A PHYLOGENETIC STUDY OF EVOLUTIONARY TRANSITIONS IN SEXUAL SYSTEMS IN AUSTRALASIAN *WURMBEA* (COLCHICACEAE)

Andrea L. Case,[1,2,]* Sean W. Graham,[1,]† Terry D. Macfarlane,‡ and Spencer C. H. Barrett§

*Department of Biological Sciences, Kent State University, Kent, Ohio 44242, U.S.A.; †University of British Columbia Botanical Garden and Centre for Plant Research, Faculty of Land and Food Systems, and Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada; ‡Manjimup Research Centre, Department of Environment and Conservation, Brain Street, Manjimup, Western Australia 6258, Australia; and §Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

Using phylogenies to make sound inferences about character evolution depends on a variety of factors, including tree uncertainty, taxon sampling, and the degree of evolutionary lability in the character of interest. We explore the effect of these and other sources of ambiguity for maximum likelihood (ML)–based inferences of sexual-system evolution in *Wurmbea*, a small genus of geophytic monocots from the Southern Hemisphere. We reconstructed *Wurmbea* phylogeny using four noncontiguous regions (ca. 5.5 kb) of the plastid genome across a broad sampling of taxa, and we confirm that the genus is divided into two well-supported clades, each defined by its geography (Africa vs. Australasia) and variation in sexual system (i.e., uniformly monomorphic vs. sexually variable, respectively). We demonstrate that the predominantly Australian clade includes the sexually monomorphic species *Iphigenia novae-zelandiae*. We observe treewide uncertainty in the state of all ancestral nodes, and therefore all state transitions, when all taxa in *Wurmbea* are considered. We demonstrate that this is primarily a consequence of interspersion of terminals with gender dimorphism vs. monomorphism throughout the Australasian clade, rather than tree uncertainty or the presence of very short internal branches. We accounted for tree uncertainty by randomly sampling alternative resolutions of branches that are poorly supported by ML bootstrap analysis, effectively interpreting these as soft polytomies. Under the assumption that well-supported aspects of our gene tree accurately depict organismal phylogeny, there is a marked evolutionary lability in the sexual systems of Australasian *Wurmbea*. A more problematic issue is that our results contradict the monophyly of two sexually polymorphic Australian species, *Wurmbea dioica* and *Wurmbea biglandulosa*. If this reflects paraphyly at the species level, lateral gene transfer, or failed coalescence, then the interpretations of character transitions will need to be adjusted. Our analysis provides an example of the impediments to linking macroevolutionary pattern with microevolutionary processes for evolutionarily labile traits in recently evolved plant groups that possess a high degree of variation in sexual characters.

Keywords: ancestral-state reconstruction, cosexuality, dioecy, gynodioecy, noncoding DNA, paraphyletic species, gender strategies, polyphyly, sexual-system evolution, species concepts, subdioecy.

Introduction

Evolutionary transitions between hermaphroditism (gender monomorphism) and separate sexes (gender dimorphism) have occurred frequently in the history of flowering plants (e.g., Weiblen et al. 2000; Vamosi et al. 2003). Understanding the evolutionary history of sexual systems and the mechanisms responsible for such transitions is thus an important issue in plant evolutionary biology (reviewed in Geber et al. 1999). The conditions for the evolution and maintenance of gender dimorphism have been the subject of much theoretical and empirical research, aimed primarily at explaining the sporadic but taxonomically widespread occurrence of this condition in the angiosperms (reviewed in Thomson and Brunet 1990;

Renner and Ricklefs 1995; Sakai and Weller 1999; Heilbuth 2000). Phylogenies can provide us with windows into the past history of organisms, enabling investigation of the long-term significance of evolutionary transitions and ecological processes that otherwise can be observed only on local or instantaneous time frames. For example, because phylogenies span multiple speciation events, they can be used to identify convergent traits that arose under similar selective regimes in distantly related taxa, to develop chronological sequences of character transitions by reconstructing states at ancestral nodes (e.g., Kohn et al. 1996), and to measure cross-taxon correlations among characters of interest (e.g., Friedman and Barrett 2008; Sargent and Vamosi 2008).

A number of studies have employed phylogenies to investigate evolutionary transitions in plant reproductive characters (e.g., Weller and Sakai 1999 and references therein; Weiblen et al. 2000; Renner and Won 2001; Krahenbuhl et al. 2002; Miller 2002; Vamosi et al. 2003; Graham and Barrett 2004; Givnish et al. 2005; Gleiser and Verdú 2005; Levin and Miller 2005). These studies are primarily aimed at estimating

---

the number of times particular traits arose and/or the ecological or morphological context in which traits may have evolved, which provide indirect evidence of the proximate mechanisms responsible for phenotypic change and the adaptive significance of trait transitions. Although character correlations and estimates of transition rates do not necessarily require precise localization of character state changes on a phylogenetic tree, it is often useful to know the local direction of change (e.g., on which terminal or near-terminal branches particular transitions occurred). Pinpointing transitions on particular branches can guide further sampling efforts and subsequent microevolutionary studies that delve into the local-scale processes responsible for observed macroevolutionary patterns. In practice, mapping traits onto trees with precision is not necessarily straightforward, particularly for characters that show a high degree of evolutionary lability and/or intraspecific polymorphism (Weller and Sakai 1999). In this article, we describe a phylogenetic analysis of transitions in the sexual systems of the Australasian members of *Wurmbea* (Liliales: Colchicaceae), a widespread genus that displays considerable variation in gender strategies. In doing so, we address some of the challenges associated with inferences of historical transitions in variable characters.

Recent advances have been made in developing and implementing model-based methods for studying character evolution using phylogenies (e.g., Pagel 1994, 1999; Cunningham et al. 1998; Mooers and Schluter 1999; Huelsenbeck et al. 2003; Pagel et al. 2004; Ronquist 2004; Maddison and Maddison 2005). This has led to more nuanced approaches than maximum parsimony (MP), which accommodate uncertainty in how ancestral states are reconstructed on a given tree and, increasingly, underlying uncertainty about the phylogenetic tree itself. MP estimates of character evolution are still widely used but could be misleading because they do not take account of available branch-length information (e.g., multiple changes along a branch are not permitted in MP reconstructions). Maximum likelihood (ML) estimates of character states and their transitions offer the advantage that they are explicitly model based and can incorporate branch-length information (Pagel 1994, 1999). A Bayesian approach that stochastically maps character evolution (Nielson 2002; Huelsenbeck et al. 2003; Pagel et al. 2004; Bollback 2006) can accommodate uncertainty in assignment of ancestral states, in addition to uncertainty in the tree itself, using Markov chain Monte Carlo (MCMC) samples from the posterior distribution of trees, substitution model parameters, and character ancestral states (Bollback 2006). Stochastic character mapping requires that the prior probability of the rate parameter of character state change be assigned, although usually this will not be known. In this method, tree uncertainty can be accounted for by using post-burn-in trees sampled by MrBayes using MCMC (Huelsenbeck and Ronquist 2001). The MCMC tree output has also been used to account for tree uncertainty in MP and ML ancestral-state reconstructions in recent studies (e.g., Miadlikowska and Lutzoni 2004; Lewis and Lewis 2005; Jones and Martin 2006; Vanderpoorten and Goffinet 2006; Galley and Linder 2007). However, MCMC samples may lead to inflated estimates of clade confidence (e.g., Suzuki et al. 2002; Cummings et al. 2003; Erixon et al. 2003; Simmons et al. 2004), potentially biasing reconstructions of ancestral states.

The most common method of accounting for tree uncertainty in phylogenetic analysis is the bootstrap (Felsenstein 1985). Bootstrapping provides a nonparametric estimate of the effect of sampling error on tree topology and is performed by resampling the characters used to infer the tree (Felsenstein 1985). Using the bootstrap profile of trees for character mapping (Jones and Martin 2006) arguably provides a more conservative approach for taking account of tree uncertainty than using an MCMC tree sample. Instead of using the raw bootstrap profile for character mapping in our study of *Wurmbea*, we retained branches that were moderately to strongly supported by ML bootstrap analysis and randomly sampled possible resolutions of poorly supported branches (i.e., we considered branches with <70% support to represent soft polytomies). These trees were used to perform ML and MP reconstructions of the ancestral states of sexual systems in *Wurmbea*. It is not our intent to compare the efficacy of different character mapping approaches, although recent empirical studies suggest that reconstructions based on MP, ML, and stochastic mappings may be quite closely related (e.g., Lewis and Lewis 2005; Jones and Martin 2006; Vanderpoorten and Goffinet 2006; Leschen and Buckley 2007).

The diversity of gender strategies within and among species of *Wurmbea*—ca. 48 species of perennial, geophytic herbs (Nordenstam 1978, 1986, 1998; Macfarlane 1980, 1986, 1987; Bates 1995, 2007; Macfarlane and van Leeuwen 1996)—has drawn much recent attention (Barrett 1992; Vaughton and Ramsey 1998, 2002, 2003, 2004; Barrett et al. 1999; Case and Barrett 2001, 2004a, 2004b; Jones and Burd 2001; Ramsey and Vaughton 2001, 2002; Barrett and Case 2006; Ramsey et al. 2006a, 2006b). Most of this work has been conducted on *Wurmbea dioica* and *Wurmbea biglandulosa*, two wide-ranging polymorphic species that exhibit considerable intraspecific variation in sexual systems, including hermaphroditism, gynodioecy, subdioecy and dioecy (Barrett 1992; Vaughton and Ramsey 2002; Case and Barrett 2004a). Biogeographical surveys and experimental studies have investigated the role of resource availability and pollination biology in the evolution and maintenance of gender dimorphism in these taxa (Case and Barrett 2001, 2004a, 2004b; Vaughton and Ramsey 2004). However, evaluating these selective hypotheses in an ahistorical context could be misleading, not least because of the potential problems of species circumscription that are common in morphologically diverse groups (e.g., Funk and Omland 2003).

Although *Wurmbea* has a relatively even African-Australian distribution (Goldblatt 1978; Nordenstam 1978; Conran 1985), the types of sexual system represented in the two continental regions are strikingly different. Gender dimorphism is present only among the Australian species, while African *Wurmbea* and all other members of Colchicaceae are uniformly monomorphic for gender (Nordenstam 1978; Macfarlane 1980; Dahlgren et al. 1985). *Wurmbea* probably originated in southern Africa and arrived in western Australia via long-distance dispersal (Nordenstam 1978; Barrett 1992; Vinnersten and Bremer 2001). The nested position of *Wurmbea* in Colchicaceae, along with the probable monophyly of the Australian taxa (Vinnersten and Reeves 2003), tends to support the idea that gender dimorphism is evolutionarily derived in *Wurmbea* and that one or more origins of dimorphism followed its arrival and establishment in

Australia (Nordenstam 1978; Barrett 1992). Within Australia, gender and sexual dimorphism are more prevalent in eastern states, with a larger proportion of gender-dimorphic taxa and greater sexual dimorphism there than in western Australia. This may indicate region-specific selection for gender and sexual dimorphism.

Biogeographical signals evident in our phylogenetic analysis may inform hypotheses for variation in sexual systems as *Wurmbea* spread across the continent and perhaps beyond. We were also interested in investigating whether an additional eastward dispersal event from Australia to New Zealand occurred in *Wurmbea*. *Iphigenia novae-zelandiae* is the sole New Zealand member of Colchicaceae, and it may be a taxonomically misplaced member of *Wurmbea* (Moore and Edgar 1970; T. D. Macfarlane, personal observation). Plants of *I. novae-zelandiae* are small and have few leaves and tiny, solitary, and "poorly-formed" flowers (Moore and Edgar 1970); firm decisions on its generic position based on morphology alone are difficult because of its floral instability and reduced stature. The high degree of developmental instability of flowers may indicate that *I. novae-zelandiae* is self-compatible and predominantly autogamous (e.g., Barrett 1985). Although no mating system information is available for *I. novae-zelandiae*, some *Wurmbea* species are self-compatible (Vaughton and Ramsey 1998, 2003; Case 2000; Ramsey and Vaughton 2002). Baker's Law (Baker 1967) predicts an increased likelihood of successful establishment after long-distance dispersal by plants capable of autonomous selfing. Thus, an additional goal of our study was to use molecular data to determine whether *I. novae-zelandiae* is indeed a species of *Wurmbea* whose New Zealand distribution resulted from a more recent long-distance dispersal event from Australia.

We used phylogenetic evidence from four noncontiguous regions of the plastid genome to investigate evolutionary transitions between monomorphic and dimorphic sexual systems in Australian species of *Wurmbea*, the biogeographical structure of gender dimorphism within Australia, and the phylogenetic status of *I. novae-zelandiae*. We encountered several possible sources of uncertainty in our inferences of character evolution. These included pronounced interspersion of monomorphic and dimorphic taxa on inferred trees, suggesting substantial evolutionary lability in character shifts; tree uncertainty (reflected in several polytomies and short branches with limited bootstrap support); and evidence of the nonmonophyly of at least two of the species that are polymorphic for sexual system. We explored the implications of these sources of uncertainty on the inferred evolutionary history of gender variation in the Australian *Wurmbea*. Our exploration provides general insights into the challenges associated with the reconstruction of evolutionary labile characters on phylogenetic trees, particularly for groups with a recent origin or those that include classically described species that may not be monophyletic.

Methods

Taxon Sampling and Outgroup Selection

Wurmbea has been the subject of recent taxonomic study; species delimitations are generally well accepted (Nordenstam 1978, 1986; Macfarlane 1980, 1986, 1987; Bates 1995, 2007; Macfarlane and van Leeuwen 1996). Of the ca. 48

species of *Wurmbea*, 18 occur in Africa and 30 in Australia. Our sampling focused on the latter because the former are exclusively sexually monomorphic. We include 16 Australian species and three species from the Cape Province of South Africa. The Australian sample includes representatives of all five dimorphic species of *Wurmbea* (see appendix). According to the most recent circumscription (Bates 2007), the widespread species *Wurmbea dioica* consists of four subspecies (*alba*, *dioica*, *brevifolia*, and *lacunaria*), and the species *Wurmbea biglandulosa* consists of two (*biglandulosa* and *flindersica*). *Wurmbea dioica* subspecies are uniformly dimorphic for gender, except in western Australia, where monomorphic and dimorphic populations of *W. dioica* ssp. *alba* co-occur (Macfarlane 1980; Barrett 1992; Case and Barrett 2001, 2004a, 2004b). Both subspecies of *W. biglandulosa* have monomorphic populations, whereas ssp. *biglandulosa* also contains dimorphic populations (Vaughton and Ramsey 2002). Our analysis included one population of each of the following: *W. biglandulosa* ssp. *flindersica*, both subspecies of *Wurmbea latifolia*, and two subspecies of *W. dioica*. We included both monomorphic and dimorphic populations of the two polymorphic species, one population of each sexual system in *W. dioica* ssp. *alba*, and two populations of each sexual system in *W. biglandulosa* ssp. *biglandulosa*.

We sampled multiple putative outgroups representing three of the four tribes of the Colchicaceae: *Onixotis punctata* and *Onixotis stricta* (Anguillarieae); *Baeometra uniflora* (Baeometreae); *Iphigenia indica*, *Iphigenia novae-zelandiae*, and *Gloriosa superba* (Iphigenieae); and *Colchicum* (Colchiceae). Among these outgroups, the nearest relatives to *Wurmbea* are predicted to belong to *Onixotis* because *Wurmbea* and *Onixotis*, together with South African *Neodredgea*, make up tribe Anguillarieae (Nordenstam 1978, 1986). Vinnersten and Manning (2007) recognize *Onixotis* and *Neodredgea* under *Wurmbea* (see also Vinnersten 2003), but for the purposes of this article we follow the narrower circumscription. We also included *Burchardia multiflora*, a species of previously uncertain taxonomic position that is now well supported as a member of Colchicaceae (Chase et al. 1995; Rudall et al. 2000; Vinnersten and Bremer 2001; see also Nordenstam 1998).

Molecular Data

We extracted DNA from frozen or silica-gel-dried leaf tissue using either CTAB (Doyle and Doyle 1987) or Qiagen DNEasy isolation kits (Qiagen, Valencia, CA). We sequenced four segments of the large single-copy region of the chloroplast genome possessing a range of evolutionary rates (e.g., Graham et al. 2006): a cluster of four photosystem II genes (*psb*B, *psb*T, *psb*N, and *psb*H; hereafter *psb*BTNH), two tRNA genes with an intervening spacer region and a single intron (*trn*L-UAA and *trn*F-GAA; hereafter *trn*LF), the intergenic spacer region between *atp*B and *rbc*L (hereafter *atp*B-*rbc*L), and the 3′ end of the coding region of NADH dehydrogenase, subunit F (hereafter *ndh*F). These regions were amplified and sequenced using primers described by Taberlet et al. (1991) for *trn*LF, Graham and Olmstead (2000) for *psb*BTNH, Mannen et al. (1994) and Savolainen et al. (1994) for *atp*B-*rbc*L, and Olmstead and Sweere (1994) and Graham et al. (1998) for *ndh*F. Two of the internal sequencing primers

of *atp*B-*rbc*L did not work for *Wurmbea*, so we designed two additional primers for sequencing (IGS4A = 5′-AATTGTGA-GTAAATGTGTTTAT; IGS4B = 5′-GATTCATTATTTCGATC-TTACC). A few regions could not be recovered for individual taxa, as is noted in the appendix.

We conducted PCR amplifications, using standard methods and profiles (e.g., Graham and Olmstead 2000). PCR products were purified with QiaQuick PCR purification columns (Qiagen) following manufacturer instructions; for cycle sequencing we used primers located internally to those used for amplification. For each gene region, we compiled and base-called contiguous sequence fragments using Sequencher (Gene Codes, Ann Arbor, MI). We used Clustal W (Thompson et al. 1994) to generate initial sequence alignments and manually adjusted alignments using Se-Al 1.0 (Rambaut 1998), following criteria described by Graham et al. (2000). Alignment gaps were coded as missing data. The aligned matrix is available on request from the first author.

Phylogenetic Inference Methods

We inferred trees with ML, using PhyML, version 2.4.4 (Guindon and Gascuel 2003), and MP, using PAUP*, version 4.0b10 (Swofford 2002), and we used Mesquite, version 1.12 (Maddison and Maddison 2005) for ML reconstructions of ancestral character states. The optimal model of DNA sequence evolution, chosen using hierarchical likelihood ratio tests (hLRTs) in ModelTest, version 3.06 (Posada and Crandall 1998), is TVM + Γ + I (see ModelTest documentation for model details). We performed heuristic searches in PhyML while determining optimal model parameters from the data but otherwise using default settings. We assessed branch support using 100 bootstrap replicates (Felsenstein 1985), using 10 random-addition replicates for each bootstrap replicate for the MP analysis.

Reconstructions of Sexual-System Evolution

We performed ML optimization of sexual systems on the best ML tree to trace their evolutionary history, using Mesquite (equivalent to the "global" option in Pagel's Discrete). We coded sexual system as a binary character (monomorphic vs. dimorphic) rather than assigning dimorphic taxa as gynodioecious, subdioecious, or dioecious, because extensive surveys indicate a substantial degree of gender variation among populations of all dimorphic taxa (e.g., Barrett 1992; Barrett et al. 1999; Vaughton and Ramsey 2002). Branch lengths were estimated from the data using PAUP*. The best ML tree contained four polytomies, which we interpret as "soft," reflecting a lack of evidence for resolving nodes (Maddison 1997). Because Mesquite cannot fully accommodate zero-length branches in ML ancestral-state reconstructions, we randomly resolved these four polytomies using the "create trees" tool in MacClade, version 4.03 (Maddison and Maddison 2001) and assigned them very short nonzero branch lengths (0.000001 substitutions per site; see also Ferrer and Good-Avila 2007). We refer to this tree as the "resolved best tree."

We estimated the overall likelihoods of one-rate and two-rate (asymmetric) models of evolution; the one-rate model (the Mk1 model in Mesquite, equivalent to the Markov model of Lewis [2001]) was the better-fitting one based on likelihood-

ratio tests (Pagel 1999). We considered ancestral states of individual nodes with a difference in log-likelihood scores greater than 2.0 to be statistically significant (Edwards 1972). Preliminary reconstructions indicated numerous transitions in sexual system in *Wurmbea* and uncertain reconstructions (monomorphic vs. dimorphic) for all nodes subtending *Wurmbea*. Although dimorphism is restricted to *Wurmbea*, the rest of Colchicaceae (and possibly related families) are uniformly sexually monomorphic. It is therefore quite possible that the one-rate model or its rate parameter does not apply consistently across the whole family. Most implementations of ML-based inference of ancestral states assume that a single, consistent evolutionary model applies across the tree under consideration. Mooers and Schluter (1999) describe a method for taking account of a shift in the evolutionary model on a specific branch. However, it is not clear how to pinpoint where (or whether) shifts in the evolutionary model or model parameters occurred. Simply placing a rate shift between an ingroup and outgroup is an unwarranted assumption. In the absence of knowledge of whether or where a rate shift occurred, we conducted separate ML reconstructions on *Wurmbea* alone and on Australasian *Wurmbea* alone (an approach suggested by W. Maddison, personal communication). We assessed both cases because it is not clear whether we should include the monomorphic African species in ML ancestral-state reconstructions; like the outgroups, the African species of *Wurmbea* are sexually monomorphic.

We used an additional approach to account for uncertainty in tree topology (phylogenetic uncertainty) and its effect on our character reconstructions. We used MacClade to collapse four branches with <70% ML bootstrap support in the best ML tree (fig. 1), taking 70% support as an indication of moderately well-supported branches (e.g., Graham et al. 1998). We generated a random sample of >10,000 resolutions of the eight resulting polytomies (the original four plus the four additional collapsed branches). We then used the "condense trees" function in PAUP* to remove duplicate trees, leaving 10,000 unique topologies for character reconstructions. After recalculating ML branch lengths for all trees using PAUP*, we assigned very short nonzero branch lengths (0.000001 substitutions per site), as we did for the resolved best tree. It is unlikely that this small departure from the ML branch lengths has a substantive effect on our overall conclusions from character reconstructions (see "Results").

Because zero-length or very short branches with poor support may make it difficult to make robust ancestral-state reconstructions, we repeated our reconstructions on trees with all branches set to unit length (e.g., Mooers and Schluter 1999) and also on trees with all zero-length branches assigned the median length estimated within the *Wurmbea* clade (0.001445 substitutions per site). The former ignores branch-length information altogether and reflects solely the number of speciation events separating species (Pagel 1994; Mooers et al. 1999); the latter does not reflect actual evolution on the tree but permits us to assess how robust inferences of ancestral states at individual nodes would be, had there been a more even timing of speciation events.

We used the "trace character over trees" function in Mesquite to determine how many of the 10,000 topologies had significant ancestral-state reconstructions for the nodes considered in the
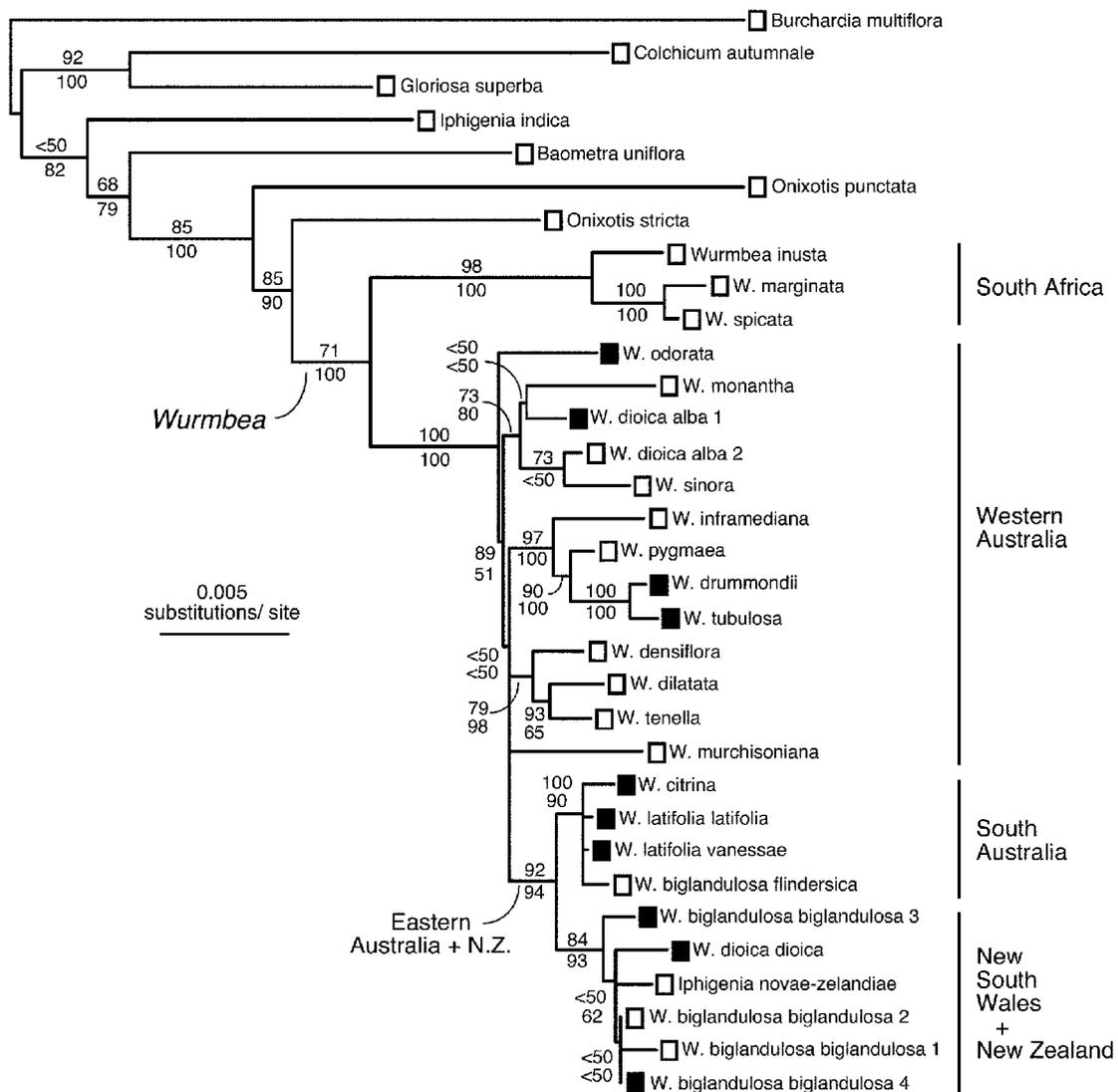
Fig. 1 Phylogenetic relationships in *Wurmbea* and relatives based on four disjunct plastid genome regions. Maximum likelihood (ML) analysis yields a single tree ($-\ln L = 13{,}084.47$); branch lengths are ML estimates. Bootstrap values are noted near branches (ML above, maximum parsimony below). Boxes at the ends of terminal branches indicate the sexual system of each sampled taxon: white indicates monomorphic, black indicates dimorphic. Multiple populations sampled within *Wurmbea dioica* ssp. *alba* and *Wurmbea biglandulosa* ssp. *biglandulosa* are distinguished using numbers.

resolved best tree, also noting the number of trees in which each node appeared. In addition, we used MP to estimate the minimum total number of transitions between monomorphism and dimorphism in either direction. We estimated total parsimony changes (Fitch 1971; Hartigan 1973) using the summary table option of the "state changes and stasis" tool in MacClade.

The best ML tree indicates that at least two species of *Wurmbea* are not monophyletic (see "Species Nonmonophyly"). We evaluated whether enforcing the monophyly of each species results in a significant increase in tree score by running heuristic ML searches in PAUP* with topological constraints enforced. The constraints enforced the monophyly of all (or subsets of) the populations for these species. We evaluated the significance of difference in scores of the shortest unconstrained and constrained trees, using

the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999), with resampling estimated log likelihoods (RELL) bootstrap, using ML estimates of TVM $+ \Gamma + I$ parameters as determined on an MP tree (base frequencies were estimated empirically) and otherwise using default settings. All tree score comparisons were made simultaneously (see Goldman et al. 2000).

Results

Data Characteristics

The total data set, including all outgroups, consists of 5337 aligned nucleotides (233 are parsimony informative), excluding a short (<15 bp) homopolymer region in the *trn*LF

intergenic spacer region. The data set for all *Wurmbea* samples contains 131 informative sites, with 79 informative sites for the Australia/New Zealand samples. For the full taxon set, the four chloroplast regions consist of 2090 (*psb*BTNH), 1262 (*trn*LF), 857 (*atp*B-*rbc*L), and 1128 bp (*ndh*F), which contribute 24%, 30%, 17%, and 28% of the informative sites, respectively. A 41-bp inversion in the *trn*LF intergenic spacer of one taxon (*Wurmbea inframediana*; see table 3 in Graham et al. 2000) was reinverted before alignment, following Graham and Olmstead (2000).

Phylogenetic Relationships in Wurmbea *and Relatives*

We rooted the best ML tree such that *Burchardia multiflora* is sister group to all other sampled Colchicaceae (Vinnersten and Reeves 2003). *Wurmbea* is then nested within a grade of *Onixotis*, a South African genus (fig. 1). *Iphigenia novae-zelandiae* is supported as a misclassified member of *Wurmbea* because of its nested position within a well-supported clade of eastern Australian members of the genus. *Wurmbea* is therefore monophyletic only if it is considered to include *I. novae-zelandiae*. A formal combination has not yet been made, but for simplicity in the rest of this article we will refer to *Wurmbea* as if it includes *I. novae-zelandiae*. There is a strongly supported basal division of *Wurmbea* into an African and an Australia/New Zealand clade. *Wurmbea odorata* is sister to the rest of the Australasian taxa, with weak to moderate support (fig. 1). A broadly eastern Australian clade of *Wurmbea* is also well supported and contains all taxa sampled from South Australia (which constitute a well-supported subclade), plus all taxa from New South Wales and New Zealand (another well-supported subclade). Relationships among four distinct *Wurmbea* lineages in western Australia are unresolved and poorly supported: (1) *W. murchisoniana*; (2) *W. inframediana + W. pygmaea + W. drummondii + W. tubulosa*; (3) *W. densiflora + W. dilatata + W. tenella*; and (4) *W. monantha + W. sinora + W. dioica* ssp. *alba*. Clades 2 and 3 are well supported; clade 4 is moderately supported.

Inference of Ancestral States

The resolved best tree shown in figure 2 includes all nodes in the best ML tree and one possible set of resolutions of the four polytomies in the best ML tree (fig. 1). No internal nodes on the resolved best ML tree for *Wurmbea* have significantly supported ancestral-state reconstructions (fig. 2). In large part, this appears to be because the 10 dimorphic taxa (terminals) in our taxonomic sample are widely interspersed among monomorphic Australian taxa. All but one of the major well-supported subclades within eastern and western Australia (see above) contain both dimorphic and monomorphic taxa. Deletion of the African clade of *Wurmbea* had no effect on the lack of significant reconstructions (data not shown).

Logically, the interspersion of dimorphic and monomorphic taxa must require multiple origins and/or losses of gender dimorphism. Mesquite does not provide an estimate of the ML number of transitions, but lower bounds to the number of transitions can be estimated using MP. The MP estimate should be close to the minimum possible number of transitions, because it permits at most one state change per branch to explain the observed distribution of character states; the true number of changes may be higher. The MP estimate of the range of total transitions across the 10,000 alternative resolutions of the weakest parts of the tree (as revealed by ML bootstrap analysis) is five to eight. This spans zero to eight gains versus zero to eight losses of gender dimorphism. The same ranges are obtained for reconstructions made across *Wurmbea* as a whole and for Australasian *Wurmbea* alone. This indicates that there were frequent evolutionary transitions in the sexual systems of *Wurmbea*.

As stated above, the resolved best tree has no significantly supported ancestral-state reconstructions (fig. 2). We explored whether any nodes on the resolved best tree might have significant ancestral reconstructions, given a large sampling of alternate resolutions of eight nodes—the four original polytomous nodes, plus the additional four nodes with relatively weak (<70%) ML bootstrap support, which were collapsed for this analysis. Below, we describe the outcome of these analyses. As a rule of thumb, we consider an assignment of an ancestral state in these reconstructions noteworthy if two conditions are met: (1) the node is present in all of the sampled alternate resolutions (indicated by "10,000" in the second column of table 1) and (2) it is assigned a significant ancestral state in the majority (>50%) of these 10,000 resolutions (indicated by values >50% in cols. A–F of table 1; see Miadlikowska and Lutzoni 2004).

First, we address the tree resolutions where the collapsed branches are resolved and assigned very short lengths (cols. A and D in table 1). Of all nodes that are strongly supported in the best ML tree (i.e., present in all 10,000 resolutions; second column in table 1), significant inferences of ancestral state were assigned in less than 5% of the sampled resolutions (all values for these nodes are ≤4.1 in cols. A and D of table 1). In only one case, which represents a random resolution of a polytomy in the best tree (node q; fig. 2; table 1), is there unambiguous assignment of an ancestral state in more than 50% of cases (underlined numbers in col. A of table 1); however, this node is present in only 1126 of the 10,000 sampled resolutions (second column in table 1), and this level of significant ancestral-state assignments is seen only in trees that include African *Wurmbea* (cf. cols. A and D in table 1).

When branch-length information is ignored (i.e., all branches are assumed to be equal; cols. B and E in table 1), the proportion of resolutions with significant ancestral states is generally higher than when inferred branch lengths are considered (cf. cols. A and B, D and E in table 1). However, the improvement is modest; reconstructions are still ambiguous in most cases. Only three strongly supported nodes (i, w, and y; fig. 2; table 1) are significantly assigned a state in >50% of the sampled resolutions (monomorphism in all three cases; underlined numbers in col. B in table 1). Of these, only i is unambiguous when Australian *Wurmbea* are considered alone (underlined numbers in col. E in table 1).

When zero-length branches in the 10,000 resolutions of the best tree are artificially inflated to the median branch length, the significance of ancestral-state reconstructions is also generally higher (cf. cols. A vs. C and D vs. F in table 1). However, short branch lengths cannot be the primary explanation for treewide uncertainty, because after branch-length inflation, most tree resolutions still do not result in significant assignments (i.e., most values in cols. C and F in table 1 are
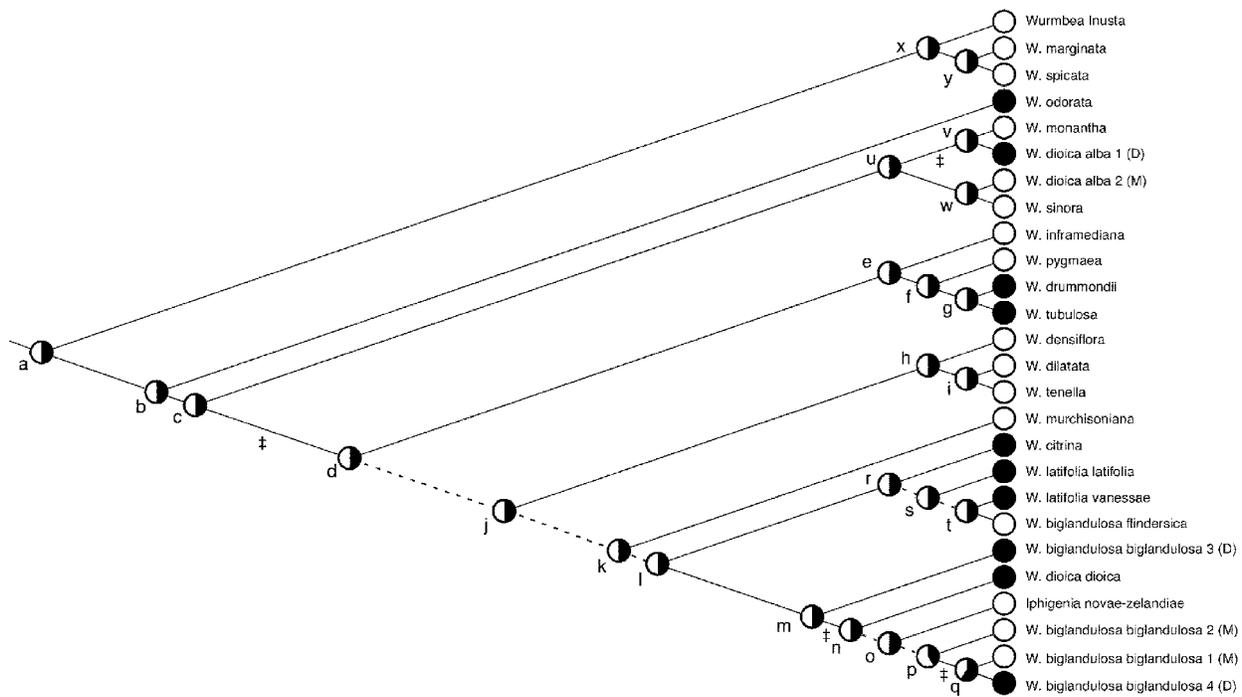
Fig. 2 Maximum likelihood ancestral-state reconstruction of sexual-system evolution on the shortest tree shown in fig. 1, with four polytomies arbitrarily resolved (the "resolved best" tree; see text). Branch lengths are assumed to be proportional to inferred DNA changes (fig. 1) but are presented as nonproportional lengths. Dashed lines denote the branches that collapse to polytomies in fig. 1; daggers indicate branches with <70% ML bootstrap support. The character reconstruction is limited to the genus *Wurmbea* (including *Iphigenia novae-zelandiae*). State inferences at nodes are represented as proportional likelihoods, shown as pie charts. The marginal probability reconstruction with model Mk1 in Mesquite has a rate of 760,745 ($-\ln L = 18.03$). Gender dimorphism is indicated in black, gender monomorphism in white. No nodes have significant ancestral-state assignments. Multiple populations sampled within *Wurmbea dioica* ssp. *alba* and *Wurmbea biglandulosa* ssp. *biglandulosa* are distinguished using numbers and the letters *M* and *D* (for monomorphic vs. dimorphic populations).

low) most of the time (i.e., most nodes have low values in cols. C and F in table 1).

A further indication of uncertainty is evident for several nodes (e.g., c, m, and r) that have significant reconstructions of *both* sexual-system states in at least some tree resolutions (occurrences of both M and D in cols. A–F in table 1). None of these findings is substantially affected by inclusion or exclusion of African *Wurmbea* from the reconstructions (cf. cols. A–C vs. D–F in table 1), although the proportion of nodes with high levels of significance is generally higher with these taxa included (cols. A–C in table 1).

Note that in order to pinpoint a transition between sexual systems with confidence, we would need to detect a change in sexual states at consecutive nodes in which each has a significant inference of ancestral state. Although we detected significant reconstructions in >50% of the sampled resolutions in a few instances (see underlined values in table 1), these particular branches are all terminal or near-terminal, and most do not indicate transitions between monomorphism and dimorphism because the inferred ancestral state in each case is identical to the states of the terminals it subtends (nodes g, i, w, y; see fig. 2). Nodes q and t provide evidence of transitions in some reconstructions, predicting a recent reversal from D to M in *Wurmbea biglandulosa* ssp. *biglandulosa* (population M1) and *W. biglandulosa* ssp. *flindersica*, respectively (fig. 2; table 1).

Species Nonmonophyly

Within the ingroup, we found evidence for nonmonophyly of two species containing both monomorphic and dimorphic populations (*Wurmbea dioica* and *W. biglandulosa*). When the monophyly of *W. dioica* as a whole is enforced, the score of the constrained tree is significantly higher than that of the best unconstrained tree (difference in $-\ln L = 111.92$; $P < 0.001$); however, when the two populations of *W. dioica* ssp. *alba* sampled here are constrained to form a small clade, this does not result in a significant increase in tree length (difference in $-\ln L = 4.03$; $P = 0.785$). *Wurmbea biglandulosa* is also not monophyletic in our analyses, but forcing the four sampled populations of *W. biglandulosa* ssp. *biglandulosa* together as a clade did not result in a significant increase in tree length (difference in $-\ln L = 5.56$; $P = 0.81$). *Wurmbea biglandulosa* ssp. *flindersica* is well supported as the sister group of *Wurmbea citrina* and *Wurmbea latifolia*, and constraining the monophyly of *W. biglandulosa* as a whole resulted in significantly longer trees (difference in $-\ln L = 85.9$; $P < 0.001$).

Discussion

We address the implications of our data for understanding sexual-system evolution in *Wurmbea*. Several strong biogeographic patterns are evident, and our reconstructions indicate

Table 1

Percentage of Nodes with Significant Maximum Likelihood (ML) Assignments of Ancestral States in 10,000 Resolutions of Eight Polytomies in the ''Resolved Best'' ML Tree

Node in figure 2	No. trees with node	All *Wurmbea* species			Australasian *Wurmbea*		
		Shortest branches = .000001 (A)	All branches equal (B)	Shortest branches = median length (C)	Shortest branches = .000001 (D)	All branches equal (E)	Shortest branches = median length (F)
a	10,000				na	na	na
b	10,000			M: .02		D: .1	
c	10,000		M: .8	M: .2		M: .01; D: .02	M: .1
d	1154		M: 5.0	M: .9		M: .7	M: .2
e	10,000		M: 12.3	M: 4.0		M: 5.7	M: 1.4
f	10,000			M: 3.0			M: 1.2
g	10,000	D: 2.1	D: .6	<u>D: 86.2</u>	D: .2		<u>D: 59.6</u>
h	10,000	M: .1	M: 47.8	<u>M: 30.8</u>		M: 25.3	M: 13.6
i	10,000	M: .3	<u>M: 77.9</u>	M: 49.3		<u>M: 50.6</u>	M: 21.8
j	489		<u>M: 5.7</u>	M: .8		M: 2.7	M: .4
k	506		M: 6.5	M: 1.6		M: 2.4	M: 1.0
l	1092		M: 1.7	M: 1.2; D: 1.3		M: .5	M: .2; D: .4
m	10,000	M: .7; D: 1.2	M: 6.5; D: .4	M: 10.5; D: 7.2	M: .3; D: .8	M: 3.7; D: .3	M: 7.8; D: 5.3
n	1099	D: .3	D: 2.5	M: .7; D: 7.7		D: 1.8	M: .3; D: 6.7
o	500		M: .2	M: .8; D: 14.4			D: 7.4
p	473		M: .4	M: 1.3			
q	1126	<u>D: 57.2</u>		M: .1	D: 29.3		
r	10,000	D: 4.1	D: .9	M: .03; D: 38.3	D: 3.6	D: .8	D: 26.4
s	2002	D: 3.6	D: 1.6	<u>D: 58.8</u>	D: 3.4	D: 1.2	D: 36.1
t	1911	D: 4.0		<u>D: 63.9</u>	D: 3.5		D: 34.7
u	10,000		M: 4.9	<u>M: .7</u>		M: 1.6	M: .1
v	3288			M: .2			
w	10,000	M: .4	<u>M: 57.7</u>	M: 43.9	M: .03	M: 34.3	M: 19.0
x	10,000		<u>M: 20.3</u>	M: 2.0	na	na	na
y	10,000	M: 3.0	<u>M: 75.5</u>	<u>M: 88.8</u>	na	na	na

Note. The eight resolved polytomies comprise the four original polytomies plus four branches with <70% bootstrap support collapsed post hoc (fig. 2). Lowercase letters correspond to nodes present in this tree, which shows one possible resolution of the four original polytomies seen in figure 1. Numbers in the second column indicate the number of resolutions in which a node was observed. The last six columns (A–F) represent reconstructions for each of two taxon sets (with analyses limited either to *Wurmbea* as a whole [A–C] or to Australasian *Wurmbea* alone [D–F]) but considering branch lengths in three different ways. The percentage of cases with a particular state at a node is noted in columns A–F (M = monomorphic; D = dimorphic); underlined numbers indicate cases for which at least 50% of the resolved trees had a significant ancestral state. Analyses were performed with zero-length branches assigned a very short length (A, D), with all branches assigned equal lengths (B, E), or with all zero-length branches assigned the median branch length within *Wurmbea* (C, F). na = not applicable.

that sexual systems in the Australian clade are evolutionarily labile. However, we were unable to pinpoint where sexual-system transitions occurred with any statistical confidence. We address the source of this uncertainty and discuss how the nonmonophyly of individual species may further complicate attempts to make inferences about the evolution of gender strategies using phylogenies. The inference-related problems that we encountered are unlikely to be restricted to *Wurmbea* and may be commonplace (but poorly recognized) in morphologically variable groups with a recent origin or in those that contain wide-ranging polymorphic taxa with evolutionarily labile traits.

Geography and Phylogeny

Species of *Wurmbea* tended to be geographically clustered within well-supported clades and subclades. The existence of African and Australasian clades received strong statistical support, as did an eastern Australian/New Zealand subclade. The relationships among western Australian subclades and of these to the eastern Australian/New Zealand subclade were poorly supported. However, there was weak support for the eastern subclade being nested in a western Australian grade. This arrangement, if correct, is consistent with migration and diversification eastward from the coast of western Australia. The Australasian clade of *Wurmbea* is nested in a larger African grade that includes the African clade of *Wurmbea* (mostly from the Cape Province of South Africa), a grade of *Onixotis* (two of three species in the genus are sampled here, all from the Cape Province), and *Baeometra uniflora* (the sole species in this genus, native to the Cape Province but collected in western Australia). Vinnersten and Manning (2007) recognize *Onixotis* and *Neodredgea* as part of *Wurmbea*; the former genus is paraphyletic with respect to *Wurmbea*, and the latter is monotypic and sister to *Onixotis* + *Wurmbea* s.s. (Vinnersten and Reeves 2003). Arguably, *Baeometra* might also

be recognized under *Wurmbea* in a broader sense (*Wurmbea* would have taxonomic priority in this situation) because it is monotypic and sister to all of these taxa (see Backlund and Bremer 1998 for a rationale). Regardless of the classification used, the taxa that we accept as *Wurmbea* are clearly nested in a grade of South African taxa. Thus, our results (and those of Vinnersten and colleagues) support a single origin of Australasian *Wurmbea*, following a relatively recent long-distance dispersal event from southern Africa.

Paradoxically, such long-distance dispersal is inconsistent with several features of colchicoid life history. Seed shadows are probably very local in Colchicaceae; most plants have a diminutive stature, and seeds are generally shaken from dry dehiscent capsules. Moreover, aside from the possibility of rafting, fruits and seeds are unlikely to be dispersed by water, and many are ant dispersed (Nordenstam 1978). Although some species of *Wurmbea* have the capacity for limited clonal reproduction, daughter corms remain enclosed within the tunics covering the parent corm and apparently do not disperse readily (Nordenstam 1978; Macfarlane 1980; A. L. Case, personal observation). Hence, the mechanism(s) by which long-distance dispersal was achieved in *Wurmbea*, and in Colchicaceae as a whole, remains an enigmatic problem for biogeographical research (Vinnersten and Bremer 2001). However, this predicted long-distance dispersal was clearly not a unique event in *Wurmbea* biogeographic history, as exemplified by our finding that *Iphigenia novae-zelandiae* dispersed from within the Australian *Wurmbea* clade to the South Island of New Zealand.

Dynamic Evolutionary History of Sexual Systems in Wurmbea

Previous phylogenetic analyses of the evolution of gender strategies have been aimed at identifying independent transitions to evaluate the selective mechanisms responsible for gender dimorphism (e.g., Hart 1985; Wagner et al. 1995; Weller et al. 1995; Soltis et al. 1996; Sakai et al. 1997; Sakai and Weller 1999; Renner and Won 2001; Levin and Miller 2005). These findings, in conjunction with population-level studies, can support hypotheses that specific ecological factors favor transitions from gender monomorphism to dimorphism (reviewed in Ashman 2006; Barrett and Case 2006). If our plastid-based estimate of phylogeny represents species relationships accurately, then there were numerous transitions in sexual system in the Australian clade of *Wurmbea*. This means that the high degree of sexual variation in this clade mirrors high evolutionarily lability of sexual systems. This makes evaluating ecological correlates of dimorphic sexual systems more difficult within a phylogenetic framework, although uncertainty in ancestral states is not necessarily problematic when among-character correlations are assessed using ML analysis (Pagel 1999). Our analyses indicate that there were a minimum of five changes in sexual system, but they do not indicate the balance or direction of these changes. For example, we cannot distinguish whether dimorphism evolved repeatedly within Australia or just once after dispersal to Australia, followed by repeated reversions to monomorphism. Moreover, the possibility that an unknown dimorphic taxon was dispersed from Africa, although it might be considered

less parsimonious (and less probable because it would usually require dispersal of both sexes), is not altogether unlikely, as indicated in the ML reconstructions (see the root node of the Australian taxa in figs. 2, 3).

Reversals from gender dimorphism to monomorphism are not commonly reported and are traditionally thought of as being exceedingly unlikely to occur, particularly in the case of dioecy, which has previously been thought generally irreversible (Bull and Charnov 1985). Bull and Charnov's arguments for the irreversibility of dioecy are probably appropriate for animal systems, but sex expression in flowering plants is subject to very different constraints (e.g., modular body plans, sexual inconstancy). Gender dimorphism has arisen independently from cosexual ancestors in almost half of flowering plant families (Renner and Ricklefs 1995), suggesting that it is not necessarily difficult to evolve. Although there are a few notable exceptions (e.g., *Cotula*, Lloyd 1975; *Fuchsia*, Sytsma et al. 1991; *Mercurialis*, Obbard et al. 2006; *Sagittaria*, Dorken et al. 2002; Cucurbitaceae, Zhang et al. 2006), reversals to cosexuality from gender dimorphism are still typically presumed to be less likely in plants (note, however, that many gender-dimorphic groups that include dioecious species exhibit a continuum of sex expression between unisexuality and hermaphroditism; Delph and Wolf 2005; Ehlers and Bataillon 2007). In the few cases (i.e., nodes) where we detected a high proportion of significant ancestral-state assignments in *Wurmbea*, our inferences of sexual-system transitions indicated reversals from dimorphism to monomorphism. These cases all included a reversal in *Wurmbea biglandulosa* ssp. *flindersica* and often also included reversals in *W. biglandulosa* ssp. *biglandulosa* and *I. novae-zelandiae*. This is not surprising, given the extensive sexual inconstancy within *Wurmbea* species (Barrett et al. 1999; Ramsey and Vaughton 2001; Barrett and Case 2006). It would be interesting to know what features of Australian *Wurmbea* biology make the clade so unusually sexually labile. Comparative microevolutionary studies would be particularly informative, as would broadscale comparative analyses of the ecology and biology of African versus Australian species.

One possible reversal to monomorphism may be associated with the long-distance dispersal event that yielded *I. novae-zelandiae*. Losses of dimorphism have been associated with long-distance dispersal in other plant groups in the Southern Hemisphere (Baker and Cox 1984; Sytsma et al. 1991). Because *I. novae-zelandiae* is nested within a local clade that includes some dimorphic taxa (*Wurmbea dioica* ssp. *dioica* and *W. biglandulosa*), a dimorphic common ancestor could have given rise to it via (or after) dispersal. A scenario in which a polleniferous plant with sex inconstancy from a gender-dimorphic species could have established a monomorphic population is consistent with ecological evidence from several species. First, there is ample evidence of labile sex expression in *Wurmbea*; individual plants with sex inconstancy reproduce as hermaphrodites in some years but as males in others (Barrett et al. 1999; Ramsey and Vaughton 2001). A single founding individual would need to reproduce as a hermaphrodite to establish a monomorphic population. Second, this type of dispersal event would also require self-compatibility for successful establishment. Self-compatibility has been reported in several *Wurmbea* species (Barrett 1992; Ramsey and Vaughton 2002;
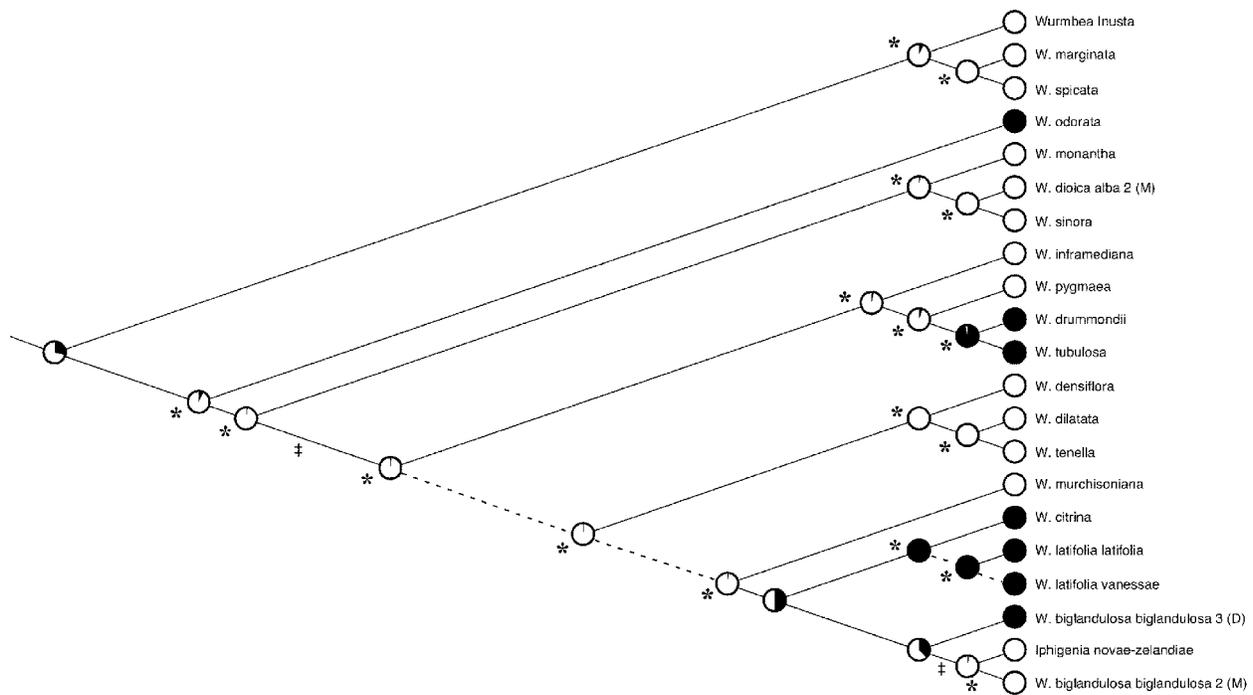
Fig. 3 Sensitivity of maximum likelihood ancestral-state reconstructions of sexual-system evolution to taxon sampling. Five population subsamples were deleted relative to fig. 2 (i.e., *Wurmbea dioica* ssp. *alba* [D], *Wurmbea dioica* ssp. *dioica*, *Wurmbea biglandulosa* ssp. *flindersica*, and *W. biglandulosa* ssp. *biglandulosa* 1 [M] and 4 [D]; see text for details). The character reconstruction is limited to the genus *Wurmbea* (including *Iphigenia novae-zelandiae*); branch lengths are assumed to be proportional to inferred DNA changes (fig. 1) but are presented as nonproportional lengths. Dashed lines denote the branches that collapse to polytomies in fig. 1; daggers indicate branches with <70% ML bootstrap support. State inferences at nodes are represented as proportional likelihoods, shown as pie charts. The marginal probability reconstruction with model Mk1 in Mesquite has a rate of 102.5 ($-\ln L = 12.13$). Gender dimorphism is indicated in black, gender monomorphism in white. The majority of internal nodes (17 out of 20) have significant ancestral-state assignments, indicated by asterisks. Multiple populations sampled within *Wurmbea dioica* ssp. *alba* and *Wurmbea biglandulosa* ssp. *biglandulosa* are distinguished using numbers and the letters *M* and *D* (for monomorphic vs. dimorphic populations).

Vaughton and Ramsey 2003), and the biology of *I. novae-zelandiae* suggests that it is predominantly selfing. Autonomous selfing would aid in the establishment of this lineage after dispersal from Australia (Baker 1967), particularly in the context of the relatively depauperate pollinator fauna of New Zealand (Webb and Kelly 1993).

We have shown that the largest source of uncertainty in the reconstruction of sexual systems in *Wurmbea* is the intermingling of monomorphic and dimorphic taxa across the phylogenetic tree. This interspersion contributes substantially more to mapping uncertainty than does the presence of zero- or near-zero-length branches (or otherwise poorly supported branches; see table 1). Studies of groups with a high degree of gender variation are especially likely to result in ambiguous ancestral-state reconstructions, while reconstructions from only a few dimorphic taxa, where there is less interspersion of sexual systems (e.g., *Lycium*; Levin and Miller 2005), should be more straightforward. In the same way that rapid speciation can obscure phylogenetic signal for determining species relationships, the complex distribution of sexual systems across our *Wurmbea* phylogeny obscures the localization of specific character transitions on the tree.

Taxon Sampling, Short Branches, Uncertain Species Boundaries, and Their Effects on Inferences of Character-State Transitions

Some of the dispersion of dimorphic sexual systems across the tree reflects the dispersed pattern of population terminals of *W. dioica* and *W. biglandulosa*. One or more of the population terminals in these taxa may represent distinct (cryptic) species, which may provide an explanation for at least part of the scattered distribution of *W. dioica* populations on the plastid phylogeny (see Vanderpoorten and Goffinet 2006 for a potentially similar situation in mosses). As currently circumscribed, *W. dioica* has the widest distribution of Australian *Wurmbea* species and a high degree of morphological variation, particularly with regard to floral traits and sex expression. Distinct morphological characters acknowledged in taxonomic treatments of each recognized subspecies of *W. dioica* (see Macfarlane 1980, 1986; Bates 1995) lend credence to the idea that at least some of these represent distinct species, either closely related or not. Indeed, during the preparation of this article, one Australian taxon was elevated to species status (*Wurmbea citrina*) from its previous designation as a subspecies of *W. dioica* (Bates 2007). Trees with *W. citrina* and *W.*

dioica ssp. *dioica* enforced as a sister taxa are significantly longer than our best tree (difference in $-\ln L = 62.0$; $P < 0.025$), supporting this proposal. In contrast to *W. dioica*, there are few obvious morphological or ecological traits in *W. biglandulosa* ssp. *biglandulosa* that distinguish the different populations or sexual morphs, particularly in the northern part of the species range, where our samples were collected (M. Ramsey, personal communication).

It is possible that only one of the population terminals in the case of *W. dioica* or *W. biglandulosa* is the correct placement for either species. For example, chloroplast capture events or failed coalescence might explain at least some of their dispersion across the phylogeny. Different terminals for individual populations of *W. dioica* and *W. biglandulosa* are more closely related to geographically proximal species than to each other, which is consistent with local introgression. Alternatively, these geographic patterns may reflect common descent from geographically proximal ancestors in which at least one member of each descendant species pair retains a more plesiomorphic floral condition and ends up being misclassified (this situation differs from the plesiospecies concept of Olmstead [1995]; in our case the error may be associated with taxonomic definitions that focus on plesiomorphic reproductive characters, whereas Olmstead's potentially genetically cohesive plesiospecies gives rise to isolated apospecies). At present, we cannot distinguish among these scenarios. However, chloroplast capture or failed coalescence may provide the correct explanation for at least some of the dispersion of dimorphic terminals across the tree.

It is worth considering what we might have reconstructed had we included a more limited sampling of populations, as many phylogenetic studies of reproductive transitions involve restricted taxon sampling. We therefore experimented with character reconstructions on trees with reduced taxon sampling, which may incidentally delete misplaced terminals. Because the main goal of our study was to reconstruct sexual-system evolution, we maintained a reasonable density of sampling, including both monomorphic and dimorphic taxa from across Australia in our taxon-deletion experiments. We subsampled taxa from the tree shown in figure 2 and traced sexual-system transitions in Mesquite, using the same procedure as for the full taxon sample, noting changes in the significance of ancestral states at each node. These taxonomic subsamplings could easily have been the samples collected for our study, if there had been a slightly different emphasis to our field collections.

We found that it can take very few terminal deletions to dramatically increase the number of significantly reconstructed ancestral states across the tree, regardless of the short internal branches observed here. For example, in figure 3 we illustrate a taxon subsample that leaves some level of interspersion and two nonmonophyletic taxa. The number of nodes inferred to have a significant ancestral-state change varies substantially— from no ancestral nodes in figure 2 to 85% significant nodes in figure 3 (i.e., 17 of 20 internal nodes). The estimate for the character transition rate for the trace shown in figure 3 (rate = 102.5) is substantially lower than that of the resolved best tree shown in figure 2 (rate = 760,745).

We experimented with different taxon samplings and found that deletion of just one branch has a large effect on the significance of ancestral-state reconstructions. Specifically,

when population D4 or M2 of *W. biglandulosa* ssp. *biglandulosa* is removed from consideration, the number of significant nodes jumps from zero to seven or nine, respectively (these significant nodes are all near-terminal; data not shown). The greater uncertainty in ancestral-state reconstructions when both populations are included is a consequence of the populations having different sexual-system states (dimorphic vs. monomorphic) with essentially no evolutionary distance between them (fig. 1). Because they have different states, a state transition must be inferred; because there is no detectable distance between them, this also implies near-instantaneous change, requiring a substantially higher treewide transition rate with both included (760,745) than with either sample excluded (357.4 or 354.9).

The existence of closely related populations of *W. biglandulosa* with alternate sexual systems contributes to the difficulty in inferring ancestral states. However, it should be emphasized that the overall uncertainty in ancestral-state reconstructions here (i.e., very few nodes with significant ancestral-state reconstructions; table 1) is not a function of high or low numerical values for the overall character transition rate under different treatments of branch length. For example, the resolved best tree (fig. 2) has a transition rate of 248.0 when zero-length branches are replaced with branches of the median length and a rate of only 0.393 when all branches are set to equal length, and yet most reconstructions are still equivocal for all three branch-length scenarios (see table 1). Furthermore, when population M2 of *W. biglandulosa* is deleted and all branch lengths are treated as having unit length, the transition rate falls to 0.4086 from 357.4, but only two nodes are inferred to have significant states (vs. nine when the ML branch lengths are considered). Although branch length affects character reconstructions in a complex way, the biggest source of uncertainty in our reconstructions is not the overall ML rate or the very short branches subtending some taxa, but rather the interspersion of taxa with different character states on the tree (fig. 2; table 1). This interspersion requires frequent transitions in any explanation of character evolution.

Whatever the cause of the dispersion of *W. dioica* and *W. biglandulosa* terminals across the tree, it serves to underline the dramatic effect that the observed interspersion has on character reconstructions. Undoubtedly, both speciation and extinction contribute to the observed patterns of taxa and character states across any phylogeny. The effect of extinction is expected to be comparable to that of incomplete taxonomic sampling, and there is some evidence that dioecious plant lineages have a higher probability of extinction than their hermaphroditic counterparts (Vamosi and Otto 2002). This is difficult to deal with in practice because extinct taxa are usually not observable (unless very good fossil data are available).

Ancestral-State Reconstruction When Species Boundaries Are Unclear

In the analyses described above, we treated each population as if it were a valid terminal for phylogenetic inference. Phylogenetic studies often include at most one or two exemplar specimens per species and tend not to be designed to test the (implicit) assumption that species boundaries are well-defined

or readily definable (e.g., Funk and Omland 2003). In our case, we sampled four and five populations in *W. dioica* and *W. biglandulosa*, respectively, and found clear evidence for the nonmonophyly of both species. This may reflect failure of ancestral polymorphisms to coalesce within a species, chloroplast capture events, or the existence of cryptic species, as described above, or even true species-level paraphyly. These alternate hypotheses cannot be distinguished using the current evidence, and, in general, it is not clear how to deal with these contrasting possibilities in phylogenetic studies of character transitions. Approaches exist for estimating species phylogeny when different terminals from a given species are interspersed on a gene tree, but these work well only if the explanation can be ascribed to a single cause (failed coalescence, for example; Maddison and Knowles 2006). It is clear that whatever the source(s) of real or apparent nonmonophyly is for *W. dioica* and *W. biglandulosa*, there is a considerable need for population genetic, demographic, and taxonomic work to determine the limits and degree of leakiness in species boundaries in these taxa.

The observed uncertainty in species definitions for *W. dioica* and *W. biglandulosa* is problematic for interpreting character reconstructions and for linking these to results from microevolutionary or ecological studies (e.g., Barrett 1992; Vaughton and Ramsey 1998, 2002, 2003, 2004; Case and Barrett 2001, 2004*a*, 2004*b*; Ramsey and Vaughton 2001, 2002). Our sensitivity analysis using altered taxon sampling indicates that if we accept that there are multiple independent terminals within each "species," the level of taxon sampling, which is often strongly affected by sampling logistics in a study, can have a profound effect on ancestral-state reconstruction. This should be of concern for any study in which character reconstructions use only one or a few population samples from widespread, highly polymorphic species. We suggest that this would be a fertile area of investigation for other studies of character evolution in recently evolved organisms. The potential error levels in inferences of ancestral state associated with poor taxon sampling may be greater than those associated with tree uncertainty or other sources of analytical error (e.g., use of MP vs. ML in ancestral-state reconstructions). It is clear that all relevant species should be included in a study, but a strong case can also be made that funding agencies should permit (i.e., support) active field collection of multiple population samples for each species. This would at least provide insights into whether exemplar-based sampling of species (the use of one or a few samples to represent a species) may be providing a biased view of the underlying phylogeny or of reconstructions of character transitions.

The inferred lability of sexual systems in *Wurmbea* is in large part a function of the interspersion of dimorphic terminals of *W. dioica* and *W. biglandulosa* among monomorphic taxa. If the observed placement of different populations of *W. dioica* is a consequence of plastid capture or failed coalescence, this may substantially mislead inference of ancestral states. It is possible that further sampling within Australian *Wurmbea* will reveal additional problems with species definitions. However, including additional unsampled species is unlikely to overturn our main finding from the current gene tree that sexual-system transitions in *Wurmbea* are frequent, because, except for the two remaining species of *W. dioica*, the remaining unsampled taxa appear to be all monomorphic for gender. Wherever these species are located in a better-sampled phylogeny, the various dimorphic terminals will still be substantially interspersed among monomorphic taxa.

It is worth considering how often failure to subsample from across a species' geographic range may miss nonmonophyly of individual species. Two reviews of this phenomenon suggest that this may be a widespread phenomenon (Crisp and Chandler 1996; Funk and Omland 2003), and it seems especially probable in classically defined species that are geographically widespread and that have a high degree of phenotypic variation. Ironically, these kinds of groups, which provide multiple inferences of convergence for macroevolutionary studies (and therefore are most likely to cause problems in ancestral-state reconstructions as a result of character lability), are also those more likely to have been used in microevolutionary studies concerning the mechanisms responsible for evolutionary transitions. In comparisons involving recently evolved taxa, taking full account of the complexity of species boundaries is likely to be a critical problem for evolutionary biologists to resolve before using phylogenies to make accurate inferences about character transitions.

Acknowledgments

Taxon (tribe)	Geographic range	Sexual system	Collection locality	Voucher	GenBank accession numbers			
					*ndh*F	*atp*B-*rbc*L	*trn*LF	*psb*BTNH
Wurmbea (Anguillareae), Australia:								
W. biglandulosa ssp. biglandulosa	NSW, Vic, ACT	M (1)	Rocky River, NSW	MR1	EU044643	EU044623	EU044698	EU044683
W. biglandulosa ssp. biglandulosa	NSW, Vic, ACT	M (2)	Mt. Yarrowyck, NSW	MR2	EU044644	EU044618	EU044699	EU044681
W. biglandulosa ssp. biglandulosa	NSW, Vic, ACT	D (3)	Warrumbungle, NSW	MR3	EU044645	EU044621	EU044700	EU044680
W. biglandulosa ssp. biglandulosa	NSW, Vic, ACT	D (4)	Gooniwigall, NSW	MR4	EU044646	EU044620		EU044682
W. biglandulosa ssp. flindersica	SA	M	Mt. Remarkable, SA	AC9	EU044642	EU044598	EU044701	EU044667
W. citrine	SA	D	Quorn, SA	AC34	EU044639	EU044596	EU044695	EU044664
W. dioica ssp. alba	SA	M	Brookton, WA	AC53	EU044633	EU044601	EU044705	EU044671
W. dioica ssp. alba	WA	D	Eneabba, WA	AC38	EU044634	EU044600	EU044704	EU044670
W. dioica ssp. dioica	SA, Vic, NSW, ACT	D	Burrenjuck Dam, NSW	AC13	EU044647	EU044594	EU044693	EU044662
W. densiflora	WA	M	Murchison, WA	AC80	EU044632	EU044610	EU044711	EU044677
W. dilatata	WA	M	Kalbarri, WA	AC3	EU044637	EU044608	EU044712	EU044678
W. drummondii	WA	D	Duck Pool, WA	AC17	EU044636	EU044606	EU044709	EU044675
W. inframediana	WA	M	Overlander, WA	AC75	EU044638	EU044605	EU044708	EU044674
W. latifolia ssp. latifolia	SA	D	Barunga Gap, SA	AC25	EU044640	EU044597	EU044696	EU044665
W. latifolia ssp. vanessae	SA	D	Williamston, SA	AC29	EU044641	EU044599	EU044697	EU044666
W. monantha	WA	M	Windy Harbour, WA	AC22	EU044652	EU044602	EU044702	EU044668
W. murchisoniana	WA	M	Murchison, WA	AC2	EU044635	EU044604	EU044703	EU044669
W. odorata	WA	D	Coral Bay, WA	AC68	EU044649	EU044611	EU044707	EU044673
W. pygmaea	WA	M	Helena Valley, WA	AC77	AF547012	AY699143	EU044714	AY465584
W. sinora	WA, SA	M	Venus Bay, SA	AC28	EU044650	EU044603	EU044706	EU044672
W. tenella	WA	M	Murchison, WA	AC78	EU044631	EU044609	EU044713	EU044679
W. tubulosa	WA	D	Guranu, WA	AC72	EU044651	EU044607	EU044710	EU044676
Wurmbea (Anguillareae), Africa:								
W. inusta	South Africa	M	Strand-Gordons Bay, South Africa	LKJ1		EU044614	EU044692	EU044661
W. marginata	South Africa	M	Darling, South Africa	LKJ2		EU044612	EU044690	EU044659
W. spicata var. spicata	South Africa	M	Little Lions Head, South Africa	LKJ3		EU044613	EU044691	EU044660
Outgroups (tribe or family):								
Baeometra uniflora (Baeometreae)	South Africa	M	Lesmurdie, WA	AC24	EU044628	EU044622	EU044687	EU044656
Burchardia multiflora	WA	M	Helena Valley, WA	AC81	EU044625	EU044616	EU044684	EU044653
Gloriosa superba (Iphigenieae)	Africa, Asia	M	Cultivated, Toronto, ON	AC82	EU044626	EU044617	EU044686	EU044655
Iphigenia indica (Iphigenieae)	Asia, WA, NT, Qld	M	India	MWC1028		EU044593	EU044685	EU044654
Iphigenia novae-zelandiae (Iphigenieae)	NZ	M	Southland, NZ	AC83	EU044648	EU044595	EU044694	EU044663
Onixotis punctata (Anguillarieae)	South Africa	M	Dutoitskloof, South Africa	LKJ4	EU044629	EU044619	EU044688	EU044657
Onixotis stricta (Anguillarieae)	South Africa	M	Cultivated, Canberra, ACT	AC16	EU044630	EU044615	EU044689	EU044658
Colchicum autumnale (Colchicaceae)	…	M	Cultivated, Toronto, ON	AC84	EU044627	EU044624		

Note. Sexual systems are either monomorphic (M) or dimorphic (D) for gender. Locality and range abbreviations are Western Australia (WA), South Australia (SA), New South Wales (NSW), Victoria (Vic), Australian Capital Territory (ACT), Northern Territory (NT), Queensland (Qld), and New Zealand (NZ). Vouchers for all WA species are deposited in the Western Australian Herbarium, South Perth (PERTH); the voucher for *Iphigenia indica* is lodged at the Royal Botanic Gardens, Kew (K); all other vouchers are in the Vascular Plant Herbarium, Royal Ontario Museum, Toronto (TRT).

Literature Cited

Ashman T-L 2006 The evolution of separate sexes: a focus on the ecological context. Pages 204–222 in LD Harder, SCH Barrett, eds. The ecology and evolution of flowers. Oxford University Press, Oxford.

Backlund A, K Bremer 1998 To be or not to be: principles of classification and monotypic families. Taxon 47:391–400.

Baker HG 1967 Support for Baker's Law—as a rule. Evolution 21:853–856.

Baker HG, PA Cox 1984 Further thoughts on islands and dioecism. Ann Mo Bot Gard 71:244–253.

Barrett SCH 1985 Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Pontederiaceae). Biol J Linn Soc 25:41–60.

——— 1992 Gender variation and the evolution of dioecy in *Wurmbea dioica* (Liliaceae). J Evol Biol 5:423–444.

Barrett SCH, AL Case 2006 The ecology and evolution of gender strategies in plants: the example of Australian *Wurmbea* (Colchicaceae). Aust J Bot 54:417–433.

Barrett SCH, AL Case, GB Peters 1999 Gender modification and resource allocation in subdioecious *Wurmbea dioica* (Colchicaceae). J Ecol 87:123–137.

Bates RJ 1995 The species of *Wurmbea* (Liliaceae) in South Australia. J Adelaide Bot Gard 16:33–53.

——— 2007 A review of South Australian *Wurmbea* (Colchicaceae ∼ Liliaceae): keys, new taxa and combinations, and notes. J Adelaide Bot Gard 21:75–81.

Bollback JP 2006 SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88.

Bull JJ, EL Charnov 1985 On irreversible evolution. Evolution 39:1149–1155.

Case AL 2000 The evolution of combined versus separate sexes in *Wurmbea* (Colchicaceae). PhD diss. University of Toronto.

Case AL, SCH Barrett 2001 Ecological differentiation of combined and separate sexes of *Wurmbea dioica* (Colchicaceae) in sympatry. Ecology 82:2601–2616.

——— 2004a Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia. Evol Ecol 18:145–164.

——— 2004b Floral biology of gender monomorphism and dimorphism in *Wurmbea dioica* (Colchicaceae) in Western Australia. Int J Plant Sci 165:289–301.

Chase MW, MR Duvall, HG Hills, JG Conran, AV Cox, LE Eguiarte, J Hartwell, et al 1995 Molecular phylogenetics of Lilianae. Pages 109–137 in PJ Rudall, PJ Cribb, DF Cutler, CJ Humphries, eds. Monocotyledons: systematics and evolution. Royal Botanic Gardens, Kew.

Conran JG 1985 Family distributions in the Liliiflorae and their biogeographical implications. J Biogeogr 22:1023.

Crisp MD, GT Chandler 1996 Paraphyletic species. Telopea 6:813–844.

Cummings MP, SA Handley, DS Myers, DL Reed, A Rokas, K Winka 2003 Comparing bootstrap and posterior probability values in the four-taxon case. Syst Biol 52:477–487.

Cunningham CW, KE Omland, TH Oakley 1998 Reconstructing ancestral character states: a critical reappraisal. Trends Ecol Evol 9:361–366.

Dahlgren RMT, HT Clifford, PF Yeo 1985 The families of the monocotyledons: structure, evolution, and taxonomy. Springer, New York.

Delph LF, DE Wolf 2005 Evolutionary consequence of gender plasticity in genetically dimorphic breeding systems. New Phytol 166:119–128.

Dorken ME, J Friedman, SCH Barrett 2002 The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). Evolution 56:31–41.

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

Edwards AWF 1972 Likelihood. Cambridge University Press, Cambridge.

Ehlers BK, T Bataillon 2007 "Inconstant males" and the maintenance of labile sex expression in subdioecious plants. New Phytol 174:194–211.

Erixon P, B Svennblad, T Britton, B Oxelman 2003 Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst Biol 52:665–673.

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

Ferrer MM, SV Good-Avila 2007 Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. New Phytol 173:401–414.

Fitch WM 1971 Towards defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406.

Friedman J, SCH Barrett 2008 A phylogenetic analysis of the evolution of wind pollination in the angiosperms. Int J Plant Sci 169:49–58.

Funk DJ, KE Omland 2003 Species-level paraphyly and polyphyly: frequency, causes, and consequences with insights from animal mitochondrial DNA. Annu Rev Ecol Evol Syst 34:397–423.

Galley C, HP Linder 2007 The phylogeny of the *Penaschistis* clade (Danthonioideae, Poaceae) based on chloroplast DNA, and the evolution and loss of complex characters. Evolution 61:864–884.

Geber MA, TE Dawson, LF Delph, eds 1999 Gender and sexual dimorphism in flowering plants. Springer, New York.

Givnish TJ, JC Pires, SW Graham, MA McPherson, LM Prince, TB Patterson, HS Rai, et al 2005 Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms a priori predictions: evidence from an *ndh*F phylogeny. Proc R Soc B 272:1481–1490.

Gleiser G, M Verdú 2005 Repeated evolution of dioecy from androdioecy in *Acer*. New Phytol 165:633–640.

Goldblatt P 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. Ann Mo Bot Gard 65:369–436.

Goldman N, JP Anderson, AG Rodrigo 2000 Likelihood-based tests of topologies in phylogenetics. Syst Biol 49:652–670.

Graham SW, SCH Barrett 2004 Phylogenetic reconstruction of the evolution of stylar polymorphisms in *Narcissus* (Amaryllidaceae). Am J Bot 91:1007–1021.

Graham SW, JR Kohn, BR Morton, JE Eckenwalder, SCH Barrett 1998 Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. Syst Biol 47:545–567.

Graham SW, RG Olmstead 2000 Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. Am J Bot 87:1712–1730.

Graham SW, PA Reeves, ACE Burns, RG Olmstead 2000 Microstructural changes in noncoding chloroplast DNA: interpretation, evolution, and utility of indels and inversions in basal angiosperm phylogenetic inference. Int J Plant Sci 161(suppl):S83–S96.

Graham SW, JM Zgurski, MA McPherson, DM Cherniawsky, JM Saarela, EFC Horne, SY Smith, et al 2006 Robust inference of monocot deep phylogeny using an expanded multigene plastid data set. Pages 3–20 in JT Columbus, EA Friar, JM Porter, LM Prince, MG Simpson, eds. Monocots: comparative biology and evolution (excluding Poales). Rancho Santa Ana Botanic Garden, Claremont, CA.

Guindon S, O Gascuel 2003 A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704.

Hart JA 1985 Evolution of dioecism in *Lepechinia* Willd. sect. Parviflorae (Lamiaceae). Syst Bot 10:134–146.

Hartigan JA 1973 Minimum mutation fits to a given tree. Biometrics 29:53.

Heilbuth JC 2000 Lower species richness in dioecious clades. Am Nat 156:221–241.

Huelsenbeck JP, R Nielsen, JP Bollback 2003 Stochastic mapping of morphological characters. Syst Biol 52:131–158.

Huelsenbeck JP, F Ronquist 2001 MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Jones A, M Burd 2001 Vegetative and reproductive variation among unisexual and hermaphroditic individuals of *Wurmbea dioica* (Colchicaceae). Aust J Bot 49:603–609.

Jones RT, AP Martin 2006 Testing for differentiation of microbial communities using phylogenetic methods: accounting for uncertainty of phylogenetic inference and character state mapping. Microb Ecol 52:408–417.

Kohn JR, SW Graham, B Morton, JJ Doyle, SCH Barrett 1996 Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. Evolution 50:1454–1469.

Krahenbuhl M, YM Yuan, P Kupfer 2002 Chromosome and breeding system evolution of the genus *Mercurialis* (Euphorbiaceae): implications of ITS molecular phylogeny. Plant Syst Evol 234:155–170.

Leschen RAB, TR Buckley 2007 Multistate characters and diet shifts: evolution of Erotylidae (Coleoptera). Syst Biol 56:97–112.

Levin RA, JS Miller 2005 Relationships within the tribe Lycieae (Solanaceae): paraphyly *of Lycium* and multiple origins of gender dimorphism. Am J Bot 92:2044–2053.

Lewis LA, PO Lewis 2005 Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). Syst Biol 54:936–947.

Lewis PO 2001 A likelihood approach to estimating phylogeny from discrete morphological character data. Syst Biol 50:913–925.

Lloyd DG 1975 Breeding systems in *Cotula*. IV. Reversion from dioecy to monoecy. New Phytol 74:125–145.

Macfarlane TD 1980 A revision of *Wurmbea* (Liliaceae) in Australia. Brunonia 3:145–208.

——— 1986 Two new species of *Wurmbea* (Colchicaceae or Liliaceae *s. lat.*) from south western Australia. Nuytsia 5:407–413.

——— 1987 *Wurmbea* (Liliaceae). Flora Aust 45:387–405.

Macfarlane TD, SJ van Leeuwen 1996 *Wurmbea saccata* (Colchicaceae), a lepidopteran-pollinated new species from Western Australia. Nuytsia 10:429–435.

Maddison DR, WP Maddison 2001 MacClade, version 4.03. Sinauer, Sunderland, MA.

Maddison WP 1997 Gene trees in species trees. Syst Biol 46:523–536.

Maddison WP, LL Knowles 2006 Inferring phylogeny despite incomplete lineage sorting. Syst Biol 55:21–30.

Maddison WP, DR Maddison 2005 Mesquite: a modular system for evolutionary analysis, version 1.06. http://www.mesquiteproject.org.

Mannen J, FA Natalie, F Ehrendorfer 1994 Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergenic region. Plant Syst Evol 190:195–211.

Miadlikowska J, F Lutzoni 2004 Phylogenetic classification of peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. Am J Bot 91:449–464.

Miller JS 2002 Phylogenetic relationships and the evolution of gender dimorphism in *Lycium* (Solanaceae). Syst Bot 27:416–428.

Mooers AO, D Schluter 1999 Reconstructing ancestor states with maximum likelihood: support for one- and two-rate models. Syst Biol 48:623–633.

Mooers AO, SM Vamosi, D Schluter 1999 Using phylogenies to test macroevolutionary hypotheses of trait evolution in cranes (Gruinae). Am Nat 154:249–259.

Moore LB, E Edgar 1970 *Iphigenia* (Colchicaceae). Flora of New Zealand. II. Indigenous Tracheophyta. Government Printer, Wellington.

Nielson R 2002 Mapping mutations on phylogenies. Syst Biol 51:729–739.

Nordenstam B 1978 The genus *Wurmbea* in Africa except the Cape region. Notes R Bot Gard Edinb 36:211–233.

——— 1986 The genus *Wurmbea* (Colchicaceae) in the Cape region. Opera Bot 87:1–41.

——— 1998 Colchicaceae. Pages 175–185 *in* K Kubitzki, ed. The families and genera of vascular plants. Vol III. Flowering plants, monocotyledons: Lilianae (except Orchidaceae). Springer, New York.

Obbard DJ, SA Harris, RJA Buggs, JR Pannell 2006 Hybridization, polyploidy, and the evolution of sexual systems in *Mercurialis* (Euphorbiaceae). Evolution 60:1801–1815.

Olmstead RG 1995 Species concepts and plesiomorphic species. Syst Bot 20:623–630.

Olmstead RG, JA Sweere 1994 Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481.

Pagel M 1994 Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc R Soc B 255:37–45.

——— 1999 The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst Biol 48:612.

Pagel M, A Meade, D Barker 2004 Bayesian estimation of ancestral characters states on phylogenies. Syst Biol 53:673–684.

Posada D, KA Crandall 1998 MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rambaut A 1998 Se-Al (Sequence Alignment Editor), version $10\alpha1$, computer program and documentation. Department of Zoology, Oxford University, Oxford. http://tree.bio.ed.ac.uk/software/seal/.

Ramsey M, G Vaughton 2001 Sex expression and sexual dimorphism in subdioecious *Wurmbea dioica* (Colchicaceae). Int J Plant Sci 162:589–597.

——— 2002 Maintenance of gynodioecy in *Wurmbea biglandulosa* (Colchicaceae): gender differences in seed production and progeny success. Plant Syst Evol 232:189–200.

Ramsey M, G Vaughton, R Peakall 2006a Does inbreeding avoidance maintain gender dimorphism in *Wurmbea dioica* (Colchicaceae)? J Evol Biol 19:1497–1506.

——— 2006b Inbreeding avoidance and the evolution of gender dimorphism in *Wurmbea biglandulosa* (Colchicaceae). Evolution 60:529–537.

Renner SS, RE Ricklefs 1995 Dioecy and its correlates in the flowering plants. Am J Bot 82:596–606.

Renner SS, HS Won 2001 Repeated evolution of dioecy from monoecy in Siparunaceae (Laurales). Syst Biol 50:700–712.

Ronquist F 2004 Bayesian inference of character evolution. Trends Ecol Evol 19:475–481.

Rudall PJ, KL Stobart, W-P Hong, JG Conran, CA Furness, CG Kite, MW Chase 2000 Consider the lilies: systematics of Liliales. Pages 347–359 *in* KL Wilson, DA Morrison, eds. Monocots: systematics and evolution. CSIRO, Sydney.

Sakai AK, SG Weller 1999 Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. Pages 1–31 *in* MA Geber, TE Dawson, LF Delph, eds. Gender and sexual dimorphism in flowering plants. Springer, New York.

Sakai AK, SG Weller, WL Wagner, PE Soltis, DE Soltis 1997 Phylogenetic perspective on the evolution of dioecy: adaptive radiation in the endemic Hawaiian genera *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae). Pages 455–473 *in* TJ Givnish, KJ Sytsma,

eds. Molecular evolution and adaptive radiation. Cambridge University Press, New York.

Sargent RD, JC Vamosi 2008 The influence of canopy position, pollinator syndrome, and region on evolutionary transitions in pollinator guild size. Int J Plant Sci 169:39–47.

Savolainen VJ, F Manen, E Douzery, R Spichiger 1994 Molecular phylogeny of families related to Celestrales based on *rbc*L 5′ flanking sequences. Mol Phylogenet Evol 3:27–37.

Shimodaira H, M Hasegawa 1999 Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16:1114–1116.

Simmons MP, KM Pickett, M Miya 2004 How meaningful are Bayesian support values? Mol Biol Evol 21:188–199.

Soltis PE, DE Soltis, SG Weller, AK Sakai, WL Wagner 1996 Molecular phylogenetic analyses of the Hawaiian endemics *Schiedea* and *Alsinidendron* (Caryophyllaceae). Syst Bot 21:365–379.

Suzuki Y, GV Glazko, M Nei 2002 Overcredibility of molecular phylogenetics obtained by Bayesian phylogenetics. Proc Natl Acad Sci USA 99:16138–16143.

Swofford DL 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer, Sunderland, MA.

Sytsma KJ, JF Smith, PE Berry 1991 The use of chloroplast DNA to assess biogeography and evolution of morphology, breeding systems, and flavonoids in *Fuchsia* sect. *Skinnera* (Onagraceae). Syst Biol 16:257–269.

Taberlet P, L Gielly, G Pautou, J Bouvet 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109.

Thompson JD, DG Higgins, TJ Gibson 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight-matrix choice. Nucleic Acids Res 22:4673–4680.

Thomson JD, J Brunet 1990 Hypotheses for the evolution of dioecy in seed plants. Trends Ecol Evol 5:11–16.

Vamosi JC, SP Otto 2002 When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. Proc R Soc B 269:1187–1194.

Vamosi JC, SP Otto, SCH Barrett 2003 Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. J Evol Biol 16:1006–1018.

Vanderpoorten A, B Goffinet 2006 Mapping uncertainty and phylo-genetic uncertainty in ancestral character state reconstruction: an example in the moss genus *Brachytheciastrum*. Syst Biol 55:957–971.

Vaughton G, M Ramsey 1998 Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). Oecologia 115:93–101.

——— 2002 Evidence of gynodioecy and sex ratio variation in *Wurmbea biglandulosa* (Colchicaceae). Plant Syst Evol 232:167–179.

——— 2003 Self-compatibility and floral biology in subdioecious *Wurmbea dioica* (Colchicaceae). Aust J Bot 51:39–45.

——— 2004 Dry environments promote the establishment of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae). Evol Ecol 18:323–341.

Vinnersten A 2003 Tracing history: phylogenetic, taxonomic, and biogeographic research in the colchicum family. PhD diss. Uppsala University.

Vinnersten A, K Bremer 2001 Age and biogeography of major clades in Liliales. Am J Bot 88:1695–1703.

Vinnersten A, J Manning 2007 A new classification of Colchicaceae. Taxon 56:171–178.

Vinnersten A, G Reeves 2003 Phylogenetic relationships within Colchicaceae. Am J Bot 90:1455–1462.

Wagner WL, SG Weller, AK Sakai 1995 Phylogeny and biogeography in *Schiedea* and *Alsinidendron* (Caryophyllaceae). Pages 221–258 *in* WL Wagner, VA Funk, eds. Hawaiian biogeography: evolution on a hot spot archipelago. Smithsonian Institution, Washington, DC.

Webb CJ, D Kelly 1993 The reproductive biology of the New Zealand flora. Trends Ecol Evol 8:442–447.

Weiblen GD, RK Oyama, MJ Donoghue 2000 Phylogenetic analysis of dioecy in monocotyledons. Am Nat 155:46–58.

Weller SG, AK Sakai 1999 Using phylogenetic approaches for the analysis of plant breeding system evolution. Annu Rev Ecol Syst 30:167–199.

Weller SG, WL Wagner, AK Sakai 1995 A phylogenetic analysis of *Scheidea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae): implications for the evolution of breeding systems. Syst Bot 20:315–337.

Zhang L-B, MP Simmons, A Kocyan, SS Renner 2006 Phylogeny of the Cucurbitales based on DNA sequences of nine loci from three genomes: implications for morphological and sexual system evolution. Mol Phylogenet Evol 39:305–322.