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Tactics for male reproductive success in plants: contrasting insights of sex allocation theory and pollen presentation theory

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Synopsis The basic tenet of sex allocation theory is that an organism's reproductive success, through either male or female function, can be represented as a sex-specific, monotonic, increasing function of the organism's investment of resources in that function. The shapes of these curves determine what patterns of resource allocation can be evolutionarily stable. Although SA theory has stimulated creative thinking about plant sexual tactics, quantifying the shapes of male and female gain curves has proven so difficult that other approaches must be considered. I contrast sex allocation theory to a different, emerging viewpoint, pollen presentation theory (PPT), which attempts to address variation in reproductive success by measuring and modeling the quantitative fates of pollen grains. Models suggest that RS through male function depends heavily on the packaging and gradual dispensing of pollen to pollinators, even with the amount of investment held constant. Many plants do deploy pollen gradually, through morphological and "behavioral" mechanisms that range from obvious to subtle. They may thereby influence many aspects of the evolution of sexual modes in plants, including transitions between dioecy and cosexuality. After reviewing the main implications of the models, I discuss recent work aimed at testing some key assumptions and predictions by functional and comparative studies in the genus *Penstemon*. Species of *Penstemon* conform to PPT predictions that bee-adapted flowers will restrict per-visit pollen availability more than hummingbird-adapted flowers.

Introduction

This article has been derived from a symposium presentation with the goal of providing a primarily zoological audience with an overview of theoretical ideas that have been developed to understand patterns of sex expression in plants, followed by an account of some recent comparative work.

Hermaphroditism is more the rule than the exception in plants. But exceptions—in the form of dioecy and variants of dioecy—pop up regularly in many different lineages, amounting to perhaps 5% of species.

Until the mid-1970s, attempts to understand this diversity of systems, and the transitions between them, were incomplete because they were narrowly focused on genetic factors. It was clear that separate sexes obviated inbreeding, and therefore inbreeding depression was thought to be the principal evolutionary force involved in separating the sexes. The emphasis on inbreeding distracted attention from what might be called the economics of offspring production. There was a general lack of theory, and a general failure to deal explicitly with the fact that hermaphrodites achieve fitness through potentially conflicting male and female functions, however obvious that seems now.

In the 1970s, several authors did focus attention on male and female pathways (for example Horovitz and Harding 1972; Janzen 1977), with Mary Willson's treatment of "sexual selection in plants" (Willson, 1979; also, 1994) stimulating the most attention. At the same time, the development of ESS modeling allowed the development of theory that related a hermaphrodite's overall reproductive success, through both male and female pathways, to the amount of resources it invested in male and female function. Sex allocation theory matured rapidly through the efforts of Charnov (1979, 1982; Charnov and others 1976), Lloyd (1984, 1985), and Charlesworth and Charlesworth (for example 1978, 1981). Charlesworth and Morgan (1991), Brunet (1992), and Campbell (2000) provide useful, critical overviews with particular attention to plants. An excellent, comprehensive treatment of ESS approaches to plant reproductive strategies was published by de Jong and Klinkhamer (2005) after this article was written.

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A simple caricature of these ideas is as follows: an organism has a fixed amount of resources that can be invested in either male or female sexual reproduction; resources can be easily reallocated to different functions; and for each sexual mode, there is a "fitness gain curve" that describes how reproductive success through that mode increases with increasing investment. With these elements, it is possible to specify the overall fitness associated with any particular allocation, by using the male and female gain curves to calculate how much reproduction is returned for the investments in male and female function, respectively. Modelers drew possible gain curves from families of smooth monotonic functions, typically power curves. With a power curve of the form RS = $(\text{investment})^y$, the exponent y serves as a "shape parameter": if it is less than 1, reproductive success RS is a decelerating function of resources invested; if it is equal to 1, RS increases linearly with investment; and if it exceeds 1, RS accelerates with investment. On the assumption of strict negative tradeoffs between investments in male and female function, fitness sets could be constructed to show the total fitness gain of a particular allocation strategy, and from that point, the evolutionarily stable strategy (ESS) allocation could be determined. By definition, the ESS allocation would be the endpoint of evolutionary adjustment of the male:female allocation: a mutant phenotype with the ESS allocation could invade a population of phenotypes with any other allocation, and no other genotype could invade a population of ESS phenotypes. These models also allowed for selfing and inbreeding depression.

The most basic prediction was that decelerating gain curves favored the maintenance of hermaphroditism, that is, a stable intermediate level of investment in both male and female function. Accelerating gain curves, on the other hand, favored the evolution of separate sexes. For example, an accelerating female gain curve could foster conditions in which a male-sterility mutation could spread in a population of hermaphrodites because the resources destined for male function would be reallocated to female function and produce a gain in female fitness that outweighed the loss in male fitness. After male-steriles became sufficiently abundant, female-steriles could spread secondarily. Accelerating male gain curves could produce dioecy through the spread of sterility genes in the opposite sequence.

Fitness gain curves, ecology, and correlations with dioecy

This insight led a number of investigators to ask what factors influenced the shape of gain curves in nature. In the case of higher plants, this focused attention on the ecology of pollination and seed dispersal. In particular, a number of authors suggested ecological mechanisms that could explain various correlations between the frequency of dioecy and particular ecological situations. For example, Bawa (1980) and Givnish (1980) proposed that the higher frequency of dioecy in plants with fleshy fruits might be attributable to a tendency for frugivorous seed dispersers to prefer plants that made large fruit crops. If such a preference were strong enough, it could produce an accelerating female gain curve. Analogous arguments were made concerning the preferences of pollinators for larger inflorescences. Some of these arguments had several stages. For example, Bawa (1980) noted an association of dioecy with plants that had small, inconspicuous flowers. He pointed out that such flowers are often pollinated by small, generalist pollinators, such as solitary bees and flies. Bawa posited that such pollinators might be particularly sensitive to the floral "display size" of plants, thereby setting up an accelerating male gain curve that would confer greater male success on plants that diverted resources from female function to making larger inflorescences that included male-only flowers. These scenarios excited a number of ecologists, including me, because it seemed possible that field investigations of pollinator and disperser behavior could be usefully linked to trends in floral evolution.

In 1990, Johanne Brunet and I reviewed the "ecological explanations for dioecy" that had been published up to that point (Thomson and Brunet 1990). We concentrated on studies that had tried to demonstrate whether the pollinators and dispersers really behaved in ways that produce accelerating gain curves. At that time, the evidence was equivocal. Although particular dispersers or pollinators might produce such effects in particular situations, it seemed impossible to generalize. Campbell's (2000) updated survey suggests that these aspects of sex allocation theory have still largely resisted direct tests.

Difficulties in applying sex allocation theory

Doing that review contributed to my growing sense that sex allocation theory, despite its elegance, actually had relatively limited applicability to pollination systems, and very limited testability. One of the bigger stumbling blocks, it seemed to me, lay in adequately characterizing the shapes of male and female gain curves. This task is tantamount to assembling a scatterplot in which it is extremely hard to measure the x-coordinate, surpassingly hard to measure the y-coordinate, and in which the theory itself suggests that selection for an optimum allocation would depress variation along both axes. To develop these points briefly in turn, the *x*-coordinate requires assessing the amount of resources devoted to male function and the amount devoted to female function. Questions of currency immediately arise. Of course the mass of ovules or stamens can be measured, although it is not certain that biomass would be the appropriate currency. Plant growth may be more severely limited by mineral nutrients, for example. A less tractable problem is that the inflorescences of hermaphroditic plants include components, such as petals, pedicels, and nectar, that serve both male and female functions. Lloyd (1985) proposed an elegant way of quantifying the assignment of the maleness and femaleness of such shared structures, but it requires knowing the shapes of the gain curves and therefore has little practical application.

Yet another difficulty is that investment in female function in higher plants typically continues long after male investment has ceased. The gradual maturation of fruits and seeds may be fueled by new, post-flowering photosynthesis, so that male and female investments are not drawn from a single, limited pool of resources as envisioned by sex allocation theory. This issue is addressed by Seger and Eckhart (1996).

If these difficulties can be circumvented, quantifying reproductive success through male and female function is still beset with all of the problems of estimating fitness via fitness components. Considering female function, it is easy enough to count seed production, but it is also easy to imagine how the vagaries of seed dispersal, post-dispersal mortality, and competition among sibling seedlings might introduce non-linearity in the relationship between seed production and offspring success. Such non-linearity would be fatal for inferring female gain curve shapes from seed counts. Directly estimating male gain curves is even more rigorous because of the intervention of the pollen-dispersal phase.

Many of these difficulties were also acknowledged in the early synthesis by Charlesworth and Morgan (1991), but those authors remained more optimistic about establishing the shapes of gain curves. The most direct fitness estimates come from using genetic markers to establish the paternal and maternal parentage of offspring from an entire population, and then relating those reproductive successes to measures of investment. Early attempts to approach this ideal via allozymes (for example Meagher 1991) were hampered by insufficient markers, limiting successful applications to small, isolated populations. The most successful assignment of paternity with allozymes markers is achieved by selecting parents with particular combinations of alleles. Devlin and colleagues (1992) conducted such studies in populations of ca. 30 plants of the annual species *Raphanus raphanistrum*. They were able to conclude that male siring success was a positive, decelerating function of flower number. However, their estimates of the shape of the male gain curve varied substantially among populations. Campbell (1998) completed what is probably the most direct test of classical sex allocation theory, in that she actually measured allocation to androecia in the monocarpic perennial *Ipomopsis aggregata*, then used allozyme-based paternity analysis to seek the functional relationship between investment and male success. For such arduous and audacious work, the results were somewhat deflating: although a relationship between male investment and estimated siring success was statistically detectable, investment explained only 9% of the variation in fitness. Furthermore, the estimated shapes of the fitness gain curves predicted an ESS allocation that did not match the empirical measures of allocation.

Now much better assignment of paternity can be achieved by using more informative markers such as microsatellites (cf. Isagi and others 2004). However, the difficulty that individuals in natural populations may display only modest variation in the ratio of male: female investment would still have to be confronted. Unless it is possible to construct artificial populations with an augmented range of allocation ratios—perhaps by selecting for extreme ratios—it may be hard to sample a broad enough range of investments to reveal any non-linear curvature of the gain functions.

This list of practical and conceptual limitations may explain why sex allocation theory does not seem to have fully matured in the sense that its quantitative premises have been routinely tested by direct measurement. It is possible to retain an optimistic stance on this issue: Campbell (2000) and de Jong and Klinkhamer (2005) discuss the most practical approaches and compromises. But I now wish to turn to an alternative viewpoint.

Tracking the fates of pollen grains, and the emergence of pollen presentation models

I was among those who were intrigued by the prospect of measuring the shapes of gain curves, but rather than using genetic markers, I adopted the more mechanistic approach of trying to follow the fates of pollen grains during the delivery process. The bumble bee-pollinated lily *Erythronium grandiflorum* provided a pollen-color dimorphism that allowed us to track grains visually by getting bees to visit rare red-pollen flowers and then a sequence of yellow-pollen recipients. As I will explain, although I initially saw this work as an approach to quantifying male gain curves, it actually led to a different group of models that I have begun calling pollen presentation theory (PPT). These models replace the focus on *amounts* of male resource (that is pollen) with a focus on how that resource is *deployed*. The question is no longer "why be a hermaphrodite?" (as in Charnov and others 1976) but "how to be a hermaphrodite?"

In *Erythronium*, the amount of pollen in an undehisced anther can be estimated by the anther's length. We measured anthers on red-pollen donors, then let the anthers dehisce, then got a bee to visit the donor flower and a long series of recipients. By counting the number of red grains on the recipients, we could estimate probable siring success (as pollen donated to other stigmas) as a function of the amount of pollen produced (Thomson and Thomson 1989; Harder and Thomson 1989).

This work suggested that pollen delivery was a decelerating function of the number of grains presented, principally as a result of grooming of grains by bees during flights between flowers. Deposition of a large load of pollen on a bee seemed to stimulate grooming that removed grains from circulation, a link later confirmed by Lawrence Harder (1990a, 1990b).

Tracking pollen grains is not the same as tracking paternity, but demonstrating fairly strong diminishing returns at this particular step in the chain between pollen production and siring success—and finding a mechanistic basis for it in pollinator grooming—gave us an entry point for a different look at the tactics that plants could employ to maximize male reproductive success. We wrote some simple bookkeeping models, in both analytical (Harder and Thomson 1989) and simulation versions (Thomson and Thomson 1992). Basically, a focal plant produces pollen through time at a specified schedule. Pollinators visit randomly at specified rates. (Non-random visitation schedules can be addressed with simulation approaches.) Each pollinator species removes pollen and subsequently delivers it to other plants' stigmas at characteristic rates. The output is the total number of grains that the focal plant delivers to other stigmas. We assume a simple lottery among the pollen grains delivered to stigmas, so that the number of grains delivered is proportional to the number of progeny sired. These models were extended by Harder and Wilson (1994, 1998), Lebuhn and Holsinger (1998), and Thomson (2003). My term "pollen presentation theory" refers to this cluster of models.

The basic insight was that a plant could evade diminishing returns on its male investment by presenting its pollen in a number of small doses, rather than all at once (Harder and Thomson 1989; also see Lloyd and Yates 1982 for the pioneering insight). Of course, this assumed that visitation by pollinators is reliably frequent enough. For an expected rate of visitation, there will in fact be an optimal pollen presentation schedule that depends on the amount of pollen removed per visit and the shape of the delivery versus removal curve. Nevertheless, this result suggested some new predictions about floral phenotypes. The most striking of these is that a plant with gradual pollen presentation will *greatly* outsire a plant with simultaneous presentation, as long as visitation rates are sufficiently high. We might therefore expect that plants—at least those pollinated by bees—might frequently present pollen in numerous small packages, or dispense it in numerous aliquots. A moment's consideration suffices to conclude that very many plants do stagger their pollen presentation through the gradual opening of flowers within inflorescences and, less obviously, the gradual dehiscence of anthers within flowers. Firmly ascribing these microphenological characteristics to selection arising from pollen-transfer efficiency is somewhat difficult, however. Given the modular architecture of plants, the gradual maturation of sexual parts might be expected as a simple consequence of non-adaptive variation in the initiation of their development.

Therefore, more telling examples come from special cases. In the andromonoecious *Aralia hispida*, for example, the perfect flowers within an inflorescence initiate male function first; then, after the stamens have been shed, they switch to female function by extending their styles. The onset of male functioning is more protracted than the later onset of female functioning by the *same set of flowers* (Thomson and Barrett 1981). If the staggered timing of flower openings were due simply to developmental slop, the female openings would be expected to be spread more broadly in time than the male openings. Seeing the opposite pattern suggests that selection has acted differently on the 2 sexual functions. Another special case is found in some aroids, such as *Dieffenbachia longispatha* (Young 1986), in which numerous anthers release pollen simultaneously. In this case, the inflorescence serves as a "trap blossom" that attracts many scarabeid beetles over several hours. The insects stay in the inflorescence until after the anthers dehisce, then leave *en masse* after becoming dusted by pollen. Therefore, in this case the pollen is still spread over many visitors, each of which arrived individually.

Anecdotal examples like these suggest that protracted schedules of flower or anther openings may indeed have an adaptive basis. However, I can provide a more robust test based on recent collaborative work with

Paul Wilson, Maria Clara Castellanos, Scott Armbruster, and Andrea Wolfe. This work, part of which has been recently published elsewhere, contrasts pollen presentation and dispensing strategies in *Penstemon* species pollinated by different pollinators. Species within this very large genus (ca. 270 spp.) form 2 reasonably discrete clusters when ordinated by a number of floral characters (Wilson and others 2004).

These clusters correspond well to classical pollination syndromes for hummingbird and hymenopteran pollination: putatively bird-adapted species have redder flowers, longer and narrower corolla tubes, reduced landing platforms, and more exserted sexual parts; they produce larger volumes of more dilute nectar with higher sucrose:hexose ratios. The visitor spectra observed in the field follow the syndromes as expected (Wilson and others 2004): hummingbirds are always prominent visitors to the red species, sometimes accompanied by bees and other insects. Birds are almost never seen on the blue-purple species, where hymenoptera predominate. Therefore, most *Penstemon* species can be unambiguously identified as either "bird-adapted" or "bee-adapted". Phylogenetic analysis makes it clear that hummingbird-adapted species have arisen in numerous independent lineages within *Penstemon* and its sister taxon *Keckiella*, which makes this genus particularly well-suited for comparative studies (A. Wolfe and others, in press; Thomson 2003; Thomson and others 2000; Castellanos and others 2006).

Furthermore, unlike most angiosperms, *Penstemon* flowers tend to have anthers (4 in number) with tough leathery walls. Rather than spilling out their pollen by everting completely upon dehiscence, the anthers of some species open by only a narrow slit that prevents grains from falling out freely. Therefore, plants can control the dosing of pollen by gradual *dispensing* through restricted anther openings or by gradual *presentation* by staggering the openings of the anthers within a flower or the openings of flowers within an inflorescence.

We arrived at predictions to test PPT by the following rationale.

(1) **Differences in pollen-transfer efficiency of bees and birds.** We expect that bees and hummingbirds will differ substantially in their efficiencies of pollen delivery. A pollen grain that is deposited on a bee's body is likely to be groomed off rather quickly. In the case of apid bees, pollen is moistened with nectar and packed into discrete pellets in the corbicular pollen baskets on the hind legs. Those grains have virtually no chance to encounter stigmas; they are effectively dead. Hummingbirds, on the other hand, have no dietary interest in pollen, and do not seem to be discommoded by it. They do not groom during foraging bouts, as bees do, and their grooming apparatus is poorly suited for removing pollen grains. Therefore, a grain deposited on a bird should have a much better chance of eventually being delivered to a stigma than another deposited on a bee; also, diminishing returns should be less pronounced.

(2) **Possibility of conditional parasitism**. If a plant is visited by 2 pollinator species, 1 of which wastes much pollen and the other of which wastes little, it can be shown by some simple models that that less effective pollinator may become a functional parasite (Thomson and Thomson 1992, Thomson 2003). That is, more pollen would be delivered if the better pollinator visited alone, because the wasteful pollinator takes away pollen that, if left behind, would be more effectively delivered by the other visitor.

(3) **Relative ease of evolutionary shifts from bee- to bird-pollination**. If birds are so superior to bees that bees become net parasites in the presence of birds, any ecological circumstances that produce sufficient bird visits (that is higher than a threshold) will create conditions that favor the spread of floral characters that will deter bee visits.

(4) **Continuing specialization to birds.** Once bees have been deterred and the plant is committed to bird-pollination, we expect continued character evolution to produce optimum pollination by the new pollinator. We expect that the suite of characters that comprise the "hummingbird syndrome" will be a mix of "anti-bee" and "pro-bird" characters.

(5) **Pollen presentation less restricted in bird-adapted taxa**. Therefore, we expect that species with narrow red corollas, etc., will have either wider-opening anthers, or less staggering of anther dehiscence times—compared with close relatives that are bee-adapted.

Following a preliminary test by Thomson and colleagues (2000), Castellanos (2003) evaluated this chain of reasoning as the focus of her dissertation. Testing proposition 1 is tricky, because it would be preferred to compare pollen transfer by birds and bees on a primitive or generalized *Penstemon* that is not obviously adapted to either bees or birds. Lacking such a species, we made comparisons on a bee-adapted species (*P. strictus*) and a bird-adapted relative (*P. barbatus*). The results generally support the prediction: birds are as effective as bees in transferring pollen in the bee-adapted species, and far better in the bird-adapted species. Pollen carryover by bees is more limited, which is consistent with more rapid loss of pollen through grooming (Castellanos and others 2003).

Turning to the main prediction (5) that bee-syndrome species should show more extreme dosing of pollen, Castellanos and colleagues (2006) found strong support with regard to anther morphology. First, we verified that the extent of anther opening was well correlated with the gradualness of pollen delivery to pollinators: anthers that open only by narrow slits require more visits to exhaust their contents than do anthers that open widely. Second, we used a preliminary phylogeny from (A. Wolfe and others (in press) to design a series of 15 pairwise comparisons of closely related taxonomic blocks that conformed to the bird and bee syndromes. When the anthers were rank-ordered by the extent to which they opened, the bee-adapted members of the pairs had highly significantly lower ranks, as predicted. Third, we directly assessed dosing in 4 pairs of taxa, all of which showed differences in the predicted direction.

Utility and limitations of PPT

Although sex allocation theory has played essential heuristic and conceptual roles in clarifying our thinking about the quantitative economics of sex, it falls short when we ask questions about particular phenotypes. The basic ideas of sex allocation theory are no more sufficient for understanding plant mating systems than would be counting up sperm and eggs for animal systems, ignoring all of the behavioral aspects of mating tactics that are so beloved of animal behavioral ecologists.

In animal-pollinated plants, the link between a plant's investment in sexual functions and its success in reproduction is mediated by the animals that do the pollinating. The reason we cannot get very far with the black-box perspective of sex allocation theory is that the mechanical ecology of pollen transfer matters. Because different pollinators may have different needs and different constraints that govern their behavior, no single sexual phenotype can be ideally suited to all pollinators. Pollination syndromes have heretofore been thought of as primarily comprising floral characters that attract certain pollinators and direct their bodies into contact with the sexual organs. Secondarily, some characters have been seen as deterring visitors by ineffectual pollinators. The work summarized here shows that other characters—characters of pollen timing that reflect adaptation to the pollen-delivery propensities of different pollinators—also covary with these suites of adaptive features.

PPT has not been aimed at exactly the same questions as sex allocation theory, and these 2 ways of viewing plant reproductive success are neither equivalent nor interchangeable. Nevertheless, PPT may give insights even into classical sex-allocation questions like the evolution of dioecy: for example, a reported ecological correlate of dioecy is with "small flowers". A hypothetical explanation has been offered based on pollinator preferences for larger inflorescences, but its premises are shaky; an alternative PPT-based hypothesis is that "small flowers" frequently translates to "many flowers", and that having many flowers allows gradual pollen presentation tactics that may linearize otherwise saturating male gain curves (see Thomson and Brunet 1990).

PPT therefore has something to offer. Nevertheless, it is currently a narrow perspective that needs considerable further development. The present models consider pollen economy only. They include unrealistically simple assumptions such as randomly timed arrivals of pollinators. They ignore female reproduction entirely, adopting the comfortable but extremely dubious assumption that female function is limited only by resources. [This assumption actually seems to be fairly applicable in *Penstemon* species we have studied, but it is surely not true in general (Burd, 1994; Wilson and others 1994)]. Unlike sex allocation models, all of which included inbreeding depression (albeit simplistically, as fixed levels of depression), pollen presentation models have thus far ignored the genetic quality of offspring. Finally, the current models consider only the performance of focal plants against a fixed background of mating opportunities. This ignores the important effects of frequency dependence that could be better understood with an ESS approach.

The problems listed above can be solved, to varying extents, by more sophisticated modeling. A very vexing obstacle continues to limit the application of PPT to interpret the quantitative conformance of phenotypes to optimality predictions. Recall that the models specify optimal presentation schedules, *given* a particular visitation rate by a particular suite of pollinators. I do not see any non-tautological way to specify the "natural" visitation rate and the "natural" pollinator spectrum for plant populations, and this difficulty is only made worse by the universal disruption of natural communities by anthropogenic disturbance, invasions, and extirpations. I fear that the only "solution" is to accept this limitation, and to design qualitative tests and hypotheses that are robust to the vagaries of community ecology. Our analysis of anther morphology and pollen dispensing in *Penstemon* is such an attempt.

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