

Analysis of Petal Shape Variation of *Primula sieboldii* by Elliptic Fourier Descriptors and Principal Component Analysis

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• **Background and Aims** Petals are important for *Primula sieboldii* because of the commercial value of its flowers, and their form is a target characteristic for breeding. An appropriate understanding of petal form in terms of genetic mechanisms and environmental effects is necessary for improvement of this species. The aim of this study was to establish a quantitative evaluation method of petal shape by elliptic Fourier descriptors and principal component analysis (EF-PCA), and thus to investigate genotypic and environmental effects on petal morphology.

• **Methods** EF-PCA describes an overall shape mathematically by transforming coordinate information concerning its contours into elliptic Fourier descriptors (EFDs) and summarizing the EFDs by principal component analysis. To examine varietal effects on principal component (PC) scores and petal area among commercial varieties, nested ANOVAs were performed (since the samples had a hierarchical structure with four sources, i.e. variety, plant, flower and petal).

• **Key Results** Petal shape variation could be evaluated successfully and the symmetrical and asymmetrical elements of the overall shape variation could be detected. The proportions of the variance component due to varietal differences were more than 70 % in the first five PCs of the symmetrical elements and petal area. By contrast, the proportions due to varietal effects of all PCs of the asymmetrical elements were less than 20 %, and the proportions of the variation within a flower were more than 75 %. It was also demonstrated that the yearly variance of petal shape was small, and that of petal area was large.

• **Conclusions** Within a flower the major source of the symmetrical elements is genotypic and the asymmetrical elements are strongly affected by the environment. With respect to petal area, the contribution of genotypes is also large; it is, however, affected by the macro-environment more notably than is petal shape.

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Key words: *Primula sieboldii*, petal shape variation, elliptic Fourier descriptors, principal component analysis.

INTRODUCTION

Petals are important organs for *Primula sieboldii* and many other ornamental plants because of the commercial value of their flowers, and the petal's form is a target characteristic for breeding, together with petal colour and other floral characteristics. Petal form, i.e. the shape and size of the petal, varies widely within *P. sieboldii*, and an appropriate understanding of petal form in terms of genetic mechanisms and environmental effects is fundamental and necessary for improvement of this floricultural crop. The petal form of *P. sieboldii* is a key marker for registration of commercial varieties, along with petal colour and other floral morphology. In Japan, these characteristics have been used as evaluation indices of genetic resources in wild populations. Therefore, an understanding of genetic control is necessary to confirm the validity of the characterization. However, the effects of genotype and environment on morphological characteristics have not been examined in detail in ornamental plants in general, let alone for *P. sieboldii*.

Primula sieboldii is a clonally propagated perennial widely grown in Japan and north-east Asia. The plant

flowers annually in late spring with inflorescences, each of which is generally composed of 3–10 flowers. In Japan, this species has been popular as a garden herb since the Edo period, about 300 years ago (Torii, 1985). *Primula sieboldii* commercial varieties have gained wide variation in petal form through breeding manipulations such as crossing and selection (Suzuka, 1975). The total number of existent varieties is more than 300.

Petal shape is one of the most important characteristics among indices in variety registration. In spite of its importance, petal shape has been mainly evaluated qualitatively. For example, in the variety registration of *P. sieboldii* in Japan, the petal shape is categorized into discrete classes such as round type, notched type, broad type, and broad and notched type (Ministry of Agriculture, Forestry and Fisheries of Japan, 2004). This classification is based on rough estimates by human visual judgement. This qualitative measure is inadequate for the evaluation of continuous shape variation, as it cannot eliminate the subjectivity of human visual judgments, which result in unacceptable human errors. Only a few exceptional quantitative measures such as aspect ratio, petal area and perimeter are presently available. Therefore, an objective and quantitative evaluation method of petal shape is vital.

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In recent years, digital photography has become common and the costs of the related equipment (i.e. personal computer and digital camera) have decreased rapidly. Consequently, researchers have the opportunity to perform digital image analyses more easily than in the past. In fact, digital image analysis has been applied to evaluation of various plant organs, suggesting various methods to describe shape quantitatively. Among these, a method based on elliptic Fourier descriptors (Kuhl and Giardina, 1982) is the most commonly used. This method describes an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficients. Rohlf and Archie (1984) suggested principal component analysis for summarizing the elliptic Fourier descriptors. In addition, they reported that it is possible to analyse the shape quantitatively by using the principal component scores as ordinary quantitative characteristics. The application of elliptic Fourier descriptors to plant organ shape was first attempted by White *et al.* (1988), who analysed the leaf shape of *Betula*. Moreover, principal component analysis of elliptic Fourier descriptors has been used for several plant organs, such as *Begonia* leaves (McLellan, 1993), soybean leaflets (Furuta *et al.*, 1995), buckwheat kernels (Ohsawa *et al.*, 1998), yam tubers (Toyohara *et al.*, 2000), radish roots (Iwata *et al.*, 1998) and citrus leaves (Iwata *et al.*, 2002). Recently, Iwata and Ukai (2002) developed a program package, 'SHAPE', for the quantitative evaluation of biological shapes on the basis of elliptic Fourier descriptors.

Our aims were to establish a quantitative method for the evaluation of the petal shape of *P. sieboldii* and to investigate in detail genotypic and environmental effects on morphology by use of elliptic Fourier descriptors and principal component analysis. The applicability of this method to the variation of *P. sieboldii* petal shape was first justified using 12 commercial varieties that provide representative petal shape variation. Then the genotypic and environmental contributions to petal shape variation were evaluated by nested ANOVA. The variation of petal area was also analysed in a similar fashion.

MATERIALS AND METHODS

Twelve commercial varieties of *Primula sieboldii* E. Morren were examined, all grown in the Agriculture and Forestry Research Center, University of Tsukuba: 'Aobanohue', 'Usuzyanome', 'Kazaguruma', 'Gyokkobai', 'Kyoganoko', 'Shirawashi', 'Sumizomegenzi', 'Nankinkozakura', 'Nuretubame', 'Hatusugata', 'Hahanomegumi' and 'Yubae'. *Primula sieboldii* is a clonally reproduced perennial that can reproduce 1–5 new sprouts annually. Six sprouts per commercial variety were chosen randomly and transplanted into 11.5-cm pots in February 2002. All plants flowered approximately 3 months after transplanting. Three plants from each variety were chosen randomly, and three flowers per plant were sampled. Flowers were also sampled in a similar manner in 2003. The number of sampled flowers was thus 216 (2 years × 12 varieties × 3 plants × 3 flowers = 216 flowers). A flower of

P. sieboldii has five petals, and before photographing it each flower was separated into its five petals using a cutter.

The petals were photographed with a scale marker (10 mm × 10 mm) using a digital camera (COOLPIX-850, Nikon, Tokyo). Each photograph was saved as an RGB colour image (BMP format) with 256 grey levels per channel (red, green, blue). Closed contours of the petals were obtained from the binary images, which were converted from the red-channel images by a threshold method. Then, the contours were chain-coded (Freeman, 1974): namely, each contour was represented as a sequence of x and y coordinates of ordered points that were measured counter-clockwise from an arbitrary starting point. Assuming that the contour between the $(i - 1)$ -th and the i -th chain-coded points is linearly interpolated, and that the length of the contour from the starting point to the p -th point and the perimeter of the contour are denoted by t_p and T , respectively, then

$$t_p = \sum_{i=1}^p \Delta t_i$$

and $T = t_K$, where Δt_i and K are the distance between the $(i - 1)$ -th and the i -th points and the total number of the chain-coded points on the contour, respectively. Notice that the K -th point is equivalent to the starting point. The x and y coordinates of the p -th point are

$$x_p = \sum_{i=1}^p \Delta x_i$$

and

$$y_p = \sum_{i=1}^p \Delta y_i$$

where Δx_i and Δy_i are the distances along the x and y axes between the $(i - 1)$ -th and the i -th point. Thus, the elliptic Fourier expansions of the coordinates on the contour are

$$x_p = A_0 + \sum_{n=1}^{\infty} \left(a_n \cos \frac{2n\pi t_p}{T} + b_n \sin \frac{2n\pi t_p}{T} \right)$$

and

$$y_p = C_0 + \sum_{n=1}^{\infty} \left(c_n \cos \frac{2n\pi t_p}{T} + d_n \sin \frac{2n\pi t_p}{T} \right)$$

The elliptic Fourier coefficients of the n -th harmonic (a_n , b_n , c_n , d_n) are given as

$$a_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^K \frac{\Delta x_p}{\Delta t_p} \left(\cos \frac{2n\pi t_p}{T} - \cos \frac{2n\pi t_{p-1}}{T} \right)$$

$$b_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^K \frac{\Delta x_p}{\Delta t_p} \left(\sin \frac{2n\pi t_p}{T} - \sin \frac{2n\pi t_{p-1}}{T} \right)$$

$$c_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^K \frac{\Delta y_p}{\Delta t_p} \left(\cos \frac{2n\pi t_p}{T} - \cos \frac{2n\pi t_{p-1}}{T} \right)$$

and

$$d_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^K \frac{\Delta y_p}{\Delta t_p} \left(\sin \frac{2n\pi t_p}{T} - \sin \frac{2n\pi t_{p-1}}{T} \right)$$

In this study, we approximated the shape by the first 20 harmonics. Because the coefficients of an elliptic Fourier descriptor are not invariant in size, rotation, shift and starting point of chain-coding about a contour, we standardized the Fourier coefficients on the basis of the work of Kuhl and Giardina (1982). Let the standardized coefficients of the n -th harmonic be a_n^{**} , b_n^{**} , c_n^{**} , and d_n^{**} . Then,

$$\begin{bmatrix} a_n^{**} & b_n^{**} \\ c_n^{**} & d_n^{**} \end{bmatrix} = \frac{1}{E^*} \begin{bmatrix} \cos \psi & \sin \psi \\ -\sin \psi & \cos \psi \end{bmatrix} \begin{bmatrix} a_n & b_n \\ c_n & d_n \end{bmatrix} \begin{bmatrix} \cos n\theta & -\sin n\theta \\ \sin n\theta & \cos n\theta \end{bmatrix}$$

where $E^* = [(A_0 - x_q)^2 + (C_0 - y_q)^2]^{1/2}$,

$$\psi = \arctan \left[\frac{y_q - C_0}{x_q - A_0} \right] \quad (0 \leq \psi < 2\pi)$$

and

$$\theta = \frac{2\pi t_q}{T} \quad (0 \leq \theta < 2\pi)$$

In the above equations, E^* is the distance between the centre point (A_0 , C_0) and a specific point (x_q , y_q), and ψ is the spatial rotation angle. In this study, we used the proximal point on the central axis of each petal as a specific point. These two parameters are for the size invariance and the rotation invariance. θ is a parameter for chain-code starting point invariance. This standardization makes a_n^{**} , b_n^{**} , c_n^{**} and d_n^{**} independent of the size, rotation, shift and chain-code starting point of a contour. Finally, the shape of each petal was approximated by 80 standardized elliptic Fourier coefficients. The petal area was also obtained by image analysis in order to examine size variation.

The 80 coefficients were classified into two groups related to symmetrical and asymmetrical variations for the central axis of each petal (Iwata *et al.*, 1998). The coefficients a_n^{**} and d_n^{**} represent symmetrical, and b_n^{**} and c_n^{**} represent asymmetrical variations. That is, the contour reconstructed only by the former coefficients is completely symmetric, and the latter represent remaining, i.e. asymmetric, variation of the contour. To summarize the information contained in the coefficients of each group, we performed principal component analysis based on a variance–covariance matrix. Then, the scores of the components were used as characteristics of petal shape in the following analyses.

To examine the varietal effects on the principal component scores and petal area among the varieties, nested ANOVAs were performed separately for the scores and area, since the samples had a hierarchical structure with four sources, i.e. variety, plant, flower, petal. As for the

asymmetrical group, all the scores of the principal components were converted to absolute values prior to this analysis to remove the influence of the direction of curvature. Nested ANOVA were conducted using JMP4.0 (SAS Institute Inc., 2000).

RESULTS

The standardized elliptic Fourier coefficients of 1080 petals from the 12 commercial varieties were calculated. The mean petal shape of each variety was then drawn using the mean values of the standardized Fourier coefficients within each variety. Figure 1 shows the wide variation of petal shape that was observed among the varieties examined in this study.

The first five principal components of the symmetrical and asymmetrical groups provide a good summary of the data, accounting for 95.6 % and 86.1 % of the total variance, respectively (Table 1). The effect of each principal component on petal shape was visualized (Fig. 2). For this visualization, the coefficients of the elliptic Fourier descriptors were recalculated inversely using an eigen-vector matrix, letting the score on a particular principal component be equal to the mean \pm 2 s.d. (standard deviation), while the scores on the remaining components remained at the mean. Then the petal contour shape for each score condition was reconstructed on the basis of the recalculated coefficients. These reconstructed shapes indicated that the first and second principal components of the symmetrical group are good measures of the aspect ratio and the depth of head notch, accounting for 48.9 % and 32.2 % of the total symmetrical shape variation, respectively (Fig. 2A). The third

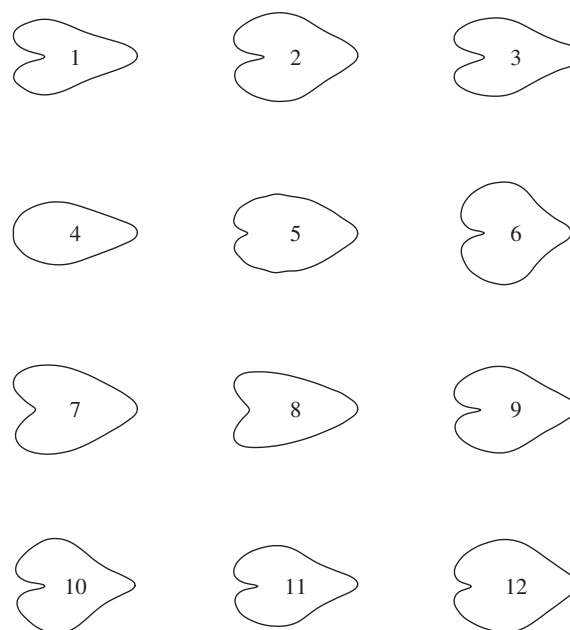


FIG. 1. Mean petal shapes of the 12 commercial varieties of *Primula sieboldii* examined in this study. 1, 'Aobanohue'; 2, 'Usuzyanome'; 3, 'Kazaguruma'; 4, 'Gyokkobai'; 5, 'Kyoganoko'; 6, 'Shirawashi'; 7, 'Sumizomegenzi'; 8, 'Nankinkozakura'; 9, 'Nuretubame'; 10, 'Hatusugata'; 11, 'Hahanomegumi'; 12, 'Yubae'.

TABLE 1. Eigenvalues and contributions of principal components of the symmetrical and asymmetrical groups

Component	Symmetrical group			Asymmetrical group		
	Eigenvalue (10^{-4})	Proportion (%)	Cumulative (%)	Eigenvalue (10^{-4})	Proportion (%)	Cumulative (%)
1	91.42	48.97	48.97	21.14	49.73	49.73
2	60.25	32.27	81.24	7.64	17.97	67.70
3	15.34	8.22	89.46	3.68	8.65	76.35
4	6.66	3.57	93.03	2.60	6.13	82.48
5	4.90	2.62	95.65	1.54	3.63	86.11
Total variance	186.70			42.50		

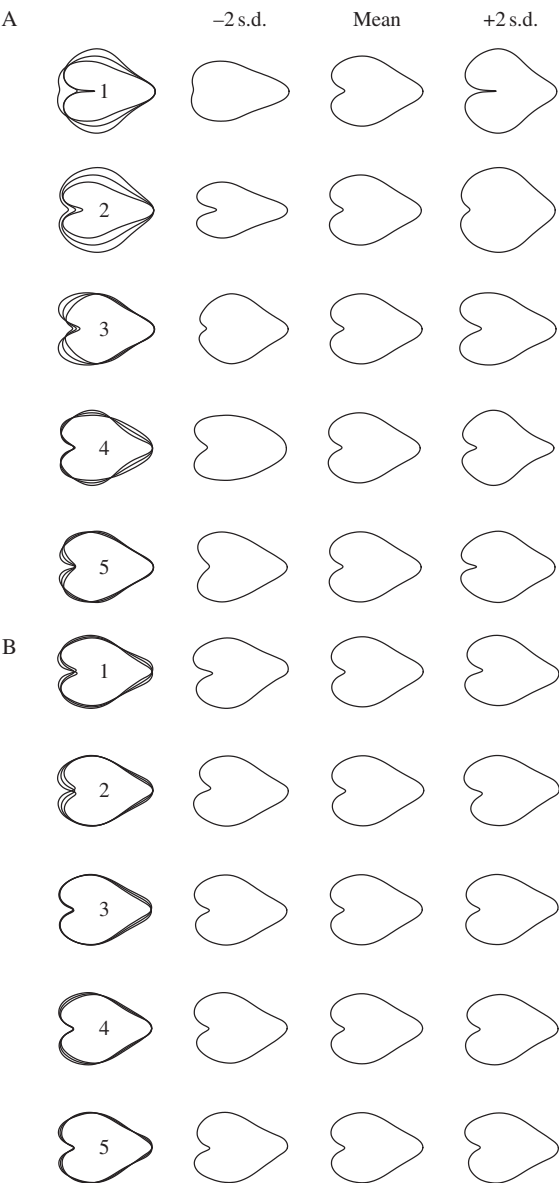


FIG. 2. Effect of each principal component on petal shape. The numbers 1–5 correspond to the first to fifth principal components, respectively. Each column shows the case where the score takes -2 s.d. (standard deviation), mean, or $+2$ s.d. as labelled, and the left-hand column shows the overlaid drawings of all three cases (see text for explanation). (A) Symmetrical variations from the symmetrical group coefficients; (B) asymmetrical variations from the asymmetrical group coefficients.

component is associated with the bluntness of the distal part of the petal, and the fourth component expresses the bluntness of the side and proximal parts of the petal. The fifth component is related to variation that cannot be ascribed to the first four components, although the meaning of this variation is not easily explained. The principal components for the asymmetrical group represent the asymmetrical variation, such as the difference of curvature and swelling in the distal and proximal parts (Fig. 2B).

Figure 3 shows the variation of the first to fifth principal component scores of the symmetrical group and the petal area among the 12 commercial varieties. Significant differences among the 12 varieties in each principal component and the petal area were observed (Table 2). From these results, we are able to characterize the commercial variety. For example, ‘Gyokkoba’ (4) has the lowest first principal component score, since it typically has a narrow petal shape with a shallow or absent head notch. ‘Shirawashi’ (6) and ‘Yubae’ (12) both show a high second principal component score. The former also shows a high first principal component score, but the latter shows an intermediate score value. Accordingly, the petal shape of ‘Shirawashi’ (6) is broad with a deep head notch, while that of ‘Yubae’ (12) is broad with a shallow head notch. Within each commercial variety, the yearly variances of the symmetrical group were smaller than that of the petal area (Fig. 3).

Table 2 shows the results of the ANOVA performed on the principal component scores of both the symmetrical and asymmetrical groups as well as the petal area. There are significant differences in all the principal components except the fourth principal component of the symmetrical group and the petal area. In this ANOVA, error variances were obtained as a pooled interaction that included variety \times year, plant \times year, flower \times year, and petal \times year interactions. The proportions of the variance component due to the varietal differences in the nested ANOVA were approximately 85 % in the first three principal components and approximately 70 % in the fourth and fifth principal components (Table 3). The proportion due to petal was the highest among the residual hierarchical sources, i.e. plant, flower and petal. By contrast, the proportions due to varietal effect of all principal components of the asymmetrical group were less than 20 %, and the proportions of the variation within a flower were more than 75 %. The proportion due to varietal effect on petal area was more than 90 %, and the proportion due to the variation among plants was largest in the remaining hierarchical sources.

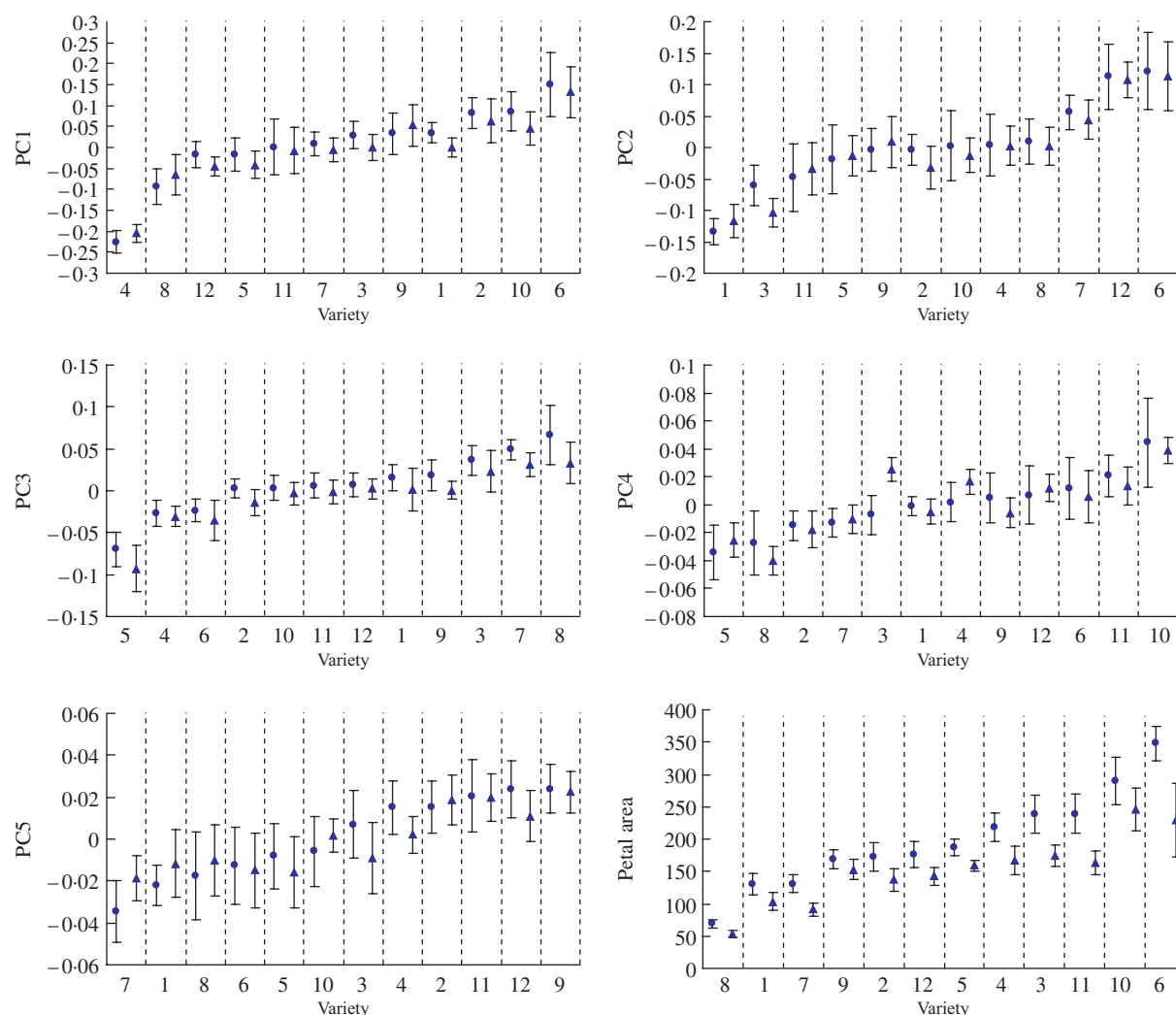


FIG. 3. Variations of the first to fifth principal component scores (PC) based on 40 symmetrical group coefficients and petal area among the 12 varieties of *Primula sieboldii*. Vertical bars indicate standard deviations. See Fig. 1 for the key to the varieties on the horizontal axes. Scores for 2002 are indicated by circles, scores for 2003 by triangles. The varieties are arranged in ascending order of the scores for 2002.

DISCUSSION

A method has successfully been established to evaluate quantitatively the petal shape of *P. sieboldii* using principal component scores obtained from standardized elliptic Fourier descriptors. The principal components are independent of each other, and it can be observed visually how each principal component affects the shape by drawing the contours under particular score value conditions, thus demonstrating that the principal component scores can be used as new shape characteristics of *P. sieboldii* petals. The petal shape variation of *P. sieboldii* can be divided into symmetrical and asymmetrical portions. The symmetrical variation had a larger proportion of the total variation than the asymmetrical variation (Table 1). Regarding the symmetrical variation, the first and second principal component scores, which indicate the aspect ratio and the depth of the head notch (Fig. 2), showed continuous variations of the petal shape, from a narrow type to a broad type, and from a shallow notched type to a deep notched type (Fig. 3).

The third principal component score represented the bluntness of the distal part of petal, and the fourth principal component score represented the bluntness of the side part of the petal. By contrast, the principal components of the asymmetrical variation were related to varying curvature and swelling in the distal and proximal parts.

The use of elliptic Fourier descriptors and principal component analysis (EF-PCA) has two major advantages. First, this method can accurately detect small shape variations. The contributions of the third, fourth and fifth principal components to the total variance were 8.22 %, 3.57 %, and 2.62 %, respectively (Table 1). It is difficult for humans to detect such small variations, but the ANOVA based on the component scores could clearly detect significant variations among commercial varieties. Secondly, EF-PCA can evaluate the shapes of objects independently of size. This independence is a great advantage because human visual judgment of shape is often deceived and misled by size factors. Although EF-PCA can detect small variations, it

TABLE 2. Results of ANOVA for the first five principal components (PC) of (A) the symmetrical and (B) the asymmetrical group coefficients, and (C) petal area

		PC1		PC2		PC3		PC4		PC5	
Source	df	SS	F	SS	F	SS	F	SS	F	SS	F
(A) Symmetrical group											
Year	1	0.042	20.273**	0.008	4.908*	0.052	148.885**	0.000	1.203 ns	0.000	0.805 ns
Variety	11	7.262	319.821**	4.800	262.069**	1.078	280.823**	0.358	112.94**	0.265	94.921**
Plant	25	0.105	2.042**	0.145	3.490**	0.034	3.913**	0.021	2.925**	0.010	1.606*
Flower	72	0.239	1.606**	0.168	1.404*	0.039	1.542**	0.038	1.832**	0.022	1.229 ns
Petal	432	0.616	0.691 ns	0.494	0.687 ns	0.121	0.805 ns	0.074	0.592 ns	0.077	0.703 ns
Error	538	1.111		0.896		0.188		0.155		0.137	
(B) Asymmetrical group											
Year	1	0.088	121.493**	0.019	60.963**	0.003	19.911**	0.001	11.600**	0.004	62.021**
Variety	11	0.042	5.362**	0.009	2.730**	0.014	7.730**	0.012	8.491**	0.007	9.210**
Plant	25	0.022	1.219 ns	0.009	1.168 ns	0.006	1.383 ns	0.004	1.407 ns	0.002	1.256 ns
Flower	72	0.054	1.036 ns	0.027	1.206 ns	0.013	1.095 ns	0.009	0.967 ns	0.005	0.952 ns
Petal	432	0.332	1.067 ns	0.135	1.023 ns	0.056	0.790 ns	0.041	0.747 ns	0.027	0.874 ns
Error	538	0.388		0.164		0.088		0.068		0.039	
Source				df		SS				F	
(C) Area											
Year				1		570188				608.418**	
Variety				11		3013929				292.364**	
Plant				25		140986				6.018**	
Flower				72		89088.6				1.320*	
Petal				432		31584.7				0.078 ns	
Error				538		504194					

df, degrees of freedom; SS, sum of squares; F, F-values.

* $P < 0.05$; ** $P < 0.01$; ns, not significant.

sometimes misses particular types of shape characteristics. For example, the petal of 'Kyoganoko' (5) is serrated, but none of the principal components could represent this feature. The elliptic Fourier descriptors for each individual petal can describe this feature, but because the phase of the serration does not coincide even within the same variety, the serration is cancelled as a characteristic by averaging within the variety. EF-PCA cannot handle this issue and it needs to be combined with another method, for example a measure called 'margin roughness' to measure small changes in direction of an outline (McLellan and Endler, 1998).

The nested ANOVA indicated significantly large varietal (i.e. genotypic) effects in the first, second and third principal components of the symmetrical group and in petal area (Table 3). It also indicated that the genotypic effects of the fourth and fifth principal components were moderately large. Organ shape is the result of a coordination of cell division and cell expansion, and several genes are known to alter these processes. In *Arabidopsis* leaves, genes that control the two-dimensional growth of leaves have been identified (Tsukaya, 1995; Tsuge *et al.*, 1996; Kim *et al.*, 1998). Tsukaya (1998) reported that the polar elongation of leaf cells in the lateral and longitudinal directions in *Arabidopsis* is controlled by two independently acting genetic systems. Similar genetic systems may regulate petal length and width in *P. sieboldii*. However, these two factors (i.e. the lateral and longitudinal directions) are not sufficient to explain all the variations detected by EF-PCA, especially by the fourth

and fifth principal components (Fig. 2). Kessler and Sinha (2004) reviewed recent mutant analyses about leaf development. They concluded that the genes that regulate the acquisition of stem-cell fate and meristem maintenance could have significant effects on leaf shape and size when the genes expressed ectopically in the leaf. In addition, Yamanaka *et al.* (2001) identified different QTLs for several shape characteristics in soybean leaflets. Uga *et al.* (2003) also performed a QTL analysis and identified different QTLs for several shape characteristics in rice glumes. Similarly, it is possible that many genes or loci are involved in the characteristics of petal shape of *P. sieboldii* detected in this study.

By contrast, the variations of all principal components of the asymmetrical group were related mainly to environmental effects (Table 3). In analyses of soybean leaves (Furuta *et al.*, 1995) and citrus leaves (Iwata *et al.*, 2002), the authors reported that the heritability of asymmetrical variation is low. However, there were significant differences at the 1 % level in some principal components of the asymmetrical group in this study. Van Valen (1962) grouped asymmetrical development in an organism, or part of an organism, into three categories: (1) directional asymmetry; (2) antisymmetry; and (3) fluctuating asymmetry. The asymmetrical variations of *P. sieboldii* petal shape in this study meant that the curvature or swell of one side of a petal was different from the other side, and thus these can be classified as antisymmetry or fluctuating asymmetry, or

TABLE 3. Proportions (%) of variance components for the principal components of (A) the symmetrical and (B) asymmetrical groups, and (C) petal area, due to four hierarchical sources estimated from nested ANOVA

Source	PC1	PC2	PC3	PC4	PC5
(A) Symmetrical group					
Variety	88.66**	85.57**	84.71**	72.81**	71.33**
Plant	0.36 ns	2.07**	2.18**	2.17*	0.86 ns
Flower	2.30**	2.14**	2.03**	7.38**	3.62**
Petal	8.67	10.22	11.08	17.64	24.19
(B) Asymmetrical group					
Variety	7.86**	3.17*	13.95**	16.10**	16.27**
Plant	1.04 ns	0.00 ns	1.90 ns	3.00 ns	1.87 ns
Flower	0.00 ns	3.34 ns	6.02*	4.51 ns	1.44 ns
Petal	91.09	93.49	78.13	76.39	80.42
(C) Area					
Variety					90.87**
Plant					4.47**
Flower					3.55**
Petal					1.11

* $P < 0.05$; ** $P < 0.01$; ns, not significant.

both. Mather (1953) first demonstrated experimentally the genetic control of fluctuating asymmetry for the lateral asymmetry of sternopleural chaeta number in *Drosophila*. Among plants, Sakai and Shimamoto (1965) and Bagchi *et al.* (1987) reported that there is a varietal or genotypic variation in the degree of asymmetry in tobacco leaves and teak leaves, respectively. In *P. sieboldii* petals, the degree of asymmetry could be inherited as it is in tobacco and teak leaves. For example, there may be a gene or genes related to the environmental response.

Environmental variation can be divided into two levels: general environmental variation, which arises from permanent or non-localized circumstances such as nutritional and climatic factors; and special environmental variation, which arises from temporary or localized circumstances operating during development (Falconer and Mackay, 1996; Ukai, 2002). In this study, we investigated the variation between two years, assuming the former level of variation. Moreover, we divided the latter into three hierarchical sources of the nested ANOVA, i.e. plant, flower and petal. Within each variety, the yearly variance of the symmetrical group was smaller than that of the petal area (Fig. 3). The yearly variance of the asymmetrical group was also small (data not shown). This pattern of differences between petal shape and area can be explained by the data in Table 2. The F -value by year for petal area is by far the largest of all variables. This indicates that petal shape is more stable and less influenced by the environment than petal area. The variations within a flower were largest in the special environmental variation of all the principal components for petal shape from both the symmetrical and asymmetrical groups (Table 3). The variation among plants was largest, and the variation within flowers was smallest, in petal area. These results indicate that petal shape variation is brought about mainly by developmental instability within a flower, which is considered to result from heterogeneity of the

internal micro-environment that occurred during developmental processes. Meanwhile, petal area is mainly influenced by the external macro-environment around each plant, such as fertility and weather conditions.

In conclusion, EF-PCA is an effective and powerful method for the quantitative evaluation of petal shape in *P. sieboldii*. Symmetrical and asymmetrical elements of the overall shape variation could be detected, and it was demonstrated that the major source of symmetrical shape variation is genotypic. The environmental shape variation of both symmetrical and asymmetrical elements is possibly due to developmental instability. The contribution of genotype to petal area is also large. However, petal area is affected by the environment more notably than is petal shape. The results confirm that petal shape is effective as an index for variety registration, as well as in genetic resource evaluation. However, petal area as such an index should be employed carefully, because the environmental effect on petal area is considerably larger than that on petal shape.

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LITERATURE CITED

- Bagchi SK, Sharma VP, Gupta PK. 1987. Development instability in leaves of *Tactona grandis*. *Silvae Genetica* 38: 1–6.
 Falconer DS, Mackay TFC. 1996. *Introduction to quantitative genetics*, 4th edn. Essex, UK: Longman.
 Freeman H. 1974. Computer processing of line drawing images. *Computing Surveys* 6: 57–97.

- Furuta N, Ninomiya S, Takahashi S, Ohmori H, Ukai Y. 1995. Quantitative evaluation of soybean (*Glycine max* L., Merr.) leaflet shape by principal component scores based on elliptic Fourier descriptor. *Breeding Science* **45**: 315–320.
- Iwata H, Nesumi H, Ninomiya S, Takano Y, Ukai Y. 2002. The evaluation of genotype × environment interactions of citrus leaf morphology using image analysis and elliptic Fourier descriptors. *Breeding Science* **52**: 243–251.
- Iwata H, Niikura S, Matsuura S, Takano Y, Ukai Y. 1998. Evaluation of variation of root shape of Japanese radish (*Raphanus sativus* L.) based on image analysis using elliptic Fourier descriptors. *Euphytica* **102**: 143–149.
- Iwata H, Ukai Y. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *The Journal of Heredity* **93**: 384–385.
- Kessler S, Sinha N. 2004. Shaping up: the genetic control of leaf shape. *Current Opinion in Plant Biology* **7**: 65–72.
- Kim GT, Tsukaya H, Uchimiya H. 1998. The *ROTUNDIFOLIA3* gene of *Arabidopsis thaliana* encodes a new member of the cytochrome P-450 family that is required for the regulated polar elongation of leaf cells. *Genes and Development* **12**: 2381–2391.
- Kuhl FP, Giardina CR. 1982. Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* **18**: 236–258.
- Mather K. 1953. Genetical control of stability in development. *Heredity* **7**: 297–336.
- McLellan T. 1993. The roles of heterochrony and heteroblasty in the diversification of leaf shapes in *Begonia dregei* (Begoniaceae). *American Journal of Botany* **80**: 796–804.
- McLellan T, Endler JA. 1998. The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology* **47**: 264–281.
- Ministry of Agriculture, Forestry and Fisheries of Japan. 2004. A characteristic table/characteristic examination standard. <http://www.hinsyu.maff.go.jp/english/index.htm>. 16 Jan. 2004.
- Ohsawa R, Tsutsumi T, Uehara H, Namai H, Ninomiya S. 1998. Quantitative evaluation of common buckwheat (*Fagopyrum esculentum* Moench) kernel shape by elliptic Fourier descriptor. *Euphytica* **101**: 175–183.
- Rohlf FJ, Archie JW. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (*Ritiera culicidae*). *Systematic Zoology* **33**: 322–317.
- Sakai K, Shimamoto Y. 1965. Developmental instability in leaves and flowers of *Nicotiana tabacum*. *Genetics* **51**: 801–813.
- SAS Institute Inc. 2000. *JMP statistics and graphics guide, version 4*. Cary, NC: SAS Institute Inc.
- Suzuka T. 1975. *Nihonsakurasou*. Tokyo: NHK-shuppan, 113–167 [in Japanese].
- Torii T. 1985. *Sakurasou*. Tokyo: Nippon Television Network Corporation, 114–123 [in Japanese].
- Toyohara H, Irie K, Ding W, Iwata H, Fujimaki H, Kikuchi F, Ukai Y. 2000. Evaluation of tuber shape of yam (*Dioscorea alata* L.) cultivars by image analysis and elliptic Fourier descriptors. *SABRAO Journal of Breeding and Genetics* **32**: 31–37.
- Tsuge T, Tsukaya H, Uchimiya H. 1996. Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh. *Development* **122**: 1589–1600.
- Tsukaya H. 1995. Developmental genetics of leaf morphogenesis in dicotyledonous plants. *Journal of Plant Research* **108**: 407–416.
- Tsukaya H. 1998. Genetic evidence for polarities that regulate leaf morphogenesis. *Journal of Plant Research* **111**: 113–119.
- Uga Y, Fukuta Y, Cai HW, Iwata H, Ohsawa R, Morishima H, Fujimura T. 2003. Mapping QTLs influencing rice floral morphology using recombinant inbred lines derived from a cross between *Oryza sativa* L. and *Oryza rufipogon* Griff. *Theoretical and Applied Genetics* **107**: 218–226.
- Ukai Y. 2002. *Genetic analysis of quantitative traits*. Tokyo: Igaku-shuppan Inc., 15–47 [in Japanese].
- Van Valen L. 1962. A study of fluctuating asymmetry. *Evolution* **16**: 125–142.
- White R, Rentine HC, Verwist T. 1988. Automated image acquisition and morphometric description. *Canadian Journal of Botany* **66**: 450–459.
- Yamanaka N, Ninomiya S, Hoshi M, Tsubokura Y, Yano M, Nagamura Y, Sasaki T, Harada K. 2001. An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. *DNA Research* **8**: 61–72.