

SPECIAL INVITED PAPER

The best of both worlds? A review of delayed selfing in flowering plants

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PREMISE OF STUDY: In a seminal body of theory, Lloyd showed that the fitness consequences of selfing will depend on its timing in anthesis. Selfing that occurs after opportunities for outcrossing or pollen dispersal can provide reproductive assurance when pollinators are limited and is expected to incur little cost, even when inbreeding depression is high. As a result, delayed selfing is often interpreted as a “best-of-both-worlds” mating system that combines the advantages of selfing and outcrossing.

METHODS: We surveyed 65 empirical studies of delayed selfing, recording floral mechanisms and examining information on inbreeding depression, autofertility, and other parameters to test the support for delayed selfing as a best-of-both-worlds strategy.

KEY RESULTS: Phylogenetic distribution of the diverse floral mechanisms suggests that some basic floral structures may predispose plant taxa to evolve delayed selfing. Delayed selfing appears to serve as a best-of-both-worlds strategy in some but not all species. While the capacity for autonomous selfing is often high, it is lower, in some cases, than in related species with earlier modes of selfing. In other delayed-selfers, low inbreeding depression and reduced investment in corollas and pollen suggest limited benefits from outcrossing.

CONCLUSIONS: Despite a growing literature on the subject, experimental evidence for delayed selfing is limited and major gaps in knowledge remain, particularly with respect to the stability of delayed selfing and the conditions that may favor transitions between delayed and earlier selfing. Finally, we suggest a potential role of delayed selfing in facilitating transitions from self-incompatibility to selfing.

KEY WORDS autonomous self-fertilization; delayed selfing; dichogamy; herkogamy; mating-system evolution; reproductive assurance; self-incompatibility.

In a series of seminal papers, David G. Lloyd (1979; 1992; Lloyd and Schoen, 1992) provided new insight into plant mating-system evolution by recognizing that the selective consequences of self- and cross-pollination and fertilization depend on the specifics of how and when they occur. In phenotypic models, Lloyd showed that the conditions that select for self-fertilization (selfing) facilitated by pollinators differ from those for selfing that occurs autonomously. Further, the selective consequences of autonomous selfing were shown to depend on its timing in anthesis in relation

to cross-fertilization (outcrossing). The theory predicts that selfing that occurs after opportunities for outcrossing have passed (delayed selfing) should be selected under the broadest range of conditions.

In general, selection of selfing is expected to be opposed by inbreeding depression, the reduced fitness of selfed progeny that results primarily from the expression of deleterious recessive alleles (Charlesworth and Charlesworth, 1987, Charlesworth and Willis, 2009). Counteracting the cost of inbreeding depression is the 50% genetic transmission advantage of selfing, which occurs because

pollen can transmit alleles through both selfed and outcrossed progeny (Fisher, 1941). Selfing that occurs autonomously can also provide reproductive assurance, a guarantee of successful reproduction even when pollinators or mates are limited (Darwin, 1876; Baker, 1955; Eckert et al., 2006; Busch and Delph, 2012).

According to Lloyd's theory (1992), while prior selfing (occurring before outcrossing) and competing selfing (concurrent with outcrossing) provide benefits, they can incur costs by reducing the number of outcrossed seeds and the amount of pollen dispersed—referred to as seed discounting and pollen discounting, respectively. As a result, prior and competing selfing are expected to be favored only when inbreeding depression is below some threshold value. Delayed selfing incurs no seed discounting, by contrast, because it does not detract from maternal fitness through outcrossing; similarly it does not incur pollen discounting or diminish male outcross success. Therefore, delayed selfing is expected to be selected for at any value of inbreeding depression < 1 (i.e., selfed offspring have fitness > 0). Because delayed selfing combines the advantages of outcrossing when it is possible (avoidance of inbreeding depression) and selfing when it is necessary (reproductive assurance), it is frequently referred to as a “best-of-both-worlds” mating system that allows plants to cope with variable pollinator environments (e.g., Bécerra and Lloyd, 1992; Lloyd, 1992; Kalisz and Vogler, 2003).

Consideration of factors not included in these models contributes further insights into the advantages of delayed selfing. When population dynamics and pollinator behavior are incorporated into theory, delayed selfing remains advantageous under all conditions (Morgan et al., 2005). The potential effects of self-pollination on floral longevity suggest an additional advantage of delayed over other forms of selfing. If pollination triggers floral senescence, as is frequently observed (Stead, 1992; van Doorn 1997), prior selfing can reduce floral longevity and therefore might reduce pollen dispersal in comparison to delayed selfing (Weber and Goodwillie, 2007).

Some costs and limitations of delayed selfing have been noted, however. When maternal resources are insufficient to provision all seeds, low-quality selfed seeds might compete with and detract from the success of outcrossed seeds fertilized earlier (Harder and Routley, 2006). When different modes of selfing act together, the costs and benefits become more complex. For example, although floral traits may prevent autonomous self-pollination until late in anthesis, pollinator-mediated selfing occurring earlier can limit the benefits of delayed selfing (Vaughton and Ramsey, 2010). These studies highlight the importance of considering the interactions and transitions between different modes of selfing.

Since the publication of Lloyd's influential papers, the compelling concept of delayed selfing as the best-of-both-worlds (BOBW) mating system has inspired observations and investigations in a myriad of plant species. Given the presumed selective advantages of this mode of selfing, we asked whether the combined evidence from the empirical studies is consistent with delayed selfing as a BOBW strategy. Here, we review reports of delayed selfing in the context of theoretical expectations, compiling information on the variety of floral mechanisms that have been reported and the distribution of delayed selfing among angiosperm families, habitats and life histories. We discuss the methods used to document delayed selfing and explore the evidence supporting the model of delayed selfing as a BOBW mating strategy. Finally, we draw on the surveyed studies to explore the broader roles of delayed selfing in mating-system evolution, identify questions that remain and suggest future directions for the study of delayed selfing.

EMPIRICAL STUDIES OF DELAYED SELFING

Using Web of Science and Google Scholar, we compiled published reports of delayed selfing in plants through May 2017, searching with the terms “delayed,” “late” and “selfing,” “self-fertilization,” “autogamy” or variants of these. A complete screening of floras, monographs, books or papers that mention floral biology only briefly is beyond the scope of this study. We note that our survey encompasses reports in the primary literature that focus on reproductive modes and is not intended to provide an estimate of the actual incidence of delayed selfing, as such information is lacking for most species.

Patterns and distribution of delayed selfing in angiosperms

Our survey yielded studies of delayed selfing in 63 angiosperm species, with reports for two varieties in two of these (for simplicity, we refer to these as 65 species hereafter). The species spanned 23 orders, 39 families, and 55 genera (Table 1, Fig. 1). Because our list is not expected to be comprehensive and undoubtedly reflects study bias, we did not attempt statistical hypothesis testing or rigorous phylogenetic correction. Nevertheless, we recorded information on life form, floral form, habitat, and pollination biology (Table 1 and Appendix S1; see the Supplemental Data with this article) and describe broad trends in the data below, using genus frequencies for traits or properties that we expected to be conserved at that taxonomic level. Although reports of delayed selfing occurred across a wide phylogenetic range, including a basal angiosperm, monocots and eudicots, their distribution was somewhat clumped (Fig. 1). For example, seven genera occurred within the Gentianaceae, whereas most families contained no more than one report. Further, the surveyed taxa showed a trend toward higher representation in the asterids than in the rosids (26 vs. 16 genera, respectively), despite the comparable sizes of these two major eudicot clades (Soltis et al., 2005; Wang et al., 2009).

Species with delayed selfing occupy diverse habitats including aquatic (Hydatellaceae: *Trithuria submersa*) and arid (Zygophyllaceae: *Zygophyllum macropterum*), and are found in both temperate and tropical regions. Arctic and alpine habitats were relatively common (7 of 55 genera) considering the overall low plant diversity found in those regions (Körner, 1995). This trend is consistent with an association between climatic extremes and selfing ability (Evans et al., 2011) and might reflect selection to cope with variable pollinator visitation in these environments (Primack, 1978; Arroyo et al., 1985; Tötland, 2001, but see García-Camacho and Tötland, 2009).

Delayed-selfing species were associated with many pollinator types, including combined wind and insect pollination in *Paris quadrifolia* (Melanthiaceae; Jacquemyn and Brys, 2008), with bee pollination the most common. Our survey is consistent with a previously reported association between specialized pollination and mechanisms for reproductive assurance (Fenster and Martén-Rodríguez, 2007; Pérez et al., 2009). For example, delayed selfing occurred in a *Clematis* species (Ranunculaceae) with tubular flowers and a specialized bee pollination syndrome, whereas its outcrossing congener had open flowers and more generalist pollination (Jiang et al., 2010). As broad evidence, we point to a prevalence of fused corollas (30 genera with fused vs. 21 with free petals, consistent with the trend for a high frequency of asterids), which are thought to be associated with pollinator specialization (Wernham,

TABLE 1. Species reported to undergo delayed selfing included in the survey, floral mechanism, and key citation. Additional sources that contributed information on parameters examined in our study (e.g., inbreeding depression) are cited in text or in Appendix S1.

Family	Species	Mechanism	Citation
Acanthaceae	<i>Ruellia subsessilis</i> (Nees) Lindau	Corolla abscission	Miranda and Vieira, 2014
	<i>Ruellia succulent</i> Small	Corolla abscission	Geiger et al., 2010
Asphodelaceae	<i>Bulbine vagans</i> E.M.Watson	Corolla closure and style straightening	Vaughton et al., 2008
Asteraceae	<i>Senecio vulgaris</i> L.	Stigma curvature	Irwin et al., 2016
Berberidaceae	<i>Podophyllum hexandrum</i> Royle	Corolla closure	Xiong et al., 2013
Bignoniaceae	<i>Incarvillea sinensis</i> Lam.	Corolla abscission	Qu et al., 2007
Boraginaceae	<i>Myosotis forsteri</i> Lehm.	Reduced herkogamy	Robertson and Lloyd, 1991
	<i>Myosotis lytteltonensis</i> (Laing et A.Wall) de Lange	Reduced herkogamy	Robertson and Lloyd, 1991
Brassicaceae	<i>Draba alpina</i> L.	Incomplete dichogamy	Brochmann, 1993
	<i>Draba oxycarpa</i> Sommerf.	Incomplete dichogamy	Brochmann, 1993
Bromeliaceae	<i>Tillandsia recurvata</i> (L.) L.	Corolla wilting	Orozco-Ibarrola et al., 2015
Campanulaceae	<i>Campanula rapunculoides</i> L.	Transient SI and stigma curvature	Vogler and Stephenson, 2001
	<i>Triodanis perfoliata</i> (L.) Nieuwl.	Stigma curvature	Goodwillie, unpublished
Cistaceae	<i>Fumana juniperina</i> (Lag. ex Dunal) Pau	Corolla closure	Carrió et al., 2008
Commelinaceae	<i>Commelina dianthifolia</i> Delile	Style curvature	Hrycan and Davis, 2005
Ericaceae	<i>Kalmia latifolia</i> L.	Release of anthers	Levri, 1998; Nagy et al., 1999
Fabaceae	<i>Crotalaria micans</i> Link	Reduced herkogamy	Etcheverry et al., 2003
	<i>Lupinus nanus</i> ssp. <i>latifolius</i> (Benth. ex Torr.) Dunn	Wilting of hairs that separate pollen and stigma	Juncosa and Webster, 1989
	<i>Trifolium fragiferum</i> L.	Incomplete dichogamy	Dhar et al., 2006
Gentianaceae	<i>Blackstonia perfoliata</i> (L.) Huds.	Incomplete dichogamy	Brys et al., 2013
	<i>Calolisianthus pendulus</i> (Mart.) Gilg.	Style curvature and incomplete dichogamy	Freitas and Sazima, 2009
	<i>Centaureum erythraea</i> Rafn	Incomplete dichogamy	Brys and Jacquemyn, 2011
	<i>Deianira nervosa</i> Cham. & Schltldl.	Incomplete dichogamy and style curvature	Freitas and Sazima, 2009
	<i>Gentianopsis paludosa</i> Ma	Reduced herkogamy	Duan et al., 2010
	<i>Sebaea aurea</i> (L. f.) Roem. & Schult.	Diplostigmaty	Kissling and Barrett, 2013
	<i>Zygostigma australe</i> (Cham. & Schltldl.) Griseb.	Corolla closure	Freitas and Sazima, 2009
Geraniaceae	<i>Geranium carolinianum</i> L.	Incomplete dichogamy and reduced herkogamy	Shirk and Hamrick, 2014
Haemodoraceae	<i>Wachendorfia brachyandra</i> W.F.Barker	Reduced herkogamy	Jesson and Barrett, 2002
Hydatellaceae	<i>Trithuria submerse</i> Hook.f.	Incomplete dichogamy	Taylor et al., 2010
Loasaceae	<i>Gronovia scandens</i> L.	Corolla closure	Raimúndez-Urrutia and Varela, 2005
Malvaceae	<i>Alcea rosea</i> L.	Style curvature	Li et al., 2012
	<i>Hibiscus laevis</i> All.	Style curvature	Klips and Snow, 1997
	<i>Hibiscus trionum</i> var. <i>trionum</i> L.	Style curvature	Buttrose et al., 1977; Ramsey et al., 2003
	<i>Hibiscus trionum</i> var. <i>vesicarius</i> (Cav.) Hochr.	Style curvature	Seed et al., 2006
	<i>Kosteletzkya virginica</i> (L.) C.Presl ex A.Gray	Style curvature	Ruan et al., 2008
Melanthiaceae	<i>Paris quadrifolia</i> L.	Incomplete dichogamy and reduced herkogamy	Jacquemyn and Brys, 2008
Onagraceae	<i>Camissoniopsis cheiranthifolia</i> (Hornem. ex Spreng.) W.L.Wagner & Hoch	Corolla closure	Dart and Eckert, 2013
	<i>Clarkia exilis</i> F.H.Lewis & Vasek	Incomplete dichogamy	Mazer et al., 2007
Orchidaceae	<i>Epipactus helleborine</i> var. <i>papillosa</i> (Franch. & Sav.) Hashimoto	Corolla closure	Suetsugu, 2013
	<i>Epipactus helleborine</i> var. <i>sayekiana</i> (Makino) Hashimoto	Corolla closure	Suetsugu, 2013
Orobanchaceae	<i>Pedicularis dunniana</i> Bonati	Corolla wilting	Sun et al., 2005
Papaveraceae	<i>Sanguinaria canadensis</i> L.	Incomplete dichogamy and filament curvature	Lyon, 1992
Pauwloniaceae	<i>Brandisia hancei</i> Hook.f.	Incomplete dichogamy and reduced herkogamy	Ren et al., 2016
Phrymaceae	<i>Mimulus guttatus</i> DC.	Corolla abscission and stigma curvature	Dole, 1990, 1992
Plantaginaceae	<i>Collinsia heterophylla</i> Buist ex Graham	Incomplete dichogamy and reduced herkogamy	Lankinen et al., 2007
	<i>Collinsia parviflora</i> Lindl.	Incomplete dichogamy and reduced herkogamy	Elle et al., 2010
	<i>Collinsia verna</i> Nutt.	Incomplete dichogamy and reduced herkogamy	Kalisz et al., 1999
Polemoniaceae	<i>Gilia achilleifolia</i> Benth.	Stigma curvature	Takebayashi et al., 2006
	<i>Leptosiphon jepsonii</i> (Schemske & Goodw.) J.M.Porter & L.A.Johnson	Transient SI and reduced herkogamy	Goodwillie et al., 2004

(continued)

TABLE 1. (Continued)

Family	Species	Mechanism	Citation
Polygalaceae	<i>Polygala lewtonii</i> Small	Pollen transferred from sterile to fertile stigma lobe	Weekley and Brothers, 2006
Portulacaceae	<i>Portulaca oleracea</i> L.	Corolla closure	Miyajima, 2006
Primulaceae	<i>Anagallis arvensis</i> L.	Corolla closure	Gibbs and Talavera, 2001
	<i>Primula halleri</i> J.F.Gmel.	Reduced herkogamy	de Vos et al., 2012
Ranunculaceae	<i>Clematis akebioides</i> (Maxim.) Veitch	Incomplete dichogamy	Jiang et al., 2010
Rutaceae	<i>Ruta graveolens</i> L.	Reduced herkogamy and stamen movement	Ren and Tang, 2012
Sapindaceae	<i>Xerospermum intermedium</i> Radlk.	Delayed breakdown of indehiscent anthers	Appanah, 1982
Scrophulariaceae	<i>Verbascum thapsus</i> L.	Incomplete dichogamy and style curvature	Gross and Werner, 1978
Solanaceae	<i>Schizanthus grahamii</i> Gill. ex Hook	Incomplete dichogamy and reduced herkogamy	Pérez et al., 2009
	<i>Schizanthus candidus</i> Lindl.	Incomplete dichogamy and reduced herkogamy	Pérez et al., 2009
	<i>Schizanthus lacteus</i> Phil.	Incomplete dichogamy and reduced herkogamy	Pérez et al., 2009
	<i>Withania somnifera</i> (L.) Dunal	Incomplete dichogamy and reduced herkogamy	Lattoo et al., 2007
Violaceae	<i>Viola pubescens</i> Aiton	Style curvature	Culley, 2002
Zingiberaceae	<i>Caulokaempferia coenobialis</i> (Hance) K.Larsen	Pollen slides in stigmatic fluid	Wang et al., 2005
	<i>Roscoea debilis</i> Gagnep.	Pollen slides in stigmatic fluid	Fan and Li, 2012
Zygophyllaceae	<i>Zygophyllum macropterum</i> C.A.Mey.	Incomplete dichogamy and reduced herkogamy	Mamut et al., 2014

1911; Faegri and Van Der Pijl, 1979; Zhong and Preston, 2015). Furthermore, a single pollinator functional group (e.g., hummingbirds, moths, bees) was reported in 27 of 60 species for which we found any pollinator information.

An association between predominantly selfing mating systems and annuality is well documented (Stebbins, 1950; Barrett et al., 1996). Accordingly, the incidence of annual or biennial species in our list (43%) was substantially higher than their overall frequency in North America (21%, Hart, 1977), though we are not aware of a worldwide estimate. A related pattern is the predominance of herbaceous species in studies reporting delayed selfing; only 12% of our surveyed species were woody compared to a recent global estimate of 45% (FitzJohn et al., 2014). This deficit may result from the high incidence of self-incompatibility (Igit et al., 2008) and dicliny (separate male and female flowers) in woody species and perhaps weaker selection for mechanisms of reproductive assurance in long-lived species. Study bias toward herbaceous species might also contribute to their predominance in our survey, because experiments and detailed floral manipulations are often difficult in woody species.

Mechanisms of delayed selfing

Diverse floral traits are reported to confer delayed self-fertilization in flowering plants (Table 1). Moreover, the taxonomic distribution of these traits suggests that most mechanisms have several origins and that multiple mechanisms have evolved within some families (Fig. 1). For example, five distinct mechanisms were reported in the Gentianaceae. Furthermore, in some species multiple traits appear to function together to promote delayed selfing in some species (see below). Mechanisms of delayed selfing can be classified broadly as mechanical, developmental, or physiological in their action.

In mechanical systems of delayed selfing, physical changes in the positioning of floral structures during anthesis result in delayed autonomous self-pollination. Mechanical processes, alone or in concert with other mechanisms, contribute to delayed selfing in 43 of

55 genera (Figs. 1 and 2). Certainly, mechanical processes are the most readily observable, which may help to explain the abundance of examples in the literature.

Changes in the positions of reproductive organs occur in a variety of ways to promote delayed selfing. For instance, in numerous species in the Malvaceae, the styles of unpollinated flowers curve backward as the flower ages, creating contact between stigmas and monadelphous stamens that surround the styles (Ruan et al., 2010). Curvature of styles, stigmas, or filaments during anthesis appears to be common, reported in 17 genera in 13 families. In an unusual example in *Viola pubescens* (Violaceae), stigmas bend downward in late anthesis to contact pollen that has fallen and collected in the anterior petal (Culley, 2002). In other cases, the changes that occur in organ position are better described as movement than as curvature; in *Ruta graveolens* (Rutaceae), for example, the stamens rise to contact the stigma late in anthesis (Ren and Tang, 2012; see below).

Progressive reduction in herkogamy (spatial separation of stigma and anthers) was reported to promote delayed self-pollination in 13 of 55 genera. This process occurs when styles and stamens elongate at different rates or when corollas expand during anthesis, causing epipetalous stamens to move upward to contact the stigma. Delayed selfing can also occur with corolla abscission. In this scenario, herkogamy prevents selfing throughout anthesis; but as the corolla detaches, epipetalous stamens make contact with the stigma as they slide past it. A special case of abscission-induced selfing occurs in *Incarvillea sinensis* (Bignoniaceae), whose senescent corollas are dragged off the inflorescence by wind (Qu et al., 2007). Finally, closure or wilting of flowers contributes to selfing by forcing stigma and anthers together in *Fumana juniperina* (Cistaceae; Carrió et al., 2008) and 11 other genera.

Dichogamy (temporal separation of stigma receptivity and pollen presentation) is present in many angiosperms in which it may function to prevent self-fertilization or pollen–pistil interference (Lloyd, 1986; Bertin, 1993; Sargent et al., 2006). A developmental mechanism of delayed selfing can occur when male and

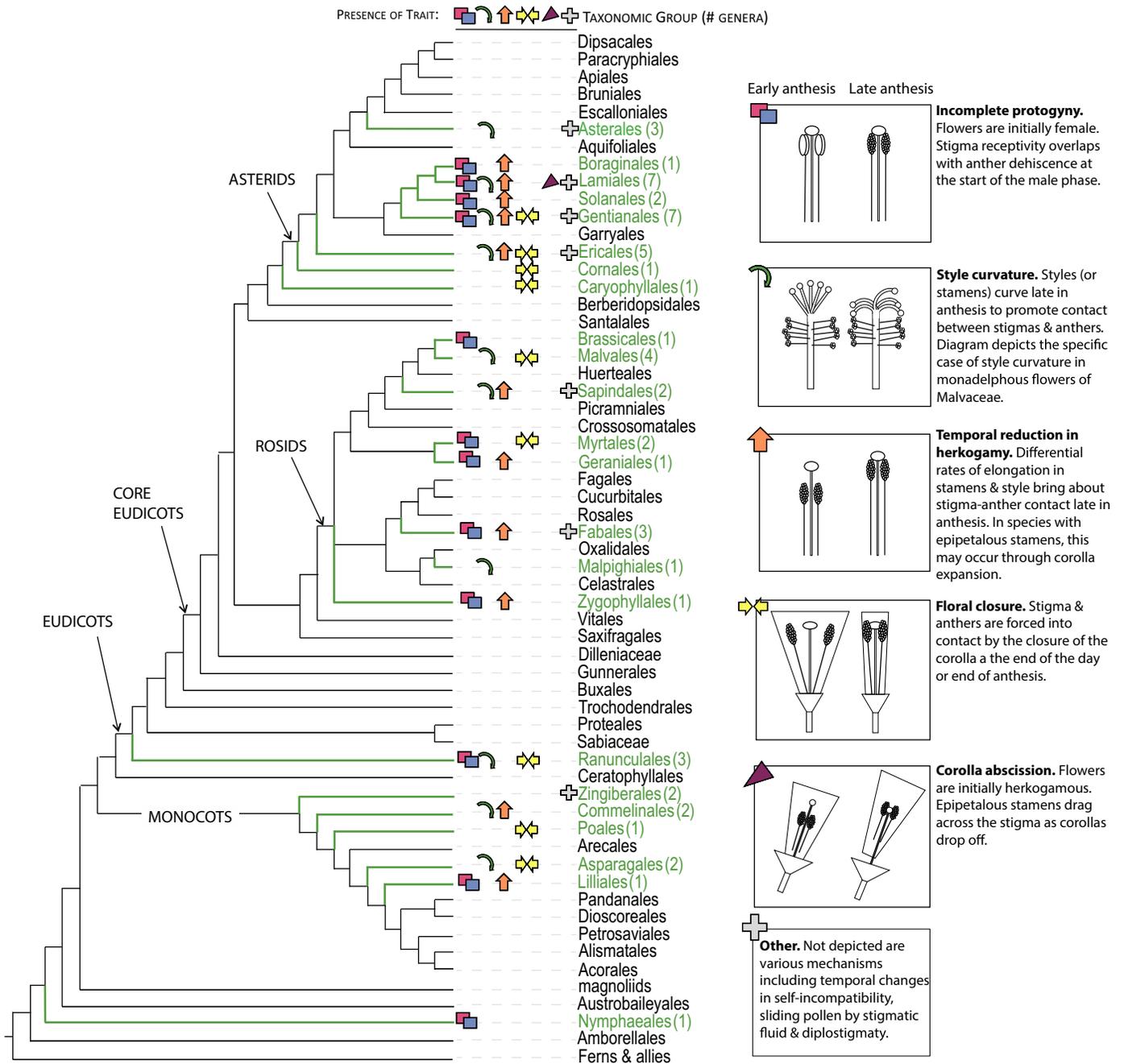


FIGURE 1. Distribution across angiosperm orders of delayed-selfing mechanisms reported in surveyed species. Number of genera with reports of delayed selfing shown in parentheses. Symbols refer to classes of delayed-selfing mechanisms shown at the right. See text for additional details. The clade of magnoliids has been collapsed because there were no cases of delayed selfing in this lineage. Tree topography follows the Angiosperm Phylogeny Website, Version 14, July 2017 (Stevens, 2001).

female phases partially overlap. In incomplete protogyny, stigmas become receptive first, allowing cross-fertilization. Subsequently, anthers dehisce while stigmas are still receptive, causing delayed selfing to occur. Mechanical and developmental processes often work together to optimize both outcrossing and delayed selfing. For example, in the protogynous desert herb *Zygophyllum macrop-terum*, herkogamy present during the phase of stigma receptivity prevents physical interference by stamens; but as anthers dehisce at

the end of the female phase, filaments elongate to reduce stigma-anther distance, and self-pollination occurs (Mamut et al., 2014). Incomplete protogyny, alone or combined with other mechanisms, was reported to promote delayed selfing in 19 of 55 genera (Fig. 2).

Physiological mechanisms include changes in self-incompatibility (SI) that allow for delayed selfing. SI systems generally prevent self-fertilization; however, a temporal breakdown in expression during anthesis, or transient SI, can be a mechanism

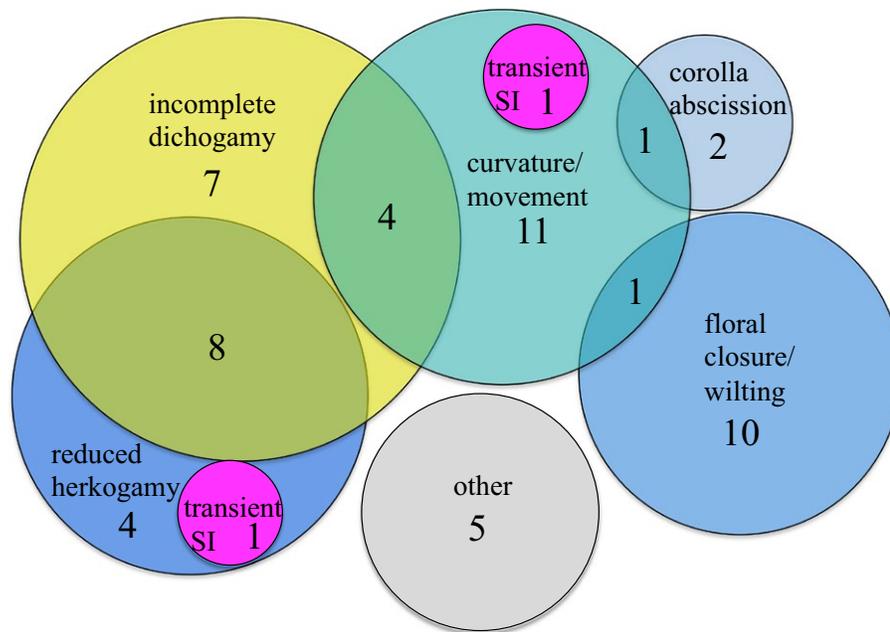


FIGURE 2. Frequency and overlap of reported delayed-selfing mechanisms. Numbers refer to counts of genera in which a mechanism or combination of two mechanisms was reported in a species in our survey (55 genera in total). Shades of blue indicate mechanical forms of delayed selfing. SI = self-incompatibility. See text for detailed descriptions of each mechanism type.

for delayed self-fertilization, as reported in *Leptosiphon jepsonii* (Polemoniaceae, Goodwillie et al., 2004) and *Campanula rapunculoides* (Campanulaceae, Vogler et al., 1999). Intriguingly, transient SI is accompanied by reduced herkogamy in *Leptosiphon* and progressive stigma curvature in *Campanula*, both of which promote late deposition of self-pollen as flowers become self-compatible (Stephenson et al., 2000; Goodwillie unpub. data). Unlike other delayed-selfing mechanisms, transient SI prevents both autonomous and pollinator-facilitated selfing early in anthesis. Our survey contained only two reports of transient SI; however, it may remain undiscovered in other species because it cannot be detected by typical tests for SI that compare seed set in hand selfed vs. outcrossed flowers.

Cryptic self-incompatibility (CSI) is a related phenomenon, in that it promotes cross-fertilization when both self- and cross-pollen are deposited on the stigma but allows for selfing when cross-pollen is absent (Bateman, 1956; Bowman, 1987; Weller and Ornduff, 1989). But CSI also allows self-fertilization to occur early in floral anthesis when cross-pollen is absent. We therefore categorize CSI as “competing selfing,” since it confers the best of both worlds, but only under a scenario in which self- and outcross-pollen are in direct competition (as described in Lloyd, 1992; Becerra and Lloyd, 1992).

Some novel mechanisms of delayed selfing fall outside our classification scheme. In two species in the Zingiberaceae, *Roscoea debilis* (Fan and Li, 2012) and *Caulokaempferia coenobialis* (Wang et al., 2005) oily fluid (originating at the stigma or anther sac) spreads during anthesis and allows pollen to travel to the stigmatic surface. Delayed selfing is promoted by diplostigmaty in *Sebaea aurea* (Gentianaceae), in which two stigmatic forms are present. An upper stigma, located above anthers, becomes receptive first and receives outcross-pollen, whereas the lower stigma matures later

and is autonomously self-pollinated, providing reproductive assurance (Kissling and Barrett, 2013). These two fascinating examples highlight the truly diverse nature of floral mechanisms.

Experimental support for delayed selfing

In the wide array of species and floral traits reported to confer delayed selfing, inferences on the timing of selfing have often been based on qualitative observation alone, such as visual inspection of changes in the position of reproductive organs. Observations can provide strong circumstantial evidence for delayed selfing, but some studies point to the importance of more rigorous testing. For example, in *Aquilegia canadensis* L. (Ranunculaceae) delayed selfing was initially hypothesized to occur through incomplete protogyny based on the timing of stigma receptivity and self-pollen deposition (Eckert and Schaefer, 1998); detailed studies revealed that pollen tube growth does not occur until anther dehiscence, which suggests that selfing and outcrossing are simultaneous (Griffin et al., 2000). In another cautionary example, delayed selfing was hypothesized in *Polygala lewtonii* (Polygalaceae), based on observations of self-pollen transfer to fertile stigmas late in anthesis; however, when tested with floral bagging experiments, autonomous selfing was negligible in all but a few individuals (Weekley and Brothers, 2006).

Although quantitative data such as counts of self-pollen deposited through time lend strength to observational reports in many species (e.g., Kalisz et al., 1999; Qu et al., 2007), experimental approaches using floral manipulations provide the most rigorous evidence for delayed selfing. These may not be feasible for all study species, yet they are necessary to fully assess the contribution of delayed selfing to reproductive success. Schoen and Lloyd (1992) proposed experimental methods to quantify the extent of selfing by different mechanisms (e.g., autonomous or facilitated by pollinators; within- or between-flower selfing) and occurring at different times (prior, competing and delayed). Seed production through delayed selfing is quantified as $(P_0 - P_3)/P_0$, where P_0 is the number of seeds produced by unmanipulated control flowers and P_3 is the number of seeds produced by flowers in which delayed selfing is prevented by stigma removal. Genetic marker-based analysis can also be used in combination with floral manipulations, such as stigma removal at different times, to estimate the contribution of delayed selfing to the overall selfing rate (Schoen and Lloyd, 1992).

Schoen and Lloyd's (1992) genetic method, arguably the most rigorous approach for quantifying delayed selfing, has been employed for few species to date. Genetic studies of *Camissoniopsis cheiranthifolia* (Onagraceae; Dart and Eckert, 2013) and *Sebaea aurea* (Kissling and Barrett, 2013), using floral manipulations confirmed substantial delayed selfing due to corolla closure and diplostigmaty, respectively. In *Mimulus guttatus* (Phrymaceae), however, genetic assays found no evidence for delayed selfing (Leclerc-Potvin and Ritland, 1994), although floral manipulations

indicate that corolla abscission and stigma curvature promote autonomous self-pollination (Dole, 1990, 1992).

Experimental studies are limited, reported for 14 of the 65 species in our survey. For example, stigma removal in late anthesis was used to quantify delayed selfing in *Collinsia parviflora* (Plantaginaceae; Elle et al., 2010). Another experimental approach quantifies seed set in flowers that have been emasculated at different intervals during anthesis—the emasculation time point after which increases in seed set are observed provides a measure of the timing of autonomous selfing (Vaughton and Ramsey, 2010; Fan and Li, 2012). The effect of transient SI on delayed selfing was estimated using mixed self- and outcross-pollen loads and genetic markers; the proportion of selfed offspring produced by mixed pollen loads was shown to increase with the floral age at which pollen was applied, demonstrating the potential for delayed selfing (Vogler and Stephenson, 2001; Goodwillie et al., 2004). Still other experimental tests have eliminated the presumed agent of delayed selfing and quantified effects on seed set. For example, Sun et al. (2005) secured corollas with threads to prevent pendulous wilting in a species of *Pedicularis* and found reduced self seed set, and a wind-exclusion experiment provided evidence for wind-dragged corolla abscission in *Incarvillea sinensis* (Qu et al., 2007).

Several experimental studies were carried out in a pollinator-free greenhouse (e.g., Dole, 1990; Goodwillie et al., 2004; Lankinen et al., 2007). Greenhouse studies can provide important information about the potential for delayed selfing, confirming that autonomous selfing can occur late in anthesis. However, field studies are necessary to determine the actual extent and fitness consequences of delayed selfing. For instance, with consistently high pollinator visitation, floral mechanisms that allow for delayed selfing might contribute little to seed set.

IS DELAYED SELFING THE BEST OF BOTH WORLDS?

Under the BOBW model, floral traits should promote outcrossing when pollinators are present, providing benefits through the avoidance of inbreeding depression. Selfing should act primarily as a back-up strategy, assuring reproduction when mates or pollinators are scarce. Thus, if delayed selfing is a BOBW mating strategy, species will (1) produce flowers that attract and reward pollinators, (2) have substantial inbreeding depression, (3) have a high capacity for autonomous selfing, and (4) experience occasional limitation in pollinator service or mate availability, resulting in variable outcrossing rates. To explore the strength of support for the BOBW concept, we examined the reported cases of delayed selfing for these properties (see Appendix S1). Here, we present our findings at the species level because the parameters measured (e.g., inbreeding depression, outcrossing rate) are likely to vary among species within a genus or even among populations. Because the methods for estimating parameters were inconsistent and some data were available for only a small subset of the species, we again present only descriptive summaries of the data.

Allocation to and benefits of outcrossing

Consistent with the BOBW concept, most species with reports of delayed selfing were described or could be characterized as having showy floral displays and often nectar rewards. In contrast, six species clearly displayed a “selfing syndrome” (Ornduff, 1969; Sicard and

Lenhard, 2011), including small flowers, low pollen production and low pollen/ovule ratios (e.g., *Fumana juniperina*, Carrió et al., 2008; *Geranium carolinianum* (Geraniaceae), Shirk and Hamrick, 2014). To gain more insight, we asked how flower size or pollen production in delayed-selfers compared with their obligately outcrossing relatives. In four of the ten such studies available, traits promoting cross-pollination appear to be maintained by selection consistent with the BOBW hypothesis; delayed-selfers in *Leptosiphon* (Goodwillie, 1999), *Primula* (Primulaceae, de Vos et al., 2012), *Schizanthus* (Solanaceae; Pérez et al., 2009) and *Camissoniopsis* (Dart et al., 2012) produce flowers that are similar in size to highly outcrossing congeners or conspecific populations. In contrast, delayed-selfing species of *Wachendorfia* (Haemodoraceae) and *Tillandsia* (Bromeliaceae) produce flowers only about half and one quarter the size, respectively, of closely related outcrossers (Jesson and Barrett, 2002; Orozco-Ibarrola et al., 2015). Similarly, in *Clarkia* (Onagraceae), *Anagallis* (Primulaceae), *Trifolium* (Fabaceae), and *Myosotis* (Boraginaceae), delayed-selfers were substantially reduced in petal size, pollen-to-ovule ratio or both, compared to more outcrossing sister species (Robertson and Lloyd, 1991; Gibbs and Taverna, 2001; Dhar et al. 2006; Mazer et al., 2007). Contrary to the BOBW model, the reduced allocation of resources to pollinator attraction suggests that selection for cross-pollination is diminished in some delayed-selfing species.

Do species with delayed selfing benefit from the opportunity for outcrossing? The primary advantage of outcrossing is thought to be the avoidance of inbreeding depression. Estimates of fitness of selfed and outcrossed offspring at multiple life stages were available for only 14 of 65 delayed-selfing species. In more than half of these, cumulative inbreeding depression was < 0.3 . Inbreeding depression estimates based on seed stages only were reported for an additional nine species, but they provide less information; of these, six showed inbreeding depression < 0.3 and three showed values close to 0. The paucity of data on inbreeding depression in delayed selfers limits conclusions; however, relatively low inbreeding depression argues against a strong fitness benefit to outcrossing in some species.

Capacity for and benefits of selfing

At the core of the BOBW concept lies reproductive assurance, which occurs if pollinator visitation is limited and autonomous self-pollination is effective. Although a survey of visitation rates was beyond the scope of this study, absence, scarcity, or high variability of pollinator visitation was frequently reported in the species surveyed (30%). The efficacy of autonomous selfing, or autofertility, can be quantified as the reproductive output of a flower that is bagged or in a pollinator-free environment divided by that of a hand-pollinated flower (Lloyd and Schoen, 1992). Autofertility was the most frequently available of the parameters examined (44 of the 65 species), although the methods used varied (see Fig. 3 caption; Appendix S1). Consistent with the BOBW model, most species showed a high capacity for autonomous selfing, with over half having an autofertility index > 0.7 (Fig. 3). However, values ranged widely. For example, in *Draba oxycarpa* (Brassicaceae) delayed selfing was relatively ineffective, with mean seed set in autonomously selfed flowers only 10% of that in hand-pollinated flowers (Brochmann, 1993).

A delay in selfing appears to result in reduced autofertility in some cases. Studies of variation in the timing of selfing among populations in *Blackstonia perfoliata* (Gentianaceae; Brys et al., 2013) and *Collinsia parviflora* (Scrophulariaceae; Elle et al., 2010) and among

species in the genera *Centaurium* (Gentianaceae; Brys et al., 2011) and *Draba* (Brochmann, 1993) indicate that delayed-selfers have lower autofertility than their earlier-selfing relatives. These findings suggest constraints on the ability to simultaneously maintain outcrossing success and maximize reproductive assurance through selfing.

The contribution of delayed selfing to reproductive success depends on both autofertility and the pollinator context. Under natural pollination, reproductive assurance can be quantified experimentally as $(1 - \text{seed or fruit set of emasculated flowers/seed or fruit set of unmanipulated flowers})$ (Eckert et al., 2006). Estimates

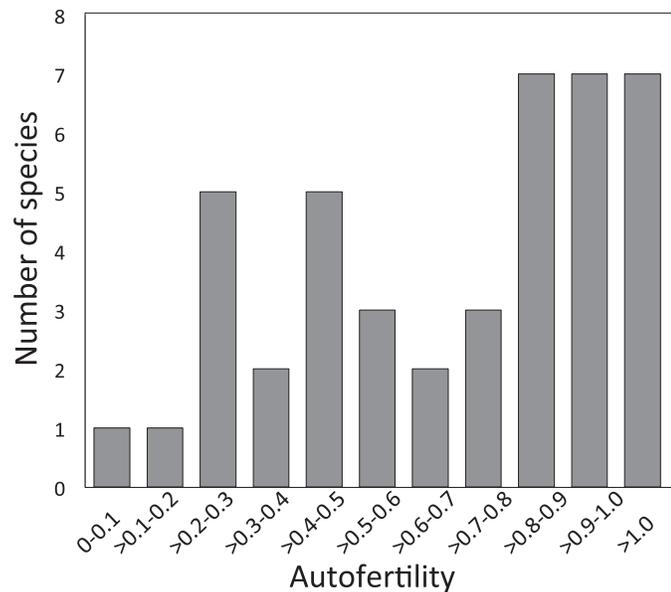


FIGURE 3. Distribution of autofertility index values in species reported to undergo delayed selfing. Autofertility = reproductive success of flowers with pollinators excluded/reproductive success of flowers with supplemental pollination. Measures used to quantify reproductive success (seeds per flower, fruits per flower, seeds per fruit or seeds per ovule) varied among species. Supplementation was either by self-pollen (35 species) or outcross-pollen (8 species). When more than one estimate was available for a species, a single value was randomly chosen for inclusion in the histogram.

were available for 26 of 65 species, and substantial values (> 0.3) were reported for at least one population or sampling time in 15 of these. Such information must be interpreted with caution, however. Emasculation can detract from pollinator attraction and shorten floral longevity, which can inflate estimates of reproductive assurance by reducing cross-pollination (Schoen and Lloyd, 1992; Dart and Eckert, 2013). Furthermore, temporal and spatial replication of experiments is necessary to assess whether selfing provides seed set during occasional pollination scarcity. Well-replicated studies of this kind, though few in number, provide some evidence that delayed selfing can provide backup when pollination fails because of weather, season, small population size or geography (Table 2).

If the degree of reproductive assurance varies with pollinator visitation, the rate of selfing should also vary. Genetic marker-based outcrossing rates were reported for only 17 of 65 delayed-selfing species and ranged from 0.1 to 1. As with reproductive assurance, replicated studies are necessary to confirm or reject the BOBW tenet that selfing will vary with pollinator visitation. The few such studies available for delayed-selfing species revealed substantial variation in outcrossing rate (e.g., Kalisz et al., 2004), sometimes in association with known factors (Table 2). For example, in *Verbascum thapsus* (Scrophulariaceae), tall plants had higher outcrossing rates than shorter ones that received fewer pollinator visits (Carromero and Hamrick, 2005).

In summary, some evidence supports a BOBW strategy in delayed-selfing species, but information on all the parameters we examined is available for only three: *Collinsia verna*, *Kosteletzkya virginica*, and *Leptosiphon jepsonii*. At least 24 species appear to lack substantial benefits from or allocation to one of the two “worlds.” In some, floral traits that promote outcrossing are reduced and/or strong benefits of outcrossing are lacking, as indicated by low inbreeding depression. Inbreeding depression was reported for only one species with a reduced floral display (*Fumara juniperina*) and, notably, outcrossed offspring were found to have no fitness advantage over selfed (Carrió et al., 2008). On the selfing side, most species have high capacity for autonomous selfing, but in some cases autofertility is lower than that of related species with earlier modes of selfing. Finally, consistent with the BOBW model, measures of reproductive assurance are high for many of the surveyed species, but information on variability in pollinator service or outcrossing rates is available for only a few. Indeed, a key finding of our survey is that evidence critical to testing the BOBW model is lacking for a large proportion of species in which delayed selfing has been reported.

TABLE 2. Evidence for variability in reproductive assurance by self-fertilization in delayed selfing species. In each case an ecological factor has been identified as a primary source of variation. RA = reproductive assurance; O = reproductive success of unmanipulated open-pollinated flowers; EO = reproductive success of emasculated open-pollinated flowers; t = outcrossing rate.

Source of variation	Species	Parameter measured	Range observed	Citation
Weather: fair vs. inclement days, pollinator visitation	<i>Bulbine vagans</i>	RA: $1 - (O/EO)$	0.54–1.0	Vaughton and Ramsey, 2010
Distance to conspecific individual	<i>Paris quadrifolia</i>	RA: $1 - (O/EO)$	0.16–0.23	Jacquemyn and Brys, 2008
Pollinator failure rate	<i>Schizanthus grahamii</i>	RA: $1 - (O/EO)$	–0.04–0.76	Pérez et al., 2009
Pollinator visitation rate	<i>Collinsia parviflora</i>	RA: $O - EO$	0.1–1.69	Kennedy and Elle, 2008b
Population size and pollinator availability	<i>Centaurium erythraea</i>	RA: $1 - (O/EO)$	0.19–0.87	Brys et al., 2011
Population size	<i>Sebaea aurea</i>	t	0.33–1.0	Kissling and Barrett, 2013
Pollinator failure rate	<i>Collinsia verna</i>	t	0.73–1.0	Kalisz et al., 2004
Altitude	<i>Schizanthus grahamii</i>	t	0.19–0.93	Pérez et al., 2013
Plant height	<i>Verbascum thapsus</i>	t	0.66–0.8	Carromero and Hamrick, 2005

Best-of-both-worlds: a case study

We conclude this section by highlighting perhaps *the* single species for which rigorous data on the factors we investigated are available and fully consistent with the BOBW model. In *Kosteletzkya virginica*, experimental emasculation of flowers demonstrated that progressive stigma curvature in unpollinated flowers confers delayed selfing (Ruan et al., 2009b). Large, showy flowers and nectar appear to be adapted for pollinator visitation, and inbreeding depression is substantial at 0.5 (Ruan et al., 2009a, 2009b). The selfing mechanism is highly effective, with autofertility estimated at 0.99 (Ruan et al., 2008). Delayed selfing appears to confer reproductive assurance when pollinators are limited, although studies were carried out in China in naturalized populations of this North American species. Pollinator visitation was found to vary in response to weather conditions, as did the magnitude of reproductive assurance (Ruan et al., 2009b). Moreover, pollinator failure, or (1 – fruit set of emasculated flowers/fruit set of hand-outcrossed flowers) (Kalisz et al., 2004) and selfing rate were positively correlated, providing further evidence for delayed selfing as a BOBW mating system (Ruan et al., 2009a).

EVOLUTION OF DELAYED SELFING TRAITS

How do traits that promote delayed selfing evolve? In some cases, the trait appears to be a novel feature that has been selected specifically to promote delayed selfing. A compelling example is a remarkable floral ballet in *Ruta graveolens*; sequential movement of individual stamens from the petal to the floral center and then back allows for presentation of pollen to pollinators in doses. In unpollinated flowers, the stamens finally rise together to contact the stigma, which simultaneously elongates during anthesis (Ren and Tang, 2012).

Perhaps more often the floral feature involved was present in outcrossing ancestors, where it may have been neutral or served a different role, and secondarily took on the function of delayed selfing as an exaptation (Gould and Vrba, 1982). Delayed selfing by corolla closure or abscission clearly represents a secondary function for a pre-existing phenomenon. For example, selfing occurs in *Fumana juniperina* when flowers close at senescence, yet floral closure is widespread in the predominantly SI family (Carrió et al., 2008). When a trait takes on a different function in a derived species, we might expect it to have been altered in some way to accomplish the new task (Gould and Vrba, 1982). Sun et al. (2005) raise this possibility with respect to the corolla-wilting mechanism of delayed selfing in *Pedicularis dunniana* (Orobanchaceae). Among a number of species studied in the largely outcrossing genus, *P. dunniana* is unusual in that its corolla remains attached for a longer period, perhaps extending the opportunity for delayed selfing. Certain trait combinations appear to be effective for delayed selfing in the surveyed species (Fig. 2), a pattern that has been demonstrated more broadly in plant mating systems (Armbruster et al., 2014; Törang et al., 2017). This, too, suggests a process of evolutionary modification in one trait to accommodate the new function of delayed selfing in another. Traits that promote delayed selfing might also serve other functions simultaneously. For example, style curvature is widespread in the Malvaceae, yet it promotes delayed selfing in only some species and appears to also prevent male–female interference and promote outcrossing (Ruan et al., 2010).

Some basic floral plans appear to predispose plant taxa toward the evolution of delayed selfing. The ancestral presence of herkogamy and dichogamy in some groups may promote the evolution of delayed selfing, perhaps explaining its high incidence in asterid families (Freitas and Sazima, 2009). Our survey suggests that epipetalous stamens might also play a role. The feature is present in all species with a corolla abscission mechanism and also in nine of 13 species that show a temporal reduction in herkogamy. In the latter mechanism, expansion of the corolla during anthesis causes the anthers of adnate stamens to rise to the level of the stigma. Although the high frequency of asterids and species with fused corollas among delayed-selfers could reflect pollinator specialization and strong selection for reproductive assurance (see above), it might also indicate simple mechanical requirements of some modes of delayed selfing. As evidence, we note that the two mechanisms of delayed selfing facilitated by epipetalous stamens—reduced herkogamy and corolla abscission—as well as incomplete dichogamy are well represented in asterid orders (Fig. 1).

EVOLUTIONARY STABILITY OF DELAYED SELFING

The evolutionary stability of delayed selfing is related to broader questions about the processes that maintain mixed mating-systems (Goodwillie et al., 2005). Early models of mating system evolution predicted only complete selfing or outcrossing as stable endpoints. The concurrent evolution of inbreeding depression and selfing rate is expected to contribute to divergent selection on mating systems; as selfing occurs, recessive deleterious alleles expressed in homozygotes can be removed by selection, purging inbreeding depression (Lloyd, 1979; García-Dorado, 2017) and favoring higher selfing rates (Lande and Schemske, 1985). Incorporation of additional factors into theory, such as functional relationships between male and female fitness components (Johnston et al., 2009), resource limitation of seed set (Harder and Routley, 2006), and pollinator behavior (Devaux et al., 2014), has uncovered conditions under which mixed mating can be stable. However, other models suggest that, although reproductive-assurance selfing is advantageous when pollen is limited, it is not an evolutionarily stable strategy (Harder et al., 2008). Thus, the stability of delayed selfing remains a question of interest.

Transitions to earlier modes of selfing could be facilitated by changes in the magnitude of inbreeding depression, destabilizing delayed selfing. If purging occurs through delayed selfing, inbreeding depression might decrease enough to select for earlier modes of selfing (Fig. 4). Although Lande and Schemske (1985) did not explicitly consider the timing of selfing, the evolution of increased selfing rates that they modeled could occur through transitions from delayed to competing to prior selfing. Inbreeding depression was low in more than half of the delayed-selfing species for which estimates were available (see above), consistent with purging. In contrast, a larger survey of angiosperms found similar mean inbreeding depression in mixed-mating and outcrossing species, which suggests that inbreeding depression can be maintained with partial selfing under many genetic scenarios (Winn et al., 2011). Clearly, more studies of inbreeding depression in delayed-selfing species are warranted.

Evolutionary transitions could also be driven by benefits of earlier modes of selfing not considered in simple models, such as more effective reproductive assurance or reduced costs of maintaining flowers. Furthermore, early self-fertilization could provide a benefit in habitats with seasonally deteriorating climatic conditions if it

allows fruits to mature more rapidly. This time-limitation hypothesis has been proposed as an advantage of selfing over outcrossing (Aarssen, 2000; Snell and Aarssen, 2005) but may also have relevance for the selection of prior vs. delayed selfing. Finally, early selfing might be selected for if it reduces interspecific pollen transfer. In contrast to delayed selfing, earlier selfing can reduce loss of fitness through production of hybrid offspring and would be favored under conditions in which hybridization invokes a high fitness cost (Goodwillie and Ness, 2013; Brys et al., 2016).

Studies of variation among populations provide evidence for transitions from delayed to earlier modes of selfing, perhaps indicating evolutionary instability. For example, in *Blackstonia perfoliata*, populations in pollinator-poor regions show evolution toward earlier selfing and a reduction in flower size (Brys et al., 2013). Drier habitats have favored the evolution of rapid development, reduced protogyny and earlier selfing in populations of *Collinsia parviflora* (Elle et al., 2010). In *Leptosiphon jepsonii*, early self-compatibility has evolved at sites where the species co-occurs with congeners,

perhaps because selfing prevents production of low fitness hybrids (Goodwillie and Ness, 2005, 2013). In these examples, autofertility is substantially higher in the early-selfing populations, suggesting benefits of increased reproductive assurance. In addition, lower inbreeding depression in earlier selfing populations of *Blackstonia*, *Collinsia* and *Leptosiphon* is consistent with purging (Goodwillie and Knight, 2006; Kennedy and Elle, 2008a; Brys et al., 2013). Even if transitions to earlier modes of selfing have occurred within a species, however, delayed selfing might be evolutionarily stable at a local scale. For example, across the range of *Camissoniopsis cheiranthifolia*, some populations are large-flowered and SI, some are large-flowered and self-compatible (SC) with delayed selfing, and still others are small-flowered with early selfing. Dart et al. (2012) argue that these forms represent different stable states of mixed mating.

At broader phylogenetic levels, our survey provides evidence both for and against long-term evolutionary stability of delayed selfing. Style curvature appears to have persisted as a stable delayed selfing strategy in the Malvaceae. The trait has been reported in at

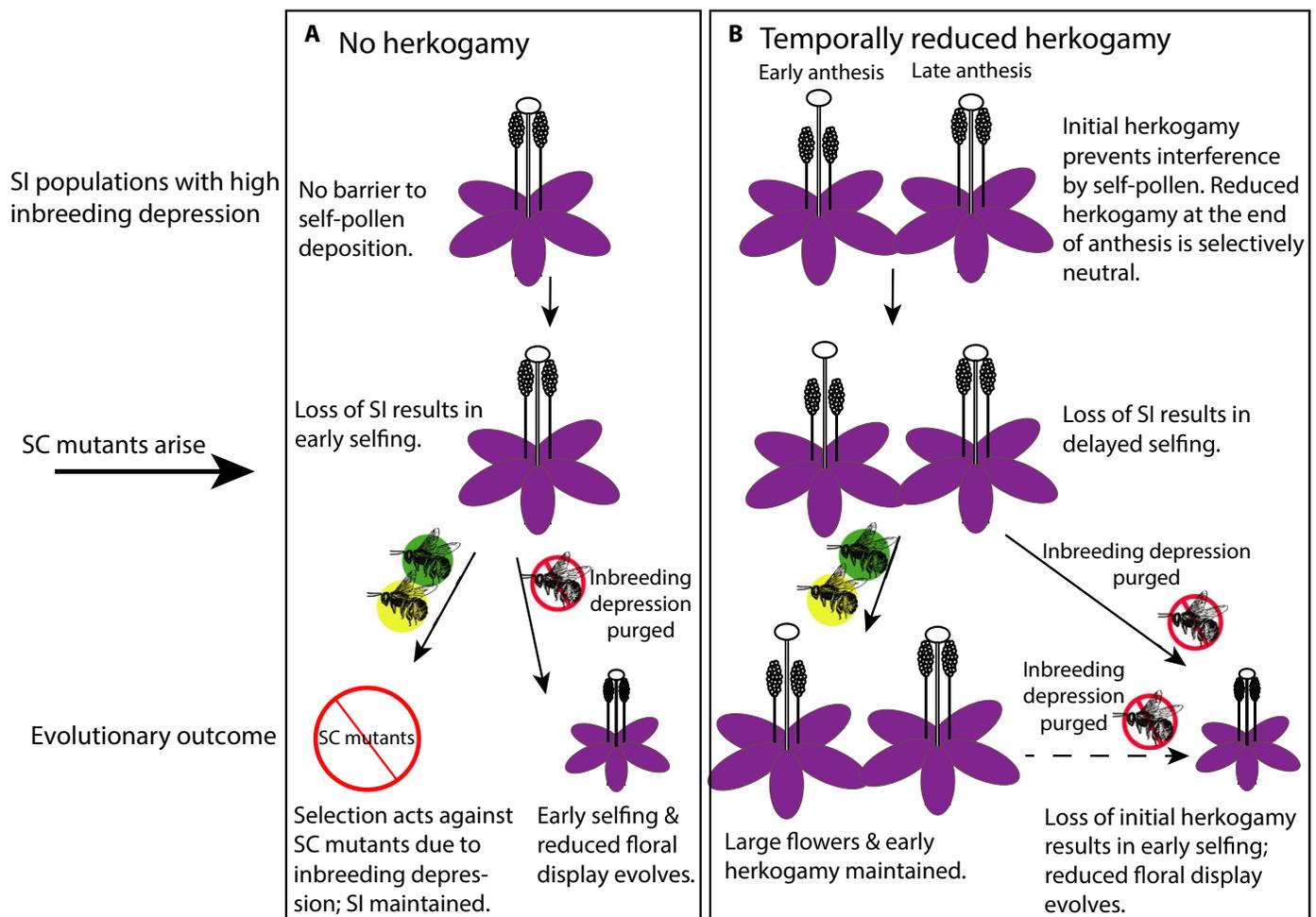


FIGURE 4. Potential pathways for the evolution of selfing from a self-incompatible (SI) ancestor under varying pollinator scenarios. (A) With no barrier to self-pollination, an SC mutant will give rise to early self-fertilization. High inbreeding depression will selectively eliminate SC mutants except with very low pollinator visitation (red circle), with subsequent purging of inbreeding depression possible. (B) When pre-existing traits (e.g., temporally reduced herkogamy) prevent early self-pollination, an SC mutant gives rise to delayed selfing, which can be selected for under all pollinator scenarios and levels of inbreeding depression. At variable (yellow) & high (green) pollinator visitation rates, delayed selfing may be maintained. If pollinator visitation rates are consistently low, inbreeding depression is purged and selection favors early selfing, either directly from the initial SC ancestor or via a delayed selfing ancestor.

least 23 species in eight genera where, in most cases, it appears to confer delayed selfing (Ruan et al., 2010, 2011). Phylogenetic analysis indicates that style curvature has evolved multiple times within the family, but some origins have given rise to large clades in which the trait is conserved (Ruan et al., 2011). Diplostigmaty, which promotes delayed-selfing in *Sebaea aurea* (Kissling and Barrett, 2013), is ancestral and widely distributed in the genus (Kissling et al., 2009) and thus, appears also to be an evolutionarily stable strategy. Furthermore, delayed stigma bending to contact pollen grains is widely, though not universally, distributed in *Viola* (Culley, 2000). Phylogenetic evidence for *Leptosiphon*, however, argues against stability. Small-flowered, highly selfing species have arisen multiple times from large-flowered, SI ancestors (Goodwillie, 1999). *Leptosiphon jepsonii* appears to be unique in the genus in having a transient form of SI that confers delayed selfing.

DELAYED SELFING AND THE EVOLUTION OF SELF-COMPATIBILITY

A consequence of delayed selfing that has received little consideration is its potential role in facilitating evolution from SI to SC (Fig. 4), a common transition in angiosperms (Stebbins, 1950; Kohn et al., 1996; Schoen et al., 1997; Goodwillie, 1999; Igic et al., 2008). If the loss of SI results in competing or prior selfing, SC mutants might be selected against because of high seed discounting and strong inbreeding depression (Lloyd, 1992; Layman et al., 2017; Fig. 4A). Only under conditions of pollinator failure might the benefits of SC outweigh its costs. But now imagine an SI species in which herkogamy declines during anthesis by slight differences in elongation rates (Fig. 4B). This late-anthesis loss of herkogamy might be effectively neutral in an SI species as it would have little effect on the amount of pollen received or dispersed. But if an SC mutation arises, temporally reduced herkogamy could take on a new function of delayed selfing. Importantly, SC would then be advantageous even with high inbreeding depression and high or variable pollinator visitation (Fig. 4B). Incomplete protogyny in an SI species might similarly facilitate the evolution of SC through delayed selfing. Several lines of evidence support a role of delayed selfing in breeding system transitions. Temporally reduced herkogamy appears to have been present before the evolution of selfing in *Wachendorfia* and *Leptosiphon*, given that it occurs in both SI and derived selfing species of each genus (Jesson and Barrett, 2002; C. Goodwillie, unpubl). More generally, herkogamy is commonly observed in SI species, where it is thought to reduce interference by self-pollen (Webb and Lloyd, 1986). Even more suggestive, a broad phylogenetic analysis of angiosperms found that transitions from SI to SC were especially frequent in protogynous species (Routley et al., 2004), perhaps reflecting a role of incomplete protogyny in promoting delayed selfing. Finally, transient SI, with flowers becoming SC in late anthesis, could also serve as a bridge to complete SC (Weber and Goodwillie, 2009).

CONCLUSIONS AND FUTURE DIRECTIONS

Continued interest in delayed selfing has been inspired by Lloyd's insightful work and the compelling idea that delayed selfing allows a species to enjoy "the best of both worlds." Our survey supports

the BOBW concept in some species but also finds reasons to be cautious in assuming that delayed selfing always serves this role. Indeed, low autofertility in some delayed selfers compared to early selfing relatives and low allocation to pollinator attraction in others suggests instead that "you can't be good at everything." We argue for rigorous examination of floral traits and evaluation of their functional and fitness consequences to avoid the temptation of an unexamined "just-so-story" (Gould and Lewontin, 1979; Pigliucci and Kaplan, 2000). Consideration of traits in a phylogenetic context can be used to determine whether and how delayed selfing has evolved from preexisting traits. Indeed, given that it seems often to arise as a secondary function, delayed selfing might provide an opportunity to study the processes by which traits are modified for novel or multiple functions (see Ruan et al., 2010).

Understanding of delayed selfing will benefit from experimental validation of the timing and extent of selfing and outcrossing and how each contributes to fitness. Two inherent challenges face researchers on this topic, however. First, extensive replication of experiments and observations in space and time will be required to test the central concept of the BOBW, that selfing provides occasional reproductive assurance. Second, the incredible variety of floral features that captures the interest of botanists also precludes one-size-fits-all experimental methods to test for delayed selfing (Schoen and Lloyd, 1992). Emasculation or style removal experiments feasible for one species may be impractical or have unintended side effects in another. Innovative and diverse experimental methods will be needed as more species are investigated, but the lack of standardization will continue to hamper rigorous meta-analysis approaches.

A major open question in the study of delayed selfing concerns its stability and broader roles in mating system evolution. Phylogenetic analysis combined with detailed studies of floral biology will contribute critical information on the distribution of delayed selfing in evolutionary lineages. Studies of this type have been attempted for only a few large taxa, such as the tribe *Collinsieae* (Armbruster et al., 2002) and sections of the Malvaceae (Ruan et al., 2010, 2011). Comprehensive studies of floral biology in families with SI can provide additional insights into the role of delayed selfing in breeding-system transitions. Further, theoretical work is needed that expands on Lloyd's models (1979, 1992) to explore the conditions under which transitions between selfing modes occur and the consequences of such transitions.

If delayed selfing combines advantages of selfing and outcrossing, we might expect it to be common. The distribution of published outcrossing rates suggests that mixed mating species are relatively common (Kalisz and Vogler, 2001; Goodwillie et al., 2005; Moeller et al., 2017) but we do not know how many are delayed selfers. A comprehensive survey of floral mechanisms, including monographs and historical botanical literature, might yield a rough estimate of the frequency of delayed selfing. Nevertheless, delayed selfing can easily go unnoticed, and detailed information on reproductive biology is limited. Rigorous experimental approaches will be necessary to quantify delayed selfing and its fitness consequences, yet simple observational studies of floral biology are also valuable and are currently lacking for many species. Natural history studies such as these will undoubtedly uncover more extraordinary floral mechanisms, provide starting points for in-depth examinations of delayed selfing, and contribute to broader understanding of the distribution and evolution of plant mating systems.

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SUPPORTING INFORMATION

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

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