A PHYLOGENETIC ASSESSMENT OF BREEDING SYSTEMS AND FLORAL MORPHOLOGY OF NORTH AMERICAN *IPOMOEA* (CONVOLVULACEAE)

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ABSTRACT

A phylogenetic investigation of 68 species and two varieties of tropical and temperate North American *Ipomoea* (Convolvulaceae) using sequence data from the internal transcribed spacer region (ITS) with parsimony and Bayesian analyses revealed multiple origins of autogamy. By assessing breeding systems and floral morphological characters in the context of this phylogeny, we estimate 16 independent origins of autogamy and 4 subsequent reversions to xenogamy. Transitions to autogamy are associated with reduced pollen-ovule ratios, decreased anther-stigma distance, and small flower size. Although the relationship between floral traits and breeding systems has been described in previous studies, this is the first investigation to examine this association in *Ipomoea*.

RESUMEN

Una investigación filogenética sobre 68 especies y dos variedades de *Ipomoea* (Convolvulaceae) en las zonas tropicales y templadas de Norteamérica, empleando datos de secuencias de ADN (ITS) con análisis de parsimonia y Bayesianos, demuestran orígenes múltiples de la autogamia. Basándose en la evaluación de los sistemas reproductivos y las características florales en el contexto de los resultados filogenéticos, estimamos 16 derivaciones independientes de la autogamia y cuatro reversiones a la xenogamia. Las transiciones a la autogamia se asocian con relaciones bajas de polen/óvulo, la disminución de la distancia entre las anteras y el estigma, y corolas pequeñas. Aunque estudios previos han tratado de las relaciones entre los sistemas reproductivos y los rasgos florales en las angiospermas, el presente estudio representa el primero que investigua estas relaciones en *Ipomoea*.

INTRODUCTION

Ipomoea, the largest genus in the Convolvulaceae is known globally for the sweet potato (*I. batatas*), the sixth most important starch crop of the world (faostat.fao.org). In addition to the sweet potato, the genus contains 600 to 1000 species (Austin & Huáman 1996; Manos et al. 2001), most of which occur in the New World with approximately 167 (25%) native to temperate and tropical North America (including Mexico). These species display a wide range of floral morphologies indicative of different pollinators and/or breeding systems (autogamy versus xenogamy; Fig. 1A–F, 2A–D). While recent molecular phylogenetic studies have included *Ipomoea* species (Miller et al. 1999; Manos et al. 2001, 2004; Miller et al. 2004), they have not used phylogenetic hypotheses to examine changes in breeding system. Similarly, numerous studies have described the breeding systems of various *Ipomoea* species (cf., Ennos 1981; Bullock et al. 1987; Devall & Thien 1992; Chang & Rausher 1998; Chemás & Bullock 2002) but have not set these studies into a comparative framework. Here we combine a phylogenetic study of North America *Ipomoea* with greenhouse studies of breeding systems of all taxa included to assess the occurrence of different breeding systems in an evolutionary context and examine the relationship between breeding systems and floral traits.

An historical and prevailing assumption that outcrossing (xenogamy) in flowering plants is responsible

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for the remarkable diversity and evolutionary success of the angiosperms (Darwin 1877; Stebbins 1974; Faegri & van de Pijl 1979) has been tempered in recent years by the recognition of high rates of autogamy in herbaceous groups (Barrett et al. 1996; Kohn et al. 1996; Schoen et al. 1997; Goodwillie1999; Richman & Kohn 2000; Lu 2001; Barrett 2002; Allem 2003; Igic et al. 2003) and the frequent occurrence of apomixis in tropical tree groups (Kaur et al. 1978; Allem 2003; Ashman & Majestic 2006). Since 20–30% of all flowering plant species produce offspring by means of self-fertilization (Barrett 2002; Allem 2003), there is ample evidence that self-compatibility confers a competitive edge over self-incompatibility under certain environmental conditions. Nevertheless, cross-fertilization is the favored mode of reproduction in most angiosperm groups, leading to a general consensus that cross-fertilization promotes heterozygosity and genetic polymorphisms in plant populations (Grant 1958; Faegri & van der Pijl 1979; Liu et al. 1998; Allem 2003), thereby facilitating adaptability to changing environments over the long course of evolutionary time (Holsinger 2000).

Evolutionary biologists recognize two different explanations for high rates of self-pollination among herbaceous angiosperm groups. The 'automatic selection' hypothesis states that alleles for self-compatibility within gene pools of outcrossing individuals will be inherited in larger proportions than self-incompatibility alleles (3:2) due to increased rates of transmission, this owing to the additive effects of both selfing and cross-fertilization mechanisms (Fisher 1941; Jain 1976; Schoen et al. 1996; Holsinger 2000). Alternatively, the 'reproductive assurance' hypothesis ascribes high rates of autogamy in plant populations of transitory and insular habitats (Baker 1955; Webb & Kelly 1993; Bernardello et al. 2001) to a filter mechanism that favors self-compatible disseminules and their ability to establish viable colonies in the absence of mates or pollinators after long-distance dispersal events (Baker 1955, 1967; Stebbins 1974; Lloyd 1980; Pannell & Barrett 1998).

Theoreticians agree that selfing advantages are counter-balanced by genetic benefits that derive from the process of cross-fertilization by increasing rates of pollen discounting and diminishing the expression of lethal homozygous deleterious alleles (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Harder & Wilson 1998). These predictions are substantiated by studies of natural plant populations, many of which conclude that perennial plant species are more susceptible to inbreeding depression than annuals on account of higher genetic loads (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Lloyd & Schoen 1992; Barrett et al. 1996; Liu et al. 1998; Holsinger 2000; Barrett 2002). While experimental crossing studies on both plants and animals support this perspective (Crnokrak & Barrett 2002), Lande and Schemske (1985) predict plant populations should be able to purge themselves of deleterious alleles by means of occasional selfing events within outcrossing populations. Populations must apparently pay, however, an initial competitive price in progeny success rates during the purgation process.

Shifts from outcrossing to inbreeding modes of reproduction are believed to be initiated by the malfunction of self-incompatibility mechanisms (Barrett 2002). If selfing behaviors prove advantageous, then selective forces will no longer favor floral characteristics that are associated with cross-pollination syndromes. Hence Barrett et al. (1996) recognize that outcrossing is usually associated with a number of floral features and syndromes that are attractive to pollinators, just as self-compatible flowers exhibit dysfunctional vestiges of these same features. They also note that co-variation of two or more floral characters between taxa can inform us about the adaptive significance of such features if we understand these attributes in the context of their phylogenetic and functional linkages.

In the present study we generate a phylogenetic hypothesis based on existing and newly-collected ITS sequence data for 70 North American *Ipomoea* taxa and use this phylogeny to pinpoint changes in breeding systems and associated changes in floral traits. Breeding systems for the species included were determined by experimental greenhouse studies. We also use these data to examine the impact of breeding system changes on floral structures. Most *Ipomoea* species present large and showy flowers and produce substantial amounts of nectar and pollen as attractants (Figs. 1, 2). In shifts from xenogamy to autogamy, one might expect the relaxation of selection pressures that maintain showy floral features and the increased influence

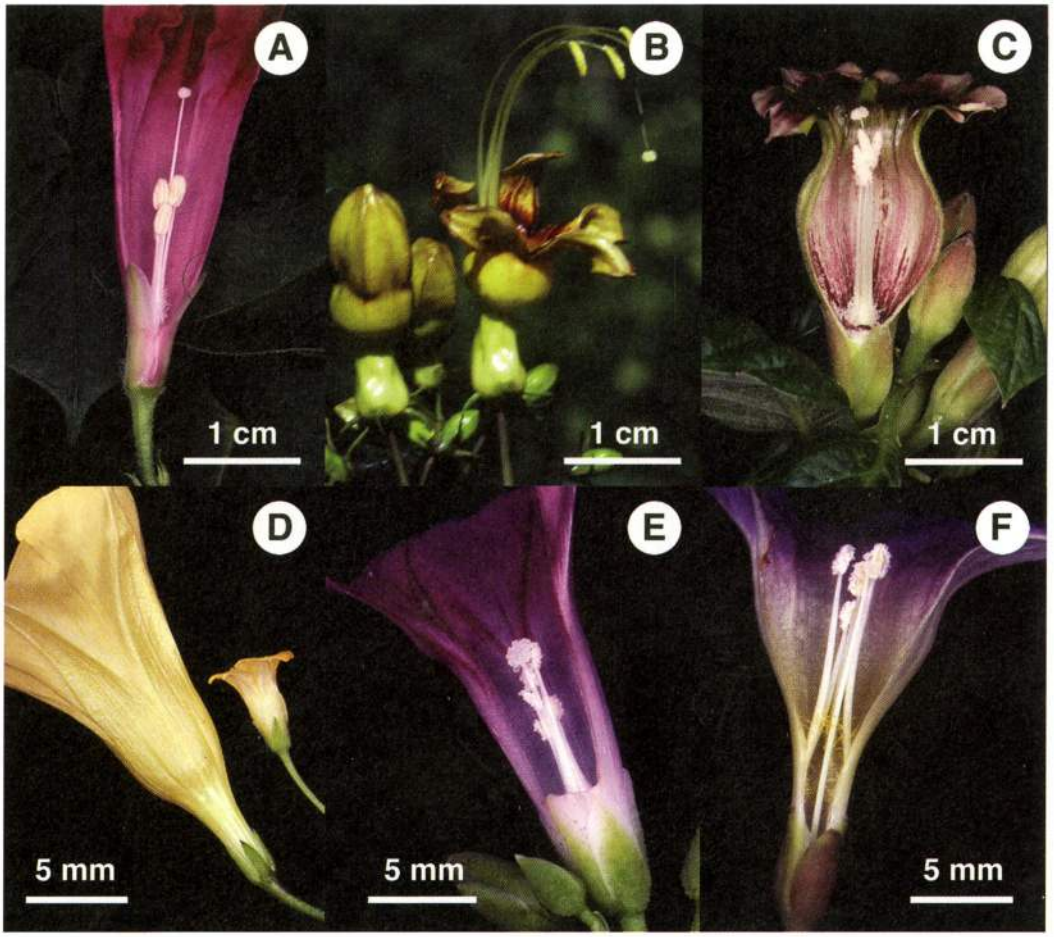

Fig. 1. Floral structures of assorted xenogamous (1–3) and autogamous (4–6) *Ipomoea* Species from North America. A. *Ipomoea orizabensis* var. *collina* is melitophilous, self-incompatible, and strongly herkogamous. B. The hummingbird pollinated species, *I. neei*, is both self-incompatible and strongly herkogamous. C. *Ipomoea gesneriodes* is somewhat unique in exhibiting herkogamous flowers, urceolate corollas, and subequal stamens. D. *Ipomoea microsepala* and *I. minutiflora*, the sole members of *Ipomoea* ser. *Microsepalae*, exhibit strong differences in flower sizes as xenogamous and autogamous species (respectively). E. *Ipomoea dumetorum* exhibits typical features of an autogamous species derived from a melitophilous ancestor. Note flowers are small (ca. 2.5 cm long) and stamens are subequal. The stigma maintains close contact with several anthers. F. *Ipomoea aristolochiaefolia* is an autogamous species that belongs to a group that is predominantly pollinated by hummingbirds or long-tongued flies (*I. Exogonium* s.l.). Note the flowers are small (ca. 2.5 cm long), the vestigial tube pronounced with adnate filaments, and the stamens subequal. The stigma maintains close contact with most anthers.

of mechanisms that enhance selfing abilities. We point out the relationships between breeding system and three floral traits: flower size (length), pollen:ovule ratio, and anther-stigma distance. The costly production of large flowers and large amounts of pollen is hypothesized to decrease in selfing lineages (Goodwillie et al. 2009) while the distance between anthers and stigma is hypothesized to decrease in order to facilitate selfing.

Although many studies have discussed relationships between breeding system and floral evolution (e.g., Cruden 1977, 2000; Lord & Eckhard 1984; Plitman & Levin 1990; Gallardo et al. 1994), this study represents the first attempts to understand these relationships within a phylogenetic context in *Ipomoea*. With an aim to examine the multiple origins of inbreeding in North American *Ipomoea*, we obtained DNA

Fig. 2. Contrastive floral features of closely related xenogamous and autogamous sister species of *Ipomoea* in North America. **A.** *Ipomoea variabilis* (*I.* sect. *Exogonium*) is xenogamous and shares a number of synapomorphic characteristics with the sister species, *I. meyeri* (late leaf primordia sub-bullate; sepals foliose and hispid, corolla tube basally yellow-pigmented). Like other outcrossing *Ipomoea*, this species has relatively large and colorful flowers, heteromophic stamens and style (herkogamous flowers), and high pollen-ovule ratios (Table 1). **B.** *Ipomoea meyeri* is autogamous and sister to *I. variabilis*. Flowers are relatively small (<2.5 cm long), stamens and style subequal, and pollen-ovule ratios low (Table 1). **C.** *Ipomoea tricolor* (*I.* sect. *Tricolores*) is xenogamous and shares a number of synapomorphic characteristics with the sister species, *I. cardiophylla* (sepals small, deltoid-elongate, papillate, corolla tube yellow). Like other outcrossing *Ipomoea*, this species has relatively large and colorful flowers, heteromophic stamens and style (herkogamous flowers), and relatively high pollen-ovule ratios (Table 1). **D.** *Ipomoea cardiophylla* is autogamous and exhibits flowers that are relatively small (<3 mm long), the stamens and style subequal, and low pollen-ovule ratios (Table 1).

sequences of about 90% of all known autogamous species in this region of the world and sampled xenogamous species that demonstrate phylogenetic affinities with inbreeding taxa on the basis of morphological characters. We measured several floral characteristics that have long been associated with inbreeding species in *Ipomoea*, including small corollas, subequal stamens and styles, and low pollen-ovule ratios, to examine the distribution of such these characteristics in a phylogenetic context and to discern multiple origins of these associative characteristics. The multiple and correlative origins of these characteristics are considered in an evolutionary context.

MATERIALS AND METHODS

Taxon Sampling

In order to obtain a broad sample of North American species and their close relatives, published ITS sequences of 50 species (47 *Ipomoea* and 3 outgroups) were acquired from GenBank (Manos et al. 2001; Miller et al. 2004) and an additional 23 xenogamous species of *Ipomoea* that demonstrate close relationships with inbreeding taxa on the basis of morphological synapomorphies, representing 21 species and 2 varieties, were sequenced. As a whole, these species comprise a substantial complement of taxa that fall within two subgenera of the genus (*I.* subg. *Eriospermum* and *Quamoclit*), representing 17 different sections and series (Table 1). The former subgenus comprises a paraphyletic assemblage of clades that include both New and Old World elements while the latter is monophyletic and consists predominantly of tropical North American taxa. Voucher specimens for a total of 73 Convolvulaceae are listed in Appendix 1 along with their collection localities and GenBank accession numbers.

We initially included samples from two or three populations of 25 species in our phylogenetic investigations, but duplicate accessions did not alter the topologies of trees from those including only a single population of each taxon. We therefore include only one sequence for each taxon in our final results.

Because various Mexican species of *Ipomoea* occupy a basal phylogenetic position within the Ipomoeae tribe (Miller et al. 1999, 2004), we employed three different outgroups of the sister tribe, Merremiaea (Stefanovic et al. 2002): *Merremia dissecta*, *Merremia tuberosa*, and *Operculina pteripes* (Appendix 1).

For newly sequenced species, DNA was extracted from fresh leaf material grown in greenhouse facilities at The University of Texas. Seeds were collected by the first author in the field or obtained from the USDA Southern Regional Plant Introduction Station, Griffin, Georgia.

DNA Extraction and Amplification

Whole genomic DNA was extracted by following a modified Doyle and Doyle (1987) protocol using a CTAB extraction buffer with 0.2% (v:v) beta-mercaptoethanol. The internal transcribed spacer (ITS) region of the 18S–26S nuclear ribosomal repeat was chosen for phylogeny reconstruction. We employed primers ITS 1A (5'GGA AGG AGA AGT CGT AAC AAG G 3') and ITS 4 (5'TCC TCC GCT TAT TGA TAT GC 3') as primers to amplify the ITS I, the 5.8s region, and ITS II via the polymerase chain reaction (PCR). Reaction volumes of 25 ul included 2.5μL of 10x Triton-X PCR buffer (final concentration 1x), 2μL 10mM dNTP mix (final concentration 0.8 mM dNTPs), 2 μL 25mM MgCl (final concentration 2mM MgCl), 1.25 μL DMSO (final concentration 5% v:v), 1 unit of Taq enzyme, and 0.25 μL 20μM forward and reverse primers (0.2μM final concentration of each primer). After initial denaturation at 95° C for 5 min, amplification proceeded at 94° C for 3 min, 46–52° C for 1 min, 72° C for 1 min, followed by 35 cycles of 94° C 1 min, 46–52° C 1 min, 72° C for 45 sec + 3 sec/cycle, with a final 7 min extension at 72° C. Amplification products were visualized on a 1.5% agarose gel stained with ethidium bromide and viewed with UV on a transilluminator. PCR products were cleaned prior to sequencing with QIAquick spin columns (Qiagen Inc.) according to the manufacturer's instructions.

Cycle sequencing was performed using Big Dye terminator chemistry and either the forward or reverse amplification primer. Centri-sep columns were used to remove residual salts and unincorporated nucleotides from the sequencing products. Automated sequencing was performed on a BaseStation sequencer (MJ GeneWorks, San Francisco, CA). Forward and reverse sequence strands were assembled and edited

TABLE 1. Tropical and Temperate North American Convolvulaceae observed under greenhouse conditions, including 86 species and two varieties of *Ipomoea* and three outgroup species of *Merremia* and *Operculina*. Detailed floral and reproductive characteristics of a sampling of *Ipomoea* and outgroups included in phylogenetic analyses (73 species and 2 varieties) are provided. Species followed by an **X** are recognized as xenogamous (< 20% viable seed set of non-treated flowers), those followed by an **A** as autogamous (>50% viable seed set of non-treated flowers), and those with **X/A** have a mixed mating system. Pollen/Ovule ratios (P/O) are recorded for selected species, most of which are based on the average measurement of 2–10 flowers. Floral characteristics of each species are scored as follows: **L** = large corollas, **S** = Small corollas; **He** = Herkogamous flowers (unequal stamens and style); **Ho** = Homomorphic (subequal) stamens and style. ITS sequence data reported by Miller et al. (1999; 2004) and Huang et al. (2002) are marked by a single asterisk (*) while new ITS sequence data are marked by two asterisks (**). Appendix 1 provides author names of binomials, voucher information, and GenBank numbers. The classification scheme follows a modified system of McDonald (1991) based on recent molecular studies by Miller et al. (1999; 2004).

	Breeding System	P/O	Corolla	Stamens
Subgenus Eriospermum				
Section *Eriospermum* H. Hallier				
Series *Anisomeres* (House) D.F. Austin				
I. squamosa	**X**		L	He
Series *Batatas* (Choisy) D.F. Austin				
I. batatas var. *batatas* *	**X**	922	L	He
I. batatas var. *apiculata***	**X**	744	L	He
*I. cordatotriloba**	**X/A**	476	L	He
*I. lacunosa***	**A**	159	S	Ho
*I. ramosissima***	**A**	198	S	Ho
*I. tabascana**	**X**	694	L	He
*I. tenuissima***	**A**	196	S	Ho
*I. tiliacea***	**X**	1069	L	He
*I. trifida***	**X**	741	L	He
*I. triloba**	**A**	151	S	Ho
*I. umbraticola**	**X**	1111	L	He
Series *Jalapae* (House) D.F. Austin				
*I. amnicola**	**A**	604	S	Ho
*I. fimbriosepala***	**A**	458	S	Ho
*I. jalapa***	**X**	1950	L	He
*I. leptophylla**	**X**	2175	L	He
*I. pandurata**	**X**		L	He
Series *Arborescentes* (Choisy) D.F. Austin				
*I. arborescens**	**X**		L	Ho
I. murucoides	**X**		L	Ho
*I. pauciflora***	**X**		L	Ho
I. populina	**X**		L	He
I. praecana	**X**		L	He
I. wolcottiana	**X**		L	Ho
Series *Setosae* (House) D.F. Austin				
*I. setosa***	**X**	1378	L	He
*I. sepacuitensis***			L	
Series *Microstictae* (unpubl.)				
*I. conzattii**	**X**		L	Ho
I. suaveolens	**X**		L	He
Series *Bombycospermae* (Presl) D.F. Austin				
I. bombycina	**X**		S	He
I. gesnerioides	**X**		L	Ho
Section *Erpipomoea* Choisy				
*I. pes-caprae**	**X**	2028	L	He
*I. imperati**	**X**	1873	L	He
Section or Series Unknown/Unpublished				
I. carnea Jacq. var. *fistulosa**	**X**	4584	L	He

TABLE 1. continued

	Breeding System	P/O	Corolla	Stamens
*I. clavata***	**A**	1172	L	Ho
I. corymbosa	**X**		L	He
*I. crinicalyx**	**X**		L	He
*I. hartwegii***	**X**		L	He
I. lenis	**X**		L	He
*I. pedicellaris**	**X**	1291	L	He
Subgenus *Quamoclit* (Moench) Clark				
Section *Pharbitis* (Choisy) Griseb.				
Series *Pharbitis* (House) D.F. Austin				
*I. ampullacea**	**X**	2413	L	He
*I. hederacea**	**A**	152	L	Ho
*I. indica**	**X/A**	887	L	He
I. laeta	**X**		L	He
*I. lindheimeri**	**X**	263	L	He
*I. mairetii**	**X**		L	He
*I. nil**	**A**	151	L	Ho
*I. neurocephala**	**A**		L	Ho
*I. pubescens**	**A**	155	S	Ho
*I. purpurea**	**X/A**	126	L	He
I. villifera	**X**		L	He
Series *Tyrianthinae* (House) D.F. Austin				
*I. ancisa***	**X**		L	
I. orizabensis var. *orizabensis**	**X**	905	L	He
I. orizabensis var. *collina***	**X**		L	He
*I. parasitica**	**A**	223	S	Ho
*I. stans**	**X**		L	He
*I. sesscossiana**	**X**		L	He
Section *Calonyction* (Choisy) Griseb.				
*I. alba**	**X**	1064	L	Ho
*I. santillanii**	**X**	3177	L	He
*I. muricata**	**A**	135	L	Ho
Section *Exogonium* (Choisy) Griseb.				
*I. aristolochiaefolia***	**A**	112	S	Ho
I. bracteata	**X**	405	L	Ho
*I. dumetorum**	**A**	90	S	Ho
I. dumosa	**X**	L		Ho
*I. expansa**	**X**	L		He
*I. meyeri***	**A**	118	S	Ho
I. puncticulata	**X**	L		He
*I. purga**	**X**	L		Ho
*I. seducta**	**X**	L		Ho
I. signata	**X**	L		He
*I. simulans***	**X**	591	L	He
I. suffulta	**X**		L	He
*I. variabilis***	**X**	1147	L	He
Section *Leptocallis* (G. Don) J.A. McDonald				
*I. chamelana**	**X/A**	108	S	He
*I. costellata***	**A**	63	S	Ho
I. tenuiloba var. *tenuiloba*	**X**	143	L	He
*I. ternifolia**	**X**	719	L	He

TABLE 1. continued

	Breeding System	P/O	Corolla	Stamens
Section *Mina* (Cerv.) Griseb.				
*I. cholulensis***	**A**	51	L	Ho
*I. coccinea**	**A**	80	L	Ho
*I. funis**	**X**		L	He
*I. hastigera**	**X**		L	Ho
*I. hederifolia**	**X/A**	134	L	Ho
*I. lobata**	**A**	383	S	Ho
*I. lutea**	**X**		L	Ho
*I. neei**	**X**		L	He
*I. quamoclit**	**A**	92	L	Ho
Section *Microsepalae* (House) D.F. Austin				
*I. microsepala***	**X**	735	L	He
*I. minutiflora***	**A**	51	S	Ho
Section *Tricolores* D.F. Austin				
*I. cardiophylla**	**A**	165	S	Ho
*I. tricolor**	**X**	726	L	He
Outgroups				
*Merremia tuberosa**	X	2674	L	He
*Merrmia dissecta**	X	5114	L	Ho
*Operculina pteripes**	X	11,871	L	He

in Sequencher 4.5. The sequences were aligned manually in MacClade (Maddison & Maddison 2000). Six variable sites were excluded due to ambiguous alignment (Treebase submission SN2687; Pin #6910). New sequences are available from GenBank (accession submission numbers DQ33303-355325, Appendix 1).

Phylogenetic Analyses

For phylogeny reconstruction, the ITS data set was analyzed in PAUP* 4.0b10 (Swofford 2002) using parsimony as the optimality criterion. Heuristic tree searches included 1,000 random addition replicates using MULPARS and tree-bisection-reconnection branch swapping (TBR). These trees were saved and used as the basis for another round of TBR swapping up to an arbitrary maximum of 20000 trees. A majority-rule and strict consensus tree were generated from these. Support for internal branches was determined by 1000 bootstrap replications (Felsenstein 1985) using the same heuristic search strategy.

The ITS data set was also analyzed using Bayesian methods implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The best-fitting model of sequence evolution was determined using ModelTest 3.7 (Posada & Crandall 1998) in PAUP 4.0b10 (Swofford 2002). Analyses in MrBayes 3.1.2 consisted of two Markov Chain Monte Carlo runs, each composed of four linked chains. The runs proceeded for 4 million generations, sampling every 100 generations. We identified the burn-in phase by examining the plot of log-likelihood scores for the two runs. Convergence was assessed by examining the standard deviation of split frequencies across chains and, more importantly, by similarities in topology, branch lengths, and clade credibilities between the two independent runs (Huelsenbeck et al. 2002).

Breeding System and Floral Morphology Data Collection

Reproductive behaviors of 89 taxa were observed directly in greenhouse facilities at The University of Texas (Table 1), accounting for more than half of North America species. Breeding systems were determined by observing spontaneous seed-set of untreated flowers (without emasculation or pollinators) within greenhouse settings. Species that consistently produced fewer than 20% seed set in the absence of pollinators were scored as xenogamous; those with over 50% seed set without pollinators were scored as autogamous.

Ten measurements of flower length and anther-stigma distance (herkogamy) were collected from either fresh or herbarium materials (TEX, UNAM). Pollen-ovule ratios for 51 of the 68 species were determined by counting the total number of pollen grains in five anthers of each flower and then dividing this sum by the total number of ovules (i.e., four in most *Ipomoea*, six for members of *I.* series *Pharbitis*; Table 1). When live material was available, an average pollen-ovule ratio was measured by directly observing and counting every pollen grain from a total of 10 flowers. Ratios obtained from herbarium specimens were based on measurements of 1–3 flowers. The herbarium sheet approximations of pollen-ovule ratios should prove reliable for the present survey, as formerly published measurements indicate pollen number within species varies from 1–10% (McDonald 1982; Chemás Jaramillo & Bullock 2002) while pollen-ovule ratios between sister autogamous and xenogamous species usually differ by factors that range from 100–2000% (Table 1).

We also collected data of all 68 species for three floral traits coded as discrete characters. Based on observation of living and herbarium materials at TEX, flowers with corolla tubes that exceed 2.5 cm in length were scored as 'large' while those that range from 0.5–2.5 cm in length were classified as 'small' (Table 1). Species were deemed herkogamous if anthers and stigma are separated spatially (usually by several millimeters).

We were not able to use methods currently available for predicting ancestral traits or for rigorous comparative analyses because we were able to sample only 41% of the species of North America and our sample is not truly random. However, we did code the floral and reproductive characters in Table 1 (autogamy versus xenogamy, herkogamy vs. homomorphic sexual structures, floral length, and pollen:ovule ratios) as discrete characters and then optimized them on the tree shown in Figure 3 using MacClade 4.08 (Maddison & Maddison 2005) under a parsimony criterion (Fig. 4A, B, Fig. 5 A, B).

RESULTS

Phylogeny estimation

The aligned matrix of ITS sequence data totaled 676 bp. Across these characters, 355 were variable and 221 were parsimony-informative. For the parsimony analysis, we excluded uninformative characters, leaving 221 characters analyzed. The parsimony analysis generated 120,950 equally most-parsimonious trees. The consensus of these trees was similar to that obtained from the Bayesian analysis.

For Bayesian analysis, the best-fitting model of sequence evolution for this dataset was a general time-reversible model with gamma-distributed rate variation and the number of invariant sites estimated. Both independent runs produced similar consensus topologies, suggesting convergence on the most probable area of tree space. The average standard deviation of split frequencies was less than 0.01 after the burn-in phase (30%), also indicating good convergence. The resulting majority-rule consensus of 56,000 trees is used (Fig. 3) for the assessment of the breeding system and floral morphology across the species.

Breeding System and Floral Trait Data

Greenhouse studies confirmed that *Ipomoea* species range from completely selfing (ca. 100% viable seed set in non-treated flowers) to completely outcrossing (0% viable seed set in non-treated flowers). Based on our scoring methods for an ingroup of 87 taxa (Table 1), 59 species were scored as xenogamous, 23 as autogamous, and 5 as having a mixed breeding system (self-compatible but facultatively herkogamous). Flowers of 69 taxa were scored as large and 18 as small. Distances between the anthers and stigma yielded 51 species being scored as herkogamous and 38 as homogamous.

Pollen:ovule ratios for the 51 species counted (Table 1) exhibited remarkable variability, ranging from 51–3177:1 (x = 597). High extremes of autogamous pollen-ovule ratios in *I. amnicola* (604:1) and *I. clavata* (1172:1) occasionally exceed the low extreme of pollen-ovule ratios in various xenogamous species (Table 1), such as those of *I. bracteata* (405:1) and *I. simulans* (591:1). In a similar fashion, several self-compatible, outcrossing species that produce herkogamous flowers, such as *I. chamelana* (P/O = 108), *I. cordatotriloba* (P/O = 476), and *I. hederifolia* (P/O = 134), exhibit pollen-ovule ratios that fall within limits of many autogamous species (Table 1).

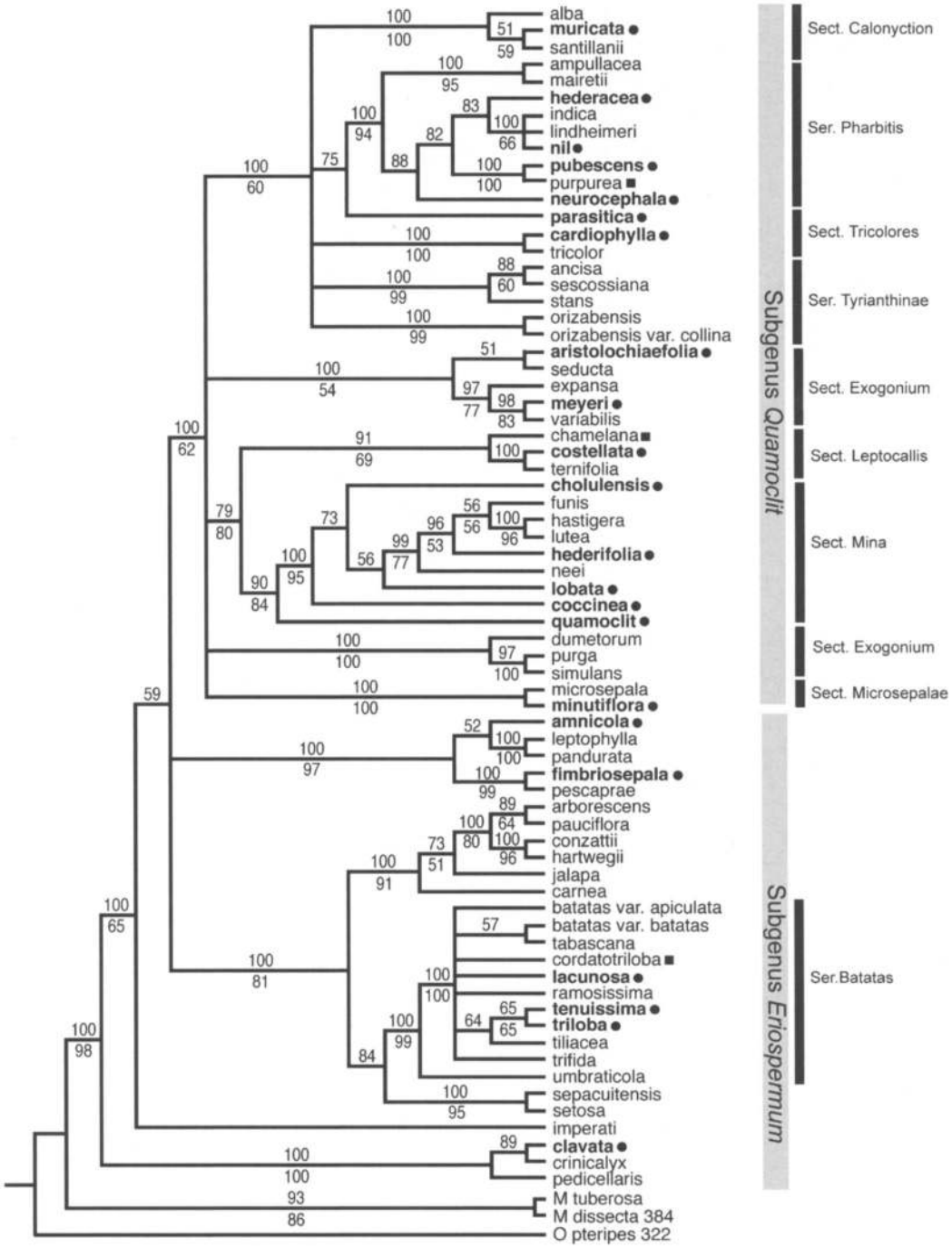

Fig. 3. Bayes consensus tree based on an analysis of ITS sequence data. Bold type and solid circles indicate autogamous lineages while xenogamous lineages are indicated by regular type and and no symbols. Names followed by a solid square box indicate self-compatible species that outcross facultatively on account of herkogamous flowers. Numbers above the branches are posterior probabilities from the Bayesian analysis. Numbers below the branches indicate bootstrap support for 1000 replicates. Sectional and series dispositions of species are indicated by bars to the right of the tree.

The results of the character optimization are shown in Figures 4 and 5. These optimizations reveal considerable congruency in character evolution. Herkogamy, long flowers and large pollen:ovule ratios usually occur on outcrossing lineages while homomorphic sexual structures, short flowers and small pollen:ovule ratios trace primarily along inbreeding clades.

DISCUSSION

The present study uses a new phylogenetic reconstruction for North American *Ipomoea* to explore breeding systems and their floral traits. Our analyses indicate multiple (potentially 16) independent transitions to autogamy and possibly reversals (Fig. 3). We found that apparent transitions between xenogamy and autogamy were strongly associated with changes in a suite of floral traits including flower size, distance between the anthers and stigma within a flower, and pollen:ovule ratio. Here we discuss the results in the context of existing studies of breeding system evolution and suggest avenues for future work.

Although a few phylogenetic studies based on morphological data suggest xenogamy may be a derived feature (Olmstead 1989; Armbruster 1993; Kelly 1997), almost all studies based on molecular evidence indicate that inbreeding lineages are derived from, and rarely revert back to, outcrossing habits (Barrett et al. 1996; Goodwillie 1999; Weller & Sakai 1999; Vieira & Charlesworth 2002; Igic et al. 2003). Similarly, our estimates based on a sample of 68 *Ipomoea* species suggest strong directionality in transitions, with many shifts from xenogamy to autogamy and a few in the reverse direction (notably *Ipomoea* sers. *Mina* and *Pharbitis*; Fig. 3).

Although most autogamous species appear as isolated tips in xenogamous clades, in a few cases autogamous species are closely related to other autogamous species, as observed among members of *Ipomoea* ser. *Pharbitis* (*I. pubescens* is sister to *I. purpurea*, *I. nil* is sister to *I. hederacea*), *Ipomoea* ser. *Batatas* (*I. lacunosa*, *I. cordatotriloba*, *I. ramosissima*), and particularly species in *Ipomoea* sect. *Mina*. Apart from these exceptional examples, however, autogamous lineages in *Ipomoea* appear to undergo cladogenesis less often than their most closely related xenogamous lineages, this being consistent with the notion that transitions to selfing may decrease speciation or increase extinction, leading to an evolutionary dead-end (Stebbins 1957; Takebayashi and Morrell 2001). Substantiating this pattern across *Ipomoea* will require, however, a broader sampling of taxa for statistical analyses, as discussed below.

The lack of species-rich clades in terms of autogamous taxa in *Ipomoea* compares closely with those of the Polemoniaceae but contrast significantly with inbreeding lineages of the Solanaceae. Self-compatible lineages have arisen at least 16 times in the Polemoniaceae and a large majority of these have failed to diversify over the course of time (Barrett et al. 1996). In contrast, an estimated 60% of species in the Solanaceae (ca. 1200 spp.) are identified as self-compatible, and these autogamous nightshades are often closely related to one another, comprising clades of exclusively inbreeding taxa (Igic et al. 2003). This distinction is noteworthy (and as yet unexplained in terms of their genetic architecture and evolutionary histories), as the Convolvulaceae are sister to Solanaceae and phylogenetically distant to the Polemoniaceae (Savolainen et al. 2000; Soltis et al. 2000).

Similar to evolutionary trends of autogamy in the Polemoniaceae (Barrett et al. 1996), selfing has arisen in distantly related groups of *Ipomoea* clades that exhibit a variety of pollination syndromes: i.e., hummingbird pollination (*I. cholulensis*, *I. hederifolia*, *I. neei*, *I. quamoclit*), bee pollination (*I. amnicola*, *I. cardiophylla*, *I. costellata*, *I. fimbriosepala*, *I. meyeri*, *I. leptophyla*, *I. microsepala*, *I. minutiflora*, *I. orizabensis*, *I. pubescens*, etc.), hawk-moth pollination (*I. neurocephala*, *I. muricata*), and long-tongued fly pollination (*I. aristolochiaefolia*, Fig. 1; McDonald 1982, 1991). Consequently, there is little evidence that specific animal vectors predispose or preclude a plant population's ability to become autogamous.

Inbreeding species in *Ipomoea* commonly possess a suite of floral characteristics that apparently arise convergently. These characteristics include relatively small (tubes usually < 2.5 cm long) and pale corollas, subequal stamens, anthers that make direct contact with the stigma (Fig. 1 E & F, Fig. 2 B & D), and low pollen:ovule ratios. Similar floral syndromes typify autogamous elements in various genera of the

Polemoniaceae (Grant 1981; Barrett et al. 1996), *Dalechampia* (Armbruster 1993), *Scutellaria, Mazus* and *Hosta* (Ushimaru & Nataka 2002), and often concomitant with a reduction in flower size in *Amsinckia*, *Limnanthes*, and *Epilobium* (Ornduff & Crovello 1968; Olmstead 1989; Parker et al. 1995; Schoen et al. 1997).

Our results suggest that shifts between autogamy and xenogamy are strongly correlated with simultaneous changes in flower size, pollen-ovule ratio, and anther-stigma distance (Table 1, Fig. 4A, B, 5A, B). Small autogamous flowers with anthers that touch stigmas and relatively low pollen-ovule ratios have arisen independently in numerous unrelated clades, including those comprising *Ipomoea* ser. *Batatas* (*I. triloba*), *I.* sect. *Calonyction* (*I. muricata*), *I.* sect. *Exogonium* s. lat. (*I. aristolochiaefolia*, *I. dumetorum*, and *I. meyeri;* Fig. 1 F&E, Fig. 2 B), *I.* sect. *Microsepalae* (*I. minutiflora*; Fig. 1 D), *I.* sect. *Mina* (*I. hederifolia*), *I.* ser. *Pharbitis* (*I. neurocephala*), *I.* sect. *Tricolores* (*I. cardiophylla*; Fig. 2 D), and *I.* sect. *Jalapae* (*I. amnicola*; see Table 1, McDonald 1982, 1991; Chemás Jaramillo & Bullock 2002). Such characteristics contrast with typical floral features of xenogamous relatives, whose flowers are normally large, brightly pigmented, herkogamous (Fig. 1 A–C, Fig. 2 A, C), and exhibit high pollen-ovule ratios (Table 1).

Pollen:ovule ratios can serve as a reliable indicator of breeding system. High pollen-ovule ratios are normally associated with obligate outcrossing behaviors, moderate pollen-ovule ratios with facultatively xenogamy, and low ratios with obligate autogamy (Cruden 1977, 2000; Lord & Eckhard 1984; Plitman & Levin 1990). As noted earlier, pollen-ovule ratios in our broad sampling of both autogamous and xenogamous taxa within *Ipomoea* varied considerably from 51–3100 grains per ovule (McDonald 1982; Chemás Jaramillo & Bullock 2002, Erbar & Langlotz 2005), and therefore provide a model system to examine this variation in a phylogenetic context. Most xenogamous taxa produced more than 400 pollen grains per ovule, roughly 2–14 times their autogamous relatives (Table 1). Lower pollen-ovule ratios (51–223:1) were invariably associated with autogamous relatives and lineages (Table 1; Fig. 3). While some autogamous species, such as *I. amnicola*, *I. clavata*, and *I. fimbriosepala* in *Ipomoea* subg. *Eriospermum*, exhibited relatively high pollen-ovule ratios (458–1172:1), these values are still 4–5 times lower than their closest xenogamous relatives [i.e., *I. leptophylla* (2175:1) and *I. setifera* (2971:1)]. Hence the quantitative relationship between pollen-ovule ratios of outcrossing and inbreeding taxa is not measurably precise, but predictably disparate in relative terms between closely related autogamous and xenogamous species.

While this study has focused on the evolution of floral traits associated with autogamy, other traits, such as life histories, are likely to be associated with shifts in breeding system. For example, a considerable portion of selfing *Ipomoea* species are annuals and share close ancestry with a xenogamous and (usually) perennial species (Fig. 3). Such associations are exemplified by the following species-pairs: *I. costellata* and *I. ternifolia*; *I. muricata* and *I. santillanii*; *I. purpurea* and *I. pubescens*; I. *cardiophylla* and *I. tricolor* (Fig. 2 C & D); *I. minutiflora* and *I. microsepala* (Fig. 1D); *I. meyeri* and *I. variabilis* (Fig. 2 A & B); *I. dumetorum* and *I. simulans/I. purga*; *I. fimbriosepala* and *I. pes-caprae*, and *I. amnicola* and *I. pandurata/I. leptophylla*.. Exceptions to this pattern include several selfing perennials, such as *I. amnicola*, *I. clavata*, and *I. fimbriosepala*, which belong to the woody and hairy seeded *Ipomoea* subgenus *Eriospermum* (*sensu lato*, Fig. 3; Table 1).

Additional research might consider the genetic and evolutionary mechanisms by which these autogamous lineages have arisen from xenogamous ancestors. Self-compatibility probably arises independently by unique point mutations at the S-locus (Kowyama et al. 2000), and the convergent evolution of floral syndromes associated with inbreeding habits likely arises secondarily and independently on account of shifts in selective forces on flower phenotypes. Without positive selection for floral features that attract and reward animal vectors, selection will favor floral reductions that allow more resource allocation for growth and seed production (Cruden & Miller-Ward 1981; Charnov 1982; Williams & Rouse 1990; Kirk 1992; Barrett et al. 1996; Goodwillie 2009). Therefore, autogamous species tend to produce relative smaller flowers that are less vivid in color (Richards 1997; Tate & Simpson 2004). The repeated evolution of the same suite of floral characters in association with selfing presents the opportunity to examine how often parallel trait evolution is caused by parallel genetic mechanisms.

Finally, *Ipomoea* offers a prime system for testing the effects of breeding system transitions on the fate

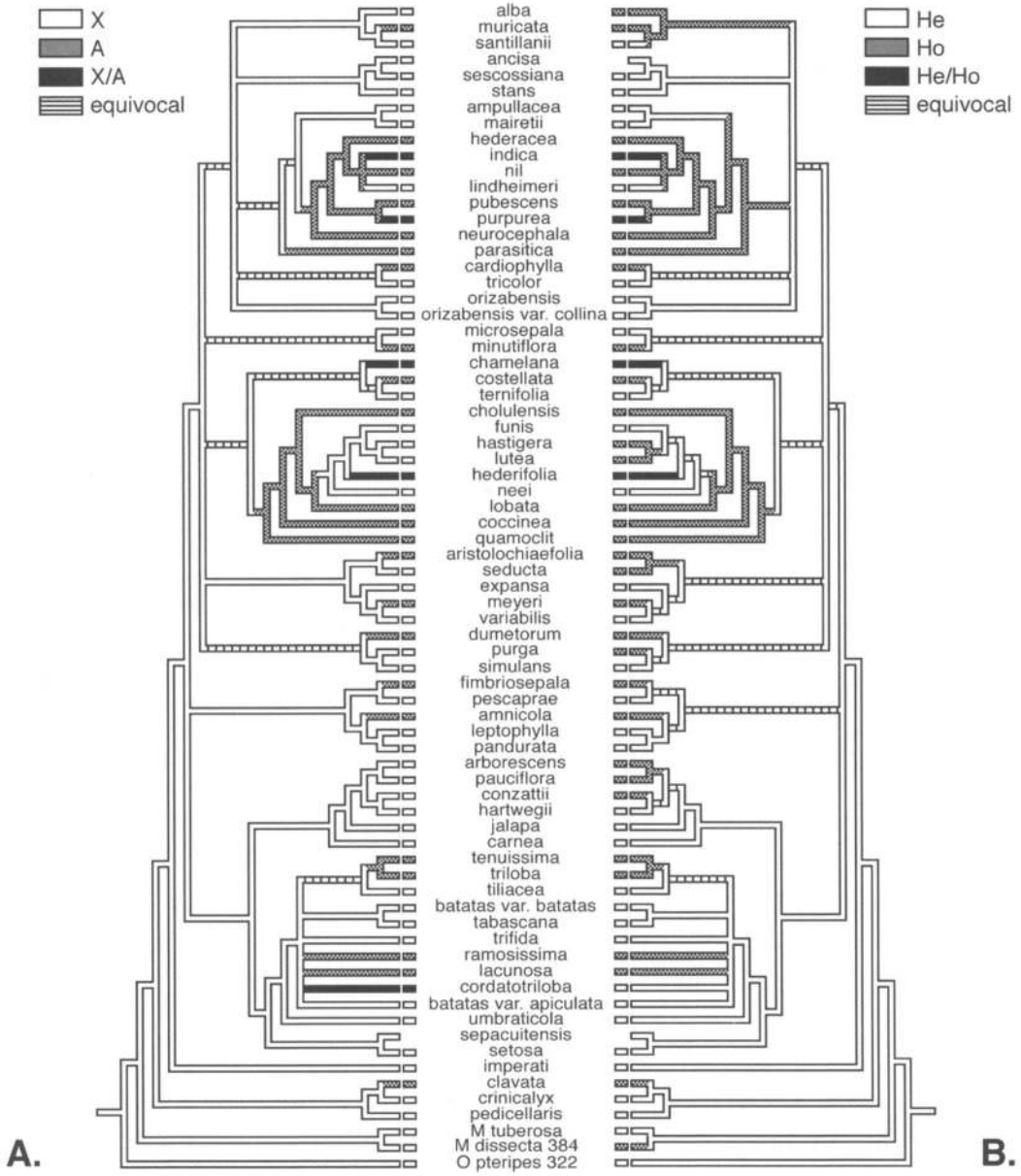

Fig. 4. Optimizations of breeding system and floral characters on the tree shown in Fig. 3. A. Breeding systems: xenogamy (X), autogamy (A), or mixed (XA). B. Anther and stigma separation: herkogamy (He), Homogamy (Ho), or mixed (He, Ho).

of lineages. Recently developed models by Maddison et al. (2007) make it possible to test statistically the effect of a binary character on rates of speciation and extinction. With such methods, one can determine whether selfing lineages diversify less than outcrossing lineages and whether the differences in species richness between the selfers and outcrossers is attributable to differential extinction, speciation, or both of these processes. Although these analyses will require more intensive taxon sampling, they hold the possibility of determining whether transitions to selfing represent evolutionary dead-ends.

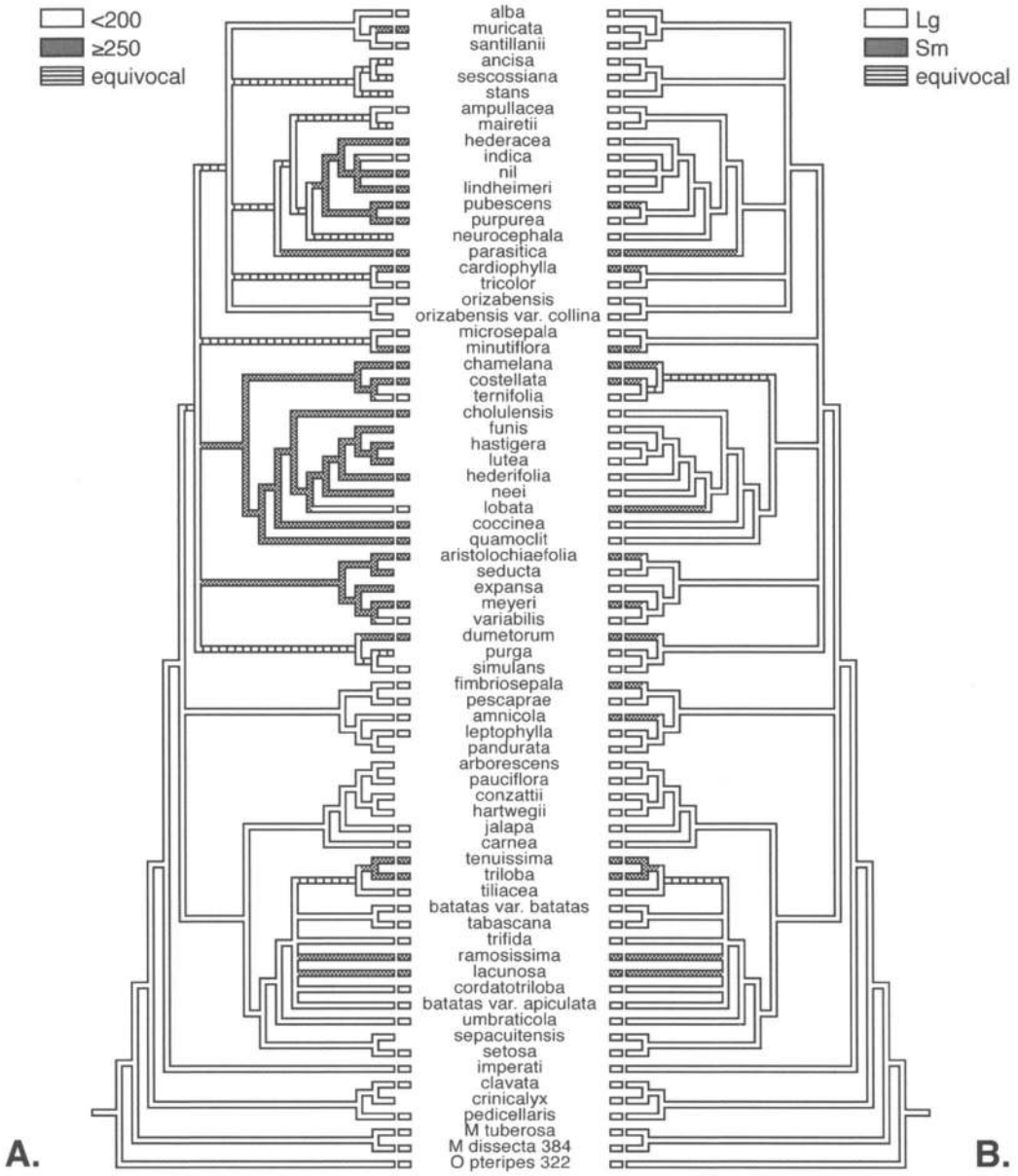

Fig. 5. Optimizations of breeding system and floral characters on the tree shown in Fig. 3. A. Pollen:ovule ratio with ratios below 250 (Table 1) as one discrete class, values above 250 as the second class, and a third class as equivocal values. B. Corolla size scored as large, small, or equivocal.

The correspondence of outcrossing features on outcrossing clades as shown in figures 4 and 5 appears to be strongest with respect herkogamy vs. homomorphic styles and stamens, while a relatively weaker correspondence is observed in pollen:ovule ratios and corolla lengths. Exceptions to the evolution of correlative characters in the latter characteristic can be attributed to various factors. As regards corolla length, for example, a less consistent correspondence between relatively large corollas and inbreeding behaviors in *I. muricata* (*Ipomoea* sect. *Calonyction*; Figs. 3, 5) and various species in *Ipomoea* sect. *Mina* (i.e., *I. quamoclit, I.*

coccinea, I. cholulensis; Figs. 3, 5) can be attributed to the unique pollination syndromes of these two groups (hawkmoth and hummingbird pollination syndromes, respectively), whose outcrossing relatives generally present extraordinarily long and narrow corolla tubes. Alternatively, relatively less congruency is observed in pollen:ovule ratios (Fig. 5) and breeding systems on account of our incomplete data set for pollen counts and an exceedingly wide range of variation in this floral parameter. Nevertheless, character optimization analyses generally reveal consistent phylogenetic correlations between autogamy, homomorphic sexual structures and low pollen:ovule ratios.

APPENDIX 1

Voucher and DNA sample information on *Ipomoea* species examined for ITS sequence variation, including (in order): taxon examined, DNA accession number, plant locality, collector, and GenBank accession number. McDonald vouchers are located at XAL or TEX; Miller vouchers are maintained at Southeastern Louisiana University (SLU), Hammond, Louisiana. Germplasm from SRPIS (USDA-Southern Regional Plant Introduction Station, Griffin, Georgia) provided fresh material. GenBank accession numbers for new sequences are presented in **boldface.**

Outgroup. *Merremia tuberosa* (L.) Rendle, REM 19, Unknown, Miller 8 (SLE), AF110909; *Merrmia dissecta* (Jacq.) Hallier f., REM 384, Chihuahua, Mexico, *Miller 305*, GQ388262; *Operculina pteripes* (G. Don) Odonell, REM 322, Jalisco, Mexico, *Miller 365*, GQ388263.

Ingroup. *I. alba* L., REM 129, Oaxaca, Mexico, *McDonald 1926* (SLE), AF538275; *I. amnicola* Morong., REM 3, SRPIS-538265, Texas, USA, *Miller 3* (SLE), AF110928; *I. ampullacea* Fernald, REM 124, Jalisco, Mexico, *Lott 2362* (MEXU), AF538277; *I. ancisa* H. H. House, REM 11, Sonora, Mexico, *van Devender 97-1263* (TEX), **DQ355304;** *I. arborescens* (H.B.K.) G. Don, REM 38, SBE Universal Seedbank, *Miller 84* (SLE), AF110924; *I. aristolochiifolia* G. Don, JAM 6, Jalisco, Mexico, *McDonald 238* (TEX), **DQ355309;** *I. batatas* (L.) Lam. var. *batatas,* REM 1, SRPIS- 561558, Mexico, *Miller 39* (SLE), AF110938; *I. batatas* (L.) Lam. var. *apiculata* (M. Martens & Galeotti) J.A. McDonald & D.F. Austin, JAM 33, Veracruz, Mexico, *McDonald 1949* (TEX), **DQ355319;** *I. cardiophylla* A. Gray, REM 134, New Mexico, USA, *McDonald 141* (SLE), AY538280; *I. carnea* Jacq. var. *fistulosa* (M. Mart. ex Choisy) D.F. Austin, B&T World Seeds – 1472, *Miller 6* (SLE), AF 110920; *I. chamelana* J.A. McDonald, REM 153, Jalisco, Mexico, *McDonald 1930* (SLE), AY538281; *I. cholulensis* H.B. & K, JAM 2, Veracruz, Mexico, *McDonald 1886* (TEX), **DQ355305**; *I. clavata* v. Ooststr. ex Macbride, JAM 30, Veracruz, Mexico, Lascurrain s.n. (TEX), **DQ355325**; *I. coccinea* L., REM 13, North Carolina, USA, *Miller 47* (SLE), AF110941; *I. conzattii* Greenm., REM 44, B&T World Seeds-32404, *Miller 71* (SLE), AF110927; *I. cordatotriloba* Dennstedt, REM 52, B&T World Seeds-74931, *Miller 73* (SLE), AF110939; *I. costellata* Torr., JAM 1, Sonora, Mexico, *van Devender 20001-859* (TEX), **DQ335306**; *I. crinicalyx* S. Moore, WMC 8329, Jalisco, Mexico, *Bullock 2000* (Kew), AF309164; *I. dumetorum* Willd. ex Roem. & Schult., REM 147, New Mexico, USA, *McDonald 140* (SLE), AF538284; *I. expansa* J.A. McDonald, REM 135, Guerrero, Mexico, *McDonald 1910* (SLE), AY538285; *I. fimbriosepala* Choisy, JAM 44, Veracruz, Mexico, *Calzada 5985* (XAL), **DQ355315;** *I. funis* Schlecht. & Cham., REM 123, Guerrero, Mexico, *McDonald 1895* (SLE), AY538286; *I. hastigera* H.B.K., REM 139, Veracruz, Mexico, *McDonald 1998* (SLE), AY538287; *I. hartwegii* Benth., JAM 13, Querétaro, Mexico, *Carranza & Silva 6355* (TEX), **DQ335313**; *I. hederacea* Jacq., REM 163, Georgia, USA, *Miller 330* (SLE), AY538291; *I. hederifolia* L., REM 183, Mexico, Miller 318 (SLE), AY538294; *I. imperati* (Vahl) Griseb., North Carolina, USA, *Miller 99* (SLE), AF110917; *I. indica* (Burm. f.) Merr., REM 168, Madagascar, *Miller 256* (SLE), AY538297; *I. jalapa* (L.) Pursh, JAM 29, Tamaulipas, Mexico, *McDonald 1288* (XAL), **DQ335316**; *I. lacunosa* L., JAM 20, Missouri, USA, *Brant 4182* (TEX), **DQ335324**; *I. lindheimeri* A. Gray, REM 25, SPRIS-553011 (Texas, USA), *Miller 31* (SLE), AF110944; *I. leptophylla* Torr., REM 4, SRPIS-303327, *Miller 30* (SLE), AF110929; *I. lobata* (Cerv.) Thellung, REM 39, B & T World Seeds – 34004, *Miller 7* (SLE), AF110940; *I. lutea* Hemsl., REM 141, Chiapas, Mexico, *McDonald 1994* (SLE), AF538299; *I. mairetii* Choisy, REM 137, Chiapas, Mexico, *McDonald 2024* (SLE), AY538300; *I. meyeri* (Spreng.) G. Don, JAM 3, Jalisco, Mexico, *McDonald 1939* (TEX), **DQ335311;** *I. microsepala* Benth., JAM 4, Oaxaca, Mexico, *Elorsa 2801* (TEX), **DQ335307;** *I. minutiflora* (M. Mart. & Galeotti) House, JAM 5, Mexico, Mexico, *McDonald 174* (TEX), **DQ335308**; *I. muricata* Cav. REM 144, Jalisco, Mexico, *McDonald 1940* (SLE), AY538332; *I. neei* (Spr.) O'Donell, REM 140, Jalisco, Mexico, *Bullock & Martijena 2098* (SLE), AF538302; *I. neurocephala* Hallier, REM 145, Michoacan, Mexico, *McDonald 1963* (SLE), AY538303; *I. nil* (L.) Roth, REM 195, New Mexico, USA, *Miller 264* (SLE), AY538308; *I. orizabensis* (Pell.) Led. ex Steudl. var. *orizabensis,* REM 142 (SLE), Veracruz, Mexico, *McDonald 2434*, AY538309; *I. orizabensis* (Pell.) Led. ex Steudl. var. *collina* (H. H. House) J.A. McDonald, JAM 12, Nuevo Leon, Mexico, *Hinton 23668* (TEX), **DQ335303;** *I. pandurata* (L.) G. Mey., REM 48, Durham Co., North Carolina, *Miller 98* (SLE), AF110930; *I. parasitica* (H.B.K.) G. Don, REM 179, Morelos, Mexico, *Miller 333* (SLE), AY538313; *I. pauciflora* M. Mart. & Galeotti, JAM 25, Puebla, Mexico, *McDonald 2010* (XAL), **DQ335314**; *I. pedicellaris* Benth., REM 97, Mexico, *Miller 185* (SLE), AF309165; *I. pes-caprae* L., REM 8, Kew Botanical Garden (Mali), *Miller 51* (SLE), AF110932; *I. pubescens* Lam., REM 76, Mexico, *Miller 113* (SLE), AF538314; *I. purga* (Wender.) Hayne, REM 133, Veracruz, Mexico, *Linajes s.n.* (SLE), AY538315; *I. purpurea* (L.) Roth, REM 171, North Carolina, USA, *Miller 254* (SLE), AY538322; *I. quamoclit* L., REM 167, Surinam, *Miller 181* (SLE), AF538323; *I. ramosissima* Choisy, JAM 56, Guanacaste, Costa Rica, *Moraga 604* (MO), **DQ335323;** *I. santillanii* O'Donell, REM 138, Veracruz, Mexico, *McDonald 1946* (SLE), AY538324; *I. seducta* House, REM 146, Chiapas, Mexico, *McDonald 1986* (SLE), AY538325; *I. sepacuitensis* Donn. Sm., JAM 61, Chiapas, Mexico, *E. Martinez S. 16636* (TEX), **DQ335317;** *I. sescossiana* Baillon, REM 143, Zacatecas, *McDonald s.n.*, AY538326; *I. setosa* Ker Gawl., JAM 47, SRPIS 6162, *Griffin 6162*, **DQ335318;** *I. simulans*

Hanbury, JAM 9, Nuevo Leon, Mexico, *Patterson 6095* (TEX), **DQ335312;** *I. stans* Cav., REM 136, Puebla, Mexico, *McDonald s.n.* (SLE), AY538327; *I. tabascana* J.A. McDonald & D.F. Austin, Tabasco, Mexico, SRPIS (518479), AF256622, AF256623; *I. tenuissima* Choisy, JAM 49, Florida, USA, SRPIS (553012), **DQ355322;** *I. ternifolia* Cav., REM 47, B & T World Seeds – 64552, *Miller 78* (SLE), AF110942; *I. tiliacea* (Willd.) Choisy, JAM 34, Quintana Roo, Mexico, *McDonald 2020* (TEX), **DQ335321;** *I. tricolor* Cav., REM 16, Shepherd's Garden Seed, *Miller 52* (SLE), AF110936; *I. trifida* (H.B.K.) G. Don, JAM 38, Oaxaca, Mexico, *McDonald 2222* (TEX), **DQ335320;** *I. triloba* L., DLP2943, Mexico, Huang 14, AF256636, AF256637; *I. umbraticola* H.H. House, REM 29, SRPIS-561557, *Miller 24* (SLE), AF110836; *I. variabilis* (Cham. & Schlecht.) Choisy, JAM 10, Chiapas, Mexico, *McDonald s.n.* (TEX), **DQ335310.**

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