

Adaptive associations among life history, reproductive traits, environment, and origin in the Wisconsin angiosperm flora

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PREMISE: We tested 25 classic and novel hypotheses regarding trait–origin, trait–trait, and trait–environment relationships to account for flora-wide variation in life history, habit, and especially reproductive traits using a plastid DNA phylogeny of most native (96.6%, or 1494/1547 species) and introduced (87.5%, or 690/789 species) angiosperms in Wisconsin, USA.

METHODS: We assembled data on life history, habit, flowering, dispersal, mating system, and occurrence across open/closed/mixed habitats across species in the state phylogeny. We used phylogenetically structured analyses to assess the strength and statistical significance of associations predicted by our models.

RESULTS: Introduced species are more likely to be annual herbs, occupy open habitats, have large, visually conspicuous, hermaphroditic flowers, and bear passively dispersed seeds. Among native species, hermaphroditism is associated with larger, more conspicuous flowers; monoecy is associated with small, inconspicuous flowers and passive seed dispersal; and dioecy is associated with small, inconspicuous flowers and fleshy fruits. Larger flowers with more conspicuous colors are more common in open habitats, and in understory species flowering under open (spring) canopies; fleshy fruits are more common in closed habitats. Wind pollination may help favor dioecy in open habitats.

CONCLUSIONS: These findings support predictions regarding how breeding systems depend on flower size, flower color, and fruit type, and how those traits depend on habitat. This study is the first to combine flora-wide phylogenies with complete trait databases and phylogenetically structured analyses to provide powerful tests of evolutionary hypotheses about reproductive traits and their variation with geographic source, each other, and environmental conditions.

KEY WORDS community assembly; convergence; flower color; flower size; life history; phylogenetically structured analyses.

The life history, growth form, and reproductive traits of angiosperms—especially flower size, color, sexuality, and mode of seed dispersal—should be shaped by whether species are introduced or native (Baker, 1965, 1974), by the other traits that they bear (Bawa, 1980; Givnish, 1980, 1982a; Barrett, 2002; Friedman and Barrett, 2009, 2011), and by the ecological conditions they inhabit (Whitehead, 1969, 1983; Regal, 1982; Givnish, 1999; Givnish et al., 1999, 2009; Givnish and Patterson, 2000). A comprehensive understanding of the distribution of reproductive traits in a flora should thus depend proximally on the trait–origin, trait–trait, and trait–environment associations observed, and ultimately on whether these accord with a priori evolutionary hypotheses.

Here, we take a first step toward such an understanding by testing classic and novel hypotheses about how life history, growth form, and reproductive traits should differ between native and introduced species, and how they should vary with each other and with habitat openness within native species, across the angiosperm flora of Wisconsin. Our aim is to determine whether trait-based hypotheses suffice to account for substantial amounts of the variation in these ecologically important traits across an entire flora, based on the hypothesized effects of species origin, other traits, and habitat openness. This study makes novel contributions by testing the impact of habitat openness on floral and fruit traits and examining the cascading effects of habitat openness

on breeding systems via such traits over an entire regional flora, while controlling for phylogenetic relationships. Previous studies, restricted to individual clades, predicted and found that open habitats favor larger flowers and dry, wind-dispersed fruits while shady habitats favor smaller, visually less conspicuous flowers and fleshy, animal-dispersed fruits in order Liliales (Givnish and Patterson, 2000; Patterson and Givnish, 2002) and in monocots as a whole (Givnish et al., 2005; Givnish et al., 2006). One important question is whether such clade-specific patterns emerge from broader, flora-wide analyses. A second question is whether the expected differences between native and introduced species in life history, growth form, and reproductive traits are seen when analyzed across a flora using a highly resolved phylogeny. A third question is whether we observe the predicted cascade of effects: from habitat openness, to floral size, floral color, and fruit dispersal type, to breeding system.

Wisconsin is an excellent venue for such studies, given its ecologically, morphologically, and phylogenetically diverse large flora with a nearly complete, highly resolved molecular phylogeny (Curtis, 1959; Spalink et al., 2018a,b; Table 1). Wisconsin lies at the crossroads of four biomes: the deciduous forests of the eastern United States, the needle-leaved evergreen forests of the boreal zone, the prairies of the Great Plains, and the oak savannas of the Midwest (Curtis, 1959; Epstein, 2017). Its glaciated landforms harbor many wetland communities, and the windswept and wave-washed shores of the Great Lakes support beach and dune communities like those found on the Atlantic Coast. Human activities have disturbed, fragmented, or destroyed natural vegetation on a wide scale, with >99.9% of the original prairies and savannas now lost (Nuzzo, 1986; Waller and Rooney, 2008) and with ~800 introduced species, one-third of the total angiosperm flora (Table 1). Wisconsin thus provides outstanding opportunities for studying trait–origin, trait–trait, and trait–environment correlations (e.g., Leach and Givnish, 1996, 1999; Amatangelo et al., 2014; Li et al., 2017; Ladwig et al., 2018).

Rigorous biota-wide tests of evolutionary hypotheses are uncommon (see Crawley et al., 1996; Sutherland, 2004; Creese et al., 2011; Anacker and Strauss, 2014; Givnish and Kriebel, 2017; Cortés-Flores et al., 2019) but can provide more generality than clade-specific studies because they can (1) detect shared patterns across multiple lineages, despite filtering among and within those clades by dispersal limitations and the environment; (2) focus on ecological variation in traits among a pool of species

that potentially can colonize the full set of ecologically distinctive habitats; (3) encompass a larger and statistically more powerful set of species than most clade-specific analyses; and (4) permit the simultaneous testing of a larger set of interrelated hypotheses than possible with most clade-specific analyses. Both clade- and biota-specific analyses require a phylogeny of the species involved on which to base valid statistical tests (Harvey and Pagel, 1991; Cavender-Bares et al., 2009; Li and Ives, 2017). Both kinds of analyses can suffer from poor resolution or support in the underlying phylogeny.

Our hypotheses are summarized in Table 2. We first predict that introduced species—by being associated with human disturbance and transport—should have a greater frequency than natives of the following character-states:

- *Annual life history, herbaceous growth form, and occurrence in open habitats*, based on the trade-off between allocation to reproduction and vegetative growth, the contribution of growth to competitive ability in crowded, infrequently disturbed habitats, greater seedling survival in frequently disturbed, less crowded habitats (Baker, 1965, 1974; Crawley et al., 1996; Sutherland, 2004; Pyšek and Richardson, 2007), and the role of frequent disturbance to maintaining an open canopy;
- *Visually conspicuous flower colors and larger flower sizes in animal-pollinated species*, given that both traits should be linked to life in open habitats, by being favored by strong illumination by broad-spectrum light, which should increase the visual signal to pollinators created by both traits and, thus, the reproductive benefit obtained (Givnish and Patterson, 2000; Patterson and Givnish, 2002; Givnish, 2010);
- *Hermaphroditic breeding system*, which should yield an advantage in colonizing open sites because they provide opportunities to breed at low density (or even alone via selfing (Baker, 1965, 1974; Van Etten et al., 2017). Hermaphroditism should also be favored in the larger, more conspicuous, and hence more costly flowers favored in open habitats (see previous point), because the advantage of bisexual flowers grows with flower cost because they use the same structures to attract *and* disperse pollen via animals, while unisexual flowers must build separate structures to accomplish both tasks (Givnish, 1982a);
- *Passively dispersed seeds* (including seeds moved by wind and gravity, or moved short distances by scatter-hoarding rodents)—due to a greater functional advantage of fleshy fruits dispersed

TABLE 1. Summary of traits and environments of Wisconsin angiosperms of native and introduced origins.

Origin	Native (66.2%, 1547 spp.)	Introduced (32.8%, 789 spp.)
Growth form	Herbs 59.1%, graminoids 24.4%, shrubs 10.0%, trees 3.7%, vines 2.7%	Herbs 73.4%, graminoids 12.9%, shrubs 6.7%, trees 3.5%, vines 3.4%
Dominant life history	Annuals 14.1%, biennials 2.2%, perennials 83.7%	Annuals 40.3%, biennials 6.0%, perennials 53.7%
Flower colors	Red 10.3%, yellow/orange 12.8%, blue 5.9%, cream 2.5%, white 23.5%, green/brown/none 41.2%, combination 3.7%	Red 18.9%, yellow/orange 21.4%, blue 10.1%, cream 2.0%, white 17.4%, green/brown/none 25.9%, combination 4.3%
Flower size	Petal/ligule length 0–125 mm; mean 6.6 ± 10.0 mm	Petal/ligule length 0–160 mm; mean 9.5 ± 14.1 mm
Pollinator	Biotic 64.4%, abiotic 34.2%, both 1.4%	Biotic 78.2%, abiotic 21.0%, both 0.8%
Breeding system	Hermaphroditism 73.9%, monoecy 18.9%, dioecy 4.7%, other 2.2%	Hermaphroditism 88.1%, monoecy 6.3%, dioecy 3.5%, other 1.9%
Dispersal type	Fleshy fruits 12.0%, other 88.0%	Fleshy fruits 9.6%, other 90.4%
Habitat occurrence	Open 75.4%, mixed 46.0%, closed 24.2%	Open 93.8%, mixed 26.0%, closed 4.1%
Understory herb	Sunny 44.4%, shady 55.6%	—
flowering season ^a		

^aFor herbaceous species occurring only in closed habitats.

TABLE 2. Hypotheses regarding associations among life history, growth form, floral and fruit traits, breeding system, and habitat openness in Wisconsin angiosperms.

1—Introduced plant species should have a higher frequency than natives of the following character-states:	
1.1	Annual life history
1.2	Herbaceous growth form
1.3	Larger flower sizes
1.4	Visually conspicuous flower colors
1.5	Hermaphroditic breeding system
1.6	Passively dispersed seeds
1.7	Occurrence in open habitats vs. closed or mixed habitats
2—Trait–trait associations expected in the native flora include the following:	
2.1	Hermaphroditism should be more common in animal-pollinated species and in species with larger, visually more conspicuous flowers
2.2	Unisexual flowers should be smaller and have less conspicuous colors
2.3	Dioecy should be more common in woody species
2.4	Dioecy should be more common in species with small, visually inconspicuous flowers
2.5	Dioecy should be more common in species with fleshy fruits
2.6	Dioecy should be more common in species combining small, inconspicuous flowers with fleshy fruits, and its incidence should decrease with flower size
2.7	Monoecy should be more common in species combining small, inconspicuous flowers with dry fruits and its incidence should decrease with flower size
3—Trait–environment associations expected in the native flora include the following:	
3.1	Abiotic (wind) pollination should be more frequent in open habitats than in non-open, less windy habitats
3.2	Larger flowers should be more frequent in open vs. non-open habitats
3.3	Visually conspicuous flower colors should be more frequent in open habitats than elsewhere
3.4	Fleshy fruits should be less frequent in open vs. non-open habitats
3.5	Dioecy should be less common in open habitats, which favor larger, more conspicuous flowers and dry fruits
3.6	Abiotic (wind) pollination should be more frequent in closed vs. non-closed habitats
3.7	Larger flowers should be less frequent in closed vs. non-closed habitats
3.8	Visually conspicuous flower colors should be less frequent in closed habitats than elsewhere
3.9	Fleshy fruits should be more frequent in closed vs. non-closed habitats
3.10	Dioecy should be more common in closed vs. non-closed habitats, which favor smaller, less conspicuous flowers and fleshy fruits
3.11	Alternatively, dioecy should be more common in open habitats, if the high incidence of wind pollination favored in such habitats compensates for the low incidence of fleshy fruits

long distances by birds in shaded, relatively windless habitats, and the greater windiness of open habitats favoring wind-dispersed seeds (Givnish, 1999; Givnish et al., 1999, 2005, 2009; Patterson and Givnish, 2002); and

- *Occurrence in open habitats vs. closed or mixed habitats*, due to the association of open canopies (especially in mesic climates) with the frequent disturbances that favor introduced weeds (Baker, 1965, 1974).

Within the native flora, we predict the following trait–trait correlations based on arguments regarding ecological drivers of breeding systems summarized by Givnish (1982a). These are based on how larger flowers (or more costly attractants and rewards) should increase the cost disadvantage of unisexual vs. hermaphroditic flowers in attracting pollinators, favoring hermaphroditic flowers; and on how dispersal effectiveness should show plateauing returns to investment for passively dispersed seeds or those dispersed just short distances (i.e., declining returns to scale due to local saturation of appropriate germination sites) and greater than linear returns to investment for fleshy fruits due to larger crops attracting disproportionately more birds (i.e., increasing returns to scale). These considerations imply that:

- *Hermaphroditism* should be more common in animal- vs. wind-pollinated species, and its frequency should increase in species with larger, more costly flowers regardless of seed dispersal mechanism;
- *Unisexual flowers* should be smaller (Givnish, 1982a; Friedman and Barrett, 2009, 2011) and have less conspicuous colors (Givnish and Patterson, 2000; Givnish, 2010);

- *Dioecy* should be more common in woody species, in order to avoid selfing via geitonogamy (based on crosses among different flowers on the same individual), given that chances of geitonogamy should increase with plant size (Bawa, 1980; Lloyd, 1982; de Jong, 2000; Barrett, 2002), or because a greater range of fruit crop sizes might attract seed-dispersing birds to an ever more disproportionate degree;
- *Dioecy* should be more common in species with fleshy fruits (Bawa, 1980; Givnish, 1980, 1982a), given the likely advantage of unisexual females producing larger fruit crops via resource reallocation from male function, and thereby obtaining a disproportionate advantage in seed dispersal by birds attracted to large fruit crops;
- *Dioecy* should be more common in species with small, visually inconspicuous flowers (see above);
- *Dioecy* should be most common in species combining fleshy fruits with small, inconspicuous animal- or wind-pollinated flowers (Bawa, 1980; Givnish, 1980, 1982a); the incidence of dioecy should be low in species with passively dispersed seeds and should decrease with flower size or visual conspicuousness (Givnish, 1982a); and
- *Monoecy* should be most common in species combining small, inconspicuous flowers with passively dispersed seeds, and its incidence should decrease with flower size or visual conspicuousness (Givnish, 1982a).

Similarly, in regard to trait–environment correlations within the native flora, we predict that:

- *Abiotic (wind) pollination* should be more frequent in *open habitats*—which are exposed to more wind—than in more closed, less windy habitats, which provide less impetus for transport of pollen via air movement (Whitehead, 1969, 1983; Regal, 1982; Weller et al., 1998; Culley et al., 2002);
- *Visually conspicuous flower colors and larger flower sizes* should be more frequent among animal-pollinated species in *open habitats* than elsewhere, given their greater value under brighter, broader-spectrum light (Givnish and Patterson, 2000; Patterson and Givnish, 2002; Givnish, 2010), and *should occur in closed habitats mainly among species flowering when the canopy is open* (e.g., before leaf-out in spring);
- *Fleshy fruits* should be more common in shaded, nearly windless understories of *closed habitats* where they provide a greater advantage over wind-dispersed seeds (Givnish, 1999; Givnish et al., 1999, 2005, 2009; Patterson and Givnish, 2002);
- *Dioecy* should be more common in *closed habitats*, which favor fleshy fruits and smaller, less conspicuous animal-pollinated flowers (see above); and
- Alternatively, *dioecy* should be more common in open habitats, if the high incidence of wind pollination favored there (see above) compensates for the low incidence of fleshy fruits.

Here, we test the preceding hypotheses among all native and introduced angiosperms in Wisconsin. To do this, we combine a molecular phylogeny for almost all these species with data on their functional traits and ecological distributions and then use phylogenetically structured analyses of these data to evaluate each hypothesis.

MATERIALS AND METHODS

Phylogeny

We used the RAxML phylogeny for the vascular flora of Wisconsin and nearby parts of northeastern North America derived by Spalink et al., 2018a,2018b), based on aligned sequences of plastid *rbcL*, *matK*, *atpB*, *atpF-atpH*, *ndhF*, *rpl32*, and *trnH-psbA*, and here restricted to species of angiosperms that are native or introduced to Wisconsin (Appendix S1). This phylogeny was calibrated against time using treePL (Smith and O'Meara, 2012), using 59 dates from Magallon et al. (2013) and Rothfels et al. (2015) as calibration points (see Spalink et al., 2018a,2018b), and is deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.qfttdz0fb> [Givnish et al., 2020]).

Trait and environment database

We scored each species for *origin* (native or introduced), *life history* (annual, biennial, perennial, or combinations thereof), *growth form* (graminoid, non-graminoid herb, shrub, tree, or vine), *flower size* (petal or ligule length in millimeters), *flower color* (blue, red/pink, yellow/orange, green/brown/absent, cream, white, or combinations thereof), *pollinator* (biotic or abiotic), *breeding system* (hermaphroditism, monoecy, dioecy, or other), *seed dispersal type* (fleshy fruit or other—including seeds dispersed by wind, gravity, scatter-hoarding rodents, or no evident means), *presence in open habitats* (e.g., prairie,

sedge meadow, marsh, bog, beach, dune), *presence in closed habitats* (e.g., deciduous forest, needle-leaved forest), *presence in mixed habitats* (e.g., oak savanna, cedar glade, thicket, shrub carr, with a mosaic of open and closed microsites), and *flowering season* (under open [spring] or closed [summer] canopies in closed habitats). Non-open habitats include both closed and mixed habitats; non-closed habitats include both open and mixed habitats. Graminoids were considered to be species in Cyperaceae, Eriocaulaceae, Juncaceae, and Poaceae. Data were drawn from Flora North America Editorial Committee (1993+), Gleason and Cronquist (1991), the Minnesota Wildflowers website (<https://www.minnesotawildflowers.info/>), the USDA PLANTS database (<https://plants.sc.egov.usda.gov/java/>), the Calflora website (<https://www.calflora.org/>), Flora of Michigan (Voss and Reznicek, 2012), and personal observations. Character-states of individual traits or environments were non-overlapping and exhaustive except for those pertaining to life history, pollinator, and flower color. Several species are annual/biennial, biennial/perennial, annual/perennial, or annual/biennial/perennial; several species in genus *Salix* are potentially pollinated by both wind and insects; and a small number of species (especially Asteraceae) bear flowers with two dominant colors. The database is deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.qfttdz0fb> [Givnish et al., 2020]).

Character mapping

To visualize the place of origin (native vs. introduced) of plants across the phylogeny, stochastic character mapping was conducted with the R package “phytools” (Revell, 2012) for the R statistical environment (R Core Team, 2018).

Statistical tests

Tests were based on analyses of phylogenetic correlation as well as simulation-based phylogenetic analyses of variance (ANOVAs; Garland et al., 1993) implemented in phytools. To evaluate phylogenetic correlations, we divided each of the two characters involved into binary states (e.g., occurrence vs. absence in open habitats, graminoids vs. non-graminoids, dioecy vs. non-dioecy, or dominant life history annual [annual, annual-biennial, annual-perennial, annual-biennial-perennial] vs. biennial [biennial, biennial-perennial] vs. perennial). Phylogenetically structured *t*-tests and ANOVAs—and in one instance, ordinary *t*-tests and χ^2 tests—were used to evaluate quantitative differences in flower size between two or more alternative states of individual characters. Only flower size had missing data; species with missing data were excluded from analyses involving flower size. To avoid false positives for multiple familywise tests, we used the Benjamini-Hochberg procedure for the trait–origin, trait–trait, and trait–environment families of comparisons to adjust the *P*-value required for significance (McDonald, 2014). Using a false discovery rate of 0.01, no changes in nominal significance were detected for the comparisons in Tables 3–5. In some analyses, we restricted our attention to species with a particular character-state (e.g., fleshy fruits) while analyzing variation in one or two additional traits. For certain tests, we aggregated flower color into two categories: conspicuous (blue, red/pink, yellow/orange) vs. inconspicuous (all other colors), based on their ability to reflect substantial amounts of light only in open habitats and distinction from the green and brown colors of ubiquitous leaves and litter. We used both unstructured logistic regression with the glm function of the R package “stats” version 3.5.2 (R Core Team, 2018)

TABLE 3. Tests of predictions regarding trait states in introduced vs. native Wisconsin angiosperms, and observed frequencies of states in the entire flora and those species found in the molecular phylogeny. Phylogenetically structured tests are based on the latter. Test statistics shown are the Pagel log-likelihood ratio for frequency data, or the phylogenetically structured ANOVA *t* for variation in quantitative traits. Application of the Benjamini-Hochberg procedure for detection of false positives does not change the nominal significance of any findings.

Prediction	Observed in entire flora	Observed in phylogeny	Pagel LLR, pANOVA <i>t</i> , or logistic slope <i>b</i>	Significance
More annuals ^a	40.3% vs. 14.1%	40.7% vs. 14.0%	122.8	$P < 1.4 \times 10^{-25}$
More herbs	73.4% vs. 59.1%	72.0% vs. 59.4%	30.6	$P < 3.8 \times 10^{-6}$
More conspicuous flower colors	54.6% vs. 32.5%	53.6% vs. 33.0%	76.4	$P < 9.9 \times 10^{-16}$
Larger flowers ^b	9.5 mm vs. 6.6 mm	9.6 mm vs. 6.6 mm	$t = 5.90$	$P > 0.097$
More hermaphrodites	88.1% vs. 73.9%	88.3% vs. 73.7%	31.3	$P < 2.7 \times 10^{-6}$
More occurrences in open habitats	93.8% vs. 75.4%	94.1% vs. 75.4%	136.6	$P < 1.6 \times 10^{-28}$
Fewer occurrences in closed habitats	4.1% vs. 24.2%	4.2% vs. 24.0%	151.3	$P < 1.1 \times 10^{-31}$
Fewer occurrences in mixed habitats	26.0% vs. 46.0%	26.8% vs. 46.1%	76.0	$P < 1.3 \times 10^{-15}$
More grasses than other graminoids	40.6% vs. 5.0%	39.9% vs. 4.4%	62.3	$P < 9.6 \times 10^{-13}$

^aIncludes obligate annuals as well as species that can be annuals (e.g., annual-biennial species).

^bZoophilous species only.

and phylogenetically structured logistic regression with the function `phylglm` of the package “`phylolm`” (Ho and Ané, 2014) to relate shifts in the probability of alternative states (e.g., dioecy vs. non-dioecy) to continuous variation in flower size. A report providing all statistical details (including degrees of freedom), and the R code used for the analyses, are deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.qfttdz0fb> [Givnish et al., 2020]).

Following Creese et al. (2011), we calculated percipience, predictive power, and explanatory power for each familywise set of tests. *Percipience* is the proportion of significant findings out of the total number of tests; *predictive power* is the proportion of directional predictions supported at $P < 0.025$; and *explanatory power* is the number of supported predictions divided by the number of significant findings, compared with the random expectation of 0.5 (see also Pasquet-Kok et al., 2010). We also calculated *qualitative predictive power* as the proportion of correct directional predictions, and *predictive magnitude* as $|(value_1 - value_2)/(value_1 + value_2)|$, where $value_i$ is the proportion of a qualitative trait (or magnitude of a continuous trait) in species pool *i*. All five of these metrics can vary from 0 to 100%.

RESULTS

Flora and phylogeny

The angiosperm flora of Wisconsin comprises 1547 native species and 789 introduced species (Table 1; Appendix S1, Table S1),

representing roughly half the flowering plants of the northeastern United States and adjacent parts of Canada. Native species are distributed in Poisson lognormal fashion among 124 families and 510 genera, with seven families and nine genera having exceptionally large numbers of species, and accounting for 48% and 21% of the total native flora, respectively (Fig. 1). Our plastid RAXML phylogeny contains 1509 (or 97.5%) of the native species and 690 (or 87.4%) of the introduced species. Introduced species are scattered throughout the tree but are especially common in grasses (Poaceae) and in several superasterid and superrosid lineages; they are rare in monocots outside grasses, and in several small eudicot lineages (Appendix S1, Fig. S1). Appendix S1, Fig. S2 provides a detailed chronogram for Wisconsin angiosperms, with node ages and family and ordinal relationships.

Traits and environments

For all 2336 Wisconsin angiosperms, Table 1 summarizes the spectra of character-states for growth form, life history, flower color, petal or ligule length, pollinator, breeding system, mode of seed dispersal, occurrence in open, mixed, or closed habitats, and understory flowering season for native vs. introduced species. The overall trait database includes 28,032 entries, with only 0.8% missing data; all missing entries are for petal/ligule length (237 of 2336, or 10.1%). For each trait, character-states are mutually exclusive, except for those involving life history and, to a much lesser degree, biotic vs. abiotic pollination and

TABLE 4. Tests of predictions regarding trait–trait patterns in native Wisconsin angiosperms. Application of the Benjamini-Hochberg procedure does not change the nominal significance of any finding.

Prediction	Observed in entire flora	Observed in phylogeny	Pagel LLR or pANOVA <i>t</i>	Significance
More hermaphrodites in species with biotic vs. abiotic pollination	93.9% vs. 42.5% ^a	93.8% vs. 41.9% ^a	49.7	$P < 4.2 \times 10^{-10}$
Unisexual species				
Smaller flowers	0.8 mm vs. 8.7 mm	0.9 mm vs. 8.8 mm	$t = 24.2$	$P < 0.001$
Less conspicuous flower colors	2.5% vs. 43.1%	0.7% vs. 32.3%	68.1	$P < 5.8 \times 10^{-14}$
Dioecious species				
More woody species	75.0% vs. 13.6%	74.6% vs. 13.3%	46.1	$P < 2.4 \times 10^{-9}$
More fleshy fruits	40.3% vs. 10.6%	39.4% vs. 10.4%	46.0	$P < 2.5 \times 10^{-9}$
Smaller flowers	1.5 mm vs. 6.9 mm	1.5 mm vs. 6.9 mm	$t = 5.03$	$P < 0.12$
Less conspicuous flower colors	4.2% vs. 33.9%	4.2% vs. 34.4%	24.6	$P < 6.2 \times 10^{-5}$
More wind pollination	6.5% vs. 3.6% ^a	6.8% vs. 3.6% ^a	13.5	$P < 9.2 \times 10^{-3}$

^aPolymorphic species scored as wind-pollinated.

TABLE 5. Tests of predictions regarding trait–environment patterns in native Wisconsin angiosperms. Predictions contradicted by the data are shown in *italics*. Application of the Benjamini-Hochberg procedure does not change the nominal significance of any findings.

Prediction	Observed in entire flora	Observed in phylogeny	PageLLR or pANOVA <i>t</i>	Significance
Open habitats				
More anemophily	38.7% vs. 26.1%	38.7% vs. 26.1%	21.2	$P < 3.0 \times 10^{-4}$
	49.5% vs. 23.1% ^a	49.3% vs. 22.2% ^a	41.2	$P < 2.5 \times 10^{-8}$
Larger flowers	10.6 mm vs. 9.8 mm	9.8 mm vs. 9.8 mm	<i>t</i> = 0.08	$P > 0.97$
More conspicuous flower colors	33.6% vs. 29.2%	34.3% vs. 29.1%	24.7	$P < 5.7 \times 10^{-5}$
	29.8% vs. 17.5% ^a	30.0% vs. 17.0% ^a	42.9	$P < 1.2 \times 10^{-8}$
Fewer fleshy fruits	10.1% vs. 18.2%	9.5% vs. 18.8%	20.9	$P < 3.3 \times 10^{-4}$
Less dioecy	5.1% vs. 3.2%	5.2% vs. 3.3%	13.3	$P < 0.01$
Closed habitats				
More zoophily	68.7% vs. 63.0%	68.5% vs. 63.1%	13.7	$P < 8.3 \times 10^{-3}$
	76.9% vs. 50.5% ^a	77.8% vs. 50.7% ^a	41.2	$P < 2.5 \times 10^{-8}$
Smaller flowers	9.2 mm vs. 10.7 mm	9.3 mm vs. 9.8 mm	<i>t</i> = 1.32	$P > 0.60$
Less conspicuous flower colors	24.9% vs. 35.0%	24.8% vs. 35.6%	34.9	$P < 4.8 \times 10^{-7}$
	17.5% vs. 29.8% ^a	17.0% vs. 30.0%	42.9	$P < 1.2 \times 10^{-8}$
More fleshy fruits	17.9% vs. 10.1%	18.1% vs. 9.8%	31.6	$P < 2.4 \times 10^{-6}$
More dioecy	2.9% vs. 5.2%	3.1 vs. 5.3%	21.9	$P < 2.2 \times 10^{-4}$

^aBased on comparisons involving species found only in open habitats and species found only in closed habitats. Note that the second such comparison for each trait, listed under closed habitats, is simply an identical repeat of that shown under open habitats, and does not count toward the total of familywise comparisons.

flower color. Life history shows somewhat of a continuum, involving annuals, biennials, and perennials and various combinations thereof (Appendix S1, Fig. S3). However, across native Wisconsin angiosperms, all but 6.3% of species are solely annuals (10.0%) or perennials (83.7%). For native and introduced species, Venn diagrams of occurrence in open, mixed, and closed habitats show the distribution of species numbers along a coarsely defined light availability gradient (Fig. 2). Open habitats have the greatest total number of native species (1165, or 75.3%); closed habitats, the least (372, or 24.0%). Smaller sets of native species occur in more than one habitat type; 70 (4.5%) species occur in all three. Introduced species are slanted far more to brightly lit conditions, with 93.7% of these species occurring, at least part of the time, in open habitats, 26.0% in mixed habitats, and 3.9% in closed habitats (Fig. 2). Overall, 81.6% of Wisconsin angiosperms occur, at least part of the time, in open habitats, 39.2% in mixed communities, and 17.4% in closed communities (Fig. 2).

Native and introduced species account for 66.2% and 33.8% of the angiosperm flora, respectively (Table 1). Non-graminoid herbs comprise 63.9% of all species; graminoids, 20.5%; shrubs, 8.9%; trees, 3.6%; and herbaceous and woody vines, 3.0% (the differing frequencies of states in native vs. introduced species are shown in Table 2 for all traits). Introduced species show a much larger representation of annuals (30.4%) and a much smaller representation of perennials (53.7%) (Appendix S1, Fig. S3). For dominant life history across all species, we get 22.9% annuals by grouping together all species that can be annuals, 3.5% biennials by grouping together biennials and biennial-perennials, and 73.6% that are perennials only (Table 1). Red, rose, red-purple, or pink flowers (here grouped as “red”) are borne by 13.1% of Wisconsin angiosperms; yellow or orange flowers (here grouped as “yellow”) by 15.7%; blue, violet, or blue-purple flowers (here grouped as “blue”) by 7.3%; cream, light yellow, or greenish yellow (here grouped as “cream”) by 2.4%; white by 21.4%; green or brown flowers (as well as flowers with no corolla; here grouped as “green”) by 36.1%; and flowers with combinations of colors (e.g., *Symphotrichum novae-angliae* with blue ligules and yellow disc florets) by 4.0%. As can be seen in Table 1, native and

introduced species differ substantially from each other in these and most other traits.

Biotic pollination (zoophily) characterizes 69.1% of the angiosperm flora; abiotic pollination (anemophily, or wind pollination, in almost all cases), 29.7%; and both, 1.2% (all in genus *Salix*). Flower size (petal or ligule length) varies from 0 to 160 mm, with a mean of 7.5 ± 11.4 mm (SD). Hermaphroditism typifies 78.7% of all species; monoecy, 14.6%; dioecy, 4.3%; and other mating systems (e.g., gynodioecy, sequential monoecy, polymorphisms), 2.1%. Fleshy fruits dispersed by vertebrates are borne by 11.2% of all species, while 88.8% bear non-fleshy, often capsular fruits with seeds dispersed by passive or only locally effective means (e.g., wind, gravity, ectozoochory, ants, scatter-hoarding rodents; see Table 1).

Among animal-pollinated herbs found only in closed habitats, 45.4% bloom under open canopies in spring, while 54.6% bloom under closed canopies in summer. Across all traits and environments, the frequencies of character-states in species included in the phylogeny closely track those for all species (see Appendix S1, Table S2).

Traits in introduced vs. native angiosperms

Phylogenetically structured tests support all but one of our hypotheses at a very high level of statistical significance (Table 3). Introduced species, as predicted, have a higher fraction of annuals (40.3% vs. 14.1% of native species), of herbs (73.4% vs. 59.1%), of more conspicuous flower colors (54.6% vs. 32.5%, including blue, red/pink, and yellow/orange as conspicuous and white, cream, green, brown, or absent as inconspicuous), of larger animal-pollinated flowers (mean petal or ligule length of 9.5 mm vs. 6.6 mm, $P > 0.1$), of hermaphroditism (88.1% vs. 73.9%), and of occurrence in open habitats (93.8% vs. 75.4%). Introduced species also have a significantly lower incidence in closed habitats (4.1% vs. 24.2%) and mixed habitats (26.0% vs. 46.0%). Interestingly, among graminoids, grasses have a much higher incidence of introduced species (40.6% vs. 5.0%) than do other graminoid families.

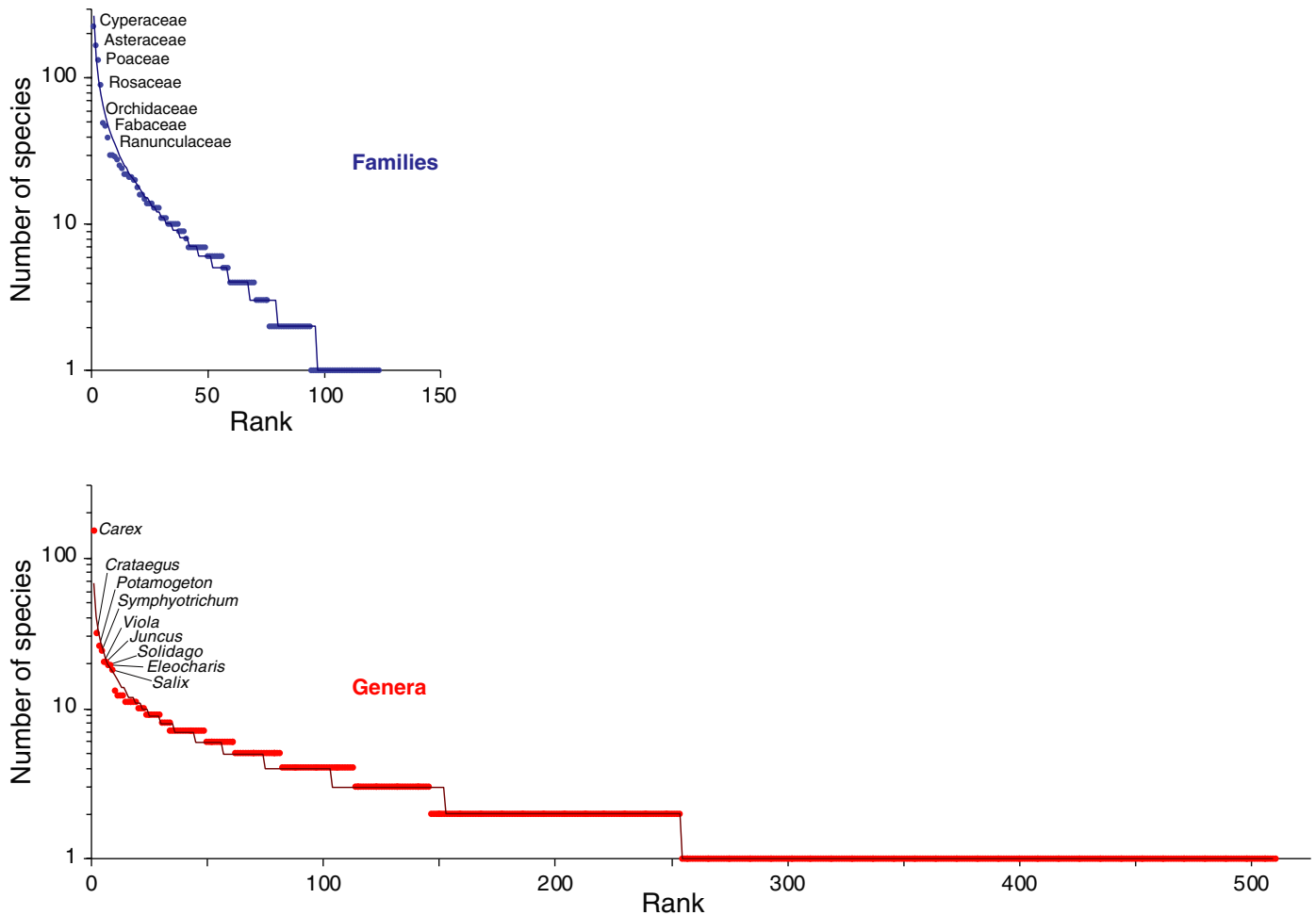


FIGURE 1. Dominance-diversity graphs for families and genera of angiosperms native to Wisconsin, showing the logarithm of the number of native species plotted against the rank of each family (or genus) based on the number of native species. Note the close approach to a log-series distribution of diversity for all but the largest families and genera. The regression curves are Poisson lognormal distributions, which represented better fits to the data than ordinary log-series or lognormal distributions (see Supplemental Material).

Trait–trait relationships in native angiosperms

Most of our hypotheses are strongly supported. Hermaphroditism is, as predicted, significantly more common than unisexuality in animal-pollinated species; flower size is smaller and conspicuous colors less frequent in unisexual vs. hermaphroditic flowers; and dioecious species are more frequently woody than hermaphrodites, more frequently bear fleshy fruits; and have flowers that are smaller, and more frequently inconspicuous in color and wind-pollinated (Table 4). Dioecy occurs in 6.5% vs. 3.6% of species with abiotic vs. biotic pollination, but fully half of all dioecious species have abiotic pollination. These patterns accord with our predictions, and almost all are statistically highly significant and explain substantial variation in trait distribution. The sole exception is that the mean flower size seen in dioecious species is not significantly smaller than that seen in other species, even though the difference in means (1.5 mm vs. 6.9 mm) is substantial.

Our data provide the first support for the predicted distribution of all three major breeding systems based on fruit type and quantitative variation in flower size. First, the incidence of unisexual flowers drops with petal or ligule length, and that of hermaphroditism rises, as

shown by highly significant logistic regressions (Fig. 3). This pattern is stronger if grasses—with their minute, wind-pollinated, but often hermaphroditic flowers—are excluded. Second, the incidence of dioecy increases with decreasing flower size in species with fleshy fruits, and is essentially absent among those with passive seed dispersal (Fig. 4A, B). Third, the incidence of monoecy increases with decreasing flower size in species with passive seed dispersal, and is essentially absent in species with fleshy fruits (Fig. 4C, D). Taken together, these results strongly support the predictions by Givnish (1982a) regarding the importance of ecological drivers of plant breeding systems.

Trait–environment relationships in native angiosperms

We predicted that, in open habitats, wind pollination should be more frequent, flowers should be larger and have more conspicuous colors, and fleshy fruits and dioecy should be less common, unless the greater incidence of wind pollination in open habitats favors enough cases of dioecy to overwhelm the trend expected in animal-pollinated plants (Table 1). In closed habitats, the converse propositions should be true (Table 2). Our data support nine of these 11 predictions, seven in significant fashion (Table

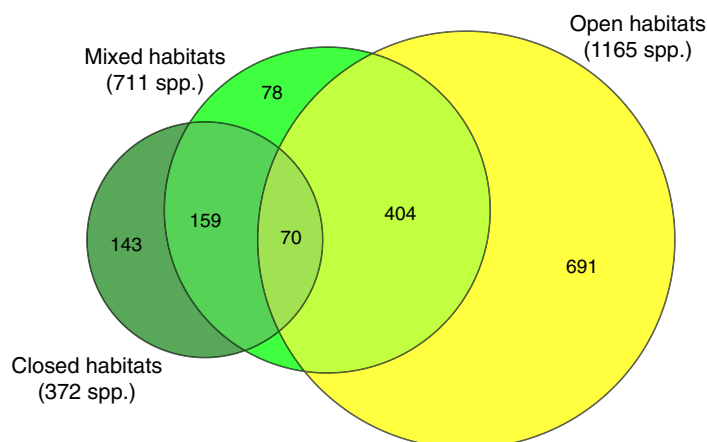
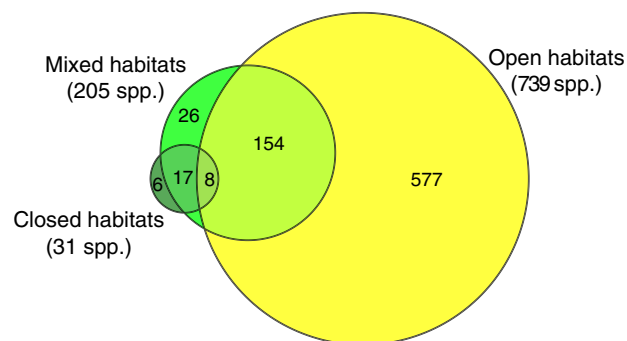
A Native**B Introduced**

FIGURE 2. Venn diagrams of occurrence in open, mixed, and closed habitats for native and introduced species. The areas and the enclosed numbers show the distribution of species in each habitat type or combination thereof, along a coarsely defined light availability gradient from open to closed habitats. Note the much higher incidence of introduced species in open habitats, and the much lower incidence in closed habitats, in relation to the distributions of native species.

5). The greater frequency of wind pollination in open habitats is substantially more marked when comparing species found in open habitats *only* vs. closed habitats *only* (49.5% vs. 23.1%) than when comparing species found in open vs. non-open (i.e., closed and mixed) habitats. Similarly, the greater frequency of animal pollination is more marked when comparing species from open habitats *only* vs. closed habitats *only* (76.9% vs. 50.5%), as is the greater frequency of conspicuous flower colors in open habitats *only* vs. closed habitats *only* (29.8% vs. 17.5%) (Table 5). Animal-pollinated flowers are slightly larger in open habitats, and smaller in closed habitats, but not significantly so. The two, closely related deviations from our predictions are that (1) dioecy is significantly *more*

frequent in open vs. non-open habitats and (2) dioecy is significantly *less* frequent in closed vs. non-closed habitats (Table 5). These contradictions might initially seem related to the greater incidence of wind pollination there (Table 4). Calculations, however, appear to exclude this as a possibility, and other factors that might favor higher levels of dioecy in open habitats are evaluated below.

DISCUSSION

We found strong statistical support for eight of nine a priori tests regarding traits in introduced vs. native species (Table 3), and for

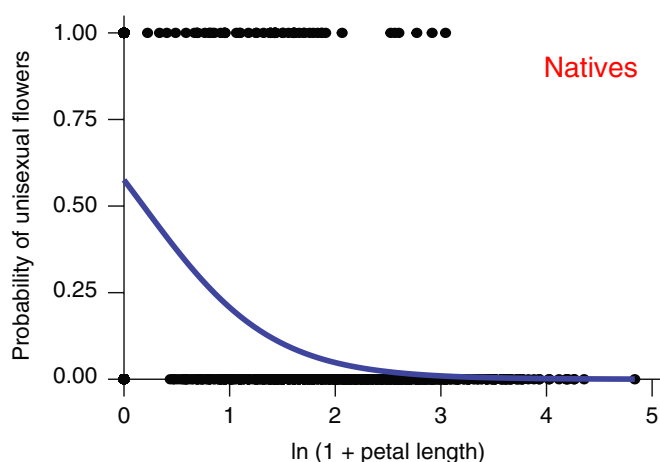
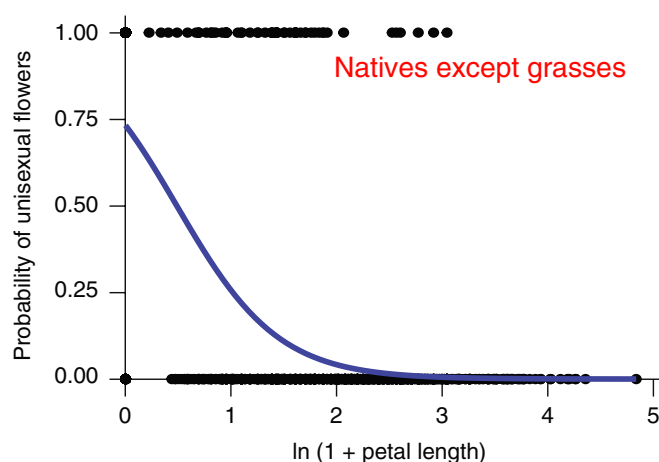
A**B**

FIGURE 3. Unstructured logistic regressions of percent unisexual species as a function of the logarithm of $z = 1 + \text{flower size (petal or ligule length)}$ for (A) all native species and (B) all natives except members of the grass family Poaceae. The grasses are mostly wind-pollinated, lack a perianth, and bear bisexual (hermaphroditic) flowers. Equations are of the form $\ln(p/(1-p)) = \alpha + \beta \ln z$, where p = probability of a species being unisexual. Values of α are 0.232 ($P < 5.6 \times 10^{-3}$) and 0.938 ($P < 2 \times 10^{-16}$) for A and B, respectively. Values of β are -1.635 ($P < 2 \times 10^{-16}$) and -2.072 ($P < 2 \times 10^{-16}$) for A and B, respectively. The values of α and β for phylogenetically structured versions of these equations are also significant, at $P < 0.00015$ and $P < 0.0052$ for A and $P < 0.05$ and $P < 2 \times 10^{-16}$ for B, respectively. The unstructured models are shown here because they are more responsive to z and provide a more easily interpretable view of the percent unisexual species as a function of $\ln z$.

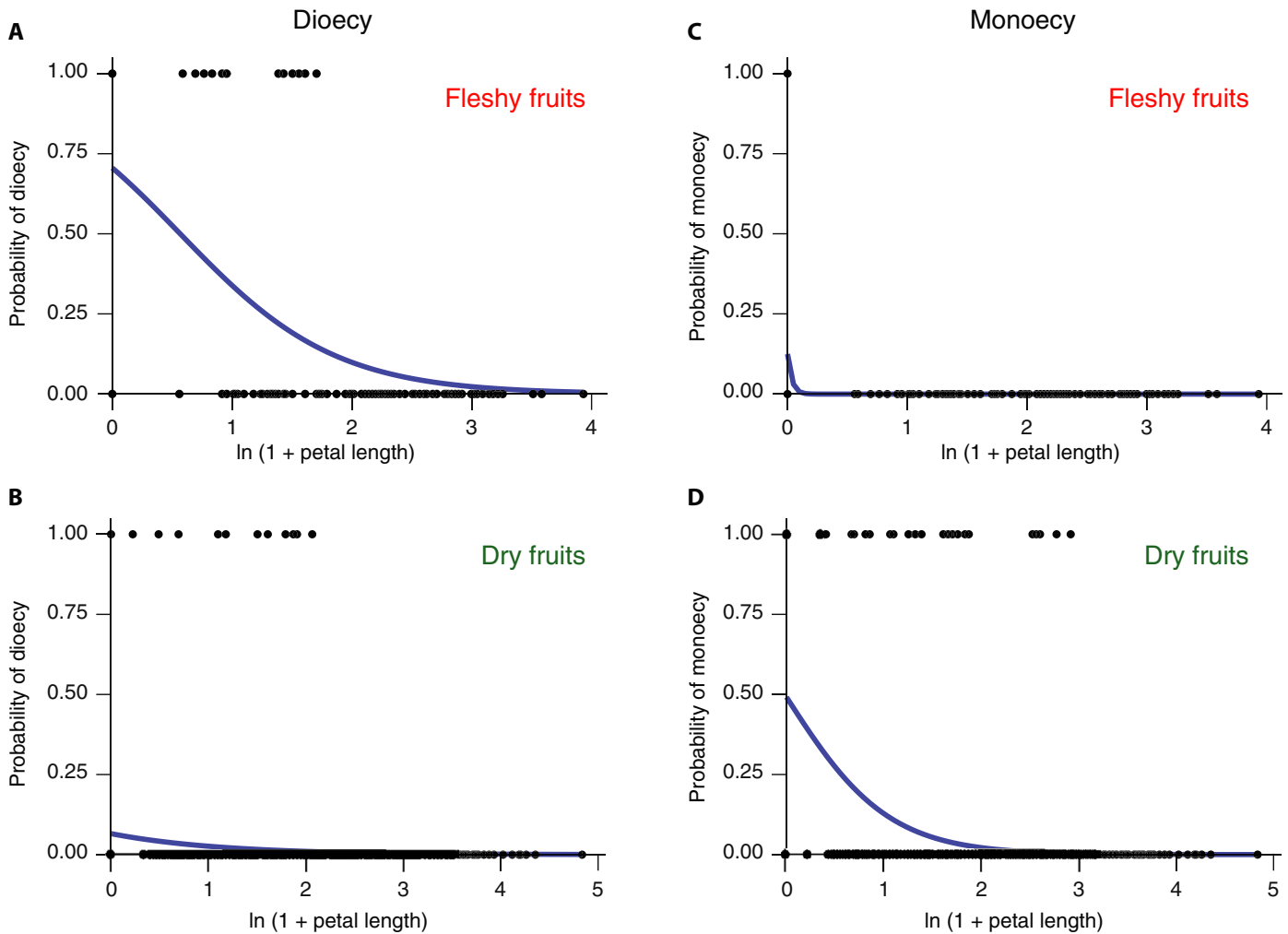


FIGURE 4. Unstructured logistic regressions of percent dioecious and monoecious species as a function of the logarithm of $z = 1 + \text{flower size (petal or ligule length)}$ for all native species with (A, C) fleshy fruits involving vertebrate seed dispersal and (B, D) dry fruits involving other forms of dispersal. Note the shift to dioecy with decreasing flower size in species with fleshy fruits, and to monoecy with decreasing flower size in species with dry fruits. (A) Unstructured $\alpha = 0.874$ ($P < 0.092$), $\beta = -1.543$ ($P < 3.4 \times 10^{-6}$); structured $\alpha = 0.210$ (NS), $\beta = -0.673$ ($P < 0.016$). (B) Unstructured $\alpha = -2.656$ ($P < 2 \times 10^{-16}$), $\beta = -0.954$ ($P < 7.0 \times 10^{-6}$); structured $\alpha = -2.784$ ($P < 0.0024$), $\beta = -0.049$ ($P > 0.73$). (C) Unstructured $\alpha = -1.946$ ($P < 0.069$), $\beta = -30.224$ ($P > 0.99$, essentially a flat line); structured $\alpha = -5.118$ ($P > 0.34$), $\beta = 0.000$ ($P = 1$, a flat line). (D) Unstructured $\alpha = -0.0338$ ($P > 0.69$), $\beta = -1.887$ ($P < 2 \times 10^{-16}$); structured $\alpha = -2.004$ ($P < 1.3 \times 10^{-6}$), $\beta = -0.459$ ($P < 0.009$).

12 of 13 tests regarding trait–trait associations in the native flora (Table 4; Figs. 3 and 4); both exceptions involved patterns in the predicted directions that were not statistically significant. Ten of 12 independent tests regarding trait–environment associations were also supported, eight significantly, with the two exceptions involving the incidence of dioecy in open vs. closed habitats (Table 5). Percipience ranged from 83.3% to 92.3%; predictive power ranged from 67% for trait–environment relationships to 92.3% for trait–trait relationships; qualitative predictive power ranged from 83.3% to 100%; and explanatory power ranged from 80% to 100%. Average predictive magnitude—a measure of relative effect size—for comparisons in Tables 3–5 varies, from 19.1% for trait–environment relationships, to 27.6% for trait–origin relationships, to 63.8% for trait–trait relationships (Appendix S1, Table S3).

Our findings on trait–origin associations represent an advance in supporting several classic hypotheses using phylogenetically

structured analyses across a whole flora for the first time; in demonstrating the statistical significance of an association of a large number of traits simultaneously; in confirming our new hypotheses regarding flower size and color and mode of seed dispersal; and in discovering a much higher incidence of grasses vs. non-grass graminoids among introduced species. Our findings on trait–trait associations in the native flora, while confirming several classic hypotheses that have largely been supported by clade-specific analyses in the past, also provide the first demonstration of the predicted relationship of breeding system to seed dispersal mechanism and quantitative variation in flower size. Finally, our results on trait–environment associations in the native flora present the first tests of a new conceptual framework linking habitat openness to flower size, flower color, and seed dispersal mechanism—and, ultimately, to breeding system (see below).

The strong statistical support for most of our hypotheses, and their generality across the Wisconsin angiosperm flora, illustrates

the power of big data (Allen et al., 2019) for addressing broad ecological and evolutionary questions—here involving phylogenetics, traits, and coarse habitat distributions scored across native and introduced angiosperms in Wisconsin. Increasingly, big data and thoughtful analyses are being used to assess many ecological and evolutionary issues at regional and global scales in a phylogenetic framework, yielding insights of unprecedented geographic and phylogenetic scope (e.g., Ordóñez et al., 2010; Du et al., 2015; Givnish et al., 2015, 2016; Thornhill et al., 2016; Baldwin et al., 2017; Razanajatovo et al., 2017; Scherson et al., 2017; Seebens et al., 2017; Bruehlheide et al., 2018; Moser et al., 2018; Burns et al., 2019a,b). The novel contributions contributed by this study are summarized below.

Traits in introduced vs. native species

Introduced Wisconsin angiosperms are substantially more likely than natives to have an annual life history, herbaceous growth form, and hermaphroditic flowers, and occurrence in open but not mixed or closed habitats (Table 3). These patterns agree with early theory about the traits favored in colonizing or invasive species (Baker, 1965, 1974) and with one or more empirical findings in several papers on local to continental floras in Europe and North America (e.g., Crawley et al., 1996; Cadotte and Lovett-Doust, 2001; Pyšek et al., 2003; Sutherland, 2004; Kuster et al., 2010; Kuester et al., 2014; Bourgeois et al., 2019). However, only one of these studies (Crawley et al., 1996, for the British Isles) assesses whole floras using a phylogenetically structured analysis, based on family-level relationships based on one gene. The traits surveyed by Crawley et al. (1996) either do not overlap with those we studied or exhibit contrary patterns with low levels of support (introduced species showed no significant excess of annuals, and a significantly lower incidence of passive seed dispersal). Crawley et al. (1996) also does not advance or test any trait predictions, or even refer to those advanced by Baker. Many papers comparing introduced and native plants still fail to use phylogenetically structured analyses (e.g., Bourgeois et al., 2019), calling into question the validity of the “classic” associations they report. The present study is thus an important contribution to the literature on trait differences between introduced vs. native species. It is the only one to use phylogenetically structured analyses based on a well-resolved, multigene tree, and in so doing identify significant excesses of all four major correlates (annuals, herbs, hermaphroditism, open habitats) of introduced vs. native angiosperms. The novelty of our results on flower size, color, and dominance of grasses vs. non-grass graminoids is described at the end of this section.

As theory regarding introduced species has matured, it has become apparent that different contexts (e.g., different climates, habitats, propagule pressures, times since introduction, disturbance regimes, and functional and phylogenetic divergences from existing native species) should favor different suites of traits (Pyšek et al., 2003; Lloret et al., 2005; Moracová et al., 2015). Thus, in general terms, it should not be surprising that, for example, in some other regions introduced species tend to be taller, woodier, or longer-lived than native species (Thompson et al., 1995; Williamson and Fitter, 1996).

But the situation can be more complex in specific ways. Although the data strongly support our hypotheses, by no means do all native or introduced species exhibit only the predicted character-states. Partly this is because plant adaptations and ecological success are shaped by multiple factors in the environment. But the lack of

all-or-none differences between all introduced species and all native species may also reflect a diversity of environmental pressures that favor specific traits in some particular kinds of species and other traits in other kinds of species.

For example, if human disturbance provides an opening for introduced species, such species should be short in stature, short-lived, and highly fecund, in order to succeed in regional competition (via enhanced dispersal) while losing in local competition for photosynthetic resources (Givnish, 1982b; Platt and Weis, 1985). But some introduced species may spread because they bear novel anti-herbivore or anti-pathogen defenses (Keane and Crawley, 2002; Mitchell and Power, 2003; DeWalt et al., 2004; Cappuccino and Carpenter, 2005; Bodawatta et al., 2019; but see Schultheis et al., 2015) or novel anti-competitor weapons (Bais et al., 2003; Callaway and Ridenour, 2004; Rudrappa et al., 2007; Callaway et al., 2008; Lankau et al., 2009; Evans et al., 2016)—their success is tied not to disturbance but to eco-evolutionary novelty *per se* (Simberloff et al., 2012; Seabloom et al., 2015). Many such species—like *Rhamnus cathartica* and *Typha angustifolia*—are tall, long-lived, and/or woody, and thereby can succeed in local competition against native species for photosynthetic resources in less disturbed sites after their novel defenses or weapons give them an initial edge. We propose that, in general, novel defenses or weapons should favor introduced species with taller stature, greater life span, and lower fecundity (the EICA hypothesis posits that novel defenses should, in turn, select for further increases in such traits via reallocation of energy from defense to them; Blossey and Nötzold, 1995; Lau and Schultheis, 2015), while adaptation to human disturbances should favor introduced species with shorter stature and life span and greater fecundity.

Furthermore, any model that asks only how plants should converge to maximize adaptation to external conditions will be blind to the advantages of *divergence* from the dominant competitors in any particular environment. Phylogenetic and/or ecological divergence from natives as an important factor driving success of introduced species has been argued and supported by many, including Darwin (1859), Strauss et al. (2006), Diez et al. (2008), van Kleunen et al. (2010), Park and Potter (2013), and Burns et al., 2019a,b). The tension between divergence (in traits and/or phylogenetic relationships) to minimize competition and *convergence* to maximize growth in the absence of competition in a particular environment is one that ecologists and evolutionary biologists have yet to resolve (e.g., Cavender-Bares et al., 2004a,b; Cavender-Bares et al., 2018; Strauss et al., 2006; Cadotte et al., 2009; Evans et al. 2009; Cleland et al., 2011; Weber et al., 2018; Bernard-Verdier et al., 2012; Givnish et al., 2014; Cavender-Bares, 2019; Chang and HilleRisLambers, 2019), and involves a dynamic that potentially ensures phenotypic diversity in any guild or community.

Our results provide the first support for the hypothesis that introduced animal-pollinated species should have larger and more conspicuously colored flowers, as a result of being associated with open habitats that receive bright, broad-spectrum light—habitats in which investment in visual signals is likely to be especially useful in attracting pollinators (Table 3; see Givnish and Patterson, 2000). The surprisingly higher incidence of non-natives in grasses than in other graminoids is probably attributable to the deliberate introduction of many grasses for forage, whereas very few species of sedges or other non-grass graminoids have been introduced. An important question is whether the latter are of lower food value than grasses—perhaps because they have tougher leaf tissue, lower N content, more chemical or endophytic defenses, or less effective

regrowth after grazing—or whether the dearth of introduced non-grass graminoids results from oversight.

Trait–trait relationships in native species

All our predictions were confirmed, and all but one had very strong statistical support: hermaphroditism is more common than unisexuality in animal-pollinated species; flower size is smaller and conspicuous colors less frequent in unisexual vs. hermaphroditic flowers; and dioecious species are more frequently woody than hermaphrodites, more frequently bear fleshy fruits, and have flowers that are smaller ($P < 0.15$) and more frequently inconspicuous in color (Table 4). These patterns accord with our predictions, and all receive highly significant statistical support and explain substantial variation in trait distribution. Vamosi et al. (2003) found similar support for associations of dioecy with woody habit, fleshy fruits, inconspicuous flowers, and abiotic pollination scored for 491 angiosperm genera (<4% of the total; Christianhusz and Byng, 2016) using a phylogenetically structured analysis. Some or all of these associations have been found in a number of phylogenetically unstructured analyses sampling angiosperm families or genera fairly comprehensively (Givnish, 1982a; Renner and Ricklefs, 1995; Renner, 2014) or a variety of local habitats, floras, or small clades (e.g., Bawa and Opler, 1975; Bawa, 1980, 1994; Fox, 1985; Muenchow, 1987; Sakai et al., 2003; Vary et al., 2011; Schlessman et al., 2014).

The observed relationship of breeding system to flower and fruit morphology—with dioecy common only in plants with small flowers and fleshy fruits, monoecy common only in plants with small flowers and passively dispersed seeds, and hermaphroditism common in plants with larger, more costly flowers regardless of seed dispersal mechanism (Figs. 3 and 4)—provide the first support for the predicted relationship of all three common plant breeding systems to fruit type and quantitative variation in flower size. The observed patterns support arguments by Givnish (1982a) regarding the importance of ecological determinants of plant breeding systems, based on how the higher costs of attracting and dispersing pollen in unisexual vs. bisexual flowers would themselves increase with flower size, and how seed dispersal might increase at a greater than linear rate in species with fleshy fruits and at lower than linear rates in species with passively dispersed seeds.

Trait–environment relationships in native species

As predicted, open habitats have significantly higher frequencies of abiotic pollination and conspicuous flower colors, and lower frequencies of fleshy fruits, than other habitats. Similarly, closed habitats have significantly higher frequencies of biotic pollination, less conspicuous flower colors, and higher frequencies of fleshy fruits (Table 5). These results—as well as those for the incidence of dioecy—diverge from the conclusion of Moeller et al. (2017) that local environments are not a primary driver of mating systems along latitudinal gradients. By contrast, our results demonstrate a substantial sorting, within an ecologically heterogeneous region, of pollination-related traits and breeding systems based on habitat openness. This suggests that local ecological conditions are indeed important drivers of floral and fruit traits, operating at substantially smaller spatial scales than those that shape latitudinal patterns. The kind of approach we have taken might be used to parse local ecological drivers of other key evolutionary traits (e.g., polyploidy) that are often seen as being shaped by growth form and large-scale climatic

gradients (e.g., Rice et al., 2019), by analyzing the relationship of such traits to, say, the range of growth forms present in different local habitats.

We will conclude this discussion by briefly addressing possible explanations for the lack of support for two pairs of hypotheses regarding trait–environment relationships, involving flower size and dioecy in open vs. closed habitats.

Although we confirmed the expectations for larger average flower size in animal-pollinated species in open vs. non-open habitats, and smaller size in closed vs. non-closed habitats, the differences were small and not statistically significant (Table 5). No doubt this partly reflects the noise introduced by conflicting expectations based on variation among species in growth form, time of flowering, and distributional position and breadth along the light gradient. However, if we focus on animal-pollinated herbs that grow in open habitats only vs. in closed habitats only, and separate the latter into species flowering in sunny conditions under open canopies in spring vs. shady conditions under closed canopies in summer, the expectations involving flower size–habitat relationships become more clearly supported.

As predicted by Givnish and Patterson (2000) and Givnish (2010), herbs of open habitats (like *Gentiana* or *Lilium*) or those that flower in forest understories under open canopies in spring (like many *Trillium* and *Viola*) have large, visually attractive flowers, while those that flower under closed canopies (like *Neottia* and *Moneses*) have smaller, visually less attractive flowers (Fig. 5). Within this species pool, species found in open habitats only, as well as those found in closed habitats only and flowering before canopy closure, have flowers larger than those found in closed habitats but flowering after canopy closure (Appendix S1, Table S4). In phylogenetically structured *t*-tests, there is no significant difference between flower size in open and closed-sunny conditions, as expected; flowers are marginally significantly larger in species that flower before vs. after canopy closure in closed habitats; and flowers are larger but not significantly so in open vs. closed-shady conditions (Appendix S1, Table S4). Based on unstructured *t*-tests, flower size does not differ significantly between open vs. closed-sunny conditions, and is significantly larger in open vs. closed-shady conditions and vs. closed-sunny conditions, as expected in all three cases (Fig. 5; Appendix S1, Table S4).

Based on these same species pools and tests of phylogenetic correlation, conspicuous flower colors are significantly more frequent in open habitats than in closed habitats among species that flower before or after canopy closure (Appendix S1, Table S4). While we found that conspicuous flower colors are significantly more common in open vs. closed habitats across all native species (Table 5), and that they are also significantly more common in native animal-pollinated herbs found only in open habitats (59.7%) than only in closed habitats and flowering in sunny conditions before canopy closure (22.2%) or after canopy closure (27.0%), it is surprising that the incidence of such colors before vs. after canopy closure hardly differs in understory herbs. This may reflect the widespread occurrence of white flower color (which we categorized as inconspicuous) in forest understories. Before canopy closure, these can be highly visible when brightly lit and combined with large flower size (e.g., *Dicentra*, *Sanguinaria*, *Trillium*); after canopy closure, when dimly lit in green light, small white flowers may be effectively more visible to pollinators than larger red or blue flowers. Among all animal-pollinated species—not merely herbs—flowers are significantly larger if they are blue, red, pink, orange, or yellow than if they are

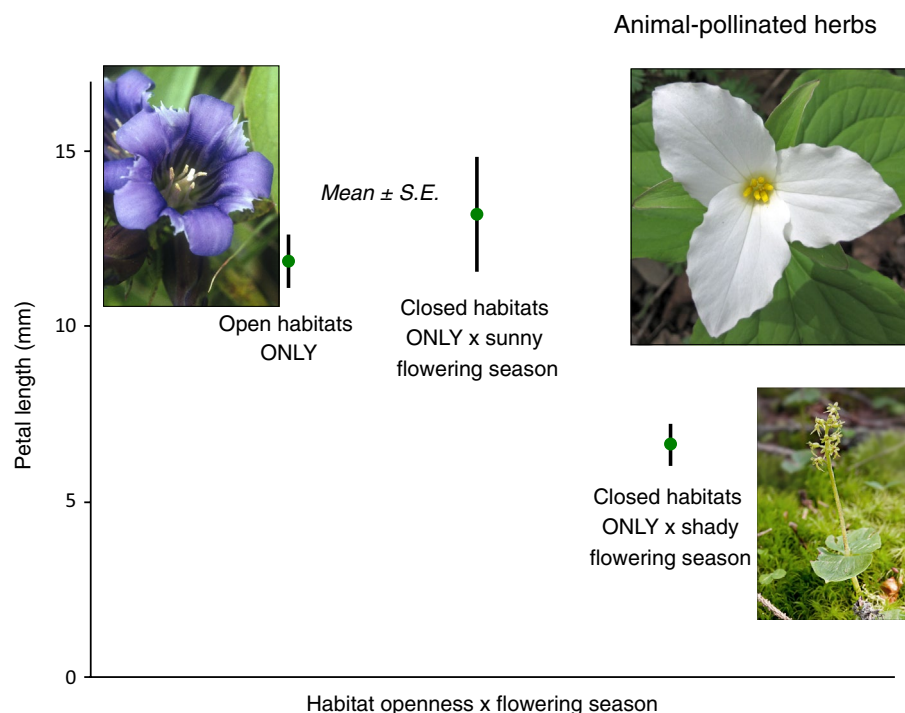


FIGURE 5. Mean ($\pm$ SE) flower sizes of native animal-pollinated herbs found in open habitats only, in closed habitats only and flowering before canopy closure in sunny conditions, and in closed habitats only and flowering after canopy closure in shady conditions. Photographs of exemplar species for each category are shown. Credits: *Gentiana puberulenta*, Gentianaceae (Thomas Givnish); *Trillium grandiflorum*, Melanthiaceae (James St. John); *Neottia cordata*, Orchidaceae (Amadej Trnkoczy).

cream or white, and all are significantly larger than green or brown flowers (Fig. 6), as expected from theory and the distribution of larger flowers in more brightly lit sites (see above).

Finally, the higher incidence of dioecy in open vs. closed habitats contradicted predictions based on closed-habitat animal-pollinated species having smaller flowers and more fleshy fruits. The deviation of theory from observation might plausibly involve three factors: (1) the greater incidence of wind-pollinated plants with tiny flowers in open habitats; (2) the deviation of the relative abundances of species with different combinations of biotic/abiotic pollination and fleshy/dry fruits from expectations; and (3) the variation in the observed and expected frequencies of dioecy associated with different floral/fruit combinations.

First, across all native species, regardless of growth form or pollination mechanism, average flower size is smaller (but not significantly so) in open vs. non-open habitats (7.1 mm vs. 8.4 mm). This reflects the higher incidence of wind pollination and smaller flowers of non-herb species. But the greater incidence of abiotic pollination in open habitats cannot explain the higher incidence of dioecy there. When we tabulate species in open vs. non-open habitats by biotic vs. abiotic pollination and fleshy vs. dry fruits, and score them for dioecy vs. other breeding systems (Appendix S1, Table S5), we find that almost all species fall into three of four categories: biotic pollination + fleshy fruits (11.9%), biotic pollination + dry fruits (52.8%), and abiotic pollination + dry fruits (35.2%). The near absence of species that combine abiotic pollination with fleshy fruits (two species, or 0.1%) across habitats means that the high incidence of wind pollination in open habitats (Table 5) cannot account for the higher incidence of dioecy there, at least in terms of the predicted

linkage of dioecy to floral and dispersal traits predicted by Givnish (1982a).

Second, as expected, abiotic pollination is more common in open vs. non-open habitats (38.3% vs. 26.0%), and biotic pollination more common in non-open vs. open habitats (74.1% vs. 61.7%) (Appendix S1, Table S5A). Given that wind pollination is favored in open habitats, and fleshy fruits in closed habitats (Table 1; Appendix S1, Table S5A), the “forbidden” combination of wind pollination and fleshy fruits may reflect adaptive conflict. For canopy trees, the puzzle remains: the best time for wind pollination is the spring, whereas the best time for producing fleshy fruits would be late summer–early fall, to coincide with avian migration or, at least, less territoriality. It is unclear why many more trees do not pursue this combination of strategies.

Third, across habitats, the frequency of dioecy varies with different combinations of floral and fruit traits. Dioecy accounts for 16.1% of species with biotic pollination and fleshy fruits, 1.0% of species with biotic pollination and dry fruits, 6% of species with abiotic pollination and dry fruits, and 100% of species with abiotic pollination and fleshy fruits (Appendix S1, Table S5A). These data are generally in line with predictions. When

each of these values is multiplied by the *actual* fraction of species having the corresponding pollination $\times$ fruit type and summed, the results can be considered projections of the incidence of dioecy in open vs. non-open habitats: 4.5% vs. 5.1% (Appendix S1, Table S5B), reversing the order of actual incidence of 5.1% vs. 3.2% (Table 5). But clearly the matter

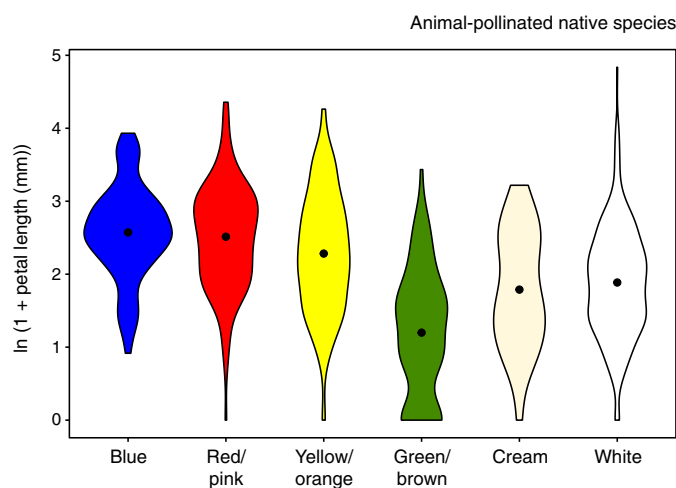


FIGURE 6. Violin plots show the distribution of flower size (log scale) for each flower-color category across native animal-pollinated angiosperms. Mean flower sizes for blue, red, and yellow categories are significantly larger than those for white or cream categories, and flower sizes for both of the latter categories are significantly larger than those for the green/brown category.

is a close one, involving deviations of only 0.8% and 2.5%. A second projection (Appendix S1, Table S5C) uses the *theoretical* fractions of species having each pollination × fruit type combination expected from the marginal frequencies of each flower and fruit type, assuming that the latter are random associated—thereby eliminating the effect of the “forbidden combination” of wind pollination and fleshy fruits (Appendix S1, Table S5A). This results in a predicted frequency of dioecy of 5.6% in open habitats and 6.3% in non-open habitats, with the difference again in the expected direction but quite small—0.7%, compared with 0.6% for the first projection and −2.6% as observed.

The unexpectedly higher incidence of dioecy in open habitats partly reflects the different frequencies of dioecy in the cells defined by flower × fruit combinations in open vs. non-open habitats. The decisive differences appear to be the incidence of 1.3% vs. 0.0% dioecy in open vs. non-open habitats for biotic pollination combined with dry fruits, and especially the incidence of 7.1% vs. 3.1% dioecy in open vs. non-open habitats for abiotic pollination combined with dry fruits (Appendix S1, Table S5A). The cause of the first small difference is unknown. The cause of the second reflect largely just one lineage, the family Salicaceae, which combines dioecy with abiotic (or abiotic + biotic) pollination and dry fruits in open habitats. Excluding that family would drop the incidence of dioecy in open habitats from 5.1% to 3.8%, and the frequency of dioecy in species combining abiotic (or abiotic and biotic) pollination with dry fruits there from 7.1% to 3.2%. Roughly half of the difference in the incidence of dioecy between open and non-open habitats is thus traceable to a single lineage whose combination of traits runs counter to that seen in other lineages. The remainder reflects slightly higher values of low levels of dioecy in species combining biotic and abiotic pollination with dry fruits in open vs. non-open habitats. These may simply be due to chance, given the small numbers of dioecious species involved. If there are nonrandom causes for these subtle differences, they remain unknown, and our calculations reveal a new set of questions to be addressed if we are to understand shifts in the incidence of dioecy in moving from open to closed habitats in temperate plant communities.

CONCLUSIONS

In conclusion, our findings support almost all of 25 classical and novel predictions regarding how flower size, flower color, breeding system, fruit type, and growth form should vary in response to each other and to variation in habitat openness and native vs. introduced origin. This is the first study to combine a flora-wide phylogeny with complete trait databases and phylogenetically structured analyses to provide powerful tests of evolutionary hypotheses about reproductive traits and their variation with geographic source, each other, and environmental conditions. It would now be important to conduct similar studies in other regions dominated by temperate forests, woodlands, and grasslands with independently evolved floras (e.g., California, Eastern Australia, Central and Eastern Europe, China) to determine whether the same patterns observed in Wisconsin apply globally.

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AUTHOR CONTRIBUTIONS

T.J.G., R.K., K.J.S., K.M.C., and D.M.W. designed the overall study. T.J.G. developed the hypotheses in consultation with all other authors. R.K. conducted all the statistical analyses, with additional input from K.J.S. and T.J.G. T.J.G. and R.K. compiled the trait database, with additional input from J.P.R., J.G.Z., D.S., and K.J.S. T.J.G. wrote the initial draft, with substantial input from all other authors on the final draft.

DATA AVAILABILITY

The phylogeny, trait database, report, and the R code used for the analyses have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.qfttdz0fb> [Givnish et al., 2020]).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Supplementary material. Includes supplemental information on method.

TABLE S1. Native and introduced species of the Wisconsin angiosperm flora. Ordinal and family names follow Angiosperm Phylogeny Group (2016).

TABLE S2. Summary of traits and environments for the 2199 Wisconsin angiosperm species included in the molecular phylogeny. All proportions and means are close to those observed across all 2357 Wisconsin angiosperm species (see Table 1).

TABLE S3. Phylogenetically structured ANOVA and unstructured t-tests (unequal variances) for significant pairwise differences in mean ± s.d. flower size and % incidence of conspicuous flower colors in native animal-pollinated herbs found in open habitats only, in closed habitats only and flowering before canopy closure, and in closed habitats only and flowering after canopy closure.

TABLE S4. Phylogenetically structured ANOVA and unstructured t-tests (unequal variances) for significant pairwise differences in mean flower size and incidence of conspicuous flower colors in native animal-pollinated herbs found in open habitats only, in closed habitats only and flowering before canopy closure, and in closed habitats only and flowering after canopy closure.

TABLE S5. Absolute and relative abundances of species with different combinations of pollination mechanism × fruit type and of dioecy in open and non-open habitats, and observed vs. projected abundance of dioecy as a fraction of the total species pool in each habitat, overall and by pollination mechanism × fruit type.

FIGURE S1. Summary chronogram of plastid phylogeny including 96% of native species and 87% of introduced species of Wisconsin angiosperms. Branch color represents a stochastic mapping of introduce/native origin on the tree. Major groups of angiosperms are indicated by brackets.

FIGURE S2. Chronogram of the Wisconsin angiosperm flora. Branch lengths are proportional to time to inferred common ancestors; red numbers indicate absolute time in millions of years (see also scale below the tree). Brackets indicate families, orders, and higher categories.

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