THE ORIGINS AND MAINTENANCE OF SPECIES BOUNDARIES IN *JAMESBRITTENIA* O. KUNTZE (SCROPHULARIACEAE: MANULEAE)

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Submitted in partial fulfillment of the degree of Bachelor of Science (Honours)
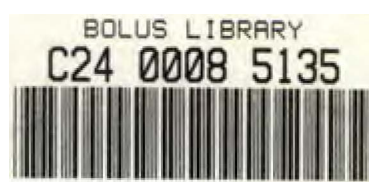
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Abstract

The genus *Jamesbrittenia* contains 83 species distributed throughout southern Africa. Many species produce attractive flowers and consequently their horticultural potential is currently being explored. Speciation patterns and reproductive isolation were investigated in order to identify trends that may apply at broader scales. Bayesian phylogenetic analysis was performed using plastid (*rps*16 and *psb*A-*trn*H) and nuclear (GScp) sequence data. Relative divergence times were calculated using a relaxed clock method. Prezygotic isolation, measured as seed set resulting from interspecific crosses, correlated with divergence time. However, recently diverged, highly sympatric taxa deviated from the overall trend. This provides circumstantial evidence for reinforcement of reproductive barriers. Floral dissimilarity and divergence time were found to be useful in predicting hybridization reported in the wild ($p < 0.0001$). Species pairs susceptible to hybridization were identified on the basis of their floral dissimilarity and divergence time in order to prevent potentially hybridizing species from being brought into contact. The inability to detect the dominant mode of speciation confounded interpretation of the results, as it was not possible to determine if the influence of geographic patterns on the evolution of reproductive isolation was a result of the mode of speciation or post-speciation evolutionary changes.

Introduction

The flora of southern Africa is remarkably diverse, comprising 18,000 plant species, of with 80% are endemic to the region (Goldblatt 1978). This diversity far exceeds that of other temperate regions of comparable size. The Cape Floristic Region is the center of this diversity, containing around 9,000 species, and is recognized as one of the six floral kingdoms of the world (Takhtajan 1969). Much research has been conducted into the evolution of the Cape flora, attempting to understand and identify the underlying causes of speciation and radiation that gave rise to the remarkable diversity (Cowling *et al* 1996, Johnson 1995, Goldblatt and Manning 2000, Linder 2003, Verboom *et al* 2003). Despite the prevalence of research into the evolution of the Cape flora, few studies have sought to explore the broader scale evolutionary patterns and processes of the southern African sub-continent.

Examination of phylogenetic hypotheses for taxa distributed throughout southern Africa, as has been done for numerous Cape clades (eg. *Ehrhata,* Verboom *et al* 2003; *Protea*, Barraclough and Reeves 2006; *Moraea*, Goldblatt *et al* 2002), may elucidate the evolutionary parallels that have resulted in high levels of diversity in both southern Africa and the Cape, as well as the departures that have caused the Cape to be far more diverse. These phylogenetic hypotheses, in conjunction with geographical distributions can be used tentatively to reconstruct historical species-level geographic patterns (Barraclough and Vogler 2000, Fitzpatrick and Turelli 2002). The ability to infer the geographic pattern of historical evolutionary events may provide a basis drawing inferences concerning the underlying process producing these patterns.

Historically, authors have attempted to infer the geographical pattern of speciation by examining the degree of range overlap exhibited by sister species (Mayr 1963). The problem with these methods is that the present range of a species or clade need not necessarily reflect that of its ancestors at the time of speciation (Losos and Glor 2003). In recent years there has been a proliferation of studies utilizing and developing methods

that attempt to infer the geographical pattern of speciation by relating reconstructions of species' ranges to the ages of sister species or clades (Perret *et al* 2007, Barraclough and Reeves 2006; Barraclough and Vogler 2000; Fitzpatrick and Turelli 2002). These methods are collectively known as 'age-range correlation' (or ARC). The suggestion is that if speciation is predominantly allopatric, then range overlap between sister species or clades will start at 0% for the most recent nodes and will increase through time as species ranges shift. However, if speciation is predominantly allopatric then overlap will start at 100% and gradually decrease for older nodes (Fitzpatrick and Turelli 2002). If species ranges change rapidly and are highly labile the pattern of speciation becomes obscured (Barraclough and Vogler 2000).

The geographic mode by which speciation proceeds influences the evolution of reproductive isolating mechanisms (Levin 1971, Grant 1994). Classically, speciation has been equated to the evolution of reproductive isolation, and thus any study investigating the former, implicitly investigated the latter (Mayr 1942, Coyne and Orr 1989). More recently there has a been a recognition that reproductive isolation can evolve as a by-product of speciation (Frost and Kluge 1994), although, few would dispute that reproductive isolation is essential for sympatric speciation to occur (Wiens 2004). Even if intrinsic reproductive isolation is not required for speciation, its evolution still has major implication for the maintenance of incipient species. The processes and traits that influence the rate and mode of reproductive isolation are arguably as important in generating species diversity as the underlying causes of speciation itself (Wiens 2004).

Incipient species would not remain distinct entities for very long if there were not mechanisms operating to restrict gene exchange between them and sympatric congeners. Isolating mechanisms can restrict gene flow at various stages of reproduction between two divergent organisms. A distinction is made between mechanisms which act before ovule fertilization (prezygotic) and those which act after ovule fertilization (postzygotic). One such mechanism is the isolation brought about by mechanical and ethological barriers to pollen transfer between flowers (Levin 1971; Grant 1994). It has been shown that floral assemblages can be structured such that floral dissimilarity prevents the

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transfer of pollen amongst congeners (Armbruster *et al* 1994; Hansen *et al* 2000). There are however multiple pathways that may result in the formation of structured dissimilarity in floral communities, and discriminating amongst alternative pathways can be complicated. Reinforcement of isolating mechanisms can occur if, when coming into secondary contact, interspecific matings result in a reduction in fitness of the parental plants. This may take the form of floral character displacement or the rapid evolution of intrinsic barriers (Armbruster *et al* 1994, Hendry *et al* 2000). Reinforcement has been shown to occur frequently between sister species in the Cape (van der Niet *et al* 2006).

Reproductive isolation is not an easy trait to measure and quantify. Identifying all possible barriers and identifying those that are disproportionately influential requires rigorous examination at a fine taxonomic scale (Ramsey *et al* 2003). Attempts have been made to find correlated characteristics and make generalizations (Edmands 2002, Fitzpatrick 2002, Moyle *et al* 2004). Reproductive isolation has been shown to correlate with genetic divergence in many taxa (Tilley *et al* 1990, Knowlton *et al* 1993, Coyne and Orr 1997, Sasa *et al* 1998). This is in accordance with theory that divergence accumulating over time results in reproductive isolation (Muller 1942). Other generalizations often made are that shorter generation times and reinforcement of reproductive barriers in sympatry accelerate the evolution of reproductive isolation (Coyne and Orr 1989, 1997, Hostert 1997, Archibald *et al* 2005). Despite evidence for these generalizations in certain groups of organisms, their application to plant taxa is seldom ubiquitous and often contradictory (Edmands 2002, Whittle and Johnston 2003, Moyle *et al* 2004).

The genus *Jamesbrittenia* occupies a variety of habitats and occurs over a broad range of climatic conditions. This, and the fact that it is distributed throughout southern Africa, provides an excellent opportunity to gain insight into the patterns and processes underlying diversity. Moreover, its distribution facilitates examination of process driving speciation at a larger scale than has been attempted previously. *Jamesbrittenia* contains 83 species of sub-shrubs, shrubs and herbs. All species except one are restricted to southern Africa, with *J. dissecta* occurring through Egypt, Sudan, India and Bangladesh.

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South Africa and Namibia are the centre of *Jamesbrittenia* diversity, containing most of the species (Hilliard 1994). With the exception of a few species restricted to limestone outcrops, no species are found in the Cape Floristic Region. Species can be found in the extremely arid Namib desert and on mesic mountain slopes in the Drakensberg. Flowering occurs sporadically, and appears to be related to light and moisture availability. Phenological isolation (or temporal allopatry) does not appear to be an isolating mechanism of much importance. Many species are adapted to highly specialized microhabitats, and are represented by small local populations. Herron (2006) demonstrated that *Jamesbrittenia* was monophyletic on the basis of a phylogeny obtained from two plastid (*rps*16 and *psb*A-*trn*H) and one nuclear (GScp) gene region sampling 42 species. She inferred that the genus arose in the arid west of southern Africa. Three major clades were well supported, two being centred in the arid west (Namibia and Namaqualand) and the other broadly distributed throughout southern Africa. Lineage divergence occurred in the Miocence, followed by diversification in the Pliocene-Pleistocene. The establishment of a drier, Mediterranean type climate and a shift to regions of higher rainfall and novel soil types were postulated as possible drivers of diversification (Herron 1994).

Since most *Jamesbrittenia* species are florally divergent and are often highly localized, the degree of intrinsic isolation amongst species is unclear. *Jamesbrittenia* displays a great range in floral morphology, which many species producing attractive, vividly colour flowers. Horticulturalists have taken an interest in this genus and a project attempting to produce horticultural varieties is underway at Kirstenbosch national botanical gardens. The horticulturalists responsible have had success in hybridization trials and it appears as though many species interbreed readily (A. Harrower pers. comm.). The possibility exists that through horticultural activities, species will come into contact with novel sympatriates, with unknown consequences. Introgression of species with congeners introduced through anthropogenic influence is well documented (Rieseberg 1991, Callaway 1992, Levin *et al* 1996, Antilla *et al* 1998). The outcome of sustained hybridization can range from stable coexistence, to total loss of populations and possibly species (Brochmann 1984). Key ecological and genetic parameters that

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influence that risk of species and populations to extinction through introgression include the strength of isolating mechanisms, the degree of self incompatibility, hybrid fitness, population sizes and population growth rates.

In considering a combination of data regarding geographic overlap, divergence time, reproductive compatibility and floral morphology, I hope to draw conclusions concerning speciation, reproduction and hybridization that can be applied specifically to *Jamesbrittenia,* and perhaps provide a framework for future studies and generalization to broad scale patterns of speciation in other southern African plant groups. With an understanding of these patterns, I will asses the risk of extinction through hybridization and introgression in *Jamesbrittenia.*

Firstly, using age-range correlation methods I aim to determine the predominant geographic mode of speciation in *Jamesbrittenia*. This will provide a geographical context for understanding the evolution of intrinsic isolating mechanisms. Allopatric speciation is most likely to be the dominant mode, as it is unlikely that almost 100 species, distributed across such a wide range of habitats, were all subject to the very specific conditions needed for sympatric speciation to occur.

Secondly, I intend to explore whether correlative relationships exist between measures of reproductive isolation (such as floral dissimilarity or intrinsic prezygotic isolation) and geographic overlap. Through comparison of geography and the isolating mechanisms, we will investigate the influence of range overlap on the evolution of reproductive isolation. Correlations between measures of isolation and relative divergence date will also be investigated. This will aid in determining which isolating mechanisms are important in *Jamesbrittenia* and assessing whether divergence time is a good proxy for intrinsic isolation, as this cannot be assumed *a priori*. If allopatric speciation is dominant, then the relationship between relative divergence time and intrinsic prezygotic isolation ought to be strong. If speciation is primarily sympatric, closely related species should be either florally dissimilar or intrinsically incompatible.

Lastly, I aim to evaluate the relative roles played by mechanical and etholgoical barriers to gene flow and intrinsic prezygotic isolation in preventing hybridization

between related species. Species complexes vulnerable to hybridization and introgression will be identified on the basis of relatedness and floral morphology. Recommendations will be made concerning the movement of species around the country for horticultural purposes.

Materials and Methods

Phylogenetic analyses

Aligned nucleotide sequence data were acquired for 70 species of *Jamesbrittenia* and one outgroup (*Teedia pubescens*) from two plastid (*rps*16, 852bp; *psb*A-*trn*H, 481bp) and one nuclear (GScp, 631bp) gene region. Phylogenetic analyses done in this study were based on existing alignments resulting from Herron (2006) and the Verboom lab (*unpubl. data*). The standard primers *rps*16F and *rps*16R were used for the *rps*16 region (Oxelman *et al* 1997), and *psb*A and *trn*H used for *psb*A-*trn*H region (Sang *et* al 1997). Primers used for the nuclear region GScp were designed by Herron (2006). These primers were: GS38F 5' TGA GCC (CT)TT CTT GTT TCG TG 3'; GS784R 5' ATA CTT GTT A(AG)T GAT TTT GCC 3' and GS681R 5'AGC TTG TTC TGT TAT TCT CTG 3'. All taxa included are listed with the associated type specimen and locality of the sample in table 1. Taxa for which symmetrical conflict existed between plastid and nuclear regions were identified by constructing separate phylogenies for the plastid and nuclear regions using parsimony implemented in PAUP v. 4 (Swofford 2003). The strict consensus tree was computed from the parsimony analysis using a heuristic search with TBR branch swapping and MULTREES in effect. A random addition sequence was used with 10,000 random addition replicates and holding 10 trees at each step. The maximum number of trees was not limited. Node support was evaluated using nonparametric bootstrapping (Felsenstein 1985). Bootstrap searches were also heuristic, using a simple addition sequence with 500 bootstrap replicates. The maximum number of trees was set to 300. Bayesian phylogenetic analysis was performed on the combined dataset using BEAST v1.4.4 (Drummond and Rambaut 2006). The GTR + Γ + I model of sequence evolution was assumed, with each gene region being modelled separately. A uniform distribution

was used for all priors except the tree prior, for which the prior was constructed using a Yule process speciation model. The starting tree was constructed using UPGMA clustering. Relative divergence dates were estimated using the relaxed clock method of Drummond *et al* (2006). This method does not assume correlated rates of sequence evolution across branches. Rather, rate variation is estimated from a lognormal distribution. The posterior probability distribution of parameters was sampled using Marcov Chain Monte Carlo (MCMC). A single MCMC chain ran for 50,000,000 generations with a burn-in of 500,000 generations. Parameter estimates were logged every 100 generations. The MCMC output was analysed using Tracer v1.3 (Rambaut and Drummond 2007). The effective sample size of all parameters was greater than 100 and the posterior probability trace had stabilized, indicating that the MCMC had mixed and converged adequately. The Maximum clade credibility tree was constructed from the final 10000 trees sampled by the MCMC in FigTree v1.0 (Rambaut 2006). The maximum clade credibility tree is the tree that contains the maximum possible sum of posterior probabilities over its nodes. Parsimony was also used to analyse the combined dataset in order to verify the results obtained using the novel approaches employed by BEAST. The settings used to construct the strict consensus tree and calculate bootstrap support were identical to those used to calculate the separate plastid and nuclear trees and the analysis was also implemented in PAUP v. 4.

The geographic pattern of speciation

The geographic distributions and ranges of 80 *Jamesbrittenia* species were constructed from 3793 georeferenced herbarium accessions from 4 herbaria (BOL, NGB, NY, PRE). Species or clade range was estimated by counting the number of quarter degree grid cells in which one or more records of a species or clade exist (Fig. 1). The degree of species or clade sympatry was defined as the number of quarter degree grid cells in which the two species or clades in question overlap / the total number of quarter degree grids cells occupied by the species or clade with the smaller range (Barraclough and Vogler 2000). Thus, if the distribution of a species occurring in a single quarter degree grid cell is nested within the distribution of a species with a much larger distribution, the degree of overlap is defined as 100%. The degree of overlap between

sister species or clades was plotted against relative node age estimated from the phylogenetic analyses. The relationship between node age and range overlap was used to infer the predominant geographic pattern of speciation in the genus (Barraclough and Vogler 2000). Only nodes with a posterior probability greater than 50% were used in the age-range correlation. Data were separated into three classes: nodes with 0.9 posterior probability, nodes with between 0.9 and 0.75 posterior probability and nodes with between 0.75 and 0.5 posterior probability. Because the assumption of homoscedastisity is invalid for age-range correlations, standard regression significance tests are not applicable. Hence, a simple test, similar to that of Perret *et al* (2007) was devised in R v2.5.1 to estimate the significance of the intercept of the age-range correlation curve. The intercept obtained from the age-range correlation was compared to the intercept obtained for the same analysis with species or clade ranges randomly assigned to nodes. This procedure was permutated 10000 times. The proportion of permutations that gave an intercept more extreme than the observed intercept was multiplied by 2 (two-sided test) and used as the p-value. The null hypothesis being tested is that the current ranges of sister species or clades do not contain information regarding the geographic pattern of speciation.

Floral geographic structuring

Floral morphological structuring of communities and the genus *Jamesbrittenia* as a whole was investigated in order to determine if mechanical and ethological barriers to hybridization are important in natural populations. The morphological characters analysed were the length and width across the corolla lateral lobes, the length and width of both the posticous and anticous corolla lobes, the length of posticous anthers and filaments, the length of anticous anthers and filaments, the style length and the stigma length. Values for each character were obtained by calculating the median values of the ranges reported by Hilliard (1994). Geographic structuring of floral communities was investigating by plotting the percentage sympatry against floral similarity for all species pairs. Similarity was calculated using square-root transformed normalized Euclidean distance between species in Primer v. 5. The significance of the relationship between floral similarity and sympatry was tested using Mantel's test (Mantel 1967). This test

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accounts for both the spatial and phylogenetic autocorrelation in the data by comparing the calculated correlation between the floral similarity and sympatry matrices with the correlation calculated from matrices with their rows and columns randomly rearranged. The test was implemented using the PopTools extension for Microsoft Excel® with matrix randomization being permutated 10000 times.

Reproductive isolation and hybridization

The relationship between prezygotic isolation and phylogenetic distance was explored in order to examine correlates of reproductive isolation and to validate the use of phylogenetic distance as a proxy for prezygotic isolation. Crossing experiments were conducted using 6 species of *Jamesbrittenia* chosen to represent a range of relatedness and sympatry. The chosen species were *J. tenuifolia, J. racemosa, J. pedunculosa, J. thunbergii, J. argentea* and *J. grandiflora*. For each species, five intraspecific outcrosses and five self fertilizations were performed to gauge the maximum potential fecundity and the potential for autogamy to bias results. For each species pair, 10 crosses were conducted with each species involved acting as the maternal parent for half the crosses. It is important to evaluate the crossing success with different species acting as the maternal parents, as the presence of asymmetrical crossing barriers is commonplace in angiosperms (Tiffen *et al* 2001). Due to the small size of seeds and the structure of infloresceses, capsules were harvested four weeks after treatment to prevent seed loss. Seed set was enumerated by counting the number of swelled ovules present within the capsule. Reproductive isolation was measured as the seed set resulting from interspecific crossing divided by seed set resulting from outcrossing in the maternal parent. Relative divergence time and reproductive isolation were plotted, and linear regression performed. It was indicated on the graph if species pairs had a geographic overlap of greater than 75%. In order to highlight any bias in the overall pattern caused by reinforcement of mating barrier brought about in allopatry.

Determinants of hybridization

In her revision of the tribe Manuleae, Hilliard (1994) reports putative hybridization between a number of species. In the absence of comprehensive

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experimental data, this is the best information available on hybridization within *Jamesbrittenia* amongst natural populations. In order to test the utility of floral similarity and divergence time in predicting hybridization, hybridizing and non-hybridizing species pairs were compared in terms of their standardized floral similarity and relative divergence dates. All at least partially sympatric species pairs for which data on relative divergence dates, floral similarity and geographic distribution existed were plotted. Allopatric species pairs were excluded because the possibility of hybridization does not exist in allopatry. A significance test was designed to test the importance of floral similarity and divergence time in predicting hybridization, and implemented in R v2.5.1. The test compares the distance from the origin of hybridizing and non-hybridizing species pairs by calculating the cumulative distance from the origin of all hybridizing species pairs. It then tests whether this distance is shorter than the cumulative distance calculated for the same number of species, chosen at random, as there are hybridizing species pairs. This procedure is repeated 10000 times and the p-value calculated as the proportion of permeations in which the distance calculated for randomly chosen pairs exceeds that calculated for the 14 hybridizing species pairs. The same test was repeated using, in the first instance, only relative divergence date and secondly, using only floral similarity.

The probability of hybrid formation between two species of a certain genetic distance and floral dissimilarity was calculated by dividing floral dissimilarity into classes of 0.1 and relative divergence date into classes of 0.05 width. The number of putatively hybridizing species pairs was divided by the total number of species pairs in the same class for which data in floral dissimilarity, geographic distribution and relative divergence was available. This proportion was taken as the probability of hybrid formation between species in that floral dissimilarity and relative divergence time class. Non-linear regression was then performed on the data using floral dissimilarity and geographic distribution as independent variables and the probability of hybrid formation as the dependent variable. This was done in order to obtain an equation and parameters with which to predict the probability of hybridization between any two species of *Jamesbrittenia*. Curve estimation was performed with Graphis[©] v. 2.9.09 (Kylebank

Software Ltd, 2003). The convergence criterion was set to 1×10^{-8} with a maximum of 200 iterations. It was decided that the exponential function given in equation 1 was most appropriate to perform regression analysis with.

$$(1) \quad z = a \times e^{(bx + cy^2)}$$

Where z = the probability of hybrid formation, x = standardized relative divergence date and y = standardized floral dissimilarity.

After inspection of the graph of the resulting function, a critical limit of hybrid formation probability was decided upon arbitrarily, based on visual inspection of the graph, above which the risk of hybrid formation rose rapidly. All species pairs of *Jamesbrittenia*, be they allopatric or sympatric, whose relative divergence date and floral dissimilarity result in a probability of hybrid formation in excess of this limit were then determined.

Table 1. Taxa and localities of specimens included in phylogenetic analyses.

Collection number	Species	Locality
M34_Teedi	Teedia	Locality uncertain
V808_ramo	ramosissima	Pella, N. Cape
V806_arid	aridicola	Aggeneys, N. Cape
V870_ampl	amplexicaulis	Okiep, N. Cape
V856_bico	bicolor	Witputs, Namibia
V815_majo	major	Ai-Ais, Namibia
V864_frut	fruticosa	Steinkopf, N. Cape
V854_sess	sessilifolia	Witputs, Namibia
V805_maxi	maxii	Aggeneys, N. Cape
V874_pedu	pedunculosa	Kamieskroon, N. Cape
V878_race	racemosa	Grootvlei Pass, N. Cape
V882_thun	thunbergii	Vanrhyns Pass, W. Cape
V859_mega	megaphylla	Vioolsdrif, N. Cape
V830_prim	primuliflora	Seeheim, Namibia
V847_fimb	fimriata	Sossusvlei, Namibia
V814_glut	glutinosa	Ai-Ais, Namibia
V823_mega	megadenia	Klein Karas, Namibia
V1102_ten	tenella	Windhoek, Namibia

Table 1. cont.

V1108_spn	sp nov	Erongo Mts, Namibia
V1109_her	hereroensis	Bloedkoppie, Namibia
V851_inte	integerrima	Aus, Namibia
V1120_heu	heucherifolia	Epupa Falls, Namibia
V1124_ele	elegantissima	Popa Falls, Namibia
V1065_ber	bergae	Thabazimbi, ***
V1062_mac	macrantha	Roossenekal, ***
V1057_acc	accrescens	Sudwala, Mpumalanga
V1036_bre	breviflora	Sani Pass, Lesotho
V1030_den	dentatisepala	Garden Castle, Kwazulu-Natal
V1069_aur	aurantiaca	Jagersfontein, Free State
V1122_con	concinna	Tsumeb, Namibia
V1039_mon	montana	Dundee, Kwazulu-Natal
V1022_mul	multisecta	Engcobo, E. Cape
V1012_fil	filicaulis	Cathcart, E. Cape
V1018_asp	aspleniifolia	Clifford, E. Cape
V835_flec	fleckii	Kuiseb Canyon, Namibia
V1106_pal	pallida	Erongo Mts, Namibia
V1101_lyp	lyperioides	Windhoek, Namibia
V1112_bar	barbata	Swakop, Namibia
V833_cane	canescens var seineri	Kuiseb Canyon, Namibia
V1128_can	canescens var laevior	Otavifontein, Namibia
V1115_che	chenopodioides	Brandberg, Namibia
V817_cane	canescens var canescens	Ai-Ais, Namibia
V1048_gra	grandiflora	Barberton, Mpumalanga
V1002_mar	maritima	Alexandria, E. Cape
V866_merx	merxmuelleri	Alexander Bay, N. Cape
M36_albom	albomarginata	Locality uncertain
M38_stell	stellata	Cape Peninsula, W. Cape
H1679_cal	calciphila	Still Bay, W. Cape
V1056_hui	huillana	Barberton, Mpumalanga
V915_tenu	tenuifolia	Seclgefield, W. Cape
MH50_arge	argentea	Locality uncertain
V1070_atr	atropurpurea	Jagersfontein, Free State
TVPA2_tor	tortuosa	Prince Albert, W. Cape
V885_inci	incisa	Calvinia, N. Cape
DGE_tyson	tysonii	Locality uncertain
V1066_alb	albiflora	Jagersfontein, Free State
H1695_asp	aspalathoides	Locality uncertain
B1453_mic	microphylla	Sundays Mouth, E. Cape
H552_foli	foliolosa	Locality uncertain
V1008_alb	albanensis	Ecca Pass, E. Cape
V1011_phl	phlogiflora	Peddie, E. Cape
V1023_kra	kraussiana	Oribi Gorge, Kwazulu-Natal
V1125_dol	dolomitica	Otavi, Namibia
V1132_acu	acutiloba	Waterberg, Namibia
V829_adpr	adpressa	Seeheim, Namibia

Table 1. cont.

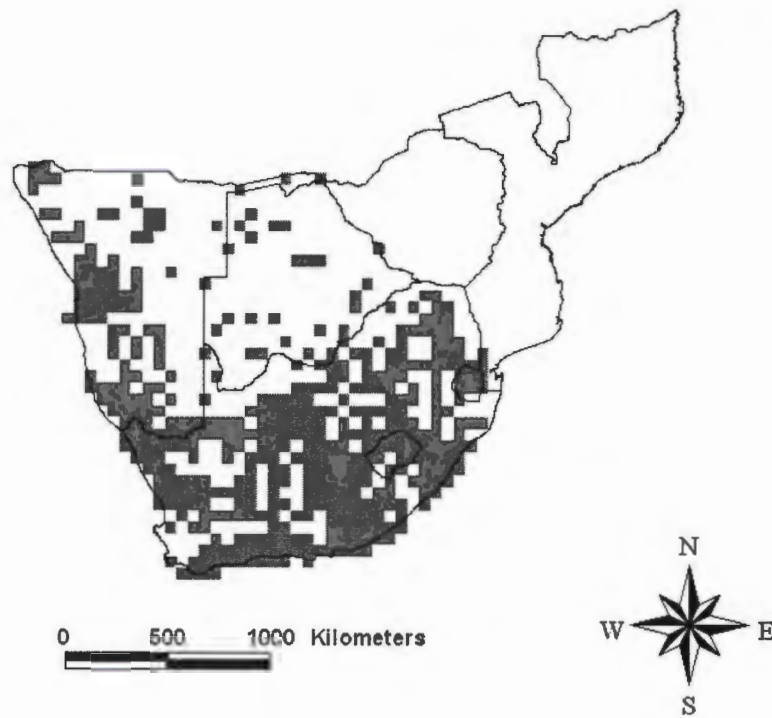| |
| --- |
| Collectors: |
| DGE=D. Gwynne Evans |
| M or MH=M. Herron |
| V=G. A. Verboom |
| H=A. Harrower |
| B=N. Bergh |

Figure 1. The distribution of *Jamesbrtittenia* in southern Africa used in geographical analyses.

Results

Phylogenies

The strict consensus of 97,940 equally parsimonious trees, based on 631 nuclear characters, 124 of which were parsimony informative is shown in figure 2. The tree length is 370 and it has a consistency index of 0.727 and a retention index of 0.915. The strict consensus of 100,000 equally parsimonious trees, based on 1334 plastid characters, 80 of which were parsimony informative is shown in figure 3. The tree length is 175, with a consistency index of 0.928 and a retention index of 0.990. Symmetrical conflict between these trees exists for 6 taxa, *J. crassicaulis, J. jurassica, J. pristisepala, J. burkeana, J. stricta* and *J. silenoidies*. In the nuclear phylogeny this group is well supported as sister to the rest of *Jamesbrittenia*, while in the plastid phylogeny it is included with within the major *Jamesbrittenia* clade, with 100% bootstrap support. These taxa were thus excluded from subsequent analysis in order to facilitate the combination of plastid and nuclear sequence data into a single phylogeny.

The topology obtained from parsimony analysis of the combined dataset (Fig. 4) is in general agreement with the topology obtained from the Bayesian analysis of the same dataset (Fig. 5). The strict consensus parsimony tree is constructed from 95,910 equally parsimonious trees, based on 1965 characters includes 187 parsimony informative characters. The tree length is 320, the consistency index for this tree is 0.738 and the retention index is 0.932 .The Bayesian tree resolves *J. ramosissima* as sister to *Teedia pubescens* with 0.99 posterior probability, rendering *Jamesbrittenia* paraphyletic. The relationship between *J. ramosissima*, *Teedia pubescens* and the rest of *Jamesbrittenia* remains unresolved in the parsimony tree. Although not resulting in topological conflict, the basal node combining all species of *Jamesbrittenia* (except *J. ramosissima*) into a single clade is supported far better by the bootstrap support (100%) than by the posterior probability (0.6). *Jamesbrittenia* is divided into three major clades in both analyses. One of these clades (labelled clade A) is centred in the Namaqualand region of southwestern Africa. Another (labelled clade B) is found mainly in Namibia, extending down to the Orange river. The other major clade (labelled clade C) is broadly

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distributed throughout South Africa, Namibia and Botswana. The diversity within this clade is generally much younger than that of the other clades. Recent diversification is evident in the group labelled clade D.

Geographic patterns

The age-range correlation provides little evidence in support of either allopatric or sympatric speciation (Fig. 6). Nodes are distributed quite randomly in the Cartesian plane and this does not change with the posterior probability support of nodes. The lack of signal is confirmed by the insignificance of the test designed to asses the significance of the intercept (p=0.379). Although the method of range estimation employed is biased by incomplete taxon sampling, most species of *Jamesbrittenia* excluded from the analysis form a monophyletic assemblage, and thus bias will only be introduced to nodes deep in the tree. These range overlap for these deeper nodes is already assumed to be biased by range shifts. Initially it appears as though the there is little or no relationship between floral dissimilarity geographic overlap (Fig. 7). However, although the correlation between floral dissimilarity was low, Mantels test confirmed that there is a trend of decreasing similarity with increasing geographic overlap and that this trend is significant (r= -0.0509, p=0.013). This indicates floral similarity is increases significantly with increasing range overlap.

Reproductive isolation

The degree of prezygotic reproductive isolation, measured as seed set, increased with increasing relative divergence date (R^2=0.27, p=0.013, Fig. 8). Crossing barriers were not asymmetric generally, as crossing success was similar when different maternal parents are used. An exception to this rule was *J. grandiflora*. In the crosses with *J. argentea* and *J. tenuifolia*, crossing success was much higher in both instances when *J. grandiflora* was the paternal parent. Deviating from the overall trend are the two most recently diverged species pairs. These are *J. racemosa - J. pedunculosa* and *J. argentea - J. tenuifolia*. All other species pairs are entirely allopatric, while these two species pairs have medium (0.75 for *J. racemosa* and *J. pedunculosa*) to high (0.88 for *J. argentea* and

J. tenuifolia) degrees of range overlap. With these species pairs removed R^2 improves to 0.57 (p<0.01).

Hybridization was reported by Hilliard (1994) for species pairs with a relative divergence date ranging between 0.00075 and 0.0012 and floral dissimilarity ranging between 0.382 and 1.38. Figure 9 shows that most hybridizing species pairs appear quite close to the origin of the graph plotting standardized relative divergence date against standardized floral dissimilarity. This bias is highly significant (p<0.0001), indicating that hybridization is significantly more likely in species that are both recently diverged and florally similar. Significance was also found when each of these was tested individually (divergence time, p<0.0001; floral dissimilarity, p=0.0002). The nonlinear regression indicates that the probability of hybridization is very low for most species pairs, but close to the origin the probability rapidly increases to a maximum of about 0.4. (a=0.4211, b=4.032, c= 8.099, R^2=0.27, p<0.0001, Fig. 10). Using a cut-off probability of 0.1, a list of likely hybridizing species pairs was generated (Table 2). On average, each species of *Jamesbrittenia* exceeded a 0.1 probability of hybridization with 10 congeners.

Table 2. A listing of all species of *Jamesbrittenia* for which the probability of hybridization exceeds 0.1.

J. accrescens	J. acutiloba	J. adpressa	J. albanensis	J. albiflora	J. albomarginata
J. acutiloba	J. adpressa	J. acutiloba	J. kraussiana	J. albanensis	J. acutiloba
J. albomarginata	J. accrescens	J. albomarginata	J. albiflora	J. aspalathoides	J. adpressa
J. dentatisepala	J. albomarginata	J. barbata	J. calciphila	J. foliolosa	J. albiflora
J. dolomitica	J. candida	J. candida	J. grandiflora	J. kraussiana	J. calciphila
J. merxmuelleri	J. chenopodioides	J. chenopodioides	J. integerrima	J. phlogiflora	J. dolomitica
J. pallida	J. dentatisepala	J. dentatisepala	J. lyperioides	J. albomarginata	J. huilana
J. heucherifolia	J. dolomitica	J. dolomitica	J. macrantha	J. argentea	J. incisa
	J. fleckii	J. elegantissima	J. stellata	J. barbata	J. tysonii
	J. heucherifolia	J. fleckii	J. tortuosa	J. calciphila	J. accrescens
	J. huilana	J. heucherifolia	J. tysonii	J. candida	J. barbata
	J. merxmuelleri	J. lyperioides		J. dentatisepala	J. candida
	J. pallida	J. pallida		J. fleckii	J. chenopodioides
		J. stellata		J. grandiflora	J. dentatisepala
				J. lyperioides	J. fleckii
				J. macrantha	J. grandiflora
				J. maritima	J. heucherifolia
				J. merxmuelleri	J. lyperioides
				J. stellata	J. merxmuelleri
				J. tenuifolia	J. pallida
				J. tortuosa	

J. aspalathoides	J. aspleniifolia	J. aurantiaca	J. barbata	J. bergae	J. bicolor
J. phlogiflora	J. micrantha	J. aspalathoides	J. adpressa	J. macrantha	J. major
J. albiflora	J. concinna	J. concinna	J. albiflora		J. sessifolia
J. aurantiaca	J. montana	J. foliolosa	J. albomarginata		
J. candida	J. multisecta		J. candida		
J. fleckii			J. chenopodioides		
J. grandiflora			J. dolomitica		
J. integerrima			J. merxmuelleri		
J. lyperioides			J. elegantissima		
J. macrantha			J. fleckii		
J. maritima			J. heucherifolia		
J. merxmuelleri			J. lyperioides		
J. tysonii			J. pallida		

Table 2 cont.

J. breviflora	J. calciphila	J. candida	J. chenopodioides	J. concinna	J. dentatisepala
J. concinna	J. albanensis	J. acutiloba	J. acutiloba	J. aspleniifolia	J. acutiloba
J. montana	J. albiflora	J. adpressa	J. adpressa	J. montana	J. adpressa
J. multisecta	J. foliolosa	J. albiflora	J. albomarginata	J. multisecta	J. albiflora
	J. kraussiana	J. albomarginata	J. dolomitica	J. aurantiaca	J. albomarginata
	J. phlogiflora	J. argentea	J. huilana	J. breviflora	J. calciphila
	J. tenuifolia	J. aspalathoides	J. merxmuelleri		J. candida
	J. tortuosa	J. calciphila	J. barbata		J. fleckii
	J. tysonii	J. chenopodioides	J. candida		J. grandiflora
	J. albomarginata	J. foliolosa	J. elegantissima		J. huilana
	J. candida	J. grandiflora	J. fleckii		J. lyperioides
	J. dentatisepala	J. merxmuelleri	J. heucherifolia		J. merxmuelleri
	J. filicaulis	J. stellata	J. pallida		J. pallida
	J. fleckii	J. tortuosa			J. stellata
	J. grandiflora	J. tysonii			J. tortuosa
	J. integerrima	J. barbata			J. tysonii
	J. lyperioides	J. dentatisepala			J. accrescens
	J. macrantha	J. filicaulis			J. heucherifolia
	J. maritima	J. fleckii			J. integerrima
	J. merxmuelleri	J. integerrima			J. macrantha
		J. lyperioides			
		J. macrantha			
		J. pallida			

J. dolomitica	J. elegantissima	J. filicaulis	J. fimbriata	J. fleckii	J. foliolosa
J. acutiloba	J. adpressa	J. calciphila	J. primuliflora	J. dentatisepala	J. kraussiana
J. adpressa	J. barbata	J. candida	J. glutinosa	J. elegantissima	J. albiflora
J. accrescens	J. chenopodioides	J. fleckii	J. megadenia	J. filicaulis	J. aurantiaca
J. albomarginata	J. dolomitica	J. grandiflora		J. heucherifolia	J. calciphila
J. barbata	J. fleckii	J. lyperioides		J. integerrima	J. candida
J. chenopodioides	J. merxmuelleri	J. maritima		J. acutiloba	J. grandiflora
J. elegantissima	J. heucherifolia	J. stellata		J. adpressa	J. integerrima
J. fleckii		J. tenuifolia		J. albiflora	J. lyperioides
J. heucherifolia		J. tortuosa		J. albomarginata	J. macrantha
J. merxmuelleri		J. macrantha		J. argentea	J. merxmuelleri
J. pallida				J. aspalathoides	J. micrantha
				J. barbata	J. stellata
				J. calciphila	J. tysonii
				J. candida	
				J. chenopodioides	
				J. dolomitica	
				J. grandiflora	
				J. huilana	
				J. merxmuelleri	
				J. stellata	
				J. tortuosa	
				J. tysonii	

Table 2 cont.

J. grandiflora	J. heucherifolia	J. huilana	J. incisa	J. integerrima	J. lyperioides
J. albanensis	J. accrescens	J. acutiloba	J. tysonii	J. albanensis	J. adpressa
J. albiflora	J. acutiloba	J. incisa	J. albomarginata	J. argentea	J. albanensis
J. albomarginata	J. adpressa	J. albomarginata	J. huilana	J. aspalathoides	J. albiflora
J. argentea	J. albomarginata	J. chenopodioides	J. stellata	J. calciphila	J. albomarginata
J. aspalathoides	J. barbata	J. dentatisepala		J. candida	J. argentea
J. calciphila	J. chenopodioides	J. fleckii		J. dentatisepala	J. aspalathoides
J. foliolosa	J. dentatisepala	J. heucherifolia		J. fleckii	J. barbata
J. merxmuelleri	J. dolomitica	J. merxmuelleri		J. foliolosa	J. calciphila
J. stellata	J. elegantissima	J. pallida		J. grandiflora	J. candida
J. tenuifolia	J. fleckii	J. stellata		J. kraussiana	J. foliolosa
J. tortuosa	J. huilana			J. lyperioides	J. grandiflora
J. tysonii	J. merxmuelleri			J. macrantha	J. kraussiana
J. candida	J. pallida			J. maritima	J. merxmuelleri
J. dentatisepala				J. merxmuelleri	J. phlogiflora
J. filicaulis				J. phlogiflora	J. stellata
J. fleckii				J. stellata	J. tortuosa
J. integerrima				J. tenuifolia	J. tysonii
J. lyperioides				J. tortuosa	J. dentatisepala
J. macrantha					J. filicaulis
J. pallida					J. integerrima
					J. macrantha
					J. pallida

J. kraussiana	J. macrantha	J. major	J. maritima	J. merxmuelleri	J. megadenia
J. albanensis	J. albanensis	J. racemosa	J. albiflora	J. acutiloba	J. fimbriata
J. albiflora	J. albiflora	J. bicolor	J. argentea	J. albiflora	J. primuliflora
J. calciphila	J. argentea		J. aspalathoides	J. albomarginata	
J. foliolosa	J. aspalathoides		J. calciphila	J. argentea	
J. integerrima	J. calciphila		J. stellata	J. aspalathoides	
J. lyperioides	J. candida		J. tenuifolia	J. calciphila	
J. macrantha	J. dentatisepala		J. tortuosa	J. dolomitica	
J. stellata	J. filicaulis		J. tysonii	J. foliolosa	
J. tortuosa	J. foliolosa		J. filicaulis	J. huilana	
J. tysonii	J. grandiflora		J. integerrima	J. stellata	
	J. kraussiana		J. macrantha	J. tenuifolia	
	J. lyperioides			J. tortuosa	
	J. maritima			J. tysonii	
	J. phlogiflora			J. accrescens	
	J. stellata			J. barbata	
	J. tenuifolia			J. candida	
	J. tortuosa			J. chenopodioides	
	J. tysonii			J. dentatisepala	
	J. bergae			J. elegantissima	
	J. integerrima			J. fleckii	
				J. grandiflora	
				J. heucherifolia	
				J. integerrima	
				J. lyperioides	
				J. pallida	

Table 2 cont.

J. micrantha	J. montana	J. multisecta	J. pallida	J. pedunculosa	J. primuliflora
J. foliolosa	J. aspleniifolia	J. aspleniifolia	J. acutiloba	J. thunbergii	J. fimbriata
J. aspleniifolia	J. micrantha	J. breviflora	J. adpressa		J. glutinosa
J. montana	J. multisecta	J. concinna	J. albomarginata		J. megadenia
	J. breviflora	J. montana	J. barbata		
	J. concinna		J. candida		
			J. chenopodioides		
			J. dolomitica		
			J. grandiflora		
			J. huilana		
			J. lyperioides		
			J. merxmuelleri		
			J. stellata		
			J. accrescens		
			J. dentatisepala		
			J. heucherifolia		

J. phlogiflora	J. primuliflora	J. racemosa	J. stellata	J. sessifolia	J. tenuifolia
J. albiflora	J. fimbriata	J. thunbergii	J. adpressa	J. bicolor	J. albiflora
J. aspalathoides	J. glutinosa	J. major	J. albanensis		J. tysonii
J. calciphila	J. megadenia		J. albiflora		J. calciphila
J. integerrima			J. foliolosa		J. filicaulis
J. lyperioides			J. huilana		J. grandiflora
J. macrantha			J. incisa		J. integerrima
J. stellata			J. kraussiana		J. macrantha
J. tortuosa			J. phlogiflora		J. maritima
J. tysonii			J. tortuosa		J. merxmuelleri
			J. tysonii		
			J. candida		
			J. dentatisepala		
			J. filicaulis		
			J. fleckii		
			J. grandiflora		
			J. integerrima		
			J. lyperioides		
			J. macrantha		
			J. maritima		
			J. merxmuelleri		
			J. pallida		

Table 2 cont.

J. thunbergii	J. tortuosa	J. tysonii
J. pedunculosa	J. albanensis	J. albanensis
J. racemosa	J. albiflora	J. aspalathoides
	J. kraussiana	J. foliolosa
	J. phlogiflora	J. kraussiana
	J. calciphila	J. phlogiflora
	J. candida	J. albomarginata
	J. dentatisepala	J. calciphila
	J. filicaulis	J. candida
	J. fleckii	J. dentatisepala
	J. grandiflora	J. fleckii
	J. integerrima	J. grandiflora
	J. lyperioides	J. incisa
	J. macrantha	J. lyperioides
	J. maritima	J. macrantha
	J. merxmuelleri	J. maritima
	J. stellata	J. merxmuelleri
		J. stellata
		J. tenuifolia
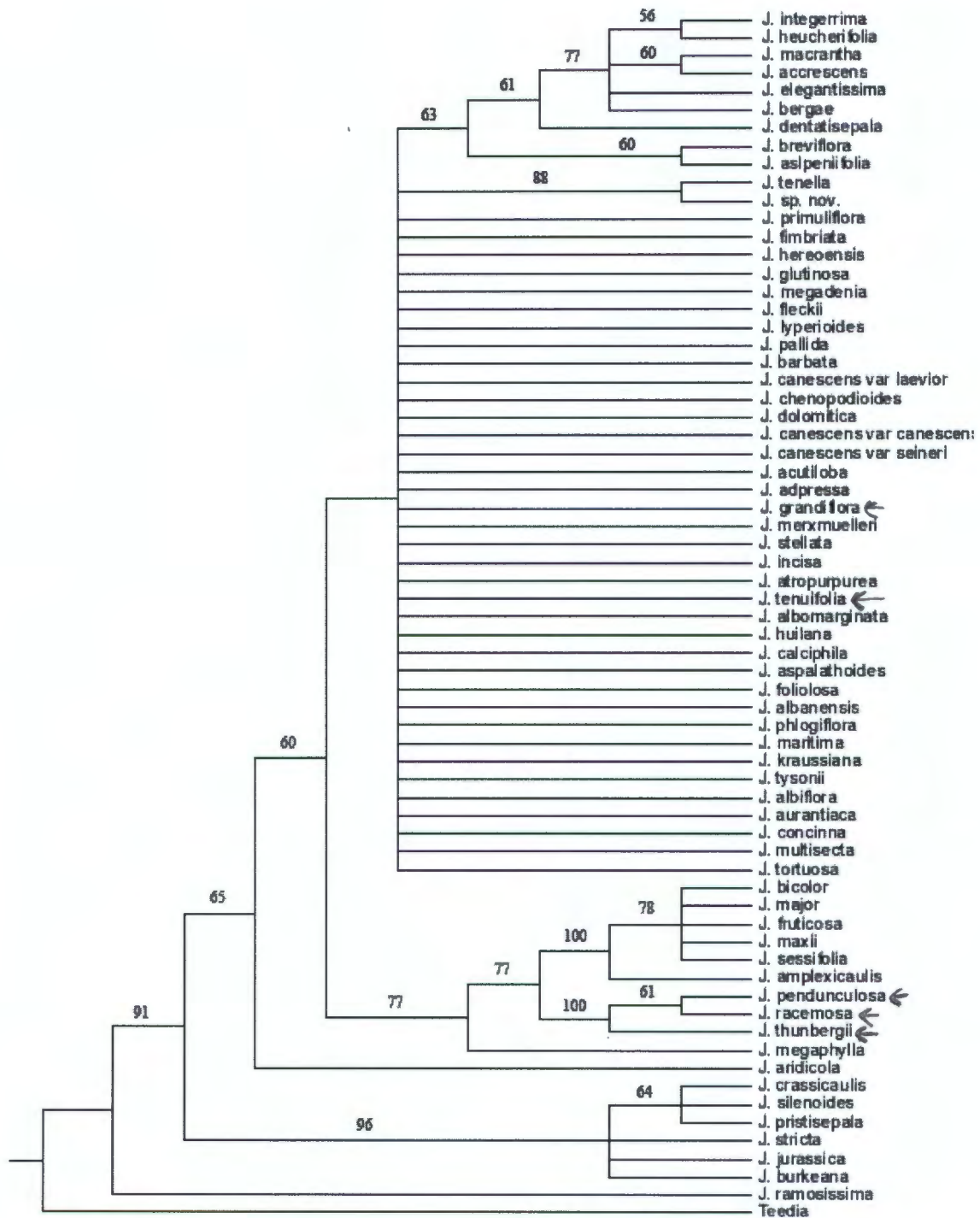
Figure 2. Strict consensus of 97, 940 equally parsimonious trees obtained from analysis of GScp sequences. Bootstrap support values are written above branches for which there is greater than 50% bootstrap support.
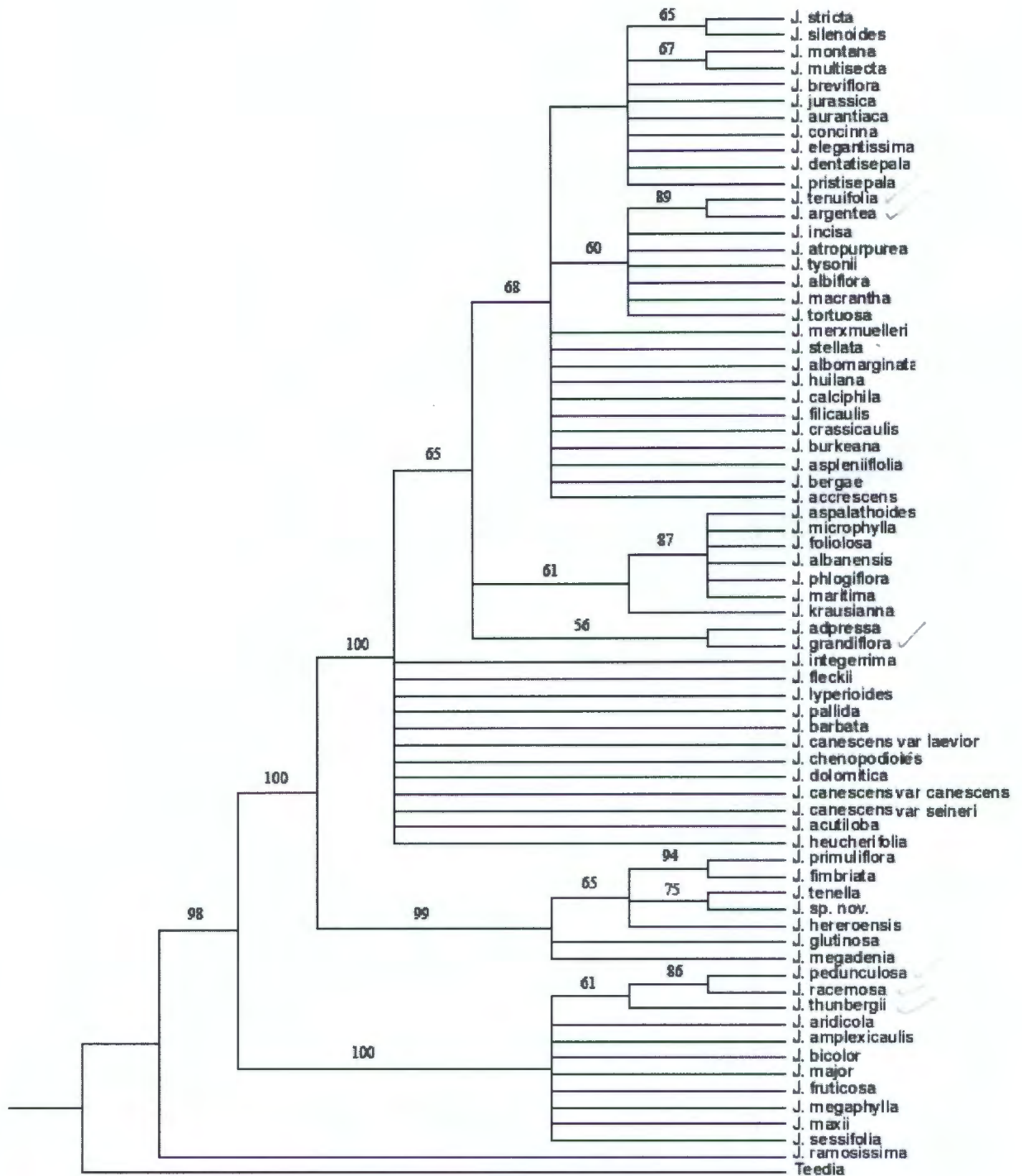
Figure 3. Strict consensus of 100,000 equally parsimonious trees obtained from analysis of *rps*16 and *psb*A-*trn*H sequences. Bootstrap support values are written above branches for which there is greater than 50% bootstrap support.
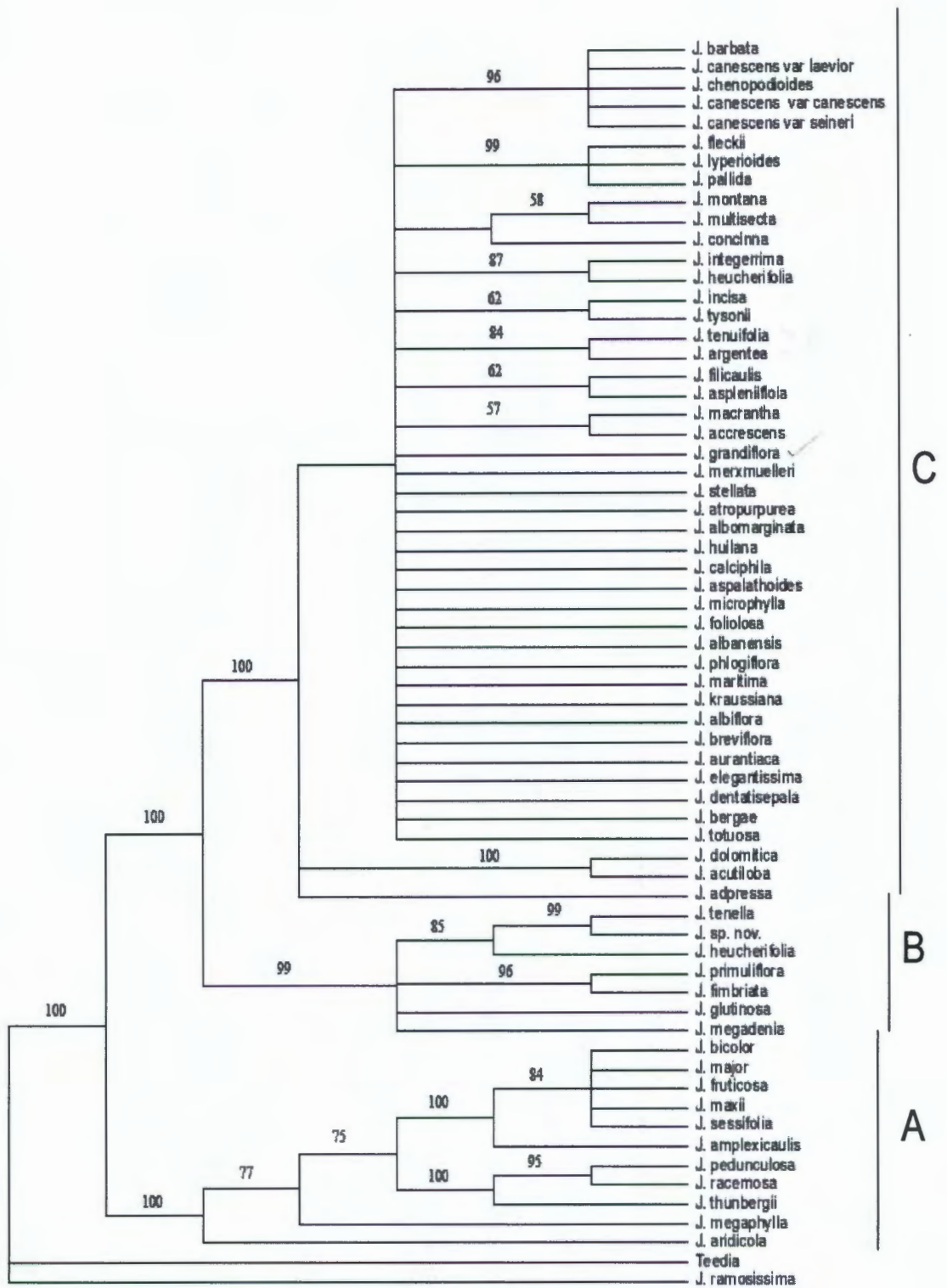
Figure 4. Strict consensus of 95,910 equally parsimonious trees obtained from analysis of the combined dataset. Bootstrap support values are written above branches for which there is greater than 50% bootstrap support.
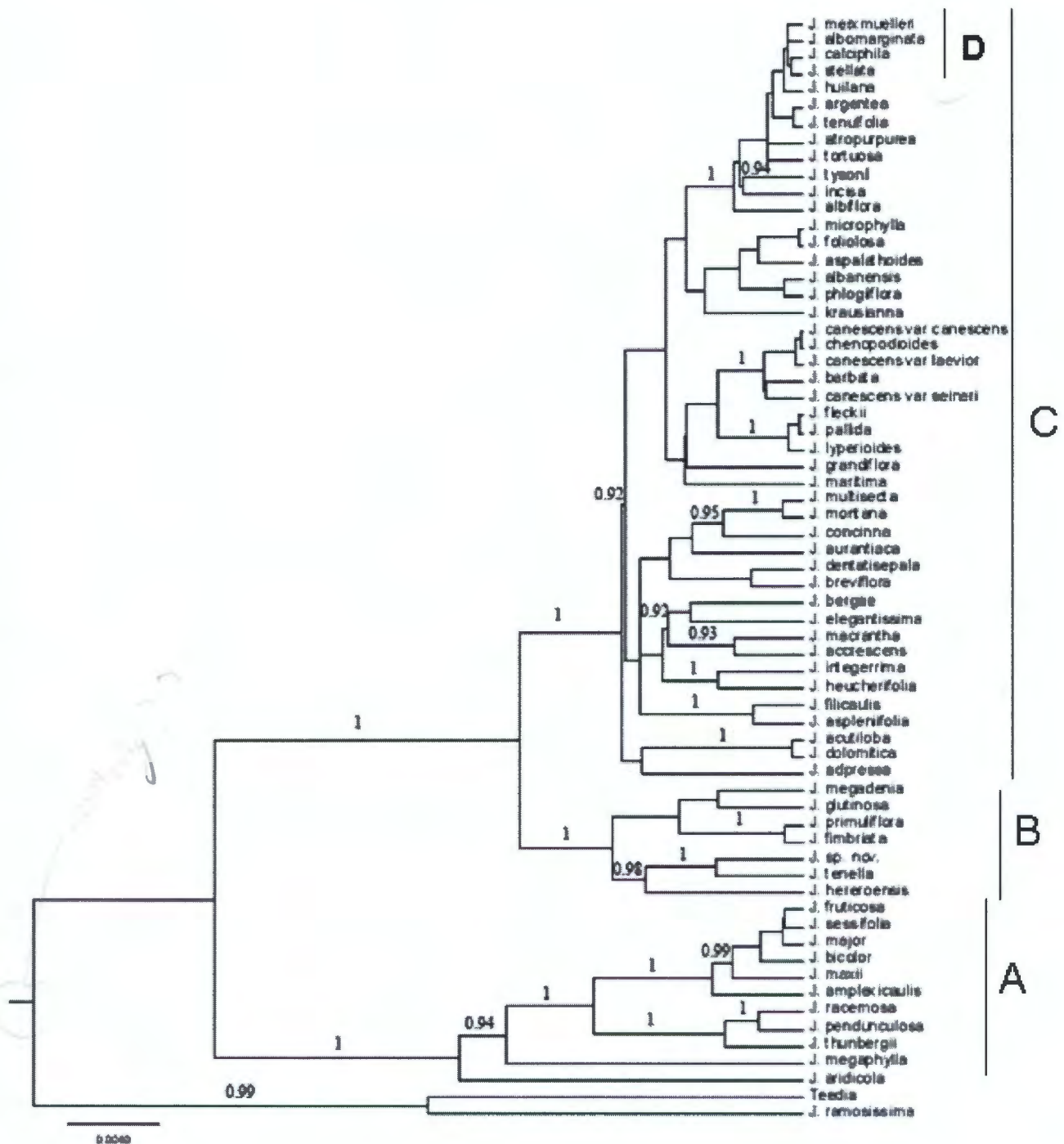
Figure 5. Phylogram of the maximum clade credibility tree obtained from Bayesian analysis of the combined dataset. Posterior probabilities greater than 0.9 are indicated above branches.
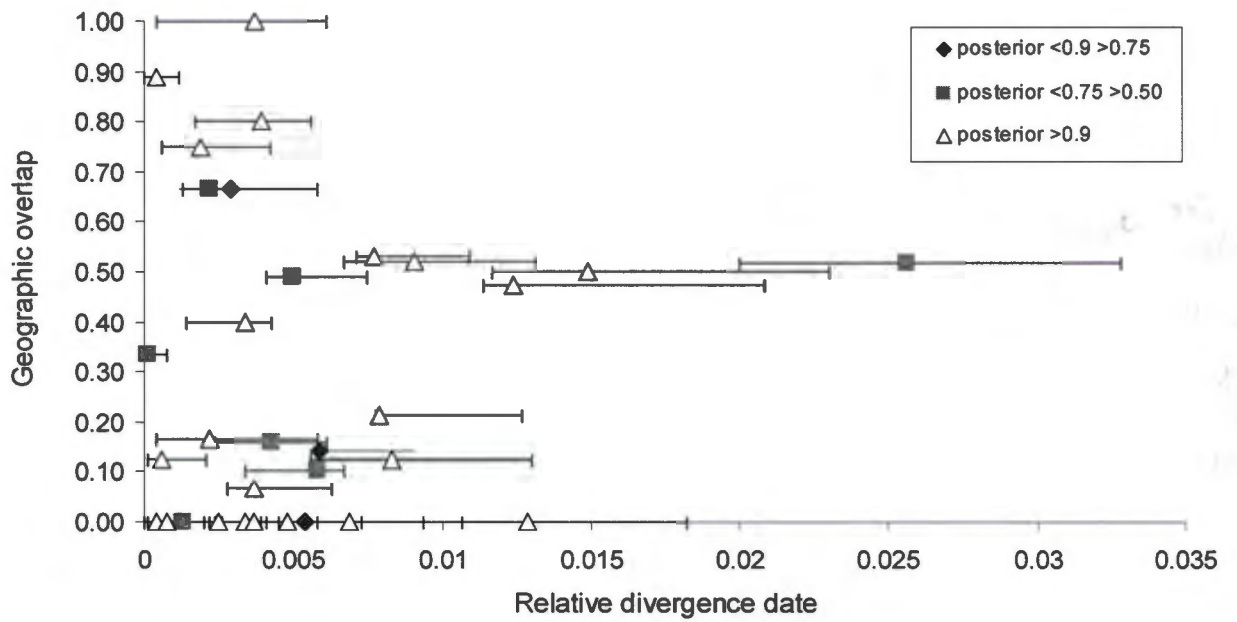
Figure 6. Age-range correlation of for all nodes in the maximum clade credibility tree with greater than 0.5 posterior probability (n=34).
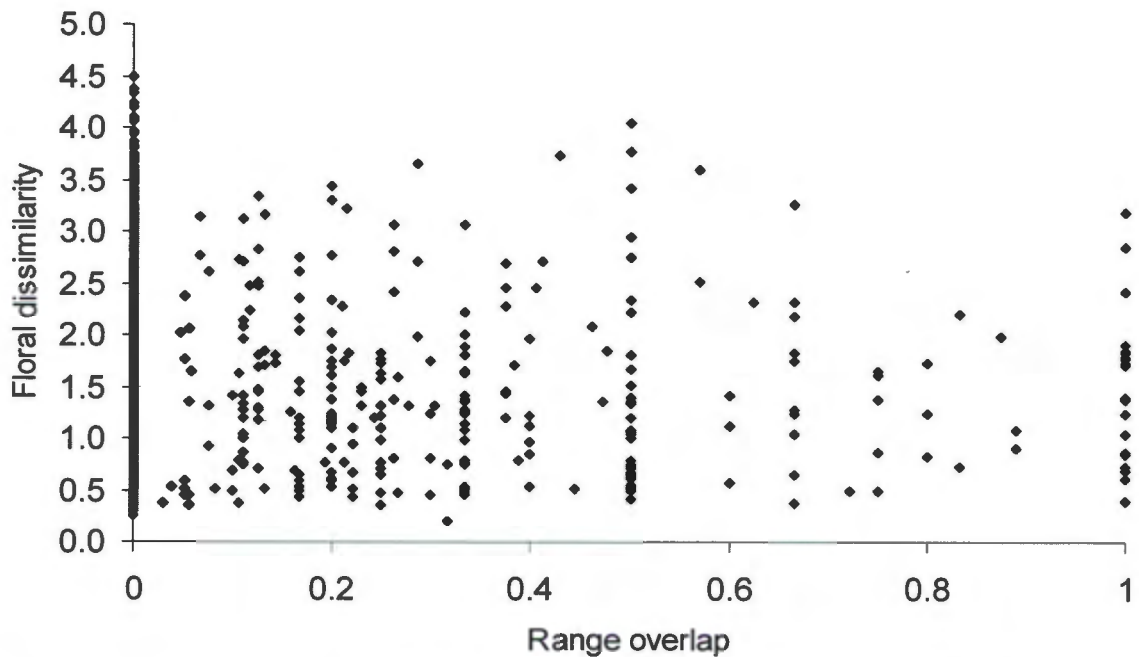
Figure 7. The geographic pattern of floral dissimilarity (r= -0.0509, p=0.013, n=2926).

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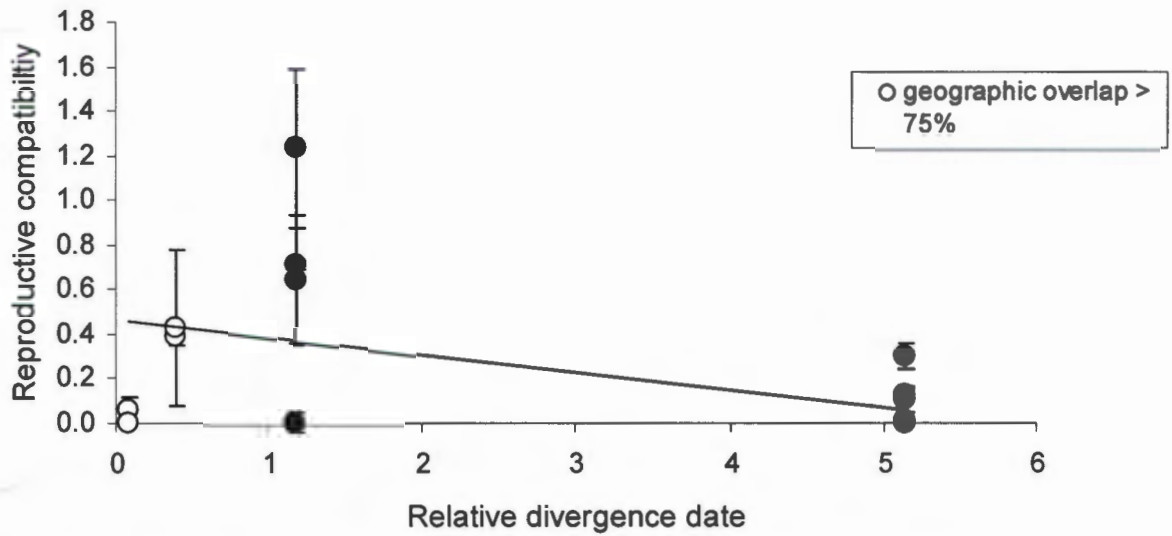
Figure 8. Relative divergence date (x10) plotted against reproductive compatibility measured as seed set relative to outcrossing seed set (R^2=0.27, p=0.013, n=83). When highly sympatric species pairs are removed R^2 improves to 0.57 and the regression remains significant (p<0.01).
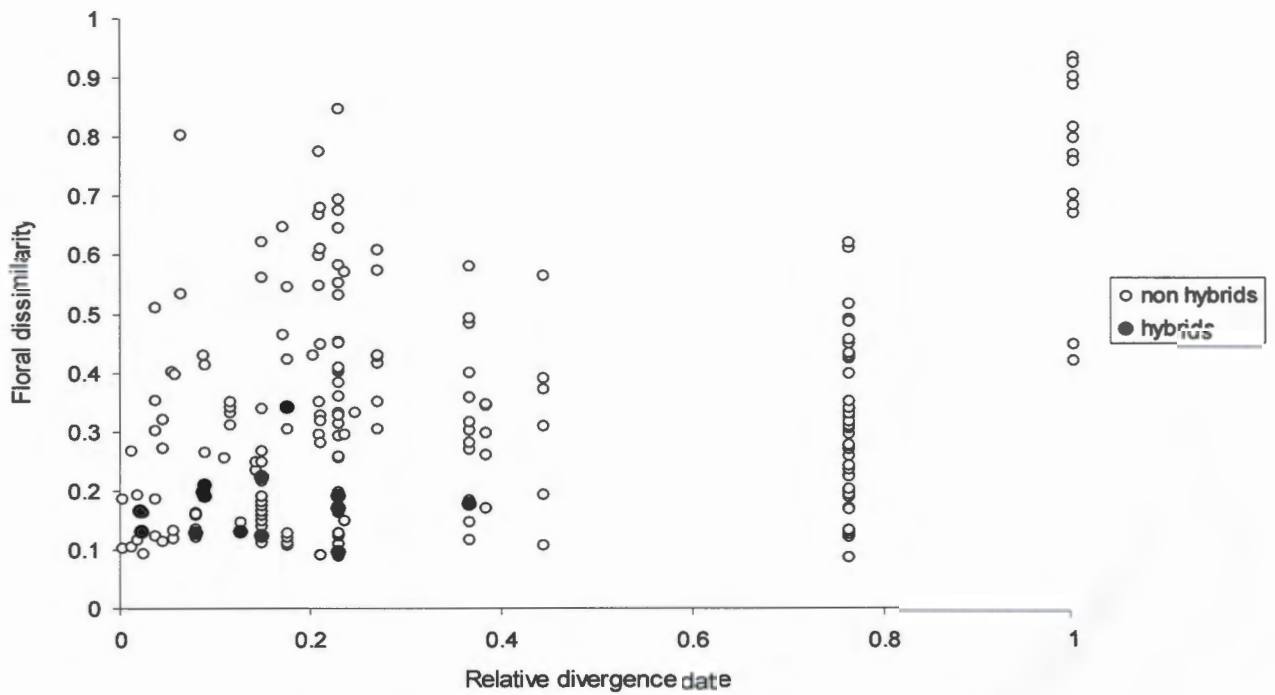
Figure 9. The pattern of relative divergence date and floral dissimilarity for all non-allopatric hybridizing (solid circles, n=14) and non-hybridizing (empty circles, n=202) species of *Jamesbrittenia*.

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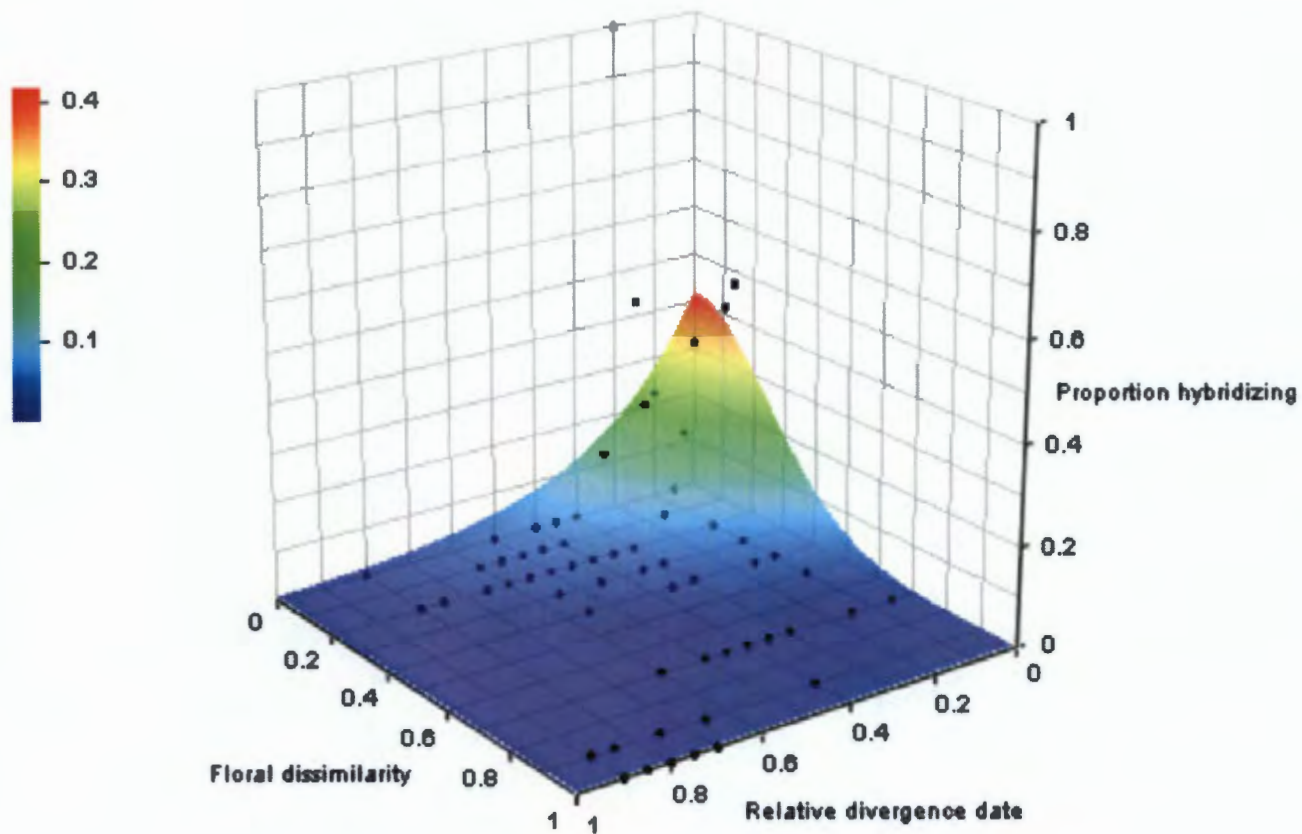
Figure 10. Non-linear exponential regression of relative divergence date and floral dissimilarity against the proportion of species pairs hybridizing in each divergence date and dissimilarity class ($y=0.4211*\exp(4.032x+8.099y^2)$), $R^2=0.27$, $p<0.001$, $n=61$).

Discussion

The results show that the attributes used as predictors of reproductive isolation are useful predictors of interspecific compatibility. Patterns of range overlap between species influence the evolution of these isolating mechanisms. The inability to detect the dominant mode of speciation confounds interpretation of the results, as it is not possible

to determine if the influence of geographic patterns on the evolution of reproductive isolation are a result of the mode of speciation or post-speciation evolutionary changes.

Phylogenetic conflict

The phylogenetic trees obtained from Parsimony and Bayesian analysis are in general agreement. The topologies are almost identical, with only minor difference in support for some clades. This is encouraging as the approach to molecular dating used by BEAST is novel. The tree obtained using Bayesian analysis does however conflict with the topology obtained by Herron (2006). It is the relationship between *J. ramosissima* and the rest of *Jamesbrittenia* that is the cause of this conflict. Herron (2006) found *J. ramosissima* to be sister to the rest of *Jamesbrittenia* in both parsimony and Bayesian analysis. It almost certainly the rooting of the tree performed by BEAST in the implementation of the relaxed clock method of Drummond *et al* (2006) that is the source of this conflict.

Evolution and diversification

It appears as thought the tempo of diversification in *Jamesbrittenia* was initially slow, with most of the species diversity evolving recently. The most rapid diversification has occurred in the species associated with limestone outcrops in the southern Cape (clade D). These outcrops are estimated to be 2-3 million years old, and have been subjected to successive periods of inundation as sea level fluctuated (Willis *et al* 1996). The colonization of islands and fragmentation of populations appears to have promoted speciation. It is interesting that an increased tempo of evolution is associated with the species that occur in the Cape floristic region, where edaphic specialization is thought to be an important driver of speciation (Linder 2003). Thus both biotic properties of *Jamesbrittenia* and abiotic factors particular to the cape can be implicated in the diversification of *Jamesbrittenia* in the Cape. In this case specialization to limestone habitats, paired with the insular distribution of these habitats have promoted barriers to gene flow leading to speciation.

The apparent patterns of diversification in *Jamesbrittenia* may simply be a result of patterns of extinction in the genus. Older lineages have had far more time for extinction to prune off taxa than the more recent lineages. It is possible that the pattern evident in *Jamesbrittenia* may simply be a result of bias induced by the age of lineages (Nee 2001).

Geographic patterns

The dominant mode of speciation is not apparent in our analyses. Three possible explanations exist for the observed pattern of node age and geographic overlap. Firstly, if ranges are highly labile the mode of speciation in even the most recent nodes will be obscured (Losos and Glor 2003). The observed pattern in the age-range correlation corresponds to a pattern which would be expected if post-speciation range shifts have obscured the predominant pattern of speciation (Barraclough and Vogler 2000). Alternately lack of signal may be due to the spacial scale at which sympatry was defined (1/4 degree grid cells). This scale may be too coarse to detect patterns of speciation in *Jamesbrittenia*, which may be occurring at the micro-habitat scale. Indeed, many species of *Jamesbrittenia* have very specific microhabitat requirements, and may be reproductively separated from other species that occur only a few kilometres away. If speciation has occurred mainly at this scale, the scale used here would be too liberal in its definition of sympatry. Another explanation is that speciation has been both sympatric and allopatric. Nonetheless, many of the most recently diverged taxa are completely allopatric. The failure to find significance in this result may be an artefact of the significance test designed to test for the significance of the mode of speciation. Perret *et al* (2007) used a similar test and failed to find significance when the dominant mode of speciation is allopatric. An alternative type of significance test, such as that used by Fitzpatrick and Turelli (2002) may be more appropriate when allopatry is common.

The negative correlation between floral dissimilarity and range overlap suggests that floral divergence is limited by somehow. This may be due to pollinator scarcity. If the available suite of pollinators at any given site is narrow and individuals are scarce plant species that occurring at that site would have to utilize all available resources in the

form of pollinators (Bierzychudek 1981). Pollinator scarcity has been suggested as being important in the evolution of the Cape flora (Johnson 1996). This is of course dependent on the scale at which communities are defined. The most important factor in determining the appropriate grain at which overlap should be defined is the mobility of pollinators. As most species of *Jamesbrittenia* have small, gracile flowers it is not likely that pollen vectors operating over long distances, such as birds or bats, are responsible for pollinating these plants. The most likely pollination syndrome is entomophily, which would imply that pollen dispersal distances are short. Thus it may be that the spacial scale at which sympatry is defined is one again too coarse to make any solid conclusions. If the spacial scale used is appropriate, then it is possible to conclude that ethological and mechanical isolation are not important isolating mechanisms in *Jamesbrittenia*, as communities are not florally divergent. Although this conclusion is based on analyses over a broad range of phylogenetic relatedness, it nonetheless has relevance to the evolution of reproductive isolation in closely related species. The likelihood of sympatric speciation would be far lower in an environment with a limited pollinator pool, as many studies have cited pollinator shifts as a cause of sympatric speciation (Dressler 1968, Paulus and Gack 1990). Another possible source of error in this analysis is the morphological characters used to define floral similarity. It was not possible to account for variation in colour and fragrance, as well as phenological variation. These may be important in attracting pollinators and remains unaccounted for in the index of similarity used. Phenological variation is probably not important in limiting gene flow between sympatric species because *Jamesbrittenia* flowering patterns appear to be determined by environmental conditions rather than intrinsic signals (A. Harrower *pers. comm.*). However, it cannot be ruled out that diel variation in receptivity isolates co-occurring species.

Isolating mechanisms and hybridization

      The significant relationship between divergence time and prezygotic isolation justifies the use of divergence time as a measure of reproductive isolation. This corroborates the results of earlier studies (Tilley *et al* 1990; Knowlton *et al* 1993; Coyne and Orr 1997; Sasa *et al* 1998). However, this relationship is weakened by the

asymmetries in reproductive success and low compatibility of recently diverged species with a high degree of sympatry. Asymmetries are a result of crosses involving *J. grandiflora*. In both instances crossing success was lower when *J. grandiflora* was maternal. A possible explanation for this is that *J. grandiflora* has an abnormally long pistil, much longer than that of either *J. argentea* or *J. tenuifolia*. Pollen tube growth rates have been found to be correlated with pistil lengths (Williams and Rouse 1990, Carney *et al* 1996), suggesting that the pollen tubes of *J. argentea* or *J. tenuifolia* may not grow rapidly enough to successfully fertilize *J. grandiflora*. Manipulating the length of the pistil in *J. grandiflora* has been observed to increase the success of interspecific crossing (A. Harrower pers. comm.). The low seed set resulting from crosses between recently diverged species with high degrees of overlap further contributes to the variability in the overall trend. The possibility exist that the apparent rapid origin of reproductive isolation in these two species pairs is an artefact of the sympatric origin of species. The evolution of reproductive isolation is assumed to be essential for sympatric speciation to occur (Wiens 2004). A more likely scenario is that speciation was allopatric and that secondary contact between these species has resulted in the reinforcement of isolating mechanisms. Indeed this process of reinforcement upon secondary contact is believed to be very common in the Cape flora (van der Niet 2006). This suggestion requires further investigation. It may be possible that neither of these two species pairs display any real sympatry, depending on their microhabitats and pollinators. Reinforcement is likely to occur when the cost of hybridization is high (Hostert 1997), and thus an investigation into the seed set occurring in natural populations of these two species pairs and the fitness of hybrid progeny would yield valuable information

Relative time since divergence and floral dissimilarity appear to be good predictors of the ability to hybridize, and thus their use in predictive context is justified. Although the nonlinear regression is based on a weak correlation and the proportion of hybridizing species in each relative divergence date and floral similarity class is based on only speculative reports of hybridization, the recommendations made are done so with the best available data. As the likelihood of species being moved beyond their natural range for horticultural purposes is high, these recommendations are urgently needed to

guide the movement of species across the region. Certain attributes of *Jamesbrittenia* are cause for further concern: most species appear to be self-infertile and occur as small isolated populations. Both of these traits were found by Wolf *et al* (2001) to increase the probability of introgression between two species upon secondary contact. Thus, the movement of any species into the range of another listed as having a high probability of hybrid formation in table 2 ought to be avoided. The problem of hybridization and introgression between closely related species brought into secondary contact through anthropogenic influences may be widespread. Recently diverged species in many floras of the world that have diversified predominantly through allopatric speciation may be at risk.

Conclusions

General patterns in the origins and strength reproductive isolation in *Jamesbrittenia* are evident and it is clear that divergence time and floral morphological characters do influence the degree of isolation between two species. The use of correlates of reproductive isolation in predicting hybridization occurring as a result of anthropogenic influences has yet to be fully exploited. However, deviations from the overall trend are to be expected and need to be taken into account when considering the mechanisms responsible for the observed correlation and their implications. The role of reinforcement in the evolution of the flora of southern Africa requires further investigation; it may be that the processes occurring in the Cape are manifested throughout southern Africa.

The importance of scale in determining patterns of speciation in *Jamesbrittenia* cannot be overemphasized. Vastly different pattern will emerge when evolutionary patterns are studies at different scales. The coarse phylogenetic and spatial scale at which this study was conducted, and the inability to identify speciation trends in *Jamesbrittenia* illustrates the need to study speciation at the phylogenetic and spatial scale at which it occurs. This implies investigation at the population-species interface and at the spatial scale at which gene flow occurs.

Acknowledgements

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