

Seed paternity in flowering plants: an evolutionary perspective

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Abstract

The ultimate importance of paternal contributions to fitness and of post-pollination selection in flowering plants have remained elusive, largely because of the technical difficulty of assigning paternity. I review empirical studies that use heritable markers to investigate per-fruit seed paternity in natural populations and after experimental multiple-donor pollination. Thirty-one studies covering 23 species from 16 plant families document that in natural populations seeds from a single fruit are often fathered by multiple pollen donors (5 species from 5 families), that donors can differ significantly in seed-siring success (8 species from 6 families), that variation in pollen tube growth rates can be heritable ($n = 1$ out of 4 studies), that donor and recipient genotypes can simultaneously affect paternity ($n = 2$), and that temporal order of pollen deposition ($n = 1$) and environmental effects ($n = 2$) affect the outcome of pollen competition. These studies also investigate the role of post-pollination selection in the avoidance of inbreeding and for species boundaries. Most studies of male reproductive success in plants to date base on isozyme electrophoresis. The availability in the last decade of highly polymorphic molecular markers such as microsatellite DNA has been expected to open new possibilities to investigate competition and selection during the gametophytic phase. Yet, to date, there is still need for greater data wealth on seed paternity to test theories of sex allocation and to gain deeper understanding of floral trait evolution and of the evolutionary consequences of post-pollination selection in flowering plants.

Key words: genetic markers, paternity analysis, pollen competition, post-pollination sexual selection

Introduction

In the analysis of mating systems, the aim is to reconstruct the number and identity of mates through the assignment of parentage to offspring (He & Smouse 2002). Assignment of paternal parentage is particularly important because paternity reflects availability and origin of pollen and measures reproductive success via

the male function. Also, paternity can be the end result of competition for fertilization among pollen from one or several donors, selection processes occurring between pollen deposition and fertilization, and post-zygotic events such as seed abortion.

Pre-zygotic competition and selection processes in flowering plants are relevant under several aspects (e.g. Snow & Lewis 1993; Delph & Havens 1998). First,

selection on pollen tubes can lead to efficient purging of deleterious mutations. The efficiency of this selection arises from haploidy of the gametophytic phase (gametophytically-expressed deleterious mutations are directly exposed to selection even if they are recessive) and from the large population sizes of pollen. Selection on pollen tubes will be more intense under ecological conditions that lead to pollen competition (Mulcahy 1979; Box 1). Mutation purging may be particularly relevant for small, isolated populations suffering from loss of genetic variability and increased probability of inbreeding. Levels of inbreeding within populations also depend on whether pollen competition will result in higher success of outcross pollen over self pollen in self-compatible plants. Where this is the case, fewer inbred offspring are produced, reducing inbreeding depression (Charlesworth & Charlesworth 1992; Baker & Shore 1995; Souto et al. 2002). In self-incompatible species, on the other hand, interference competition can occur between compatible and incompatible conspecific pollen (Cowan et al. 2000; de Nettancourt 2001).

If pollen competition and pre-zygotic selection favour outcross pollen and result in genetically more diverse offspring, or if the competitive ability of pollen tubes is genetically correlated to the success of the diploid, pollen-donating plant (but see Delph et al. 1998), these processes can effectively contribute to enhance female fitness by increasing offspring quality compared to offspring from single-donor pollinations (Schlichting et al. 1990). Also, paternity of developing seeds within fruits is relevant to the potential for evolutionary conflicts among maternal, paternal and progeny interests (Ellstrand 1984; Haig & Westoby 1989; Bañuelos & Obeso 2003). Pollen competition is also relevant for species boundaries, as it may affect the degree of hybridization between species (Hauser et al. 1997) or cytotypes (e.g. Husband et al. 2002). Finally, the fate of pollen, together with seed dispersal, is essential to gene flow in these sedentary organisms (Ellstrand & Marshall 1985; Kaufmann et al. 1998; Nason et al. 1998; Richards et al. 1999; Rogli et al. 2000).

Box 1. Definitions for analysis of seed paternity in flowering plants.

Pollen competition: The simultaneous deposition on one pistil of an excess number of viable pollen grains relative to the number of ovules that are available for fertilization during the receptivity period. In this definition, pollen competition refers to the pool of potential mates at the time of pollen deposition, and is distinct from the competitive ability of pollen, pollen tubes and donors. Pollen and pollen tube competitive ability, together with mate choice in the pistil and incompatibility of pollen and pistil, are potential mechanisms determining seed paternity. Mate choice in the pistil, and pollen and pollen tube competitive ability can cause a difference in allele or genotype frequency between the pool of potential mates and the pool of successfully mating individuals, i.e. result in sexual selection.

Paternity shares: The proportion of seeds from a given fruit that have been fathered by each of different donors following pollen competition, either experimental or natural. With experimental pollen deposition, it is possible to choose donors of distinct genotypes and thus to identify also donors not obtaining paternity. Paternity shares could also be defined for groups of pollen grains (e.g. having a given gametophytically-expressed allele) when pollen competition occurs among the pollen of a given donor. The term is equivalent to male fertility at the level of the progeny in one fruit, when pollen was in excess of ovules before fertilization, and is related to reproductive success (the absolute number of seeds fathered by one donor).

Effective paternity: When the proportions of seeds from a given fruit that have been fathered by different donors are unequal, an index K_E can be constructed for the effective number of fathers (after Starr 1984):

$$K_E = \frac{1}{\sum (p_i)^2}$$

whereby p_i = proportion of the seeds in a fruit fathered by the i -th pollen donor, and $i = 1 \dots k$, the number of pollen donors competing for fertilization. This index depends on the relative skew among paternity shares, as illustrated below for the following examples for a three-donor case. The index is maximized and equals the number of donors ($K_E = K_{Max} = k$) when shares are equal ($p_i = p_j$ for all $i = 1 \dots k$ and $j = 1 \dots k$).

| Donor 1 | Donor 2 | Donor 3 | K_E | Donor 1 | Donor 2 | Donor 3 | K_E | Donor 1 | Donor 2 | Donor 3 | K_E |
|---------|---------|---------|-------|---------|---------|---------|-------|---------|---------|---------|-------|
| 0.1 | 0 | 0.9 | 1.22 | 0.1 | 0.1 | 0.8 | 1.52 | 0.1 | 0.3 | 0.6 | 2.17 |
| 0.2 | 0 | 0.8 | 1.47 | 0.2 | 0.1 | 0.7 | 1.85 | 0.2 | 0.3 | 0.5 | 2.63 |
| 0.3 | 0 | 0.7 | 1.72 | 0.3 | 0.1 | 0.6 | 2.17 | 0.3 | 0.3 | 0.4 | 2.94 |
| 0.4 | 0 | 0.6 | 1.92 | 0.4 | 0.1 | 0.5 | 2.38 | 0.4 | 0.3 | 0.3 | 2.94 |
| 0.5 | 0 | 0.5 | 2.00 | 0.5 | 0.1 | 0.4 | 2.38 | 0.5 | 0.3 | 0.2 | 2.63 |

In studies of plant reproductive biology, the assignment of paternity has often been neglected, as has been pointed out since the mid-eighties (Marshall & Ellstrand 1986; Snow & Lewis 1993; Smouse & Meagher 1994). A possible reason why relatively few studies assess seed paternity within fruits is that measuring male fertility and paternal contribution to fitness requires heritable markers, while female fertility can be more easily estimated through seed set (Meagher 1986; Snow & Lewis 1993). The focus on female contribution to fitness in a group of organisms that are prevalently hermaphroditic possibly reinforced a paradigm highlighting the dichotomy between selfing and outcrossing, rather than exploring continuous variation in the contribution by each of different potential mates to fertilization (Barrett 2003). An additional difficulty in the study of paternity in flowering plants is that seed development occurs within the maternal sporophytic environment. Because of this, the maternal environment may have a strong influence on embryo development and survival (including seed abortion, e.g. Havens & Delph 1996), and as a result it may be difficult to attribute the events occurring between pollen deposition and fruit maturation unambiguously to paternal or maternal influences, to sporophytic or gametophytic traits.

Here, I review empirical studies that investigate per-fruit seed paternity in natural populations or after experimental pollination using heritable markers to directly infer paternal parentage of offspring. I discuss studies that address the ultimate importance of post-pollination events, either by inferring multiple paternity in the field, investigating the mechanisms of differential seed-siring success, comparing single- and multiple-donor pollination, and directly competing self- vs. outcross or heterospecific vs. conspecific pollen.

Inclusion criteria

Among the studies that I was able to retrieve, I selected those investigating seed paternity within fruits, rather than within maternal plants (e.g. Meagher 1986; Smouse & Meagher 1994; Nason et al. 1998; Smouse et al. 2001; White et al. 2002). Per-fruit multiple paternity can be more directly compared to multiple paternity following sperm competition within one reproductive period in animals (Marshall & Ellstrand 1985; Birkhead & Møller 1998). Moreover, considering per-fruit paternity (rather than the number of mating partners at the plant level) reflects pollen competition events that are affected by the fusion among the carpels of a single flower. The fusion of carpels (syncarpy) has been interpreted as a key innovation contributing to the ecological success of flowering plants

(Mulcahy 1979; Endress 1982). Syncarpy intensifies pollen competition and, thus, may increase the chances of obtaining pollen from the best male parent. In support of this idea, and consistent with an adaptive function, a recent phylogenetic analysis estimates that syncarpy has evolved independently 17–26 times in the flowering plants (Armbruster et al. 2002).

As a second criterion of inclusion, I restricted the search to studies using heritable markers to estimate seed paternity. Heritable markers include phenotypic markers with a mendelian genetic basis, such as flower and leaf colour (e.g. Stanton et al. 1986; Snow & Mazer 1988; Cowan et al. 2000), allozymes and other molecular markers. To date, it is isozyme electrophoresis that still makes the major methodological contribution to the body of published studies on paternity shares within fruits in plants (Table 1). Although the use of highly polymorphic, neutral markers has been invoked already early on (Snow & Lewis 1993), it may have found less wide application than in zoology (Birkhead & Møller 1998). Thus a potential caveat for the interpretation of results is that both phenotypic markers and isozymes have potential limitations, due to the fact that the markers themselves may directly affect paternity success. For instance, in *Raphanus raphanistrum*, pollinators preferentially visit the yellow petal-colour morph over white-petal flowers (Stanton et al. 1986). Similar effects can occur for different alleles at isozyme loci, that can affect paternity, via linked loci or directly (Travers & Holtsford 2000; Travers & Mazer 2001). Also, the assignment of paternity can require statistical approaches (genetic exclusion, likelihood and fractional likelihood methods, reviewed in Snow & Lewis 1993), and the variability of the markers affects the possible resolution for paternity analysis.

Multiple paternity in natural populations

Isozyme electrophoresis has been widely used to estimate the rate of selfing and also the occurrence of multiple paternity in natural populations (Ellstrand 1984; Smouse & Meagher 1984; Meagher 1986; Campbell 1998; Smouse et al. 2001), while some more recent studies used DNA-based methods (Krauss 2000; Reusch 2000; White et al. 2002). For instance, in *Raphanus sativus*, multiple paternity has been documented under natural conditions, involving all individuals and 85% of all fruits in one study population (Ellstrand 1984). Such a pattern of multiple paternity may result from pollen carry-over or from multiple pollinator visits (Ellstrand 1984).

Multiple paternity within fruits does not necessarily require multiple pollinator visits, if pollinators carry

over pollen from flower to flower and thus deposit mixed pollen loads. This appears to be common in nature (Marshall & Ellstrand 1985). Evidence suggests that carry over is sufficient to explain high levels of multiple paternity in natural populations of the hummingbird-pollinated *Ipomopsis aggregata* (Campbell

1998). In this species, movement patterns of and pollen carry over by the hummingbird pollinators were used to construct a simulation model for multiple paternity. The simulations were consistent with paternity data obtained from isozyme analysis. Isozyme markers revealed multiple paternity in the majority of

Table 1. Overview of pollen competition studies investigating seed paternity using heritable markers (A, allozymes; M, microsatellite DNA or AFLP; *, parents homozygous at diagnostic loci). Studies classified by type: E, experimental vs. O, observational; F, field vs. G, greenhouse or garden. Donor effects: +, evidence for significant differences among individual donor plants in siring ability.

| Reference | Species | Family | Study | Type | Marker | Loci | Donor effects |
|---|--|------------------|-------|------|--------------|------|---------------|
| Paternity in natural populations | | | | | | | |
| Ellstrand (1984) | <i>Raphanus sativus</i> | Brassicaceae | O | F | A | 6 | |
| Meagher (1986) | <i>Chamaelirium luteum</i> | Liliaceae | O | F | A | 8 | |
| Smouse & Meagher (1994) | <i>Chamaelirium luteum</i> | Liliaceae | O | F | A | 8 | |
| Campbell (1998) | <i>Ipomopsis aggregata</i> | Polemoniaceae | O | F | A | 10 | |
| Krauss (2000) | <i>Persoonia mollis</i> | Proteaceae | E, O | F | AFLP | | + |
| Reusch (2000) | <i>Zostera marina</i> | Potamogetonaceae | O | F | M | 8 | |
| Mechanisms of differential seed siring success | | | | | | | |
| Marshall & Ellstrand (1985) | <i>Raphanus sativus</i> | Brassicaceae | E | G | A* | 1 | |
| Stanton et al. (1986) | <i>Raphanus raphanistrum</i> | Brassicaceae | E | F | petal colour | 1 | |
| Snow & Mazer (1988) | <i>Raphanus raphanistrum</i> | Brassicaceae | E | G | petal colour | 1 | + |
| Cruzan (1990) | <i>Erythronium grandiflorum</i> | Liliaceae | E | F | A* | 1 | |
| Delph et al. (1998) | <i>Silene vulgaris</i> | Caryophyllaceae | E | G | A* | 1 | |
| Pasonen et al. (1999) | <i>Betula pendula</i> | Betulaceae | E | G | A* | 1 | + |
| Skogsmyr & Lankinen (1999) | <i>Viola tricolor</i> | Violaceae | E | G | A* | 1 | + |
| Marshall et al. (2000) | <i>Raphanus sativus</i> | Brassicaceae | E | G | A | 1 | + |
| Skogsmyr & Lankinen (2000) | <i>Viola tricolor</i> | Violaceae | E | G | A* | 1 | + |
| Snow et al. (2000) | <i>Hibiscus moscheutos</i> | Malvaceae | E | G | A | 1 | |
| Marshall & Diggle (2001) | <i>Raphanus sativus</i> | Brassicaceae | E | G | A | | + |
| Travers & Mazer (2001) | <i>Clarkia unguiculata</i> | Onagraceae | E | G | A* | 1 | + |
| Single/multiple donors | | | | | | | |
| Marshall & Ellstrand (1986) | <i>Raphanus sativus</i> | Brassicaceae | E | G | A | 1 | + |
| Marshall (1991) | <i>Raphanus sativus</i> | Brassicaceae | E | G | A* | 2 | |
| Taylor et al. (1999) | <i>Silene alba</i> | Caryophyllaceae | E | G | A | 2/6 | + |
| Self/outcross pollen | | | | | | | |
| Bertin & Sullivan (1988) | <i>Campsis radicans</i> | Bignoniaceae | E | F | A* | 1 | |
| Montalvo (1992) | <i>Aquilegia caerulea</i> | Ranunculaceae | E | F | A | 1/7 | |
| Broyles & Wyatt (1993) | <i>Asclepias exaltata</i> | Asclepiadaceae | E | G | A* | 2 | |
| Rigney et al. (1993) | <i>Erythronium grandiflorum</i> | Liliaceae | E | F | A* | 1 | |
| Baker & Shore (1995) | <i>Turnera ulmifolia</i> | Turneraceae | E | G | A* | 6 | |
| Mitchell & Marshall (1995) | <i>Lesquerella fendleri</i> | Brassicaceae | E | G | A* | 1 | + |
| Mahy & Jacquemart (1999) | <i>Calluna vulgaris</i> | Ericaceae | E | G | A | 1 | |
| Steiner & Gregorius (1999) | <i>Alnus glutinosa</i> | Betulaceae | E | G | A | 1 | |
| Cowan et al. (2000) | <i>Trifolium repens</i> | Fabaceae | E | G | leaf colour | | |
| Conspecific/heterospecific or conspecific/hybrid pollen | | | | | | | |
| Hauser et al. (1997) | <i>Brassica campestris</i> <i>Brassica napus</i> | Brassicaceae | E | G | RAPD / A | 1(A) | |
| Campbell et al. (2003) | <i>Ipomopsis aggregata</i> <i>Ipomopsis tenuituba</i> | Polemoniaceae | E | G | A* | 1 | |

fruits, which contain two to 14 seeds. The average number of fathers per fruit was four, but up to nine fathers were represented in a single fruit (Campbell 1998). However, mixed pollen loads are unlikely to represent a random mixing from different donors. Smouse & Meagher (1994) suggest that in entomophilous species we should expect some clustering of male fertilization, because insect-vectored pollen is rarely delivered as single grain units (see also Snow & Spira 1991).

Although many plants suffer from pollen limitation at the plant level, pollen competition within single flowers has been documented in natural populations, suggesting that multiple paternity may often result from pollen competition. In a study of several natural populations of *Phlox drummondii*, Levin (1990) found for instance that most pistils investigated contained more pollen tubes than there were ovules to be fertilized. Indirect inference on the occurrence of multiple paternity in the field can also be drawn by observing pollinator behaviour (e.g. Winsor et al. 2000), or by measuring increase in variance in seed mass within seed families following multiple-donor pollinations as a circumstantial indication of multiple-donor siring within fruits (e.g. Bañuelos & Obeso 2003).

Variation among donor plants in seed siring success

Allozymes may not always be sufficiently variable for unambiguous assignment of paternity in natural populations, yet they have been widely used to assess paternity following hand-pollination. A major approach involves hand pollinations (but see Richards et al. 1999) between pollen-receiving and pollen-donating plants of known, distinct genotypes, typically homozygotes for alternative alleles at one or more isozyme loci (e.g. Marshall & Ellstrand 1985, 1986; Bertin & Sullivan 1988; Broyles & Wyatt 1993; Baker & Shore 1995; Mitchell & Marshall 1995; Delph et al. 1998; Mahy & Jacquemart 1999; Skogsmyr & Lankinen 1999, 2000; Steiner & Gregorius 1999; Roggli et al. 2000; Travers & Mazer 2001).

Some of these studies reveal differences in seed siring success among different pollen-donating individuals (Table 1). For instance, Pasonen et al. (1999) compared the seed-siring success of competing pollen donors in *Betula pendula* after two-donor hand-pollinations. Seed-siring success was significantly non-random in 20 out of 29 crosses involving six donors and eleven recipient plants selected for their specific isozyme marker. Ranking of paternity was consistent across recipient plants. There was a significant positive correlation between seed-siring success and pollen-

tube growth rate measured both in vivo and in vitro. In vitro pollen tube growth rates were also correlated with paternity shares in *Viola tricolor*, where the former was found to be a heritable trait (Skogsmyr & Lankinen 2000).

Non-random seed siring success has been documented among others in *Chamaelirium luteum* (Smouse & Meagher 1994), *Lesquerella feindleri* (Mitchell & Marshall 1995), *Alnus glutinosa* (Steiner & Gregorius 1999) and *Viola tricolor* (Skogsmyr & Lankinen 2000). That pollination with equal amounts of pollen from different donors can result in unequal paternity has also been shown in *Persoonia mollis* (Krauss 1999, 2000). The ranking of seed-siring success after hand pollination was consistent across recipient plants; however, it did not correlate with seed-siring success following natural mating (Krauss 2000). There are several possible explanations for such a lack of correlation, and reasons may vary between studies and species. In hand pollination experiments, care must be taken to control pollen viability and stigma receptivity as these potentially introduce spurious variation when evaluating pollen performance within and across treatment groups. In a survey of hand-pollination experiments, Stone et al. (1995) found that only half of the published studies on pollen competition reported pollen viability assessment. In the total survey of hand pollination studies, only 8% formally tested for stigmatic receptivity. Also, the timing of pollen deposition and inter-pollination interval in hand pollinations can affect paternity. In wild radish, the proportion of seeds fathered by the second donor declines rapidly with distance between pollinations (Marshall & Ellstrand 1985). Cowan et al. (2000) used a leaf-colour marker of white clover to infer paternity following hand pollination. In their study, after a first compatible pollination, both the success of a second pollen donor and seed yield decreased the longer the inter-pollination interval. In *Hibiscus moscheutos*, experimental manipulation of first and second pollen arrival indicates that inter-pollination interval plays a crucial role for fertilization success (Snow et al. 2000).

In addition to significant differences among pollen donors in seed siring ability, paternity can depend on the identity of the pollen-receiving individual, or on the interaction between recipient and donor plants. In *Raphanus sativus*, seed paternity is simultaneously affected by maternal plant identity and pollen donor identity, suggesting genotypic interactions (Marshall et al. 2000), and by environmental factors such as experimentally manipulated levels of water stress (Marshall & Diggle 2001).

Although differential seed siring success among different donor individuals suggests genetic variation for pollen competitive ability, we need more data to ap-

precipitate the extent to which pollen competitive ability (and, accordingly, variation among recipient plants affecting paternity) is heritable, and how such variation may be maintained (Delph & Havens 1998).

Pollen competition and sex ratio distortion

One limitation of the approach to employ selected homozygotes as parent plants is that paternity analysis fails to detect potential non-mendelian segregation (Ellstrand & Devlin 1989; Marshall 1991). Segregation distorters (meiotic drive alleles) can be responsible for instance for a deviation from the random expectation of 1:1 sex ratio among the progeny. It has been suggested that polyandry (i.e. sampling numerous pollen donors) may be beneficial to females as a defence against mating with males carrying a meiotic drive allele (Haig & Bergström 1995). Males heterozygous for a meiotic drive allele typically produce fewer functional sperm or pollen than do normal males, thus the transmission advantage of meiotic drive allele is diminished by sperm or pollen competition. From the female's perspective, selection should favour the avoidance of males carrying drive alleles because drive-allele homozygosity often results in embryo death, sterility or reduced fertility.

This idea found support from studies in animals (e.g. Wilkinson & Fry 2001) but also in plants. Taylor et al. (1999) analyzed the outcome of pollen competition between males that produced different sex ratios in the white campion (*Silene alba*), a dioecious plant with XY-sex determination. They estimated how the sex-ratio bias influenced the transmission properties of the sex chromosomes, using a combination of single-male pollinations and pollen mixtures to evaluate the effects of multiple paternity. Paternity following multiple-donor pollinations was estimated using allozymes. Males that produced biased sex ratios among their offspring in single pollinations fathered far fewer offspring of either sex in pollen mixtures. In crosses involving single males, however, these males produced the same number of offspring as other males. This study reveals how multiple-donor pollination can substantially affect the relative performance of pollen from different males.

Competition between self- and outcross pollen

A number of studies involved experimental multiple-donor pollinations, in which self and outcross pollen were simultaneously applied to the stigma. This should provide a test of the hypothesis that pollen

competition favours less related pollen, and leads to fewer inbred offspring being produced (e.g. Souto et al. 2002). Proportions of developed seeds fathered by self-pollen following self/outcross mixed pollinations vary widely, ranging from 0.6% in *Asclepias exaltata* (Broyles & Wyatt 1993), to 22% in *Calluna vulgaris* (Mahy & Jacquemart 1999) and 33% in *Campsis radicans* (Bertin & Sullivan 1988). To resolve whether post-pollination selection results in fewer inbred progeny, we need on one hand a comparison of seed set after single and mixed pollination. In *Campsis radicans*, up to 33% of viable seeds from mixed pollinations were fathered by self pollen, while nearly none would be fathered using loads of purely self pollen (Bertin & Sullivan 1988). In *Aquilegia caerulea* (Montalvo 1992) and *Echium vulgare* (Melser et al. 1997), the proportion of selfed seeds after mixed pollinations reflects the ratio of seed set after pure self and pure outcross pollinations. Thus, none of these studies provides strong evidence that post-pollination selection excludes self pollen from fertilization. However, lack of evidence is not evidence for a lack of effect, and more studies are needed to resolve this question. On the other hand data must be reported for independent replicates. In some cases, these proportions are reported as the average over several replicates. For instance in *Calluna vulgaris*, the proportion of 22% is calculated for the overall number of selfed progeny (9) in the total sample (46) of seedlings genotyped; however, we do not know how the selfed progeny was distributed among the five fruits investigated. In *Asclepias exaltata*, all selfed progeny recorded were produced in two flowers on the same plant (Broyles & Wyatt 1993). Paternity after competitive pollination involving self and outcross pollen has also been investigated for a number of crop species (see references in Marshall & Ellstrand 1986).

Mixed pollinations, comparing the success of self vs. within- or between-population pollen using allozymes, were carried out in *Turnera ulmifolia* (Baker & Shore 1995). In this neotropical species, populations are often small and genetically depauperate, and inbreeding depression has been reported (Baker & Shore 1995). Two pollination methods were compared. Competitive pollinations were carried out by applying mixtures of pollen from two donors on all stigmas of one flower, or of each donor separately on one single stigma within the same flower. Within populations, there was no evidence for a competitive advantage to outcross pollen. Thus, pollen competition is unlikely to significantly reduce the production of selfed progeny in these populations. Between populations, a marked advantage to self pollen occurred for most populations, and the extent of this was correlated with increasing morphological divergence of the outcross

pollen donor population. Populations diverged significantly in morphological traits, including style length and pollen size.

Based on these studies, it is not possible to draw general conclusions. An important future direction for studies comparing the fate of self- vs. outcross pollen would be to simultaneously measure the extent of post-pollination selection and the fitness benefits of excluding related pollen from fertilization.

Competition between conspecific and heterospecific pollen

Where pollinators are generalists, mixed pollen loads can contain heterospecific pollen (e.g. Guéritaine et al. 2003). In *Brassica*, hybridization can occur between the weed *B. campestris* and oilseed rape *B. napus*, suggesting that under natural conditions the stigma is likely to receive a mixture of pollen from the two species. Hauser et al. (1997) investigated mixed pollinations in variable ratios (1:2, 1:1, 2:1, 5:1) and scored the resulting progeny (seedlings) with isozymes. In addition, ungerminated seeds were also scored by using species-specific markers to infer the proportion of hybrids among aborted seeds. Pollen of *B. campestris* was at about a 10-fold disadvantage in *B. napus* styles, whereas no significant difference was found in *B. campestris* styles. Hybrid zygotes had lower survival than conspecific zygotes in both species after mixed pollinations, but this was not the case after pollinations using pure heterospecific pollen, suggesting that post-zygotic selection occurred in this case, and that seed abortion rates responded to the average relatedness within seed families.

Post-pollination selection can contribute to reproductive isolation when conspecific pollen fathers more seeds either compared to heterospecific pollen or to hybrid pollen in mixed pollinations. A very recent study found evidence for conspecific pollen advantage over hybrid pollen in mixed pollination in *Ipomopsis* (Campbell et al. 2003).

Future directions

Clearly, we need more studies on seed paternity and on the genetic diversity of mates at the level of pollen loads, fruits and recipient plants, to account for variation among species in breeding system and to document the relative importance of pollen competition and pollen limitation in natural populations. PCR-based methods such as multilocus microsatellite genotyping provide suitable tools (Snow & Lewis 1993). One advantage of these techniques is that they require

only small amounts of DNA. For natural populations, developing seeds of recently fertilized ovules were typed in the eelgrass, *Zostera marina* (Reusch 2000). This example illustrates the possibility of gathering information on paternity patterns at various stages, including early seed development. Thus, these techniques may provide a tool to disentangle pre- and post-fertilization processes, and to distinguish between effects due to competition among pollen donors and maternal choice of developing embryos (see also Korbecka et al. 2002). A second advantage is that high variability increases resolution, so that in some cases only a few loci are sufficient to assign parentage (e.g. Bernasconi et al. 1997).

For experimental pollinations, a fruitful direction to pursue would be to combine a range of donor diversities with estimates of effective paternity. Effective paternity can be expressed by 'skew indices' that take into account the relative success of pollen donors in the proportion of a fruit seeds that they have fertilized (see Box 1). Including more than two males (e.g. Marshall 1991) allows to sample a more realistic range and to draw inferences on the mechanisms that relate donor diversity to female reproductive success and offspring vigour (Bernasconi et al. 2003). To date, few of the experimental studies involving hand pollination explore competition among a diversity of pollen donors (e.g. Marshall & Ellstrand 1986), while most consider competition between two donors. Observational evidence for a positive correlation between seed set and number of donors, that naturally varied between four and nine fathers per fruit, was found in *Ipomopsis aggregata* (Campbell 1998). However, under natural conditions, increased seed set with increasing number of donors may also be due to variation in pollen load. A recent experimental study exploring the effect of pollen donor diversity on female reproductive success, while keeping pollen load constant, is a study using the rare plant *Cochlearia bavarica*. Paschke et al. (2002) observed a strongly positive log-linear effect of the experimentally controlled number of pollen donors, increasing from one to three to nine, on female reproductive success (measured as seed set). The increase in seed set with pollen donor diversity is likely to be relevant for the survival of *C. bavarica*, as it could overcome the biparental inbreeding depression observed in small populations of this rare endemic species (Paschke et al. 2002). However, since paternity was not estimated, it is unknown whether the effect arises through a higher chance of including a 'good' or compatible donor in the higher donor diversity treatment, or through a different mechanism (Bernasconi et al. 2003).

Other important aspects for future studies include accounting for potential environmental effects on seed paternity and pollen competitive ability (e.g. Lau &

Stephenson 1994; Lankinen 2000; Pasonen et al. 2000), and investigating possible simultaneous effects on paternity of both the recipient genotype and the donor genotype. Finally, future directions may include exploring the correlation of floral traits, floral phenology and plant architecture with male reproductive success and competitive ability. Pollinator attraction depends on floral display (Snow & Lewis 1993; Elle & Meagher 2000; Morgan & Conner 2001; Jesson & Barrett 2002; Barrett 2003), including the availability of rewards. Not only pollinator density, but also floral traits such as floral longevity, duration of receptivity (G. Bernasconi & Å. Lankinen, unpubl. results), resting stage in the pistil (Dahl & Fredrikson 1996) and timing of pollen dispersal potentially affect male-male competition and female choice in flowering plants (Barrett 2003).

In conclusion, the availability of highly polymorphic markers (Snow & Lewis 1993) should allow more studies to estimate not only seed set (fitness via the female function) but also paternity shares (fitness via the male function) under realistic and naturally relevant conditions of among-donor pollen competition, and to relate male fertility to the intensity of competition and floral traits. Such an approach should help clarify the role of post-pollination events and post-pollination sexual selection in plants and to fill the gap that apparently exists compared to the research effort devoted to post-mating events in the zoological literature.

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Added in proofs. A recent study examines the influence of pollen origin (self pollen, open pollination and interspecific pollination) on the extent of apomixis in facultative apomictic *Rubus* using DNA markers (Werlemark & Nybom 2003).

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