FUNCTION AND EVOLUTION OF AGGREGATED POLLEN IN ANGIOSPERMS

Lawrence D. Harder[1] and Steven D. Johnson

Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada; and School of Biological and
Conservation Sciences, University of KwaZulu-Natal, Postal Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

The evolution of different forms of pollen aggregation (tetrads, polyads, pollen threads, pollinia) from individual monads is a recurring transition in angiosperm history, having occurred independently at least 39 times. Aggregation should evolve only under special circumstances, because diminishing returns associated with pollen removal and receipt instead favor monads that act largely independently. All forms of aggregation result in sibling pollen grains acting together, but they seem to evolve to ease different limitations on siring success: tetrads may evolve most commonly when pollinators visit infrequently, pollen threads may be most beneficial when ovules become available synchronously, and pollinia greatly increase the probability that a pollen grain removed by a pollinator reaches a conspecific stigma. Once pollen aggregation evolves, its implications for gametophytic competition and the relatedness of seeds within fruits probably influence further reproductive evolution, especially the frequency with which pollen from a single donor sires all seeds in a fruit. This latter effect, rather than improvements in pollination efficiency, probably accounts for the common association of pollen aggregation with low pollen : ovule ratios. The ability of orchid pollinia to reduce diminishing returns during pollination may explain both the floral diversity and the widespread occurrence of deceit pollination in this clade.

Keywords: gametophytic competition, pollen-transfer efficiency, pollinia, pollination, polyad, tetrad, viscin threads.

Introduction

Outcrossing angiosperms produce several orders of magnitude more pollen grains than seeds. Each seed represents prior fertilization by a successful pollen grain, so that large pollen : seed ratios illustrate that the vast majority of pollen grains fail in their primary function. To the extent that siring success differs among pollen-producing plants, this low success rate should impose strong fecundity and sexual selection on pollen characteristics. Not surprisingly, pollen exhibits extensive interspecific variation in size, form, and physiology (Muller 1979), which undoubtedly represents the outcome of such selection. Among the many pollen adaptations, pollen aggregation stands out as a mechanism that enhances the chance that a pollen grain will contribute genes to a seed. Whereas most angiosperms shed pollen as relatively independent monads, various forms of aggregation (tetrads, polyads, viscin threads, pollinia) have evolved repeatedly within angiosperms. Species with aggregated pollen tend to have much lower pollen : ovule ratios than species with monads (Cruden 2000), indicating that individual grains of species with aggregated pollen have a comparatively high chance of contributing to the next generation of sporophytes. Thus, the evolution of pollen aggregation from individual monads represents a significant functional transition in angiosperm reproduction.

How aggregation enhances siring success is unclear for two general reasons. First, aggregation has manifold effects, as it can affect the rate of pollen removal, pollen transport, pollen

deposition on individual stigmas, postpollination processes that result in ovule fertilization, and the relatedness of developing seeds. Disentangling the relative importance of aggregation for these processes presents a challenge, given the formidable difficulties of studying pollen dispersal and siring success in natural populations. Furthermore, different forms of pollen aggregation may generate contrasting advantages, so the benefits and costs of aggregation may not be universal to all types of aggregation. The second source of uncertainty arises because the diverse hypotheses for the adaptive benefits of pollen aggregation remain largely unexplored theoretically and untested empirically.

Almost 30 years ago, Willson (1979) proposed that variation in pollen aggregation among plants traits reflects sexual selection through a process akin to sperm competition in animals. Since then, understanding of the function of male floral traits and their evolution has progressed considerably. However, pollen aggregation has not been considered thoroughly in the context of the general theory that has developed, and this important transition in angiosperm evolution has not been reviewed thoroughly.

In this article, we consider the variety of pollen aggregation, its occurrence among angiosperms, its influence on pollen performance, and its associated consequences for reproductive evolution in angiosperms. We begin by reviewing the various types of pollen aggregation, the incidence of transitions from monads to aggregation among angiosperm families, and the association between pollen aggregation and clade diversity. Next, we consider general consequences of sibling pollen grains acting together which both explain the widespread production of distinct, rather than aggregated, grains and
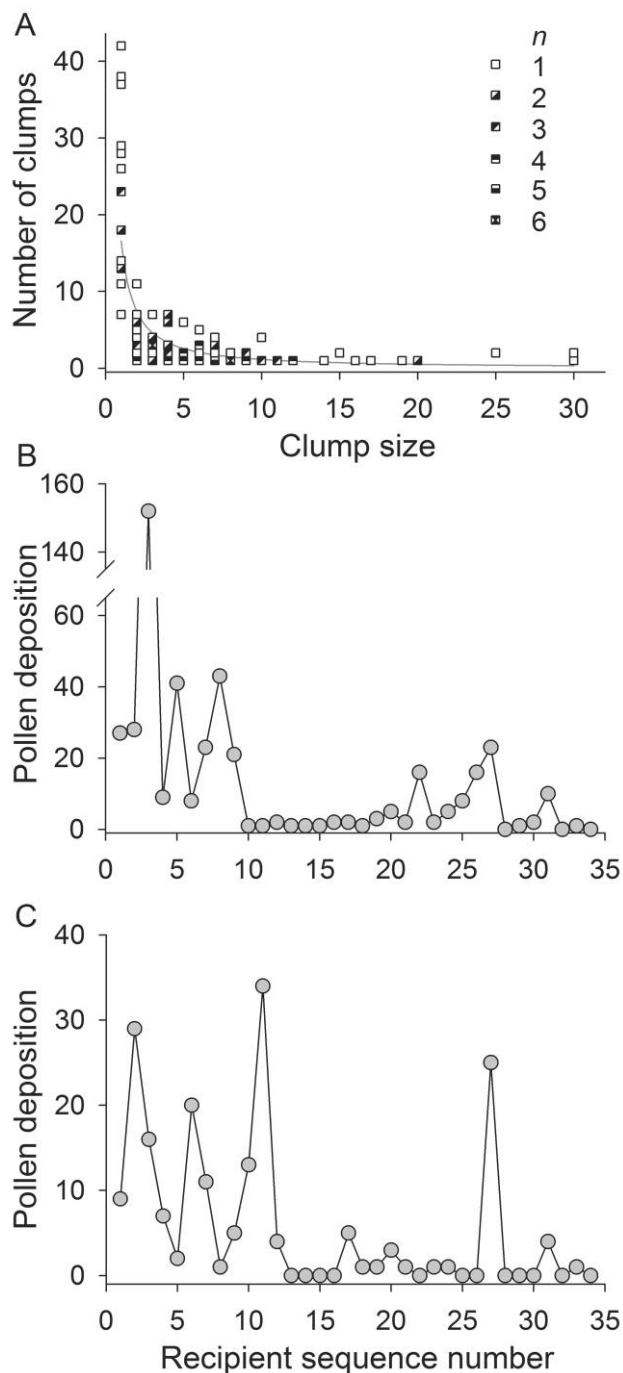
[1] Author for correspondence; e-mail: harder@ucalgary.ca.

identify specific outcomes that must be realized for aggregation to be selected. Given this conceptual framework, we then consider empirical evidence for the influences of aggregation on pollen fates, including pollen removal, pollen transport, and postpollination success, and their likely roles in the origin and elaboration of aggregation. Finally, we consider several aspects of plant reproduction that may evolve once aggregation is established. This review illustrates that pollen aggregation has extensive and sometimes fundamental consequences for the diversification of angiosperm reproduction.

Types and Phylogenetic Distribution of Aggregated Pollen

Pollenkitt and Tryphine

Since adhesion of pollen both to vector and stigma plays such a central role in angiosperm reproduction, it is surprising that so little is known about the exine coatings which confer adhesive properties on the grains of many species. (Dickinson et al. 2000, p. 302)

During the final stages of pollen production, the tapetal cells lining the inner surface of an anther degenerate, coating the pollen grains with viscous pollenkitt or tryphine (Dickinson et al. 2000; Pacini and Hesse 2005). In most angiosperms, degeneration is complete and produces lipid-rich pollenkitt, whereas less complete degeneration in the Brassicaceae produces chemically more complex tryphine, which contains tapetal organelles (Dickinson et al. 2000). Pollenkitt is produced by almost all animal-pollinated species other than Brassicaceae, but it is absent, or at least much reduced, in many wind-pollinated and buzz-pollinated species (Pacini and Hesse 2005). Many functions have been proposed for pollenkitt and tryphine, including roles during pollen dispersal and pollen-pistil interactions (Dickinson et al. 2000; Pacini and Hesse 2005). However, most of these functions remain to be assessed experimentally, and their possible implications have not been examined theoretically.

Owing to their viscosity, pollenkitt and tryphine cause pollen grains to adhere to anther walls, to each other, and to pollinators (see photographs in Hesse 1980). Consequently, pollen grains of animal-pollinated plants tend not to travel independently, even if they are produced as separate monads. For example, figure 1A illustrates that 86% of *Narcissus assoanus* pollen grains on the tongues of butterfly pollinators were being transported in clumps, rather than as separate grains, with a median clump size of seven grains and a maximum of ca. 150 grains. Pollenkitt and tryphine result in rather haphazard aggregation of pollen grains, with considerable variation in clump size (e.g., fig. 1A). In addition, clump size probably changes during dispersal as the action of pollen vectors breaks large clumps apart and combines individual grains and small clumps (see Lisci et al. 1996). Such aggregation and

Fig. 1 Clumping of individual pollen grains and its effect on pollen dispersal. *A*, Frequency distribution of clump sizes of *Narcissus assoanus* pollen on the proboscides of 16 *Cleopatra* butterflies (*Gonepteryx cleopatra*) collected north of Montpellier, France. Different symbol types indicate the number of butterflies with the same number of clumps of a given size (e.g., two butterflies each had one clump of 20 grains). Five clumps with >30 grains (maximum = 150 grains) were not included. *B, C*, Two examples of dispersal of monads from individual donor inflorescences to sequences of recipient flowers by individual bumblebees, illustrating extensive stochasticity in the dispersal process around a generally declining trend. *B*, Dispersal of pollen from a long-styled *Pontederia cordata* inflorescence to short-styled recipient flowers, tracked by morph-specific differences in pollen

size (L. D. Harder and S. C. H. Barrett, unpublished data). *C*, Dispersal of *Brassica rapa* pollen from a donor plant with a β-glucuronidase reporter gene to wild-type recipients (N. M. Williams and L. D. Harder, unpublished data).

the considerable variation in clump size undoubtedly contribute to the extensive stochasticity in pollen dispersal by individual pollinators from individual donor inflorescences to successively visited flowers (e.g., fig. 1B, 1C).

Tetrads and Polyads

Lack of separation of pollen grains during pollen production can generate structural aggregations, typically involving four pollen grains (tetrads) but with up to 32 grains in polyads of some African *Acacia* species (fig. 2A; Knox and Kenrick 1983). Some angiosperms, primarily noneudicots, undergo successive microsporogenesis, during which callose is deposited after each meiotic division, producing tetrads for varying shapes (including linear; Furness et al. 2002). In contrast, most eudicots undergo simultaneous microsporogenesis, whereby the two meiotic divisions occur before callose is deposited, to produce the four microspores of each tetrahedral pollen tetrad (Furness et al. 2002). Typically, the tetrads separate to produce monads; however, if this dissociation does not occur, mature pollen grains remain as permanent tetrads. Some species, especially in the Mimosoideae (Fabaceae), produce polyads consisting of multiple tetrads (fig. 2A), which arise because the sporogenous cell divides mitotically before the resulting pollen mother cells undergo meiosis (Knox and Kenrick 1983; Seijo and Solís Neffa 2004). Polyad production can be associated with considerable reduction in pollen production per anther. For example, the anthers of Australian *Acacia* species are divided
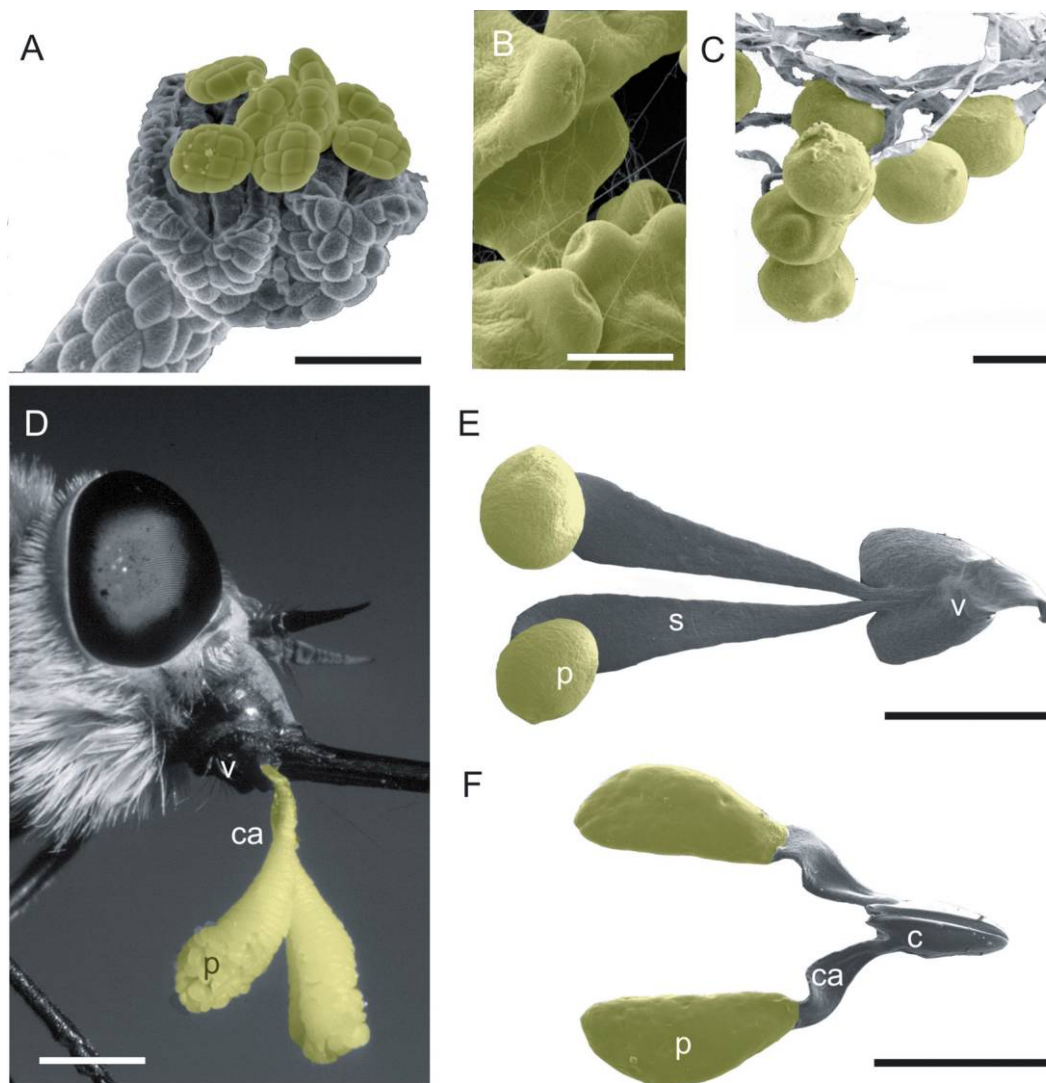

Fig. 2 Representative examples of pollen aggregation in angiosperms. *A*, Polyads consisting of 16 pollen grains in an *Acacia ataxacantha* anther (Mimosoideae [Fabaceae]). *B*, Viscin threads of *Oenothera biennis* (Onagraceae) pollen. *C*, Nonsporopollenin threads entangling *Strelitzia nicolai* (Strelitziaceae) pollen. *D*, Pollinarium of the orchid *Disa harveyana* with two sectile pollinia attached to the proboscis of *Philoliche rostrata* (Tabanidae). *E*, Pollinarium of the orchid *Cyrtorchis arcuata*, with two solid pollinia. *F*, Pollinarium of the milkweed *Pachycarpus grandiflorus*, with two solid pollinia (Asclepiadoideae [Apocynaceae]). False yellow color is added to pollen structures. *c* = corpusculum, *ca* = caudicle, *p* = pollinium, *s* = stipe, *v* = viscidium. Scale bars: *A*, 50 μm; *B*, *C*, 10 μm; *D–F*, 1 mm.

into eight loculi, each of which produces a single polyad, with typically 16 pollen grains per polyad (Knox and Kenrick 1983: also see fig. 2*A*).

Pollen Threads

The pollen of species in a few families is aggregated by various types of threads that entangle grains. Hesse et al. (2000) distinguished threads composed of sporopollenin (viscin threads; fig. 2*B*), which are projections from the outer layer of the pollen wall (ectexine), from those composed of other substances, primarily pollenkitt, that adhere to pollen grains (fig. 2*C*). The production of viscin threads is an integral process of microsporogenesis for species that possess them (see Rowley and Skvarla 2006), so they are produced by every grain in an anther. These threads are flexible but not elastic (Hesse et al. 2000). Only species in the Onagraceae (fig. 2*B*) and Rhodoreae [Ericaceae] produce viscin threads, and pollen in these clades is also shed as tetrads. In contrast, nonsporopollenin threads develop after pollen grains have been produced and are often not associated with each pollen grain. These threads occur in scattered species or genera in the Annonaceae, Araceae, Aristolochiaceae, Caesalpinioideae [Fabaceae], Heliconiaceae, Hydrocharitaceae, Marcgraviaceae, Passifloraceae, Potamogetonaceae, and Strelitziaceae (fig. 2*C*; Hesse et al. 2000).

Pollinia and Pollinaria

Pollinia are cohesive masses of many pollen grains that are removed as a unit from anthers (Johnson and Edwards 2000; Verhoeven and Venter 2001). They are the most evolutionarily derived form of pollen aggregation and are known from only two families, Orchidaceae and Apocynaceae. In derived orchids (Epidendroideae and Orchidoideae) and milkweeds (Secamondoideae and Asclepiadoideae), two or more pollinia and accessory structures for attachment to pollinators constitute a pollinarium (fig. 2*D*, 2*E*).

Orchids exhibit diverse types of pollen aggregation, from a sticky smear of monads in most Cypripedioideae to various forms of pollinia with monads or tetrads (fig. 3*A*). The simplest pollinia, found in the Vanilloideae, consist of a loose sticky mass of monads that adheres to the pollinator without a viscidium. Analogous loose pollinia lacking detachable viscidia also occur in some cypripedioid and epidendroid orchids, notably the tribe Neottiea. In some Neottiea, such as *Epipactis* and *Listera*, attachment of loose pollinia to pollinators is aided by a droplet of glue secreted by the rostellum (an elaboration of the stigma). Mealy pollinia with detachable viscidia occur in the Diurideae and Cranichideae. Sectile pollinia, in which distinct subunits of pollen known as massulae are held together by viscin, occur in several tribes, including the Orchideae and Diseae (fig. 2*D*). For mealy and sectile pollinia, clumps of pollen or wedge-shaped massulae, rather than the whole pollinium, adhere to individual stigmas, so that numerous flowers can be pollinated by a single pollinium (Peakall 1989; Johnson and Nilsson 1999). Hard pollinia (fig. 2*E*) predominate in the largest orchid clade (Epidendroideae) and are deposited on stigmas as an entire unit. Multiple pollinia can allow a single pollinarium to pollinate more than one flower; however, pollen deposition on stigmas

of epidendroid orchids usually involves simultaneous deposition of all pollinia from a single pollinarium (Nilsson et al. 1992; Alexandersson 1999).

The accessory structures of an orchid pollinarium include a sticky viscidium derived from stigmatic tissue, which attaches the pollinarium to a pollinator, and a connecting structure known as a "caudicle," if it is derived from sporogenous tissue, or a "stipe," if it is derived from stylar or stigmatic tissue (Dressler 1993; Johnson and Edwards 2000). Caudicles and stipes of many orchids bend or twist slowly after a pollinarium is removed from the anther. This reorientation reduces the likelihood of geitonogamous self-pollination, as pollinators have usually moved to a different plant by the time pollinia are positioned so that they can contact stigmas (Johnson et al. 2004; Peter and Johnson 2006). Caudicles, in particular, are highly elastic, which makes the attached pollinaria resistant to loss by pollinator grooming.

The Apocynaceae also exhibit various forms of pollen aggregation, although with less diversity than in the orchids (fig. 3*B*). Most Periplocoideae produce free tetrads, but some genera in this subfamily possess pollinia deposited onto spoon-shaped translators, which adhere to pollinators by a sticky viscidium similar to that of orchids. Flowers of Asclepiadoideae and Secamonoideae produce five pollinaria, each with two or four hard pollinia covered in a hard outer wall, giving the entire mass a smooth bony appearance (fig. 2*F*). Each pollinium connects to a clasping corpusculum, which attaches the pollinarium to a pollinator via a translator (sometimes termed a "caudicle"; fig. 2*F*). Both corpusculum and translator are derived from stigmatic secretions (Verhoeven and Venter 2001). Curiously, the pollinia attached to a single corpusculum are derived from adjacent halves of different anthers. Sideways torsion is usually required for pollinia to be inserted into the stigmatic grooves, which are orientated vertically at 90° relative to the anthers. Thus, as with orchid pollinia, many milkweed pollinia undergo gradual reconfiguration after removal from a flower (e.g., Wyatt 1976), and variation in the timing of this process is also likely to reduce geitonogamous self-pollination.

Phylogenetic Distribution of Pollen Aggregates

Angiosperm history has involved recurring experimentation with pollen aggregation, as the transition from monads to various types of aggregation has occurred repeatedly. Most angiosperms, especially animal-pollinated species, have mechanisms that aggregate pollen, so the evolution of derived forms of aggregation (tetrads, polyads, viscin threads, and pollinia) must be considered in the context of ancestral populations of plants with monads that adhered to each other to varying degrees owing to the presence of pollenkitt. Forty-two of the 457 families recognized by the APG (2003) include species with tetrads, polyads, pollen threads, or pollinia, and these families are scattered throughout the angiosperms (table 1). Maximum parsimony analysis (Mesquite, ver. 1.12; Maddison and Maddison 2006) of the occurrence of pollen aggregation based on the phylogenic tree of Davies et al. (2004) suggests that aggregated pollen has evolved independently at least 39 times. This evolution typically occurred within families, as 34 of the families with aggregated pollen also include species with monads. In addition, the evolution of aggregated pollen usually resulted in
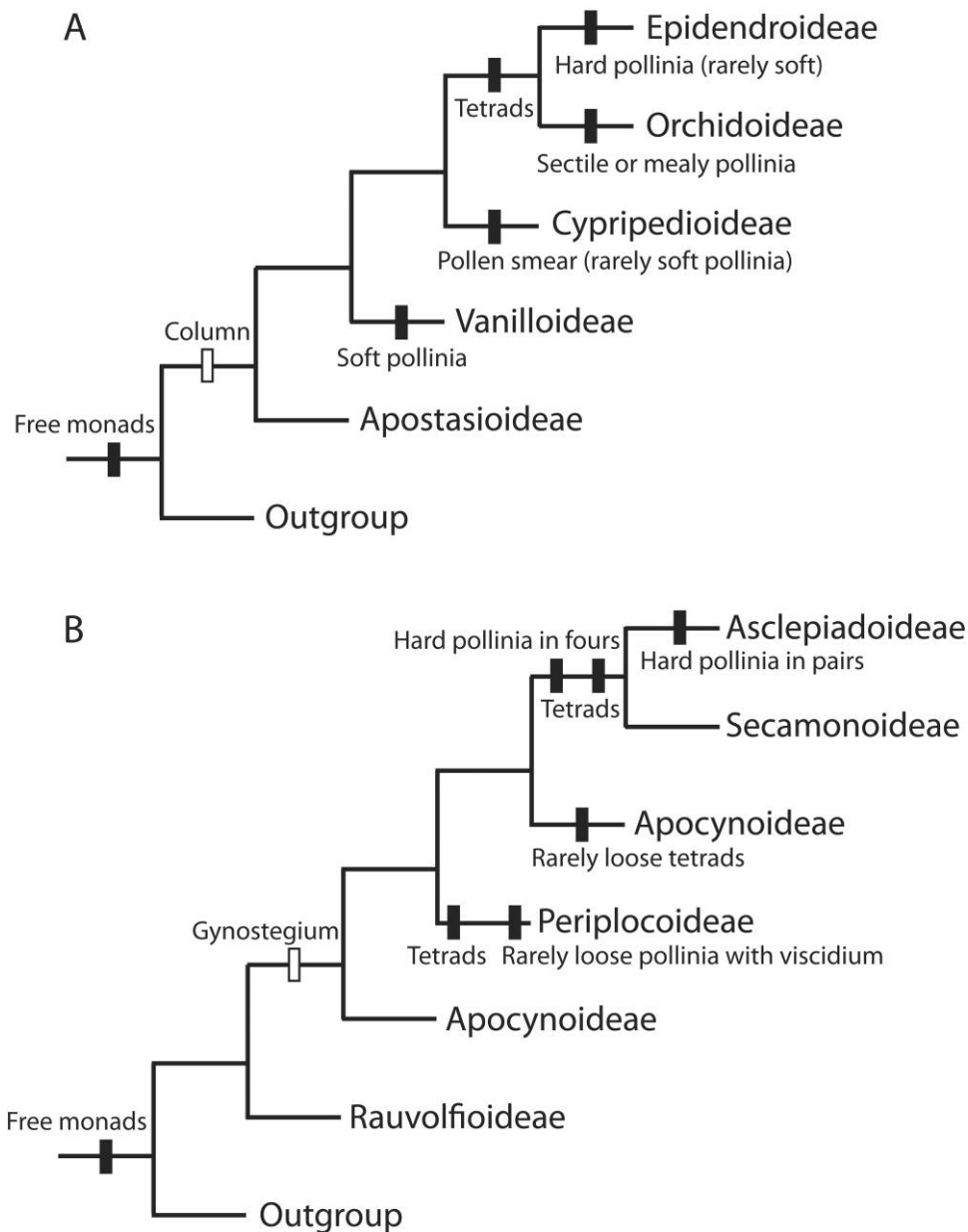
Fig. 3 Phylogenetic hypotheses for transitions in the evolution of pollen aggregation in (*A*) the Orchidaceae and (*B*) the Apocynaceae. Phylogenetic trees of subfamilies and character mapping (based on maximum parsimony) are adapted from Kocyan et al. (2004; Orchidaceae) and Livshultz et al. (2007; Apocynaceae). Filled bars indicate transitions of pollen traits, whereas open bars indicate the fusion of the androecium and gynoecium. In *B*, branches representing the Apocynoideae simplify the relationships identified by Livshultz et al. (2007), who proposed that this paraphyletic group comprises at least seven clades.

the production of tetrads (39 families). Thus, most origins of aggregated pollen probably involved the relatively easy retention of an intermediate stage in pollen production, with little additional evolutionary innovation (table 1). In contrast, the rare evolution of viscin threads (Onagraceae and Rhodoreae [Ericaceae]) and pollinia (Orchidaceae and Apocynaceae) suggests strong evolutionary constraints that have seldom been overcome. Aggregated pollen occurs primarily in animal-pollinated taxa, although it is also found in the water-pollinated Hydrocharitaceae (including Najadaceae) and four wind-pollinated clades

(Thurniaceae + Cyperaceae + Juncaceae, Myrothamnaceae, Scheuchzeriaceae, and Typhaceae).

Although apparently rare, reversions from tetrads to monads are known (Walker and Doyle 1975; Freudenstein 1999; Doyle et al. 2004). For example, the pseudomonad of Cyperaceae represents a tetrad in which three grains do not develop (Walker and Doyle 1975). In contrast, the true monads of *Orthilia* (Pyroloideae, Ericaceae) seem to represent a case in which the terminal stage of pollen development has reevolved (Freudenstein 1999). Such examples suggest that, at least for tetrads, the advantages of

Table 1

Taxonomic Distribution of Different Types of Pollen Aggregation among Angiosperm Families Recognized by APG (2003)

Aggregation type	Family
Dyads	Ericaceae (Styphelioideae), Podostemaceae, Scheuchzeriaceae
Tetrads	Actinidiaceae, Agavaceae, Anisophylleaceae, Annonaceae, Apocynaceae (Periplocoideae), Araceae, Berberidaceae, Bromeliaceae, Celastraceae, Clusiaceae, Cornaceae, Cyperaceae, Droseraceae, Ericaceae, Escalloniaceae, Fabaceae, Gentianaceae, Goodeniaceae, Gunneraceae, Hydrostachyaceae, Juncaceae, Lactoridaceae, Meliaceae, Monimiaceae, Muntingiaceae, Nepenthaceae, Onagraceae, Orchidaceae, Pedaliaceae, Philydraceae, Rubiaceae, Sarcolaenaceae, Solanaceae, Tamaricaceae, Thurniaceae, Torricelliaceae, Typhaceae, Velloziaceae, Winteraceae
Polyads	Annonaceae, Celastraceae, Fabaceae, Hydrocharitaceae
Viscin threads	Ericaceae (Rhodoreae), Onagraceae
Pollinia	Apocynaceae (Secamonoideae + Asclepiadoideae), Orchidaceae

Note. Based on data from Watson and Dallwitz (2006).

pollen aggregation depend on the prevailing conditions for pollination and/or mating, rather than being universal.

Pollen evolution in both the Orchidaceae and Apocynaceae has proceeded from monads through increasing aggregation, culminating in alternate forms of pollinaria (fig. 3, filled bars). In both clades, the evolution of pollinia was preceded by the fusion of the androecium and the gynoecium, producing the column (orchids) or gymnostegium (Apocynaceae: fig. 3, open bars), which initially allowed stigmatic fluid to participate in adhering pollen to pollinators. This fusion and the resulting functional integration may represent an essential transition that facilitated the subsequent evolution of pollinia.

Within orchids, tetrads and pollinia originated at different times (fig. 3A), suggesting that they serve different functions. Species in the basal Apostasioideae have free pollen monads, are nectarless, and are pollinated by pollen-collecting bees. The vanilloid orchids, most of which have soft pollinia of monads, are generally regarded as a sister clade to the remainder of the orchids (e.g., Kocyan et al. 2004), although in some phylogenies, this position is occupied by the Cypripedioideae (e.g., Cameron et al. 1999). The Cypripedioideae mostly deposit a sticky smear of monads onto flower visitors, so the soft pollinia in some derived taxa in this subfamily, notably *Phragmidium longifolium*, presumably evolved independently of soft pollinia in other orchid clades. The Orchidoideae, which dominate the orchid floras of temperate regions, tend to have either mealy or sectile pollinia. Within the Epidendroideae, the largest orchid clade, the number of pollinia per pollinarium seems to have generally been reduced from eight to two (Cameron et al. 1999), but examples of increases in pollinium number are also known (e.g., Whitten et al. 2000). Although most Epidendroideae produce hard pollinia, species in some, probably derived, tribes, such as the Neottieae (*Cephalan-*

thera, *Epipactis*, and *Listera*), produce soft pollinia (Dressler 1993).

The evolution of pollen aggregation within the Apocynaceae is not fully resolved, because of uncertainty about the affinities of the Periplocoideae. Some analyses proposed that the Periplocoideae constitute a sister clade to the Asclepiadoideae-Secamonoideae (e.g., Fishbein 2001), but the most recent analyses suggest that the Periplocoideae is a monophyletic clade within the paraphyletic Apocynoideae (Livshultz et al. 2007; fig. 3B). Early-branching lineages in the Apocynoideae have independent monads, whereas most Periplocoideae produce tetrads (Verhoeven and Venter 2001). Tetrads in *Apocynum*, which in Livshultz et al.'s (2007) phylogenetic tree is in the sister clade to Asclepiadoideae-Secamonoideae, are clearly not homologous with those of other species in the family (Nilsson et al. 1993) and are thus considered to be independently derived (fig. 3B). Rudimentary pollinia appear to have evolved at least three times within the Periplocoideae (Ionta and Judd 2007). Pollinaria with hard pollinia connected to a clasping corpusculum appear to have originated once in the common ancestor of the Asclepiadoideae and Secamonoideae. Reconstructions of likely ancestors by Livshultz et al. (2007) support Fishbein's (2001) suggestion that pollinaria with four pollinia (as found in the Secamonoideae) represent the ancestral condition in the Asclepiadoideae-Secamonoideae clade, whereas pollinaria with two pollinia (all Asclepiadoideae) are derived (fig. 3B).

On average, families with aggregated pollen contain more species (mean = 1200 species, lower SE = 286 species, upper SE = 376 species, based on ln-transformed data) than families with only monads (mean = 304 species, lower SE = 24 species, upper SE = 26 species; fig. 4A). This association is also evident in comparisons of sister clades that contain only species with monads and those that include species with aggregated pollen (fig. 4B). Thus, aggregated pollen may facilitate species diversification. Alternatively, given that not all species within families with aggregated pollen exhibit this trait, large families may simply provide more opportunities for the evolution of aggregation (or any other trait).

Diminishing Returns in Pollen Function

Aggregation of pollen necessarily causes pollen grains to be removed together, to travel together, to be deposited together on individual stigmas, and to increase the chance of seeds in a fruit being full siblings. Each of these consequences of aggregation has distinct implications for plant reproduction, so pollen aggregation will be selected only if the cumulative effects of aggregation improve reproductive performance. If success during any of these events depends on the number of sibling pollen grains that participate, the resulting "density dependence" will influence whether pollen aggregation is advantageous. Therefore, before considering the circumstances that may permit and facilitate the evolution of pollen aggregation, we review two reproductive processes for which outcomes depend on the number of sibling pollen grains involved: pollen export and competition for ovule fertilization. Both processes can involve diminishing returns, which arise when an investment of 1 unit of a "resource" produces an outcome of magnitude
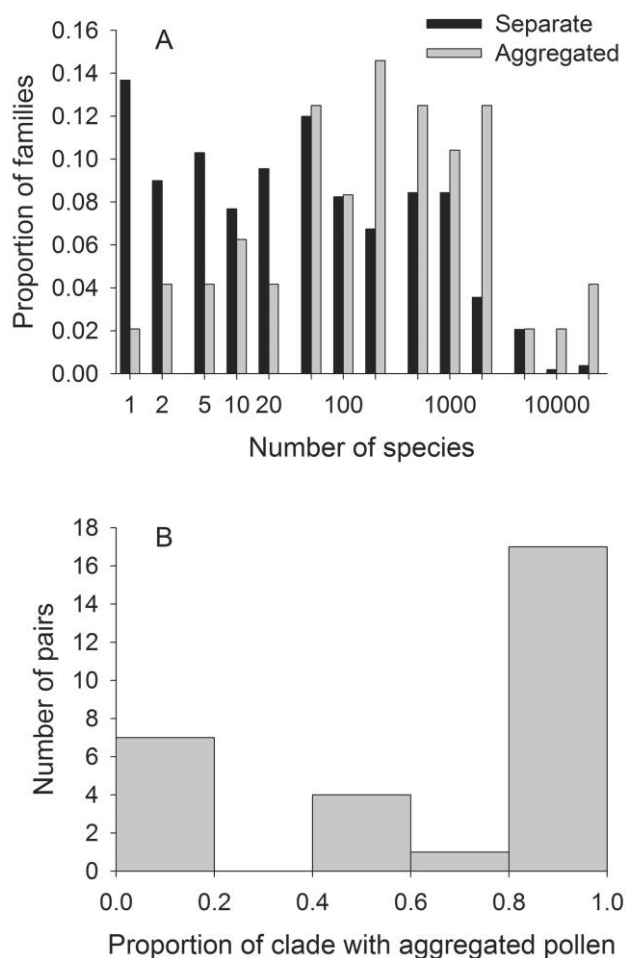
Fig. 4 Associations of species richness with separate versus aggregated pollen, based on data from Watson and Dallwitz (2006). *A,* Relative frequency distributions of species richness for families that include only species with separate pollen grains or some species with aggregated pollen. The two types of families differ significantly in species richness (likelihood ratio test, $G_1 = 33.61$, $P < 0.001$; generalized linear model, with negative binomial distribution and log-link function). *B,* Proportions of the total number of species in clades represented by subclades (usually a family) that include species with aggregated pollen. The complementary sister subclade in each clade includes only species with separate pollen grains. Sister subclades were based on Davies et al. (2004). A one-tailed Wilcoxon signed-rank test based on these proportions found higher species richness in subclades with aggregated pollen ($P < 0.05$).

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r, whereas the outcome from a single investment of $n > 1$ units is less than nr. In such situations, division of R resources into multiple smaller investments produces a greater total outcome than fewer, larger subdivisions.

Pollen Export

Pollen dispersal by individual plants is probably often subject to diminishing returns on increased pollen removal per pollinator (Harder and Thomson 1989; Harder and Barrett 1996). At least three nonexclusive processes can create this re-

lation. First, bees groom during most flights between flowers (Harder 1990), which dislodges pollen from pollinators' bodies and thus excludes it from subsequent dispersal (Thomson 1986). Because both the chance of a bee grooming and grooming intensity increase with the amount of pollen removed from the last-visited flower (Harder 1990), removal of many grains from a flower decreases the chance of an individual grain reaching a conspecific stigma (Harder and Wilson 1997). Second, layering of pollen on the bodies of pollinators that groom infrequently, such as hummingbirds and Lepidoptera, can cause diminishing returns, because the rate of pollen burial (and its exclusion from deposition on stigmas) increases with the amount of pollen that is removed from each flower and occupies the outer pollen layer (Harder and Wilson 1997). Diminishing returns caused by layering can be particularly severe if the accumulation of pollen eventually causes all of the pollen carried by a pollinator to fall off (see Johnson et al. 2005). The third process, self-pollination between flowers (geitonogamy), depends on the number of flowers that a pollinator visits on a plant. The more flowers with functional stigmas that a pollinator visits after picking up pollen from the same plant, the smaller the proportion of removed pollen that leaves the plant for potential export (Harder and Barrett 1995, 1996). The diminishing returns caused by this pollen discounting are aggravated if a pollinator removes much pollen from each flower (Harder and Barrett 1996). Geitonogamy is probably a widespread cause of diminishing returns during pollen dispersal, as it occurs commonly and can be the main mode of self-pollination, regardless of the type of pollinator (reviewed by Harder et al. [2004]; see also Harder and Johnson 2005; Johnson et al. 2005). If any or all of these three processes are active, each pollinator will export a smaller proportion of the pollen it removes from a plant if it removes many grains than if it removes few grains.

The general consequences of diminishing returns on increased pollen removal can be appreciated by considering a simple model of pollen dispersal (for a full analysis, see Harder and Wilson 1994; LeBuhn and Holsinger 1998). Suppose a plant exposes P pollen grains during a brief period, all of which are removed by n pollinators. The amount of pollen exported by pollinator i (e_i) increases with pollen removal (p_i) according to

$$e_i = ap_i^b, \tag{1}$$

where a depicts the chance of a pollen grain reaching a stigma, regardless of the number of grains with which it is removed, and b represents removal-dependent effects on dispersal. For equation (1), $b = 1$ indicates a linear relation between removal and export, whereas $0 < b < 1$ represents diminishing returns (fig. 5A). Two studies of this relation for species with monads reported $b \approx 0.3$ (Harder and Thomson 1989; M. B. Routley and L. D. Harder, unpublished data). For simplicity, suppose that each of the n pollinators removes P/n pollen grains, so total pollen export is

$$E = \sum_{i=1}^{n} e_i = na\left(\frac{P}{n}\right)^b = aP^b n^{1-b} \tag{2}$$

(fig. 5B). In equation (2), n^{1-b} represents the ratio of total export by n pollinators to that realized if a single pollinator
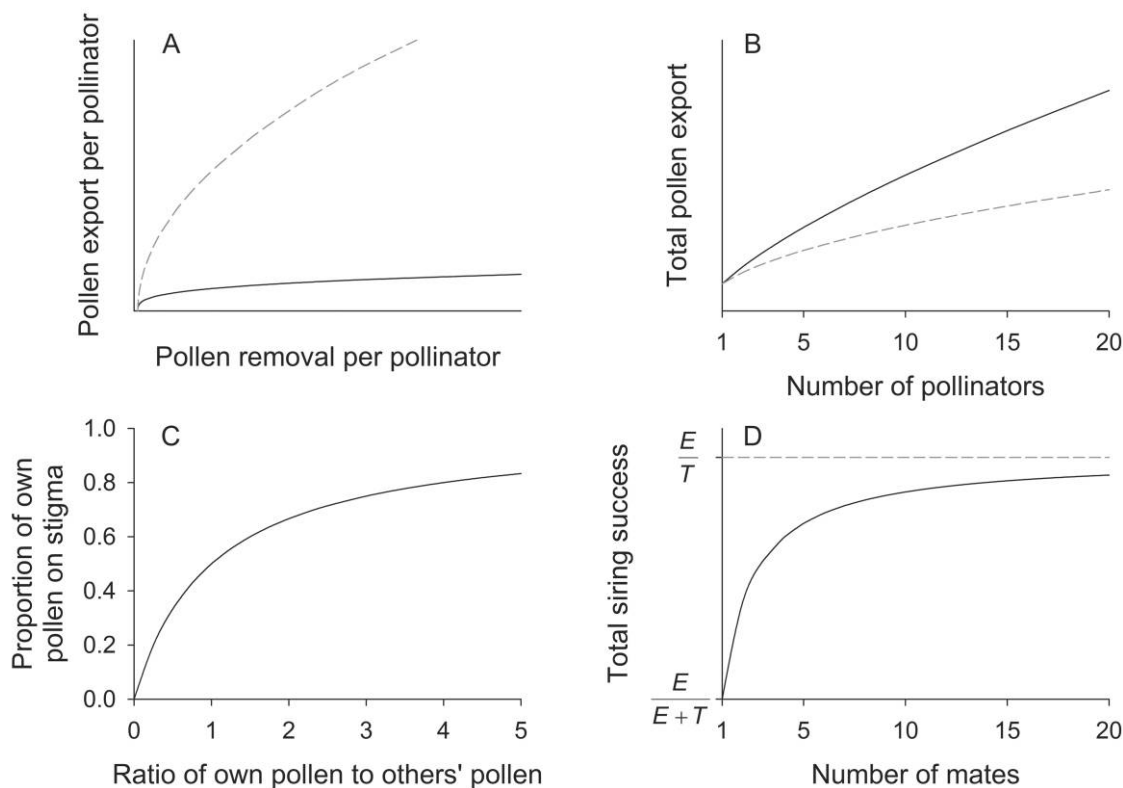
Fig. 5 Theoretical examples of diminishing returns for a plant's paternal success. *A* and *B* depict the effects of pollen removal per pollinator on pollen export per pollinator (eq. [1]) and total export (eq. [2]), respectively. Solid curves: $a = 1$, $b = 0.3$; dashed curves: $a = 1$, $b = 0.5$. *C* and *D* illustrate the consequences of increased deposition of a focal donor's pollen on individual stigmas for the donor's share of pollen tubes (eq. [3]) and total siring success on all stigmas (eq. [4]), respectively.

removed all of a plant's pollen. This model exposes three predictions about animal-pollinated plants when pollen export is subject to diminishing returns (i.e., $0 < b < 1$). First, P^b in equation (2) proposes that total pollen export exhibits diminishing returns with increased pollen production. This relation favors the evolution of hermaphroditism and also controls the evolutionary allocation of reproductive investment in pollen versus ovules (Charnov 1982; Zhang 2006; see "Pollen : Ovule Ratios"). Second, total pollen export increases (in a decelerating manner) with the number of pollinators attracted to a plant (n^{1-b}) if they all share in pollen removal (fig. 5*B*). Therefore, selection for increased siring success should increase pollinator attraction, within constraints imposed by the costs of attraction. The diverse and elaborate signaling and rewarding traits that distinguish animal-pollinated angiosperms from abiotically pollinated species are consistent with this expectation. Third, diminishing returns should favor restricted pollen removal by individual pollinators, which probably explains the diverse mechanisms for pollen packaging and dispensing exhibited by angiosperms (Harder and Thomson 1989; Thomson et al. 2000). The optimal restriction of pollen removal depends on the mean and variation in pollinator availability and on time-dependent aspects of pollen viability and ovule availability (Harder and Wilson 1994). Thus, the diminishing returns associated with pollen dispersal probably influence many aspects of floral evolution.

Gametophytic Competition for Ovule Fertilization

Once pollen reaches a stigma, it can again be subject to diminishing returns during competition to fertilize ovules, as another simple model illustrates. Suppose a plant contributes t_j pollen tubes to the pistil of recipient flower j, which compete with a total of T pollen tubes from other plants. If the $T + t_j$ pollen grains exceed the number needed to fertilize all ovules and all pollen tubes have an equal chance of fertilizing ovules, the proportion of ovules fertilized by the plant of interest in flower j will be

$$f_j = \frac{t_j}{T + t_j}. \tag{3}$$

If the number of pollen tubes from other plants is fixed, then two aspects of competition affect the siring success of the focal donor in this pistil. First, an increase in a donor's contribution of pollen to a stigma, t_j, enhances its share of pollen tubes compared to those from other donors, so its siring success increases in a decelerating manner with increases in its contribution of pollen tubes to flower j (fig. 5*C*). Second, an increase in t_j also intensifies competition between the focal donor's own pollen grains, reducing the chance of any specific pollen grain fertilizing an ovule (also see Queller 1984). This local mate competition creates diminishing returns for increases in a plant's contributions of pollen to individual stigmas, as long as pollen import is sufficient to maximize ovule fertilization.

The diminishing returns associated with pollen tube competition promote dispersal of pollen to many recipients, rather than a few. For example, suppose a focal donor exports a total of E pollen grains to m recipient flowers, so $t_j = E/m$. Therefore, this donor's total siring success,

$$S \propto \sum_j^m f_j = \sum_j^m \frac{E/m}{T + E/m} = \frac{mE}{E + mT}, \qquad (4)$$

increases from a minimum of $E/(E + T)$ when a donor plant mates with only one recipient to an asymptote of E/T if it mates with many recipients (fig. 5D).

General Implications

The preceding models demonstrate that diminishing returns select for subdivision, rather than aggregation. In particular, diminishing returns select for both restricted pollen removal per pollinator to diversify pollen dispersal among many pollinators (eq. [2]) and diversification in the number of mates to which pollen is dispersed (eq. [4]). The occurrence of aggregated pollen contradicts both of these expectations. Therefore, aggregated pollen must evolve in special circumstances with either no diminishing returns or countervailing influences. With these expectations in mind, we now consider what circumstances could be responsible for the origin and maintenance of aggregated pollen in angiosperms.

Conditions Promoting Pollen Aggregation

As a male character, pollen aggregation can evolve only if it enhances siring success. Therefore, we now examine specific conditions under which pollen aggregation may enhance siring; these are summarized in table 2A.

Pollen Removal

Aggregated pollen must generally increase the number of pollen grains removed by individual pollinators. In many monandrous orchids, both pollinaria share a viscidium (e.g., *Cyrtorchis arcuata*; fig. 2E) or have two closely adjacent viscidia on the rostellum, such that a single visit usually results in removal of all of a flower's pollen. According to the preceding overview of the implications of diminishing returns during pollen dispersal, permissive pollen removal is expected if ecological circumstances do not allow plants to benefit from restricting pollen removal by individual pollinators. We now consider three such circumstances.

Despite diminishing returns during pollen dispersal, pollen removal per pollinator should not be restricted when flowers are visited infrequently (Harder and Thomson 1989; Harder and Wilson 1994), either because pollinators are rare in the environment or because the species is less attractive than other coflowering species. The extent to which infrequent pollinator visits prompted the evolution of pollen aggregation is unclear. Orchids are notoriously pollen limited (Darwin 1877; Tremblay et al. 2005), and their flowers commonly wilt with pollinia remaining in anthers (Harder 2000); however, as we describe below ("Deceit Pollination"), pollen removal failure in orchids may often be an indirect consequence of reproductive strategies associated with pollen aggregation. Some of the families listed in table 1 include species that experience pollen limitation because they either are unrewarding (e.g., Podophyllaceae [=Berberidaceae]; Laverty 1992) or occupy environments with few or unpredictable pollinators (e.g., Ericaceae; Kudo and Suzuki 2002). More generally, in his review of pollen limitation, Burd (1994) included data for species from 14 of the families listed in table 1, and pollen limitation was detected in 12 of these families. For the two families in which pollen limitation was not observed, the single species studied in the Agavaceae and at least one of the three species in the Rubiaceae do not produce pollen aggregates (pollen state of the two other

Table 2

Aspects of Reproduction That Could Influence the Evolution of Pollen Aggregation

Reproductive phase, condition/consequence	Benefit of pollen aggregation
A. Conditions promoting aggregation:	
Pollen removal:	
Infrequent pollinators	Increased chance of dispersal; access to many vacant stigmas
Brief pollen viability	Increased chance of dispersal while viable
Synchronous ovule availability	Increased access to ovules
Pollen transport:	
Low transfer efficiency	Increased access to stigmas
Stigmas susceptible to usurpation	Priority access to ovules
Pollen tube growth and fertilization:	
Many ovules per ovary	Increased access to ovules, given successful dispersal
B. Accentuating consequences of aggregation:	
Pollen transport:	
Weak diminishing returns during transport	Enhanced transfer efficiency; total export becomes independent of number of pollinators
Lower variance in export to individual stigmas	Reduced local mate competition for fertilization
Pollen mixtures on stigmas involve lower male diversity	Reduced intermale competition for fertilization
Seed development:	
Developing seeds are mostly full siblings	Reduced embryo competition; opportunity for altruistic suicide

Rubiaceae is undetermined). This association is admittedly crude and warrants more detailed analysis, but it is consistent with pollen aggregation serving as one adaptive response to insufficient pollinator visitation.

Pollen removal should also not be restricted if a species' pollen has brief viability (Harder and Wilson 1994). In general, binucleate pollen, in which the germinative cell has not divided into sperm when the pollen is shed, is less active metabolically during dispersal and remains viable longer than trinucleate pollen (Hoekstra and Bruinsma 1975), so aggregated pollen might be favored in trinucleate clades. However, data from Watson and Dallwitz (2006; based largely on Brewbaker 1967) do not support this expectation. In particular, the incidence of families with aggregated pollen does not differ between families with only binucleate pollen (10.1% of 238 families) and those with only trinucleate pollen (8.7% of 69 families; Fisher's exact test, $P = 0.822$). Furthermore, orchid and milkweed pollen remains viable for several days (e.g., Morse 1987; Proctor 1998; Luyt and Johnson 2001), even after removal from anthers. Thus, limited pollen viability is probably not a common influence on the evolution of pollen aggregation.

If ovules become available relatively synchronously within a population, permissive pollen removal by a flower's first few visitors increases siring chances, despite diminishing returns during dispersal (Harder and Wilson 1994). Although such temporal patterns are not widespread, they may have played a role in the evolution of viscin threads in the Onagraceae. Anyone who has observed the rapid anthesis in populations of moth-pollinated *Oenothera* species followed by the brief period (ca. 1 h) of crepuscular activity by hawk moths can appreciate the advantage of permissive pollen removal in these species. However, these genera occur in a derived clade within the Onagraceae (Levin et al. 2003) and so need not represent conditions during the evolution of viscin threads, which is a synapomorphy for this family (Levin et al. 2003). Nevertheless, *Ludwigia*, the earliest-branching extant genus in this family (Levin et al. 2003), also exhibits relatively synchronous anthesis. For example, Gimenes et al. (1996) reported that the one-day flowers of *Ludwigia elegans* open between 0800 and 0900 hours, with anther dehiscence 30–60 min later. Specialized bees begin visiting once ambient temperature exceeds 18°C, and they cease pollen collection before 1200 hours. Such temporal limitation of mating opportunities should strongly favor rapid pollen removal to facilitate prompt access to newly available ovules. Pollen aggregation, such as viscin threads, provides one mechanism for rapid dispersal. However, many species with aggregated pollen do not have synchronous anthesis (notably orchids and milkweeds), so this mating circumstance does not provide a general explanation for the evolution of pollen aggregation.

Pollen Transport

Aggregated pollen could be beneficial if it enhances the probability that a removed pollen grain reaches a conspecific stigma (pollen-transfer efficiency). Pollen-transfer efficiency can be estimated for a plant population as the ratio of the average number of pollen grains on stigmas to the average pollen removal from flowers after pollination is complete (removal =

production − remaining). For hermaphroditic plants, this measure does not distinguish between self- and cross pollination and so tends to overestimate pollen export between plants. Nevertheless, comparison of estimates of pollen-transfer efficiency for 16 species with monads, 54 orchid species, and 28 asclepiad species reveals that pollinia enhance average pollination efficiency by up to two orders of magnitude (fig. 6*B*; comparison of species with monads and those with pollinia, $F_{1, 92} = 92.60$, $P < 0.001$). We found estimates of pollen removal and deposition for only one species with tetrads, *Drosera tracyi* (Droseraceae; Wilson 1995), which had somewhat elevated transfer efficiency, compared to species with granular pollen, but lower
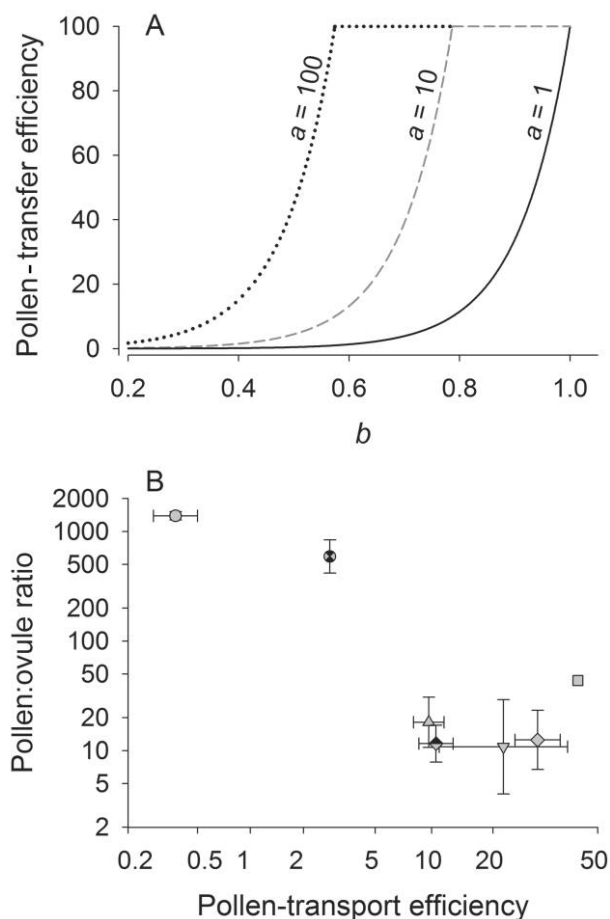

Fig. 6 Influences on the percentage of pollen removed from flowers that reaches conspecific stigmas (pollen-transfer efficiency) and its relation to pollen : ovule ratios. *A*, Theoretical effect of the diminishing-return parameter, *b*, on pollen-transfer efficiency, ap^{b-1}, based on equation (1), for three values of the scaling parameter, *a*. Total pollen removal = 50,000 grains. *B*, Relation of mean ($\pm$SE) pollen : ovule ratio to mean ($\pm$SE) pollen-transfer efficiency for species that present their pollen as monads (gray circle) or tetrads and polyads (half-filled circle); orchids with smear (*Cypripedium*: square), solid (gray diamond), mealy (down-pointing triangle), and massulate pollinia (up-pointing triangle); and asclepiads with pollinia (half-filled diamond). Based on published records and unpublished observations. Data for pollen-transfer efficiency and pollen : ovule ratios were collected from different sources (see appendix) and generally do not involve the same species. Note logit-scaled abscissa and ln-scaled ordinate.

efficiency than species with pollinia (fig. 6B; $P > 0.05$ in both cases). In contrast, for *Leucothoe racemosa* (Ericaceae), another species with tetrads, only 0.88% of pollen production was found on stigmas (a conservative measure of efficiency; L. D. Harder, unpublished data), which is similar to the pollen-transfer efficiency for species with monads (fig. 6B). Thus, whether tetrads enhance pollen-transfer efficiency remains to be clarified by the collection of more data. We found no measurements of pollen-transfer efficiency for species with viscin threads, but 7.1% of the pollen produced by *Chamerion angustifolium* (Onagraceae) was found on stigmas (L. Grinevitch and L. D. Harder, unpublished data), which exceeds the average efficiency for species with monads by an order of magnitude (fig. 6B).

Pollen aggregation could increase pollen transport either without affecting diminishing returns (e.g., larger *a* in eq. [1]; fig. 6A) or by alleviating diminishing returns (e.g., larger *b* in eq. [1]; fig. 6A). These nonexclusive alternatives are difficult to assess with existing information, because the relation of pollen export to removal by individual pollinators has been measured so seldom. Removal-independent improvements in pollen-transfer efficiency may underlie the evolution of aggregated pollen in some species with underwater pollination. Ackerman (1995) demonstrated that many such species produce either linear pollen monads (e.g., *Zostera*, Zosteraceae) or linear pollen aggregates (e.g., *Thalassia* and *Halophila*, Hydrocharitaceae). The linear form of pollen dispersal units induces tumbling in a moving current, which increases the probability of contact with a nearby stigma. Unfortunately, pollen-transfer efficiency has not been measured for such species, so whether aggregated pollen improves efficiency for submerged pollination remains a matter of conjecture.

The greatly enhanced pollination efficiency of orchids and milkweeds undoubtedly results because they glue their pollen, or pollinaria, to pollinators (fig. 2D), thereby reducing transport losses and relaxing diminishing returns during export. For milkweeds and derived orchids, this function is served by accessory structures of pollinaria (corpusculum and viscidium, respectively; fig. 2D), which evolved after pollen aggregation. However, the high pollen-transfer efficiency for the smear pollinium of *Cypripedium acaule* in figure 6B (O'Connell and Johnston 1998), which represents a basal clade in the Orchidaceae (see Freudenstein et al. 2004), compared to that of species with more independent monads, indicates that high efficiency probably predated the evolution of these elaborations. Both the stickiness of a *C. acaule* pollen mass and its transport on the thoracic dorsum of bees greatly reduce losses associated with bee grooming. As grooming is an important cause of diminishing returns for bee-pollinated species (see "Pollen Export"), the *Cypripedium* pollinium probably improves pollen-transfer efficiency by alleviating this dependence of export probability on the number of pollen grains removed per pollinator (e.g., larger *b* in eq. [1]; fig. 6A). The remarkable improvement in pollination efficiency associated with such an innovation would result in strong selection for this particular type of pollen aggregation.

Fertilization Opportunities

It is probably because of the variable number of ovules per ovary that different types of pollen dispersing units

are present in angiosperms. (Pacini and Hesse 2005, pp. 399–400)

Ovaries with multiple ovules provide more siring opportunities when multiple pollen grains from a donor reach a stigma, so it is not surprising that most families listed in table 1 have multiovulate ovaries. However, in contrast to Pacini and Hesse's assertion (and similar claims by Darwin [1877]), multiovulate ovaries per se are neither a necessary nor a sufficient condition for the evolution of pollen aggregation. For example, Kress (1981) found little difference in mean or median ovule number between genera in the Annonaceae with pollen monads and those with tetrads. Indeed, in the Annonaceae, tetrads have evolved in several uniovulate clades (e.g., *Pseudoxandra*, *Fusaea*), and uniovulate ovaries may have evolved from multiovulate ancestors in clades with aggregated pollen (Doyle and Le Thomas 1997). Furthermore, aggregation has not evolved in most clades with profuse ovule production, such as the Cucurbitaceae and Phyrmaceae. Therefore, although multiovulate ovaries undoubtedly facilitate the evolution of pollen aggregation, some other advantage is probably needed for its establishment.

Consequences of Aggregation That Promote Elaboration

The evolution of pollen aggregation precipitates several reproductive consequences that could favor additional evolution and further improve siring ability. These consequences largely involve postpollination processes, which determine fertilization success and the relatedness of seeds within individual fruits (table 2B).

Success of Male Gametophytes in Pistils

Pollen aggregation affects the dispersion of pollen grains among recipient stigmas, especially the frequency with which a specific donor plant is the sole contributor of pollen to individual stigmas, which in turn influences the extent and nature of competition between pollen tubes for access to ovules (fig. 5C). For a given pollen production, aggregation necessarily reduces the number of plants to which a plant can export pollen. The pollen removed by a single pollinator or gust of wind can reach only the subset of potential recipient plants in the population located along the vector's subsequent path. By reducing the number of "visits" required to remove a plant's pollen, aggregation restricts the number of recipient subsets that can be accessed. In addition, for animal-pollinated species, aggregation modifies the distribution of pollen from a donor plant on a single pollinator among the potential recipient flowers. For species with monads, a visit to a recipient flower usually removes a fraction of the donor pollen on the pollinator (Morris et al. 1994), so donor pollen is carried over to be delivered to multiple recipients (e.g., fig. 1B, 1C). Aggregation restricts the opportunity for pollen carryover (see Galen and Plowright 1985; Peakall 1989; Peakall and Beattie 1991; Nilsson et al. 1992; Johnson and Nilsson 1999; Johnson et al. 2005), especially for species with solid pollinia (e.g., Nilsson et al. 1992). This restriction can be compounded in species subject to geitonogamy, because self-pollination discounts the pollen available to be carried over for export to other plants.

Despite restricting the number of plants that receive a plant's pollen, pollen aggregation should generally reduce variation in the pollen contributed to each recipient, which may ease local mate competition among a donor's pollen grains. For species with monads, dispersal of pollen from a specific donor plant by a single pollinator creates a general decline in export to successive recipient flowers (Morris et al. 1994). To appreciate the implications of this pattern for siring success, consider figure 1B, which illustrates the dispersal of monad pollen from a *Pontederia cordata* inflorescence by a single pollinator. Several of the initial recipient flowers in this sequence received >40 donor pollen grains, with one flower receiving 152 grains. However, these large pollen contributions to individual stigmas largely represent lost siring opportunities, because *P. cordata* flowers contain only one ovule, so that each of these successfully dispersed pollen grains has a small chance of fertilizing an ovule. In contrast, production of pollinia, and perhaps viscin threads, probably moderates the variance in pollen contributed to individual stigmas, thereby reducing siring opportunities lost to local mate competition. This advantage of aggregation may also apply to some polyads (see *Acacia* examples described below) but not to pollen tetrads, which likely exhibit dispersal patterns similar to those observed for monads.

Restricted pollen carryover should also tend to reduce competition for fertilization between pollen tubes from different donors. Owing to carryover, stigmas of species with monads generally receive pollen from several donors during a single pollinator visit (e.g., Karron et al. 2006), resulting in multiple paternity among seeds in individual fruits of multiovulate species (see Campbell 1998; Mitchell et al. 2005). In contrast, if aggregation limits pollen export to fewer recipient stigmas, then recipient stigmas must also import pollen from fewer donors, on average. For example, a milkweed flower has five stigmatic chambers and so could receive pollinia from up to five donors, but Queller (1984) found that 59% of *Asclepias exaltata* flowers and 82% of *Asclepias viridiflora* flowers received only one pollinarium, Gold and Shore (1995) found no *Asclepias syriaca* fruits sired by more than one male, and Broyles and Wyatt (1990) found only a few *A. exaltata* fruits with multiple paternity. Similarly, Trapnell and Hamrick (2006) found no evidence of within-fruit multiple paternity in 15 populations of an epiphytic orchid, *Laelia rubescens*. Indeed, stigmas of epidendroid orchids rarely receive pollinia from more than a single pollinarium (Nilsson et al. 1992; Dressler 1993; Alexandersson 1999).

Aggregated pollen may further reduce gametophytic competition among pollen donors if pollen receipt shortens floral longevity, thereby reducing the chance of subsequent receipt of pollen from other donors. Lankinen et al. (2006) proposed that pollination-induced wilting, which occurs throughout orchids and is also known from other clades with aggregated pollen (see van Doorn 1997), is caused by pollen-borne chemicals and represents a male strategy to limit gametophytic competition. The benefits of such a strategy generally increase with the number of pollen grains from a single donor that arrive simultaneously on a stigma and so should be particularly favored for species with aggregated pollen (Lankinen et al. 2006). However, if mate choice is possible and sufficiently advantageous and pollen arrives relatively frequently, selection should favor female counterstrategies.

How might local mate and intermale competition affect selection on pollen aggregation? In general, while ovules remain unfertilized, a specific donor's siring success should increase roughly linearly with its pollen export to a stigma, regardless of the number of other donors contributing pollen. In contrast, if pistils receive enough pollen to fertilize most ovules, increased export to individual stigmas imposes diminishing returns (fig. 5C), so pollen should instead be dispersed to more recipients (fig. 5D). Thus, postpollination benefits of pollen aggregation should be greatest in two nonexclusive types of species: those in which insufficient pollen receipt commonly limits seed production during a flowering season and those in which only single donors typically contribute pollen to individual stigmas. As discussed above, both of these characteristics are expected outcomes of the dispersal of aggregated pollen, so postpollination benefits of aggregation may often reinforce selection on dispersal characteristics. Such synergy between pollination and postpollination outcomes would increase the selective advantage of pollen aggregation.

The preceding overview of the postpollination consequences of pollen aggregation focused on the male perspective, but the pattern of pollen dispersal also depends on stigmatic characteristics. In particular, if each stigma removes much of the pollen carried by a pollinator, then little pollen will be carried over to other recipients, and each pollinator will deliver pollen from few donors. For example, the rostella of many orchids scrape individual pollinia from pollinators (Nazarov and Gerlach 1997), and the mechanism of insertion of pollen into a stigmatic chamber of a milkweed flower allows receipt of only one pollinium per stigmatic chamber, so that a pollinarium can contribute pollen to a maximum of only two (Asclepiodeae) or four (Secamonoideae) stigmas (Kunze 1991). Thus, limited mate diversity in such species may involve both the mechanism of pollen deposition and pollen aggregation. Alternatively, small stigmas may limit mate diversity, despite the possibility of extensive pollen carryover, if they cannot capture many pollen grains. For example, the stigmas of many acacias have a terminal cup that is large enough to accept one polyad comfortably (Kenrick and Knox 1982; Tandon et al. 2001). Data from two *Acacia* species indicate that stigmas received a single polyad more frequently than expected from a random (Poisson) distribution of polyads among stigmas (*Acacia retinodes*: $X_2^2 = 20.78$, $P < 0.001$, Knox and Kenrick 1983; *Acacia senegal*: $X_3^2 = 46.99$, $P < 0.001$, Tandon et al. 2001). Similarly, for *Acacia melanoxylon*, a species with 16-grain polyads, Muona et al. (1991) found that only 8% and 15% of the pods assayed from two populations contained seeds sired by two donors rather than one. These orchid, milkweed, and *Acacia* examples reveal that the postpollination implications of pollen aggregation must be considered in the context of the floral mechanisms responsible for both female and male influences on pollen dispersal.

Seed Development

Pollen aggregation increases the chance that seeds in a multiovulate ovary are full siblings, rather than half-siblings, which influences opportunities for sibling competition, altruism, and parent-offspring conflict (Hamilton 1964; Kress 1981; Uma Shaanker et al. 1988; Lloyd 2000). A maternal plant is equally related to all of its outcrossed offspring and so should

invest resources equally among them, unless they differ in quality because of the paternal genetic contribution. In contrast, if the chance of seedling establishment increases with seed size, an individual seed benefits directly from appropriating as much of the maternal resources as possible. However, if maternal resources are limited, the resulting competition reduces the number of siblings that can mature and may waste resources if some less competitive seeds consume some resources but then die. Such waste reduces both the direct fitness of the maternal (and paternal) plant and the indirect (collective) fitness of offspring realized through the transmission of shared alleles (Hamilton 1964; Lloyd 2000). High relatedness among offspring reduces the individual benefits of an offspring's competition with its siblings and maternal parent and so should promote resource sharing among siblings, reducing competitive waste. Consequently, pollen aggregation could enhance total seed production and reduce seed size variance within fruits.

Of course, such selection depends on seed production consequences for entire plants, rather than individual flowers. To the extent that pollen aggregation limits pollen carryover, it should increase mate diversity among flowers on individual plants. Such a negative association of within- and among-fruit mate diversity has been observed for an acacia (Muona et al. 1991) and two milkweed species (Broyles and Wyatt 1990; Gold and Shore 1995). High mate diversity among fruits, but not within fruits, provides opportunities for maternal plants to implement "family planning" by allocating resources preferentially to fruits with genetically superior offspring (e.g., Bookman 1984; Torres et al. 2002). This mechanism of offspring choice would be more economical than preferential allocation among seeds within individual fruits, because the fixed costs of fruit production (e.g., investment in pericarp) are expended primarily on fruits with many viable seeds (see Bookman 1984). However, family planning requires fertilization of more flowers than mature into fruits, whereas pollen limitation is common for many species with aggregated pollen (Burd 1994; Tremblay et al. 2005). Thus, pollen aggregation does not generally provide opportunities for offspring selection, and so this consequence of aggregation will not contribute universally to its further evolutionary elaboration.

The low diversity of mates contributing pollen to individual flowers associated with pollen aggregation also influences the consequences of self-pollination. If most flowers receive pollen from only one donor plant, then self-pollination in species with either self-incompatibility mechanisms (e.g., *Acacia*: Kenrick and Knox 1989; Asclepiadoideae: Wyatt and Lipow 2007) or strong inbreeding depression (e.g., some Onagraceae: Husband and Schemske 1996; some Orchidaceae: Smithson 2006) will greatly depress fruit set. For example, Shore (1993) found that self-pollination accounted for 66% of pollinia on stigmas in a population of *A. syriaca*, a species with notoriously low fruit set (Kephart 1987; Morse 1994).

Such observations led Wyatt and Lipow (2007) to propose "that the evolution of postzygotic self-incompatibility (in the Apocynaceae) created conditions in which compatible cross pollen was wasted whenever it occurred in mixture with incompatible pollen, because such mixed pollen loads would likely cause the entire fruit to abort.... This maladaptive condition may have been the stimulus for the evolution of pollinia" (p. 477). We find this hypothesis unsatisfying for several

reasons. First, as a male character, pollen aggregation must evolve to improve siring success, so that it can evolve in species with strong self-incompatibility or subject to strong inbreeding depression only if it increases pollen export and/or competitive ability in pistils on other plants. Second, although pollinia apparently evolved several times in the Apocynaceae, they have not evolved in the myriad other dicot families with self-incompatibility or inbreeding depression. Third, the pollinia of the Apocynaceae bear many similarities to those of orchids, but orchids mostly lack self-incompatibility systems (Johnson and Edwards 2000), causing Wyatt and Lipow (2007) to invoke different causes for pollinium evolution in these two families. In contrast, a common cause (enhanced pollen-transfer efficiency) provides a more parsimonious explanation, especially given the unusual fusion of the androecium and gynoecium before pollinia evolution in both families (fig. 3), which we interpret as a necessary step in pollinium evolution. Finally, Wyatt and Lipow's hypothesis led them to propose "that the greater efficiency of pollen delivery is an epiphenomenon, a fortunate happenstance of the evolution of pollinia," because "the likelihood of selection for pollination efficiency being so strong and consistent across so many derived taxa of the Apocynaceae . . . seems remote," especially "given the wide range of environments, including pollinator diversity, that various Apocynaceae experience" (Wyatt and Lipow 2007, p. 481). In contrast, figure 6B illustrates that pollinia improve pollen-transfer efficiency by almost two orders of magnitude for milkweeds and orchids, compared to species with monads, even though the 29 milkweed species represented in this figure occupy different continents and are pollinated by diverse pollinators, including bees, butterflies, wasps, and birds. Together, these arguments favor enhanced pollen-transfer efficiency as the primary influence on pollinium evolution in both milkweeds and orchids.

Consequences of Aggregated Pollen for Floral Evolution

The preceding review indicated that pollen aggregation has diverse direct effects on plant reproduction. Such influences should in turn affect selection on other floral traits that influence reproductive performance, several of which we now consider.

Pollinaria and Floral Diversification

Among clades with aggregated pollen, orchids and milkweeds are unique in that aggregation has not been the final stage in the evolution of pollen dispersal units. Instead, in derived groups, pollinia are combined with unique structures for attachment to pollinators that often can also reorient to reduce self-pollination (fig. 2D–2F; see "Pollinia and Pollinaria"). In both orchids and milkweeds, these accessory structures are produced in whole or in part by the stigma, so pollen dispersal units comprise both male and female tissues. This sexual cooperation in pollen dispersal is one manifestation of unusual flower structures in both groups, in which the gynoecium and androecium are fused into a common structure (orchid column, milkweed gynostegium). In contrast, the rarity of ancillary elaboration of pollen aggregation in other clades may largely reflect the widespread structural and functional independence of

gynoecium and androecium, which serves to reduce sexual interference in most angiosperms (Barrett 2002; secondary pollen presentation [Yeo 1993] being a notable exception).

The precision in pollen exchange with pollinators afforded by the combination of adhesive pollinia or pollinaria and close proximity of anthers and stigma on the column/gynostegium probably plays a key role in the remarkable floral diversification among both the ca. 22,000 species of orchids and the ca. 2400 species of Secamonoideae and Asclepiadoideae. Together, these traits greatly increase the precision of pollen placement on pollinators. For example, Maad and Nilsson (2004) described the pollination of two sister species of *Platanthera* that differed in the position of attachment of pollinaria on moth pollinators: proboscis versus eye. Such precision allows these sympatric species to use the same pollinators with limited hybridization (see Kephart and Theiss 2004 for a milkweed example). Similarly, because of precise pollen placement, simple changes in traits that affect where pollinators contact the column/gynostegium, such as the length of the nectar spur, can facilitate evolutionary transitions between different pollinators (e.g., Steiner 1989; Johnson 1997; Johnson and Steiner 1997; Johnson et al. 1998). Thus, pollen aggregation in the orchids and milkweeds may directly facilitate floral diversification and speciation and so represent a key innovation.

Deceit Pollination

Deceit pollination, in which pollinators are not rewarded for their services, is rare among angiosperms as a whole but occurs in ca. 8000 orchid species, which is roughly one-third of the family (Dressler 1990). We propose that this high incidence of deceit pollination among orchids evolved because of the relaxed diminishing returns during pollen dispersal resulting from reduced pollen loss during transport and limited geitonogamy in species with pollinarium reorientation (see "Pollen Transport"). As equation (2) indicates, relaxed diminishing returns during pollen dispersal (i.e., as b approaches 1) cause total pollen export to become increasingly independent of whether a few or many pollinators disperse a plant's pollen. Given the costs of flower maintenance (Ashman and Schoen 1996) and pollinator attraction (showy corollas, rewards), plants subject to weak or absent diminishing returns on pollen removal should allow the first few pollinators that visit to remove all of their pollen. Because such plants do not need to be as attractive as those subject to strong diminishing returns, deceit pollination becomes a practical option. Of course, deceit pollination involves risks, because pollinators learn to avoid unrewarding plants (Smithson and MacNair 1997). As a result, deceitful species generally experience low pollinator visitation, as pollen is left in the anthers of deceitful orchid species when flowers wilt more frequently than in those of rewarding orchids, on average (Harder 2000). However, lack of rewards also reduces the number of flowers visited by individual pollinators, which reduces geitonogamy (Johnson et al. 2004; Jersakova and Johnson 2006), further easing diminishing returns and reinforcing the evolution of deceit.

Unlike orchids, all milkweeds studied to date provide nectar and so do not engage in purely deceitful pollination, although some species use misinformation to attract or trap flies (e.g., Meve and Liede 1994; Masinde 2004). Indeed,

some milkweeds produce much more nectar than species with monads and equivalent flower size and number per inflorescence (Harder and Barrett 1992). Presumably, the excessive nectar production of some milkweeds and the trap flowers of *Ceropegia* (Masinde 2004) are necessary to retain pollinators on flowers for long periods to increase the chance of pollinarium removal and/or pollinium insertion from the complex flowers (see Kunze 1991). Long visits likely also caused the high frequency of self-pollination observed by Shore (1993) in an *Asclepias syriaca* population. As self-pollination in this species requires insect activity, the incidence of self-pollination likely involves geitonogamy and so should impose strong diminishing returns. Thus, the high transfer efficiency for milkweeds (fig. 6B) probably reflects improved aspects of dispersal that arise independently of the number of pollen grains removed per pollinator (e.g., increased a in fig. 6A).

Pollen : Ovule Ratios

If the Orchideae had elaborated as much pollen as is produced by other plants, relatively (sic) to the number of seeds which they yield, they would have had to produce a most extravagant amount, and this would have caused great exhaustion. Such exhaustion is avoided by pollen not being produced in any great superfluity owing to the many special contrivances for its safe transportal from plant to plant, and for placing it securely on the stigma. (Darwin 1877, pp. 288–289)

Outcrossing species with monads typically produce more that 1000 pollen grains per ovule (Cruden 2000), which greatly exceeds the mean pollen : ovule ratios of 570 for 17 genera with tetrads or polyads, 166 for eight genera with viscin threads, and 10 for 23 genera with pollinia (based on ln-transformed data from Erbar and Langlotz 2005; fig. 6B). Darwin's intuition that species with aggregated pollen produce relatively few pollen grains per ovule because their pollination systems deliver pollen efficiently to fertilize available ovules is a recurring theme in the pollen aggregation literature (Cruden 1977; Cruden and Jensen 1979; Kenrick and Knox 1982; Nazarov and Gerlach 1997; Erbar and Langlotz 2005), even though no published study of pollen : ovule ratios has also reported pollen-transfer efficiency. Nevertheless, the summary of available data in figure 6B illustrates the anticipated negative association between pollen : ovule ratio and pollination efficiency ($r = 0.83$, df = 5, $P < 0.025$).

The association depicted in figure 6B seems at odds with theoretical analyses of pollen : ovule ratios. Charnov (1982) rejected Cruden's (1977) suggestion "that P/O's reflect the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set" (p. 32) as a proposal that pollen grain production is governed by ovule production. Instead, Charnov correctly noted that pollen grains and ovules are alternate means of contributing genes to offspring for hermaphrodites, and so selection should act on allocation of reproductive resources in the production of both pollen and ovules. Theory that incorporates Charnov's perspective predicts that the optimal pollen : ovule ratio equalizes the rate of change in maternal and paternal fitness contributions with increasing investment (marginal fitness; Charnov 1982; Lloyd

1984). This result led Lloyd (1984) to conclude that "it is not the efficiency of pollination as such that controls gender allocations. It is the way in which pollinator actions affect the shape of the paternal fitness curve (with increased paternal investment) that is important" (p. 299). How can this conclusion be reconciled with figure 6*B*?

We proposed above that, at least in orchids, pollen aggregation improves pollen-transfer efficiency by alleviating the diminishing returns associated with pollen dispersal, which should strongly influence "the shape of the paternal fitness curve." However, the resulting more linear relation of paternal fitness to allocation in pollen should increase relative investment in pollen relative to ovules, whereas figure 6*B* illustrates that pollen : ovule ratio declines with increased efficiency. Thus, the explanation for low pollen : ovule ratios in plants with aggregated pollen must lie elsewhere.

Instead, resolution of the apparent conflict between theory and observation may be found in Lloyd's (1984) conclusion that "[a]n upper limit on paternal fitness offers the most promise of explaining the observed deviations (in sex allocation) emphasizing maternal expenditure" (p. 298). Lloyd identified restriction in the number of fertilizations resulting from individual pollen-removing visits as one cause of such an upper limit. As discussed above, pollen aggregation both reduces the number of visits required to remove all of a flower's pollen and can result in strong local mate competition if more sibling pollen grains reach individual stigmas than are needed to fertilize all available ovules. In this case, individuals that allocate more reproductive resources to ovule production and less to pollen production should contribute more genes to offspring, resulting in selection for a lower pollen : ovule ratio. Such selection should be particularly intense if individual donor plants are the sole contributors of the pollen received by individual stigmas, which seems to occur commonly for species with aggregated pollen (see also Wyatt et al. 2000). In turn, relatively exclusive access to individual stigmas should select for correlation between the typical number of pollen grains deposited on stigmas and ovule number. According to this interpretation, the association of low pollen : ovule ratios with high pollination efficiency in many species with polyads or solid pollinia is not causal but instead evolves as a correlated consequence of aggregation for low mate diversity within fruits.

Conclusion

The preceding overview suggests that the different types of pollen aggregation are not alternate solutions to a single prob-

lem but instead may originate to mitigate contrasting limitations on siring ability. In particular, tetrads may be especially advantageous for species subject to infrequent pollinator visits, viscin threads promote rapid removal when ovules become available synchronously (at least for Onagraceae), and aggregation enhances pollen-transfer efficiency for species with submerged pollination, or viscin threads, or pollinia that limit grooming losses. These alternate influences on aggregation may act together, as some species have two means of aggregating pollen (e.g., viscin threads and tetrads in *Rhododendron*), underscoring the conclusion that aggregation probably serves several functions. Consequently, pollen aggregation cannot be considered a single recurring trait with different degrees of elaboration. Indeed, even the pollinia of orchids and milkweeds seem not to be functionally comparable. Given the relative frequency of transitions from monads to different types of pollen aggregation, adaptations that enhance pollen-transfer efficiency seem to originate less often than those that contend with infrequent pollination or limited ovule availability.

Once aggregation evolves, it may precipitate further evolution in reproductive traits, restriction of mate diversity within fruits, increased relative allocation in female function, and pollination by deceit. These far-reaching effects arise because pollen aggregation influences all phases of reproduction from pollen removal to seed production. As a result, the transition from monads to aggregated pollen can influence subsequent phenotypic, functional, and phylogenetic diversification. However, these changes must occur in the context of overall reproductive function, and so they are realized to differing extents among the lineages in which pollen aggregation has originated.

Appendix

Sources of the Observations of Pollen-Transfer Efficiency and Pollen : Ovule Ratios Summarized in Figure 6*B*

Pollen-Transfer Efficiency

Table A1

Sources for Measurements of the Percentage of Pollen Removed from Flowers That Was Found on Conspecific Stigmas

Aggregation type	Sources
Monads	Ornduff 1970*a*, 1970*b*, 1971; Webb and Bawa 1983; Snow and Roubik 1987; Galen and Stanton 1989; Harder and Thomson 1989; Young and Stanton 1990; Wilson and Thomson 1991; van der Meulen 1992 (two species); Aizen and Raffaele 1996; Freitas and Paxton 1998; Harder 2000 (three species); Hiei and Suzuki 2001; Thomson and Goodell 2001 (two species)
Tetrads	Wilson 1995
Pollinia:	
Asclepiad	Wyatt 1976; Bertin and Willson 1980 (two species); Kunze and Liede 1991; Pleasants 1991; Liede 1994 (three species); Broyles and Wyatt 1995; Lipow and Wyatt 1998; Pauw 1998; Vieira and Shepherd 2002 (seven species); Ollerton et al. 2003 (nine species); Tanaka et al. 2006; S. D. Johnson and L. D. Harder, unpublished data[a]
Orchid massulate	Harder 2000 (seven species); S. D. Johnson and L. D. Harder, unpublished data[b]
Orchid mealy	Harder 2000 (two species)
Orchid smear	O'Connell and Johnston 1998
Orchid solid	Boyden 1982; Nilsson et al. 1986; Ackerman and Montalvo 1990; Christensen 1992; Pettersson and Nilsson 1993; Ackerman et al. 1994; Bartareau 1995; Singer and Cocucci 1997; Alexandersson 1999; Harder 2000; S. D. Johnson and L. D. Harder, unpublished data[c]

Note. In analyses, the observation for *Calypso bulbosa* was the average of results from Boyden (1982), Alexandersson (1999), and Harder (2000).

[a] *Gomphocarpus fruticosus*, 15.2%.

[b] *Anacamptis pyramidalis*, 7.6%; *Brownleea macroceras*, 12.1%; *Coeloglossum viride*, 7.7%; *Dactylhoriza incarnata*, 17.6%; *Disa cephalotes*, 7.3%; *Disa chrysostachy*, 6.8%; *Disa cooperi*, 6.8%; *Disa ferruginea*, 8.9%; *Disa graminifolia*, 11.9%; *Disa hircicornis*, 6.8%; *Disa pulchra*, 16.9%; *Disa uniflora*, 6.5%; *Disa versicolor*, 11.3%; *Gymnadenia conopsea*, 16.6%; *Ophrys sphegodes*, 14.3%; *Orchis militaris*, 8.7%; *Orchis morio*, 8.1%; *Orchis ustulata*, 1.6%; *Platanthera bifolia*, 4.6%; *Platanthera chlorantha*, 9.3%; *Satyrium bicorne*, 6.7%; *Satyrium coriifolium*, 9.0%; *Satyrium erectum*, 17.6%; *Satyrium hallackii*, 6.9%; *Satyrium longicauda*, 12.7%; *Satyrium longicolle*, 8.3%; *Satyrium membranaceum*, 10.8%; *Satyrium microrynchum*, 6.0%.

[c] *Eulophia cucullata*, 20.5%; *Eulophia parviflora*, 30.3%; *Eulophia welwitschii*, 52.6%; *Eulophia zeyheri*, 11.8%; *Mystacidium capense*, 56.2%; *Mystacidium gracile*, 35.2%; *Mystacidium venosum*, 42.8%; *Rangaeris muscicola*, 23.4%.

Pollen : Ovule Ratios

Information on pollen : ovule ratios was drawn from three published sources. The estimate for species with pollen monads is the average (based on log-transformed data) for xenogamous and facultative xenogamous species reported in table 1 of Cruden (1977). Most observations for species with aggregated pollen were extracted from Erbar and Langlotz's (2005) compilation, as summarized in table A2.

We used means for species for which Erbar and Langlotz (2005) presented multiple observations. Finally, we included observations for *Cypripedium calceolus* (orchid smear pollinium), *Calypso bulbosa*, and *Corallorhiza striata* (orchid solid pollinia) from Lukasiewicz (1999) and observations for *Earina aestivalis*, *Earina autumnalis*, *Earina mucronata*, and *Winika cunninghamii* (orchid solid pollinia) from Lehnebach and Robertson (2004).

Table A2

Species for Which Pollen : Ovule Ratios Were Obtained from Erbar and Langlotz (2005)

Aggregation type, family	Species
Tetrads:	
Annonaceae	Three *Asimina* spp.
Apocynaceae = Asclepiadaceae	*Periploca aphylla*
Clusiaceae	*Kielmeyera coriacea*
Ericaceae	*Andromeda polifolia*, *Calluna vulgaris*, *Moneses uniflora*, *Pernettya rigida*, three *Pyrola* spp., four *Vaccinium* spp.
Lactoridaceae	*Lactoris fernandeziana*
Winteraceae	*Pseudowintera colorata*

Table A2

(*Continued*)

Aggregation type, family	Species
Polyads:	
Ericaceae (=Pyrolaceae)	*Chimaphila umbellata*
Fabaceae	Four *Calliandra* spp., seven *Inga* spp., *Mimosa bimucronata*
Pollinia:	
Asclepiad:	
Apocynaceae (=Asclepiadaceae)	28 *Asclepias* spp., two *Calotropis* spp., *Ceropegia woodii*, two *Cynanchum* spp., *Hoya carnosa*, *Huemia* sp., five *Matelea* spp., *Oxypetalum caerulea*, *Pergularia daemia*, two *Sarcostemma* spp., *Vincetoxicum officinale*
Orchid massulate:	
Orchidaceae	*Comperia comperiana*, five *Dactylorhiza* spp., *Goodyera repens*, three *Ophrys* spp., six *Orchis* spp., two *Platanthera* spp., *Steveniella satyroides*
Orchid mealy:	
Orchidaceae	*Isotria* spp., *Listera ovata* (Orchidaceae)
Orchid solid:	
Orchidaceae	*Coryanthes senghasiana* (Orchidaceae)
Viscin threads:	
Ericaceae	Eight *Rhododendron* spp.
Onagraceae	*Calyophus serralatus*, two *Camissonia* spp., *Circaea canadensis*, seven *Clarkia* spp., 14 *Epilobium* spp., *Gaura drummondii*, *Oenothera biennis*

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