Int. J. Plant Sci. 160(5):917–929. 1999.

1058-5893/1999/16005-0013$03.00

EVOLUTIONARY CHANGES IN FLORAL STRUCTURE WITHIN *LEPIDIUM* L. (BRASSICACEAE)

John L. Bowman,[1,]* Holger Brüggemann,† Ji-Young Lee,* and Klaus Mummenhoff†

*Section of Plant Biology, University of California, Davis, California 95616, U.S.A.; and †Universität Osnabrück, Fachbereich Biologie, Spezielle Botanik, Barbarastrasse 11, 49069 Osnabrück, Germany

The basic floral ground plan is remarkably constant across Brassicaceae. However, within *Lepidium* (ca. 175 species), deviations from this ground plan are common, with over half of *Lepidium* species having only two stamens rather than the usual six and a further eighth of the species having only four stamens. Furthermore, petals are reduced in size in a majority of *Lepidium* species. In order to determine the frequency and direction of changes in floral structure within *Lepidium*, we have inferred the phylogeny within the genus from sequences of the internal transcribed spacers of the nuclear ribosomal DNA. On the basis of this inferred phylogeny, we conclude that floral structure within *Lepidium* is relatively fluid. In order to account for the phylogenetic distributions of the different floral ground plans, at least two independent reductions to the two-stamen condition and at least one reversal to flowers with increased organ numbers are likely to have occurred. To account for the frequency of morphological evolution observed within the genus, we propose that some clades within *Lepidium* may be predisposed to changes in floral structure. In addition, several transoceanic dispersals are needed to explain the geographic distributions of the clades inferred from the phylogeny.

Keywords: Lepidium, flower structure, flower evolution, internal transcribed spacers, Brassicaceae.

Introduction

While floral structure varies greatly among angiosperms as a whole, at lower taxonomic levels floral structure is usually highly conserved. This conservation suggests that floral structure is under strict genetic control. One approach to understanding the genetic control of floral ground plans is to analyze patterns in floral ground plan variation between closely related species. This approach assumes that the differences between species arise from the fixation of relatively few genetic determinants. Brassicaceae provide a tractable model because the floral ground plan is remarkably conserved among the family's more than 3000 species in ca. 350 genera (Schulz 1936; Cronquist 1981; Endress 1992). The stereotypical Brassicaceae flower consists of four sepals, four petals, six stamens, and two carpels. The six stamens are arranged so that four are in medial positions and two are in lateral positions within the flower. While deviations from this basic ground plan are rare within Brassicaceae as a whole (for review, see Endress 1992), reductions in floral organ numbers are common within the genus *Lepidium*, which consists of ca. 175 species (fig. 1; Thellung 1906; Hewson 1981; Al-Shehbaz 1986; Rollins 1993). Stamens are reduced from six to two in ca. one-half of the species, while a further one-eighth have only four stamens (Al-Shehbaz 1986). In the case of species with two stamens, stamens develop in medial positions only. Species with four stamens can have four medial stamens or two lateral and two medial stamens. In addition, petals are reportedly absent from ca. one-quarter of the *Lepidium* species, and they are rudimentary in many others. Thus, the basic ground plan of Brassicaceae is found in less than half of the *Lepidium* species.

Lepidium has a widespread distribution, with species endemic to all continents (with the exception of Antarctica) and many oceanic islands, such as New Zealand and Hawaii. Thellung (1906) classified species of *Lepidium* into seven sections. In this classification made on the basis of morphological fruit characters, most of the species with two stamens were placed in the section *Dileptium*, whose distribution includes all inhabited continents. Likewise, species with four medial stamens are found in North and South America and Australia. These distributions suggest that there have been independent parallel reductions in floral ground plan on several continents, or that there have been multiple intercontinental migrations, or a combination of both. To resolve this issue we have inferred the phylogenetic relationships of species within the genus *Lepidium* from sequences of the internal transcribed spacers (ITS) of the nuclear ribosomal DNA. These sequences have been shown to be very useful for resolving phylogenetic relationships within and among plant genera (reviewed in Baldwin et al. 1995). The results indicate that there have been at least two independent reductions in floral ground plan, and one or more reversals to increased floral organ number. Our results further suggest that multiple transoceanic migrations have occurred.

Material and Methods

On the basis of phylogenies constructed from ca. 100 *Lepidium* species analyzed (H. Brüggemann and K. Mummenhoff, unpublished data), a critical assemblage of *Lepidium* species was chosen to reflect changes that have occurred in floral

[1] Author for correspondence and reprints; e-mail jlbowman@ucdavis.edu.

Manuscript received March 1999; revised manuscript received June 1999.

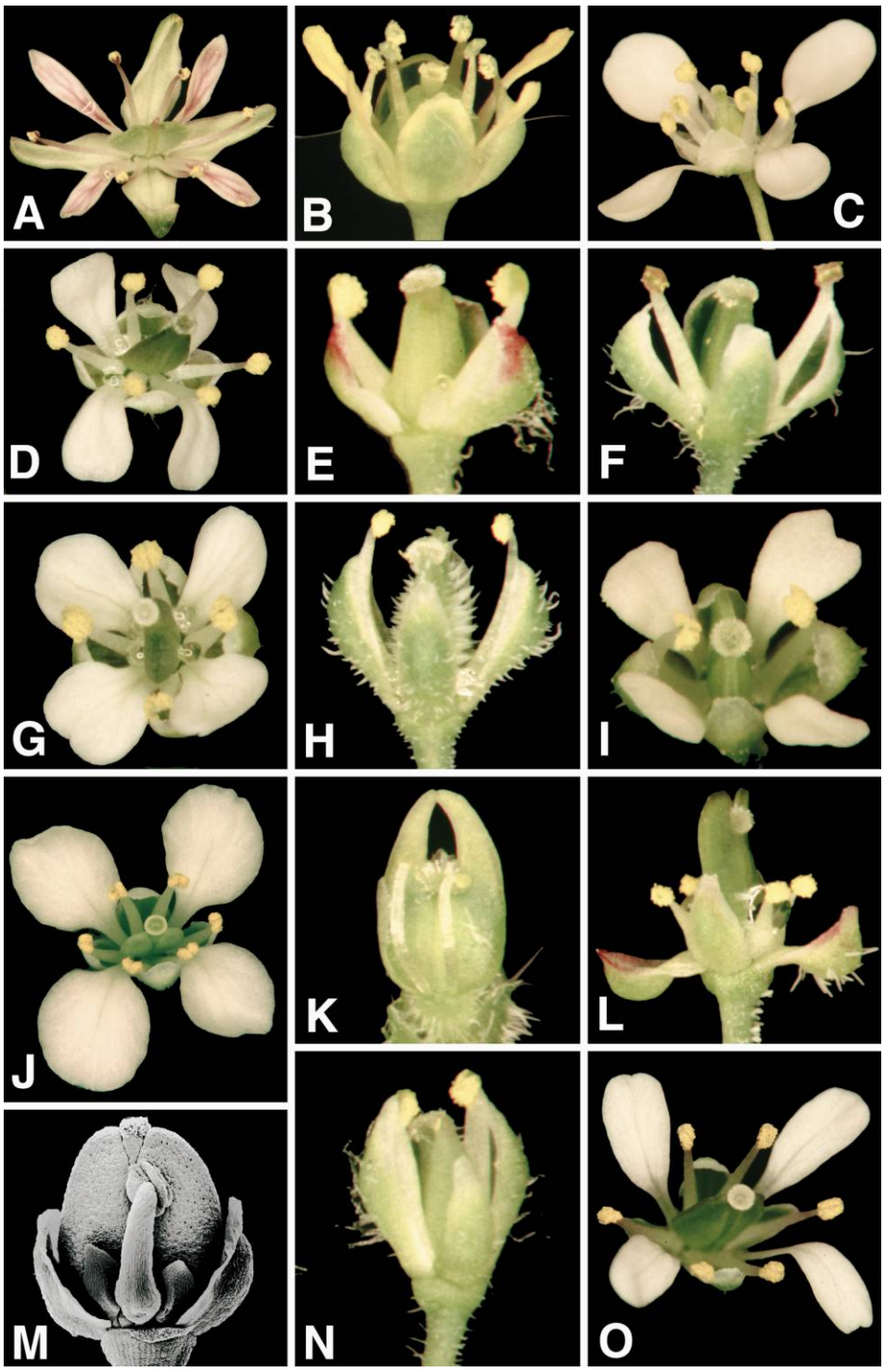

Fig. 1 Floral structure. *A*, *Lepidium phlebopetalum* (2 lateral + 4 medial stamens); *B*, *Lepidium perfoliatum* (2 + 4); *C*, *Lepidium vesicarium* (2 + 4); *D*, *Lepidium graminifolium* (2 + 4); *E*, *Lepidium africanum* (0 + 2); *F*, *Lepidium hyssopifolium* (0 + 2); *G*, *Lepidium oleraceum* (2 + 2); *H*, *Lepidium lasiocarpum* (0 + 2); *I*, *Lepidium virginicum* (0 + 2); *J*, *Lepidium fremontii* (2 + 4); *K*, *Lepidium dictyotum* (0 + 4); *L*, *Lepidium oxytrichum* (0 + 4); *M*, *Lepidium fasciculatum* (0 + 2); *N*, *Lepidium ruderale* (0+2); *O*, *Lepidium sativum* (2 + 4). One sepal has been removed in *E* and *M*, and two sepals have been removed in *K*.

ground plan. This assemblage was chosen for several reasons. First, the present taxon sample represents a broad spectrum of the variation in *Lepidium* including members of six (out of seven) sections and 11 (out of 11) greges *sensu* Thellung (1906). Second, these species reflect all types of changes that have occurred in floral ground plan. Third, the ratio of the distribution patterns of the different flower types within each clade corresponds to the ratio in the phylogenies constructed using ca. 100 *Lepidium* species. Thus, ancestral character state reconstruction on this critical assemblage should reflect that which was supported by sampling the full 100 species data set. Collection data and sources of plant material for obtaining ITS sequences are presented in table 1.

Because *Lepidium* may not clearly be separated from closest related genera, i.e., *Cardaria* (treated by Thellung as a section of *Lepidium*), *Coronopus*, *Stroganowia*, and *Andrzeiowskia* (all from subtribe Lepidiinae, tribe Lepidieae), we used the outgroup species, *Hornungia alpina* (L.) O. Appel and *Hornungia procumbens* (L.) Hayek (Appel and Al-Shehbaz 1997). In a recent chloroplast DNA analysis of tribe Lepidieae (K. Zunk, K. Mummenhoff, and H. Hurka, unpublished data). *Hornungia* is sister to the *Lepidium/Cardaria/Coronopus* clade.

Fresh or dry leaves (from herbarium specimens) were taken from individual plants. Total DNA was isolated following the procedure of Doyle and Doyle (1987). Double stranded DNA of the ITS-1 and ITS-2 regions was amplified using the polymerase chain reaction (PCR) protocol given in Mummenhoff et al. (1997). Primer 18 F was modified as described in Mummenhoff et al. (1997, fig. 1). Amplification products were purified using the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany). Purified DNAs were sequenced by the dideoxy chain termination method (Sanger et al. 1977) using the fmol kit (Serva, Heidelberg), following the protocol in Mummenhoff et al. (1997). The four primers used for sequencing both strands of the ITS-1 and ITS-2 regions were 18 F, 5.8 F, 5.8 R, and 25 R (for details, see Mummenhoff et al. 1997).

DNA sequences were aligned visually by sequential pairwise comparison (Swofford and Olsen 1990). Ambiguity was encountered only for *Lepidium phlebopetalum* for nucleotides 287–300. Exclusion of this region from the analysis did not alter the topology of the tree, although bootstrap support levels were moderately affected for some nodes. The aligned sequences are presented in figure 2. While many of the species within *Lepidium* are polyploid, there is no clear evidence in the literature for allopolyploid speciation. In addition, we did not detect any polymorphisms at individual nucleotide sites that could indicate previous hybridization/polyploidization events. To evaluate the nonrandom structure of the ITS-sequence data, the skewness test (g_1 statistic) of Hillis and Huelsenbeck (1992) was implemented using PAUP (Phylogenetic Analysis Using Parsimony), version 4.0β1 (Swofford 1993). Parsimony analysis of the aligned sequences assumed unordered and unweighted character states (i.e., Fitch parsimony) and used the heuristic search strategy in PAUP with MULPARS, TBR (tree bisection-reconnection) branch swapping, and 100 random taxon additions. Sets of equally parsimonious trees were summarized by the strict consensus approach. A bootstrap analysis (Felsenstein 1985) with 1000 replicates was performed saving no more than 300 trees per replicate to obtain estimates of reliability for each monophyletic group. The sequences were also analyzed using a maximum likelihood search with the values for ti/tv and γ distribution (with four rate categories) estimated from the 15 most parsimonious trees. A single tree was found after 10 random taxon addition replicates, starting with the 15 most parsimonious trees, using the Hasegawa-Kishino-Yano model of sequence evolution (Hasegawa et al. 1985).

Ancestral state reconstruction was performed on the 15 most parsimonious trees using the parsimony, Acctran, and Deltran options on MacClade 3.07 (Maddison and Maddison 1992), with the exception of one tree in which a four-clade polytomy existed; this tree was analyzed using only the parsimony option. The most parsimonious trees suggest that the character state of two stamens has evolved multiple times. To test the robustness of this conclusion, we constrained the topology to group the two-stamen species in a single clade by joining node 10 with either node 5 or node 7. MacClade 3.07 was used to determine the length of resultant shortest trees. Complete rearrangement searches above and below the branch leading to the single two-stamen clade in both of the artificial trees were performed to discover the shortest possible topology given this constraint. To assess whether this constrained topology was significantly worse than the shortest trees recovered without this constraint, a series of nonparametric tests was conducted using PAUP 4.0β1. Tree length was used as an evaluation criterion for these tests. Twenty-five thousand trees were generated through a heuristic search of the ITS dataset, and all trees $\leq$10 steps longer than the shortest trees were saved. A series of tree filters was used to segregate trees into files containing 30 trees of the shortest length, one step longer than the shortest trees, two steps longer than the shortest trees, and so on. Each tree file was pruned in a second filter such that no more than 30 trees remained at each length. The tree files were assembled and tested with pairwise nonparametric comparisons to discover how long trees must be to be significantly worse, in terms of their lengths, based on the Wilcoxon signed-rank test (Templeton 1983) and the winning-sites test (Sokal and Rohlf 1995).

To analyze flower structure, plants of 19 species were grown in 18-h days at 21°C. Seeds for the *Lepidium* were obtained from the wild by J. L. Bowman (*L. phlebopetalum*, *Lepidium perfoliatum*, *Lepidium africanum*, *Lepidium virginicum*, *Lepidium dictyotum*, *Lepidium oxytrichum*), from C. Gomez-Campo, Instituto Nacional de Investigaciones Agrarias, Madrid, Spain (*Lepidium vesicarium*, *Lepidium fremontii*, *Lepidium lasiocarpum*, *Lepidium lyratum*), from USDA North Central Regional Plant Introduction Station, Ames, Iowa (*Lepidium graminifolium*, *Lepidium ruderale*, *Lepidium sativum*, *Lepidium spinosum*, *Lepidium campestre*, *Lepidium meyenii*), from Peter de Lange (*Lepidium oleraceum*; see table 1), or as previously described (Bowman Smyth 1998; *Lepidium hyssopifolium*). The floral structure of *Lepidium fasciculatum* was determined from field samples (Bowman and Smyth 1998). Other floral structures presented in figure 3 are inferred from floras of the respective regions (Thellung 1906; Hitchcock 1945; Al-Shehbaz 1986).

Table 1

Origin and Systematic Treatment (Thellung 1906) of *Lepidium* Species under Study

Species	Provenance	Source	Collector or accession number[a]	Section/grex[b]
Lepidium sativum L.	Denmark, Jersie	Botanical Garden, Copenhagen, Denmark	s.n.	*Cardamon* DC.
L. spinosum Ard.	Turkey, Central Anatolia, west of Gaziantep	Ministerio de Agricultura Pesca y Alimentacion	6229-83	*Lepiocardamon* Thell.
L. aucheri Boiss.	Iran, Semnan Province, Touran Protected Area, southeast of Sharud	Frank Hellwig, Göttingen, Germany	Freitag 15072	*Lepiocardamon* Thell.
L. campestre (L.) R. Br.	France, Meurthe-et-Moselle, Villers-lès-Nancy, 370 m	Botanical Garden, Nancy, France	s.n.	*Lepia* (Desv.) DC.
L. bipinnatifidum Desv.	Bolivia, La Paz, Railway Station	University of Osnabrück, Germany	K. Mummenhoff and H. Brüggemann	*Dileptium*/ Bipinnatifida
L. dictyotum Gray	U.S.A., California, San Luis Obispo County	Tucker Herbarium, University of California, Davis	32057	*Dileptium*/ Oxycarpa
L. fasciculatum Thell.	Australia, Victoria, River Road near Lake Walla Walla	La Trobe University, Australia	J. H. Browne	*Dileptium*/ Pseudoruderalia
L. ruderale L.	Germany, Borkholzberg near Oldenburg, near railway	Botanical Garden, Oldenburg	s.n.	*Dileptium*/ Ruderalia
L. lasiocarpum Nutt.	U.S.A., California, Mojave Desert, Joshua Tree	C. Gomez-Campo, Germplasm Collection of Crucifers, Madrid, Spain	430-1738-69	*Dileptium*/ Virginica
L. virginicum L.	Mexico, Carrizal Chico, 1300 m, dry riverbed	University of Osnabrück, Germany	R. Stöckmann and K. Bosbach	*Dileptium*/ Virginica
L. africanum (Burm. f.) DC. ssp. *africanum*	South Africa, Cape Province, Williston District	National Herbarium Pretoria, South Africa	G. Germishuizen 6232	*Dileptium* or *Lepidium*/—
L. cordatum Willd. ex Stev.	Cultivated, native in Asia	Botanical Garden, Copenhagen, Denmark	s.n.	*Lepidium*/—
L. graminifolium L.	Italy, Genoa, Monte Borriga	Botanical Garden, Göttingen, Germany	s.n.	*Lepidium*/—
L. lyratum L.	Iran, mountains near Abadeh	C. Gomez-Campo, Germplasm Collection of Crucifers, Madrid, Spain	433-3758-75	*Lepidium*/—
L. perfoliatum L.	Origin unknown	Botanical Garden, Vácrátót, Hungary	s.n.	*Lepidium*/—
L. vesicarium L.	Iran, abandoned fields between Karadj and Teheran	C. Gomez-Campo, Germplasm Collection of Crucifers, Madrid, Spain	438-3705-75	*Lepidium*/—
L. fremontii Wats.	U.S.A., California Mojave Desert, Joshua Tree	University of Osnabrück, Germany	H. Hurka	*Monoploca*/ Alyssoidea
L. meyenii Walp.	Peru, Dpto. Junin, Prov. Junin, Dist. Junin, Huayre	M. Hermann, International Potato Center, Quito, Ecuador	JTA 106	*Monoploca*/ Gelida

Table 1

(*Continued*)

Species	Provenance	Source	Collector or accession number[a]	Section/grex[b]
L. phlebopetalum (F. Muell.) F. Muell.	Australia, Western Australia, Millstream	Kings Park and Botanic Garden, West Perth, Western Australia	L. Sweedman	*Monoploca*/ Monoplocoidea
L. cyclocarpum Thell.	Peru, Dpto. Arequipa, 40 km south of Chala	Herbarium, Missouri Botanical Garden, St. Louis	C. R. Worth and J. L. Morrison 15701	*Monoploca*/ Oblanceolata
L. arbuscula Hillebr.	U.S.A., Hawaii, Oahu, Makua Valley, Ohikilolo Ridge	National Tropical Botanical Garden, Lawai, Kauai, Hawaii	S. P. Perlman	—/*Oleracea*
L. oleraceum Sparrm.	New Zealand, Port Waikato, Ngatutura Point	Peter de Lange, Department of Conservation, Auckland Conservancy, New Zealand	Auck 941265	—/Oleracea
L. oxytrichum Sprague	Australia, New South Wales, 10 km west of Broken Hill	University of California, Davis	J. L. Bowman	—/Papillosa
L. hyssopifolium Desv.	Australia, Victoria, Beveridge	La Trobe University, Australia	N. H. Scarlett et al.	—/Pseudoruderalia

[a] s.n. = *sine numero*.

[b] The taxonomic category grex, based on geographical distribution, was established by Thellung (1906) only for species of sections *Lepidium* L., *Dileptium* (Raf.) D.C., and *Monoploca* (Bunge) Prantl (see Hewson 1981; Mummenhoff et al. 1992). Dashes indicate that the species was not assignable to section or grex, respectively (see Hewson 1981; Mummenhoff et al. 1992).

Results

The g_1 statistic was -1.16 ($P = 0.01$), indicating that there is significant nonrandom structure in the sequences (Hillis and Huelsenbeck 1992). Of 466 total characters, 121 were parsimony informative. The size, sequence variation, and sequence divergence of the ITS among the *Lepidium* species studied will be presented in a separate report, as will a detailed phylogeny of the genus including ca. 100 species (K. Mummenhoff, H. Brüggemann, and J. L. Bowman, unpublished data).

A heuristic search based on parsimony yielded the 15 most parsimonious trees of tree length 412 steps on one island (Consistency Index = 0.740 and $-\ln$ likelihood = 2831.63). The strict consensus of the most parsimonious trees is shown in figure 3 with bootstrap values indicated on the nodes. In this context it may be noted that the preliminary tree generated with the full assemblage of ca. 100 *Lepidium* species is in agreement with the topology derived from the reduced taxon sample presented here, with the exception that node 13 is not resolved in the more comprehensive tree (H. Brüggemann, K. Mummenhoff, and J. L. Bowman, unpublished data). Several higher order nodes are reasonably well supported (=70% bootstrap value; 1, 2, 3, 4, 6, 7, 9, 10), while other higher order nodes are less so (5, 8, 13). The single maximum-likelihood tree was an exact match to the strict consensus based on parsimony analysis.

The floral structures of extant species and of the hypothetical ancestors of each clade are shown in figure 3. The hypothetical ancestors of clades 1, 2, 3, 4, 6, 10, and 12 were unambiguous using the parsimony, Acctran, and Deltran options of MacClade. Since the Acctran option of MacClade accelerates the transition between character states (minimizing reductions and maximizing reversals in the present case) and the Deltran option delays transitions (minimizing reversals and maximizing reductions in the present case), these options should represent the extremes in the transitions in stamen number. The hypothetical ancestor of clade 5 is always six stamens using the Deltran option, whereas using the Acctran option, either six-stamen or two-stamen ancestors are predicted with approximately equal frequency. Likewise at node 7, the Acctran option always predicts a two-stamen ancestor, whereas the Deltran option predicts two- and six-stamen ancestors with

```
                          10         20         30         40         50         60         70         80
L. phlebopetalum      TCGATACCTG TTCAAAACAG AACGACCCGT GAACCAA-CG ATCATCACTT GCGGTGGACA GGTTTCTTAA CGGATCCATC
L. campestre          .......... .C........ .........C .......-.T .......... ....C..G.C .........G .A.....CG.
L. perfoliatum        .......... .C........ .........C ....T..-.T .......... .......G.C .........G .A.....CGT
L. aucheri            .......... .C........ .......... .......-.T .......... .......G.C .........G .T....TTAT
L. vesicarium         .......... .C........ .T.A.....C .......A.T .........C .......G.T .........G .T.....C.T
L. lyratum            .......... .C........ .........C .......-.T .......... .......G.C .........G .T.....CGT
L. graminifolium      .......... .C..G..... .........C .......-.T .......... .......G.C .........G .T.....CG.
L. africanum          .......... .C........ .........C .......-.T .......... .......G.C .........G .T.....CGT
L. oleraceum          .......... .C........ .........C .......-.T .......... .......G.C .........G .T.....CGT
L. hyssopifolium      .......... .C........ .........C .......-.T .......... .......G.C .........G .T.....CGT
L. bipinnatifidum     .......... .C......C. .........C .......-.T .......... C......G.C .........G .A.....CGT
L. meyenii            .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. arbuscula          .......... .C........ .........C .......-GT .......... .......G.C ......G..G .A.....CGT
L. virginicum         .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. lasiocarpum        .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. cyclocarpum        -......-.. .C........ .......... .......-.T .......... .......G.C ......C.TG .A.....CGA
L. fremontii          .......... .C........ T........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. dictyotum          .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. oxytrichum         .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. fasciculatum       .......... .C........ .........C .......-.T .......... .......G.C ......C..G .A.....CGT
L. cordatum           .......... .?..G..... .........C .......-.T .........C ....C..G.C .........G .A.G...CGT
L. ruderale           .......... .C........ .........C .......-.T .......... .......G.C ..G......G .A.....CGT
L. sativum            .......... .C........ .........C .......-.T .......... .......G.C .........G .A.....CGT
L. spinosum           .......... .C........ .........C .......-.T .......... .......G.C .........G .A.....CGT
Hornungia alpina      ....AT.... ..T....... ..T....... .......-A. .........C A......G.C .T.......G .T....TCGT
Hornungia procumbens  -......... -C........ .......T.. ....T..-.. .........C AT.....G.C ..-CA....G .T....TCGT

                          90        100        110        120        130        140        150        160
L. phlebopetalum      GTCCACCGAA TCCGTGGTTT CGCGTGCTGT CCCAGTTAGG AGAGCAATCT --CCGTTGGG TCGTGCGCGA T-TCTTCCGG
L. campestre          .C..G.T... ...TAT..G. .......... T..G..CG.. ...T.TC..C --.G.AC... .G.......T A-G...AT..
L. perfoliatum        .C..G.T... ...TG..... .......... T.TGC.CG.. ...T.-C..C --.G.AC... AA.....T.T A-G...AT..
L. aucheri            .C..G..... ...T...... .......C.. T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. vesicarium         AC..G....G ...T...... ..T.C..C.. T.GGAACT.. ...T.TC..C --.G.ACC.. A........T A-A.CGAA..
L. lyratum            .C.TT..... ...T...... .......C.. T.GGAACGA. ...T.TC..C --.G.ACC.T .........T AAG...AA..
L. graminifolium      .C..G..T.. ...T...... .......C.. T.GGAACG.. ...T.TC... CT.G.ACC.. .........T A-G..GAA..
L. africanum          .C..G..... ...T...... .......C.. T.GGAACG.. ...T.TC... --.G.ACC.. ....C....T A-G..GAA..
L. oleraceum          .C..G..... ...T...... .......C.. T.GGAACG.. ...T.TC... --.GTACC.. ....C....T A-G..GAA..
L. hyssopifolium      .C..G..... ...T...... .......C.. T.GGAACG.. ...T.TC... --.G.ACC.. ....C...TT A-G..GAA..
L. bipinnatifidum     ....G..... ...T...... G......GT. T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. meyenii            ....T..... ...T.....G .....A.C.. T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. arbuscula          ....T..... ...T.....G .....AT... T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. virginicum         ....T..... ...T.....G .....AT... T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. lasiocarpum        ....T..... ...T.....G .....AT... T..G.ACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. cyclocarpum        ...TT..... ...A.....G .....T.C.. T..GAACG.. G..T.TC... CC.G.ACC.. .......G.C A-G..CAT..
L. fremontii          ....T..... ...T.....G .....A.A.. T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. dictyotum          ....T..... ...T...... .....AGC.. T..GAACG.. ...T.TC..C --.G.ACC.. ..C......T A-G..GAT..
L. oxytrichum         ....T..... ...T...... .....A.C.. T..GAACG.. ...T.TC..C --.G.ACC.. ..C......T A-G..GAT..
L. fasciculatum       ....T..... ...T...... .....AT... T..GAACG.. ...T.TC..C --.G.ACC.. ..C......T A-G..GAT..
L. cordatum           ....G..... ...T....C. .......CC. T..GAAAG.. ...T.TC..C --.G.ACC.. .........T A-G...A...
L. ruderale           ....G..... ...T...... ......ACC. T..GAACG.. ...T.TC..C --.G.ACC.. .........T A-G..GAT..
L. sativum            ....G..... ...T...... .....T.C.. T..GAACG.. ...T.TC..C --.G.A.C.. .........T A-G..GAT..
L. spinosum           ....G.G... ...T...... .A...T.C.. T..GAACG.. ...T.TC..C --.G.A.C.. .........T A-G..GAT..
Hornungia alpina      .C.TG..... .......... ..AT.T.... T.TG..CG.. .TCA...... --.G.C...T ......T..G A-G.......
Hornungia procumbens  .C.TG..TG. .......... T.TA.A.... .T.TT.C... .TTTTT.... --AGTCA... A....T..TT G-G.......
```

Fig. 2 Alignment of ITS sequences. ITS-1 is nucleotides 1–225; ITS-2 is nucleotides 227–466. The only region of ambiguous alignment consisted of nucleotides 287–300; the ambiguity is only for taxon *Lepidium phlebopetalum*. Hyphens = gaps. (*Continued on following pages.*)

equal frequency. Node 8 is predicted to have a four-stamen ancestor using the Acctran option, whereas this node is ambiguous (two- or four-stamen) using the Deltran option. In the tree presented in figure 3, we chose a four-stamen ancestor to represent clade 8, based on the surrounding topology of the tree including ca. 100 species of *Lepidium* (see "Discussion"). Likewise, node 9 is predicted to have a two-stamen ancestor with Acctran, whereas it is ambiguous (two- or six-stamen) using Deltran. Node 11 is ambiguous using either option. Finally, in all trees, node 13 parallels the prediction for node 9. Since this node is weakly supported in the tree presented here, and this node breaks in the more comprehensive phylogeny (H. Brüggemann, J. L. Bowman, and K. Mummenhoff, unpublished data), the hypothetical ancestor of this clade is ambiguous.

Analysis of the constrained topologies suggests that the two-stamen condition has evolved independently in a minimum of two clades. Trees that were constrained by joining node 10 with either node 5 or node 7 were judged to be significantly less parsimonious as compared with the shortest trees on the

```
                          170        180        190        200        210        220        230        240
L. phlebopetalum     AAATCACAAA AACCCGGCAC GAAAAGTGTC AAGGAACATT CAAATGATGA GCCAGCCTCT GCCTC-CCCG GAGACGGTGT
L. campestre         .T.......C .......... .......... .........G ...CC..ACG ......T.AC .G...-.... .........C
L. perfoliatum       .T.......C .......... .......... .........G ...CC..ACG ......T.TC .T...-.... .........C
L. aucheri           .T.......C .T.A...... .......... .........G ...CC..AC. ......G.TC ....T-.... .........C
L. vesicarium        .T.......C ...A...... .......... ........AG A..C...ACG ....T.G.TC ....T-.... .........C
L. lyratum           .T........ .C.A...... .......... .........G ...CC..ACG ....-.G.TC ....T-.... .........C
L. graminifolium     .T........ .C.A...... .......... .........G ...CC..ACG ...G..G.TC ....T-.... ..A......C
L. africanum         .T........ .C.A...... .......... .........G ...CC..ACG ...G..G.TC ....T-.... .........C
L. oleraceum         .T........ .C.A...... .......... .........G ...CC..ACG ...G..G.TC ....T-.... .........C
L. hyssopifolium     .T........ .C.A...... .......... .........G ...CC..ACG ...G..G.TC ....T-.... .........C
L. bipinnatifidum    .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. meyenii           .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. arbuscula         .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. virginicum        .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. lasiocarpum       .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. cyclocarpum       .T.......C ...A...... .......... .........G ...CC..ACG ....-.G.TC ....T-.... .........C
L. fremontii         .T.......C ...A...... .......... .........G ...CC..AC. ....T.G.TC ....T-.... ..........
L. dictyotum         .T.......C .......... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. oxytrichum        .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. fasciculatum      .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. cordatum          .T.......C .......... .......... ........CG ...CC..ACG ...G-.G.TC ....T-.... .........C
L. ruderale          .T.......C ...A...... .......... .........G ...CC..ACG ......G.TC ....T-.... .........C
L. sativum           .T.......C ...A...... .......... .........G ...CC..ACG .....TG.TC ....T-.... .........A
L. spinosum          .T.......C ...A...... .......... .........G ...CC..ACG .....TG.TC ....T-.... .........A
Hornungia alpina     .T........ .C........ .......... .........G ...CAT.ACG ......T... .....-.TT. ...-------
Hornungia procumbens .T........ .C........ .......... .........G ...CA..AC. .T....T.T. ..T..-.... ..........

                          250        260        270        280        290        300        310        320
L. phlebopetalum     GAGTGTGGAT GCTGTGCGGA AATCTAAATT CTATCGTCGT CCCCCCAT-- --CCTTT-AA GGATAAGGGA CGGAAGCTGG
L. campestre         ....CC.T.. ....C..T.C G.......G. .......... .....TCAA- TT.T...-GC ..T..TT... .....A....
L. perfoliatum       .....C.A.. ....C..T.C G..G....G. .......... .....TCAA- TT.T...-GC ..G..TT... ..........
L. aucheri           .T...C.A.. .T.....T.T GT......G. .......... .....ACACA AAAT...-GC .AG.GTT... .....T....
L. vesicarium        ...C.C.A.. .T.....T.C G.......G. .......... .....TCACG AAATA..-TC .AGCGT.... ..........
L. lyratum           ...?.C.A.. .......T.C G.......G. .......... .....TCACG AAAT..C-.C .AGCGT.... ..........
L. graminifolium     ...C.C.A.. .......T.C G.......G. .......... .....TCACG AAAA...-TC .AG.GT.... ..........
L. africanum         ...C.C.A.C .......T.C G.......G. .......... .....TCACG AAAT...-TC .AG.GT.... ..........
L. oleraceum         ...C.C.A.C .......T.C G.......G. .......... .....TCACG AAAT...-TC .AG.TT.... ..........
L. hyssopifolium     ...C.C.A.C .......T.C G.......G. .......... .....TCACG AAAT...-TC .AG.TT.... ..........
L. bipinnatifidum    ...C.C.A.. .......T.C G.......G. .......... .....TCATG AAAT...-GC .AG.GT.... ..........
L. meyenii           ...C.C.A.. .......T.C G.......G. .......... T....TCACA AAAT...-GC .AG.GC.... ..........
L. arbuscula         ...C.C.A.. .......T.C G.......G. .......... T....TCACG AAAT.A.-GC .AG.GC.... ..........
L. virginicum        A..C.C.A.. .......T.C G.......G. .......... T....TCACA AAAT.A.-GC .AG.GC.... ..........
L. lasiocarpum       A..C.C.A.. .......T.C G.......G. .......... T....TCACA AAAT.A.-GC .AG.GC.... ..........
L. cyclocarpum       ...C.C.A.. .......T.C G.......G. .......... ....A..CGC GAGA...TGT .AGAGC.... ..........
L. fremontii         ...C.C.A.C .......T.C G.......G. .......... ...T.TCCCA AAAT...TGC .AG.GC.... ..........
L. dictyotum         ...C.C.A.. .......T.C G.......G. .......... .....TCACG AAAT...-GC .AG.GC.... ..........
L. oxytrichum        ...C.C.A.. .......T.C G.......G. .......... ...A.TCACG AAAT...-GC .AG.GC.... ..........
L. fasciculatum      ...C.C.A.. .......T-C G.......G. .......... ...A.TCACG AAAT...TCC .AG.GC.... ..........
L. cordatum          T..C.C.A.. .......T.C G...A...G. .......... .....TCACG AAATA..-GC .AG.GT.... ..........
L. ruderale          T..C.C.A.. .......T.C G...A...G. .......... .....TCACG AAATA..-GC .AG.GT.... ..........
L. sativum           ...C.C.... .......T.C G.......G. .......... .....TCACG AATT..C-.C .AG.GT.... ..........
L. spinosum          ...C.C.... .......T.C G.......G. .......... .....TCACG AATT..C-.C .AG.GT.... ..........
Hornungia alpina     ---..C.... T......T.T G.......G. .......... ..AAA.T.AT ......A-CG .ATA.C.... ..........
Hornungia procumbens .....CA..A .T.....T.T G.......G. .......... ......T.AT ......A-C. .ATAGT.... ..........
```

Fig. 2 (*Continued*)

basis of both the Wilcoxon signed-rank test and the winning sites test. On the basis of these analyses and the MacClade analyses, there appears to have been multiple reductions as well as reversals in the evolution of floral structure within the *Lepidium*.

Discussion

The tree presented in figure 3 represents one interpretation made on the basis of accepting the strict consensus of the most parsimonious trees and then reconstructing ancestral character states at each of the nodes. Only those ancestral character states that are unambiguous are shown. Relative to the species considered here, *Lepidium* forms a natural monophyletic assemblage, though few of the sections or greges *sensu* Thellung (1906) and Hewson (1981) represent monophyletic groups, corroborating previous studies (Mummenhoff et al. 1992, 1995). However, suggestions on the delimitation of *Lepidium* and the closely related genera *Cardaria* and *Coronopus* await the inclusion of these taxa in molecular studies. With respect to the evolution of floral ground plan, several conclusions can be drawn on the basis of the inferred phylogeny presented in figure 3. As expected, the primitive condition is a floral ground plan with six stamens (two lateral and four medial) and well-developed petals, as in *Lepidium phlebopetalum* (fig. 1*A*), *Lepidium perfoliatum* (fig. 1*B*), and *Lepidium campestre*. This

```
                          330        340        350        360        370        380        390        400
L. phlebopetalum          TCTCCCGTGT GTTACCGCAC GCGGTTGGCC AAAATACGAG CTAAGGATGC TTGGAGCGTC TTGACATGCG GTGGTGAACT
L. campestre              .......... .......... .......... ......T... .......... G......... CC........ ..........
L. perfoliatum            .......... ..A....... .......... .....C.... .......... G......... CC........ ........TA
L. aucheri                .......... .......... ..-....A.. .......... .......... .G........ CC........ .......T..
L. vesicarium             .......... .......... .......A.. .....CT... ..G....... .G........ CC........ .......T.A
L. lyratum                .......... .......... .......... G....CT... ..G....... .-........ CC........ .......T..
L. graminifolium          .......... .......... ....C..A.. .....CT... ..G....... .-........ CC........ .......T..
L. africanum              .......... .......... .......A.. .....CT... ..G....... .-........ CC........ .......T..
L. oleraceum              .......... .......... .......A.. .....CT... ..G....... .-........ CC........ .......T..
L. hyssopifolium          .......... .......... .......A.. .....CT... ..G....... .-........ CC........ .......T..
L. bipinnatifidum         .......... .......... .......A.. .....CT... ..G....... .G........ CC........ .......T..
L. meyenii                .......... .......... .......... .....CT... ..G....... .......... CC........ .......T..
L. arbuscula              .......... .......... .......... .....CT... ..G....... .G........ CC........ .......T..
L. virginicum             .......... .......... .......... .....CT... ..G....... .......... CC........ .......T..
L. lasiocarpum            .......... .......... .......... .....CT... ..G....... .G........ CC........ .......T..
L. cyclocarpum            .T........ .......... .......... .....CT... .CG....... .G........ CC........ .......TT.
L. fremontii              .......... .......... .......... .....CT... ..G....... .GC....... C........ .......T..
L. dictyotum              .......... .......... .......... .....CT... ..G....... .G........ CC........ .......T..
L. oxytrichum             .......... .......... .......... .....CT... ..G....... .G........ CC........ .......T..
L. fasciculatum           .......... .......... .......... .....CT... ..G....... .G........ CC........ .......T..
L. cordatum               .......... .......... .......A.. .....CT... ..G....... .G.T...... CC........ .......T..
L. ruderale               .......... .......... .......A.. .....CT... ..G....... .......... CC........ .......T..
L. sativum                .......... .......... .......A.. .....C.... ..G......T .......... CC........ .......T..
L. spinosum               .......... .......... .......A.. .....C.... ..G......T .......... CC........ .......T..
Hornungia alpina          .......... .......... .......... .....C.... .C........ .AT....... CC........ .......GT.
Hornungia procumbens      .......... .........T .......... .....T.... .C.......T CATA...... CC........ .......GT.

                          410        420        430        440        450        460
L. phlebopetalum          -CAAGCCTCT TC--ATTCAG TCGACCACTC CTATCCGAAA GCTCATGATG ATCCAAAATC CTCAAA
L. campestre              -.......... ..--..AT.. ...GT.G... .......T.. ....TC.... .C....TG.. ......
L. perfoliatum            -G......... ..--..AAC. ..TGT.G... .......T.. ....TC.... .C....TG.. ......
L. aucheri                -A......... ..--..ATC. C..GT.G... ..G...AT.. ....T..T.. .C...--T.T ......
L. vesicarium             -A..A...... ..--..ATT. C..GT.G... ..G....T.T ....TC.T.. .C....TG.. ......
L. lyratum                -A......... ..--..ATT. C..GT.G... ..G....T.T ....TC.T.. .C....TG.. ......
L. graminifolium          -A......... ..--..ATT. C..GT.G... ..G....T.T ....TC.T.. .C....TG.. ......
L. africanum              TA......... ..--..ATT. C..GT.G... ..G....C.T ....TC.T.. .C....TG.. ......
L. oleraceum              TA......... ..--..ATT. C..GT.G... ..G....C.T ....TC.T.. .C....TG.. ......
L. hyssopifolium          TA......... ..--..ATT. C..GT.G... ..G....C.T ....TC.T.. .C....TG.. ......
L. bipinnatifidum         -A......... ..--G.ATT. C.TGT.G... ..G....C.. ....TC.T.. .C....TG.. ......
L. meyenii                -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C....TG.. A.....
L. arbuscula              -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C....TG.. A.....
L. virginicum             -A......... ..--..ATT. C..GT.G... ..G...AT.. ....TC.T.. .C....TG.. A.....
L. lasiocarpum            -A......... ..--..ATT. C..GT.G... ..G...AT.. ....TC.T.. .C....TG.. A.....
L. cyclocarpum            -A......... ..--..AAT. C..GT.G... ..G....T.. ....-C.T.. .C....TG.. A.....
L. fremontii              -A......... ..--..ATT. C..GT.G... ..G....T.. .....A.T.. .C....T... A.....
L. dictyotum              -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C....TG.. ......
L. oxytrichum             -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.TC. .C....TG.. ......
L. fasciculatum           -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C....TG.. ......
L. cordatum               -A......... ..--..ATT. C.TGT.G... ..G....T.. ....TC.T.. .C....TG.. ..A...
L. ruderale               -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C....TG.. ......
L. sativum                -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C.....G.. ......
L. spinosum               -A......... ..--..ATT. C..GT.G... ..G....T.. ....TC.T.. .C.....G.. ......
Hornungia alpina          -T..CAGG.. ..TTG.A.T. .T.TT.GT.- ..G....... ....TM.... .C.....G.. T.....
Hornungia procumbens      -A..CAAG.. ..TTG.ATT. ...GA.GT.- T.GA.T.... ....TA.... .C.....G.. T.....
```

Fig. 2 (*Continued*)

is the basic ground plan for the Brassicaceae as a whole (Endress 1992). Given that four different floral structures, in terms of number and position of stamens, occur in *Lepidium*, at least three events are required to account for the variation. The events could consist entirely of reductions in stamen number, or, alternatively, could consist of a combination of reductions and reversals of the reductions. The inferred phylogeny suggests the latter. There have been at least two independent reductions to the two-stamen condition, and there have likely been reversals from reduced floral structures to floral structures with increased organ number. Finally, several transoceanic migrations are required for the phylogenetic distributions of *Lepidium* species.

Occurrence of Multiple Independent Alterations of Floral Structure

On the basis of the inferred phylogeny, reductions to two stamens have occurred a minimum of two times: once within clade 6 and once within clade 5. Let us first consider the reduction in clade 6. In each of the 15 most parsimonious trees, the ancestral condition of clade 10 is predicted to be two stamens. This is further corroborated in the analysis with a larger number of *Lepidium* species (H. Brüggemann, J. L. Bowman, and K. Mummenhoff, unpublished data). In this case, the small clade of four-stamen New Zealand species (including *Lepidium oleraceum*) nests within a much larger clade of two-stamen

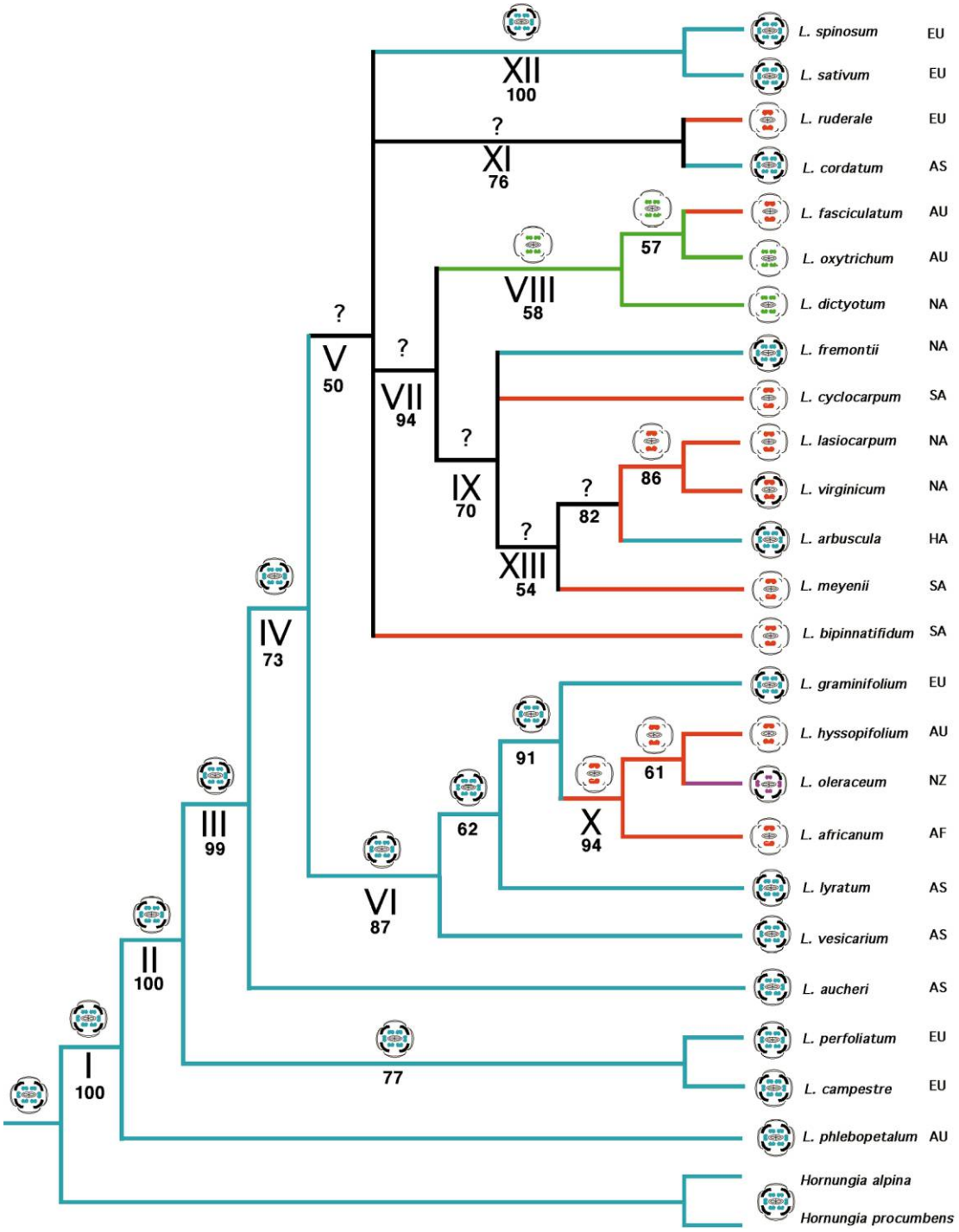

Fig. 3 Phylogenetic relationships within the genus *Lepidium* based on ITS sequences, highlighting the changes in floral ground plan within the genus. This article includes representatives of six out of seven sections and 10 out of 11 greges *sensu* Thellung (1906), and therefore the taxon sample represents a broad range of the variation within *Lepidium*. The tree presented represents the strict consensus of 15 most parsimonious trees with nodes for which the bootstrap support was 50% collapsed. Nodes/clades referred to in the text are denoted by arabic numerals and bootstrap values supporting the nodes are indicated. The floral structure of each of the extant species as well as some of the hypothetical ancestors, are represented by floral diagrams. Full-sized petals are indicated within the floral diagrams by thick lines; reduced petals are indicated by thin lines; petals absent are denoted by dots. Blue indicates lineages with 2 + 4 stamens; green indicates lineages with 0 + 4 stamens; purple indicates lineages with 2 + 2 stamens; orange indicates lineages with 0 + 2 stamens. The continent/island of origin is listed at the far right (*AU* = Australia; *AF* = Africa; *AS* = Asia; *EU* = Europe; *HA* = Hawaii; *NA* = North America; *NZ* = New Zealand; *SA* = South America).

species native to Africa and Australia. Thus it appears a reduction from 2 + 4 stamens and well-developed petals (e.g., *Lepidium vesicarium*, fig. 1*C*; *Lepidium graminifolium*, fig. 1*D*) to 0 + 2 stamens and reduced petals (e.g., *Lepidium africanum*, fig. 1*E*; *Lepidium hyssopifolium*, fig. 1*F*), and a subsequent reversal from 0 + 2 stamens and reduced petals to 2 + 2 stamens and well-developed petals, as in *L. oleraceum* (fig. 1*G*), occurred within clade 6.

The second reduction, within clade 5, is less straightforward. Within clade 5, two-stamen species occur in clades 7, 11, and the clade represented by *Lepidium bipinnatifidum*. However, the lack of resolution at node 5 precludes a simple reconstruction of the phylogeny within this clade, and a number of scenarios can be envisioned. Given the topology of the tree, multiple reductions, and possibly reversals, are required to account for the phylogenetic distribution of species within clade 5. For example, if the ancestral condition of clade 5 was six stamens, and reversals are eliminated, based on the 15 most parsimonious trees, a minimum of seven independent reductions are required to account for the distribution of two-stamen species. Conversely, if the ancestral condition of clade 5 was two stamens, and reductions are minimized to a single event, based on the 15 most parsimonious trees, three to five independent reversals are required to account for the distribution of four- and six-stamen species. Unfortunately, broader sampling of *Lepidium* species does not resolve the majority of these ambiguities (H. Brüggemann, J. L. Bowman, and K. Mummenhoff, unpublished data).

We postulate that the ancestral condition of clade 8 is a four-stamen species rather than a two-stamen species. This is based on the observation that the small clade of Australian two-stamen species (e.g., *Lepidium fasciculatum*, fig. 1*M*) nests within a larger clade of four-stamen species native to North America (e.g., *Lepidium dictyotum*; fig. 1*K*) and Australia (e.g., *L. oxytrichum*; fig. 1*L*) in trees based on wider sampling of *Lepidium* species (H. Brüggemann, J. L. Bowman, and K. Mummenhoff, unpublished data). If this is the case, another reduction to two stamens from a four-stamen ancestor is implied within clade 8. In summary, it is likely that two independent reductions to the two-stamen condition occurred within clade 5, and in addition, other reductions and/or reversals are necessary to account for the phylogenetic distribution of floral structures within this clade.

Strikingly, in most cases, reductions in stamen number are accompanied with reduction in petal stature. Likewise, in those taxa that may have experienced reversals, the reappearance of stamens is accompanied by a corresponding increase in petal stature. However, these processes can be genetically separated as exemplified by the occurrence of well-developed petals without an increase in stamen number in *Lepidium virginicum* (fig. 1*I*), whose ancestors might have had a floral structure similar to that of *Lepidium lasiocarpum* (fig. 1*H*). Additional evidence comes from the observation that there is a large variation in petal structure observed among species with two stamens. Thus, while most of the changes in floral structure within the genus involve both the petals and the stamens, changes in petal development can occur independently of alterations in stamen development.

Another indication that floral structures are relatively fluid is the variation observed within some *Lepidium* species. For example, flowers with three or four medial stamens have been reported in *L. virginicum* (Thellung 1906) and *Lepidium aschersonii* (Bowman and Smyth 1998), and a variety of *L. virginicum* with six stamens (2 + 4) has been reported (var. *tepicense*; Rollins 1993). Flowers of *L. virginicum* and *L. aschersonii* normally have only two medial stamens. Conversely, flowers with two or three stamens are occasionally observed in *L. dictyotum*, which normally has four medial stamens (J.-Y. Lee and J. L. Bowman, unpublished data). In all the cases we could examine in detail, the variation observed was infrequent and within individual plants, indicating a variable and low penetrance of the trait. Nonetheless, further sampling of wild isolates, such as *L. virginicum* var. *tepicense*, could provide potentially informative intraspecific variation.

Homoplastic Tendencies?

That reductions, and reversals of reductions, in the floral ground plan have occurred multiple times within *Lepidium* suggests that there might exist homoplastic tendencies for this character within the genus. By this we mean a tendency toward convergent evolution of the same character state within the phylogeny (reviewed in Maynard Smith et al. 1985; Wake 1991). In this case, the character states are reduced number of stamens (e.g., 0 + 2) and a reduced petal stature. A survey of Brassicaceae reveals that only three genera (*Lepidium*, *Coronopus*, and *Papuzilla*) exhibit reductions from 2 + 4 to 0 + 2 stamens (Schulz 1936; Endress 1992). However, the New Guinean genus *Papuzilla* has been treated by Hewson (1982) as *Lepidium*, perhaps closely allied to *L. aschersonii*, and preliminary molecular analyses indicate that *Coronopus* does not represent a natural group but rather that these species also nest within *Lepidium* (H. Brüggemann, K. Mummenhoff, and J. L. Bowman, unpublished data). This suggests that in the Brassicaceae reductions from 2 + 4 to 0 + 2 stamens occur exclusively within *Lepidium*. That changes rarely occur in the family, but appear to be clustered within *Lepidium*, suggests that there might be a predisposition for species within *Lepidium* to experience such changes as noted by Endress (1992). That a homoplastic tendency exists should be tested statistically (Sanderson 1991) once a more complete phylogeny of Brassicaceae becomes available. From a mechanistic standpoint, a homoplastic tendency could be explained by a genetic change occurring near the base of the lineage; this initial change would not result in morphological alterations but rather would predispose descendent taxa to exhibit morphological evolution due to subsequent genetic changes.

Mechanisms of Floral Structure Reduction

Floral organ reduction can occur either by the failure to initiate organ primordia or, alternatively, by suppression of development subsequent to primordia initiation (Tucker 1988). Developmental analyses of *Lepidium* species reveal that in this genus reductions in stamen number are the result of a failure to initiate organ primordia. In contrast, reduction of petals occurs by suppression of growth subsequent to primordial initiation, even in those species in which petals are reportedly absent (Bowman and Smyth 1998; J. L. Bowman, unpublished data). Because the reductions (and most putative reversals) appear to involve both stamens and petals, it has

been hypothesized that alterations in the expression or function of B-class genes, which specify petal and stamen identity in the angiosperm flower (Bowman et al. 1991; Weigel and Meyerowitz 1994), may be the cause of the coordinate reduction of organs in these two whorls (Endress 1992; Bowman and Smyth 1998). Loss of function alleles in the B-class gene lead to the second and third whorls of the flower differentiating inappropriately into sepals and carpels, instead of petals and stamens, respectively (Bowman et al. 1991). Conversely, gain of function alleles in which these genes are ectopically expressed lead to ectopic development of stamens in the fourth floral whorl (Krizek and Meyerowitz 1996). In addition, the B-class genes, and genes involved in B-class gene regulation (e.g., *LFY*, *UFO* and *SUP*), have roles in regulating the meristematic activity of the flower meristem, such that their misregulation can result in increases or decreases in numbers of organs per whorl and numbers of whorls of floral organs, particularly in those whorls in which petals and stamens develop (e.g., Schultz et al. 1991; Bowman et al. 1992; Weigel et al. 1992; Sakai et al. 1995; Lee et al. 1997).

While the B-class genes, genes directing B-class gene regulation, and targets of the B-class genes are obvious candidates to control floral structure in the second and third whorls of the flower, it is unlikely that simple loss-of-function alleles of these genes could account for the variation in *Lepidium* floral structure. This is supported by several observations of which three are mentioned here. First, loss-of-function alleles in these genes cause dramatic phenotypic alterations beyond those observed in *Lepidium* floral morphology. Second, the *DEFICIENS* protein of *Antirrhinum* can largely complement *apetala3* mutations in *Arabidopsis* (Irish and Yamamoto 1995) suggesting that the orthologous coding regions of this B-class gene are functionally equivalent in these two distantly related eudicots, despite major differences in the numbers and positions of petals and stamens between these species. Third, in the case of variation in floral symmetry within the Asteridae, the majority of instances of evolutionarily derived actinomorphy do not appear to be equivalent to the actinomorphic flowers generated by loss-of-function alleles in the *CYCLOIDEA* gene of *Antirrhinum* (Donoghue et al. 1998). It appears more likely that variation is generated by altering the temporal and spatial pattern of regulatory genes or their targets because of changes in the promoter regions of the corresponding genes (reviewed in Doebley and Lukens 1998). Among metazoans, alterations in the cis-regulatory elements of either the regulatory genes themselves or their targets have been proposed to be the cause of morphological variation among related species (reviewed in Gellon and McGinnis 1998; Akam 1998; Gibson 1999). For example, within arthropods variation in the expression patterns of the Hox genes, or other regulatory genes, has been shown to be the basis of morphological diversification in butterfly wing eyespot morphology and *Drosophila* bristle number (Brakefield et al. 1996; Stern 1998). That changes in the spectrum of downstream targets of the homeotic genes can also be the cause of changes in morphology has been shown for *Ultrabithorax* in its control of wing and haltere morphology in flies and butterflies, respectively (Warren et al. 1994; Weatherbee et al. 1999). Similar changes in the targets of Hox genes have also been proposed to be causative in the regulation and evolution of wing number in insects (Carroll et al. 1995). Analysis of candidate genes within *Lepidium* should clarify at which level, if any, changes in the B-class gene hierarchy are responsible for the observed changes in floral structure.

A more direct approach to identifying genes pertinent to changes in morphological evolution is to analyze the genetics of morphology in interspecific hybrids of closely related species with different structures (Gottlieb 1984). This has been particularly fruitful in identifying the genes responsible for the morphological evolution of maize from teosinte (Beadle 1939; White and Doebley 1998). If candidate genes can be mapped in this manner, they may then be identified, assuming a reasonable level of synteny exists between the genomes of *Lepidium* and *Arabidopsis*.

Occurrence of Multiple Transoceanic Migrations within the Evolution of Lepidium

Assuming that the diversification within *Lepidium* occurred after the dissolution of Gondwana, as suggested by the pollen fossil record (Muller 1981), multiple intercontinental dispersals are needed to account for the phylogeny presented in figure 3. For example, three phylogenetically distinct groups of *Lepidium* species are endemic to Australia. One clade, represented by *L. phlebopetalum* (fig. 1*A*), is likely to be ancient, while the other two, represented by *L. oxytrichum* (fig. 1*L*), *L. fasciculatum* (fig. 1*M*), and *L. hyssopifolium* (fig. 1*F*), respectively, are more recent introductions. The group of Australian endemics that nests within clade 10 (e.g., *L. hyssopifolium*) evidently shares common ancestry with species native to Africa. One attractive hypothesis is that this clade has its origins in Africa, with a genetic constitution similar to that of *L. africanum* (fig. 1*E*), presently a widely distributed weed. A transoceanic migration from Africa to Australia/New Zealand could then account for the present distribution of species within this clade. While an alternative model in which the African and the Australian/New Zealand species were independently derived from a Eurasian stock cannot presently be ruled out, wider sampling within clade 6 supports the former scenario. A third clade with Australian endemics, represented by *L. oxytrichum*/*L. fasciculatum*, might have had its ancestry in the Americas, perhaps similar to *L. dictyotum* (fig. 1*K*) in genetic constitution. Alternatively, if the American members of clade 7 were derived from Australia, the Australian species would have their derivation in Eurasia. Regardless of which scenarios are accepted, several transoceanic migrations are required to account for the geographic distributions of *Lepidium* species. One feature common to these and other implied intercontinental dispersals is that the putative ancestral species exhibited a reduced floral structure. Most of the extant species with reduced floral structures are characterized by autogamous breeding systems and are often polyploid, both typical features of colonizing plants. It is tempting to speculate that the Eurasian ancestors of species such as *Lepidium ruderale* (fig. 1*N*) represent the basic stock from which the species in clades 8 and 9 have evolved, and the lack of resolution at node 5 might reflect a rapid adaptive radiation into the Americas and Australia from such a stock.

Acknowledgments

We would like to thank the many people and institutions who kindly provided plant material, and we would also like to thank Ulrike Coja for her valuable technical help; Bob Kuzoff, Mike Sanderson, and Marty Wojciechowski for helpful discussions; and Ellen Dean for assistance in the Tucker Herbarium (Davis, Calif.). This article was supported by a grant of the German Research Foundation to K. Mummenhoff and a Beckman Young Investigator award to J. L. Bowman.

Literature Cited

Akam M 1998 From master genes to micromanagers. Curr Biol 8: R676–R678.

Al-Shehbaz IA 1986 The genera of Lepidieae (Cruciferae; Brassicaceae) in the southeastern United States. J Arnold Arbor Harv Univ 67:265–311.

Appel O, IA Al-Shehbaz 1997 Generic limits and taxonomy of *Hornungia*, *Pritzelago*, and *Hymenolobus* (Brassicaceae). Novon 7: 338–340.

Baldwin BG, MJ Sanderson, JM Porter, MF Wojciechowski, S Campbell, MJ Donoghue 1995 The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Mo Bot Gard 82:247–277.

Beadle G 1939 Teosinte and the origin of Maize. J Hered 30:245–247.

Bowman JL, H Sakai, T Jack, D Weigel, U Mayer, EM Meyerowitz 1992 *SUPERMAN*: a regulator of floral homeotic genes in *Arabidopsis*. Development 114:599–615.

Bowman JL, DR Smyth 1998 Patterns of petal and stamen reduction in Australian species of *Lepidium* L. (Brassicaceae). Int J Plant Sci 159:65–74.

Bowman JL, DR Smyth, EM Meyerowitz 1991 Genetic interactions among floral homeotic genes of *Arabidopsis*. Development 112: 1–20.

Brakefield P, J Gates, D Keys, F Kesbeke, P Wijngaarden, A Monteiro, V French, S Carroll 1996 Development, plasticity and evolution of butterfly eyespot patterns. Nature 384:236–242.

Carroll S, S Weatherbee, J Langeland 1995 Homeotic genes and the regulation and evolution of insect wing number. Nature 375:58–61.

Cronquist A 1981 An integrated system of classification of flowering plants. Columbia University Press, New York.

Doebley J, L Lukens 1998 Transcriptional regulators and the evolution of plant form. Plant Cell 10:1075–1082.

Donoghue MJ, RH Ree, DA Baum 1998 Phylogeny and the evolution of flower symmetry in the Asteridae. Trends Plant Sci 3:311–317.

Doyle JJ, JL Doyle 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15.

Endress PK 1992 Evolution and floral diversity: the phylogenetic surroundings of *Arabidopsis* and *Antirrhinum*. Int J Plant Sci 153(suppl):S106–S122.

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

Gellon G, W McGinnis 1998 Shaping animal body plans in development and evolution by modulation of *Hox* expression patterns. Bioessays 20:116–125.

Gibson G 1999 Redesigning the fruitfly. Curr Biol 9:R86–R89.

Gottlieb LD 1984 Genetics and morphological evolution in plants. Am Nat 123:681–709.

Hasegawa M, H Kishino, T Yano 1985 Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 21:160—174.

Hewson HJ 1981 The genus *Lepidium* L. (Brassicaceae) in Australia. Brunonia 4:217–308.

——— 1982 The genus *Lepidium* L. (Brassicaceae) in New Guinea. Brunonia 5:73–78.

Hillis DM, JP Huelsenbeck 1992 Signal, noise and reliability in molecular phylogenetic analyses. J Hered 83:189–195.

Hitchcock CL 1945 The South American species of *Lepidium*. Lilloa 11:75–134.

Irish VF, YT Yamamoto 1995 Conservation of floral homeotic gene function between *Arabidopsis* and *Antirrhinum*. Plant Cell 7: 1635–1644.

Krizek BA, EM Meyerowitz 1996 The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide B class organ identity function. Development 122:11–22.

Lee I, DS Wolfe, O Nilsson, D Weigel 1997 A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS*. Curr Biol 7:95–104.

Maddison WP, DR Maddison 1992 MacClade Version 3: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Mass.

Maynard Smith J, R Burian, S Kauffman, P Alberch, J Campbell, B Goodwin, R Lande, D Raup, L Wolpert 1985 Developmental constraints and evolution. Q Rev Biol 60:265–287.

Muller J 1981 Fossil pollen records of extant angiosperms. Bot Rev 47:1–142.

Mummenhoff K, A Franzke, M Koch 1997 Molecular phylogenetics of *Thlaspi* s.l. (Brassicaceae) based on chloroplast DNA restriction site variation and sequences of the internal transcribed spacers of nuclear ribosomal DNA. Can J Bot 75:469–482.

Mummenhoff K, H Hurka, H-J Bandelt 1992 Systematics of Australian *Lepidium* species (Brassicaceae) and implications for their origin: evidence from IEF analysis of Rubisco. Plant Syst Evol 183: 99–112.

Mummenhoff K, E Kuhnt, M Koch, K Zunk 1995 Systematic implications of chloroplast DNA variation in *Lepidium* sections *Cardamon, Lepiocardamon* and *Lepia* (Brassicaceae). Plant Syst Evol 196:75–88.

Rollins RC 1993 The Cruciferae of continental North America: systematics of the mustard family from the Arctic to Panama. Stanford University Press, Stanford, Calif.

Sakai H, LJ Medrano, EM Meyerowitz 1995 Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. Nature 378: 199–203.

Sanderson MJ 1991 In search of homoplastic tendencies: statistical inference of topological patterns in homoplasy. Evolution 45: 351–358.

Sanger F, S Nicklen, AR Coulsen 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Schultz E, FB Pickett, GW Haughn 1991 The *FLO10* gene product regulates the expression domain of homeotic genes *AP3* and *PI* in *Arabidopsis* flowers. Plant Cell 3:1221–1227.

Schulz EO 1936 Cruciferae. Pages 227–658 *in* A Engler and K Prantl, eds. Die Natürlichen Pflanzenfamilien. Vol 17*b*. 2d ed. Engelmann, Leipzig.

Sokal RR, FJ Rohlf 1995 Biometry. 3d ed. Freeman, New York.

Stern DL 1998 A role of *Ultrabithorax* in morphological differences between *Drosophila* species. Nature 396:463–466.

Swofford DL 1993 Phylogenetic analysis using parsimony (PAUP), version 3.1.1. Illinois Natural History Survey, Champaign, Ill.

Swofford DL, GJ Olsen 1990 Phylogeny reconstruction. Pages 411–501 *in* D M Hillis, C Moritz, eds. Molecular systematics. Sinauer Associates, Sunderland, Mass.

Templeton AR 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

Thellung A 1906 Die Gattung *Lepidium* (L) R Br. Eine monogra-

phische Studie. Neue Denkschr Allg Schweiz Naturforsch Ges 41: 1–340.

Tucker SC 1988 Loss versus suppression of floral organs. Pages 69–82 *in* P Leins, SC Tucker, PK Endress, eds. Aspects of floral development. Cramer, Berlin.

Wake DB 1991 Homoplasy: the result of natural selection, or evidence of design limitations. Am Nat 138:534–567.

Warren R, L Nagy, J Selegue, J Gates, S Carroll 1994 Evolution of homeotic gene regulation and function in flies and butterflies. Nature 372:458–461.

Weatherbee SD, HF Nijhout, LW Grunert, G Halder, R Galant, J Selegue, S Carroll 1999 Ultrabithorax function in butterfly wings and the evolution of insect wing pattern. Curr Biol 9:109–115.

Weigel D, J Alvarez, DR Smyth, MF Yanofsky, EM Meyerowitz 1992 *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell 69: 843–859.

Weigel D, EM Meyerowitz 1994 The ABC's of floral homeotic genes. Cell 78:203–209.

White S, J Doebley 1998 Of genes and genomes and the origin of maize. Trends Genet 14:327–332.