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ANOTHER APPROACH TO LEAF SHAPE COMPARISONS

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Summary

Methods for shape comparisons among groups originally developed in zoological studies are found to be useful in examining variation in leaf shape. These methods (sampling outlines by means of truss networks connecting landmarks or pseudolandmarks, and sheared principal components analysis) take advantage of electronic methods of data capture and multivariate data analysis. They have the desirable properties of discriminating between shape variation and variation in size, and of permitting their results to be related directly to the original measurements from which they were obtained. The utility of these methods is demonstrated with respect to a search for possible leaf shape intermediacy in putative *Crataegus* hybrids, and an examination of geographic variation in the cross-sectional shape of *Larix laricina* needles.

Introduction

The title of this paper suggests the question, *why* should plant taxonomists consider yet another method for incorporating leaf shape data into their studies of group differentiation? This question is answered in what follows, by first identifying some of the reasons for an interest in shape data, then pointing out some limitations of the methods currently used to document variation in shape, and finally illustrating a method which overcomes these limitations.

Taxonomic interest in shape comparisons stems from observations that while variation in the size of plant structures often reflects variation primarily in environmental conditions, variation in shape tends to be more independent of the environment, and more heritable. Because of their availability and the wide range of morphological variation that leaves exhibit, leaf form has been a rich source of sytematic data for as long as plants have been classified (Theophrastus, 1916; Linnaeus, 1751). Leaf shape variation occurs at every hierarchical level: within and between individuals, populations, and taxa. In some taxa contrasting environmental conditions during development can induce marked phenotypic differences in leaf shape (heterophylly), e.g. between the leaves of emergent and submerged shoots of *Ranunculus flabellaris* (Bostrack and Millington, 1962). Leaf shape variation within individuals may also occur regardless of environmental conditions, as part of the normal developmental pattern, notably among sequential leaf positions on a stem (leaf heteroblasty, Greyson et al., 1982; e.g. *Gossypium,* Hammond, 1941, Stephens, 1945; *Nicotiana,* Paxman, 1956). Similar differences in leaf shape within and between shoot types are known in numerous but less well studied genera such as *Ulmus* (Melville, 1937), *Betula* (Dancik and Barnes, 1974), *Crataegus* (Dickinson and Phipps, 1984) and *Larix* (Gathy, 1954).

Once sources of within-individual variation in leaf shape have been accounted for, other patterns of variation may be revealed. It may be possible to meaningfully quantify and compare within-individual variability in leaf shape in relation to the genetic control of

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morphogenesis and the degree of heterozygosity (Paxman, 1956). Having controlled for heteroblastic development, Melville (1937, 1960) examined leaf shape in a number of genera, comparing hybrids and their known or supposed parents. Similar studies have been carried out on *Crataegus* taxa in Europe by Gostyńska-Jakuszewska (1975) and in North America by Wells (1985; similar studies of other *Crataegus* hybrids by Love and Feigen (1978) and Christensen (1982, 1984) did not control for short shoot leaf heteroblasty).

Leaf variation has been studied within species complexes of diverse genera by Dancik and Barnes (1975), West and Noble (1984) and Dickinson (1983, 1986; Dickinson and Phipps, 1984), among species within genera (Marshall, 1978; Parker et al., 1979, 1981; El-Gazzar, 1980; Phillips, 1983; Phipps, 1983; Parker and Maze, 1984), and in taxonomically diverse collections (Hill, 1980).

Most of these studies have used shape descriptions derived more or less directly from the existing shape terminology which, in turn, derives more from the human propensity to *recognize* shape, rather than analyze it (Bookstein, 1978). Thus (leaf) shape has been described largely by reference to well-known plants (e.g. apple, pear; Theophrastus, 1916) or to human artifacts or organs (e.g. awl-shaped, fiddle-shaped, trowel-shaped, heart-shaped, etc.; Linnaeus, 1751; Stearn, 1969, 1983). The use of most of these terms has recently been standardized, by reference to length-width ratio classes and the relative position of the widest point (Systematics Association Committee for descriptive Biological Terminology (SACBT) 1962; Stearn 1969). Quantitative studies of leaf shape variation have thus usually employed merely the same dimensions or similar ones, either as the original measurements (Melville, 1937, 1960; Stephens, 1945; Greyson et al., 1982; Dancik and Barnes, 1975; Marshall, 1978; Phillips, 1983; Dickinson and Phipps, 1984; Parker et al., 1981; Parker and Maze, 1984) or else combined as ratios that are treated as continuous variables (Bradshaw, 1953, 1971; Paxman, 1956; Byatt, 1975, 1976; El-Gazzar, 1980; Christensen, 1982, 1984), although in some cases both measurements and ratios are used (Gostyńska-Jakuszewska, 1975; Parker et al., 1979; Hill, 1980; Phipps, 1983; West and Noble, 1984).

The difficulty with most of these approaches is that, with fewer dimensions measured (leaf width, and length above and below the widest point; Stephens, 1945; Greyson et al., 1982; Marshall, 1978; Phillips, 1983; Dickinson and Phipps, 1984), leaf shape is greatly oversimplified (Fig. 1). Moreover, ratios of leaf dimensions in the past have been assumed to provide a summary of shape relationships that is independent of size differences. Atchley et al. (1976) have shown, however, that ratio variables may be highly correlated with size variables. In addition, the distribution of ratio variables may be such to vitiate their statistical, and even biological analysis (Atchley et al., 1976). Dickinson (1983) found that using log-transformed ratios of leaf dimensions as descriptors in canonical variates analyses resulted in a loss of discrimination, when compared with analyses using the original measurements. Phillips (1983) has examined the use of ratios in describing leaf shape variation in *Parnassia* species, and suggests employing more valid regression-based alternatives.

Since traditional shape descriptors often represent a subset of larger multivariate data sets, even exploratory studies may benefit from electronic data collecting methods and multivariate analysis. For example, the study reported by West and Noble (1984) used digitized leaf outlines to obtain a large number of leaf descriptors (leaf area and dimensions, their ratios, and angles) for use in subsequent cluster and discriminant analyses. An alternative method based on digitized outlines is the description of their shape in terms of the coefficients of Fourier transforms (Kincaid and Schneider, 1983; Rohlf and Archie, 1984; Ferson et al., 1985). Criticisms of this approach have been advanced by Bookstein et al. (1982) and replied to by Ehrlich et al. (1983). The criticisms include the difficulty of interpreting Fourier coefficients biologically. Kincaid and Schneider (1983) suggest, however, that Fourier coefficients are natural descriptors of shape that are relevant to biophysical processes such as heat transfer, and Ehrlich et al. (1983) provide an interpretation of the results of Fourier analyses in terms of a number of historical and environmental charac-

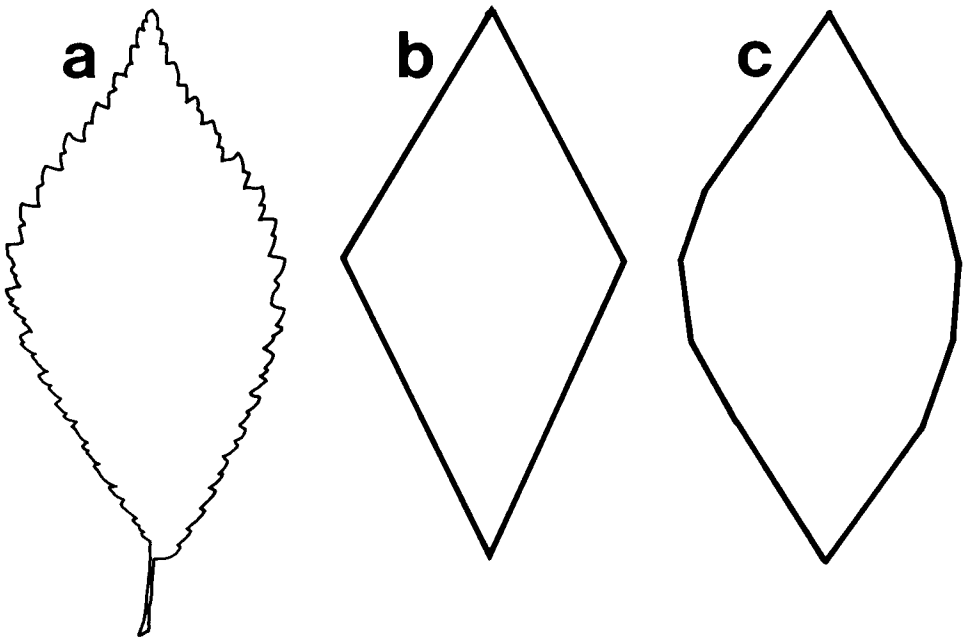

Fig. 1. Simplification of leaf shape: (a) outline of a leaf of *Ulmus nitens* (redrawn from Melville, 1937); (b) the same as (a) but described only in terms of the maximum width, and lengths above and below the widest point; (c) the same as (a) but described with reference to 12 landmarks (and pseudolandmarks) located on the margin by the method described in Fig. 2 but not shown here.

teristics of their sample of Pleistocene planktonic foraminifers. Rohlf and Archie (1984) point out that uninterpretability is neither unique to Fourier analysis nor necessarily a disadvantage. They suggest that detailed interpretations of complex summaries such as Fourier coefficients could be meaningless and in any case are unnecessary, if interest centers on describing contrasts in overall shape.

In the study reported here, however, we have chosen instead to examine the behavior of methods for studying shape variation that have been suggested by Humphries et al. (1981) and by Strauss and Bookstein (1982; Bookstein et al., 1985). Shape can be defined—and distinguished from size—in terms of the covariances of multiple linear measurements of distances between corresponding points made on a sample of objects. *Shape* is the aspect of the spatial form of the sample described by covariances among these measurements that are both positive and negative (some dimensions tend to be bigger while others are smaller). *Size* is the aspect described by covariances all of which have the same sign (all dimensions are larger in large objects than they are in small ones). In the case of leaves, size will be highly correlated with surface area. Shape is characterized as a configuration of landmarks (and pseudolandmarks; see below and Figs. 2, 3). Landmarks (K, Fig. 2; A–E and G, Fig. 3) are defined by features of the structure at the point where they are located (Bookstein et al., 1985). Alternatively, *pseudo*landmarks (A–J, Fig. 2; F and H, Fig. 3) may be defined by specifying their position on the structure in relation to each other and any landmarks present, according to a set of consistent rules (Figs. 2, 3). This represents relationship in terms of topographic homology (correspondence in relative position and composition; Jardine, 1969). Shape is described by measuring the distances between these points (Bookstein, 1978). Variation in a multi-group sample with respect to these measurements (log-transformed; see Bookstein et al., 1985, for a detailed rationale) is partitioned into inde-

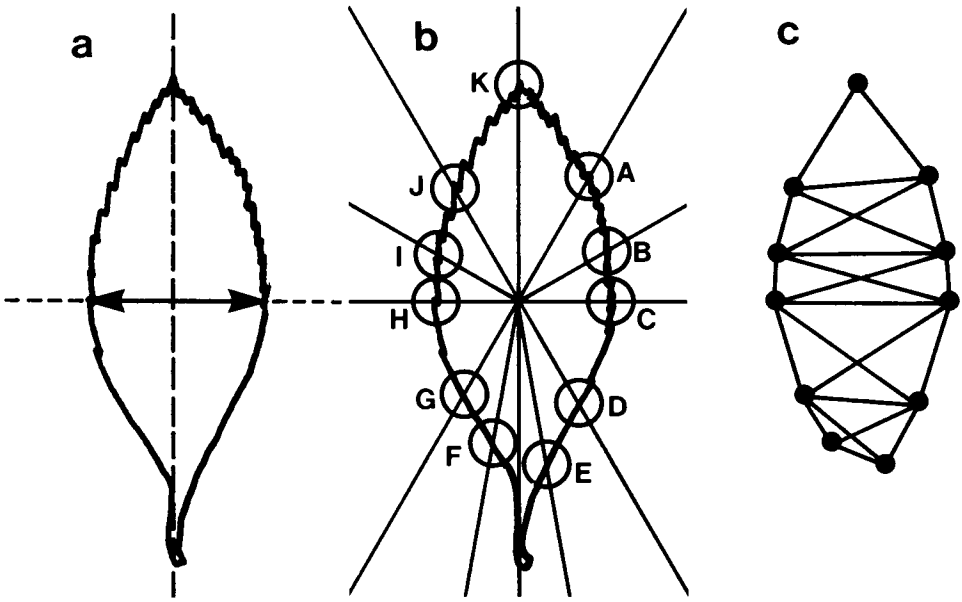

Fig. 2. Leaf of *Crataegus crus-galli* s. str. in outline, showing (a) the location of the widest point in relation to the longitudinal axis of the leaf, (b) the ray diagram as superimposed on (a) and used to sample the outline (pseudolandmarks A–J plus landmark K) and (c) the network of truss dimensions connecting A–K and employed in the multivariate analyses. In (b), the ray diagram is positioned so that CH spans the widest part of the leaf while its perpendicular passes through the leaf tip (K) and the leaf base. CH is the reference dimension used in orienting individual leaf outlines prior to finding average landmark (x, y) coordinates for each OTU. In (c) the quadrilaterals ABIJ, BCHI, CDGH and DEFG together with their enclosed diagonals are the box trusses referred to in the text.

pendent size and shape factors by means of principal components analysis (PCA). The shape factors are obtained in such a way as to be independent of between-group size differences. These factors have the further advantage that scores on them are interpretable in terms of their correlation with the original measurements, so that they can detect highly localized changes in form. In this respect they differ markedly from the way in which such local changes may be spread over a large number of Fourier coefficients. While the landmarks on which this method is based may be difficult to locate on a smooth, relatively featureless outline (that is readily described by Fourier coefficients, however), the use of pseudolandmarks as described elsewhere (Figs. 2, 3) can overcome this drawback. Consequences of the use of pseudolandmarks are discussed below.

These methods are illustrated using two data sets. The first comes from a study collection of hawthorn (*Crataegus* L., Rosaceae: Maloideae) leaves (Dickinson and Phipps, 1984; Dickinson, 1986) representing five taxa, four belonging to *C. crus-galli* L. sensu lato, plus *C. punctata* Jacq. Three of the *crus-galli* taxa could represent *crus-galli* (sensu stricto) × *punctata* hybrids (Dickinson, 1983, 1985; Dickinson and Phipps, 1985); this hypothesis is examined here by looking for leaf shape intermediacy in the putative hybrids. The second data set comes from a range-wide study of phenotypic variation in tamarack (*Larix laricina* (Du Roi) K. Koch, Pinaceae), and represents samples from nine stands in the eastern portion of the range. We are interested to know whether or not needle shape and other cross-sectional characteristics vary over this large geographic area in a manner that demonstrates population differentiation and that might be correlated with environmental or historical factors.

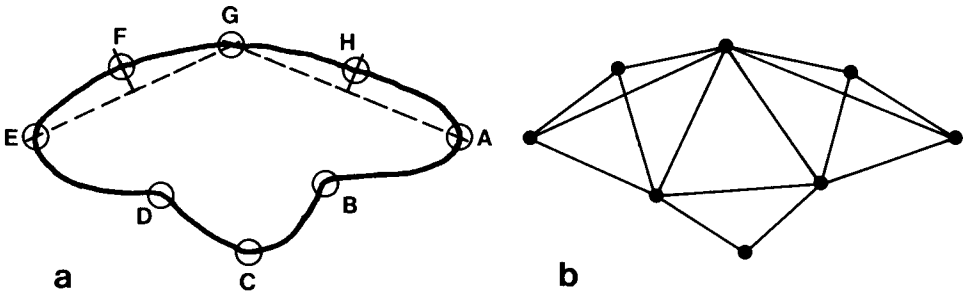

Fig. 3. Needle of *Larix laricina* in transverse section showing (a) the landmarks used (A–H), and (b) the network of truss dimensions connecting them, employed in the multivariate analysis. Landmarks A and E are lateral extremes of the outline in the direction approximately perpendicular to CG; C and G are the ad- and abaxial extremes of the outline. Landmarks B and D are inflection points on the outline associated with rows of stomata. F and H are pseudolandmarks located at the intersection of the outline and the perpendicular bisectors of EG and AG, respectively. In (b) BD is the reference dimension used in orienting individual needle outlines prior to finding average landmark (x, y) coordinates for each OTU. In (b) the quadrilaterals ABGH and DEFG, together with the enclosed diagonals, are the box trusses referred to in the text.

Materials and Methods

Sampling.—Operational taxonomic units (OTUs) are individual hawthorn or tamarack trees. *Crataegus* OTUs (N = 176) were sampled randomly at eight sites in southern Ontario (Dickinson, 1983, 1986) and may be assigned to the following five taxa: *C. crus-galli* s. str., *C. fontanesiana* (Spach) Steud., *C. ?disperma* Ashe, *C. ?grandis* Ashe, and *C. punctata,* as described elsewhere (Phipps and Muniyamma, 1980; Dickinson and Phipps, 1985). Each OTU in the study is represented by the fully expanded leaves of six to eight of its short shoots, collected as described earlier (Dickinson and Phipps, 1984) so as to retain information about the sequential position along the stem of each leaf; in order to control for variation in leaf shape due to short shoot leaf heteroblasty, data for each *Crataegus* OTU was collected only from short shoot subterminal leaves (Gostyńska-Jakuszewska, 1975; Dickinson, 1986).

The sites in the province of Ontario, Canada, at which *Larix laricina* was collected (KEN, NOB, SEV, TBY) are part of the sampling scheme for a larger study of the genecology of tamarack in northern Ontario. Other collections from the provinces of Nova Scotia (BRI) and Quebec (JOV), and the states of Maine (SPR), New York (KDY), and Pennsylvania (TVL), U.S.A., were made either by local collaborators (see acknowledgments) or by one of the authors (TAD). At each site trees were chosen so as to be separated by a minimum of one to two average tree heights, taking care to minimize any bias in the selection process. Between 14 and 22 OTUs were sampled at each site (N = 156). Herbarium vouchers (mid-crown outer branches, cone-bearing if possible) were collected from each OTU; these are held in the School of Forestry, Lakehead University, with vouchers for each site deposited in the Claude E. Garton Herbarium (LKHD).

The needles of most conifers exhibit relatively few gross morphological features for use in numerical studies. In contrast, their composition and shape in transverse section provide a large number of features, variation in which may be of considerable systematic interest (Parker and Maze, 1984). As the genus *Larix* Mill. is characterized by dimorphic shoots and winter-deciduous leaves, sampling for our study has been restricted to the leaves (needles) of short shoots on the previous year's leader increment. This is advantageous since early in the growing season *Larix* short shoots complete the expansion of a large number of exclusively pre-formed leaves. Shoot morphogenesis in *Larix* long shoots con-

tinues over a longer period and usually involves expansion of both pre- and neoformed leaves that differ in size, shape, and some anatomical features (Gathy, 1954; Owens and Molder, 1979; Macdonald et al., unpubl.).

Data collection and summarization.—Data were collected from hawthorn leaves mounted on sheets of heavy paper, as described below. Data for tamarack leaves were obtained from transverse sections, prepared as follows. For each OTU, ten attached short shoot needles were removed from the herbarium vouchers. The central third of each one was rehydrated by boiling in distilled water for 15 minutes, then dehydrated using an ethanol tertiary butanol series, and infiltrated with embedding medium. Sections of five needles were cut at 7 μm and stained with Johansen's safranin and Fast Green FCF (Clark, 1973).

The outlines of hawthorn leaves and tamarack needle sections were recorded as (x, y) coordinates for each of eleven (*Crataegus*; Fig. 2) or eight (*Larix*; Fig. 3) landmarks and pseudolandmarks, using a digitizing tablet (Houston Instruments) attached to an Apple IIe microcomputer. Digitization of the needle outlines was done using a microscope equipped with a drawing tube to superimpose their image on the digitizing tablet.

Data in the form of sets of (x, y) coordinates for several outlines were summarized, for each OTU, as follows. The array of (x, y) coordinates specifying each outline was first rotated to a common orientation by multiplying it by a matrix of sines and cosines of the angle between an arbitrary reference vector and the line connecting a pair of landmarks in each outline (Figs. 2, 3; Green and Carroll, 1976, pp. 94, 114–115). Next the centroid of each rotated outline was found and its (x, y) coordinates subtracted from those of each landmark, thus shifting each rotated outline to a common centroid at the origin of the coordinate system (0, 0). The (x, y) coordinates for each landmark were then averaged over all the outlines available for each OTU, so as to obtain a mean outline (Melville, 1937; Rohlf and Archie, 1984). The analyses described below were carried out on the $\log_{10}$-transformed inter-landmark distances (Figs. 2, 3), calculated from the mean (x, y) coordinates for each OTU. These distances make up the network of box trusses suggested by Strauss and Bookstein (1982) that samples outline shape redundantly, in more directions than are implied by traditional shape descriptions (Figs. 1–3).

Data analysis.—Most of the analyses which follow, as well as the data summarization described above, were carried out using the functions and macros provided by the data anlaysis and graphics software package S (Becker and Chambers, 1984), running on the Lakehead University Computing Centre VAX 11/750 installation. The remaining data manipulations plus some ANOVAs and outline plotting were done on Apple IIe microcomputers, using special-purpose programs written by WHP or TAD.

Sheared PCA (S-PCA) is a method for extracting from multiple distance measurements factors (shape components) which discriminate a priori groups with respect to shape independent of differences in size (Humphries et al., 1981). Recognition of groups may be based on prior analyses (cluster analyses, ordinations) or other criteria. In the *Crataegus* example five groups are recognized: the supposed parental taxa *C. crus-galli* s. str. and *C. punctata,* and the putative hybrids *C. fontanesiana, C. ?disperma,* and *C. ?grandis.* In the case of *L. laricina,* the groups are the nine study sites.

The rationale for the S-PCA method described by Humphries et al. (1981) is the observation that the correlations between scores on the first principal component, calculated from distance measurements of the kind used here, and the original data are all of the same sign, thus reflecting differences in overall size. Both positive and negative correlations between the original distances and each of the subsequent principal components reflect the more complex patterns of their covariation that result in shape differences.

S-PCA consists initially of calculating a first principal component from data where the effect of group has been removed (Fig. 4) to yield a group-independent size component. This component is then used to adjust a second or third principal component calculated from data which retain group differences, to yield shape components (H1, H2) that discriminate

(a) The basic data matrix of $\log_{10}$-transformed truss distances calculated for each OTU (Fig. 2, 3) is centered by the grand mean vector and used to calculate scores on principal components PC1, PC2, PC3, etc.

(b) The basic data matrix is centered, group by group, by each group's mean vector. The first principal component calculated from the data centered in this way is the within-group size component S.

(c) The scores on PC1, PC2, PC3, etc. are centered by group as in (b), to yield scores on shifted components PZ1, PZ2, PZ3, etc.

(d) A slope α_1 which describes the confounding of S with PZ2 is obtained from the regression of PZ2 on S. Additional α's (α_2, etc.) could be obtained using PZ3, etc.

(e) Multiple regression of S on PZ1 and PZ2 (or PZ3, etc.) yields coefficients β_{11} and β_{12} (or β_{21} and β_{22}, etc.) from which to estimate $\hat{S}$ lying in the plane of PZ1 and PZ2 (or PZ3, etc.).

(f) The residual H1 (or H2, etc.) from the regression in (d) is calculated using $\hat{S}$ instead of S. This estimate is calculated using the original component scores that retain the differences among groups as follows:

$$H1 = PC2 - \alpha_1(\beta_{11}PC1 + \beta_{12}PC2)$$

$$H2 = PC3 - \alpha_2(\beta_{21}PC1 + \beta_{22}PC3)$$

etc.

Fig. 4. Scheme for calculating scores on sheared principal components (S-PCA; Humphries et al., 1981). Note that principal components calculated in steps (a) and (b) are obtained from the covariance matrix of the log-transformed measurements either according to definitional formulae (e.g. Pimentel, 1979) or equivalently by singular value decomposition of the centered data (Chambers, 1977; Becker and Chambers, 1984).

groups but are independent of size differences. An outline of the S-PCA method based on that given by Humphries et al. (1981) is given in Fig. 4. Correlations between H1 and H2 calculated in this way and the original $\log_{10}$-transformed truss dimensions were calculated, in order to elucidate the nature of the shape contrasts represented by H1 and H2.

An alternative to S-PCA in examining the discrimination of a priori groups with respect to multivariate data (but as discussed below, one that may not be able to distinguish between size and shape) is the multivariate analysis of variance (MANOVA; Cooley and Lohnes, 1971) and ordination of the sample by means of canonical variates analysis (CVA; Cooley and Lohnes, 1971; Gittins, 1979). MANOVA tests group discrimination in terms of null hypotheses of the homogeneity of group covariance matrices and, if this first hypothesis is accepted, the equality of group mean vectors. Regardless of the outcome of these tests, CVA depicts the sample in a space of reduced dimensionality in which group separation is maximized. Dimensionality was evaluated using Roy's largest-root criterion (Gittins, 1979), by comparing the squared canonical correlations associated with each

canonical variate with critical points of the greatest characteristic root (gcr) distribution (Morrison, 1976). Since the MANOVA statistics (Wilks' Λ, Bartlett's M, and the corresponding F approximations; Cooley and Lohnes, 1971) require calculation of individual group covariance matrices and their determinants, the basic data matrix was first summarized by means of multi-group PCA (M-PCA, i.e. PCA based on eigenanalysis of the pooled within-groups covariance matrix **W**; Campbell, 1976; Pimentel, 1979). In this way problems of limited sample size (fewer OTUs than measurements), as well as of redundancy among the measurements, were avoided by restricting the MANOVA and CVA to the scores on a limited number of M-PCA axes. The axes employed were those associated with eigenvalues larger than the average eigenvalue (Legendre and Legendre, 1983). It should be noted that M-PCA corresponds to step (2) of S-PCA, except that the PCA rotation (specified by the eigenvectors of **W**) is applied to data centered by the grand mean vector rather than by the individual group mean vectors, thus preserving differences among groups.

PCA dimensionality and robustness were also investigated by calculating jackknifed eigenvalues and eigenvectors for the covariance matrices of interest (Gibson et al., 1984; Becker and Chambers, 1984). Distributional properties of the data were examined by means of normal probability plots plus the use of a correlation statistic suggested by Ryan and Joiner (undated; Ryan et al., 1981). The basic data matrix was summarized as $\mathbf{y}_M$, the generalized distance form of Van Valen's total variance quantity, calculated from M-PCA scores $\mathbf{x}_j$ (centered by the group means) and eigenvalues λ_j (Orlóci, 1978) as:

$$\overset{\frown}{y}_{iM} = \left(\sum_j (x_{ij}/\lambda_j)^2 \right)^{1/2},$$

for the ith OTU and j = 1 . . . t M-PCA axes (van Valen, 1978; Dickinson and Phipps, 1985). ANOVA of the y_{iM}s provides a supplementary and more robust test of the homogeneity of the group dispersions. As suggested by Dagnelie (1975; Legendre and Legendre, 1983) the normality of the y_{iM}s for a sample (tested as described above) is a test of the multinormality of the original data.

Group structure in the univariate descriptors of size (M-PC1) and shape (H1, H2) variation in the samples studied was tested by means of robust analysis of variance (ANOVA) methods proposed by Brown and Forsythe (1974a, b). These methods test null hypotheses of (1) the equality of group variances regardless of departures from normality, and (2) the equality of group means regardless of departures from homoscedasticity. A nested ANOVA (Sokal and Rohlf, 1981) was carried out on the *Larix* sample scores on H1 and H2 in order to examine variance components associated with needles (within OTUs), OTUs (within sites), and sites. In this analysis the experimental units were the five needles of the first 14 OTUs for each of the nine populations (N = 630).

Results

Leaf shape intermediacy of putative hawthorn hybrids.—Of the three putatively hybrid *Crataegus* taxa, only *C. ?grandis* demonstrated an appreciable degree of leaf shape intermediacy when compared with its supposed parent taxa, *C. crus-galli* s. str. and *C. punctata* (Fig. 5; Table 1). This intermediacy is only with respect to the second shape axis (H2) that relates to a contrast between leaves with a broader, more nearly rounded obovate outline (*C. punctata*) and narrower, more obtrullate leaves (*C. crus-galli* s.l.; Fig. 5; Table 2).

As a group, the taxa of the *crus-galli* complex were also differentiated from *C. punctata* along the first shape axis (H1), but without any indication of the putatively hybrid taxa being intermediate in shape (with respect to a contrast between broader leaves (*C. punctata*) and more narrow ones (*C. crus-galli* s.l.; Fig. 5; Tables 1, 2). The intermediacy of the sample of *C. ?grandis* is evident also in the results of the CVA based on M-PCA scores (Fig. 6).

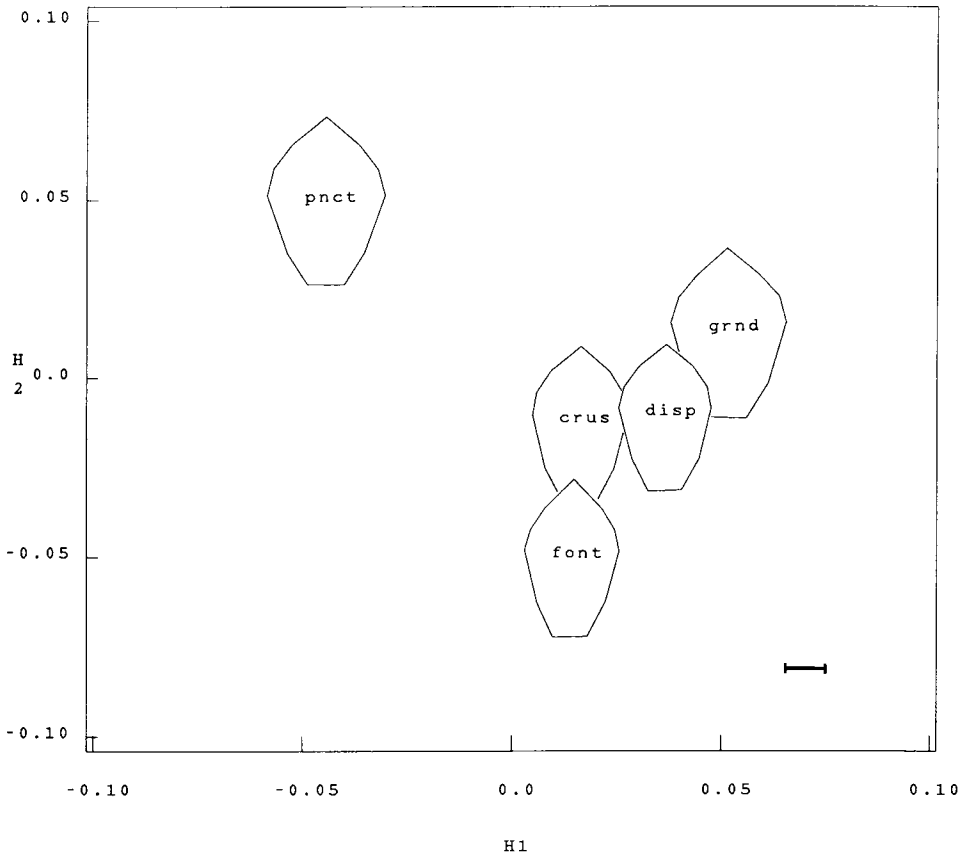

Fig. 5. Samples of five *Crataegus* taxa in the H1, H2 plane (see text and Table 2 for additional details). *C. crus-galli* s. str. (CRUS; N = 69), *C. fontanesiana* (FONT; N = 37), *C. ?disperma* (DISP; N = 10), *C. ?grandis* (GRND; N = 7), *C. punctata* (PNCT; N = 53). Correlations betwen H1 and H2 and the M-PCA axes from which they are derived (Fig. 4) are greater than 0.96. Outlines are those obtained from landmark (x, y) coordinates averaged over the entire sample of short shoot subtermial leaves for each taxon (the scale bar at the lower right represents 1 cm at the same scale as the leaf outlines).

Only the first three M-PCA axes calculated from the 23 truss dimensions were used in the CVA calculations. These three axes accounted for 96% of the total variance in the 176 OTU sample. All 23 correlations between the truss dimensions and scores on M-PC1 were negative; those with the scores on M-PC2 and M-PC3 were both negative and positive.

On the basis of comparisons of both determinants and $\bar{y}_M$ values, the dispersions of the five taxa described by their scores on the three retained M-PCA axes were heterogeneous (Table 1), owing to the contrast between the limited variability of the *C. ?grandis* sample and the heterogeneity of the one of *C. punctata.* Only one significant canonical variate was found ($p < 0.01$), but ordination of the samples along this axis (Fig. 6) demonstrates the intermediacy of the *C. ?grandis* sample with respect to the three other components of Ontario *C. crus-galli* s.l., and *C. punctata.* The contributions of M-PC1 and M-PC3 to this ordination are approximately equal, and considerably in excess of that due to M-PC2 (Fig. 6).

Geographic variation in needle cross-sectional shape.—Based on data summarized as

Table 1. Distributional and statistical parameters of the *Crataegus* sample.

Taxon	N	$\bar{y}_M$[a]	det[b]	M-PC1[c]	H1[d]	H2[e]
crus-galli s. str.	69	4.47	916.3	86.7	0.016	−0.012
fontanesiana	37	5.07	880.1	86.3	0.014	−0.050
?disperma	10	3.87	41.1	188.2	0.037	−0.010
?grandis	7	3.81	9.8	−171.0	0.052	0.014
punctata	53	4.90	1120.6	−186.0	−0.045	0.050

[a] Entries are taxon means, which differ significantly ($p < 0.001$). The distribution of y_{iM} values was found to depart from normality ($p < 0.01$), being long-tailed to the right.

[b] Determinants of the taxon dispersion matrices ($\times 10^{-9}$) calculated from scores on M-PC1 . . . 3, and found to be heterogeneous ($p < 0.001$).

[c–e] Entries are taxon means which differ significantly (M-PC1, $p \ll 0.001$; H1, $p < 0.001$; H2, $p \ll 0.001$). The three eigenvalues associated with these axes accounted, respectively, for 75–79%, 10–13%, and 7–8% of the trace of either **W** or the total sample covariance matrix. Jackknifing the first three eigenvalues of these matrices demonstrated them to be resolutely non-zero ($p \ll 0.001$), with non-overlapping 95% confidence intervals. Jackknifing the corresponding eigenvectors showed all but one element (in the third eigenvector) to be non-zero ($p \ll 0.001$).

average coordinates and used to calculate average truss dimensions for each OTU, shape variation among the nine tamarack sites reflects both needle cross-sectional asymmetries (H1, Table 4) and contrasting width-thickness proportions (H2, Table 4). These two shape axes differed in the extent to which they demonstrated differentiation of the sites. One-way ANOVAs of the entire sample demonstrated much stronger differentiation among sites with respect to H2 than with respect to H1 (Table 3). Both analyses manifested a similar pattern, however (Fig. 7). Scores for the two southernmost sites (KDY, TVL) are intermediate between those of two geographically heterogeneous groups of sites (KEN, NOB, SEV; and JOV, SPR, TBY; Fig. 7). The position of the BRI centroid in these analyses was anomalous.

In the nested ANOVA, which compared variation among needles within OTUs, among OTUs within sites, and among sites, both H1 and H2 showed significant variation among sites and among OTUs within sites (Table 5).

Only the first four M-PCA axes calculated from the 15 truss dimensions were associated with eigenvalues larger than the average eigenvalue, and so were retained in the CVA calculations. These four axes accounted for 88% of the total variance in the 156 OTU

Table 2. Correlations between H1, H2 and the 23 truss dimensions (Fig. 2b, c) for the *Crataegus* sample.

	H1	H2		H1	H2
AB	−0.401	0.263	DE	0.405	−0.488
AI	−0.332	0.363	DF	0.304	−0.175
AJ	−0.444	0.283	DG	0.124	0.183
AK	−0.719	−0.165	DH	0.013	0.331
BC	−0.110	0.474	EF	0.270	−0.156
BH	−0.144	0.472	EG	0.284	−0.177
BI	−0.194	0.459	FG	0.429	−0.434
BJ	−0.339	0.367	GH	0.063	0.258
CD	0.077	0.271	HI	−0.167	0.445
CG	0.029	0.331	IJ	−0.439	0.254
CH	−0.100	0.480	JK	−0.707	−0.167
CI	−0.146	0.470			

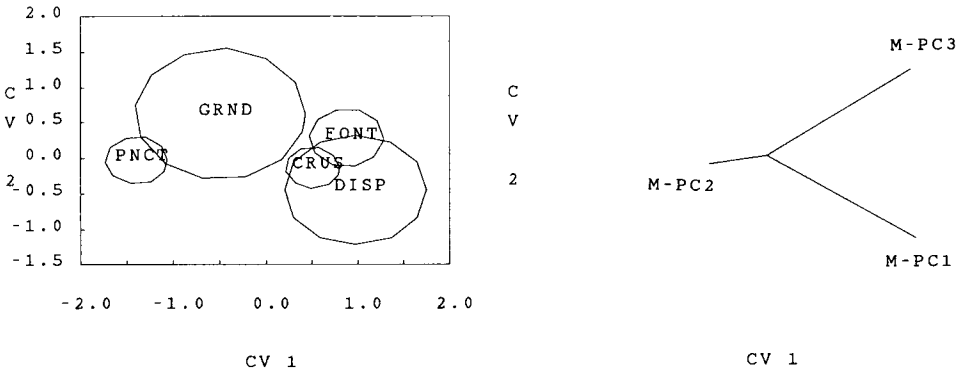

Fig. 6. Samples of five *Crataegus* taxa in the plane of the first two canonical variates (CV1, CV2) calculated from M-PC1 . . . 3. Circles indicate approximate 95% confidence regions around taxon centroids. Vectors represent the contribution of M-PC1 . . . 3 (calculated from the 23 log-transformed short shoot subterminal leaf truss dimensions) to the distribution of the sample in the CV1, CV2 plane in terms of the correlations between the canonical variates and M-PC1 . . . 3. Taxa as identified in Fig. 5.

sample. Correlations between the truss dimensions and scores on M-PC1 . . . 4 showed the same pattern as in the *Crataegus* example; only those for M-PC1 were all of the same sign (negative).

Dispersions of the nine tamarack samples were heterogeneous according to the test on the determinants, but not so with respect to the $\bar{y}_M$ values (Table 3). Ranking site variability by these two methods was not consistent, especially for the more variable sites (Table 3). CVA of these samples was found to be two-dimensional ($p < 0.01$), and demonstrated the way in which separation of the samples is a function principally of scores on M-PC1 (size variation), M-PC3 and M-PC4 (Fig. 8).

Discussion

Hybrid intermediacy.—Hybridization between *C. crus-galli* and *C. punctata* has been advanced to explain a number of North American *Crataegus* taxa (Rickett, 1937; Palmer, 1963; Phipps and Muniyamma, 1980). The limited data available on interspecific fertility in *Crataegus* (Bradshaw, 1971; Love and Feigen, 1978; Dickinson and Phipps, 1986; Wells, 1985) and on the phenology of these two taxa in particular (Phipps and Muniyamma, 1980; Smith et al., 1980; Dickinson, 1983) suggest that such an explanation is quite possible. Studies of *C. crus-galli* s.l. flower and fruit morphometrics which included *C. punctata* as an outgroup (Dickinson, 1983; Dickinson and Phipps, 1985) indicated the intermediacy of *C. fontanesiana* with respect to the supposed parent taxa. *Crataegus ?disperma* was not included in these earlier studies, but its relationships are readily summarized. Whereas *C. fontanesiana* has glabrous inflorescences (like *C. crus-galli* s. str.) and flowers with approximately 20 stamens and (1–)2–3 styles (like *C. punctata*), the flowers of *C. ?disperma* resemble those of *C. crus-galli* s. str. in composition but not pubescence. Rather, stamen and style numbers in *C. ?disperma* are low (approximately 10; 1–2(–3)) and young inflorescences are markedly pubescent. In addition, the leaves of both *C. fontanesiana* and *C. ?disperma* share a tendency toward having the secondary veins of their leaves proceed directly to the margin, a characteristic of the leaves of *C. punctata* but not of Ontario *C. crus-galli* s. str. (Dickinson, 1985).

Crataegus ?grandis is nearly indistinguishable from *C. punctata* with respect to flower and fruit descriptors and some leaf descriptors (toothing, venation) examined in earlier studies (Dickinson, 1983; Dickinson and Phipps, 1984, 1985). Nevertheless, a relationship

Table 3. Distributional and statistical parameters of the *Larix laricina* sample.

Site[a]	N	$\bar{y}_M$[b]	det[c]	M-PC1[d]	H1[e]	H2[f]
NOB	19	3.77	6.03	−73.0	−0.015	0.003
TBY	15	4.28	1.16	53.3	0.046	−0.041
KEN	18	3.58	1.86	−62.0	−0.031	0.065
SEV	19	3.94	2.02	−39.1	−0.034	0.023
JOV	22	3.68	4.95	124.3	0.021	−0.035
BRI	14	3.41	13.19	−108.9	−0.002	−0.027
SPR	15	3.86	9.29	40.5	−0.010	−0.030
KDY	15	3.42	2.07	43.8	0.034	−0.005
TVL	19	3.44	1.11	−1.5	0.000	0.033

[a] Explanation of site abbreviations: BRI, Brier I., Nova Scotia; JOV, St.-Jovite, Quebec; KDY, Kennedy Bog, Monroe Co., New York; KEN, Kenogami R., 20 km north of Mammamattawa, Ontario; NOB, North Bay, Ontario; SEV, Fort Severn, Ontario; SPR, Sprague Plantation, Penobscot Co., Maine; TBY, Thunder Bay, Ontario; TVL, Tannersville, Pennsylvania.

[b] Entries are taxon means, which were found not to differ significantly ($p = 0.3$). The distribution of y_{iM} values departed from normality ($p < 0.01$) because of six outlier OTUs representing four sites.

[c] Determinants of the site dispersion matrices ($\times 10^{-14}$) calculated from scores on M-PC1 . . . 4 and found to be heterogeneous ($p < 0.001$).

[d–f] Entries are site means which differ significantly (M-PC1, $p \ll 0.001$; H1, $p = 0.001$; H2, $p \ll 0.001$). The three eigenvalues associated with these axes accounted, respectively, for 53–56%, 13–16%, and 11–12% of the trace of either $\mathbf{W}$ or the total sample covariance matrix. Jackknifing the first three eigenvalues of these matrices demonstrated them to be resolutely non-zero ($p < 0.001$), with non-overlapping 95% confidence intervals. Jackknifing the corresponding eigenvectors showed all but one element (in the second eigenvector) to be non-zero ($p < 0.001$).

with *C. crus-galli* is strongly suggested by the texture and glossiness of the leaves of both taxa. Furthermore, *C. ?grandis* in Ontario is male-sterile and appears to be triploid (Ontario *C. crus-galli* s. str. is predominantly tetraploid, *C. punctata* diploid; Muniyamma and Phipps, 1979; Dickinson and Phipps, 1986). At the site where *C. ?grandis* occurs in Ontario it is accompanied not only by individuals of *C. crus-galli* s. str. but also by two intermediate individuals (leaves like *C. ?grandis,* flowers and fruits more like those of *C. crus-galli* s. str., and at least partially male-fertile). The present-day population of *C. crus-galli* s.l. at this site (i.e. comprising the *C. crus-galli* s. str., *C.?grandis* and intermediate individuals) could be explained as having resulted largely from the seed crop(s) of tetraploid *C. crus-galli* s. str. pollinated in part by diploid *C. punctata,* as well as from subsequent back-crosses of the *C. ?grandis* to *C. crus-galli* s. str. Such an explanation accounts for the leaf-shape intermediacy of *C. ?grandis* (Fig. 5) if in this case high stamen and style numbers are dominant over reduced numbers, whereas leaf shape is inherited quantitatively. Analysis

Table 4. Correlations between H1, H2 and the 15 truss dimensions (Fig. 3) for the *Larix* sample.

	H1	H2		H1	H2
AB	−0.224	0.218	DE	−0.109	0.280
AG	−0.503	−0.366	DF	−0.261	0.678
AH	−0.505	−0.359	DG	0.105	0.535
BC	−0.010	−0.133	EF	0.551	−0.015
BD	−0.164	0.101	EG	0.570	−0.016
BG	−0.533	0.320	FG	0.582	−0.011
BH	−0.340	0.654	GH	−0.489	−0.373
CD	−0.024	−0.101			

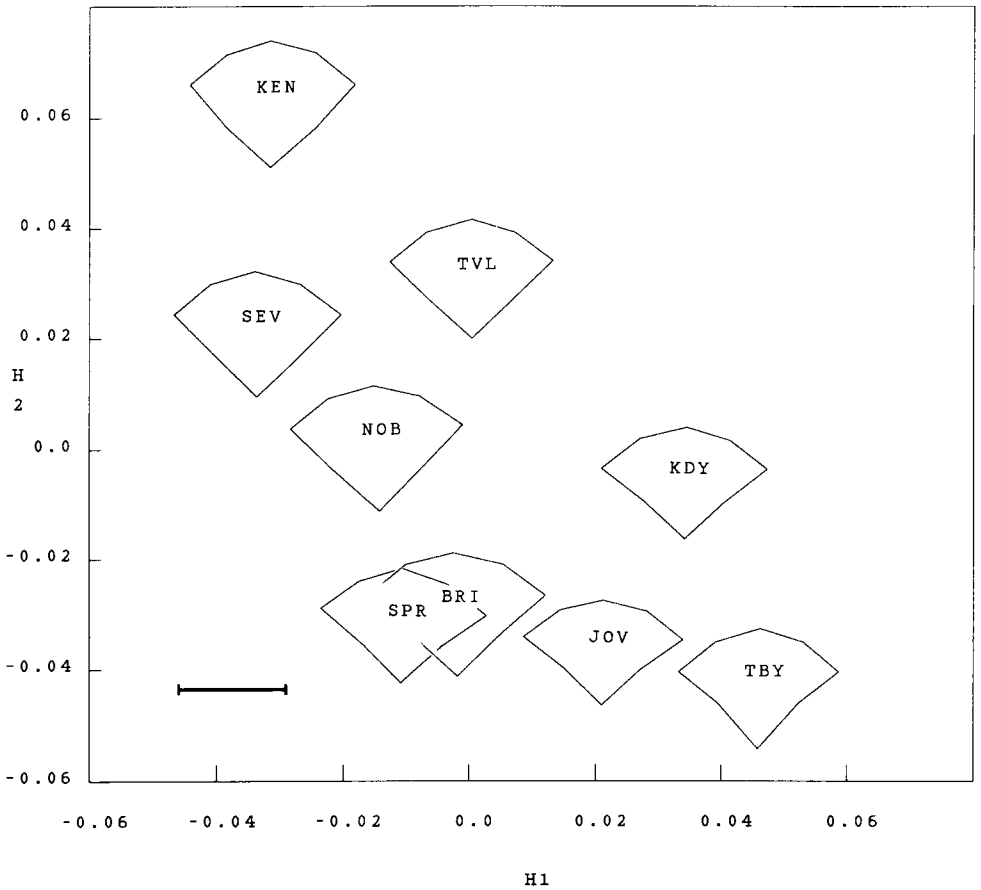

Fig. 7. Samples of *Larix laricina* from nine sites in the H1, H2 plane (see text and Table 4 for additional details). See Table 3 for explanation of the site abbreviations. Correlations between H1 and H2 and the M-PCA axes from which they are derived (Fig. 4) are greater than 0.91. Outlines are those obtained from landmark (x, y) coordinates averaged over the entire sample of short shoot needles for each population (the scale bar at the lower left represents 1 mm at the same scale as the needle outlines).

of leaf shape variation in some of the better-documented hybrids between *Crataegus* species (Bradshaw, 1971; Byatt, 1975; Love and Feigen, 1978; Christensen, 1982; Wells, 1985) as well as in other genera (Melville, 1960; Dancik and Barnes, 1975; cf. Sneath, 1971) suggests that leaf shape can in fact be inherited in this way, so that hybrids and their derivatives exhibit an array of intermediate leaf shapes by combining features from both parental taxa.

Instead of being a hybrid, *C. fontanesiana* may be derived with less modification than *C. crus-galli* s. str. from ancestral *C. crus-galli* with higher numbers of stamens and styles (as well as other primitive reproductive characteristics; Dickinson and Phipps, 1986). Such an ancestral condition for *C. crus-galli* s.l. is plausible in view of the frequency of higher stamen numbers (approx. 20 per flower) among diploid *Crataegus* taxa, as well as among the other genera of the subfamily Maloideae. Recent, more detailed studies of *C. ?disperma* (Wells, 1985) suggest that this taxon is more likely a form of *C. crus-galli* s. str. than of hybrid origin.

The results obtained here by analyzing leaf shape variation in the five *Crataegus* taxa

Table 5. Nested ANOVA of the scores on (a) H1 and (b) H2 calculated for five needles for each of 14 OTUs of *Larix laricina* at nine sites.

	df	MS	Percent	Fs	p
a) H1					
Among sites	8	0.13	4.8	3.33	0.0018
Among OTUs within sites	117	0.04	12.1	1.72	$<10^{-4}$
Within OTUs	504	0.02	83.2	—	—
Total	629	—	—	—	—
b) H2					
Among sites	8	0.11	13.2	7.14	$<10^{-6}$
Among OTUs within sites	117	0.01	15.9	2.12	$<10^{-7}$
Within OTUs	504	0.01	70.9	—	—
Total	629	—	—	—	—

by S-PCA suggest that this method is in fact capable of detecting patterns which are quite subtle (Fig. 5), but which admit to explanations supported by other data.

Geographic variation.—It appears likely that comparisons must be made over a wider area than is represented by the nine sites examined here, if readily interpreted patterns of geographic variation are to be obtained with respect to the shape axes H1 and H2 produced by S-PCA (Fig. 7). Such comparisons are underway (Parker and Dickinson, unpubl.). In the meantime we have nonetheless succeeded in detecting significant variation in needle cross-sectional shape among sites and among OTUs within sites (Figs. 7, 8; Tables 3, 4). It is of interest that variation with respect to H1 (left-right asymmetry; compare Fig. 3 and Table 4) is much more pronounced among OTUs within sites than among sites (Table 5a). This could be due to differences among trees in the direction of the phyllotactic spiral. We are unaware of studies of how this direction is determined in the axillary buds of conifers; in angiosperms it may be consistent throughout the shoot system or reverse at each branch point (Berg, 1976; references in Bible, 1976). Differentiation among sites with respect to H1 could result from differences in aspect, if this either influences leaf development or results in preferential development of one direction over another. Variation along H1 is not likely the result of specimen preparation since this was consistent over all samples, and care was taken to section at right angles to the transverse axis of the needle (i.e. perpendicular to dimension BD in Fig. 3).

Utility of the method.—Two examples have been given to illustrate the advantages of redundantly sampling leaf outline shape by means of a network of truss dimensions, and using S-PCA to efficiently summarize the resulting data. The summary obtained consists of a limited number of mutually independent (orthogonal) axes (H1, H2, . . . etc., Fig. 4) that describe shape variation among a priori groups in a manner directly referable to the original measurements, and independent of size differences among the groups. The shape differences among the groups (hawthorn taxa, tamarack study sites) are extremely subtle (Figs. 5, 7), yet significant variation in shape among these groups, indexed by H1 and H2, is detectable (Tables 1, 3, 5). Analysis of groups among which the shape of leaves or other structures varies more strikingly (e.g. *Crataegus monogyna* and some of the species with which it hybridizes; Bradshaw, 1971; Byatt, 1975; Gostyńska-Jakuszewska, 1975; Love and Feigen, 1978; Christensen, 1983; Wells, 1985) would undoubtedly be even more rewarding.

In the zoological context in which it was developed the approach described here has a number of conspicuous advantages. Allometric relationships (shape variation independent of size differences) will be extremely important in studies of (non-metameric) organisms which continue to increase in size during their life-span. Such relationships are preserved

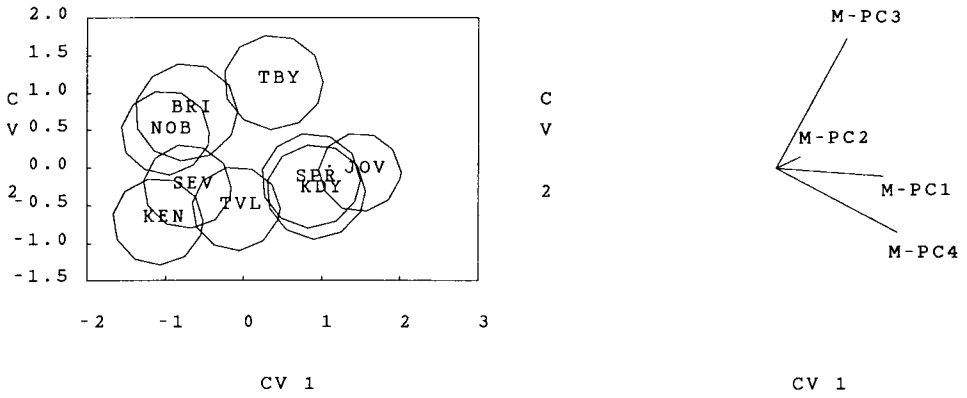

Fig. 8. Samples of *Larix laricina* from nine sites in the plane of the first two canonical variates (CV1, CV2) calculated from M-PC1 . . . 4. Circles and vector diagram as in Fig. 6. Sites as identified in Table 3.

by analyzing the covariance patterns of shape data obtained in the form of linear measurements. Patterns of shape variation may be associated with differences in feeding habit (Example 1 in Humphries et al., 1982) or other functional considerations. Consequently, it is desirable to be able to relate contrasts along shape axes to particular measurements or groups of measurements by means of component-measurement correlations, in order to explore their possible adaptive significance. It might seem unnecessary to apply methods with these virtues to botanical studies in which the plant parts measured frequently can be selected so as to have completed their increase in size. Likewise, the functional significance of shape variation in these structures may be obscure. In their study of the *Dodonaea viscosa* complex West and Noble (1984) found only leaf area to be strongly correlated with environmental variables (but see Givnish (1978) for possible approaches to this problem).

While considerations such as these are valid, nevertheless shape differences *have* accumulated as lineages have diverged. A better understanding of *how* such differences accumulate would greatly assist in understanding phylogenetic relationships. For example, most Eurasian *Crataegus* species differ from most North American ones in having strongly lobed leaves, so much so that El-Gazzar distinguished subgenera *Crataegus* and *Americanae* El-Gazzar, respectively, on this basis (together with the correlated occurrence of veins to the base of the inter-lobe sinus; the cytological criterion also used was based on out-of-date information). The North American species *C. marshallii* Egglest. (not discussed by El-Gazzar) resembles subgenus *Crataegus* in the lobing of its leaves, however. Also, the fossil record of *Crataegus* in North America appears to include forms similar to extant Eurasian taxa (*C. newberryii* Cockerell; Chaney, 1944). S-PCA comparison of shape development trajectories of leaves in *C. marshallii* and in typical Eurasian and North American species would help to establish shape homologies in these taxa, and so suggest possible phylogenetic and biogeographic hypotheses.

In other studies it may be of interest to demonstrate that shape is more or less variable in a population (Dickinson, 1986). Similarly, S-PCA provides a means of investigating the relationship postulated by Lerner (1954) between the genomic heterozygosity of an individual and its degree of developmental canalization (cf. Paxman, 1956). S-PCA of multiple measurements on developmental series provides a method for both localization and multivariate quantification of ontogenetic variability.

Comparison with other approaches.—In contrast with S-PCA, CVA axes calculated from truss data or their summaries will not necessarily reveal contrasts in shape (compare the disposition of group centroids in Figs. 5 and 6, and in Figs. 7 and 8). This is because group

discrimination may be a function of size contrasts as well as shape ones (M-PC1 correlated with M-PC3 in Fig. 6; M-PC1 weakly correlated with M-PC2, M-PC3 in Fig. 8). In addition, shape contrasts distinguished by S-PCA may be combined in a single CVA axis (Fig. 6). S-PCA is likely to be more informative than CVA whenever interest centers on contrasts in shape among, rather than overall discrimination of, groups.

S-PCA requires that the sample be dissected a priori into groups. This characteristic is shared with other multivariate methods that have been used to examine putative hybrids in relation to their supposed parents such as CVA and the polar ordination method proposed by Wells (1980; Maze, 1980). Since the arbitrariness of the a priori group hypothesis with respect to shape variation can be tested statistically, this requirement is not seen to be a drawback.

Leaf shape description by means of a network of truss dimensions differs radically from most other methods used previously. In particular, it differs from other non-Fourier multivariate approaches (e.g. Hill, 1980; West and Noble, 1984) in the way in which it does *not* require a priori specification of the possible modes of shape variation. By sampling shape redundantly, as described by Strauss and Bookstein (1982), and examining the correlations between the measurements and the shape axes obtained by S-PCA, it is possible to discover and localize shape trends among the groups under study (Figs. 5, 8). It should be noted that the *Crataegus* study described above differs from most zoological applications of the method in that leaf shape was described (with one exception) by reference to pseudolandmarks (Bookstein et al., 1985) on the outline. The effect of using pseudolandmarks appears in the pattern of variances of the elements of the jackknifed eigenvectors. In each of the first three eigenvectors (corresponding to S, H1 and H2, respectively) different, largely non-overlapping, groups of truss-dimensions were much more variable than others. Variation in the truss dimensions is constrained, however, since the positions of all of the digitized landmarks and pseudolandmarks (as well as their average positions for each OTU or sample of OTUs) lie in the plane of the digitizing tablet surface. Accordingly, with respect to a particular contrast (S, H1, H2), as the position of the pseudolandmarks varied some dimensions (e.g. the two diagonals of truss quadrilaterals) remained fairly constant while the others (the four sides) were much more variable. In the *Larix* example only points F and H are pseudolandmarks (Fig. 3), and such effects are not apparent in the jackknifed eigenanalyses.

Other applications of PCA to the study of patterns of morphometric variation have questioned the significance of variation expressed on the second and subsequent PC axes (i.e. associated with smaller and smaller eigenvalues accounting for less and less of the total sample variance; Gibson et al., 1984). The critical values tabulated by Frontier (1976; Legendre and Legendre, 1983, Table D) for the percent trace accounted for by successive eigenvalues would suggest that only the first eigenvalue (associated with size contrasts) was significant, in both studies here. Experience indicates that Frontier's test is extremely rigorous, compared with other criteria (e.g. results in Dickinson, 1983). The results of calculating jackknifed eigenanalyses (Tables 1, 3) suggest that the eigenvalues associated with H1 and H2 are in fact distinct and non-zero, each axis thus representing the detection of significantly different patterns of variation (Tables 2, 4).

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