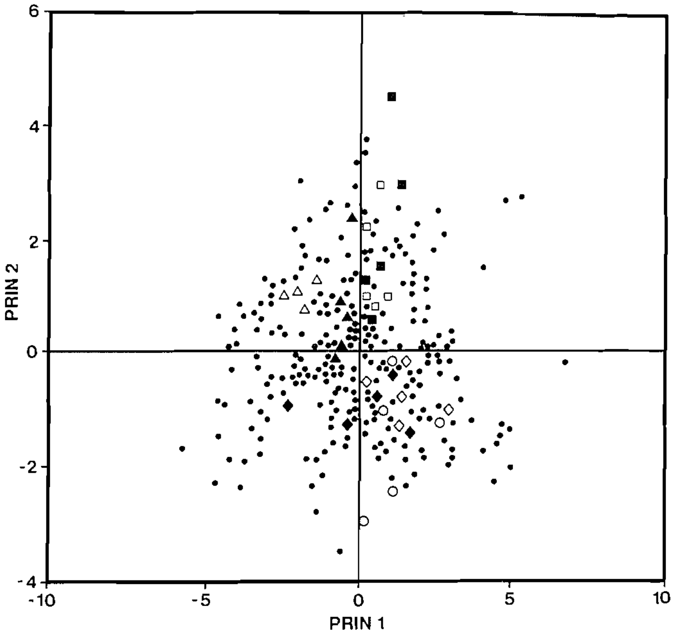

FIG. 2. Ordination of *E. randii* OTUs generated by plotting the first two principal components (not all OTUs are shown). Symbols represent the following type material: *E. randii* lectotype (◇) and isolectotype (◆); *E. randii* forma *albiflora* holotype (■) and isotype (□); *E. randii* forma *candida* holotype (○); *E. randii* var. *farlowii* holotype (△); *E. randii forma iodantha* holotype (▲). PRIN, principal component axis.

and were perceived to reflect the morphological variability exhibited by the species. Additionally, many of the herbarium specimens represented plants that were collected and described by Fernald and Wiegand (1915) and (or) annotated as either var. *randii* or var. *farlowii* by Sell and Yeo (1970). Type material, including all the previously recognized varieties and forms of *E. randii*, was also included in the numerical analyses. In all, 59 populations (Fig. 1), representing 291 individuals (operational taxonomic units or OTUs) were scored or measured for 13 characters (Table 1). The characters were selected on the basis of literature reports of distinguishing characters within the complex (Fernald and Wiegand 1915; Fernald 1950; Sell and Yeo 1970) and others that we thought might be taxonomically significant.

Since some of the methods employed in this investigation require the assumption that the data have multivariate normal distributions, the data matrix was inspected using the S199 data inspection program (Agriculture Canada 1981). Of the quantitative characters, all had normal or near-normal distributions. An analysis of variance (ANOVA) was performed using individuals as OTUs and populations as groups to select continuous characters that would be useful to differentiate populations. The higher values of these F statistics represent greater between-group than within-group variability and therefore indicate the most significant discriminating characters. All nine continuous characters, each having a probability of $F > 0.0001$, were retained in the data set. The means, ranges, variances, and F-statistics obtained for these quantitative characters are presented in Table 2.

The OTU by OTU similarity assessment was made using Gower's general coefficient of similarity (Gower 1971), which assesses two-state, multistate (ordered and qualitative), and quantitative characters simultaneously. To explore patterns of variation among the OTUs, principal components analysis (PCA) was done on standardized data, and the components extracted from the resulting correlation matrix. The data matrix was also subjected to four agglomerative clustering procedures: single linkage, complete linkage, unweighted pair-group method using arithmetic averages (UPGMA), and flexible sorting (with $\alpha = 0.625$ and $\beta = -0.25$). The four phenograms were produced using the CLUSTRIT cluster analysis program (Lefkovitch 1981). ANOVA and PCA were obtained using the procedures ANOVA and PRINCOMP, respectively (SAS Institute Inc. 1985). The computational work was done on an Amdahl 370 mainframe computer at the University of Ottawa. The data matrix is available upon request.

Results

Principal components analysis

Of the 13 principal component axes that accounted for the total variance of the data, the first three accounted for 56.2% (33.9, 13.6, and 8.7%, respectively). The remaining 10 component axes each accounted for 7.5% or less of the remaining variance. The projection of OTUs, including type material, onto the first two component axes is illustrated in Fig. 2. The five characters contributing most to the total variance in principal component axis 1 are, in decreasing order of importance, bract length (character No. 5), cauline leaf length (No. 6), capsule length (No. 13), calyx tooth length (No. 11), and stem height (No. 1). The characters contributing greatly to the ordination of OTUs in principal component axis 2 are, also in descending order of importance, the number of pairs of leaves or branches (No. 4), first flowering node (No. 3), and habit

TABLE 2. Table of continuous characters showing mean, range, variance, and *F*-statistics

Character	Mean	Range	Variance	*F*
1. Stem height (mm)	91.7	5–400	2663.81	28.16
3. First flowering node	5.0	1–15	5.26	3.31
4. Leaf or branch pairs	12.2	4–27	18.27	10.45
5. Bract length (mm)	5.8	1.8–11.5	3.62	11.01
6. Leaf length (mm)	5.9	1.2–14.0	4.22	8.28
7. No. of teeth	7.4	3–13	1.90	4.44
9. Corolla length (mm)	3.5	1.8–6.5	0.59	9.45
11. Calyx tooth length (mm)	1.2	0.4–2.3	0.17	10.42
13. Capsule length (mm)	3.4	1.8–6.2	0.58	6.34

NOTE: Characters are numbered as in Table 1.

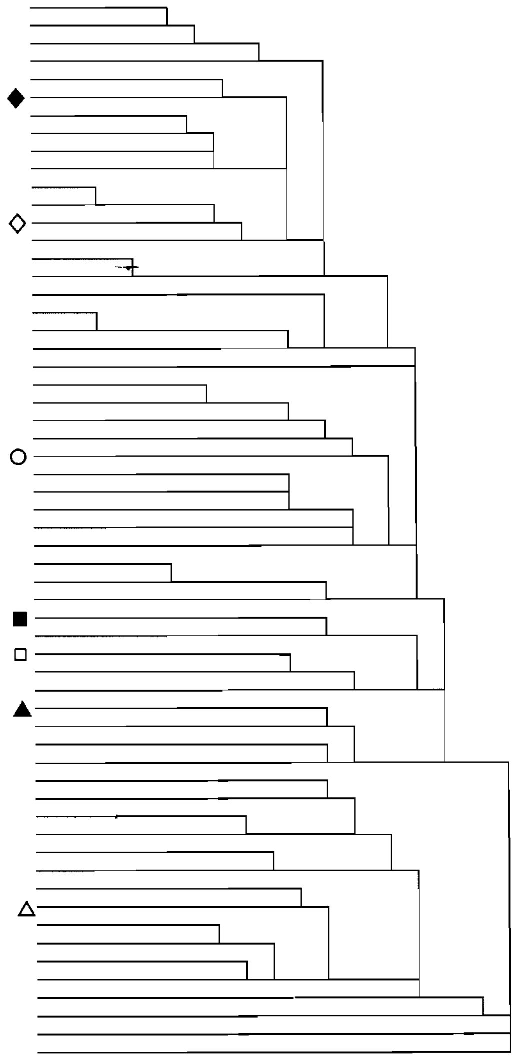

FIG. 3. Phenogram derived from agglomerative clustering with UPGMA of 59 OTUs of *E. randii*. Symbols represent the following type material: *E. randii* lectotype (◇) and isolectotype (◆); *E. randii* forma *albiflora* holotype (■) and isotope (□); *E. randii* forma *candida* holotype (○); *E. randii* var. *farlowii* holotype (△); *E. randii* forma *iodantha* holotype (▲).

(No. 2). Only one character, corolla colour (No. 10), contributed significantly to the total variance in principal component axis 3.

A continuum of morphological variation is exhibited along principal component axis 1 and reflects character size. OTUs with high negative loadings represent small individuals possessing small bracts, leaves, capsules, and calyx teeth. Conversely, OTUs with high positive loadings possess greater values with respect to these same characters. OTUs with a high positive loading on principal component axis 2 included plants with many pairs of leaves, a high first flowering node, and a much-branched habit. The OTUs having high negative loadings were plants bearing simple stems with few pairs of leaves and a low first flowering node. No separation of OTUs was achieved on any axis of the PCA between those populations previously designated by Sell and Yeo (1970) as var. *farlowii* or as var. *randii*. Much variation was found among individuals within any one population, and considerable overlap was apparent among individuals from different populations (e.g., material representing typical *E. randii* overlaps with that of *E. purpurea* forma *candida*).

Because preliminary analyses using individuals as OTUs yielded somewhat confusing results (perhaps because of the high within-population variability of many characters), character means were determined for each population, and the populations were treated as OTUs for further analyses. After subjecting this new data set to a PCA, still no separation of any group of OTUs was achieved. In this case, the first three components accounted for 64.8% of the total variation of the data. With the exception of principal component axis 3, in which both corolla colour and flowering node had two of the highest absolute eigenvector values, the characters contributing most to the total variance on each of the first three component axes were the same as in the first analysis.

Cluster analysis

To further explore any phenetic relationship among the OTUs, several agglomerative clustering procedures were chosen. Since there was little difference between the two previous PCAs, the character means for each population were treated as OTUs to produce a smaller phenogram. No meaningful discernible clusters of OTUs were apparent upon the examination of the phenograms produced by single linkage, complete linkage, and UPGMA clustering. For illustrative purposes, the phenograms derived from UPGMA clustering is shown in Fig. 3. The phenogram produced using the flexible-sort clustering procedure revealed four major clusters of OTUs (Fig. 4). It is known that flexible sorting can structure the OTUs into a smaller number of major divisions than the other three clustering procedures, with the possibility that residual clusters can be formed that may not be at all reflective of good groups (McNeill 1975). We expect that this is the case here. The OTUs in each of these four major clusters were delimited by the flexible-sort algorithm depending upon their corolla colour, the

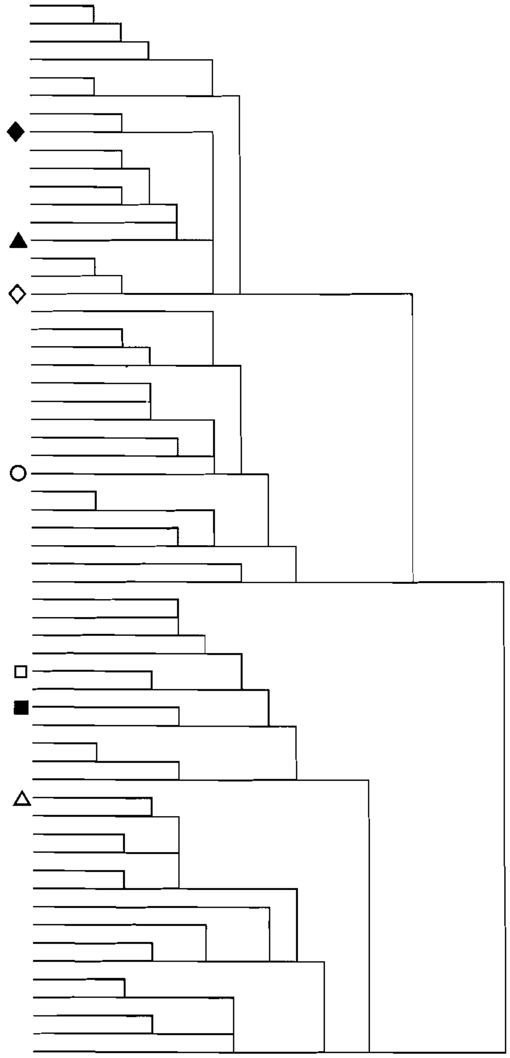

FIG. 4. Phenogram derived by the flexible-sorting method (α = 0.625 and β = −0.25) of 59 OTUs of *E. randii*. Symbols represent the following type material: *E. randii* lectotype (◇) and isolectotype (◆); *E. randii* forma *albiflora* holotype (■) and isotype (□); *E. randii* forma *candida* holotype (○); *E. randii* var. *farlowii* holotype (△); *E. randii* forma *iodantha* holotype (▲).

position of the first flowering node, and similarities in character size. The recognition of these clusters as representing good groups is spurious in the light of the PCA and other clustering results. Most importantly, however, is that the type material representing var. *farlowii* and its forma *iodantha* did not cluster together, the latter taxon clustering with typical *E. randii*. In fact, all four clustering procedures failed to separate among Sell and Yeo's (1970) previously recognized specimens of var. *farlowii* and var. *randii*.

Discussion

Karlsson (1976) described the present species concept in *Euphrasia* as quite possibly being erroneous. Characters previously regarded as being important in delimiting North American *Euphrasia* (cf. Sell and Yeo 1970) are correlated and appear to form adaptive complexes dependent on habitat and climatic factors. For example, in Sweden, *Euphrasia* branch number is largely dependent on the supply of nutrients to the plant (Karlsson 1984); the number of internodes to the lowest flower is strongly correlated with the length of the vegetation period (Karlsson 1976), and the length of the internodes is dependent on the height of the surrounding vegetation (Karlsson 1976; our own observations for North American plants). In *Euphrasia* there is often great variation within populations, and adjacent populations can often differ appreciably (Downie *et al.* 1988), with many local and regional races and infraspecific taxa being described.

Our results do not show, nor can we justify, the recognition of discernible groups within the data set. In *E. randii*, patterns of morphological variation appear to be of one continuous gradation. Fernald (1950) called attention to the nature of the variability within *E. randii* when he noted that it is "Our most variable species. . ." *Euphrasia randii* var. *farlowii* was previously characterized by its small size, much-branched habit, small, densely pubescent leaves, and minute flowers (Sell and Yeo 1970). Within *E. randii*, characters such as leaf and bract size, stem height and habit, and vestiture were found to be highly plastic and extremely variable. Vegetative organs and plant vestiture are considered phenotypically more plastic than floral organs (Stebbins 1950; Bradshaw 1965). Davis and Heywood (1963) described good characters as not being subject to wide variation nor being easily susceptible to environmental modification. The variation found in many characters of *E. randii* is undoubtedly of this nature and has confounded the taxonomy of this species in the past.

Taxonomy

Euphrasia randii B.L. Robinson, Rhodora, 3: 273. 1901
Euphrasia purpurea var. *randii* (B.L. Robinson) Fern. & Wieg., Rhodora, 17: 188. 1915
TYPE: Maine, Mount Desert Island, "Great Cranberry Isle, 17 July, 1897, E.L. Rand" (LECTOTYPE: GH!; ISOLECTOTYPES: GH!)
Euphrasia purpurea Reeks, List Fl. Pl. Ferns Nfld. 4. 1873. non *E. purpurea* Desf., Fl. Atlant. 2: 36. 1798
Euphrasia randii var. *reeksii* Fern., Rhodora, 45: 112. 1943
TYPE: Newfoundland, Cow Head, bog back of the strand, July 22, 1910. Fernald & Wiegand 3985 (LECTOTYPE: CGE)
Euphrasia purpurea forma *albiflora* Fern. & Wieg., Rhodora, 17: 188. 1915
Euphrasia randii forma *albiflora* (Fern. & Wieg.) Fern., Rhodora, 45: 112. 1943
TYPE: "Maine: turfy crests, Elwell Point, South Thomaston, August 15, 1913, Bissell, Fernald & Chamberlain (Fernald, No. 10 404)" (HOLOTYPE: GH!; ISOTYPE: GH!)
Euphrasia purpurea forma *candida* Fern. & Wieg., Rhodora, 17: 187. 1915
Euphrasia randii forma *candida* (Fern. & Wieg.) Fern., Rhodora, 45: 112. 1943
TYPE: Quebec, "Magdalen Islands: knolls at border of brackish marsh, East Cape, Coffin Island, July 19, 1912, Fernald, Bartram, Long & St. John, No. 8018" (HOLOTYPE: GH!)
Euphrasia randii var. *farlowii* B.L. Robinson, Rhodora, 3: 274. 1901
Euphrasia purpurea var. farlowii (B.L. Robinson) Fern. & Wieg., Rhodora, 17: 189. 1915

TYPE: "Dog Island, Eastport, Maine, September, 1877, W.G. Farlow" (HOLOTYPE: GH!)

Euphrasia purpurea forma *iodantha* Fern. & Wieg., Rhodora, 17: 189. 1915

Euphrasia randii forma *iodantha* (Fern. & Wieg.) Fern., Rhodora, 45: 112. 1943

TYPE: "Maine: Matinicus Island, August 22, 1905, A.H. Norton" (HOLOTYPE: GH!)

Stem simple to divergently much branched from near the base, up to 4 dm with 4–27 pairs of ascending branches or leaves gradually decreasing in size upwards. Inflorescence elongate at maturity, lower bract pairs widely separated, the mature central spike one-half to three-fourths full height of plant, flowering from 1st to 15th node. Cauline leaves 1.2–14 mm long; ovate–oblong to nearly orbicular with 3–9 (rarely 13) rounded or obtuse teeth, cauline internodes 1–3 (rarely 5) times as long as the subtending leaves, shorter upwards. Bracts similar to the cauline leaves but smaller, 1.8–11.5 mm long, floral internodes 0.5–2 times as long as the subtending bracts, cauline leaves and bracts glabrous or sparingly crisp-pubescent to densely white pubescent. Calyx lobes 0.4–2.3 mm long, narrowly acuminate or rarely acute or obtuse. Corolla 1.8–5 (rarely to 6.6) mm long, deep purple to white with purple veins and usually with a yellow throat, upper lip very shallowly bilobed. Capsule 1.8–6.2 mm long, approximately equalling or slightly longer than the calyx lobes.

DISTRIBUTION: Coastal regions from southern Labrador to Maine.

REPRESENTATIVE SPECIMENS: CANADA: NEW BRUNSWICK: Charlotte Co., White Island *Hinds 7017* (DAO), Kent Island *Hodgdon 18814* (NHA), South Wolf Island *Hodgdon & Pike 20218* (NHA), Grand Manan Island *Knowlton & Weatherby s.n.* (DAO). NEWFOUNDLAND: Table Mountain *Fernald & Weigand 4002* (GH), St. Anthony, Cremaillere Bay *Savile 2378* (DAO), Conception Bay, Kelligrew's *Fernald & Weigand 6164* (GH), Stephenville Crossing *Fernald & Wiegand 3991* (GH), St. John Island *Fernald, Wiegand, Long, Gilbert, Jr.* and *Hotchkiss 28978* (GH), Notre Dame Bay, New World Island *Fernald & Wiegand 6163* (GH), St. Margaret Bay, Dog Peninsula *Fernald, Wiegand, Long, Gilbert, Jr. & Hotchkiss 28988* (GH), Pistolet Bay, Cape Norman *Weigand & Long 29001* (GH). NOVA SCOTIA: Cape Breton Co., Scatari Island *Smith, Schofield, Sampson & Bent 5309* (DAO); Halifax Co., Lawrencetown *Roland 38078* (DAO); Kings Co., Cape Split *Schofield & Webster 5881* (DAO); Sable Island, Main Station *Catling & Freedman 4021* (DAO); Yarmouth Co., Cape Forchu *Bassett & Spicer 4670* (DAO). PRINCE EDWARD ISLAND: Prince Co., Campbell's Pond, Malpeque, *Fernald & St. John 11179* (DAO, GH), Cape Aylesbury, *Fernald, Long & St. John 8022* (DAO, GH). QUEBEC: Gaspé Co., Bonaventure Island *Fernald & Collins 1167* (DAO); Magdalen Islands, Grindstone Island *Fernald, Long & St. John 8020* (GH), Ile Grande Entrée *Brisson 5504* (DAO); Saguenay Co., Sept Iles *Robinson 943* (GH), Iles Nétagamiou *St. John 90712* (GH). ILE ST. PIERRE: Savoyard *Le Hors s.n.* (DAO). U.S.A.: MAINE: Hancock Co., Winter Harbor *Stebbins 387* (DAO); Knox Co., Elwell Point, S. Thomaston *Bissell, Fernald & Chamberlain 10404* (DAO); Washington Co., Cutler *Fernald 272* (DAO), Eastport, Dog Island *Fernald & Wiegand 271* (DAO).

Acknowledgements

This study was supported by a Natural Sciences and Engineering Research Council of Canada operating grant to J.M. Deborah Katz-Downie and Andrée Quintin are thanked for assistance in the field. We thank the curators of the various herbaria for the loans used in this study.

Agriculture Canada. 1981. Data inspection program. Statistical Research Station, Engineering and Statistical Research Institute, Research Branch, Agriculture Canada, Ottawa.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. **13**: 115–155.

Davis, P., and Heywood, V. H. 1965. Principles of angiosperm taxonomy. Oliver & Boyd, Edinburgh.

Downie, S. R., Quintin, A., and McNeill, J. 1988. Le statut des *Euphrasia borealis, E. nemorosa* et *E. stricta* dans l'est de l'Amérique du Nord : une analyse numérique. Can. J. Bot. **66**: 2208–2216.

Fernald, M. L. 1943. Two later homonyms. Rhodora, **45**: 111–112.

——— 1950. *Euphrasia. In* Gray's manual of botany. 8th ed. Van Nostrand Reinhold Co., New York.

Fernald, M. L., and Wiegand, K. M. 1915. The genus *Euphrasia* in North America. Rhodora, **17**: 181–201.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics, **27**: 857–874.

Holmgren, P. K., Keuken, W., and Schofield, E. K. 1981. Index herbariorum. Part 1. The herbaria of the world. Regnum Veg. **106**: 1–452.

Karlsson, T. 1976. *Euphrasia* in Sweden: hybridization, parallelism, and species concept. Bot. Not. **129**: 49–60.

——— 1984. Early-flowering taxa of *Euphrasia* (Scrophulariaceae) on Gotland, Sweden. Nord. J. Bot. **4**: 303–326.

Lefkovitch, L. P. 1981. CLUSTRIT (S075). A cluster analysis package. Statistical Research Station. Engineering and Statistical Research Institute, Research Branch, Agriculture Canada, Ottawa.

McNeill, J. 1975. A generic revision of Portulacaceae tribe Montieae using techniques of numerical taxonomy. Can. J. Bot. **53**: 789–809.

Robinson, B. L. 1901. The North American *Euphrasia*. Rhodora, **3**: 270–276.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 edition. SAS Institute Inc., Cary, NC.

Sell, P. H., and Yeo, P. F. 1970. A revision of the North American species of *Euphrasia* L. (Scrophulariaceae). Bot. J. Linn. Soc. **63**: 189–234.

Stebbins, G. L. 1950. Evolution and variation in plants. Columbia University Press, New York.