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**The ecology and evolution of gender strategies in plants:
the example of Australian *Wurmbea* (Colchicaceae)**Spencer C. H. Barrett^{A,C} and Andrea L. Case^{A,B}^ADepartment of Botany, 25 Willcocks St, University of Toronto, Toronto, Ontario, Canada M5S 3B2.^BDepartment of Biological Sciences, Kent State University, Kent, Ohio 44242, USA.^CCorresponding author. Email: barrett@botany.utoronto.ca

Abstract. Angiosperms possess diverse sexual systems, often with different combinations of hermaphroditic, pistillate and staminate flowers. Despite this sexual diversity, most populations are either monomorphic or dimorphic with respect to gender strategy, where gender refers to the relative contribution that individuals make to fitness through female and male function. An important problem in evolutionary biology is to determine how and why variation in gender strategies originates and is maintained. *Wurmbea* (Colchicaceae), a genus of insect-pollinated geophytes, has recently become the focus of ecological and evolutionary studies aimed at understanding these issues. Phylogenetic reconstructions suggest dispersal from Africa to Australia, then New Zealand, and multiple transitions between monomorphic and dimorphic sexual systems within Australia. Microevolutionary studies of *W. dioica* and *W. biglandulosa*, two wide-ranging taxa with monomorphic and dimorphic populations, provide insights into the selective mechanisms governing transitions between sexual systems. Dimorphic populations of these taxa likely comprise independent origins of dimorphism via the gynodioecious pathway by invasion of females into monomorphic populations. Shifts in pollination biology and flower size, and their consequent effects on mating patterns, may have contributed to the evolution of gender dimorphism. Pollinator-mediated selfing and inbreeding depression provide a sufficient fertility advantage for females to be maintained in dimorphic populations. Once dimorphism establishes, increasing gender specialisation is associated with invasion of more arid environments. Inbreeding avoidance, particularly under stressful ecological conditions, is the most likely selective mechanism maintaining gender dimorphism in *Wurmbea*. We conclude our review by suggesting avenues for future research that might provide a more comprehensive picture of the evolution of gender strategies in *Wurmbea*.

Introduction

The evolutionary forces shaping gender strategies in plants have been an active topic of research since Charles Darwin (1877) documented the rich sexual diversity among angiosperm species (Geber *et al.* 1999). Studies of plant gender are concerned with the relative contribution to fitness that individuals make through maternal *v.* paternal investment, and the patterns of gender distribution that characterise sexual systems (Lloyd 1979; Sakai and Weller 1999). Gender strategies in plants are more complex than in most animal groups because of the subdivision of reproductive effort into many flowers and fruits, each of which represents a separate unit of investment. Although most species possess hermaphroditic (perfect) flowers, some also produce unisexual flowers. This variation allows scope for the evolution of diverse sexual systems resulting from different combinations of pistillate, staminate and hermaphroditic flowers at the plant and population levels

(Richards 1996; Webb 1999; Barrett 2002). Modular growth and plasticity in reproductive allocation further complicate gender expression (Cox 1988; Seger and Eckhart 1996; Miller and Diggle 2003). Individuals can modify their sexual condition in response to altered environmental circumstances, resulting in significant ecological and developmental components to gender variation (Lloyd and Bawa 1984; Delph and Wolf 2005).

Despite the complexity of plant sexual diversity, virtually all populations can be classified into two distinct gender strategies—monomorphic or dimorphic (Lloyd 1980). In contrast to the term ‘sex,’ which reflects phenotype, ‘gender’ describes the relative genetic contribution of individuals to the next generation as maternal and paternal parents. Populations with ‘gender monomorphism’ show quantitative (unimodal) variation in gender and usually comprise individuals that produce offspring through both ovules and pollen (co-sexuality). Alternatively, populations with ‘gender

dimorphism' show strong bimodality in gender, with two distinct sexual morphs that function primarily as female or male parents. For example, morphs can be entirely unisexual (dioecy), or one morph can be hermaphroditic and the other female (gynodioecy). Dioecy often evolves from gynodioecy, and 'transitional' populations containing female, male and hermaphroditic plants (sub-dioecy) commonly occur (Lloyd 1976; Delph 1990). Much confusion has occurred in classifying and interpreting sexual systems because of the failure to appreciate the quantitative nature of gender. Moreover, because plants can often change their gender with age or size, the accurate assessment of gender strategies in populations usually requires observations through time, especially in long-lived plants (Policansky 1982; Lloyd and Bawa 1984).

The origin of dioecy from hermaphroditism is a frequent evolutionary transition in flowering plants. Because this change from gender monomorphism to dimorphism has occurred repeatedly during the evolutionary history of the angiosperms, understanding the mechanisms responsible represents an important problem in evolutionary biology. Although dioecy is relatively uncommon (~6% species) it occurs in nearly half of all angiosperm families, including both basal and derived taxa (Renner and Ricklefs 1995; Heilbut 2000). Theoretical models generally identify the following three main factors that need to be considered in the selection of dioecy: the relative fitness of progeny arising from self- *v.* cross-fertilisation in ancestral populations, the optimal allocation of resources to female and male function, and the genetics of sex determination (Charnov 1982; Lloyd 1982; Charlesworth 1999). Although genetic aspects of this transition are reasonably well understood, much less is known about the ecological mechanisms favouring the spread and maintenance of unisexual individuals in populations composed of hermaphroditic plants.

One difficulty in identifying the environmental circumstances favouring the evolution of dioecy is that this transition has occurred under diverse ecological conditions and among groups with contrasting evolutionary histories. Moreover, several different evolutionary pathways are involved in the origin of dioecy depending on the sexual system of ancestral populations (Bawa 1980; Webb 1999). These considerations make it very unlikely that a single causal agent is involved. Recent comparative and phylogenetic studies have established statistical associations between dioecy and several life-history, biogeographical and ecological correlates (Renner and Ricklefs 1995; Sakai and Weller 1999; Vamasi *et al.* 2003). This implies that the origin of dioecy is probably favoured under particular environmental and demographic conditions. Determining the microevolutionary mechanisms responsible for the ecological correlates of dioecy is currently a major challenge for understanding how and why gender dimorphism evolves.

Experimental studies on the selective mechanisms responsible for the evolution of gender dimorphism are most feasible in short-lived herbaceous species in which individual genets are easily identified, cultured and crossed. Taxonomic groups characterised by abundant interspecific, and especially intra-specific, variation in sexual systems are particularly valuable. This variation provides opportunities to examine the role of contemporary ecological factors in guiding evolutionary change. Indeed, variation of this type has been used to great advantage in studies of another important transition between sexual systems—the evolution of predominant selfing from outcrossing (Lloyd 1965; Schoen 1982; Barrett *et al.* 1989; Fishman and Wyatt 1999; Moeller and Geber 2005). Unfortunately, most dioecious species are not amenable to experimental studies as they are often long-lived woody species, or, if herbaceous, frequently large in size with prolific clonal growth. Furthermore, the evolution of dioecy is commonly associated with significant changes to floral traits, resulting in substantial differentiation and reproductive isolation from hermaphroditic ancestors. This divergence limits opportunities for microevolutionary analysis because many morphological and ecological factors are confounded with gender expression. Therefore, in contrast to studies of outcrossers and selfers (reviewed in Jain 1976; Barrett 2003; Goodwillie *et al.* 2005), relatively few closely related groups with gender monomorphism and dimorphism have the necessary prerequisites for ecological and evolutionary analysis.

Here we review research on gender strategies in *Wurmbea*, a Southern Hemisphere genus of perennial herbs. This group has recently been used with some success to investigate questions concerning the ecology and evolution of gender dimorphism in plants. The goal of this review is to highlight progress that has been made on this topic, focusing particularly on Australian species. Our treatment begins with a summary of the natural history of the genus, a necessary background for case studies in ecology and evolution. This is followed by a discussion of the patterns of gender variation and sexual dimorphism that occur within and among species of *Wurmbea*. We then summarise phylogenetic analyses investigating the migratory history and evolution of sexual systems in the genus. Next, we consider how biogeographical and ecological investigations have provided insights into the selective mechanisms responsible for the evolution of gender dimorphism. We conclude by outlining a future research agenda for addressing unresolved questions concerning the evolution of combined *v.* separate sexes in *Wurmbea*.

Natural history of *Wurmbea*

Wurmbea (Anguillaridae, Colchicaceae) is a genus of ~47 species of small, insect-pollinated geophytes, with a relatively even distribution of species between temperate Australia and Africa. The majority of species occur in Western Australia

and the Cape region (Nordenstam 1978, 1986, 1998; Macfarlane 1980, 1986, 1987, 1993; Bates 1995; Macfarlane and van Leeuwen 1996). Our phylogenetic investigations have also uncovered a dispersal event involving a single *Wurmbea* species to the south island of New Zealand (see below). Gender dimorphism occurs only among Australian species of *Wurmbea*; African species and other members of the Colchicaceae possess primarily hermaphroditic flowers and all populations are therefore monomorphic for gender. This distinct biogeographical distribution of gender strategies raises the question as to why dioecism is restricted to the Australian subcontinent, and what features of the African species make their populations resistant to invasion by unisexual plants. The answer to this question is at present unknown.

All species of *Wurmbea* have similar morphological structure and life-form. Plants are small with a simple architecture, resulting in limited morphological variation, particularly of vegetative traits. They spend summer underground as a tunicate corm; when conditions are suitable in early spring following winter rain, plants produce an annual shoot consisting of one basal and two cauline leaves and a cymose spike-like inflorescence. Of the life-history traits commonly associated with dioecy—fleshy fruits, large plant size, climbing growth, woodiness, perenniality, small inconspicuous often white flowers, and pollination by unspecialised pollinators (see Renner and Ricklefs 1995; Sakai and Weller 1999; Vamosi *et al.* 2003)—only the last three conditions characterise *Wurmbea*.

In contrast to vegetative uniformity, the flowers of *Wurmbea* species exhibit a wide range of morphological diversity (Fig. 1). They are sessile, actinomorphic and white, cream, pink or purple in colour. Flower number per inflorescence ranges from 1 to ~40 and most African species possess larger floral displays than Australian species (Fig. 2). Why this difference occurs is unclear. The pollination of Australian species is by flies, small bees and butterflies (e.g. *W. dioica*, Vaughton and Ramsey 1998; Case and Barrett 2004a), although the exceptional *W. saccata* has concealed nectar pouches unique to the genus and may be exclusively lepidopteran pollinated (Macfarlane and van Leeuwen 1996). Several of the small-flowered Australian species (e.g. *W. tenella*, *W. pygmaea*) are probably largely self-pollinating. There is no evidence of self-incompatibility in *Wurmbea*, although only two Australian species have been determined as self-compatible by using experimental pollinations (*W. biglandulosa*, Ramsey and Vaughton 2002; *W. dioica*, Vaughton and Ramsey 2003). Conspicuous nectaries are present near the middle, or at the base, of the six free perianth segments, which are always basally connate. Floral tubes are generally absent in Australian species, *W. tubulosa* being an exception, but are common in African species and are particularly well developed in *W. dolichantha*. Little is known about the

pollination biology of African species; some have distinct scents ranging from vanilla-like (e.g. *W. recurva*) to a more foetid odour (e.g. *W. marginata*, Nordenstam 1986), suggesting the possibility of pollinator-driven chemical diversification. Fruits are dry capsules, containing small black spherical seeds that are commonly shaken from capsules by wind or animals but are generally dispersed close to maternal parents.

The ecology of *Wurmbea* species has not been investigated in detail and there have been no studies of habitat preferences, demography or the physiological ecology of populations (although see Case and Barrett 2001). Reproduction is primarily by seed and most species have limited or no capacity for clonal spread. Populations occur in a wide variety of habitats including open rocky slopes, grasslands and pastures, granite and limestone outcrops, seepage slopes, stream and pool edges, *Eucalyptus* woodlands, and deep desert sands. Several of the African species occur in mountainous regions in the alpine up to 3000 m (e.g. *W. tenuis* ssp. *australis*, Nordenstam 1978). Population size and density vary considerably among species, with some (e.g. *W. dioica*, *W. tenella*) characterised by large and extensive stands containing thousands of individuals. Mass flowering of populations occurs commonly, especially after fire.

Species of *Wurmbea* often grow in sympatry with overlapping flowering times and in some cases this can result in hybridisation (Nordenstam 1986; Vaughton and Ramsey 2002; S. C. H. Barrett and A. L. Case, pers. obs.). Limited cytological data indicate that the basic chromosome number among Australian taxa is $x=10$ or 11, and $x=5$ and 7 in South African taxa (Nordenstam 1986). Polyploidy occurs in *W. dioica*, with apparently diploid and tetraploid populations (Macfarlane 1980). Studies of the relation between polyploidy and sexual systems in *Wurmbea* would be desirable because of the potential associations between polyploidy and the evolution of gender dimorphism (Miller and Venable 2000).

Gender variation and sexual dimorphism

Flowers of Australian *Wurmbea* species can be hermaphroditic, pistillate or staminate, resulting in complex sex expression depending on how they are combined at the plant or population level. This sexual diversity has led to a proliferation of botanical terms in the literature. For example, Macfarlane (1980) described *W. dioica* as dioecious, polygamodioecious, hermaphrodite or androhermaphrodite, whereas Ornduff (1985) described a population of *W. dioica* ssp. *alba* from Western Australia as a 'mixed monoclinous-andromonoecious population containing two morphs.' Patterns of gender variation in plant populations are often continuous and botanical terminology emphasising discrete categories (see table 1 in Sakai and Weller 1999) can be limited by its lack of precision. Sex classes based on morphology preclude measurement of the



Fig. 1. Selected *Wurmbea* species illustrating diversity in floral morphology, flower colour and inflorescence display. (a) *W. monopetala*, (b) *W. marginata*, (c) *W. tubulosa* female, (d) *W. tubulosa* male, (e) *W. spicata*, (f) *W. inusta*, (g) *W.* sp. nov., Thomas River WA, (h) *W. dioica* ssp. *dioica* (female left, male right), (i) *W. pygmaea*, (j) *W. dioica* ssp. *alba* (cosexual), (k) *W. graniticola*, (l) *W. tenella*, (m) *W. biglandulosa* ssp. *biglandulosa* (female). Species a, b, e and f are African species; species c, d and g–m are Australian species.

relative performance of individual plants as female and male parents, an issue of primary interest in ecological and evolutionary investigations.

To clarify patterns of sex expression in the geographically variable species *W. dioica* (now shown to be paraphyletic and composed of diverse lineages, see Fig. 5), Barrett (1992) undertook a quantitative study of gender variation in different parts of the range, employing standardised measures of

phenotypic gender developed by David Lloyd (Lloyd 1979, 1980; Lloyd and Bawa 1984), and now widely employed to describe gender variation in plants. The production of pollen and ovules (or seeds) of individuals relative to the average ratio of expenditure in the population is recorded by counting the number of hermaphroditic, pistillate or staminate flowers from a random sample of individuals. From this data, the standardised phenotypic femaleness (G_p) is

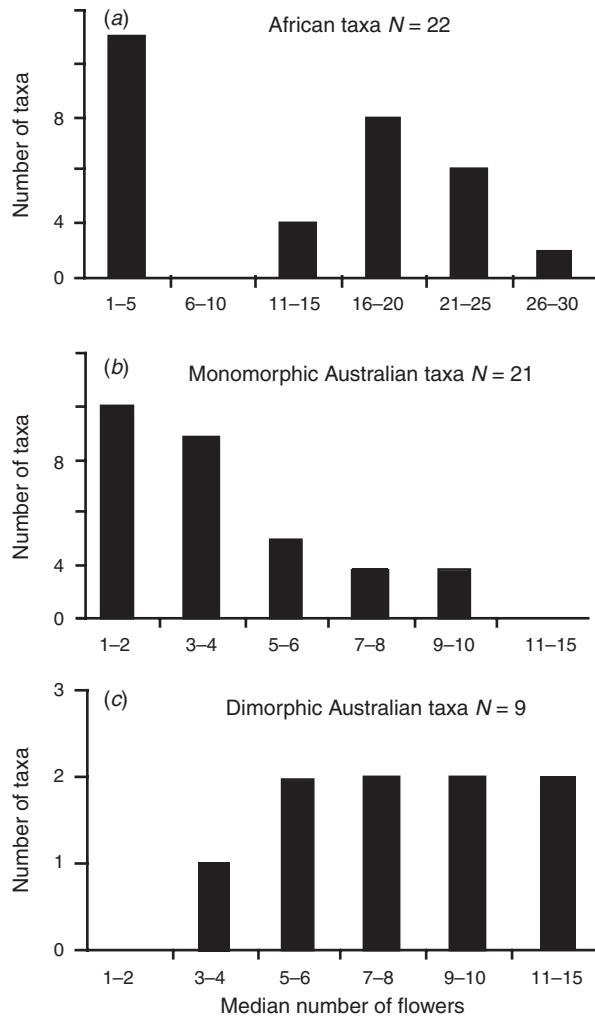


Fig. 2. Variation in flower number per inflorescence among *Wurmbea* species from Australia and Africa. All species from Africa are monomorphic for gender. Data on flower number are from taxonomic monographs by Nordenstam (1978, 1986, 1998) and Macfarlane (1980, 1986, 1987).

determined. Values can range from 0, male plants producing only pollen, to 1, female plants producing only ovules (see Lloyd 1980 for further details).

Barrett's (1992) study distilled sexual variation in *W. dioica* into two fundamental gender classes (Fig. 3g–i), regardless of the number of sexual phenotypes present. Populations of *W. dioica* are monomorphic, if individuals collectively display monotonic variation in gender, or dimorphic, if there are two classes of plants functioning primarily as either female or male parents. Geographical surveys of gender variation in *W. biglandulosa* in south-eastern Australia have recently found similar patterns of gender monomorphism and dimorphism (Vaughton and Ramsey 2002). Indeed, populations of all *Wurmbea* species fall into one or the other of these two gender classes. We

illustrate several representative examples in Fig. 3, with three gender monomorphic taxa (Fig. 3a–c), monomorphic and dimorphic populations of *W. biglandulosa* (Fig. 3d–f) and *W. dioica* (Fig. 3g–i), and three taxa with gender dimorphism (Fig. 3j–l).

In populations of *W. tenella* (Fig. 3a) all plants produce exclusively hermaphroditic flowers, and have G_p values of 0.5. However, in *W. murchisoniana* (Fig. 3b) and *W. densiflora* (Fig. 3c), deviations from $G_p = 0.5$ in the gender plots are evident. These deviations can occur for two reasons. Most commonly, distal flowers on inflorescences are staminate rather than hermaphroditic (e.g. *W. biglandulosa*, Vaughton and Ramsey 2002; Fig. 3d, e). In *W. dioica* ssp. *alba*, plants with this condition were termed the 'andromonecious morph' by Ornduff (1985) and considered distinct from plants with exclusively hermaphroditic flowers. Less often, very small plants may produce only staminate flowers. This has been reported in *W. dioica* subsp. *alba* (Barrett 1992; Fig. 3h) and in *W. biglandulosa* (Vaughton and Ramsey 2002; Fig. 3e). In *W. biglandulosa*, transfer of these plants to the glasshouse resulted in an increase in size and, in subsequent years, the production of hermaphroditic flowers. Rather than consider these two situations as involving distinct gender morphs, we believe it makes more sense to accommodate all three sexual phenotypes ('male', 'andromonecious', 'hermaphrodite') as part of the continuum of gender variation expressed by co-sexual plants in monomorphic populations. In many species, this variation is size-dependent and associated with different reproductive costs to female and male function (Lloyd and Bawa 1984; Sarkissian *et al.* 2001).

In populations of some dimorphic *Wurmbea* species (e.g. Fig. 3f, h, j, k) not all plants are exclusively female or male, as in the strictly dioecious populations illustrated in Fig. 3i, l. This commonly occurs in species where dioecy has evolved via the gynodioecy pathway and populations contain female, male and hermaphrodite individuals. These populations are referred to as gynodioecious or subdioecious, depending on the relative frequencies of the three sexual phenotypes (Lloyd 1976; Delph and Wolf 2005). Investigations of species with this pattern of gender variation commonly reveal that individuals bearing hermaphroditic flowers are actually male plants with inconstant or labile sex expression ('fruiting males', see Delph and Wolf 2005). For example, in subdioecious populations of *W. dioica* ssp. *dioica* comparisons of flower size among the three phenotypes indicate that male and hermaphrodite plants have similar-sized flowers whereas female plants possess substantially smaller flowers (Barrett *et al.* 1999; Jones and Burd 2001; Ramsey and Vaughton 2001). Demographic studies of marked plants of *W. dioica* ssp. *dioica* demonstrated that females exhibit canalised sex expression producing only pistillate flowers, whereas maternal investment in male plants was labile from year-to-year and size-dependent (Fig. 4; Barrett *et al.* 1999). In a 3-year glasshouse study of *W. dioica*,

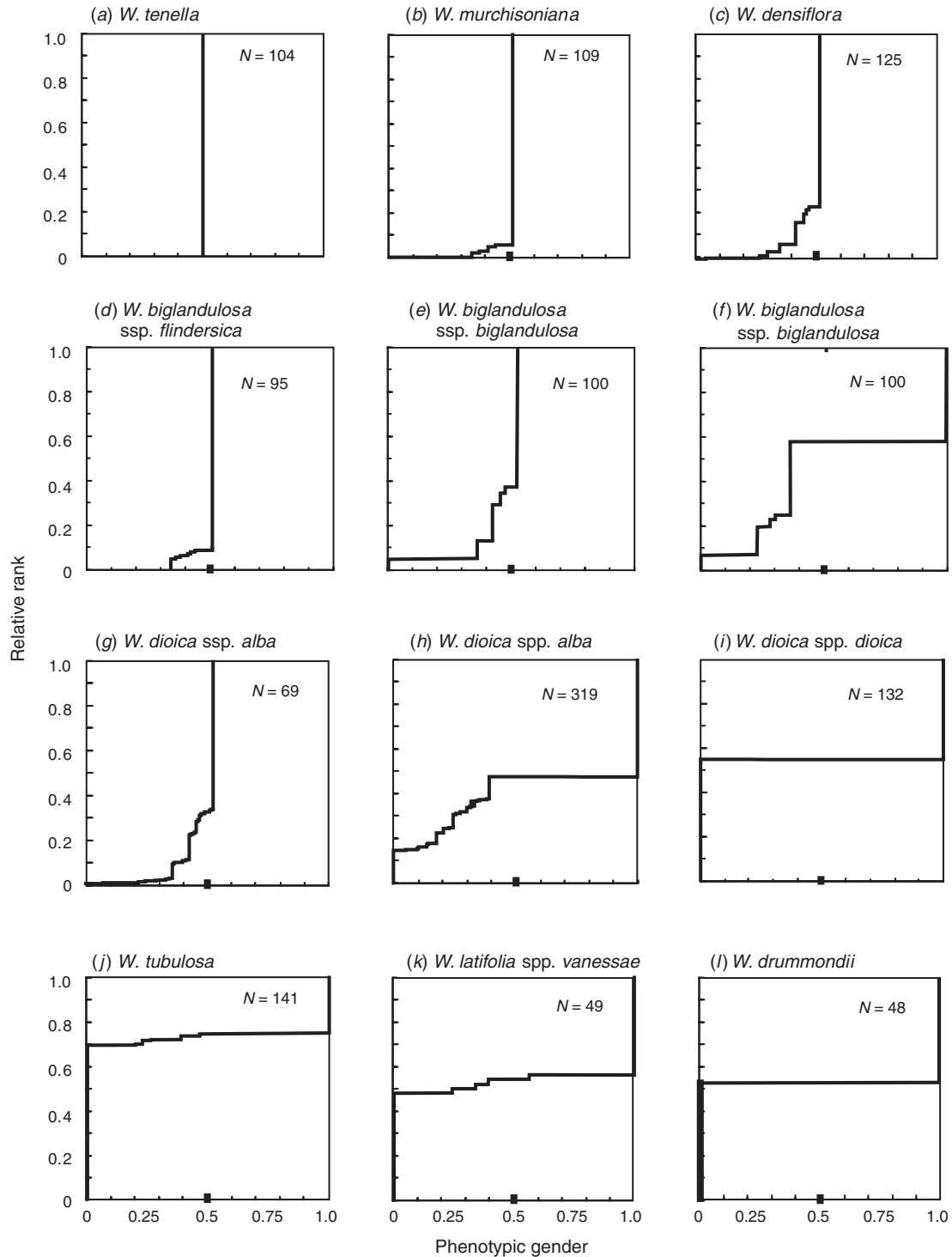


Fig. 3. Representative plots illustrating variation in phenotypic gender among populations of *Wurmbea* species. Each plot is the relative rank of standardised phenotypic femaleness (G_p) for a sample of plants obtained from each population (0 = male, 1 = female). Sample sizes are indicated. See Lloyd (1980) for details of the calculation of G_p . (a) *W. tenella*, (b) *W. murchisoniana*, (c) *W. densiflora*, (d) *W. biglandulosa* ssp. *flindersica*, (e) *W. biglandulosa* ssp. *biglandulosa*, Tenterfield population, after Vaughton and Ramsey (2002), (f) *W. biglandulosa* ssp. *biglandulosa*, Moonbi population, after Vaughton and Ramsey (2002), (g) *W. dioica* ssp. *alba*, (h) *W. dioica* ssp. *alba*, (i) *W. dioica* ssp. *dioica*, (j) *W. tubulosa*, (k) *W. latifolia* ssp. *vanessae*, (l) *W. drummondii*. Plots a–e, g are populations with gender monomorphism, the remaining populations exhibit gender dimorphism.

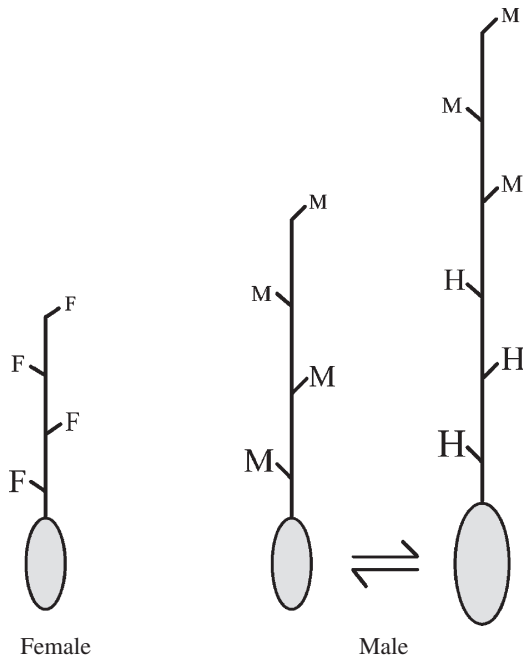


Fig. 4. Subdioecious populations of *Wurmbea dioica* ssp. *dioica* are polymorphic for size-dependent gender modification. Populations contain three sex phenotypes, female, male and hermaphrodite. Females exhibit canalised sex expression producing only pistillate flowers (F). In contrast, some males produce only staminate flowers (M) when they are small and a mixture of staminate and hermaphroditic flowers (H) when they are larger. In all three phenotypes flower size declines from the bottom to the top of the inflorescence and hermaphroditic flowers are always produced below staminate flowers. After Barrett *et al.* (1999).

Ramsey and Vaughton (2001) obtained parallel results and they have also demonstrated that male sex inconstancy and size-dependent gender expression also occur in male plants of *W. biglandulosa* (Vaughton and Ramsey 2002). Sex inconstancy may be strongly selected against in females because producing pollen could cause selfing and inbreeding depression, limiting fitness through female function. In males, such an effect may carry less of a penalty since plants obtain most of their fitness through pollen (Charlesworth and Charlesworth 1978).

The gender morphs in dimorphic *Wurmbea* species commonly differ in traits other than gender itself (sexual dimorphism), and these traits are referred to as secondary sex characters (Lloyd and Webb 1977; Geber 1999; Sakai and Weller 1999). There is considerable variation among *Wurmbea* species in the nature and degree of dimorphism in secondary sex characters. Sexual dimorphism is most evident in *W. tubulosa* (Fig. 1c, d) and *W. latifolia*, where male plants have an erect, open inflorescence but female plants have contracted inflorescences produced at ground level. Differences in flower size and number between females and males have been particularly well documented in *W. dioica* (Fig. 1h; Barrett *et al.* 1999; Jones and Burd 2001;

Ramsey and Vaughton 2001), where geographical variation in the degree of dimorphism is evident (Barrett 1992). By using pollinator observations and experimental arrays of *W. dioica*, Vaughton and Ramsey (1998) investigated the consequences of sexual dimorphism for pollination and mating. They proposed that increases in flower size and number have occurred largely through selection on male rather than female function, a suggestion consistent with Bateman's Principle and sexual selection theory (Willson 1979; Bell 1985; Queller 1997).

The striking diversity of gender strategies within and among *Wurmbea* species raises many questions concerning the evolution and functional basis of this variation. For example, what are the proximate ecological and developmental mechanisms that influence gender variation within monomorphic and dimorphic populations, and when during development is the gender of individual flowers determined? In populations with gender dimorphism, what is the relative importance of genetic and environmental factors in governing variation in maternal expenditure among male plants and do purely male plants commonly occur in gynodioecious and subdioecious populations? Finally, what are the evolutionary relationships between these different gender strategies and what selective mechanisms are responsible for the origin and maintenance of gender dimorphism. We next consider how phylogenetic reconstructions of *Wurmbea* can be used to provide insights on the evolutionary history of sexual systems.

Evolutionary history

Charting the evolutionary history of complex traits, such as those involved with gender variation, provides a unique perspective on the selective mechanisms responsible for major transitions in evolution (reviewed in Donoghue 1989; Barrett *et al.* 1996; Weller and Sakai 1999). We conducted a phylogenetic study of *Wurmbea*, on the basis of combined plastid DNA sequences and morphological data, in an effort to unravel the puzzle of why gender dimorphism has evolved only among the Australian species (Fig. 5; A. L. Case, S. W. Graham, T. D. Macfarlane, S. C. H. Barrett, unpubl. data). Although the species relationships in *Wurmbea* and the localisation of sexual-system changes were difficult to resolve, our study provided support for a strong geographical pattern to phylogenetic relationships and evidence for multiple shifts between gender monomorphism and dimorphism. However, we detected several cases of taxonomic uncertainty involving paraphyletic groups that could, if they had remained undetected, mislead conclusions about the evolution of sexual systems in the genus. We discuss these findings below.

We detected a strong geographical pattern of relatedness among species of *Wurmbea*; separate African and Australian clades are strongly supported, as is an eastern Australian clade nested within a grade of western Australian species.

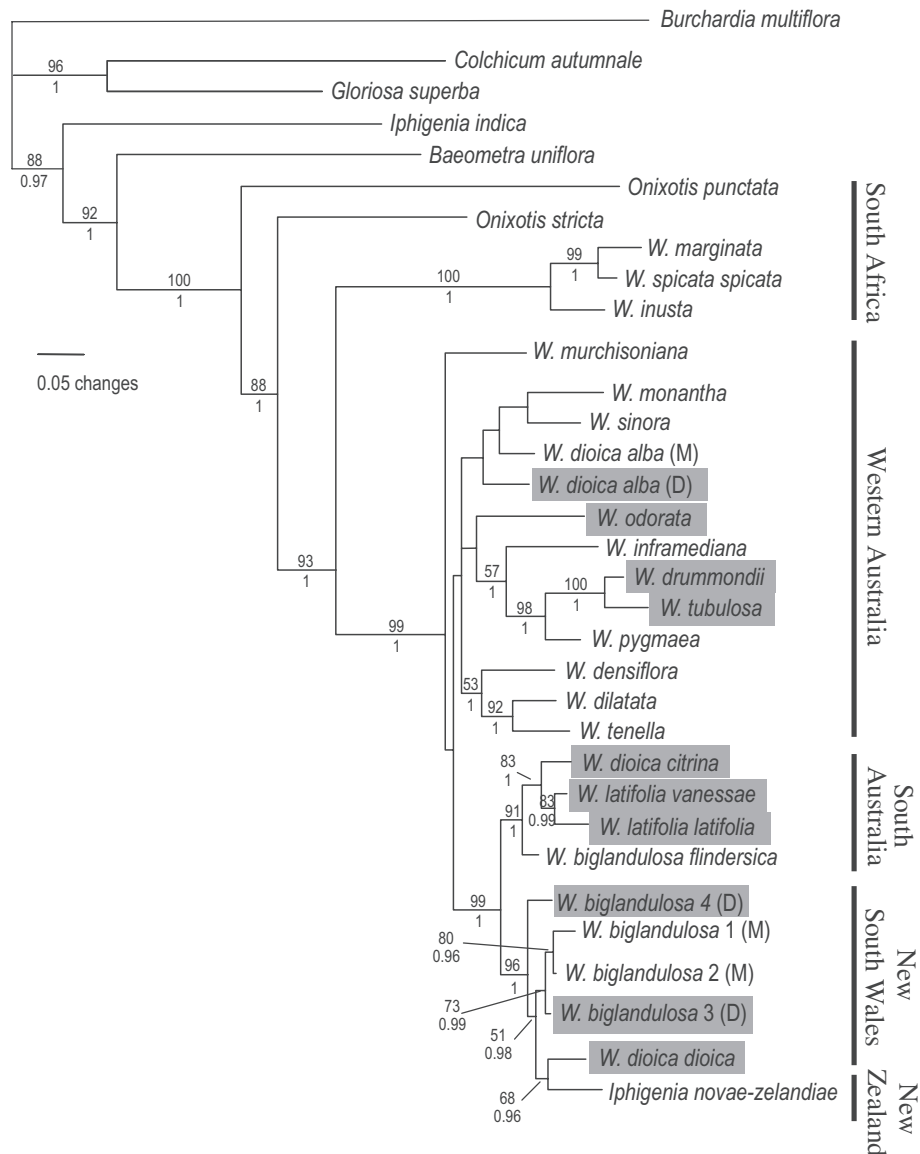


Fig. 5. Phylogenetic relationships of 25 members of the genus *Wurmbea* and eight outgroup taxa in the Colchicaceae based on Bayesian inference using combined morphological and molecular data. Taxa with gender dimorphism are indicated by the shaded boxes. Branch support is indicated by bootstrap values (% above branch) and proportional posterior probabilities (PPs, below branch); support values are shown only for branches having at least 70% bootstrap support or PPs greater than 0.95. Analytical details of phylogeny estimation are available upon request from A. L. Case (acase@kent.edu).

Robust support for a monophyletic Australian clade (Fig. 5) and estimated divergence times (see Vinnersten and Reeves 2003) imply a single dispersal event from Africa to Australia. Although the sexual system of the most recent Australian ancestor was equivocal in our analysis, it may have been gender monomorphic because the rest of the family, including South African *Wurmbea* species, are uniformly monomorphic, and because hermaphroditism confers advantages over unisexuality following establishment after long-distance dispersal (Baker 1967). Given the geographic structure within the Australian clade, the

Australian ancestor could have established in Western Australia and spread eastwards across temperate Australia, culminating in a single dispersal event to New Zealand. This historical scenario is consistent with the placement of Western Australian taxa at the base of the Australian clade and the nesting of *Wurmbea* (= *Iphigenia*) *novae-zelandiae* with its geographically closest congeners.

Although the centre of species diversity in *Wurmbea* resides in south-western Australia, relatively few (4 of 26) Western Australian taxa possess gender dimorphism, whereas half (6 of 12) of the eastern Australian taxa

have separate sexes. It is unclear why this should be so. Monomorphic species of *Wurmbea* are widely distributed from the wettest to the driest habitats throughout temperate Australia, whereas many dimorphic taxa are relatively restricted in their geographical distribution, and only one (*W. dioica* ssp. *lacunaria*) occurs in wet habitats (Macfarlane 1980; Bates 1995); the remainder is more commonly found in arid habitats. The narrower geographic distributions of dimorphic taxa could reflect reduced dispersal ability (see Heilbut *et al.* 2001), more recent speciation events, or could result from parallel ecological radiations into similar, geographically restricted environmental conditions. A key question concerns the ecological conditions that favoured the evolution of gender dimorphism during the spread of *Wurmbea* across the continent.

The expression of gender variation differs among dimorphic taxa of *Wurmbea* (Fig. 3). Several are more characteristically gynodioecious or subdioecious, with hermaphrodite plants possessing varying degrees of female function (e.g. *W. biglandulosa*, *W. dioica* ssp. *alba* and eastern populations of *W. dioica* ssp. *dioica*). Other taxa are closer to a dioecious state, with virtually no female function in polleniferous plants and sex ratios close to unity (Western Australian *W. odorata*, *W. tubulosa* and *W. drummondii*, and South Australian *W. dioica* and *W. latifolia*). Identifying evolutionary transitions to dimorphism and investigating the specific ecological and life-history context for each of these instances should give further clues concerning the adaptive significance of separate sexes and the maintenance of variable degrees of gender dimorphism in the genus.

Inferences about the specific number of transitions from gender monomorphism to dimorphism and reversals to hermaphroditism remain equivocal in Australian *Wurmbea*. Part of this uncertainty results from several branches near the base of the Australian clade being short and poorly supported, perhaps reflecting a relatively rapid radiation early on in the history of the clade in Australia. These difficulties are further exacerbated by taxonomic uncertainty concerning the circumscription of the most widespread and phenotypically diverse species. If the current taxonomic boundaries are too broad with respect to species status, as seems to be the case for *W. dioica*, then samples that are assumed to be populations of a single species may actually correspond to multiple cryptic species. Inclusion of only one representative population of each species, the common practice in most molecular systematic studies, will then inadvertently under-sample critical lineages that are relevant to making inferences about character evolution, especially in wide-ranging polymorphic complexes.

A second source of uncertainty in character reconstruction is the significant dispersion of dimorphic taxa across the phylogenetic tree (Fig. 5). Having dimorphic and monomorphic taxa interspersed requires multiple evolutionary transitions between the two sexual systems. In

the same way that rapid speciation can obscure phylogenetic signal for determining species relationships, frequent evolutionary changes between sexual systems in *Wurmbea* obscure the localisation of specific character transitions on the tree. Consequently, ecological correlates of dimorphic sexual systems will be difficult to evaluate within a phylogenetic framework (Sakai and Weller 1999).

Both taxonomic uncertainty and the dispersion of contrasting sexual systems are particularly important for understanding gender variation in *W. biglandulosa* and *W. dioica*. In *W. biglandulosa* ssp. *biglandulosa*, there are no obvious morphological or ecological traits that distinguish the populations or gender morphs (M. Ramsey, pers. comm.). The fact that the dimorphic *W. biglandulosa* populations are inferred to be paraphyletic on the plastid-derived tree (Fig. 5), and may represent separate species, makes conclusions from microevolutionary studies difficult to interpret. Contrasts between the sister taxa (e.g. *W. biglandulosa* ssp. *biglandulosa* M1 or M2 v. D3; Fig. 5) may be informative, but studies comparing the more distantly related populations (e.g. *W. biglandulosa* ssp. *biglandulosa* M1 or M2 v. D4) may confound historical signal across long periods of time and multiple evolutionary events. Without knowing more about the phylogenetic relationships (or genealogical distinctiveness) of the *W. biglandulosa* populations under study, conclusions about the proximate mechanisms promoting transitions are difficult to make.

The source of apparent paraphyly for *W. biglandulosa* (Fig. 5) could accurately reflect relationships among independently evolving populations (i.e. a paraphyletic species), additional cryptic species (as in *W. dioica*), or events such as chloroplast capture (Wolfe and Elisens 1995; Van Raamsdonck *et al.* 1997; Jackson *et al.* 1999) or incomplete coalescence (Comes and Abbott 2001). The latter two explanations would confound any interpretation of character evolution based, as here, on trees inferred primarily from a single linkage group. Without further evidence, the source of apparent incongruence between the taxonomic species ('*W. biglandulosa*') and the non-monophyletic distribution of population samples on our phylogenetic tree is impossible to identify. However, introgression can occur following hybridisation (Tsitroni *et al.* 2003) and hybrids between *W. biglandulosa* and *W. dioica* ssp. *dioica* have been observed from New South Wales on the basis of intermediate floral phenotypes and allozyme markers (S. C. H. Barrett, pers. obs.; M. Ramsey, G. Vaughton and D. Jones, pers. comm.). It is possible that hybridisation events between these two taxa have facilitated chloroplast transfer, followed by subsequent sequence divergence between extant populations. However, it is worth noting that the populations used in our analysis are from an intensively surveyed part of the range of *W. biglandulosa* in northern NSW, where hybrids have never been observed (M. Ramsey, pers. comm.).

In the case of *W. dioica*, there are distinct morphological characters for each subspecies, lending support to the idea that these represent multiple distinct species. Thus, instead of an intra-specific continuum of gender variation across Australia (Barrett 1992), this polymorphic group may represent a series of morphologically similar but independently evolving lineages with different degrees of gender and sexual dimorphism. The fact that the 'subspecies' (Macfarlane 1987) appear to be distantly related suggests that the morphological similarities used to group these taxa under a single species were misleading, perhaps reflecting retention of plesiomorphic characters among these taxa within the clade.

Our phylogenetic analysis has identified the following obstacles to understanding the evolutionary history of gender dimorphism: (1) variation in tree topology, (2) frequent changes in sexual system across the Australian clade, and (3) problems with the taxonomic delimitation of species. These various uncertainties make it difficult to relate transitions in sexual system to those in other morphological or ecological traits. Despite these difficulties, we can deduce that gender is clearly a highly evolutionarily labile character. Future research in *Wurmbea* could usefully explore explanations for the considerable lability in sexual systems of Australian *Wurmbea*, and focus on why these evolutionary transitions appear to be absent from the rest of the Colchicaceae.

Selective mechanisms

Gender dimorphism in *Wurmbea* has most likely originated and been lost on multiple occasions within the Australian subcontinent following the dispersal of a hermaphroditic ancestor from Africa. Understanding the selective mechanisms responsible for these evolutionary transitions is a major focus of current research. So far, most work has focused on the wide-ranging *W. dioica* and *W. biglandulosa*. Despite their paraphyletic status, there is evidence of significant variation in gender strategies involving both monomorphism and dimorphism within lineages of each of these groups (Fig. 5). Recent work on the biogeography and ecology of sexual-system variation has revealed strikingly similar patterns, implicating environmental stress as a candidate selective mechanism associated with the evolution and maintenance of gender dimorphism.

Environmental stress

Stressful ecological conditions have been increasingly implicated in the evolution of gender dimorphism in plants (reviewed in Sakai and Weller 1999; Delph 2003; Case and Barrett 2004b; Ashman, in press). Species with gender dimorphism are often found in drier habitats than their co-sexual relatives (e.g. Hart 1985; Weller and Sakai 1990; Weller *et al.* 1990), and dimorphic populations are often closer to a dioecious state at sites where resources,

particularly water, become limiting (e.g. Wolfe and Shmida 1997; Ashman 1999). These patterns could occur if dry conditions decrease the relative fitness of co-sexual plants, allowing female plants to invade monomorphic populations, or if harsh conditions magnify the outbreeding advantage of females when hermaphrodites experience significant self-fertilisation. Australian environments are often characterised by rainfall gradients and severe aridity, and populations of monomorphic *Wurmbea* species exposed to this variation may be vulnerable to conditions favouring the evolution of gender dimorphism.

Geographical surveys of gender variation in populations of *W. dioica* ssp. *alba* from Western Australia (Case and Barrett 2004b) and *W. biglandulosa* ssp. *biglandulosa* from New South Wales (Vaughton and Ramsey 2002, 2005; provide evidence that dry conditions play a role in the evolution and maintenance of gender dimorphism. In *W. dioica* ssp. *alba*, monomorphic and dimorphic populations tend to be both geographically (Case and Barrett 2004b) and locally (Case and Barrett 2001) segregated into wetter and drier sites, respectively. At a local scale, this ecological segregation is associated with differences in plant density, flowering time and pollinator guilds (see below). At a regional scale, plants in monomorphic and dimorphic populations of *W. dioica* exhibit contrasting patterns of gender expression and biomass allocation in areas with different amounts of precipitation. Among dimorphic populations, lower precipitation is associated with a higher frequency of female plants, and reduced allocation to female function in fruiting males (Fig. 6a). These responses to stress result in increasing gender specialisation in dimorphic populations. However, stress conditions have no effect on female allocation in monomorphic populations (Case and Barrett 2004b). Similar patterns involving seed fertility also occur in monomorphic and dimorphic populations of *W. biglandulosa* ssp. *biglandulosa* along a rainfall gradient (Fig. 6b; Vaughton and Ramsey 2002, 2005).

These results do not support the hypothesis that resource constraints on maternal allocation in co-sexual plants provide conditions that enable female invasion. However, they do indicate that once gender dimorphism establishes, stressful conditions have the potential to increase female frequencies and convert hermaphrodites into males. Increasing gender specialisation has the effect of driving dimorphic populations along the gynodioecy pathway through subdioecy to dioecy. In these circumstances, the spread of females can be aided by the occurrence of environmental gender modification in hermaphrodites (Delph 1990; Ashman 1999; Barrett *et al.* 1999). Under stressful conditions the relative advantage of females increases because hermaphrodites cannot maintain both sex functions, hence they perform largely as males. Studies of other gynodioecious taxa also report associations between increased female frequency and reduced female function of hermaphrodites in drier

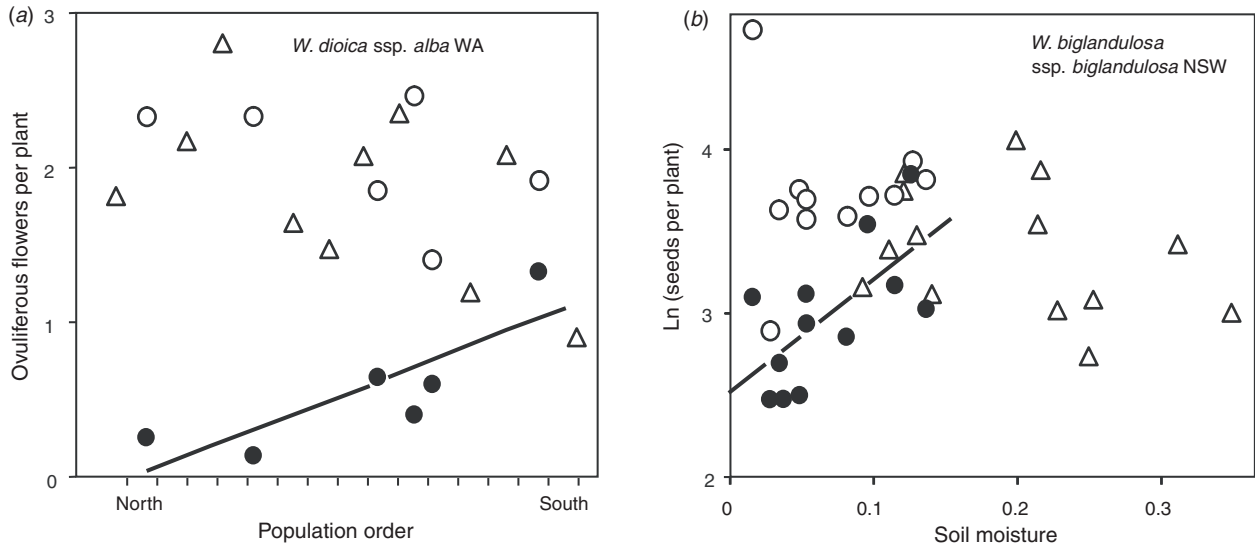


Fig. 6. The response of female function to environmental stress in two *Wurmbea* species. The three sex phenotypes compared are co-sexual plants (Δ) in monomorphic populations and female (\circ) and male (\bullet) plants in dimorphic populations. (a) Relation between increasing latitude (increasing rainfall from north to south) and the production of ovuliferous flowers in populations of *W. dioica* ssp. *alba* from Western Australia, after Case and Barrett (2004b). (b) Relation between soil moisture (g water per g dry soil) and the number of seeds per plant in populations of *W. biglandulosa* ssp. *biglandulosa*, after Vaughton and Ramsey (2005). In both species, only males significantly increased investment into maternal function with increasing soil water availability (*W. dioica* ssp. *alba*: $y = 0.09x - 0.15$, *W. biglandulosa* ssp. *biglandulosa*: $y = 6.64x + 2.52$).

or poorer site conditions (Webb 1979; Delph 1990; Wolfe and Shmida 1997; Ashman 1999; Alonso and Herrera 2001; Asikainen and Mutikainen 2003), although this is not always the case, particularly under cytoplasmic *v.* nuclear genetic systems (Barr 2004). In several of these groups, gender plasticity in hermaphrodites appears to play an important role in explaining these patterns (reviewed in Delph and Wolf 2005).

Pollination and mating

In self-compatible taxa, such as *Wurmbea*, pollinators have the potential to exert significant influence on the evolution and maintenance of sexual-system variation. This is because pollinators mediate pollen dispersal within and among plants and therefore levels of inbreeding. If monomorphic populations experience a reduction in the quality of pollinator service, causing increased selfing rates and strong inbreeding depression, selection for unisexuality should be favoured because of outbreeding advantage (reviewed in Sakai and Weller 1999). Theoretical models for the evolution of gender dimorphism provide quantitative predictions on the levels of selfing and inbreeding depression that are necessary for this scenario to occur (Charlesworth 1999). For example, when nuclear genes control male sterility, females can spread if the product of the selfing rate and inbreeding depression exceeds 0.5 (Charlesworth and Charlesworth 1978). Identifying the proximate ecological conditions that might cause elevated selfing rates in monomorphic populations represents the most difficult

element in testing this evolutionary scenario for transitions to dimorphism.

Three types of information are necessary to evaluate the hypothesis that changes in pollination service could have played a role in stimulating transitions from monomorphism to dimorphism. First, observations on the pollination biology of populations are required to determine the kinds and behaviour of pollinators visiting flowers and their effectiveness in pollen dispersal. Second, estimates of selfing rates by using genetic markers should be obtained since these will indicate the potential for inbreeding depression. Linking altered pollinator service to changes in mating pattern in plant populations has rarely been attempted (but see Kalisz *et al.* 2004); however, it is critical for testing the 'pollinator change hypothesis.' Finally, comparisons of the fitness of selfed and outcrossed progeny are necessary to reveal whether the intensity of inbreeding depression is sufficient to allow females to invade monomorphic populations, despite their disadvantage in genetic transmission.

Comparisons of sympatric monomorphic and dimorphic populations of *W. dioica* ssp. *alba* on the Darling Escarpment, Western Australia, have revealed striking differences between the two sexual systems in flower size and morphology, reproductive phenology, and the quantity and quality of floral rewards (Case and Barrett 2004a). These differences are associated with visitation by contrasting pollinator guilds, with significant consequences for levels of self-pollination. Early flowering dimorphic populations bloom at low density, have relatively small flowers and are visited primarily by

nectar-foraging flies. In contrast, the main pollinators of later-flowering, larger-flowered monomorphic populations are pollen-collecting bees. These bees are presumably attracted to the abundant floral resources that are available in monomorphic populations which 'mass flower' because of high plant density (Case and Barrett 2001). Flies do visit the flowers of monomorphic populations; however, because of their small size and tendency to forage unsystematically for long periods within individual plants, they are much less effective pollinators, particularly in promoting cross-pollination. These differences provide important clues on the role that pollination biology may have played in transitions to gender dimorphism. Here we propose an ecological scenario, by using *Wurmbea* as a model, for how changes in pollinator service may have stimulated transitions to gender dimorphism (Fig. 7). Elements of this hypothesis were proposed earlier in Barrett (1992) and Case and Barrett (2004a, 2004b) and the original idea involving pollinator shifts as a trigger for changes in sexual system was first suggested by Ganders (1978).

The starting point for our scenario involves monomorphic populations of *Wurmbea* that occur in habitats that are near optimal for growth and reproduction. Under these conditions we assume high population densities and predictable pollinator service. These demographic conditions are commonly encountered in *W. dioica* ssp. *alba* growing in areas with high precipitation (Barrett 1992; Case and Barrett 2001, 2004b). Here, most pollination is by pollen-collecting bees (Case and Barrett 2004a), which because of their size and foraging behaviour are probably effective in promoting outcrossing. Hence, we assume that these populations maintain high genetic loads and this may account for their resistance to invasion by female variants.

We now assume that migration to more arid habitats by monomorphic populations occurs, resulting in two important reproductive consequences. First, less optimal growth conditions result in reduced plant density and less floral resources for insect visitors. This could have the effect of causing a shift in pollinator service from bees to flies, resulting in elevated rates of self-pollination. We note

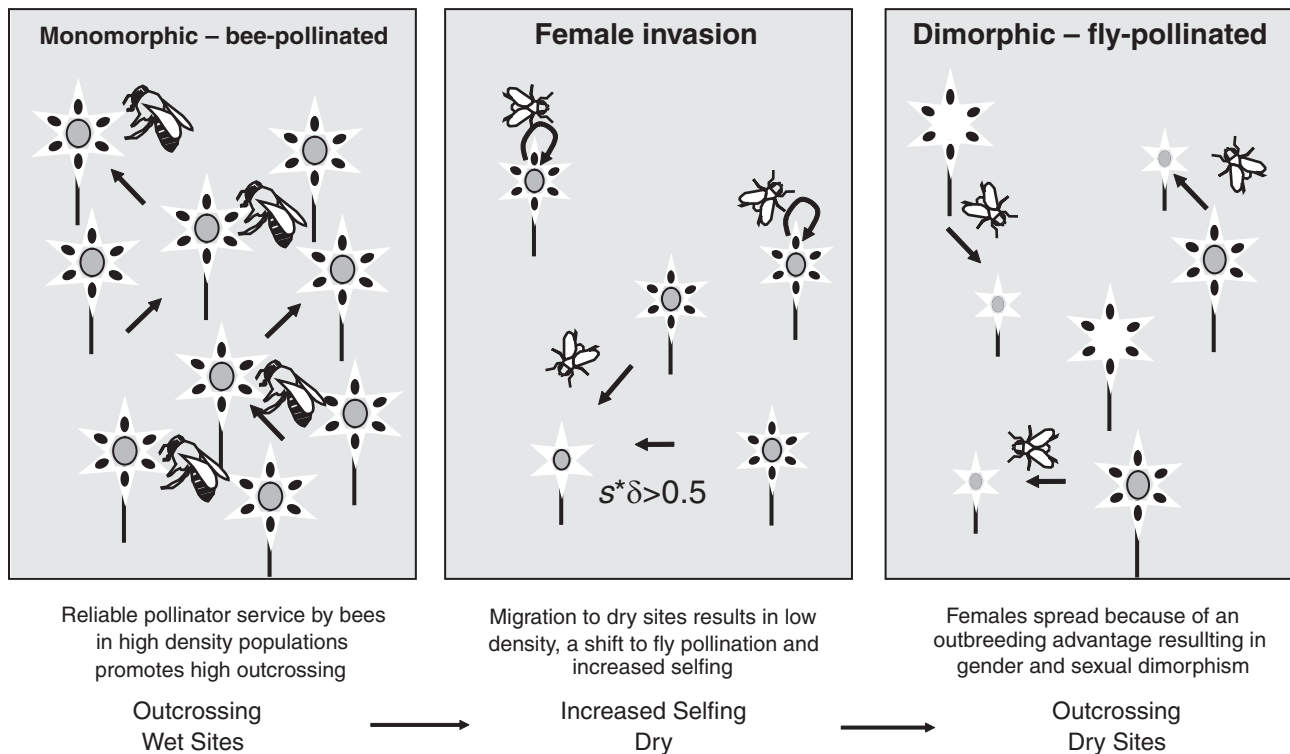


Fig. 7. Ecological model of the evolution of gender dimorphism from monomorphism in *Wurmbea* through changes in demography and pollinator service. Ancestral monomorphic populations occupy moist sites, exhibit high plant densities and are largely outcrossing because of reliable pollinator service by bees. Migration to more arid sites results in reduced density, smaller plant and flower size, and an increase in selfing rates because of a shift to fly pollination. Strong inbreeding depression facilitates the invasion of female variants, which spread as a result of outbreeding advantage. Gender dimorphic populations containing females, males and hermaphrodites (subdioecy) develop sexual dimorphism in flower size and floral adaptations favouring fly pollination. If increasing gender specialisation occurs, populations may become strictly dioecious and exhibit sex ratios of unity (not shown). Although this model emphasises the evolution of gender dimorphism from monomorphism the reverse sequence may also occur (see Fig. 5).

that even if there is no qualitative change in the kinds of pollinators visiting flowers, low density or small population size may alone result in increased selfing rates, as has been observed in several species (Barrett and Eckert 1990; Routley *et al.* 1999). Second, arid conditions also limit plant size, resulting in allometric reductions in flower size. This has been observed in *W. dioica* ssp. *alba* populations at dry sites (Case and Barrett 2004b). Reduced flower size may further increase self-pollination, since flies are now more effective as pollinators because they are able to contact both sex organs (Case and Barrett 2004a). These reductions in flower size could involve both plastic and genetic changes. The consequence of increased pollinator-mediated self-pollination would likely be elevated selfing rates and high inbreeding depression. These demographically induced pollination conditions should provide more favourable circumstances for the spread of females through outbreeding advantage. Indeed, fitness differences between selfed and outcrossed progeny of *Wurmbea* may be magnified under more stressful conditions because of environment-dependent heterosis (see Dudash 1990).

Following the establishment of gender dimorphism, selection is likely to modify patterns of resource allocation in response to altered ecological conditions, including gender-specific differences in sexual dimorphism and smaller flowers adapted primarily to fly pollination. There is supporting evidence from *W. dioica* ssp. *alba* for particular stages and conditions in this ecological scenario; however, measurements of mating patterns and inbreeding depression are not available for this taxon. Information on the pollinators that visit populations of contrasting size and density and their impact on selfing rates is critical for evaluating our ecological hypothesis on the proximate mechanisms driving sexual-system evolution in *Wurmbea*.

Recent work by Mike Ramsey and colleagues on two eastern Australian taxa (*W. dioica* ssp. *dioica* (Ramsey *et al.* 2006a) and *W. biglandulosa* ssp. *biglandulosa* (Ramsey *et al.* 2006b)) provide the first measurements of mating patterns and fitness of selfed and outcrossed offspring in *Wurmbea*. Their investigations indicate that in both species, substantial selfing in fruiting males of dimorphic populations, combined with strong early acting inbreeding depression, provide females with a sufficient fertility advantage to be maintained in populations. These data provide compelling evidence that inbreeding avoidance is likely to be the principle mechanism maintaining gender dimorphism in *Wurmbea*. Mating patterns in two monomorphic populations of *W. biglandulosa* ssp. *biglandulosa* (Ramsey *et al.* 2006a) indicated that these species were highly outcrossing ($tm = 0.97$) and both exhibited strong early acting inbreeding depression. Mating patterns in these populations should therefore make them resistant to female invasion. How frequent and under what ecological conditions ancestral monomorphic populations also experience selfing rates

similar to those measured in fruiting males from dimorphic populations is not known. A future focus on this issue could be profitably pursued.

Increased selfing rates in monomorphic populations have the potential to initiate two contrasting evolution trajectories of sexual-system evolution in *Wurmbea*. With consistently limited pollinator service, a largely autogamous mating system is likely to evolve on the basis of autonomous self-pollination. This pathway appears to have been taken in species such as *W. tenella* and *W. novae-zelandiae*, which have very small flowers and low allocation of resources to floral display. In contrast, in *W. biglandulosa* and *W. dioica* increased selfing and inbreeding depression enable the spread of females through outbreeding advantage and the establishment of gender and sexual dimorphism. Which of these pathways is followed may be linked to details of the pollination biology of populations, particularly the types of pollinator service that they experience (Harder and Barrett 1996). In monomorphic populations with *insufficient* pollinator service, selfing is likely to be selected through reproductive assurance. In contrast, where pollinator service is *inferior*, for example through a shift from bee to fly pollination (Fig. 7), increased inbreeding may provide the conditions favouring the evolution of gender dimorphism. Future work linking the pollination and mating biology of populations would be worthwhile to determine whether altered pollination conditions play a significant role in initiating these distinct alternatives in sexual-system evolution.

Future research

The diversification of sexual systems in Australian *Wurmbea* provides valuable opportunities to investigate general questions regarding the ecology and evolution of gender strategies in plants. In common with other contemporary work in this area (reviewed in Geber *et al.* 1999), diverse approaches have been used to address questions, including molecular phylogenetic and comparative analyses, biogeographical surveys and investigations of the life history, ecology, demography, physiology and genetics of populations. Much of this work has focused on populations of the two most wide-ranging polymorphic taxa, *W. dioica* and *W. biglandulosa*. Although these studies perhaps raise more questions than they have answered, they have highlighted some of the difficulties associated with historical inference, and of the complex nature of selective mechanisms, particularly the potential interactions between abiotic (aridity) and biotic (pollinators) factors. We conclude this review by summarising what has been learnt from studies on *Wurmbea* in Australia and then suggest a future research agenda that may help in addressing unresolved questions.

Gender monomorphism is basal in *Wurmbea* and it is probable that there have been multiple transitions to dimorphism with some reversals back to monomorphism.

However, current phylogenetic information for this group precludes identification of the location and number of times these shifts in sexual system have occurred. Biogeographical surveys and investigations of the population biology of *W. dioica* and *W. biglandulosa* have revealed strikingly concordant patterns of gender variation. Although composed of several lineages, these taxa both possess monomorphic and dimorphic populations. Dimorphism in each of these taxa has likely originated independently via the gynodioecious pathway by invasion of females into co-sexual populations. There is some evidence that shifts in pollination biology and their effects on mating patterns may have contributed to the evolution of gender dimorphism. Experimental work indicates that pollinator-mediated selfing combined with strong inbreeding depression provides a sufficient fertility advantage for females to be maintained in dimorphic populations of *W. biglandulosa* and *W. dioica*. Once dimorphism establishes in these groups, occupation of more arid environments is associated with increasing gender specialisation. Where particular populations lie on the continuum of gender specialisation between gynodioecy, subdioecy and dioecy may, in part, reflect gradients of drought stress. Inbreeding avoidance is the most important selective mechanism maintaining gender dimorphism in the genus, a finding consistent with much theoretical work.

Elucidating macroevolutionary patterns

Future research should involve further macroevolutionary investigations on the origin and evolution of sexual systems in *Wurmbea*. The finding that *W. dioica* and *W. biglandulosa* are paraphyletic should motivate taxonomic and population genetic/phylogeographic studies to re-evaluate species boundaries in these and other species of *Wurmbea*. These investigations are required to provide a better historical context for inferring evolutionary shifts between gender monomorphism and dimorphism.

Multiple evolutionary changes within Australia will limit our ability to localise these transitions, precluding links between particular traits and alternate sexual systems. However, more detailed environmental data on the habitat requirements of individual species should enable comparisons of ecological information between continents, and improve our understanding of the evolution of *Wurmbea* after its arrival from Africa and spread across Australia. It may also help address why dimorphism is more prevalent in eastern Australia and why it has not evolved in Africa, despite the occurrence of several African species in ecologically stressful conditions.

Historical changes in floral display between these major clades are also of interest because of their potential functional relation to the mating system component of sexual-system evolution. Floral display differs between continents and sexual systems (Fig. 2) and this may be, in part, related

to the absence of unisexuality in monomorphic African *Wurmbea*. Within Australia, gender dimorphic taxa tend to have larger inflorescences than monomorphic taxa. This could be because unisexuality relieves the constraints on flower number that geitonogamous selfing may impose in monomorphic taxa. Alternatively, selection for increased flower number may have preceded the origin of unisexuality, with the resulting increase in geitonogamous selfing leading to the evolution of gender dimorphism. Determining the polarity of character evolution is critical for distinguishing between these hypotheses. Significantly, nearly half of all African species have larger inflorescences than Australian species (Figs 1 and 2). It is possible that these species have floral mechanisms not present in Australian species (e.g. self-incompatibility, inflorescence-level dichogamy) that limit some of the mating costs associated with geitonogamy (see Harder and Barrett 1996). The clear bimodality of flower number in African species (Fig. 2) may reflect, as discussed earlier for Australian taxa, selection for few-flowered selfing taxa *v.* many-flowered outcrossers because of differences in the types of pollinator service they receive.

Style morphology and its effect on pollen capture would also be worth investigating in *Wurmbea* because of its potential to influence pollinator shifts and the evolution of sexual systems (Case and Barrett 2004a). Flowers of Australian *Wurmbea* have two distinct types of styles—straight styles with capitate stigmas (e.g. Fig. 1g) and outwardly recurved styles with elongate stigmas (e.g. Fig. 1i). All but two of the gender dimorphic species have recurved styles; most of the monomorphic Australian taxa have straight styles, and no African species bears recurved styles. Style recurvature facilitates selfing by bringing stigmas and anthers of hermaphroditic flowers closer together. It also brings stigmas closer to the tepal-borne nectaries. This makes small nectar-foraging flies effective pollen vectors, particularly in small-flowered plants. Determining the functional inter-relations between habitat quality, flower size, style morphology and pollinator effectiveness could be useful for understanding sexual-system evolution in *Wurmbea*.

Experimental studies of microevolutionary processes

Microevolutionary investigations of *Wurmbea* could focus on several unresolved questions concerning the selective mechanisms governing evolutionary transitions between sexual systems. First, how important are stress conditions, pollination biology and their interaction in governing these transitions? The data are largely correlative and future research involving experimental manipulations and reciprocal transplant experiments could profitably explore how local gradients of stress and different pollinator regimes influence the mating patterns of plants from monomorphic and dimorphic populations. The recent work by Mike Ramsey

and Glenda Vaughton is encouraging in this regard since they have established that *Wurmbea* species can be experimentally manipulated and that selfing rates can be measured directly by using genetic markers.

Second, it will be important to determine the genetic basis of sex determination through controlled crosses within and between the sex phenotypes from monomorphic and dimorphic populations of *Wurmbea* species. *Wurmbea* is not an especially suitable genus for this type of work since plants can take 3–5 years to flower (Pate and Dixon 1982; M. Ramsey, pers. comm.) and our experience has been that obtaining reliable germination and seedling establishment on non-native soil is challenging. Nevertheless, introducing this genetic dimension is critical for testing theoretical models for the evolution of gender dimorphism since these vary depending on the relative role that nuclear and cytoplasmic factors play in governing sex expression (Schultz 1994; Charlesworth 1999). Comparative studies on the genetics of sex determination among *Wurmbea* species may also be valuable for determining the number of origins of dimorphism and the pathways of sexual-system evolution (Dorken and Barrett 2004).

Finally, recent studies, including those reviewed here on *Wurmbea*, demonstrating an increase in female frequencies and concomitant reductions in fruit production by males associated with stress gradients should motivate detailed physiological studies aimed at determining how stress conditions influence trade-offs between resource acquisition and allocation. Case and Barrett's (2001) study of *W. dioica*, using carbon isotope ratios, has provided some insights into the contrasting physiological strategies exhibited by the gender morphs in monomorphic and dimorphic populations in response to local moisture conditions. Future work on photosynthetic rates, transpiration, respiration and nutrient allocation should provide more dynamic measures of physiological efficiency and provide a more accurate picture of physiological responses to aridity. Adjustment of these physiological parameters may permit females to maintain high levels of investment in seed despite reduced water availability. This work could provide the much-needed mechanistic explanation for Darwin's (1877, p. 301) prescient observation on the ecology of gender dimorphism that '[a] very dry station apparently favours the presence of the female form'.

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