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Developmental plasticity, morphological variation and evolvability: a multilevel analysis of morphometric integration in the shape of compound leaves

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Abstract

The structure of compound leaves provides flexibility for morphological change by variation in the shapes, sizes and arrangement of leaflets. Here, we conduct a multilevel analysis of shape variation in compound leaves to explore the developmental plasticity and evolutionary potential that are the basis of diversification in leaf shape. We use the methods of geometric morphometrics to study the shapes of individual leaflets and whole leaves in 20 taxa of *Potentilla* (sensu lato). A newly developed test based on the bootstrap approach suggests that uncertainty in the molecular phylogeny precludes firm conclusions whether there is a phylogenetic signal in the data on leaf shape. For variation among taxa, variation within taxa, as well as fluctuating asymmetry, there is evidence of strong morphological integration. The patterns of variation are similar across all three levels, suggesting that integration within taxa may act as a constraint on evolutionary change.

Introduction

The leaves of plants have a fantastic diversity of forms. Both genetic variation and phenotypic plasticity contribute to this variation, so that mutations have often dramatic effects on leaf morphology and even leaves on a single plant can have remarkably different forms (Jones, 1992; Kim *et al.*, 2003a,b; Tsukaya, 2005; Bensmihen *et al.*, 2008; Royer *et al.*, 2009). In particular, the modular architecture of compound leaves, where the leaf blade is subdivided into multiple leaflets, provides many opportunities for developmental variation and associated morphological differences. It is therefore not surprising that variation in the form of compound leaves is particularly rich. Developmental genetic studies are rapidly providing information about the mechanisms that regulate this diversity of forms (e.g. Kim *et al.*, 2001, 2003a; Blein *et al.*, 2008; Efroni *et al.*, 2010; Hasson *et al.*, 2010; Koenig & Sinha, 2010). Yet, many aspects of how the shapes of compound leaves evolve remain unclear.

Morphometric approaches are used more and more to investigate a wide range of questions in evolutionary and developmental biology (Klingenberg, 2010). A range of morphometric methods have been used by an increasing number of studies of leaf shape variation (e.g. Jensen, 1990; Jones, 1992; Jensen *et al.*, 2002; Meade & Parnell, 2003; Langlade *et al.*, 2005; Bensmihen *et al.*, 2008; Weight *et al.*, 2008; Feng *et al.*, 2009; Viscosi *et al.*, 2009a,b; Albarrán-Lara *et al.*, 2010). Most of these studies considered simple rather than compound leaves (but see Young *et al.*, 1995). Although the complex structure of compound leaves can be challenging for morphometric analyses, this complexity itself can be exploited to address evolutionary and developmental questions. Shape analyses of compound leaves include information on the shapes, relative sizes and arrangement of leaflets along the rachis of the leaf. To investigate whether this structure has an effect on evolutionary processes, parallel analyses can be conducted for whole leaves or individual leaflets.

Shape variation in the leaves and leaflets can be quantified to extract patterns of integration at multiple

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levels that can provide information about the origin of variation and its evolutionary potential (Klingenberg, 2010). This approach combines information about the shape variation among leaves or leaflets with data on variation at different levels, such as fluctuating asymmetry (Klingenberg, 2003a; Albarrán-Lara *et al.*, 2010) and comparative methods for studying the diversification of shape among related taxa (Miller & Venable, 2003; Sidlauskas, 2008; Klingenberg & Gidaszewski, 2010). Fluctuating asymmetry results from the developmental plasticity of leaves or leaflets and therefore can provide information about the developmental mechanisms that produce shape variation. The hypothesis that developmental processes are the principal determinants of morphological variation predicts agreement of the patterns of fluctuating asymmetry and individual variation within taxa (Klingenberg, 2008). The patterns of evolutionary integration among traits result from divergence among lineages by selection or drift. The hypothesis that constraints or biases are key determinants of evolutionary diversification (Schluter, 1996; Arthur, 2001) predicts that the patterns of variation within taxa and of diversification among taxa are congruent (Young & Badyaev, 2006; Hunt, 2007; Drake & Klingenberg, 2010). The combined analysis and comparison of variation at these different levels can provide information on the mechanisms responsible for phenotypic variation and evolutionary change, as has been demonstrated by studies in various animals (e.g. Klingenberg & McIntyre, 1998; Debat *et al.*, 2000; Willmore *et al.*, 2005; Young & Badyaev, 2006; Drake & Klingenberg, 2010; Ivanović & Kalezić, 2010; Klingenberg *et al.*, 2010; Jojić *et al.*, 2011). So far, however, this approach has not been used in studies of plants.

Here, we use this multilevel approach to investigate the patterns of morphological integration and allometry for shape variation in compound leaves. We consider three levels of variation of leaflets and whole leaves: evolutionary divergence among taxa, variation within taxa and fluctuating asymmetry (Klingenberg, 2008, 2010). We use the methods of geometric morphometrics to characterize shape variation (Dryden & Mardia, 1998; Zelditch *et al.*, 2004; Klingenberg, 2010). We demonstrate this approach in an analysis in 20 taxa of cinquefoils (*Potentilla* and related genera, Rosaceae). Because the molecular phylogeny of *Potentilla* is poorly resolved and contains some inconsistencies (Dobeš & Paule, 2010; Töpel *et al.*, 2011), we use a new method based on the bootstrap (Felsenstein, 1985a) to incorporate an assessment of phylogenetic uncertainty into tests of phylogenetic signal in morphometric data (Klingenberg & Gidaszewski, 2010). Comparisons of the patterns of variation across the different levels of variation address the question whether developmental plasticity defines the patterns of morphological integration within taxa and whether that integration acts as a constraint in the process of evolutionary diversification. We discuss the roles of plasticity and constraints in the evolution of leaf shape and relate the morphometric findings to information from developmental biology.

Materials and methods

Data collection

Leaves of 20 taxa of *Potentilla* and related genera were collected in botanical gardens (Royal Edinburgh Botanic Garden; Botanic Garden of the University of Freiburg; Royal Botanic Gardens, Kew). The taxa were chosen to represent a range of leaf shapes, including both palmate and pinnate leaves (Fig. 1). Most taxa are distinct species, but two (Gibson's Scarlet and Yellow Queen) are cultivars of *P. atrosanguinea* and a further one (*P. goldbachii*) turned out to be a synonym of *P. thuringiaca* (it can thus be considered a distinct accession for this species).

To avoid problems concerning the homology of leaflets, this study is limited to taxa with leaves that consist of five leaflets, excluding taxa with fewer or more leaflets per leaf (five is the most widespread number of leaflets in *Potentilla*). This study therefore excludes the discontinuous variation associated with differences in leaflet number. Except for this limitation, the taxa in our sample represent all the main features of leaf variation across *Potentilla* s. l. (Wolf, 1908).

As far as possible, rosette leaves from the outer whorl were collected. For each taxon, 10 leaves were collected, two each from five plants. Leaves were glued on paper, and landmarks were digitized from images obtained using a Nikon D3100.

For each leaf, the shape of the whole leaf was characterized by a set of 25 landmarks that indicate the relative sizes, approximate shapes and positions of the five leaflets (Fig. 2a). For each leaflet, landmarks were located at the base and the tip of the leaflet lamina and at the most basal tooth of the serrated part of the leaflet margin on each side. In addition, the insertion points of the petiolules of the leaflets (except the distal one) to the rachis and a landmark at the base of the petiole were also included. For palmate leaves, the landmarks at the insertion points of leaflet petiolules are located in the same point.

For 15 of the taxa (see Supporting Information), the shapes of the distal and lateral (basal) leaflets were studied in more detail. For each leaflet, a set of 15 landmarks was digitized (Fig. 2b). The landmarks are located as pairs on both margins: at the base of the leaflet, at the point where the leaflet widens to form the lamina, at the tips and in the notches associated with the first two teeth of the serrated part of the leaflet margin, at a level half-way between the second tooth and the tip of the leaflet and, finally, a single landmark at the tip of the leaflet (Fig. 2b).

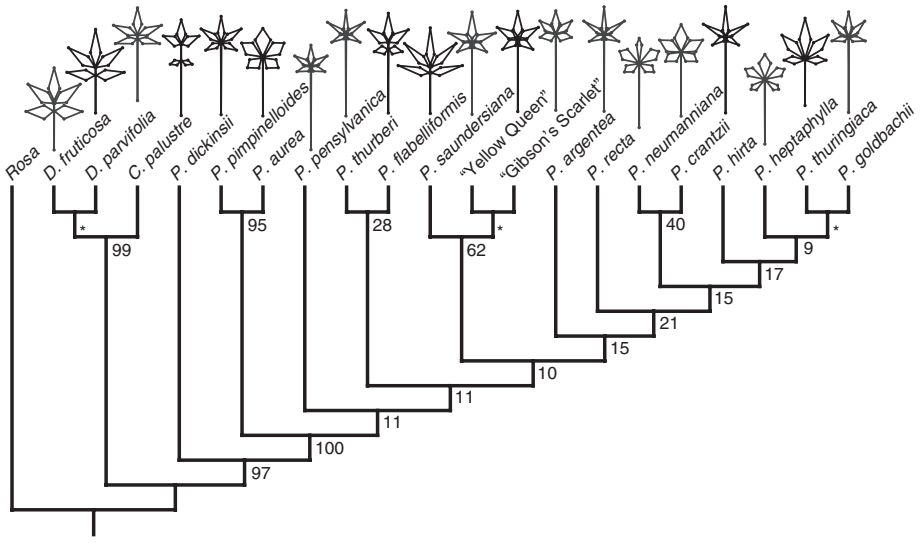

Fig. 1 Phylogenetic tree of the species included in this study and mean leaf shapes. The tree is the maximum-likelihood tree for the original sequence data, and numbers at the nodes indicate the bootstrap support of the corresponding nodes (as a percentage of the 200 bootstrap replicates; asterisks indicate sister group relationships that were assumed based on taxonomic information). The diagrams of leaf shapes show the mean of the symmetric component of shape variation, based on the landmarks included in this study. It should be noted that the diagrams have been scaled to the same centroid size, but that there also are large interspecific differences of leaf size.

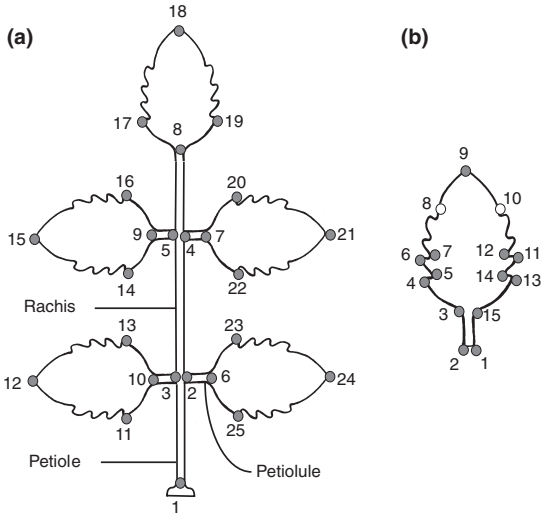

Fig. 2 Landmarks measured for the whole leaves (a) and leaflets (b). (a) For each leaf, a set of 25 landmarks characterizes the entire leaf (note that the diagram shows a pinnate leaf – for a palmate leaf, the rachis is shortened so that all five leaflets originate from a single point at the end of the petiole). (b) For the distal and one of the proximal lateral leaflets, more detailed data were collected, with 15 landmarks per leaflet.

Phylogeny

Classification of the genus *Potentilla*, long based on morphological traits (Wolf, 1908), has recently been revised in the light of new phylogenetic information (Eriksson *et al.*, 2003; Lundberg *et al.*, 2009; Dobeš & Paule, 2010; Töpel *et al.*, 2011). In particular, a number of species have been reclassified from the traditional *Potentilla* s.l. to distinct genera, such as *Comarum* and *Dasiphora*. Unfortunately, the most detailed phylogenies of *Potentilla* s.l. contain a large and mostly unresolved clade, called 'core *Potentilla*' (Dobeš & Paule, 2010) or 'Argentea clade' (Töpel *et al.*, 2011), which includes most of the species considered in this study. There also appears to be incongruence between nuclear and chloroplast sequences and differences in ploidy within and between species, which may reflect hybridization events (Goldblatt & Johnson, 1979; Lundberg *et al.*, 2009; Töpel *et al.*, 2011).

To obtain a better phylogenetic resolution for the species in our study, we ran a detailed phylogenetic analysis of the species in described here and used *Rosa majalis* as an outgroup to root the tree. Three chloroplast DNA sequences ($trnL^{uaa}$-$trnF^{gaa}$ IGS, $trnS^{uga}$-*ycf9* IGS and $trnC^{gca}$-*ycf6* IGS) and two nuclear sequences (ITS 5.8S rRNA gene and ETS 18S rRNA) were individually aligned using ClustalX (Larkin *et al.*, 2007) and concatenated to form an alignment of 2992 nucleotides, which was used for subsequent analysis. A phylogenetic tree (Fig. 1) was estimated using RAxML (Stamatakis *et al.*, 2008) under a model that partitioned the data into chloroplast and nuclear genes and allowed a different GTR + Γ model parameters and branch lengths for each partition. (Full details of phylogenetic analysis and model choice are provided in Appendix S1.) Uncertainty in the phylogenetic tree was assessed using a nonparametric bootstrap with 200 replicates (Felsenstein, 1985a; Stamatakis

et al., 2008), and these trees were stored for use in later analyses.

Because no sequence data were available for some taxa, some of the relationships had to be inferred from other sources. *Dasiphora parvifolia* was entered as sister taxon to *D. fruticosa*, assuming monophyly of the genus *Dasiphora*. Because *P. goldbachii* is a synonym of *P. thuringiaca*, we treated the corresponding accessions as sister taxa. Likewise, we treated the two cultivars of *P. atrosanguinea* as sister taxa.

Morphometric analysis

The morphometric analyses in this study are based on a definition of shape as all geometric aspects of a configuration of landmarks, except its size, position and orientation. Shape information is extracted from the landmark coordinates in a Procrustes superimposition (Rohlf & Slice, 1990; Goodall, 1991; Dryden & Mardia, 1998). The coordinates of the superimposed landmark configurations are then used in multivariate analyses to address specific biological questions.

Both the whole leaves and leaflets are bilaterally symmetric about their respective central axes (petiole/rachis or leaflet midrib). To take into account this symmetry and to gain information about asymmetry of shape, we used the method of shape analysis for object symmetry (Klingenberg *et al.*, 2002). This method uses the landmark configurations and their reflected copies (with paired landmarks relabelled) in a joint Procrustes superimposition (Dryden & Mardia, 1998). A symmetric component of shape variation is obtained from the averages of original and reflected copies, and an asymmetric component is computed from the differences between original and reflected copies (Klingenberg *et al.*, 2002).

To visualize and test the separation of leaf shapes among taxa, we used canonical variate analysis (CVA) of the symmetric component of shape variation (Mardia *et al.*, 1979; Campbell & Atchley, 1981). CVA maximizes the differences between taxa relative to the variation within taxa and is therefore the most efficient method for detecting separation among taxa. The statistical significance of pairwise differences in mean shapes was assessed with permutation tests using Mahalanobis distance as the test statistic (10 000 permutations per test).

To display the main patterns of variation in shape space, we used principal component analysis (PCA; e.g. Jolliffe, 2002). PCA, unlike CVA, does not distort the shape space and is therefore most suitable to display these patterns. The PCs are axes that successively maximize the variation for which they account, subject to the constraint that each PC must be orthogonal to all preceding PCs. The shape changes that correspond to the directions of the PCs in shape tangent space can be displayed graphically.

The phylogenetic signal in the shape data was studied by mapping the shape data onto the phylogeny (Fig. 1) using squared-change parsimony (Klingenberg & Ekau, 1996; Miller & Venable, 2003; Sidlauskas, 2008; Klingenberg & Gidaszewski, 2010). Because branch lengths were not available for all the branches in the phylogeny, we used unweighted squared-change parsimony (Maddison, 1991). As a test for the presence of phylogenetic signal in the data, we used a permutation test that simulates the null hypothesis that there is no phylogenetic signal by randomly exchanging the shapes among terminal nodes of the phylogeny (Klingenberg & Gidaszewski, 2010). We ran this test with 10 000 rounds of permutation per test.

To take into account the considerable uncertainty in the estimated phylogeny (see bootstrap support values in Fig. 1), we used a new method based on the bootstrap. The bootstrap resampling provides an estimate of the statistical uncertainty under a process of random sampling from the data (DNA sequences in this case; Felsenstein, 1985a; Efron & Tibshirani, 1993). The variation in the outcome of analyses on the resulting bootstrap trees indicates the effects of uncertainty in the estimated phylogeny. Specifically, we used all 200 trees from the bootstrap analysis, mapped the shape data onto each tree and ran the permutation test for phylogenetic signal (with 250 rounds of permutation per tree). The resulting distribution of *P*-values provides an indication whether the results of the test are robust with regard to the statistical uncertainty of the estimated phylogenetic tree. This method is implemented in the MorphoJ software package (Klingenberg, 2011). For the shape of the whole leaves, where the permutation test provided some evidence against the null hypothesis of no phylogenetic signal, we computed independent contrasts of the shape and centroid size data (Felsenstein, 1985b).

We carried out PCAs at several different levels: among the average shapes of the species or independent contrasts (corresponding to evolutionary change), the pooled within-taxon covariance matrix of the symmetric component of variation (a common estimate for shape variation among leaves within the samples) and for fluctuating asymmetry (variation within each leaf; Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2002). To avoid confounding effects from differences in directional asymmetry among taxa, the PCA for fluctuating asymmetry used the pooled within-taxon covariance matrix of the asymmetric component of variation.

To quantify the similarity of covariance structures at the different levels, we computed the matrix correlations between the corresponding covariance matrices. Because the relative amounts of variation at different landmarks are an important aspect of the covariance structure, we included the diagonal blocks of variances and covariances of coordinates of each landmark in the calculation of the matrix correlation (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2002). Matrix correlations were tested

with a matrix permutation test, as adapted for geometric morphometrics, permuting the order of landmarks and not individual coordinates (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2002).

Allometry was analysed by multivariate regression of shape on size (Monteiro, 1999). We used log-transformed centroid size as a measure of size because preliminary analyses showed that it resulted in much better linear relationships than untransformed centroid size. The statistical significance of the regressions was assessed with permutation tests with 10 000 iterations for each test. To visualize allometric relationships, we plotted the regression scores (the projection of shape data onto the direction of the regression vector in the shape tangent space; Drake & Klingenberg, 2008). All morphometric analyses were carried out with the MorphoJ software package (Klingenberg, 2011).

Results

Phylogeny

The maximum-likelihood tree contains several branches with high bootstrap support, but many others have weak support, indicating substantial uncertainty concerning a large portion of the tree (Fig. 1). This agrees with previous reports of a poorly resolved 'core *Potentilla*' (Dobeš & Paule, 2010) or 'Argentea' clade (Töpel *et al.*, 2011).

Analysis of whole leaves

The CVA indicates that all taxa and accessions included in the analysis are clearly distinct from each other (Fig. 3). Mahalanobis distances range from 3.26 (*P. goldbachii* vs. *P. pensylvanica*) to 25.10 (*P. dickinsii* vs. *P. neumanniana*), and all permutation tests indicate that mean shapes differ significantly among taxa (all $P < 0.0001$ in pairwise permutation tests between taxa). The Procrustes distances range from 0.0616 (*P. recta* vs. *P. hirta*) to 0.6136 (*D. parvifolia* vs. *P. pensylvanica*), and most are thus quite large shape differences. The scatter plot of canonical variate (CV) scores shows a gradient of pinnate to palmate leaves along the CV1, with a marked concentration of palmate groups at high CV1 scores (Fig. 3, horizontal axis). The CV2 contrasts, at one extreme, leaves with long petioles and narrow leaflets where the serrated edge extends from the tip along most

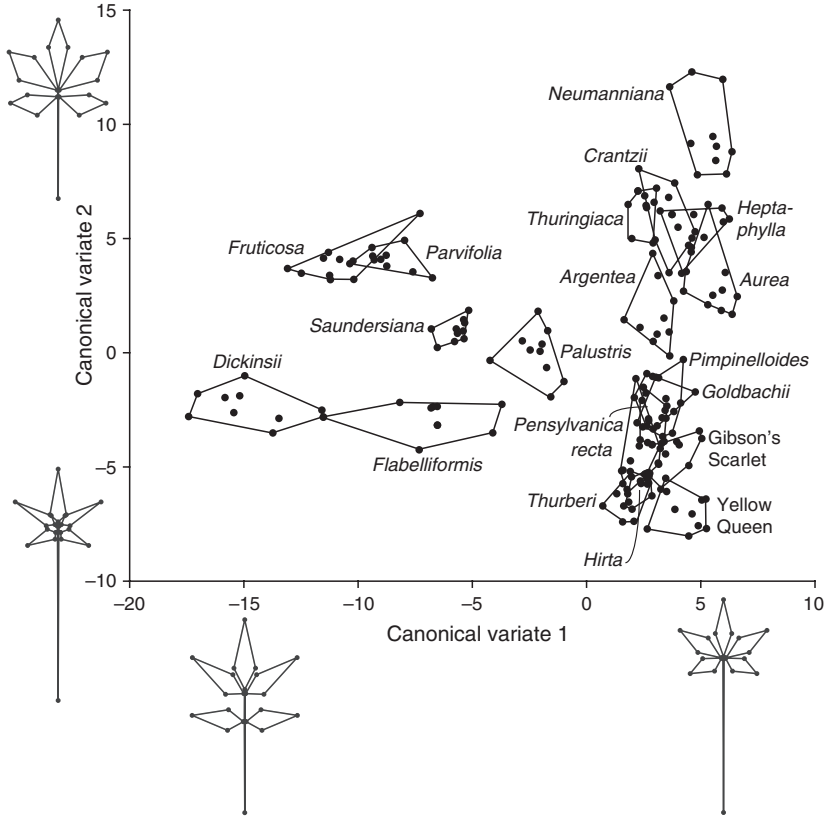

Fig. 3 Canonical variate analysis of compound leaf shape. The diagrams of leaf shapes show the shapes for CV1 scores of −15 and +5 and for CV2 scores of −8 and +10 (all other CV scores kept at value 0).

of the length of the leaflets and, at the other extreme, leaves with broad leaflets where only the distal part is serrated (Fig. 3, vertical axis).

To visualize the phylogenetic history of shape change in the leaves, we projected the phylogeny (Fig. 1) into the shape tangent space by squared-change parsimony and plotted the resulting tree in the plane of the first two PCs of the taxon mean shapes (Fig. 4a). Closely related taxa are not necessarily close to each other in shape space and some remotely related taxa have similar leaf shapes, so that there are long branches that criss-cross the plot.

The permutation test for a phylogenetic signal in the symmetric component of leaf shape variation yielded a *P*-value of 0.059. This means that the test is not statistically significant according to the conventional threshold of 0.05 but still provides some evidence against the null hypothesis that variation of leaf shape is unrelated to phylogeny. The uncertainty in estimating the phylogeny is an important factor for the tests of the phylogenetic signal. For the 200 bootstrap trees, the *P*-values of the permutation test ranged from 0.004 to 0.36 (median 0.10). Because of this uncertainty, we conducted analyses of evolutionary change in leaf shape both with independent contrasts, accounting for phylogenetic structure, and based on the mean shapes of taxa.

In the PCA of the variation among the 20 taxa means, the PC1 accounts for more than 60% and is therefore clearly the dominant pattern of diversification of leaf shapes (Fig. 4b). The shape change associated with it is a contrast between the relative length of the petiole and the sizes of the leaflets (and also the spacing of the basal pair of leaflets relative to the other leaflets). In contrast, the PC2 mainly represents a concerted change in the shapes of the leaflets, concerning their relative length vs. width, how far the serrated part of the leaf margin extends towards the base and, to some extent, the orientation of the leaflets on either side of the leaf. The PCA of independent contrasts of leaf shapes gave very

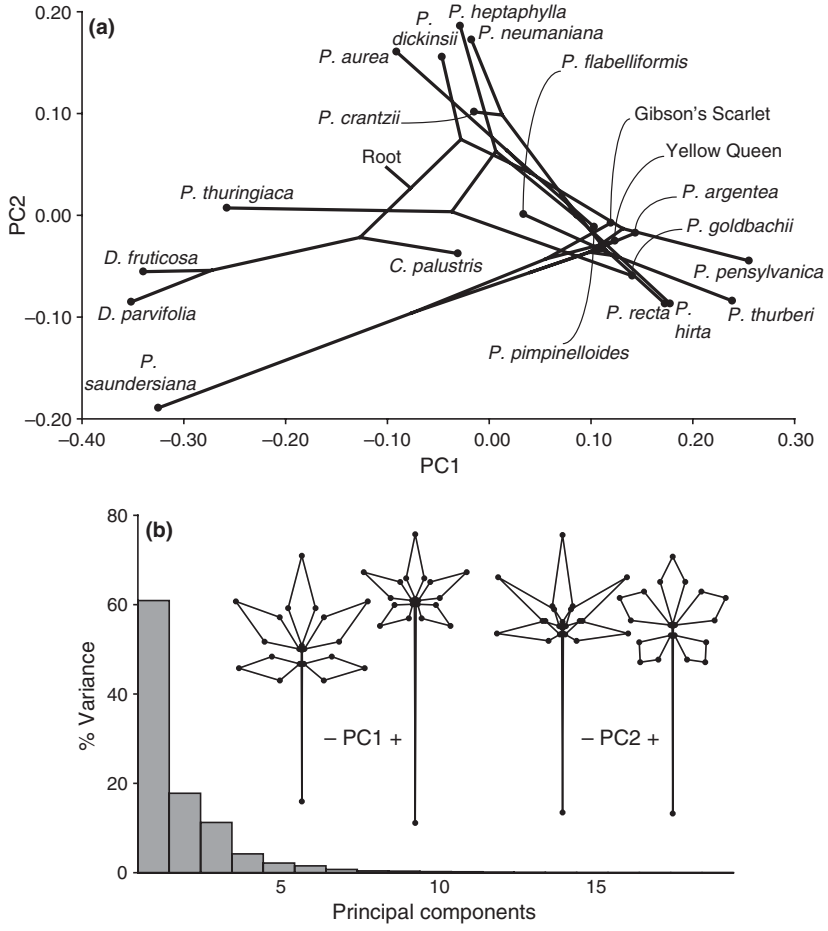

Fig. 4 Principal component analysis (PCA) of diversification in compound leaf shape. The PCA is based on the covariance matrix among the taxon means and therefore represents the evolutionary changes in shape. (a) Projection of the phylogeny (Fig. 1) onto the shape space, as represented by the first two PCs. (b) Percentages of the total variance for with the PCs account and the shape changes associated with the first two PCs. For each PC, the left and right diagrams represent the shapes for PC scores of −0.2 and 0.2, respectively.

similar results, in terms of both the shape changes associated with the PC1 and PC2 and the amounts of variation taken up by the PCs (Fig. 5a).

To examine whether these patterns of diversification may relate to patterns observed within taxa, we used a PCA of the pooled within-taxon covariance matrix, which is a joint estimate of the covariance structure within taxa (Fig. 5b). The PC1, which accounts for just under half the total variance, is associated with a pattern of shape change that is primarily a contrast between relative petiole length and the size of all leaflets. The PC2 corresponds to a shape change that concerns the orientation of the leaflets on either side of the main axis. Many aspects of these shape changes relate to features seen in the corresponding PCs for divergence among taxa (Figs 4b and 5a). As a measure of overall similarity of the covariance structures within and among taxa, we computed the matrix correlations between the pooled within-taxa covariance matrix and the covariance matrices among taxon means and among independent contrasts of leaf shapes. Both matrix correlations were 0.91 and therefore very strong, and the matrix permutation tests were highly significant ($P < 0.0001$).

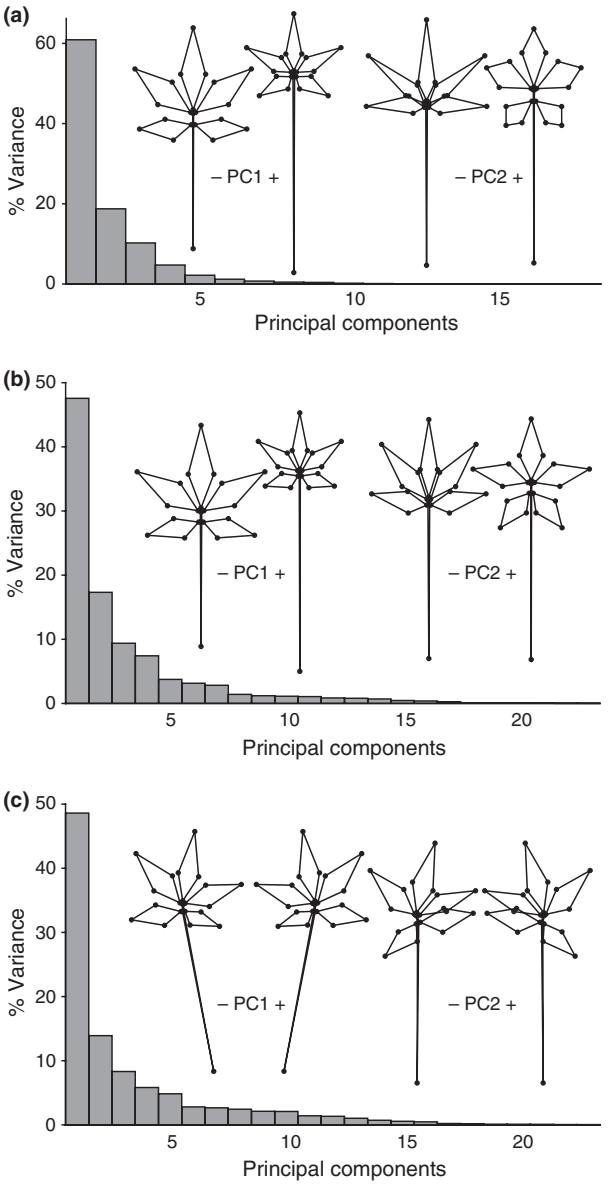

Fig. 5 Principal component analysis (PCA) of within-taxon variation and fluctuating asymmetry of leaf shape. (a) PCA of the independent contrasts of the symmetric component of leaf shape variation. (b) PCA of the pooled within-taxon covariance matrix of the symmetric component of leaf shape variation. (c) PCA of the covariance matrix for fluctuating asymmetry of leaf shape. Bar charts indicate the percentages of the total variance for with the PCs account, and the leaf diagrams show shape changes associated with the first two PCs. For each PC, the left and right diagrams represent the shapes for PC scores of −0.2 and 0.2, respectively.

The PCA for fluctuating asymmetry shows that it is concentrated in relatively few dimensions, with just under half of the total variance in the PC1 alone (Fig. 5b). The PC1 combines a bending of the leaf and petiole with differences in the relative sizes of leaflets on either side of the main leaf axis (the lateral leaflets are smaller on the inside of the bend than on the outside). The PC2 mostly features asymmetry in the orientation of the lateral leaflets (Fig. 5b). The matrix correlation between the covariance matrix for fluctuating asymmetry and the pooled within-taxon covariance matrix for the symmetric component of variation is 0.57 ($P < 0.0001$). The covariance matrix for fluctuating asymmetry is less similar to the pattern of divergence among taxa. The matrix correlation with the covariance matrix among taxon averages is 0.38 ($P = 0.0065$), and the matrix correlation with the covariance matrix for independent contrasts is 0.39 ($P = 0.0048$).

To examine whether allometry influences the patterns of variation, we regressed the independent contrasts of shape on the independent contrasts of log-transformed centroid size. The multivariate regression shows a clear relationship (Fig. 6a) that accounts for 51.4% of the shape variation and is statistically significant ($P < 0.0001$). It thus indicates strong evolutionary allometry. The shape change associated with evolutionary allometry is a relative lengthening of the petiole and a slight apical shift of the basal pair of leaflets with increasing size. The pooled within-taxon regression accounts for 10.1% of the shape variation within taxa and is statistically significant ($P < 0.0001$; Fig. 6b). This within-taxon allometry is associated with a shape change that is similar to that of evolutionary allometry, except for the basal pair of leaflets that shift towards the leaf base with increasing size (Fig. 6b). We used this within-taxon allometry to correct for the effects of size in the data by computing residuals. The results of the PCAs for these size-corrected data are similar to the analyses presented above, but the dominance of the PC1 in the

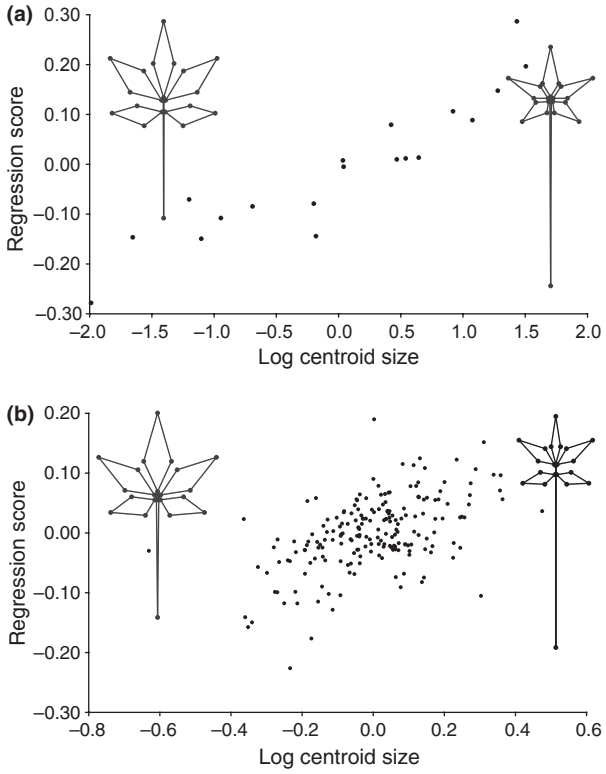

Fig. 6 Allometry of the leaves. (a) Evolutionary allometry, as characterized by the regression of the independent contrasts of the symmetric component of shape variation on independent contrasts of log-transformed centroid sizes (the leaf diagrams show the predicted shape change for an increase in log-transformed centroid size by 4 units, from −2 to 2 on the horizontal axis). (b) Allometry within taxa, characterized by a pooled within-group regression of shape of log-transformed centroid size (the leaf diagrams show the predicted shape change for an increase in log-transformed centroid size by 2 units). The scatter plot in (b) shows the individual deviations from the taxon means of shape and log-transformed centroid size.

analyses is less extreme. Importantly, the high and statistically significant matrix correlations between the different levels of variation remain almost the same.

Analysis of leaflets

Projecting the phylogenetic tree into plots of the first two PCs of the taxon means for the distal and lateral leaflets (Fig. 7a,b) shows that there are many branches that are relatively long and cross extensively (more so for the lateral leaflet, Fig. 7b, than for the distal leaflet, Fig. 7a). The permutation test for phylogenetic signal is nonsignificant for the distal leaflet ($P = 0.19$) and the lateral leaflet ($P = 0.56$). For the distal leaflet, *P*-values of the permutation test with the 200 bootstrap ML trees range from <0.004 to 0.83 (median 0.15) and therefore underscore that phylogenetic uncertainty makes a difference for the interpretation of the phylogenetic signal. In contrast, the *P*-values for the lateral leaflet range from 0.12 to 0.88 (median 0.42) and are therefore nonsignificant for all 200 bootstrap trees.

The PCAs of the taxon mean shapes show that the PC1s take up most of the variation – about 70% of the total variance (Fig. 7c,d). Comparing the shape changes associated with the first two PCs shared features in the analyses of the distal and lateral leaflets. The PC1s are associated with variation in the relative sizes of the proximal region (from the base to the first two teeth of the serrate margin) vs. the distal region. The PC2s combine variation between a more slender or broader leaflet shape with variation in the depth of the serration of the margin, but the direction of the association is different for the distal and lateral leaflets. Overall, the covariance structures for evolutionary divergence in both leaflets are similar, as indicated by the matrix correlation of 0.85 ($P < 0.0001$ in the matrix permutation test).

The patterns of within-taxon variation of the symmetric component of shape variation are quite similar to the patterns of variation among taxa, as can be seen by comparing the shape changes associated with the first two PCs for the distal (cf. Figs 7a and 8a) and for the lateral leaflet (cf. Figs 7b and 8b). Also, the PC1 is dominant and accounts for more than 60% of the total variance in the within-taxon PCAs for both leaflets (Fig. 8a,b). The matrix correlation between the within- and among-taxon covariance matrices is 0.96 ($P < 0.0001$) for the distal leaflet and 0.89 ($P < 0.0001$) for the lateral leaflet. Furthermore, the patterns of integration in the distal and lateral leaflets are also closely related, as indicated by the matrix correlation of 0.90 between the pooled within-taxon covariance matrices for the symmetric component of shape variation ($P < 0.0001$).

The PCAs of fluctuating asymmetry (computed from the pooled within-taxon covariance matrix of the asymmetric component of shape variation) feature asymmetric shifts of the serrations of the leaflet margin towards or away from the leaflet base, as well as lateral expansions or contractions that result in bending of the leaflet to one or the other side (Fig. 8c,d). There are also apparent correspondences between these patterns of asymmetry and those for the symmetric component of variation within taxa (compare the differences between the left and right sides of the leaflet diagram in Fig. 8c,d to the differences between the positive and negative scores of the corresponding PC in Fig. 8a,b). These similarities of symmetric variation and fluctuating asymmetry also are reflected in the matrix correlations between the covariance matrices for symmetric within-taxon variation and fluctuating asymmetry, which are 0.55 ($P = 0.0008$) for the distal leaflet and 0.48 ($P = 0.0016$) for the lateral leaflet. The covariance matrices of fluctuating asymmetry of the distal and lateral leaflets are very similar. Matrix correlation between them is 0.94 ($P < 0.0001$).

As in the analysis for the whole leaf, allometry is an important factor for the variation of leaflet shapes. In the

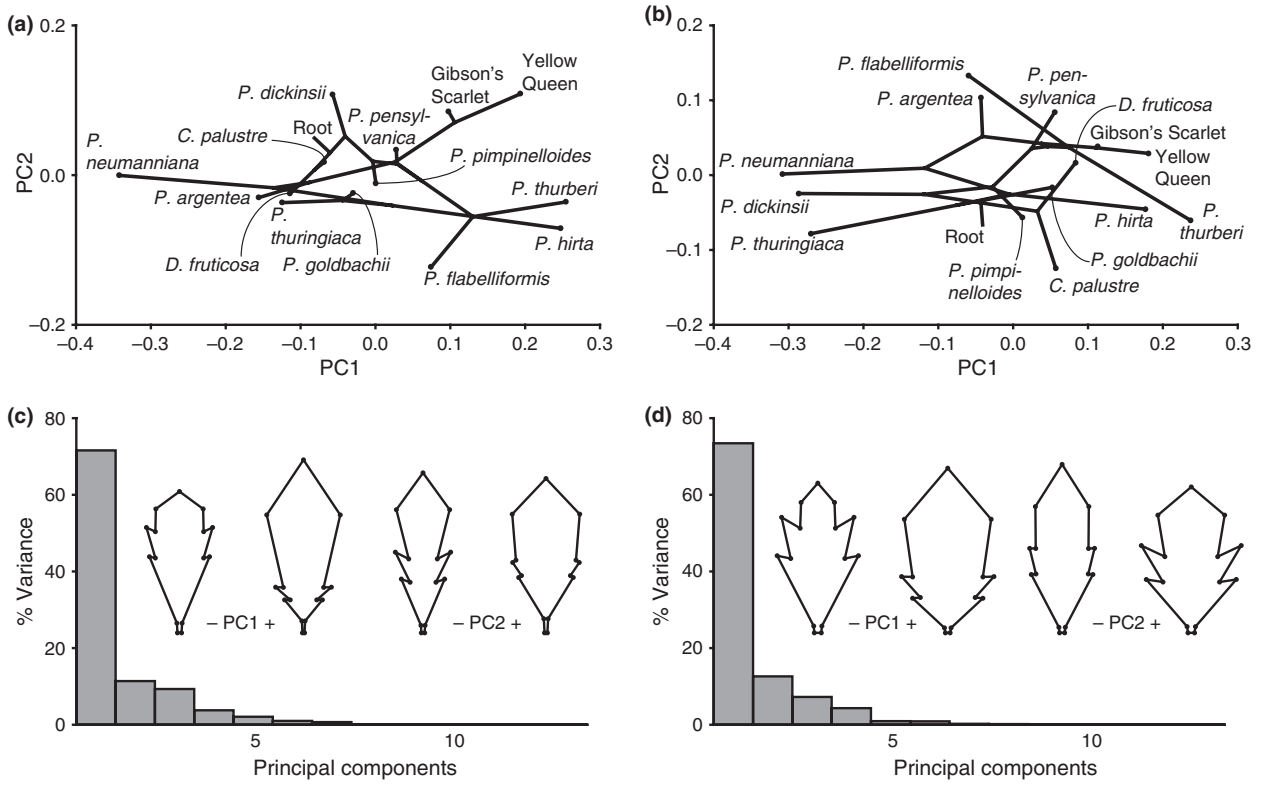

Fig. 7 Principal component analysis (PCA) of diversification in leaflet shape. These PCAs are based on the covariance matrices of the mean leaflet shapes of the taxa. (a) Projections of the phylogenies into shape space for the distal leaflets. (b) Projections of the phylogenies into shape space for the lateral leaflets. (c) Percentages of the total variance for with the PCs account and the shape changes associated with the first two PCs for the distal leaflets. For each PC, the left and right diagrams represent the shapes for PC1 scores of −0.3 and 0.3 and for PC2 scores of −0.1 and 0.1, respectively. (d) Percentages of the total variance for with the PCs account and the shape changes associated with the first two PCs for the lateral leaflets. For each PC, the left and right diagrams represent the shapes for PC1 scores of −0.25 and 0.25 and for PC2 scores of −0.1 and 0.1, respectively.

analyses for evolutionary allometry, regressions of species average shapes on log-transformed centroid size are statistically significant and account for 41.2% of the total shape variation in the distal leaflet ($P = 0.0017$; Fig. 9a) and for 39.8% in the lateral leaflet ($P = 0.0045$; Fig. 9b). Allometric shape changes associated with increasing size feature shifts of the two basal teeth of the serrated margin towards the leaflet base and relative expansions of the apical part of the leaflet (Fig. 9a,b). Pooled within-taxon regressions of leaflet shape on log-transformed centroid size account for 3.6% of the shape variation within taxa in the distal leaflet ($P = 0.0053$; Fig. 9c) and for 3.5% in the lateral leaflet ($P = 0.0029$; Fig. 9d). The allometric shape changes for the pooled within-taxon regression are similar to the corresponding shape changes for evolutionary allometry (compare Fig. 9a, c and 9b, d).

Discussion

This study has used a multilevel approach to investigate the variation of leaf shapes in cinquefoils and their evolution (Fig. 1). The results are broadly similar for the analyses of the whole leaf and of the distal and lateral leaflets and show a substantial degree of developmental plasticity and evolvability, combined with strong integration.

CVA of leaf shapes shows that most taxa are clearly separated from each other (Fig. 3; CVAs of the leaflets yield similar results, not shown). Many large shape differences between taxa (Figs 4 and 7) underscore that there is a substantial evolutionary potential in the shapes of the whole leaf and single leaflets. This result is consistent with earlier analyses that found that taxa in *Potentilla* can be distinguished with morphological traits (Hansen *et al.*, 2000), but also may relate to the considerable phenotypic plasticity found in various traits of *Potentilla* species (Huber, 1996; Stuefer & Huber, 1998).

Given this potential for evolutionary change, it may appear surprising that our analyses did not find a clear phylogenetic signal (marginally nonsignificant permutation test for the whole leaf and clearly nonsignificant tests for both leaflets). It is unlikely that these nonsignificant results are due to insufficient statistical power,

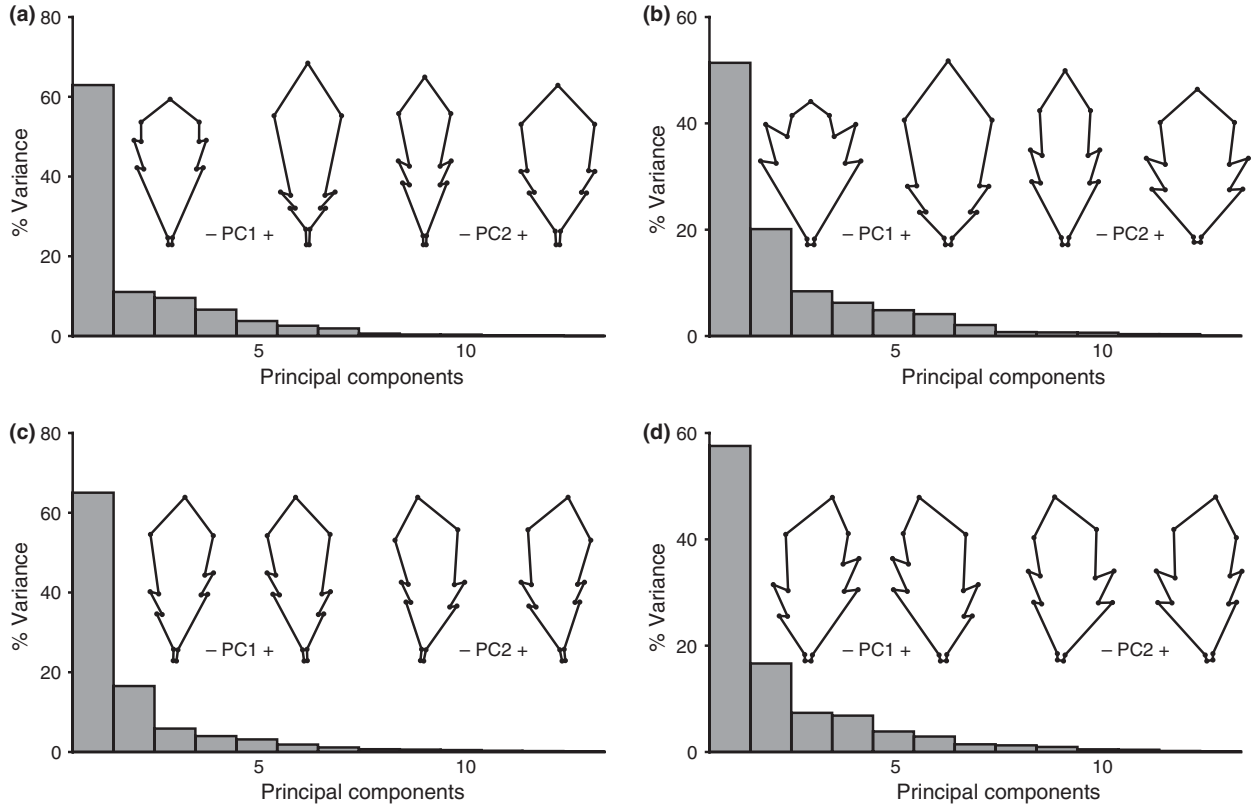

Fig. 8 Principal component analysis (PCA) of within-taxon variation and fluctuating asymmetry of shape of the distal and lateral leaflets. (a) PCA of the pooled within-group covariance matrix for the symmetric component of shape variation in the distal leaflet. The bar charts indicate the percentages of the total variance for with the PCs account, and the leaflet diagrams show shapes associated with PC1 scores of −0.3 and 0.3 and with PC2 scores of −0.1 and 0.1, respectively. (b) PCA of the pooled within-group covariance matrix for the symmetric component of shape variation in the lateral leaflet. The leaflet diagrams show shapes associated with PC1 scores of −0.3 and 0.3 and with PC2 scores of −0.1 and 0.1, respectively. (c) PCA of the pooled within-group covariance matrix for the asymmetric component of shape variation in the distal leaflet (i.e. fluctuating asymmetry). The leaflet diagrams show shapes associated with PC1 scores of −0.15 and 0.15 and with PC2 scores of −0.1 and 0.1, respectively. (d) PCA of the pooled within-group covariance matrix for the asymmetric component of shape variation in the lateral leaflet (i.e. fluctuating asymmetry). The leaflet diagrams show shapes associated with PC1 scores of −0.2 and 0.2 and with PC2 scores of −0.1 and 0.1, respectively.

because other studies with smaller numbers of taxa and less shape variation yielded significant results (Figueirido *et al.*, 2010; Klingenberg & Gidaszewski, 2010). Uncertainty in estimating phylogeny, however, appears to play a major role. For both the whole leaf and the distal leaflet, the permutation tests of phylogenetic signal for the 200 bootstrap trees yield *P*-values ranging from very significant to clearly nonsignificant. Within the margins of uncertainty due to sampling of the DNA sequences, there are therefore trees that are associated with a significant match to these morphometric data and others that are not. Results of the permutation test for the ML tree for the original sequence data (Fig. 1) should therefore be interpreted with caution. The situation is different for the lateral leaflet, however, where the permutation tests both for the tree for the original sequence data and for all 200 bootstrap trees produce nonsignificant *P*-values. Therefore, regardless of phylogenetic uncertainty, there is no indication of any phylogenetic signal for the shape of the lateral leaflet.

Uncertainty in estimating phylogeny is one possible cause of the lack of a phylogenetic signal, but it is not the only one. In *Potentilla* and its relatives, hybridization and polyploidization have been reported and may be widespread (e.g. Goldblatt & Johnson, 1979; Lundberg *et al.*, 2009; Töpel *et al.*, 2011). Such events may cause abrupt changes in morphological traits and therefore may be a factor for the evolution of leaf shape. Also, a phylogenetic tree cannot accurately represent an evolutionary history that includes hybridization events and may therefore produce a nonsignificant phylogenetic signal. Furthermore, vegetative parts such as leaves have long been known to have a high degree of phenotypic plasticity and evolutionary malleability and therefore may provide little information on phylogenetic relatedness (e.g. Darwin, 1859). Unfortunately, with the available

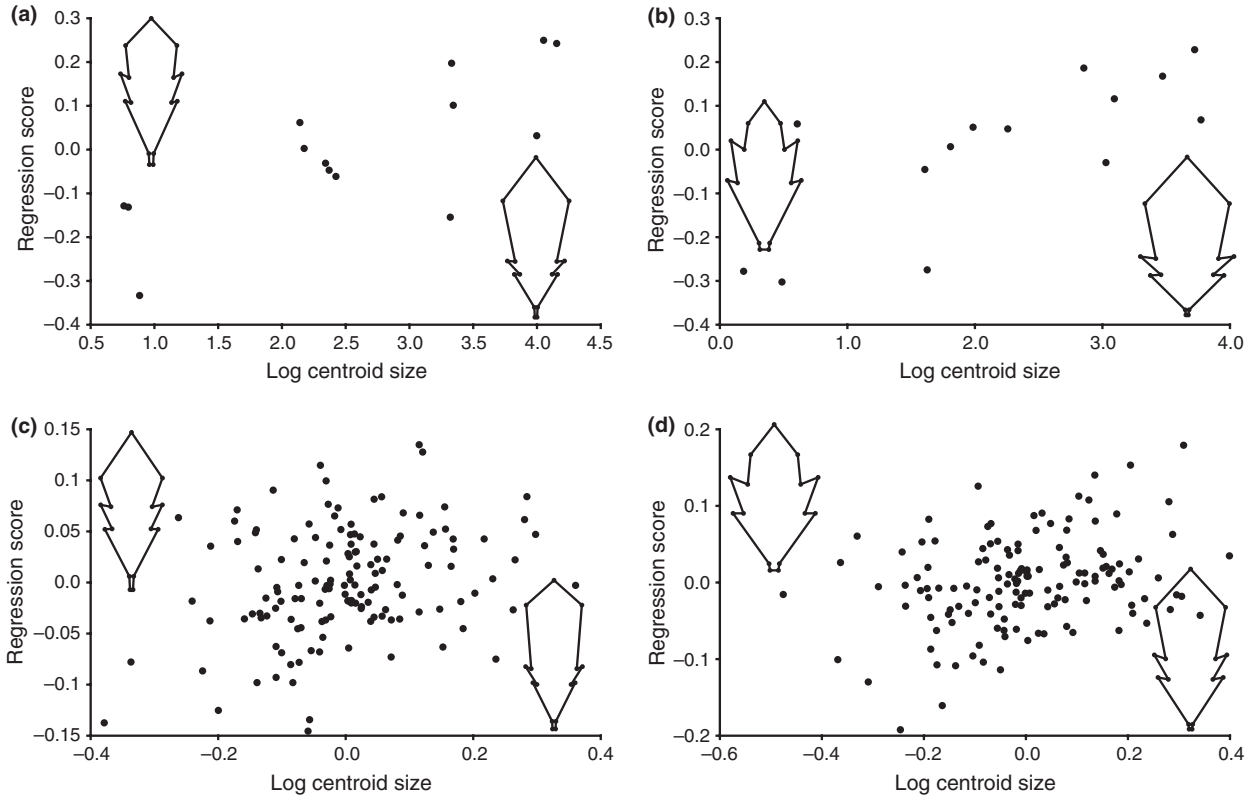

Fig. 9 Allometry of the leaflets. (a) Evolutionary allometry of the distal leaflet, computed by the regression of the mean shapes of the taxa on mean log-transformed centroid sizes (the leaflet diagrams show the predicted shape change for an increase in log-transformed centroid size by 3 units). (b) Evolutionary allometry of the lateral leaflet (the leaflet diagrams show the predicted shape change for an increase in log-transformed centroid size by 4 units). (c) Allometry within taxa of the distal leaflet, obtained by a pooled within-group regression of shape of log-transformed centroid size (the leaflet diagrams show the predicted shape change for an increase in log-transformed centroid size by 2 units). (d) Allometry within taxa of the distal leaflet (the leaflet diagrams show the predicted shape change for an increase in log-transformed centroid size by 2 units). The scatter plots in (c) and (d) show the individual deviations from the taxon means of shape and log-transformed centroid size.

evidence, it is not possible to determine conclusively why no significant phylogenetic signal for leaf or leaflet shape was found.

For all PCAs of the whole leaf and the two leaflets, the PC1 takes up at least about half of the variation, far more than any other PC (Figs 4, 5, 7 and 8). This indicates that the shape variation in each data set is highly concentrated in a single direction. Moreover, the shape changes associated with the PC1s are remarkably consistent across all three levels of analysis: evolutionary divergence among taxa (analyses with taxon means or independent contrasts), variation of the symmetric component of variation among leaves within taxa and fluctuating asymmetry of leaves (shape differences between the two halves of each leaf; Fig. 4, 5, 7 and 8). This correspondence is also apparent from the very high matrix correlations between the covariance matrices for within-taxon variation and evolutionary divergence and the moderately high ones for the covariance matrices of within-taxon variation and fluctuating asymmetry (it should be noted that only the paired landmarks can be used in the comparison of covariance matrices between the symmetric and asymmetric components of variation, and that these matrix correlations are therefore based on less information; see Klingenberg *et al.*, 2002).

Shape variation at the three levels originates from different sources: divergence among taxa results from evolution by selection and drift or processes such as hybridization and polyploidization. The symmetric component of variation within taxa is due to genetic variation and phenotypic plasticity, and the asymmetric component of shape variation reflects the combined effects of phenotypic plasticity and developmental instability. The strong dominance of a single PC and the consistency of patterns across all three levels suggest that a common process may channel variation at all three levels in a single direction of phenotypic space. Because variation within taxa is concentrated to a large extent in a single dimension of the shape spaces, evolutionary changes are

also most likely in that direction. It is thus possible that the PC1s, both for leaflets and for whole leaves, act as 'lines of least resistance' that acts as a relative constraint or bias for evolutionary change (Schluter, 1996; Arthur, 2001; Hunt, 2007; Klingenberg, 2010).

The symmetric component of within-taxon variation is of both genetic and environmental origins. In contrast, fluctuating asymmetry originates entirely from non-genetic causes. It is a mix of developmental instability, the imprecision in developmental processes based on the same genome and environmental conditions and phenotypic plasticity due to microenvironmental differences between the left and right halves of each leaf (e.g. shading; shown experimentally by Freeman *et al.*, 2003). Note that this is different from motile organisms, such as most animals, where it can be assumed that the movement eliminates microenvironmental differences by 'averaging out' their effects and where therefore fluctuating asymmetry can be interpreted as originating exclusively from developmental instability (Klingenberg, 2003b). For uses of fluctuating asymmetry as indicators of whole-plant developmental instability, measuring the asymmetry of several leaves provides a different way of averaging out the local microenvironment of individual leaves (e.g. Puerta-Piñero *et al.*, 2008; Albarrán-Lara *et al.*, 2010). These possible microenvironmental effects on leaf asymmetry preclude the interpretation of correlated asymmetries as indicating direct developmental interactions (Klingenberg, 2003a, 2008). Nevertheless, fluctuating asymmetry provides information about the patterns of variation that are produced by the developmental system spontaneously or in response to effects of the environment (Breuker *et al.*, 2006; Drake & Klingenberg, 2010). That these patterns are similar to those for the other two levels in the analysis, evolutionary divergence and within-taxon variation suggests that the developmental system has a strong effect in modulating the expression of variation from multiple origins. Furthermore, the strong integration of fluctuating asymmetry, indicated by the large proportion of the total variance for which the PC1s account (Figs 5b and 8c,d), suggests that the developmental modulation is channelling variation strongly into the corresponding direction and is therefore an important integrating factor for individual leaflets and the whole leaf.

Allometry is well known as a factor contributing to morphological integration (Klingenberg, 2009), and it has a substantial effect on shape variation in the whole leaf and in both leaflets (Figs 6 and 9). Shape changes associated with the within- and among-taxon allometries are clearly similar. Such agreements between levels of allometry have been reported before (Cheverud, 1982; Klingenberg & Zimmermann, 1992), but the modular architecture of plants makes this a particularly promising area for further analyses (Preston & Ackerly, 2004). The allometric shape changes also coincide with the PC1 patterns of shape variation at all three levels analysed in this study (Figs 4, 5, 7 and 8). It is therefore plausible that the process responsible for the common pattern of shape variation is related to size variation and growth. However, analyses correcting for allometry by regression (not shown) do not eliminate the coherent patterns of shape variation, so that allometry is clearly not the only source of integration.

The strong integration and common pattern of variation at multiple levels suggest a shared mechanism underlying this variation and raise the question what its nature may be. Developmental genetic studies have identified a number of pathways that are involved in the patterning and growth of compound leaves (Efroni *et al.*, 2010; Hasson *et al.*, 2010; Koenig & Sinha, 2010). *NAM/CUC3* and *KNOX* genes have conserved gene expression patterns and are likely to be involved in the initiation of leaflets and leaf dissection (Hareven *et al.*, 1996; Janssen *et al.*, 1998; Hay & Tsiantis, 2006; Blein *et al.*, 2008). The expression domain of the transcription factor *PHANTASTICA* specifies the spacing of leaflets that defines pinnate and palmate leaves even in groups that independently evolved compound leaves (Kim *et al.*, 2003a). Despite the apparent conservation of these mechanisms at a very broad phylogenetic scale, some authors caution that no single explanation can account for the available data across taxa (Efroni *et al.*, 2010). From the point of view of integration, it is interesting to note that the molecular pathways of leaf patterning involve plant hormones such as auxin and gibberellin (Hay *et al.*, 2006; Koenig *et al.*, 2009; Hasson *et al.*, 2010; Koenig & Sinha, 2010), and experiments have demonstrated that even messenger RNA can be transported over long distances in tomato plants to influence leaf morphology (Kim *et al.*, 2001). Such transport of growth regulators may be a direct physiological basis for morphological integration over extended distances in the leaflets and entire leaves, as it appears in our morphometric analyses. It is premature, however, to invoke specific molecular mechanisms as explanations for specific changes in leaf shape within or among species. Studies that combine specific genetic changes with the quantitative analysis of shape are a step in that direction (Langlade *et al.*, 2005; Bensmihen *et al.*, 2008; Klingenberg, 2010).

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References

Albarrán-Lara, A.L., Mendoza-Cuenca, L., Valencia-Avalos, S., González-Rodríguez, A. & Oyama, K. 2010. Leaf fluctuating asymmetry increases with hybridization and introgression between *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae) through an altitudinal gradient in Mexico. *Int. J. Plant Sci.* **171**: 310–322.

Arthur, W. 2001. Developmental drive: an important determinant of the direction of phenotypic evolution. *Evol. Dev.* **3**: 271–278.

Bensmihen, S., Hanna, A.I., Langlade, N.B., Micol, J.L., Bangham, A. & Coen, E. 2008. Mutational spaces for leaf shape and size. *HFSP J.* **2**: 110–120.

Blein, T., Pulido, A., Vialette-Guiraud, A., Nikovics, K., Morin, H., Hay, A. *et al.* 2008. A conserved molecular framework for compound leaf development. *Science* **322**: 1835–1839.

Breuker, C.J., Patterson, J.S. & Klingenberg, C.P. 2006. A single basis for developmental buffering of *Drosophila* wing shape. *PLoS ONE* **1**: e7.

Campbell, N.A. & Atchley, W.R. 1981. The geometry of canonical variate analysis. *Syst. Zool.* **30**: 268–280.

Cheverud, J.M. 1982. Relationships among ontogenetic, static, and evolutionary allometry. *Am. J. Phys. Anthropol.* **59**: 139–149.

Darwin, C. 1859. *The Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.

Debat, V., Alibert, P., David, P., Paradis, E. & Auffray, J.-C. 2000. Independence between developmental stability and canalization in the skull of the house mouse. *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 423–430.

Dobeš, C. & Paule, J. 2010. A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): implications for its geographic origin, phylogeography and generic circumscription. *Mol. Phylogenet. Evol.* **56**: 156–175.

Drake, A.G. & Klingenberg, C.P. 2008. The pace of morphological change: historical transformation of skull shape in St. Bernard dogs. *Proc. R. Soc. Lond. B Biol. Sci.* **275**: 71–76.

Drake, A.G. & Klingenberg, C.P. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. *Am. Nat.* **175**: 289–301.

Dryden, I.L. & Mardia, K.V. 1998. *Statistical Shape Analysis*. Wiley, Chichester.

Efron, B. & Tibshirani, R.J. 1993. *An Introduction to the Bootstrap*. Chapman & Hall, New York.

Efroni, I., Eshed, Y. & Lifschitz, E. 2010. Morphogenesis of simple and compound leaves: a critical review. *Plant Cell* **22**: 1019–1032.

Eriksson, T., Hibbs, M.S., Yoder, A.D., Delwiche, C.F. & Donoghue, M.J. 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the *TRNL/F* region of chloroplast DNA. *Int. J. Plant Sci.* **164**: 197–211.

Felsenstein, J. 1985a. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Felsenstein, J. 1985b. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.

Feng, X., Wilson, Y., Bowers, J., Kennaway, R., Bangham, A., Hannah, A. *et al.* 2009. Evolution of allometry in *Antirrhinum*. *Plant Cell* **21**: 2999–3007.

Figueirido, B., Serrano-Alarcón, F.J., Slater, G.J. & Palmqvist, P. 2010. Shape at the cross-roads: homoplasy and history in the evolution of the carnivoran skull towards herbivory. *J. Evol. Biol.* **23**: 2579–2594.

Freeman, D.C., Brown, M.L., Dobson, M., Jordan, Y., Kizy, A., Micallef, C. *et al.* 2003. Developmental instability: measures of resistance and resilience using pumpkin (*Cucurbita pepo* L.). *Biol. J. Linn. Soc.* **78**: 27–41.

Goldblatt, P. & Johnson, D.E. (eds) 1979. *Index to Plant Chromosome Numbers*. Missouri Botanical Garden, St. Louis, MO.

Goodall, C.R. 1991. Procrustes methods in the statistical analysis of shape. *J. R. Statist. Soc. B* **53**: 285–339.

Hansen, K.T., Elven, R. & Brochmann, C. 2000. Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). *Am. J. Bot.* **87**: 1466–1479.

Hareven, D., Gutfinger, T., Parnis, A., Eshed, Y. & Lifschitz, E. 1996. The making of a compound leaf: genetic manipulation of leaf architecture in tomato. *Cell* **84**: 735–744.

Hasson, A., Blein, T. & Laufs, P. 2010. Leaving the meristem behind: the genetic and mlecular control of leaf patterning and morphogenesis. *C. R. Biol.* **333**: 350–360.

Hay, A. & Tsiantis, M. 2006. The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. *Nat. Genet.* **38**: 942–947.

Hay, A., Barkoulas, M. & Tsiantis, M. 2006. ASYMMETRIC LEAVES1 and auxin activities converge to repress *BREVIPEDICELLUS* expression and promote leaf development in *Arabidopsis*. *Development (Camb.)* **133**: 3955–3961.

Huber, H. 1996. Plasticity of internodes and petioles in prostrate and erect *Potentilla* species. *Funct. Ecol.* **10**: 401–409.

Hunt, G. 2007. Evolutionary divergence in directions of high phenotypic variance in the ostracode genus *Poseidonamicus*. *Evolution* **61**: 1560–1576.

Ivanović, A. & Kalezić, M.L. 2010. Testing the hypothesis of morphological integration on a skull of a vertebrate with a biphasic life cycle: a case study of the alpine newt. *J. Exp. Zool. B Mol. Dev. Evol.* **314**: 527–538.

Janssen, B.-J., Lund, L. & Sinha, N. 1998. Overexpression of a homeobox gene, LeT6, reveals indeterminate features in the tomato compound leaf. *Plant Physiol. (Rockv.)* **117**: 771–786.

Jensen, R.J. 1990. Detecting shape variation in oak leaf morphology: a comparison of rotational-fit methods. *Am. J. Bot.* **77**: 1279–1293.

Jensen, R.J., Ciofani, K.M. & Miramontes, L.C. 2002. Lines, outlines, and landmarks: morphometric analyses of leaves of *Acer rubrum*, *Acer saccharinum* (Aceraceae) and their hybrid. *Taxon* **51**: 475–492.

Jojić, V., Blagojević, J. & Vujošević, M. 2011. B chromosomes and cranial variability in yellow-necked field mice (*Apodemus flavicollis*). *J. Mammal.* **92**: 396–406.

Jolliffe, I.T. 2002. *Principal Component Analysis*, 2nd edn. Springer-Verlag, New York.

Jones, C.S. 1992. Comparative ontogeny of a wild cucurbit and its derived cultivar. *Evolution* **46**: 1827–1847.

Kim, M., Canio, W., Kessler, S. & Sinha, N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* **293**: 287–289.

Kim, M., McCormick, S., Timmermans, M. & Sinha, N. 2003a. The expression domain of *PHANTASTICA* determines leaflet placement in compound leaves. *Nature (Lond.)* **424**: 438–443.

Kim, M., Pham, T., Hamidi, A., McCormick, S., Kuzoff, R.K. & Sinha, N. 2003b. Reduced leaf complexity in tomato wiry mutants suggests a role for *PHAN* and *KNOX* genes in

generating compound leaves. *Development (Camb.)* **130**: 4405–4415.

Klingenberg, C.P. 2003a. Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the developmental origins of morphological integration. In: *Developmental Instability: Causes and Consequences* (M. Polak, ed.), pp. 427–442. Oxford University Press, New York.

Klingenberg, C.P. 2003b. A developmental perspective on developmental instability: theory, models and mechanisms. In: *Developmental Instability: Causes and Consequences* (M. Polak, ed.), pp. 14–34. Oxford University Press, New York.

Klingenberg, C.P. 2008. Morphological integration and developmental modularity. *Annu. Rev. Ecol. Evol. Syst.* **39**: 115–132.

Klingenberg, C.P. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a-priori hypotheses. *Evol. Dev.* **11**: 405–421.

Klingenberg, C.P. 2010. Evolution and development of shape: integrating quantitative approaches. *Nat. Rev. Genet.* **11**: 623–635.

Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**: 353–357.

Klingenberg, C.P. & Ekau, W. 1996. A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biol. J. Linn. Soc.* **59**: 143–177.

Klingenberg, C.P. & Gidaszewski, N.A. 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* **59**: 245–261.

Klingenberg, C.P. & McIntyre, G.S. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* **52**: 1363–1375.

Klingenberg, C.P. & Zimmermann, M. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *Am. Nat.* **140**: 601–620.

Klingenberg, C.P., Barluenga, M. & Meyer, A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* **56**: 1909–1920.

Klingenberg, C.P., Debat, V. & Roff, D.A. 2010. Quantitative genetics of shape in cricket wings: developmental integration in a functional structure. *Evolution* **64**: 2935–2951.

Koenig, D. & Sinha, N. 2010. Evolution of leaf shape: a pattern emerges. *Curr. Top. Dev. Biol.* **91**: 169–183.

Koenig, D., Bayer, E., Kang, J., Kuhlemeier, C. & Sinha, N. 2009. Auxin patterns *Solanum lycopersicum* leaf morphogenesis. *Development (Camb.)* **136**: 2997–3006.

Langlade, N.B., Feng, X., Dransfield, T., Copsey, L., Hanna, A.I., Thébaud, C. *et al.* 2005. Evolution through genetically controlled allometry space. *Proc. Natl Acad. Sci. USA* **102**: 10221–10226.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. *et al.* 2007. Clustal W and Clustal X version 2.0. *Bioinformatics (Oxf.)* **23**: 2947–2948.

Lundberg, M., Töpel, M., Eriksen, B., Nylander, J.A.A. & Eriksson, T. 2009. Allopolyploidy in Fragariinae (Rosaceae): comparing four DNA sequence regions, with comments on classification. *Mol. Phylogenet. Evol.* **51**: 269–280.

Maddison, W.P. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Syst. Zool.* **40**: 304–314.

Mardia, K.V., Kent, J.T. & Bibby, J.M. 1979. *Multivariate Analysis*. Academic Press, London.

Meade, C. & Parnell, J. 2003. Multivariate analysis of leaf shape patterns in Asian species of the *Uvaria* group (Annonaceae). *Bot. J. Linn. Soc.* **143**: 231–242.

Miller, J.S. & Venable, D.L. 2003. Floral morphometrics and the evolution of sexual dimorphism in *Lycium* (Solanaceae). *Evolution* **57**: 74–86.

Monteiro, L.R. 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Syst. Biol.* **48**: 192–199.

Preston, K.A. & Ackerly, D.D. 2004. The evolution of allometry in modular organisms. In: *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes* (M. Pigliucci & K.A. Preston, eds), pp. 80–106. Oxford University Press, New York.

Puerta-Piñero, C., Gómez, J.M. & Hódar, J.A. 2008. Shade and herbivory influence fluctuating asymmetry in a Mediterranean oak. *Int. J. Plant Sci.* **169**: 631–635.

Rohlf, F.J. & Slice, D.E. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**: 40–59.

Royer, D.L., Meyerson, L.A., Robertson, K.M. & Adams, J.M. 2009. Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PLoS ONE* **4**: e7653.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* **50**: 1766–1774.

Sidlauskas, B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* **62**: 3135–3156.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **57**: 758–771.

Stuefer, J.F. & Huber, H. 1998. Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. *Oecologia (Berl.)* **117**: 1–8.

Töpel, M., Lundberg, M., Eriksson, T. & Eriksen, B. 2011. Molecular data and ploidal levels indicate several putative allopolyploidization events in the genus *Potentilla* (Rosaceae). *PLoS Curr.* **3**: RRN1237.

Tsukaya, H. 2005. Leaf shape: genetic controls and environmental factors. *Int. J. Dev. Biol.* **49**: 547–555.

Viscosi, V., Fortini, P., Slice, D.E., Loy, A. & Blasi, C. 2009a. Geometric morphometric analyses of leaf variation in four oak species of the subgenus *Quercus* (Fagaceae). *Plant Biosyst.* **143**: 575–587.

Viscosi, V., Lepais, O., Gerber, S. & Fortini, P. 2009b. Leaf morphological analyses in four European oak species (*Quercus*) and their hybrids: a comparison of traditional and geometric morphometric methods. *Plant Biosyst.* **143**: 564–574.

Weight, C., Parnham, D. & Waites, R. 2008. LeafAnalyser: a computational method for rapid and large-scale analyses of leaf shape variation. *Plant J.* **53**: 578–586.

Willmore, K.E., Klingenberg, C.P. & Hallgrímsson, B. 2005. The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. *Evolution* **59**: 898–909.

Wolf, T. 1908. *Monographie der Gattung Potentilla*. Schweizerbart, Stuttgart.

Young, R.L. & Badyaev, A.V. 2006. Evolutionary persistence of phenotypic integration: influence of developmental and

functional relationships on complex trait evolution. *Evolution* **60**: 1291–1299.
Young, J.P., Dickinson, T.A. & Dengler, N.G. 1995. A morphometric analysis of heterophyllous leaf development in *Ranunculus flabellaris*. *Int. J. Plant Sci.* **156**: 590–602.
Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier, San Diego.

Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1 Taxa and data used in the study.
Appendix S1 Phylogenetic inference and substitution model choice.

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