American Journal of Botany 91(2): 254–261. 2004.

MOLECULAR EVIDENCE FOR BICONTINENTAL HYBRIDOGENOUS GENOMIC CONSTITUTION IN *LEPIDIUM* SENSU STRICTO (BRASSICACEAE) SPECIES FROM AUSTRALIA AND NEW ZEALAND[1]

KLAUS MUMMENHOFF,[2,6] PETER LINDER,[3] NIKOLAI FRIESEN,[4]
JOHN L. BOWMAN,[5] JI-YOUNG LEE,[5] AND ANDREAS FRANZKE[2]

[2]Universität Osnabrück, Spezielle Botanik, Barbarastrasse 11, 49076 Osnabrück, Germany; [3]Institute for Systematic Botany, University Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland; [4]Universität Osnabrück, Botanischer Garten, Albrechtstrasse 29, 49076 Osnabrück, Germany; and [5]Plant Biology, University of California, Davis, California 95616 USA

Lepidium sensu stricto (s.s.) (Brassicaceae) (ca. 150 species) is distributed worldwide with endemic species on every continent. It is represented in Australia and New Zealand by 19 and seven native species, respectively. In the present study we used a nuclear ribosomal internal transcribed spacer (ITS) phylogeny in comparison with a cpDNA phylogeny to unravel the origin of Australian/New Zealand species. Although phylogenetic relationships within *Lepidium* s.s. were not fully resolved, the cpDNA data were in agreement with a Californian origin of *Lepidium* species from Australia/New Zealand. Strongly conflicting signals between the cp- and nuclear DNA phylogenetic analysis clearly indicated hybridogenous genomic constitution of Australian *Lepidium* s.s. species: All 18 studied Australian/New Zealand *Lepidium* s.s. species examined shared a Californian cpDNA type. While eleven Australian/New Zealand species appeared to harbor a Californian ITS type, a group of seven species shared a South African ITS type. This pattern is most likely explained by two trans-oceanic dispersals of *Lepidium* from California and Africa to Australia/New Zealand and subsequent hybridization followed by homogenization of the ribosomal DNA either to the Californian or South African ITS type in the two different lineages. Calibration of our molecular trees indicates a Pliocene/Pleistocene origin of *Lepidium* in Australia/New Zealand. Low levels of cpDNA and ITS sequence divergence and unresolved topologies within Australian/New Zealand species suggest a rapid and recent radiation of *Lepidium* after the hybridization event. This coincides with dramatic climatic changes in that geological epoch shaping the composition of the vegetation.

Key words: biogeography; Brassicaceae; hybridization; *Lepidium*; long-distance dispersal; molecular phylogenetics; polyploidy.

Lepidium L. is one of the largest genera in the Brassicaceae, consisting of ca. 175 species worldwide. Recent molecular studies of *Lepidium* phylogeny utilizing the nuclear rDNA internal transcribed spacer (ITS), noncoding cpDNA and single copy nuclear DNA sequences (an intron of PISTILLATA, PI), respectively, clarified only some relationships within the genus (Bowman et al., 1999; Mummenhoff et al., 2001; Lee et al., 2002). Although the studies focused on different topics and were based on different taxon coverage (ITS, evolution of floral structure, 24 taxa studied; cpDNA, phylogeny and biogeography, 73 taxa studied and PISTILLATA [PI] intron: 43 taxa studied), all phylogenies indicated that few of the infrageneric taxa as delimited in the systems of Thellung (1906) and Hewson (1981) represent monophyletic groups. All molecular phylogenies support three main lineages, corresponding to (1) section *Monoploca* sensu stricto (s.s.) (Australia), (2) section *Lepia* with *Cardaria* included (Eurasia), and (3) *Lepidium* s.s. representing the bulk of species formerly assigned to sections *Monoploca* sensu lato (s.l.), *Dileptium* and *Lepidium* (Eurasia, Africa, North and South America, Australia, New Zealand, and the Pacific region). *Monoploca* s.s. appeared as sister group to *Lepia* and *Lepidium* s.s. (Bowman et al., 1999) and thus represents an older introduction while Australian/New Zealand *Lepidium* s.s. represents a more recent introduction (Mummenhoff et al., 2001). Incongruencies between topologies based on maternally inherited cpDNA and uni- or biparentally inherited nuclear ITS sequences (Wendel et al., 1995a; Sang et al., 1995a) and the large number of polyploid species suggest reticulate evolution in *Lepidium* s.s. (Brüggemann, 2000). This was indicated also in the analysis of PISTILLATA intron sequences in which many polyploid taxa harbor two or more phylogenetically distinct sequences (Lee et al., 2002). Thus, an important unanswered question concerns the evolutionary history, i.e., the biogeographic context and time of origin, of Australian/New Zealand *Lepidium* s.s. species. Based on morphological differences among native Australian taxa, H. Hewson (CSIRO, personal communication) suggested independent introductions of *Lepidium* in Australia from South America, South Africa, and Southeast Asia.

In the present study we clarify the evolutionary history of *Lepidium* s.s. in Australia/New Zealand by comparing noncoding cpDNA and ITS sequences from 18 Australian/New Zealand species along with 38 species from the other continents. This represents the whole spectrum of variation in *Lepidium* s.s. Here we show that Australian/New Zealand *Lepidium* s.s. species are a polyploid group that descended from Californian ancestors hybridizing in Australia with *Lepidium* species originating from South Africa.

MATERIALS AND METHODS

Taxon sampling—Fifty-six taxa were chosen to represent the whole spectrum of variation in the *Lepidium* s.s. lineage and cover all major geographic

[1] Manuscript received 6 May 2003; revision accepted 18 September 2003.

The authors thank Torsten Struck for advice on relative rate tests; Ulrike Coja for technical assistance; Konrad Bachmann, Holger Brüggemann, and Herbert Hurka for fruitful discussions; two anonymous reviewers for valuable comments; and people and institutions who kindly provided plant material. This work was supported by the German Research Foundation (MU 1137/2-1).

[6] E-mail: Mummenhoff@biologie.uni-Osnabrueck.DE.

distributions areas: Europe/Asia, Africa, North and South America, and Australia/New Zealand. Australia and New Zealand harbor 19 and nine native taxa, respectively; our sampling of 10 Australian and eight New Zealand taxa comprises representatives of all taxonomic entities and thus represents the full range of variation of *Lepidium* s.s. in Australia and New Zealand. As an outgroup, species of section *Lepia* s.l., i.e., *L. campestre* and *L. hirtum* subsp. *hirtum*, were used. In previous analyses section *Lepia* appeared as sister to *Lepidium* s.s. (Bowman et al., 1999; Mummenhoff et al., 2001). Collection data for the accessions used in this study along with GenBank accession numbers are available as supplemental data accompanying the online version of this article.

Molecular methods—Methods for DNA extraction, PCR, and direct sequencing of ITS and noncoding cpDNA (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer) are given elsewhere (Bowman et al., 1999; Mummenhoff et al., 2001). To study the parental ITS units in presumed hybridogenous Australian *Lepidium*, the PCR products of selected Australian/New Zealand polyploids (*L. fasciculatum, L. pseudohyssopifolium, L. pseudotasmanicum, L. muelleriferdinandi, L. oleraceum, L. banksii*) were cloned and 2–8 clones were sequenced. The PCR products of the entire ITS region were purified with the QIAquick purification kit (Qiagen, Hilden, Germany) and directly ligated into plasmid vector pGEM T-Easy (Promega, Madison, Wisconsin, USA) and transformed into competent cells (*E. coli* strain DH α). Cells were plated on ''Luria Broth Base'' plates containing ampecillin. White colonies were selected with IPTG (Isopropyl-β-D-thiogalactopyranoside) and β-galactosidase and they were grown in the same medium. Plasmid preparation was performed using QIAprep Spin Miniprep kit (Qiagen). The same primers as for the PCR amplification were used for sequencing. Compilation of the information obtained from direct sequencing and from clone sequencing reveals one nucleotide polymorphism in *L. oleraceum* and *L. fasciculatum*, respectively, two polymorphic sites in *L. pseudohyssopifolium*, and three in *L. muelleriferdinandi*. Thus, we treated these nucleotide sites as polymorphic character states in the ITS data set used for subsequent phylogenetic analyses.

The DNA sequences were aligned by hand. Regions of ambiguous alignment were eliminated. Insertions/deletions (indels) were treated as missing data in the ITS matrix. In our recent cpDNA analysis (Mummenhoff et al., 2001) we demonstrated that indels ≥3 base pairs (bp) provided reliable phylogenetic information. Therefore, indels in the cpDNA data were coded as missing in the core data matrix but six indels ≥3 bp were recoded as additional binary characters. Alignments are available upon request.

Phylogenetic analysis—Parsimony analysis of ITS and cpDNA assumed unordered and unweighted character states (i.e., Fitch parsimony) and used the heuristic search strategy in PAUP (version 4.0b10; Swofford, 2000) with tree bisection-reconnection (TBR) branch swapping, and 100 random taxon additions. Up to 10 000 trees were kept from each random addition sequence replicate.

Bootstrap support values were obtained from 100 replicates by using an heuristic search and simple addition. The inclusion of hybrids in cladistic analyses may effect tree topology (McDade, 1992), e.g., as a consequence of recombination between divergent parental ITS types (Baldwin and Sanderson, 1998). All Australian species of presumed hybrid origin were included in the final data set because the exclusion of these species in initial analyses had no effect on topology of the remaining species.

Sequence divergence and relative rate test—Pairwise distance divergences were calculated in PAUP under Kimura's two parameter model (K2P) by using the pairwise-deletion option for gaps and ambiguous data. Rate heterogeneity among lineages in the cpDNA and ITS trees was examined by using a tree-wide likelihood ratio (LR) test (Modeltest 3.04; Posada and Crandall, 1998). This test compares the log likelihoods (determined in 4.0b10) of constrained and unconstrained hypotheses (constrained, DNA substitution rates are equal among lineages; unconstrained, rates are allowed to vary among lineages) under the most adequate substitution model that best fits the data (Huelsenbeck and Rannala, 1997).

RESULTS

The cpDNA and ITS sequence variation—The species considered here for analyzing the origin of Australian/New Zealand *Lepidium* species were included in a previous more detailed study of three noncoding cpDNA regions (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer) in the genus (Mummenhoff et al., 2001). Therefore, details on characterization of this marker system are given there. After eliminating regions with ambiguous alignment, 1435 positions were available for phylogenetic analysis. Of the 220 variable characters, 89 were potentially parsimony informative. Averaged over the three regions K2P pairwise sequence divergence among the *Lepidium* s.s. taxa varied between 0 and 2.5% and within Australian/New Zealand taxa between 0 and 1.5%.

The ITS regions were sequenced for 56 species and 34 clones of selected Australian *Lepidium* species. The alignment generated a matrix of 456 characters, of which 87 are potentially informative in parsimony analysis. The K2P pairwise sequence divergence among the *Lepidium* s.s. taxa ranged between 0 and 7.9% and within Australian/New Zealand taxa between 0 and 1.5%.

ITS sequence polymorphism in Australian/New Zealand Lepidium and presumed progenitors—Eighteen Australian *Lepidium* s.s. taxa were analyzed by direct sequencing. Known polyploids (*L. fasciculatum*, *L. pseudohyssopifolium*, *L. pseudotasmanicum*, *L. muelleriferdinandi*, *L. oleraceum*, *L. banksii*; ploidy level see Fig. 1) were also analyzed by cloning amplified ITS sequences. Three species (*L. aschersonii*, *L. muelleriferdinandii*, *L. pseudohyssopifolium*) show additivity at one nucleotide position that is variable between the Californian (clade C) and South African (clade A) species group (Fig. 1, sites 108, 297, 432). This indicates that the Australian/New Zealand species may have originated by hybridization. With respect to those sites that are variable between the California and South Africa species group (Fig. 1) the Australian species of clade C and A show exactly those character states of the Californian (clade C) or South African (clade A) species, respectively. However, at site 109 most Australian/New Zealand taxa including those of clade C (related to Californian species) are characterized by the nucleotide found in the South African species (clade A).

Phylogenetic position of Australian/New Zealand Lepidium—We analyzed noncoding cpDNA regions and nuclear ITS regions from 56 *Lepidium* s.s. taxa of all major geographic distributions to unravel the origin of Australian/New Zealand *Lepidium* in this lineage. Comparison of the phylogenetic trees (strict consensus of most parsimonious trees) generated from the two data sets is illustrated in Fig. 2. Several nodes are reasonably well supported (>70% bootstrap values), while other nodes are less so, due to low numbers of nucleotide substitutions. This is not surprising in view of a Pliocene/Pleistocene origin of *Lepidium* s.s. (Mummenhoff et al., 2001). The maximum likelihood (ML) trees generated from the same data sets (not shown) were a nearly exact match of the parsimony trees, with the exception of slight differences in branching within some terminal nodes. In the current study of *Lepidium* s.s. both phylogenies agree in grouping geographically related species, but there are strongly conflicting signals between the cpDNA and ITS topologies (Fig. 2). In the cpDNA strict consensus tree all Australian/New Zealand species are weakly

	ITS1 ITS2
	1111111112233344
	6788001344677593693 3
	8347896568024179540 2
Californian taxa, clade C	
dic (4x), lat (4x), nit (4x), oxyc	CATTAGCCCGTCATCGG-TA
Australian/New Zealand taxa, clade C	
nau (4x), oxyt (3x), pap,	
sis (3 ssp., 4x), ten	CATTACCCCGTCATCGG-TA
fasc (4x)	CATTATCCCGTCATCGG-TA
pseup	CGTTACCCCGTCATCGG-TA
asch	CATTATCCCGTCATCGG-TW
muel (6x)	CATTRCCCCGTCATCGG-TA
Australian/New Zealand taxa, clade A	
ban (4x), des, fle (4x),	
hys (4x), ole (4x), pseut (4x)	TTCGGCGTGCAACCTA-TCT
pseuh (4x)	TTCGGCGTGCAACCYA-TCT
South African taxa, clade A	
afr (2x), dese, div, myr,	
pin, schi, tri	TTCGGCGTGCAACCTA-TCT
cap	TTCGGCGTGCAACCAA-TCT

Fig. 1. Composition of the ITS sequences of Californian, Australian/New Zealand, and South African *Lepidium* species. This data matrix contains only those positions of a complete alignment (not shown) that distinguish Californian from South African *Lepidium* taxa. Californian and South African species are extant members of two different lineages suggested to have been involved in the hybridogenous origin of Australian/New Zealand *Lepidium*. Clade A and clade C refer to the ITS tree in Fig. 2. Site numbers are those of the complete alignment. R = A and G; W = A and T; Y = C and T. Taxon abbreviations; Californian taxa: dic = *L. dictyotum*; lat = *L. latipes*; nit = *L. nitidum*; oxyc = *L. oxycarpum*; Australian/New Zealand clade C taxa: nau = *L. naufragorum*; oxyt = *L. oxytrichum*; pap = *L. papillosum*; sis (three ssp.) = *L. sisymbrioides* ssp. *kawarau*, ssp. *sisymbrioides*, ssp. *matau*; ten = *L. tenuicaule*; fasc = *L. fasciculatum*; pseup = *L. pseudopapillosum*; asch = *L. aschersonii*; muel = *L. muelleriferdinandi*; Australian/New Zealand clade A taxa: ban = *L. banksii*; des = *L. desvauxii*; fle = *L. flexicaule*; hys = *L. hyssopifolium*; ole = *L. oleraceaum*; pseut = *L. pseudotasmanicum*; pseuh = *L. pseudohyssopifolium*; South African taxa: afr = *L. africanum*; dese = *L. desertorum*; div = *L. divaricatum*; myr = *L. myriocarpum*; pin = *L. pinnatum*; schi = *L. schinzii*; tri = *L. trifurcum*; cap = *L. capense*. Ploidy levels (2*x*–6*x*) are based on own chromosome counts.

grouped into a monophyletic group arising from a polytomy. However, in 60% of all maximally parsimonious trees (clade B, cpDNA, Fig. 2) four coastal Californian species are found as sister to the Australian/New Zealand clade. Within clade B species relationships are not well resolved due to lack of characters. However, within this clade two distinct groups may be recognized composed of New Zealand species, i.e., *L. sisymbrioides* with three subspecies (100% bootstrap support) and *L. naufragorum*, *L. flexicaule*, *L. banksii*, and *L. oleraceum* (53% bootstrap support), respectively. In the ITS tree Australian species are distributed among two different lineages. One group shares common ancestry with the same four Californian species mentioned above (clade C). The remaining species are nested within an unresolved clade A along with South African species and one East African species.

Relative rate test and molecular clock calibration—Rate heterogeneity among lineages in the cpDNA and ITS trees, respectively, was examined by using a tree-wide likelihood ratio (LR) test (Modeltest 3.04; Posada and Crandall, 1998). Rate constancy across lineages throughout the ITS and cpDNA tree was rejected. So we followed the recommendations given in Sanderson (1998) to perform localized LR tests on subclades of the phylogenies. As we have focused in the present study on the origin of Australian/New Zealand *Lepidium* species, we tested for rate constancy within those clades containing Australian/New Zealand species (Fig. 2: ITS tree, clade A, C; cpDNA tree, clade B). Substitution rate constancy was rejected for clade B in the cpDNA tree, but rate constancy within clades A and C of the ITS phylogeny cannot be rejected at the $\alpha = 0.01$ level.

We used fossil data (Mai, 1995) of *Rorippa* (Brassicaceae) to calibrate mutation rates in *Lepidium*. *Rorippa* was found sister to *Cardamine* in recent phylogenetic analyses (Franzke et al., 1998; Mitchell and Heenan, 2000). Assuming a correct dating for the *Rorippa* fossil, a minimum of K2P sequence divergences of 1.8% (*trn*T/L spacer, *trn*L intron) and 4.4% (ITS), respectively, observed between *Rorippa* and *Cardamine* taxa analyzed (Franzke et al., 1998), might then correspond to 2.5–5 $\times$ 10^6 (million) years ago (Myra), as a rough estimate for divergence time between *Rorippa* and *Cardamine*. Thus, 1% sequence divergence corresponds to 0.6–1.1 $\times$ 10^6 (million) years (Myr) for the ITS regions and 1.3–2.8 Myr for the cpDNA. Our calibrated ITS rates are in the same order of magnitude as recently published ITS rates, e.g., *Gossypium*: ca. 1% = 1 Myr (Wendel et al., 1995b), *Gentianalla* 1% = 0.6–1.1 Myr (von Hagen and Kadereit, 2001), *Soldanella* 1% = 0.6–1.3 Myr (Zhang et al., 2001), *Robinsonia* 1% = 0.6 Myr (Sang et al., 1995b), and are thus reasonable.

Sequence divergence among the Australian/New Zealand species ranged from 0 to 1.2% (0.5% mean) for the *trn*T/L spacer and *trn*L intron. The ITS divergence ranged from 0.2 to 3.4% (1.2% mean) and from 0 to 1.1% (0.5% mean) among the Australian/New Zealand species of lineage A and C (Fig. 2), respectively. By using the ITS substitution rate calibrated with *Cardamine*/*Rorippa* (see above), we roughly estimated an age of 0.7–1.3 Myr and 0.3–0.55 Myr for the Australian/New Zealand species of lineage C and A, respectively. As we had to reject a tree-wide constant rate of ITS, the deviating divergence values of these two lineages might result from rate heterogeneity and reticulation between basal lineages. Although noncoding cpDNA evolution (even within the Australia/New Zealand clade) was not clocklike, the estimated age for the Australian/New Zealand lineage ranges from 0.6 to 1.4 Myr. Our time estimates based on ITS and cpDNA data are in the same order of magnitude indicating a Quaternary origin of Australian/New Zealand *Lepidium* species.

DISCUSSION

Reticulate evolution in Lepidium s.s.—*Lepidium* s.s. includes species from all continents. Most of the species are characterized by reduced flowers, an autogamous breeding system, and polyploidy, all these being typical features of colonizing plants (Al-Shehbaz, 1986; Bowman et al., 1999). Our recent phylogenetic analysis of the *PISTILLATA* (*PI*) intron, a member of the MADS-box gene family involved in stamen and petal specification, indicates that many species have originated from alloploidization (Lee et al., 2002). The present ITS

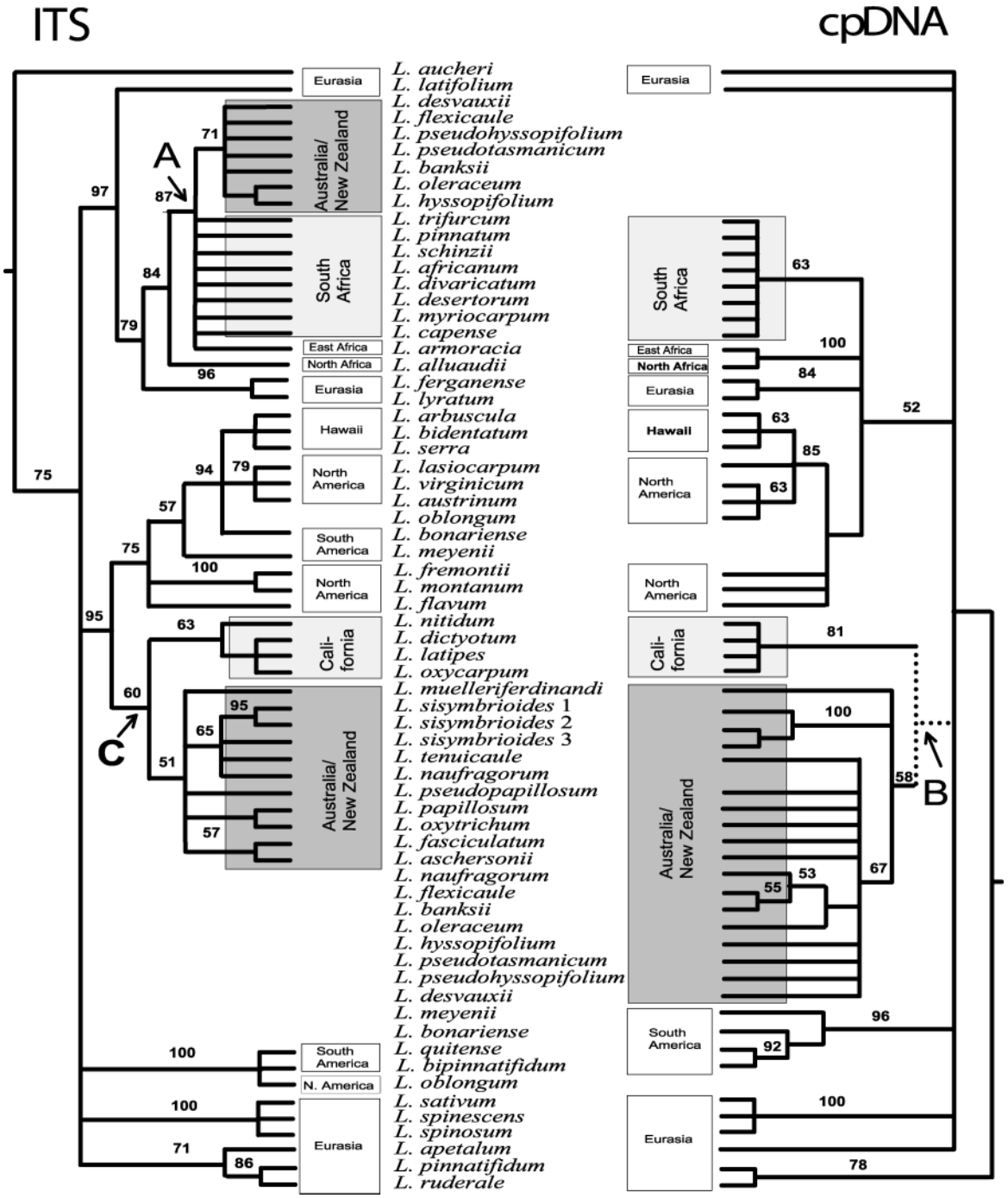

Fig. 2. Comparison of nuclear and chloroplast phylogeny of Australian/New Zealand *Lepidium* species and continental relatives based on sequence analysis of ribosomal ITS and three noncoding cpDNA regions (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer), respectively. Shown is the strict consensus each of 1369 most parsimonious ITS and 990 001 cpDNA trees with 202 and 275 steps and a consistency index (autapomorpies excluded) of 0.80 and 0.71, respectively. Dashed line in the cpDNA tree indicates a branch present in 60% of the minimum length trees. Arrows indicate clades involving *Lepidium* species of South Africa, Australia/New Zealand (clade A), and California, Australia/New Zealand (clade C, clade B). Bootstrap values >50% are given above branches. *Lepidium sisymbrioides* 1 through 3 refer to subspecies *sisymbrioides*, *kawarau*, and *matau*. Trees were rooted by outgroup comparison.

and cpDNA data reinforces this hypothesis. Strongly conflicting signals between the different genome phylogenies are easily observed throughout the trees. Such discrepancies are considered to be an indication of reticulation events (Rieseberg et al., 1996; Barrier et al., 1999). A highly reticulate evolution in *Lepidium* s.s. might explain the failure of the monographer Thellung (1906) to recognize lineages within this critical species complex, due to the variation patterns in morphology being blurred by (multiple) hybridizations. Although reticulate evolution seems to have played an important role in the phylogeny of *Lepidium* s.s. worldwide the current study focuses on the origin of Australian/New Zealand *Lepidium* s.s. species.

Two nuclear genomes in Australian/New Zealand Lepidium—Judged from the cpDNA phylogeny all Australian/New Zealand species harbor a cpDNA type most closely related to that of Californian species, and they are grouped into clade B in 60% of maximally parsimonious trees (Fig. 2). The cpDNA mean sequence divergence values of 0.48% between species from California and Australia/New Zealand compared to the 0.92% sequence divergence observed between Australian/New Zealand species of clade B and South American species, i.e., *L. bonariense*, *L. meyenii*, and *L. quitense*, also supports this close relationship between Californian and Australian/New Zealand species. However, the ITS data provides strong evidence that some Australian/New Zealand *Lepidium* species contain the nuclear genome of the Californian species (clade C), while others have the nuclear genome of the South African lineage (clade A). The predominance of a distinct ITS type within each Australian lineage (clade C and A), respectively, may be explained by rapid bidirectional concerted evolution following ancient hybridization between species from California and South Africa. The cpDNA data indicates that species of a Californian lineage represented by, e.g., *L. dictyotum* was the most probable maternal parent of the Australian /New Zealand species, both groups sharing a similar cp-genome. The ITS data confirms this Californian lineage to be among the parental taxa but also indicates that South African species (e.g., *L. africanum*, etc.) were also involved. The hybridogenous genomic constitution of Australian/New Zealand *Lepidium* species can also be observed directly in ITS sequences. Nucleotide additivity at three nucleotide sites (Fig. 1) in the ITS regions Australian/New Zealand *Lepidium* s.s. species indicates that reticulate evolution has occurred as has been demonstrated in *Paeonia* (Sang et al., 1995a) and that parental taxa of both continents were involved. The Australian/New Zealand species of clade C and A show with one exception exactly those character states of the Californian or South African species, respectively (Fig. 1). Thus, concerted evolution via gene conversion or unequal crossing-over is apparently operating. The observation of (nearly) homogenous ITS sequences (Fig. 1) indicates (nearly) complete uni- or bidirectional homogenization or transition stages in the homogenization process of the ITS region in the alloploids, respectively (Wendel et al., 1995a; Campbell et al., 1997).

Based on *PI* intron sequences Lee et al. (2002) demonstrated that many polyploid taxa harbored two or more phylogenetically distinct sequences, confirming a hybrid nature of Australian/New Zealand taxa. The *PI* intron types of presumed parental taxa of Australian/New Zealand *Lepidium* (i.e., *L. nitidum*, *L. dictyotum*) were also detected in three of nine Australian/New Zealand species, representing clade A and C taxa of the ITS phylogeny (Fig. 2). The remaining six Australian/New Zealand species covered by the *PI* study were not characterized by a Californian specific (i.e., *L. nitidum*, *L. dictyotum*) *PI* intron type. Furthermore, a South African origin of the nuclear genome of some Australian/New Zealand species demonstrated in the current ITS study was not found in the *PI* intron analysis (Lee et al., 2002), but, in the *PI* study only one South African taxon was analyzed. These discrepancies might be explained by rapid genomic change and gene loss in the polyploids (Soltis and Soltis, 1995; Song et al., 1995). For instance, restricted fragment length polymorphism (RFLP) analysis with *PI* in those Australian species with only a single *PI* sequence type (e.g., *L. hyssopifolium*, *L. oleraceum*) found two loci in both species, meaning one of the homeologues of *PI* (that putatively being related to the African lineage) was not detected by PCR due to either rapid mutation in the process of pseudogenization of sequences of two homeologous loci via concerted evolution or recombination or gene loss (Wendel, 2000; Kashkush et al., 2002). Other discrepancies of species relationships between the cpDNA/ITS and *PI* intron studies might also be explained by hybridization followed by these mechanisms of rapid genome change, although lineage sorting of *PI* intron in some *Lepidium* species cannot be ruled out as another possible cause. In the long run, in order to elucidate the entire reticulate history of *Lepidium* species, we will need to sequence further single copy nuclear genes.

Bicontinental hybrid origin of Australian/New Zealand Lepidium s.s.—We suggest that Australian *Lepidium* species are hybrids derived from a cross between colonizing species of the Californian and South African lineages C and A, respectively. Uniform/similar cpDNA and ITS sequences within the lineages containing Australian/New Zealand *Lepidium* taxa indicate that this ancient hybridization scenario predated the rapid speciation within these lineages. These speciation events probably also include later hybridization events as evidenced from grouping of species in the cpDNA tree (i.e., *L. naufragorum*, *L. banksii*, *L. flexicaule*, *L. oleraceum*) that are distributed among the two different clades A and C in the ITS tree (Fig. 2). The question of whether polyploid Australian/New Zealand *Lepidium* are homoploid or alloploid hybrids cannot be answered from our data. The very few South African and Californian species from which chromosome numbers are known are *L. africanum* ($2n = 2x = 16$) and *L. dictyotum*, *L. latipes*, *L. nitidum*, (all $2n = 4x = 32$), respectively. We generally used herbarium specimens in the current study and thus the ploidy level of Californian and South African *Lepidium* could not be analyzed. Until this point is clarified, one may suggest that the parental species were perhaps polyploid as polyploidy may have facilitated the establishment of the colonizing taxa from California and South Africa prior to hybridization. This is an attractive hypothesis because polyploidy may confer various characteristics appropriate to colonizing organisms including self-compatibility and buffering the effects of selfing (i.e., inbreeding depression), better vigor, and broader ecological tolerance (Barrier et al., 1999; Miller and Venables, 2000). Indeed, based on *PI* intron sequences, *L. nitidum* is suggested to be an allopolyploid (Lee et al., 2002).

The most attractive hypothesis is that the ancient hybridization event occurred in Australia. This implies one dispersal from South Africa and California, respectively. Dispersal from California to South Africa and subsequent hybridization with an endemic taxon (or vice versa) followed by transoceanic migration of the hybrid to Australia may not be excluded. But this scenario is less parsimonious because it requires independent introductions by long-distance dispersals of the different ITS types found in recent Australian/New Zealand taxa.

The origin of *Lepidium* and of the Brassicaceae as a whole presumably occurred in an area encompassing the Mediterranean and the Irano-Turanian territory, a region extremely diverse ecologically, altitudinally, and geologically (Thellung, 1906; Mummenhoff et al., 2001). Fossil data, easy dispersible mucilaginous seeds, widespread autogamy and polyploidy, and low levels of cpDNA divergence between species from different continents or islands suggest a rapid radiation of *Lepidium* by long-distance dispersal in the Pliocene/Pleistocene (Mummenhoff et al., 2001). As a consequence of climatic

changes in this geological epoch, arid/semiarid regions were established, providing favorable conditions for the radiation of *Lepidium* worldwide. South Africa was reached by the ''arid corridor,'' a belt of dry country that stretched from the Horn of Africa to Namibia (Hedge, 1976; Jürgens, 1997), and immigration of *Lepidium* into North and subsequently South America in Quaternary times is compatible with our estimates of divergence times, based on cpDNA sequence data (Mummenhoff et al., 2001).

There are several genera centred in southern Africa with a few species in Australia, e.g., *Bulbine* (Asphodeliaceae), *Wurmbea* (Colchicaceae), *Caesia* (Hemerocallidaceae), *Spiloxene* (Hypoxidaceae), and *Dietes* (Iridaceae). In a phylogenetic analysis of *Pelargonium* (Geraniaceae) the close relationship between the South African and Australian species is caused by long-distance dispersal to Australia, probably as recent as the late Pliocene (Bakker et al., 1998). In the other cases mentioned above the possibility of recent dispersal across the Indian Ocean has not been confirmed, but it seems unlikely that the distributions date to the Jurassic, which is when the continents were last physically connected via Antarctica.

Our data provide evidence that Australian/New Zealand *Lepidium* are polyploid descendants of two different continental ancestors, one probably immigrating from California and the other from South Africa. Long-distance dispersal of colonizing species from California and South Africa to Australia/New Zealand seems unlikely given that these areas are currently separated by more than 10 000 km, respectively. Carlquist (1983) however, demonstrated intercontinental dispersal (California to Chile) of mucilaginous *Lepidium* seeds adhering to birds (Mummenhoff et al., 1992; Norton et al., 1997) and sea bird migration pathways between coastal California and Australia/New Zealand and between South Africa and Australia/New Zealand (Lincoln et al., 1998) are fully compatible with the proposed colonization scenario.

Time of origin—Calibration of our molecular trees by using *Rorippa* fossil data yield ages of approximately 0.7–1.3 Myr and 0.3–0.55 Myr for the Australian/New Zealand species of clade C and A, respectively. These age differences might slightly modify our hypothesis that the hybridization of more or less concurrently arriving Californian and South African ancestors predates the radiation of Australian/New Zealand species. Different estimates of time of origin of Australian/New Zealand representatives of clades A and C, respectively, might indicate that the diversification of Californian ancestors in Australia predates a chloroplast transfer into a later arriving South African colonizing species. The evidence against this scenario is the additive sequences in *L. aschersonii* and *L. muelleriferdinandi* (Fig. 1). Of course, another possibility is that the A-clade nucleotides in these two species were acquired later by secondary hybridization between clades A and C in Australia and are not relicts of an original hybridization. This would be more consistent with the dating analyses. However, this discrepancy in time estimations may simply be explained by the observed rate heterogeneity between these two lineages and across the whole ITS tree. The low levels of cpDNA and ITS sequence divergence and unresolved topologies within Australian/New Zealand species (due to low numbers of nucleotide substitutions) suggest a rapid and recent radiation of *Lepidium* after the hybridization event. This coincides with dramatic climatic fluctuations of the Quaternary when a cooling climate and the formation of a more mountainous landscape in New Zealand and a drying trend in Australia may have created novel habitats and thus highly invasible terrain (Markgraf et al., 1995; McGlone et al., 2001). This could have provided the necessary ecological space into which *Lepidium* could have radiated.

Distribution of Australian/New Zealand clade A and C species coincides with rainfall distribution—One of the most important determinants of vegetation types in Australia is the total amount of rainfall (Groves, 1999). The two Australian/New Zealand lineages reflecting the different continental origins occupy more or less different habitats in Australia and New Zealand, and this reflects the habitats of these clades in their continents of origin. Californian *Lepidium* of clade C are annual plants of saline areas and are found in open habitats near streams, vernal pools, and margins of salt marshes (Hitchcock, 1936; Rollins, 1993), and this also generally applies to the Australian members of the clade. Most Australian species of group C and one New Zealand species (with three subspecies) are typically distributed in arid/semiarid areas of Australia and dry inland regions of New Zealand, respectively. Beadle (1981) suggested that the dry inland taxa (including *Lepidium*) were derived from saline and subsaline habitats along the Australian coast. In littoral habits salinity and soil type may impose physiological conditions similar to those of arid areas. One could speculate that seabirds migrating from coastal saline marshes in California might have transported seeds to equivalent habitats in Australia. This leads to the hypothesis that coastal species in Australia or New Zealand are reported to be salt tolerant (e.g., *L. aschersonii*, Hewson, 1981; Norton et al., 1997) and such species might be among the first descendants of the colonizing scenario.

The annual life form typically found in Australian species of group C is generally far more abundant in the arid areas than in any other Australian climate, and the annual habit appears to be a preadaptation to the erratic rainfall (Beadle, 1981). African clade A species occur in a wide range of habitats from subalpine areas in Lesotho to semi-desert habitats on the central plateau to winter-rainfall areas in the Cape Floristic Region. As all the South African species fall together in one unresolved group in the cpDNA tree and along with Australian/New Zealand species in the ITS phylogeny, we cannot establish which South African species is sister to the Australian/New Zealand clade. New Zealand species are coastal whereas Australian species are distributed along the mesic margins of southeast Australia. These areas receive more rain than the inland with a substantial portion of the rain in winter, similar to southern South Africa. Southeast Australia and New Zealand have a diverse invasive flora derived from South Africa (Hewson, 1981; Marchant et al., 1987; Carr, 1993). Australian/New Zealand members of group A are typically perennial herbs (Hewson, 1981) as are the South African species (Marais, 1970; Jonsell, 1975). African and Australian members of Group A are difficult to distinguish morphologically, and *L. africanum* has been repeatedly confused with *L. hyssopifolium* and *L. pseudohyssopifolium* (Ryves, 1977; Hewson, 1981). *Lepidium africanum*, a highly successful weed in Australia, is found in the same area as Australian members of clade A. African species (*L. africanum, L. divaricatum*), along with Australian relatives of clade A, are naturalized in New Zealand (Webb et al., 1988), and South African species (*L. africanum, L. divaricatum, L. capense, L. schinzii*) are even adventive in the British Islands. Thus, South African *Lepidium*

appeared to be successful colonizers both in the present and in the past, and apparently they transmitted these capabilities to their Australian descendants.

Origin of New Zealand Lepidium s.s.—There is insufficient resolution on our molecular trees to disentangle the migration patterns and directions across the Tasmanian Sea. Some species are common between New Zealand and Australia (e.g., *L. hyssopifolium*, *L. pseudohyssopifolium*, *L. pseudotasmanicum*, *L. flexicaule*, *L. desvauxii*). *Lepidium desvauxii* is native to southeast Australia, Tasmania, and probably New Zealand; *L. flexicaule* is native to New Zealand and Tasmania, whereas Australian *L. pseudotasmanicum* and *L. pseudohyssopifolium* are naturalized in New Zealand (Hewson, 1981; Webb et al., 1988). This indicates recent and possibly frequent dispersal across the Tasmanian Sea, probably by coastal and wetland birds (McGlone et al., 2001).

There is extensive documentation of dispersal between New Zealand and Australia, and possibly in both directions, for a wide range of both monocots and dicots, e.g., *Rytidosperma* (Linder and Walsh, 1995), *Hebe* (Garnock-Jones, 1993), and alpine plants in general (Raven, 1973). Recently it was suggested that the New Zealand flora has been predominantly formed by long-distance dispersal from or via Australia during the Late Miocene to the Pleistocene period followed by speciation into characteristic New Zealand types (Pole, 1994; McGlone et al., 2001; Winkworth et al., 2002) and that this applies particularly to the herbaceous species such as *Lepidium* (Mitchell and Heenan, 2000). Then the presence of New Zealand endemic taxa within ITS clades A and C, respectively, and among two subclades within clade B (cpDNA tree) would suggest that there have been at least two dispersals and colonizations to New Zealand.

Do they look like hybrids?—To seek morphological confirmation of the hybrid origin of all Australian/New Zealand *Lepidium* s.s. species from Californian and South African parental taxa, we analyzed those characters that differ between those taxa representing descendents of the parental lineages in hybrid polyploid Australian/New Zealand *Lepidium* species. Two characters showed additivity of character states. Fruit valves of South African (clade A) taxa are veinless whereas fruits of the Californian (clade C) taxa are characterized by veins. The Australian/New Zealand species show either of these contrasting character states: *L. pseudopapillosum* fruit is veined, whereas all other Australian/New Zealand taxa have veinless fruits. A second morphological fruit character distinguishing the parental lineages (stylus present or absent) is even more equally distributed among Australian/New Zealand taxa. Fruits of South African *Lepidium* taxa are characterized by the presence of a stylus whereas fruits of the Californian taxa have a sessile stigma. Ten and five species of the Australian/New Zealand taxa are characterized by either one character state, respectively, and three Australian taxa (*L. fasciculatum*, *L. desvauxii*, *L. pseudohyssopifolium*) are polymorphic for this character. This pattern does not follow the distribution of Australian/New Zealand species in the ITS tree. Such additivity of character states in the presumed hybrid taxa might therefore reflect morphologically the hybrid origin of the Australian/New Zealand *Lepidium* s.s. species.

LITERATURE CITED

AL-SHEHBAZ, I. A. 1986. The genera of Lepidieae (Cruciferae; Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum* 67: 265–311.

BAKKER, F. T., D. HELLBRÜGGE, A. CULHAM, AND M. GIBBY. 1998. Phylogenetic relationships within *Pelargonium* sect. *Peristera* (Geraniaceae) inferred from nrDNA and cpDNA sequence comparisons. *Plant Systematics and Evolution* 211: 273–287.

BALDWIN, B. G., AND M. J. SANDERSON. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences, USA* 95: 9402–9406.

BARRIER, M., B. G. BALDWIN, R. H. ROBICHAUX, AND M. D. PURUGGANAN. 1999. Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotioc gene duplications. *Molecular Biology and Evolution* 160: 1105–1113.

BEADLE, N. C. 1981. The vegetation of Australia. Fischer, Stuttgart, Germany.

BOWMAN, J. L., H. BRÜGGEMANN, J.-Y. LEE, AND K. MUMMENHOFF. 1999. Evolutionary changes in floral structure within *Lepidium* L. (Brassicaceae). *International Journal of Plant Sciences* 160: 917–929.

BRÜGGEMANN, H. 2000. Molekulare Systematik und Biogeographie der Gattung *Lepidium* L. und verwandter Sippen (Brassicaceae). Ph.D. dissertation, University of Osnabrück, Osnabrück, Germany.

CAMPBELL, C. S., M. F. WOJCIECHOWSKI, B. G. BALDWIN, L. A. ALICE, AND M. J. DONOGHUE. 1997. Persistent nuclear ribosomal DNA polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81–90.

CARLQUIST, S. 1983. Intercontinental dispersal. *In* K. Kubitzki [ed.], Dispersal and distribution, 37–47. Parey, Hamburg, Germany.

CARR, G. W. 1993. Exotic flora of Victoria and its impact on indigenous biota. *In* D. B. Foreman and N. G. Walsh [eds.], Flora of Victoria, 256–297. Inkatha Press, Melbourne, Victoria, Australia.

FRANZKE, A., K. POLLMANN, W. BLEEKER, R. KOHRT, AND H. HURKA. 1998. Molecular systematics of *Cardamine* and allied genera (Brassicaceae): ITS and non-coding chloroplast DNA. *Folia Geobotanica* 33: 225–240.

GARNOCK-JONES, P. J. 1993. Phylogeny of the *Hebe* complex (Scrophulariaceae: Veroniceae). *Australian Systematic Botany* 6: 457–479.

GROVES, R. H. 1999. Present vegetation types. *In* A. E. Orchard [ed.], Flora of Australia, vol. 1, 2nd ed., 369–401. ABRS/CSIRO,Melbourne, Australia.

HEDGE, I. C. 1976. A systematic and geographical survey of the Old World Cruciferae. *In* J. G. Vaughan, A. J. MacLeod, and B. M. Jones [eds.], The biology and chemistry of the Cruciferae, 1-45. Academic Press, London, UK.

HEWSON, H. 1981. The genus *Lepidium* L. (Brassicaceae) in Australia. *Brunonia* 4: 217–308.

HITCHCOCK, C. L. 1936. The genus *Lepidium* in the United States. *Madrono* 3: 265–320.

HUELSENBECK, J. P., AND B. RANNALA. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* 276: 227–232.

JONSELL, B. 1975. *Lepidium* L. (Cruciferae) in tropical Africa. *Botanske Notiser* 128: 20–46.

JÜRGENS, N. 1997. Floristic biodiversity and history of African arid regions. *Biodiversity and Conservation* 6: 95–514.

KASHKUSH, K., M. FELDMAN, AND A. A. LEVY. 2002. Gene loss, silencing, and activation in a newly-synthesized wheat allotetraploid. *Genetics* 160: 1651–1659.

LEE, J.-Y., K. MUMMENHOFF, AND J. L. BOWMAN. 2002. Alloploidization and evolution of species with reduced floral structures in *Lepidium* L. (Brassicaceae). *Proceedings of the National Academy of Sciences, USA* 99: 16 835–16 840.

LINCOLN, F. C., S. R. STEVEN, AND J. L. ZIMMERMAN. 1998. Migration of birds. U.S. Dept. of the Interior, U.S. Fish and Wildlife Service, Washington, D.C., USA.

LINDER, H. P., AND N. G. WALSH. 1995. A new species of *Rytidosperma* (Poaceae: Arundinae) in New South Wales and Victoria. *Muelleria* 8: 283–285.

MAI, D. H. 1995. Tertiäre Vegetationsgeschichte Europas. Fischer, Stuttgart, Germany.

MARAIS, W. 1970. *Lepidium*. *In* L. E. Codd, B. De Winter, D. J. Killick, and

H. B. Rycroft [eds.], Flora of southern Africa, vol. 13, 83–94. Government Printer, Pretoria, South Africa.

MARCHANT, N. G., J. R. WHEELER, B. L. RYE, E. M. BENNETT, N. S. LANDER, AND T. D. MACFARLANE. 1987. Flora of the Perth region. West Australian Herbarium, Perth, Western Australia, Australia.

MARKGRAF, V., M. S. MCGLONE, AND G. HOPE. 1995. Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems—a southern perspective. *Trends in Ecology and Evolution* 10: 143–147.

MCDADE, L. A. 1992. Hybrids and phylogenetic systematics II: the impact of hybrids on cladistic analysis. *Evolution* 4: 1329–1346.

MCGLONE, M. S., R. P. DUNCAN, AND P. B. HEENAN. 2001. Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography* 28: 199–216.

MILLER, J. S., AND D. L. VENABLES. 2000. Polyploidy and the evolution of gender dimorphism in plants. *Science* 289: 2335–2338.

MITCHELL, A. D., AND P. B. HEENAN. 2000. Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. *Systematic Botany* 25: 98–105.

MUMMENHOFF, K., H. BRÜGGEMANN, AND J. L. BOWMAN. 2001. Chloroplast DNA phyogeny and biogeography of *Lepidium* (Brassicaceae). *American Journal of Botany* 88: 2051–2063.

MUMMENHOFF, K., H. HURKA, AND H.-J. BANDELT. 1992. Systematics of Australian *Lepidium* species (Brassicaceae) and implications for their origin: evidence from IEF analysis of Rubisco. *Plant Systematics and Evolution* 183: 99–112.

NORTON, D. A., P. J. DELANGE, P. J. GARNOCK-JONES, AND D. R. GIVEN. 1997. The role of seabirds and seals in the survival of coastal plants: lessons from New Zealand *Lepidium* (Brassicaceae). *Biodiversity and Conservation* 6: 765–785.

POLE, M. S. 1994. The New Zealand flora—entirely long distance dispersal? *Journal of Biogeography* 21: 625–635.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

RAVEN, P. H. 1973. Evolution of subalpine and alpine plant groups in New Zealand. *New Zealand Journal of Botany* 11: 177–200.

RIESEBERG, L. H., J. WHITTON, AND C. R. LINDER. 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica Neerlandica* 75: 243–266.

ROLLINS, R. C. 1993. The Cruciferae of continental North America. Stanford University Press, Stanford, California, USA.

RYVES, T. B. 1977. Notes on wool-alien species of *Lepidium* in the British Isles. *Watsonia* 11: 367–372.

SANDERSON, M. J. 1998. Estimating rate and time in molecular phylogenies: beyond the molecular clock? *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, 2nd ed., 242–264. Chapman and Hall, New York, New York, USA.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1995a. Documentation of reticulate evolution in paeonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences, USA* 92: 6813–6817.

SANG, T., D. J. CRAWFORD, T. F. STUESSY, AND M. SILVA O. 1995b. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55–64.

SOLTIS, D. E., AND P. S. SOLTIS. 1995. The dynamic nature of polyploid genomes. *Proceedings of the National Academy of Sciences USA* 92: 8089–8091.

SONG, K., P. LU, K. TANG, AND T. C. OSBORN. 1995. Rapid genome change in synthetic polyploids of Brassicaceae and its implications for polyploid evolution. *Proceedings of the National Academy of Sciences, USA* 92: 7719–7723.

SWOFFORD, D. L. 2000. PAUP* 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

THELLUNG, A. 1906. Die Gattung *Lepidium* (L.) R. Br. Eine monographische Studie. *Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft* 41: 1–304.

VON HAGEN, K. B., AND J. W. KADEREIT. 2001. The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms Diversity and Evolution* 1: 61–79.

WEBB, C. J., W. R. SYKES, AND P. J. GARNOCK-JONES. 1988. Flora of New Zealand, vol. 4, DSIR (Department of Scientific and Industrial Research), Christchurch, New Zealand.

WENDEL, J. F. 2000. Genome evolution in polyploids. *Plant Molecular Biology* 42: 225–249.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995a. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences, USA* 92: 280–284.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995b. An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic, intergenomic introgression. *Molecular Phylogenetics and Evololution* 4: 298–313.

WINKWORTH, R. C., S. J. WAGSTAFF, D. GLENNY, AND P. J. LOCKHART. 2002. Plant dispersal N.E.W.S. from New Zealand. *Trends in Ecology and Evolution* 17: 514–520.

ZHANG, L.-B., H. P. COMES, AND J. W. KADEREIT. 2001. Phylogeny and quaternary history of Euopean montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. *American Journal of Botany* 8: 2331–2345.