STYLE PERSISTENCE, POLLEN LIMITATION, AND SEED SET IN THE COMMON PRAIRIE PLANT *ECHINACEA ANGUSTIFOLIA* (ASTERACEAE)

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Pollen limitation of seed set in flowering plants has important ramifications for the population dynamics, evolution, and conservation of plant populations. I conducted a pollen addition and exclusion experiment demonstrating that style persistence signifies pollen limitation in the narrow-leaved purple coneflower *Echinacea angustifolia*, a species native to the North American prairie and plains. I developed a measure of style persistence, SP, a novel way to quantify pollen limitation in individual plants during the flowering season. Using this measure, I investigated the relationship between pollen limitation and seed set over two years in 19 and 27 natural remnant populations in an agricultural landscape. Population mean rates of seed set per plant varied from 0% to 54% in 1997 and from 0% to 63% in 1998. I found that pollen limitation reduced annual reproductive fitness within and among the populations studied. An analysis of the relationship between floret production and the rate of seed set provided no evidence that resource limitation influenced the rate of seed set. I estimated annual fecundity per plant as the product of the rate of seed set per floret, a pollen-limited process, and floret production per plant, likely a resource-limited process. Population means of individual annual fecundity ranged from 0 to 182 in 1997 and from 0 to 156 in 1998 and were predicted by population means of SP and the rate of seed set, but not by floret production. The effect of pollen limitation, as quantified by SP, overrides the strong, fundamental relationship between fecundity and floret production. This finding shows that populations consisting of large plants with large floral displays do not necessarily produce more seeds per plant.

Keywords: *Echinacea*, pollen limitation, pollination, prairie, reproductive success, fecundity, style persistence.

Introduction

Pollen limitation of seed set in animal-pollinated plants is widely reported (Burd 1994; Larson and Barrett 2000) and has important ramifications for the demography, evolution, and conservation of plant populations. For example, pollen limitation affects population dynamics (Heithaus et al. 1982). It has been suggested as the proximate cause of local extinction of both common and rare species (DeMauro 1993; Groom 1998). In addition, pollen limitation affects population genetic structure, thereby influencing natural selection on floral traits (Galen 1985; Young et al. 1996). Pollen limitation has also been implicated in mating system evolution (Barrett and Harder 1996; Goodwillie 2001). Pollen limitation is a critical factor in the reproduction of some plant populations; it is therefore important to identify the contexts in which it naturally occurs and to quantify the magnitude of its impact on seed production.

Numerous hypotheses have been advanced to account for patterns of pollen limitation in nature. Plant traits proposed as potential contributors to pollen limitation include self-incompatibility (Byers and Meagher 1992), dependence on specialized pollinators (Rathcke and Jules 1993), pollination by generalists (House 1992), absence or paucity of floral reward (Melampy and Hayworth 1980; Burd 1995; Johnson and Bond 1997), brevity of flowering period (House 1992), small size of floral display (Mitchell 1994), and small size of plant (Dudash 1993). Researchers have also proposed ecological causes of pollen limitation, including small habitat patch size (Sih and Baltus 1987), inclement weather (Campbell 1987; Hendrix and Kyhl 2000), competition for pollination (Campbell 1985; Feinsinger and Tiebout 1991), rarity or conspecific population sparseness (Galen 1985; Feinsinger et al. 1991; Byers 1995), and depauperate pollinator diversity and abundance (Jennersten 1988; Aizen and Feinsinger 1994). Interactions between plant traits and environmental factors have been implicated as well. Examples include site size and mating type diversity (Byers and Meagher 1992; DeMauro 1993), floral rewards and soil moisture (Campbell 1987), and generalized pollination and heterospecific floral competitors (Kunin 1993). Many of these hypothetical causes of pollen limitation remain insufficiently tested, in part because measuring pollen limitation is challenging.

Two obstacles frequently impede quantification of pollen limitation in natural populations. First, to discriminate conclusively among alternative causes of seed limitation, such as resource and pollen availability, tests must meet rigorous requirements, particularly in perennials and plants with more than one flower (Calvo and Horvitz 1990; Johnston 1991; Ehrlen and Eriksson 1995; Dudash and Fenster 1997).

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However, experimental manipulations, such as the pollen addition and exclusion experiments typically used to account for variation in seed set due to resource and pollen availability, are likely to interfere with the natural dynamics of seed production in the system. For example, adding pollen to one flower may induce resource limitation in other flowers on the same plant, even in a subsequent year (Zimmerman and Pyke 1988). Investigators have tried to overcome this obstacle by combining experimental and observational approaches (Bosch and Waser 1999, 2001).

Second, pollen limitation is an imprecise term that is used to describe many phenomena. Seed production in angiosperms involves at least five pollen-related steps: (1) pollen release, (2) pollen transport, (3) pollen deposition, (4) pollen germination, and (5) fertilization of the ovule. Pollen limitation can occur at any of these steps. Current methods of assessing pollen limitation quantify limitation at one or more of these steps but often confound it with aspects of reproduction that are not related to pollination. For example, pollen limitation is sometimes inferred from seed set data. But, seed set may also reflect pollen quantity (Molano-Flores and Hendrix 1999), pollen quality (Bosch and Waser 1999; Paschke et al. 2002), seed abortion due to resource limitation, and even ovule predation (Inouye et al. 1994). Researchers have addressed the confounding of these phenomena using strategies that depend on the nature of the pollen limitation hypotheses tested and the floral biology of the species investigated (Griffin and Barrett 2002). Here, I define pollen limitation as the situation in which insufficient pollen is deposited to fertilize all ovules produced in one season, such that additional pollen deposition would result in more fertilized ovules.

I investigated the reproductive biology of the narrow-leaved purple coneflower *Echinacea angustifolia*. Distinct aspects of *Echinacea*'s floral biology independently reflect the resources available to and the pollination of the plant. First, the number of florets produced by a plant in one season, which represents the maximum possible number of single-seeded fruits, depends on the resources available to the plant. The number of florets on a head is determined before pollen is shed. If resources continue to limit reproduction after florets are produced, then small plants, i.e., those with fewer resources, are expected to produce fewer seeds per floret (Griffin and Barrett 2002). Second, the duration of style persistence depends on pollination. I present evidence that an individual style emerges receptive and withers within 24 h following deposition of compatible pollen. If no pollen arrives, the style persists up to 10 d. These two observations indicate that pollen limitation in this species may be assessed during the flowering season in natural populations.

In this article, I present first a pollen addition and exclusion experiment demonstrating that style persistence signifies pollen limitation in *E. angustifolia*. Using this finding, I then develop a measure of style persistence (SP) that can be used in the field. Employing individual plants from many natural populations over 2 yr, I verify that SP measures pollen limitation by relating SP to the rate of seed set. Then I test whether floret production, a process limited by resources, is related to SP and to the rate of seed set. Finally, I assess resource and pollen limitation of annual fecundity jointly in natural populations.

Material and Methods

Study Site and Species

The narrow-leaved purple coneflower *Echinacea angustifolia* (Asteraceae) is a common native plant in the tallgrass prairie and plains of North America. It is a long-lived perennial with a single taproot and no vegetative reproduction. Plants rarely flower before their third year (Baskauf 1993; S. Wagenius, personal observation). In western Minnesota, a flowering plant usually produces one capitulum (78%; 13% two heads; 6% three heads; 2% four heads; 1% >4 heads) but may have more than 10. The large, distinctly colored capitula (heads) stand above surrounding vegetation and are conspicuous during and after flowering. Pollen production and stigma receptivity occur from early July through early August. Most *Echinacea* populations are strictly self-incompatible (McGregor 1968; Franke et al. 1997), although one population of the same species studied in North Dakota may be partially self-compatible (Leuszler et al. 1996). Diverse generalist insects pollinate *Echinacea*, including butterflies, bees, and flies (Krombein et al. 1979; S. Wagenius, personal observation). When pollinated, each disk floret produces one uniovulate fruit, a cypsela. The seed coat expands and hardens whether or not the ovule is fertilized. Hereafter, I refer to fruits with fertilized ovules as seeds, fruits with unfertilized ovules as empty seed coats, and fruits with unknown fertilization status as achenes. *Echinacea* seeds have no specialized means of dispersal. They require specific light, temperature, and moisture conditions to break dormancy (Feghahati and Reese 1994). Plants produce basal leaves in years when they do not flower.

The study area comprises 6400 ha (25 sections) in rural western Minnesota (centered near 45°49'N, 95°42'30''W). Before European settlement in the 1870s, the entire area, except for lakes and wetlands, was potential *Echinacea* habitat. *Echinacea* now persists in remnant populations on hillsides too steep for agricultural production, in fence corners inaccessible to farm machinery, along road and railroad rights-of-way, and on abandoned pastureland. Remnant populations vary in size from one to several thousand flowering individuals each year. The largest population within the study area occupies a 45-ha virgin prairie preserve owned and managed by the Nature Conservancy.

Floral Phenology

Echinacea florets develop in concentric circular rows sequentially from the bottom outside of the capitulum to the top middle. The florets are protandrous. On one day, anthers on florets in a single row shed yellow pollen. On the next day, styles emerge from florets in that row, and their lobes open, while anthers in the adjacent inner, higher row shed pollen (fig. 1). Typically, this process continues daily to the top of the head, although on a cold or cloudy day, some plants may shed no pollen (such days occur zero to two times each season). A floret's position below the current pollen-shedding row indicates the number of days earlier that the anthers emerged from that floret. Furthermore, the shape of a style indicates its receptivity—a style shrivels within 24 h after receiving compatible pollen. I number each row sequentially
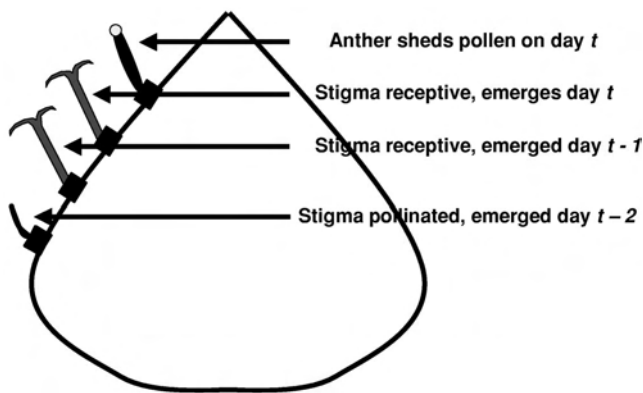
Fig. 1 Schematic cross section of *Echinacea* head with detail of four florets, representing a snapshot in time on day *t*. Each floret represents a row of florets. Floret rows emerge sequentially from bottom to top. The florets are protandrous, presenting anthers and pollen one day, and styles the next. A floret's position relative to the pollen-shedding row is revealed when the style emerges. A style's shape indicates receptivity because it shrivels within 24 h after compatible pollen lands on the stigmatic surface of the style branches.

from the bottom to the top. Each row is obvious when the anthers shed pollen, but when several rows of styles are present, rows become less obvious. To identify the row of an individual floret, I observe that there are two direct diagonal paths of florets to the sterile ray florets at the bottom of the head. The shorter of the paths (counting the florets) identifies the row of that floret. A bract subtends each floret and facilitates counting in the field.

Pollen Exclusion and Addition Experiment

On July 21, 1997, I placed pollinator exclusion bags on 16 flower heads, each on a separate plant from one population. On July 24, I isolated the florets on one half of each head from the florets on the other half with a paper or plastic divider. On July 27, I artificially pollinated all receptive florets. On one half of each head, I used self-pollen (pollen from other florets on the same head) and on the other half out-cross-pollen (pollen from one other plant). I counted the number of style rows persisting for the 3 d after the pollination treatment.

Sampling in Natural Populations

In 1997, I selected 12 plants at random from each of 19 different populations, including 28 plants from the nature preserve. In 1998, I selected up to six plants (12 on the preserve) from 28 populations, including the 19 populations sampled in 1997. From populations with fewer than six flowering plants, I chose all plants. In each year, plants were selected from all flowering plants, which were found in exhaustive searches, except at the preserve, where plants were sampled from all those observed within a 5-m-wide transect. Each plant was uniquely identified with a numbered aluminum tag and each head with a colored tag around the peduncle. An assistant or I visited each plant every third day during the summer starting on June 30. For each head on every plant at every visit we characterized each row of florets as

immature, with anthers, with receptive styles, or with post-receptive styles. If the florets in a given row were not uniform (a rare occurrence), then we characterized the proportion in each state (e.g., 80% florets receptive, 20% postreceptive). In every year some plants produced heads but no styles due to disease or damage; these heads produced no seeds.

Style Persistence

Information about style receptivity and pollen shedding of all rows on one plant over the course of the flowering season reveals the pollination status of the plant (fig. 2). My rules for interpolating style status between observation days are based on two observations of floral development: first, rows do not develop out of order, and second, no more than one style row emerges each day. Rule 1: if rows x and $x + 1$ on day t are receptive, then row x is receptive on day $t - 1$. Rule 2: if row x is receptive on day t and day $t - n$, then the row is receptive every day from $t - n$ to t. Rule 3: every row is receptive at least 1 d.

Because I observed heads only every third day, I inferred the actual beginning and end dates of receptivity. For example, styles in row 1 of figure 2 could have become receptive on July 7 or 8 and ceased being receptive on July 11, 12, or 13. I chose to calculate all SP values with the most conservative estimate of persistence duration; i.e., I relied on rules 1–3 to infer starting dates, and I assumed that the styles shriveled immediately after they were last observed to be receptive.

For each head in a given season, I calculated the average number of days that each row of styles remained receptive. Style persistence, SP, is the unweighted mean duration of persistence of all style rows on all heads of a plant. The minimum value of SP is 1, which occurred in plants when every row was observed on one day only. A value of six means that rows of styles on the plant remained receptive for an average of 6 d.
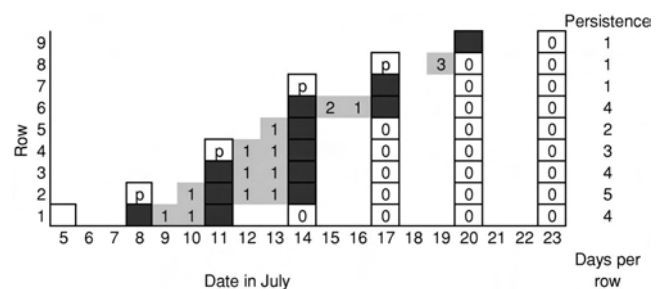

Fig. 2 Style row persistence on an *Echinacea* capitulum during a flowering season. This diagram shows the flowering status of floret rows over time. Observations were made every third day, and style receptivity was inferred on the other days. The empty square on July 5 represents an observation before any florets emerged. A square with *p* represents a row of pollen-shedding florets. Black squares represent rows of florets that were observed with receptive styles on a given day. A square with *0* represents a row of shriveled styles. Floret rows inferred to be receptive are shown in gray. Numbers within squares refer to the rule used to infer style row receptivity. The column on the right shows the duration of style receptivity in days for each row and includes black and gray squares. I define the mean style duration over all rows as style persistence (SP). Here, SP = 2.8 d.
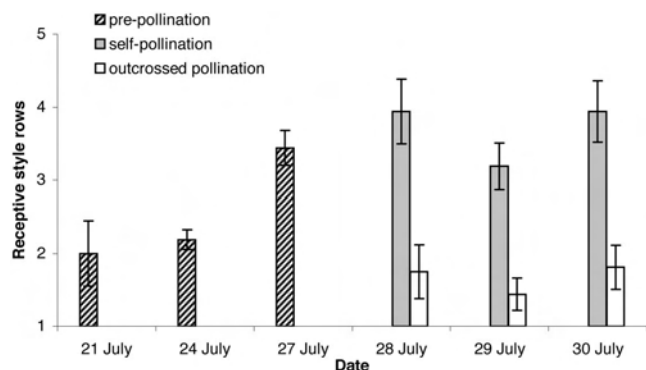
Fig. 3 Number of persistent style rows on 16 capitula over time. All pollinators were excluded by mesh bags placed over each head on July 21, 1997, thereby inducing rows of receptive styles. A selfing or outcrossing pollination treatment was applied on July 27 to one-half of each head. Data are means ± SE.

Floret Production and Seed Set

I collected every head from all observed plants in late August or September when the head was dry and the peduncle was entirely brown. Heads were stored individually at 4°C in paper bags until achenes were removed. All achenes were separated from the receptacle and then counted. This count represents the number of florets produced per head. The maximum annual potential seed yield per plant is the count of all florets from all heads on the plant (floret production). From each head I randomly selected 45 achenes for germination trials in the following January. Achenes were germinated using a pregermination and germination protocol that uniformly breaks dormancy, synchronizes emergence, and germinates almost every viable seed (Feghahati and Reese 1994). This method includes temperature, light, moisture, and ethylene treatments. I followed the published methodology except that I used no fungicide, and I placed 15 achenes on blotters in 5-cm-diameter petri plates, instead of in germination boxes. Each seed was removed from the petri dish when the radicle was at least 1 mm long.

I classified the sampled achenes as germinated, fertilized, not fertilized, damaged, or aborted. Achenes that were visibly shorter than the others, not fully expanded, and empty were classified as aborted. The remaining full-sized, nongerminated achenes were dissected, and the presence of an embryo indicated fertilization. Achenes were inspected for damage; most damage was caused by insects. The rate of seed set per plant was defined as the proportion of achenes that germinated or were fertilized divided by the sample of achenes subjected to the germination treatment. Aborted and damaged achenes were not included in the numerator or denominator of this ratio. The rate of seed set is equivalent to ovule fertilization efficiency (Inouye et al. 1994) and can be interpreted as the probability of successful completion of pollination of an individual floret. Floret production per plant was estimated by counting all achenes, including those aborted and damaged. Several heads lost a few achenes in the field before harvest, but in every case I could count the number of missing achenes.

Data analyses were conducted separately for each year. SP, the rate of seed set, and floret production were calculated for all individual plants. Using simple linear regressions, I estimated and tested the dependence of seed set on SP, SP on floret production, and seed set on floret production. To discern patterns among individual plants, plants from all populations were pooled, while population means of individual measures were used to discern patterns among populations. The total fecundity (number of fertilized achenes produced in 1 yr) for each plant was estimated as the product of the rate of seed set and floret production. I show how population mean fecundity was related to seed set, SP, and floret production. Three plants were outliers in floret production and were excluded from all regression analyses. Their exclusion did not affect the outcome of any hypothesis test (SAS Institute 2002).

Results

Pollen Deprivation and Addition Experiment

During the period of pollinator exclusion before the pollination treatment, the number of rows of receptive styles per head increased (fig. 3). Following the pollination treatment, styles remained exserted on artificially self-pollinated halves of heads, but styles shriveled rapidly on the halves that were artificially outcrossed with pollen from another plant (fig. 3). The number of rows with exserted styles is directly related to the time that the styles persisted without receiving compatible pollen. Hence, SP signifies that florets have not received compatible pollen and that the ovules have not been fertilized, i.e., pollen limitation.

Style Persistence and Seed Set in Unmanipulated Plants

Individual rates of seed set varied from 0% to 85% in 1997 and from 0% to 82% in 1998, and population means varied from 0% to 54% in 1997 and from 0% to 63% in 1998. Fewer than 10% of achenes had undetermined fertilization status; they were not included in the calculations of seed set. During both years, the rate of seed set, expressed either for individual plants or as population means, declined significantly with increasing SP (fig. 4; 1997 individuals: $N = 153$, $R^2 = 0.14$, $P < 0.0001$; 1997 populations: $N = 19$, $R^2 = 0.42$, $P = 0.0028$; 1998 individuals: $N = 129$, $R^2 = 0.34$, $P < 0.0001$; 1998 populations: $N = 27$, $R^2 = 0.72$, $P < 0.0001$). These observations demonstrate pollen limitation in natural plant populations in the absence of experimental manipulation of individuals.

Floret Production, Style Persistence, and Seed Set

Floret production, estimated by the total number of achenes per plant, varied from six to 682 in 1997 (mean 218 ± 133; outliers had 1247 and 3870) and from 37 to 754 in 1998 (mean 219 ± 118; one outlier had 1062). SP and the rate of seed set exhibited no clear relationship with floret production in either year of the study (fig. 5). These results offer no support for the hypothesis that resource limitation influences variation in seed set on a per floret basis.

Fecundity

Annual fecundity per plant is estimated as the product of two independent factors: the rate of seed set per floret and
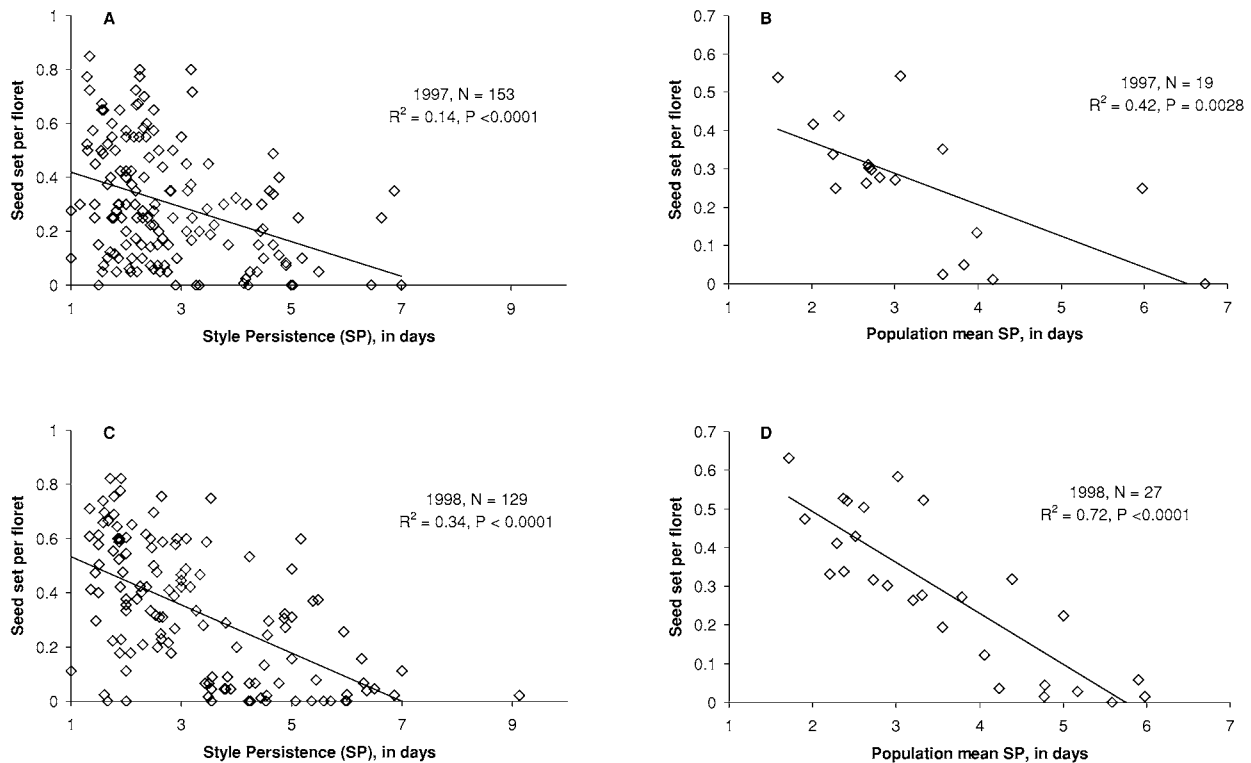
Fig. 4 Relationship between the rate of seed set and style persistence (SP) in *Echinacea angustifolia*: (*A*) 153 individuals in 1997, (*B*) 19 population means in 1997, (*C*) 129 individuals in 1998, and (*D*) 27 population means in 1998. SP is the mean duration of receptivity in days over all rows of styles on all heads of a plant.

floret production per plant. Fecundity per plant ranged from 0 to 455 in 1997 (mean 67 ± 65; outliers had 38 and 1234) and from 0 to 317 in 1998 (mean 73 ± 76; outlier had 607). Population mean estimates of fecundity ranged from 0 to 182 in 1997 (mean 60 ± 44) and from 0 to 156 in 1998 (mean 64 ± 46). Population means of fecundity per plant were predicted by population means of SP and the rate of seed set but not by floret production (fig. 6). This result shows that populations with better pollination, as quantified by SP or the rate of seed set, produced more seed. In contrast, populations with larger plants, as quantified by floret production per plant, did not predictably produce more seed. The effect of pollen limitation, as quantified by SP, overrides the strong, fundamental relationship between fecundity and floret production.

Discussion

Pollen versus Resource Limitation in Echinacea

The rate of seed set in remnant *Echinacea* populations was limited by pollen, but not by resources, whether on a basis of individual plants or population means. In both years of this study, SP, a direct measure of pollen limitation, predicted the rate of seed set (fig. 4). In each year, pollen limitation apparently reduced the rate of seed set in some populations to 0. In contrast, there was no evidence that resource limitation af-

fected the rate of seed set in either year of the study. If resources limited the rate of seed set, then small plants, ones with reduced floret production, should have a lower rate of seed set. I found that the rate of seed set was independent of floret production (fig. 5).

Annual floret production in a uniovulate species such as *Echinacea* represents the maximum number of seeds that a plant could produce in a year. Floret production was independent of pollen limitation (fig. 5). Given that all florets on a head develop before pollination begins, this finding makes sense; nevertheless, pollination levels in one year could affect floret production in a subsequent year (Calvo and Horvitz 1990; Ehrlen and Eriksson 1995; Dudash and Fenster 1997), a possibility I did not test. On the other hand, floret production likely depends on resources (Campbell and Halama 1993; Griffin and Barrett 2002). Both floret production and the rate of seed set influenced annual individual fecundity strongly. Thus, pollen and resources independently limited individual fecundity to the extent that I quantified them. It is possible that there are interactions at extreme levels of pollen or resource limitation (Griffin and Barrett 2002). Such levels were not a focus of this investigation, in part because I could not determine the fertilization status of less than 10% of all achenes, and they were not included in the calculations of rates of seed set or fecundity. About one-third of these indeterminate achenes came from two heads with damaged peduncles that produced only aborted achenes. Another third came from five diseased heads.
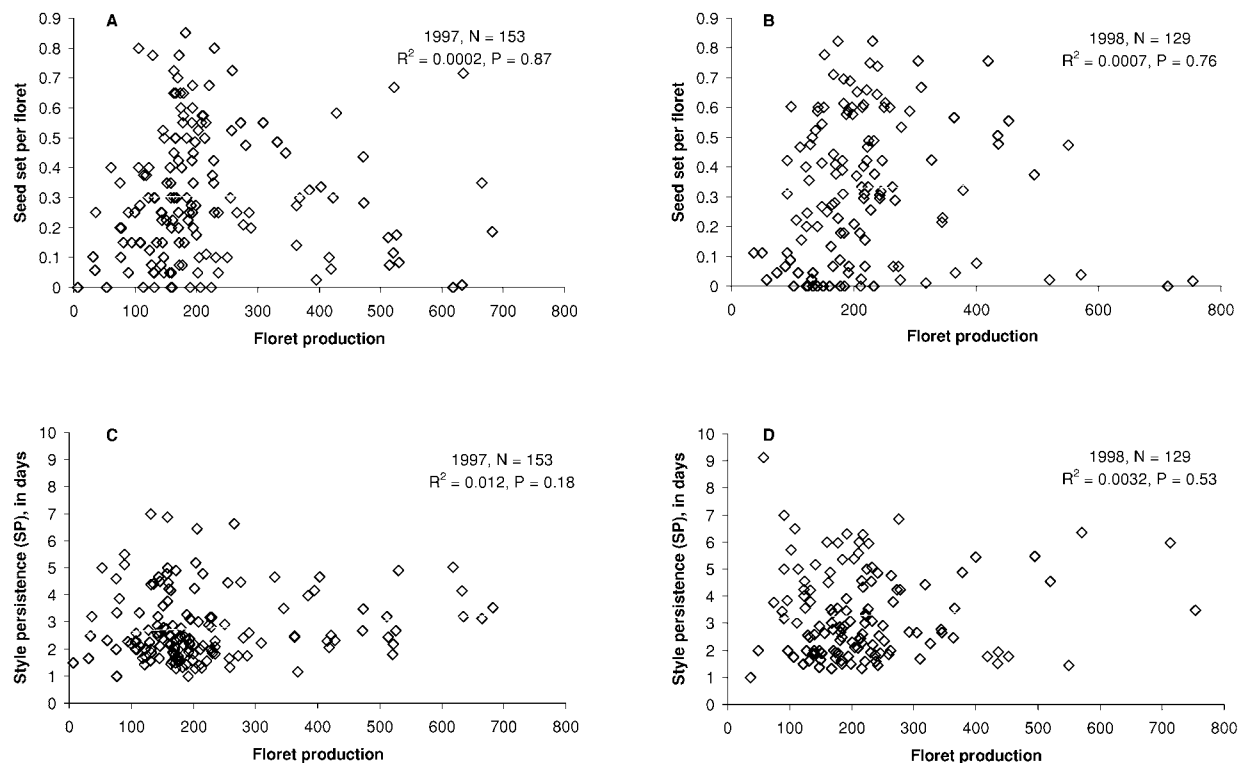
Fig. 5 Annual floret production per plant as a predictor of the rate of seed set and style persistence (SP) in *Echinacea angustifolia*: (*A*) seed set versus floret production in 1997, (*B*) seed set versus floret production in 1998, (*C*) SP versus floret production in 1997, and (*D*) SP versus floret production in 1998.

Even though floret production, a process likely limited by resources, and seed set, a pollen-limited process, together determine annual plant fecundity, the latter factor is overwhelmingly influential at the population level. The population means of annual fecundity per plant were predicted with linear regressions by population means of SP and the rate of seed set (fig. 6), which means that populations with good pollination produced more seeds per plant than more pollen-limited populations. Remarkably, populations with greater floret production per plant did not predictably produce more seeds per plant. This means that populations with large plants and plants with large floral displays do not necessarily produce more seeds per plant nor do these plants have greater annual reproductive fitness. From a management perspective, this suggests that a rapid field appraisal of an *Echinacea* population should prioritize an assessment of styles higher than an evaluation of plant size.

Causes and Consequences of Pollen Limitation

On one hand, *Echinacea* has some characteristics that are not expected in pollen-limited plants; it is a common temperate zone herbaceous plant of open areas with nectar and pollen rewards, a generalized floral architecture, and numerous generalized floral visitors (S. Wagenius, personal observation). Larson and Barrett (2000) conducted a comparative analysis of 224 species and found that herbaceous, nectariferous, and temperate plants were less likely to be pollen lim-

ited. Commonness and generalized pollinators are also hypothesized to reduce the chance of pollen limitation (Galen 1985). On the other hand, *Echinacea* is self-incompatible, a trait that was strongly associated with pollen limitation in Larson and Barrett's study. Kunin (1993) argued that generalized insects are less effective pollinators when heterospecific floral competition is high. He demonstrated this experimentally with *Brassica kaber* grown in arrays with and without other flowering species. Kunin hypothesized that self-incompatible plants with generalist pollinators and nearby heterospecific flowering plants are likely to receive poor pollination services. The *Echinacea* results are consistent with Kunin's prediction.

Pollen limitation may affect evolution and population dynamics in *Echinacea* because it causes variation in individual annual fitness and population mean fitness. Variation in individual fitness provides opportunities for natural selection to alter distributions of heritable traits associated with fecundity. Such traits may include self-compatibility, vegetative reproduction, attractiveness of floral display, duration of style receptivity, nectar quality or quantity, and pollen production. The existence of variation in mating compatibility in *Echinacea* remains an open question and should be the starting point for investigations into the evolution of self-compatibility. Most investigators state that *Echinacea* is strictly self-incompatible (McGregor 1968; Franke et al. 1997), which corroborates my observations, but these investigations do not specify sample sizes or power to detect self-compatibility. One
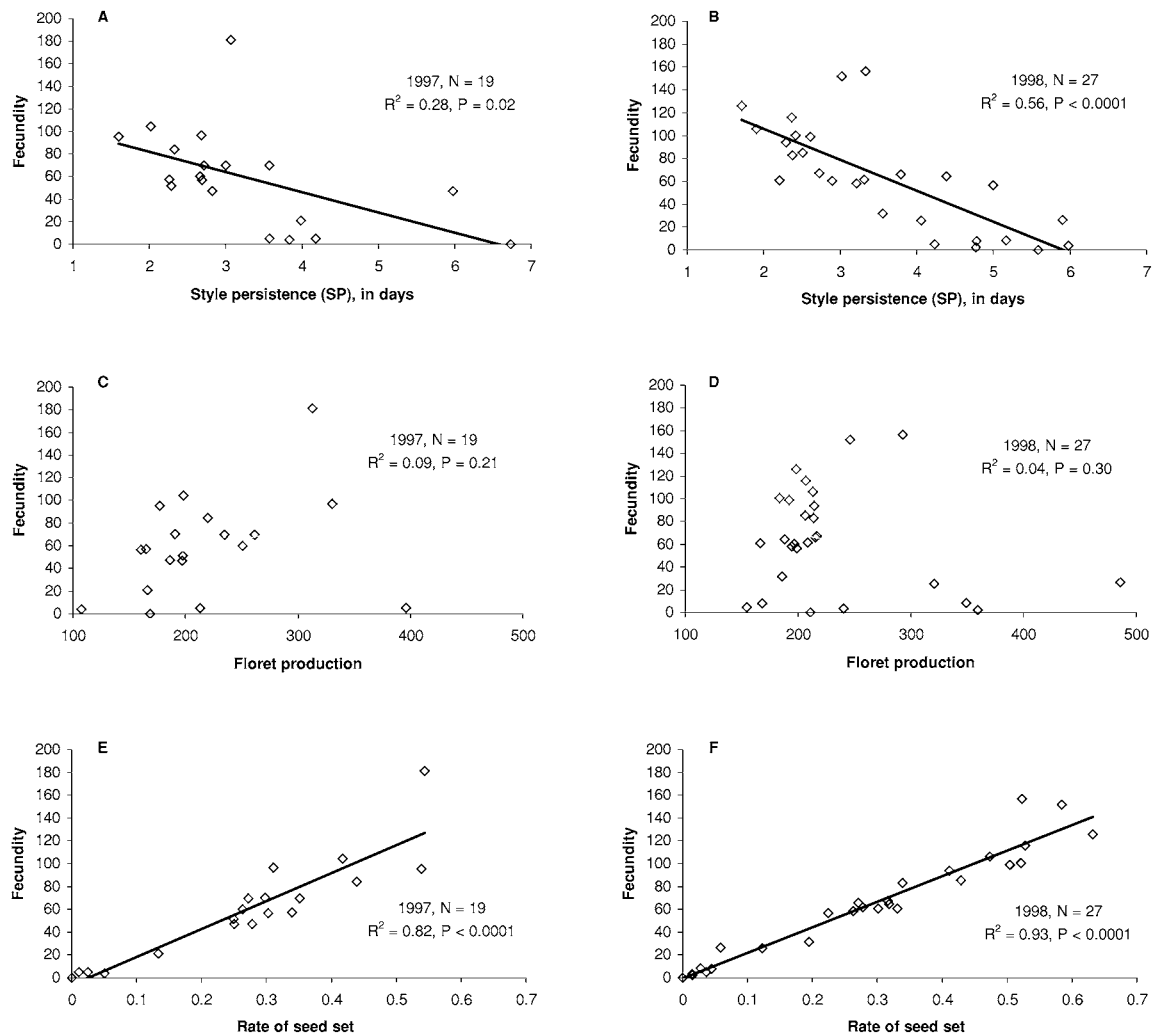
Fig. 6 Population means of annual fecundity per plant in *Echinacea angustifolia* as predicted by style persistence (SP), floret production per plant, and the rate of seed set per floret: (*A*) fecundity versus SP in 1997, (*B*) fecundity versus SP in 1998, (*C*) fecundity versus floret production in 1997, (*D*) fecundity versus floret production in 1998, (*E*) fecundity versus seed set in 1997, and (*F*) fecundity versus seed set in 1998.

study found low levels of self-compatibility (Leuszler et al. 1996) but perhaps did not control for pollen contamination.

Echinacea serves as a model for a large class of prairie plants: those that are self-incompatible, pollinated by generalist insects, and reproduce only via seeds. Kunin (1993) implicated these characteristics as potential causes of pollen limitation. The prairie, with diverse insect-pollinated plants, combines all the components of Kunin's pollen limitation scenario. A broad class of common prairie plants may experience the effects of pollen limitation, including significant reductions in annual reproductive fitness that put demographic pressure on plant populations and may affect population dynamics. Such effects may be intensified with further fragmentation of the prairie and disruption of pollinators. Even though pollen limitation of seed set is only one aspect of reproduction, at the population level, it appears to overwhelm the other factors, including resource limitation, that influence annual reproductive output in *Echinacea* through their effect on floret production.

Style Persistence Quantifies Pollen Limitation

My pollen exclusion and addition experiment showed that a row of styles persisted when it received no pollen from another plant and shriveled shortly after receipt of compatible pollen. Thus, SP is a measure of pollen limitation. In natural populations during both years of the study, SP was significantly related to the rate of seed set. This method for quantifying pollen limitation has several advantageous features. First, SP can be assessed directly in the field when plants are flowering; it requires no experimental manipulation affecting pollen, styles, or pollinators. However, it does require frequent observations. Second, SP is independent of resource limitation. Third, SP explicitly quantifies pollen limitation, instead of inferring limitation from measurements of pollination. In this respect, the SP method is similar to observing untripped flowers with trip mechanisms (Parker 1997) and counting pollen tubes (Aizen and Feinsinger 1994) but differs from observing pollinators and counting pollen loads (Bosch

and Waser 1999). Fourth, SP is a conservative measure of pollen limitation because styles can disappear by means other than the deposition of compatible pollen. For example, styles can be damaged or eaten by insects, causing them to shrivel, thus giving the investigator a false pollination signal. Fifth, SP enables comparison with pollen limitation in a treatment group to a variety of nonlimited controls, including the level of pollination found in nature (Bosch and Waser 1999), a supernatural treatment induced by the investigator (Young and Young 1992; Baker et al. 2000), or the absolute lack of limitation, a one-to-one seed-to-ovule ratio (Griffin and Barrett 2002). Finally, the precision of SP results correlates with observational frequency and focus. For example, observing daily or focusing on individual florets instead of rows would increase precision. Hence, SP may be useful for studying pollination in a variety of contexts, particularly in species with uniovulate fruits.

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