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## Genetic structure and post-pollination selection in biennial plants

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The main purpose of reproduction in all organisms is to ensure that an individual transfers its genes to the next generation. The reproductive success of an individual has two important components: the number of offspring and the quality. The number of offspring may be directly related to the amount of energy obtained by a parent. In contrast, the quality of an individual results from both the amount of energy or resources invested in it and its genetic constitution. The genetic constitution is shaped by a number of processes acting during or after mating. These processes account for sexual selection and selective embryo abortion.

In 1871, Darwin introduced the concept of sexual selection to explain the presence of characters that increase the probability of mating and getting offspring but not necessarily to increase the individual's survival. He considered mainly secondary sexual characters and behaviors. Since Darwin countless studies on animals have presented such a role for male ornaments, songs and scents attracting mates and for courtship behaviors. However, it became also clear that post-copulatory mechanisms may influence the reproductive success of an individual as well. When a female mates with two or more males, sperm from different males may compete in female reproductive tracts for fertilization (Parker, 1970). The female can also influence the paternity of her offspring. Mechanisms of post-copulatory female choice (so called *cryptic female choice*) include for example: changes in rates of oviposition, timing of ovulation, sperm dumping, digesting of sperm and selective storage of sperm (Birkhead and Møller, 1998; Eberhard, 1996).

Apart from sexual selection, post-fertilisation processes may influence genetic constitution of offspring in animals. Developing embryos may be aborted depending on their genotype. Such a selective embryo abortion has been described for example in mammals, where an embryo may be rejected by the mother just before its implantation (Haig, 1993).

Bateman (1948) used the term *sexual selection* for the first time in reference to plants. Since then it appears in plant breeding literature, although still less frequently compared to animal studies (Willson, 1994). Plants are unable to search for their mates or attract them in any way. They depend in their mating success on biotic or abiotic pollinating agents. However, as I will describe later, a number of morphological adaptations can modulate pollen transfer. After pollination, a number of processes can influence the paternity of seeds. These processes are analogous to sperm competition and cryptic female choice known in animals. Selective embryo abortion can also play a role in plants. Below, I will describe how sexual selection acts in plants during and after mating. I will also write about a potential for selective embryo abortion to act in plants.

### ***Mating in plants***

Plants can not choose their mates directly. However, they may have many adaptations that lead to an increase of pollen export or variation of pollen that lands on the surface of a stigma. Such adaptations are: prolonged receptivity of the stigma and characters increasing the number of pollinators visits like high nectar production rate and attractive floral display (Stephenson and Bertin, 1983).

Almost seventy five percent of the flowering plant species are hermaphroditic (Charnov, 1982, p. 254). Therefore, mate choice in self-compatible plants often involves the avoidance of self-pollination. Various morphological and physiological

features like: position of the stigma (herkogamy) and timing of its receptivity (protandry, protogyny) can minimize self-pollination within the same flower (autogamy). However, there may be still a lot of self-pollination, which can not be prevented, as pollinators tend to visit neighboring flowers on the same genet (geitonogamy). As a result self-compatible plants may experience a considerable degree of inbreeding depression. Apart from autogamy and geitonogamy, crosses between related individuals (biparental inbreeding) may increase inbreeding. Such crosses can be common in populations with a genetic structure because pollinators tend to visit neighboring plants. The level of biparental inbreeding depends on the genetic structure of the population.

Many studies have detected a genetic structure in plant populations (Loveless and Hamrick, 1984; Vekemans and Hardy, 2004). However, they usually aimed at estimating the dispersal distances of the species, and they frequently include juveniles into data sets. To my knowledge, there are only few recent studies with the main objective to estimate biparental inbreeding (Griffin and Eckert, 2003).

#### ***Post-pollination selection***

Because opportunities to choose mates prior to pollination are limited, post-pollination mate choice is essential for sexual selection in plants. Selection at this stage operates directly on the gametes. Bernasconi *et al.* (2004) emphasized in their review that this is in contrast to animals, where post-copulatory mechanisms discriminate among available ejaculates rather than sperms. Intra-ejaculate competition among individual sperms can not be strong because of limited gene expression at this stage.

Pollen, unlike sperm, does not move in a fluid. After a pollen grain lands on a stigma, its vegetative cell elongates producing a pollen tube, which grows into a structure of the style and delivers a generative cell to the ovule. The complexity of this process, direct interaction with the female tissues and also high levels of gene expression in pollen (Becker *et al.*, 2003) suggest that there is ample opportunity for sexual selection to act at this stage. Gametophytic self-incompatibility is a clear example of such selection favoring outbreeding (Richards, 1997).

However, sexual selection may discriminate also among compatible pollen that is genetically heterogenous. The term *pollen competition* has been widely used in pollination studies to describe differential fertilization success of such pollen.

One experimental approach to test whether pollen competition takes place is to apply different pollen loads and measure offspring quality. It is assumed that higher pollen loads lead to stronger selection. Therefore, offspring resulting from such a pollination treatment will be more vigorous. This prediction was confirmed by many studies that detected enhanced germination, vegetative growth or/and reproductive performance among plants grown from the seeds sired in higher pollen loads treatment (e. g. Bjorkman, 1995; Quesada *et al.*, 1996; Richardson and Stephenson, 1992).

Other studies use a more direct approach in which single donor pollinations with pollen from different donors are followed by measurement of the pollen tube growth. Often such measurements show high variation in pollen performance among donors (e. g. Bjorkman *et al.*, 1995; Sari-Gorla *et al.*, 1995) and pollen tube growth correlates with siring success (Pasonen *et al.*, 1999; Snow and Spira, 1991)

Pollen competition is believed to be responsible for cryptic self-incompatibility (CSI): a slow growing self-pollen may fertilize all ovules in single donor pollinations,

but when it is applied in a mixture with a faster growing outcross pollen its fertilization success is greatly reduced. Bateman (1956) reported CSI for the first time in *Cherianthus cheiri* – a species producing full seed set when pure self-pollen is applied. He performed two mix pollination treatments on one yellow flowered individual: in the first treatment he used a mixture of self-pollen and pollen from a red flowered donor, in the second treatment – a mixture of outcross pollen from two donors: yellow and red flowered. The proportion of red flowered offspring equaled 92.2% and 22.7% in the first and second treatment respectively. This result suggests a strong disadvantage of self-pollen in *C. cheiri*.

Since Bateman, CSI has been studied by means of pollen tube growth measurements or/and paternity analysis following mixed pollinations. So far, most of the evidence for CSI comes from studies on heterostylous species. In these species self-pollination is a kind of illegitimate pollination and self-pollen may have more disadvantages than only a slower growth. Other CSI studies showed a number of other methodological flaws like low number of genotypes or using morphological characters for paternity analysis (see introduction to chapter 5). Therefore, I concluded that there is a need for a study of CSI in non-heterostylous species, using many genotypes and selectively neutral markers for paternity analysis.

#### ***Embryo abortion in plants***

After fertilization, embryos can be selectively aborted depending on their genotype. This is however rather difficult to prove in plants. First one has to prove that ovules that did not develop into seeds were indeed fertilized, embryos were potentially viable and moreover, that the observed abortion was selective. A common observation in plants is that they produce more ovules than seeds (Bawa and Webb, 1984; Stephenson, 1981). Seed-ovule ratios equal on average 85% for annuals and 50% for perennials (Wiens, 1984). However, low fruit or seed production can not be attributed directly to embryo abortion, because not necessarily all of the ovules are fertilised in the field. Burd (1994) reviewed pollen limitation in field studies by comparing fruit or seed production after natural and after hand pollination with outcrossed pollen. He assumed that after hand pollination excess of pollen were applied and thus seed-set and fruit-set were not pollen limited. Burd (1994) reported that 62% out of 258 analysed species was significantly pollen limited in the field. On basis of data provided in the same paper, I made a frequency distribution of percent fruit set for the species for which pollen limitation was not found (fruit set is defined as fraction of flowers setting fruits). This distribution indicates a great potential for embryo abortion (Fig. 1a). One should keep in mind, however, that fruit abortion may be related to seed number rather than seed quality or genetic constitution, even when pollen is not limiting seed production. Although, pollen is not limiting fruit production, still 77 out of 90 species have a fruit set lower than 90% in the field (Fig. 1a). A part of this abortion under natural conditions can be explained by a post fertilisation self-incompatibility mechanism (Seavey and Bawa, 1986). However, after pollination with outcross pollen still only 23 out of 90 species have a fruit set higher than 90% (Fig. 1b).

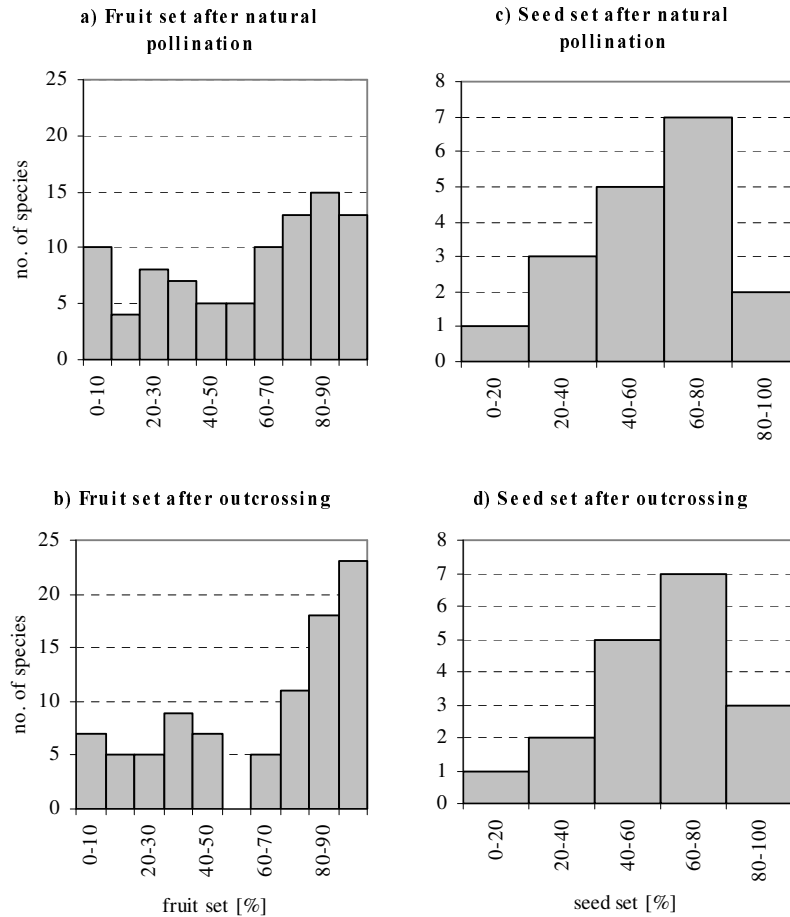


Fig. 1

Fruit set (fig. 1a and 1b) and seed set (fig. 1c and 1d) distributions after natural pollination (fig. 1a and 1c) and outcrossing by hand pollination (fig. 1b and 1d) for plant species that do not show pollen limitation according to Burd (1994). If data were available for more than one population an average was calculated for those populations that did not show pollen limitation. Fruit set was analysed for 90 species from 39 families, and seed set for 18 species from 10 families. Two species (*Erythronium propullans* and *Igna quaternata*) that did not have any fruit set after outcross pollination were excluded from the analysis.

An even better indication for embryo abortion is provided by a similar frequency distribution made for the percentage seed set (% of ovules that develops into seeds). Burd (1994) provides information about seed set in only 28 species, out of which 18 did not show pollen limitation. Figure 1c shows the frequency distribution of seed set for those 18 species after natural pollination. Only for 2 of them seed set exceeds 80% in the field. After outcrossing only three species have such a high seed set (Fig. 1d). Thus, even if pollen is not limiting seed production, many ovules do not mature seeds, indicating that many embryos are aborted.

#### *Selective embryo abortion*

There are three hypotheses explaining this apparent "overproduction" of ovules in plants. First, in hermaphroditic plants the production of "excess" flowers can result from selection on optimal division of resources into male and female function. Flowers that do not contribute to seed production may disperse pollen and contribute to siring seeds (e. g. Sutherland and Delph, 1984). Second, it has been suggested that in variable environments it is impossible for the plant to predict how much of the resources will be available during seed maturation. The overproduction of zygotes gives an opportunity of adjusting the seed set to the number that is optimal in a certain reproduction period. Such a strategy is called bet-hedging (Kozlowski and Stearns, 1989; Lloyd, 1980). Third, embryo abortion may serve to increase female fitness by providing the possibility to select for superior offspring and avoid investment of resources in seeds that produce offspring with a low fitness later in life (Willson and Burley, 1983).

I will focus on the third hypothesis, as a part of sexual selection. The selective embryo abortion (SEA) hypothesis received a lot of attention in 1980s (Casper, 1988; Stephenson, 1981; Willson and Burley, 1983). However, experiments designed to test it on the level of the phenotype were not able to discriminate between artifacts resulting from a treatment and effect of SEA (e. g. Casper, 1988).

#### *Thesis outline*

The experimental part of my thesis is based on two biennial species, tetraploid *E. vulgare* and diploid *C. officinale*. I start my thesis by characterizing microsatellites for both species (chapter 2 and 3). This characteristic includes a description of PCR conditions and a test for polymorphism of developed microsatellite loci.

In chapter 4, I used the developed microsatellites to test for a genetic structure in natural populations of *E. vulgare* and *C. officinale*. Using two species in such a test gives an opportunity for an interesting comparison. I expected to find a stronger genetic structure in *C. officinale* because of a lower ploidy level and higher selfing rate. Apart from testing for a genetic structure, I estimate the amount of biparental inbreeding in the populations of both species.

In chapter 5, I deal with aspects of post-pollination selection in *E. vulgare* only. Since, selfing rates in this species are much lower than predicted from the plant size and pollen dynamics, I tested for cryptic self-incompatibility.

In the following chapter 6, I study the potential for SEA by collecting literature data on genetic maps of plants. This chapter was greatly inspired by previous work of Melser (2001) who used phenotypic approach to study SEA in *E. vulgare* and *C. officinale*. Since the phenotypic approach has proven to have many limitations, I searched for an alternative way to test this hypothesis – on the level of a genotype.

SEA, like every selection, should show up in segregating progeny of one cross as a departure from Mendelian segregation. I assumed that if there is a considerable level of selective embryo abortion in plants—many genetic maps of plants should report non-Mendelian segregation of molecular markers. Therefore in chapter 6, I review genetic maps of plants to estimate the level of non-Mendelian segregation. I also propose an experimental design that will allow for attributing the detected non-Mendelian segregation to embryo abortion.

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## Chapter 1

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