

**Effects of Interspecific Pollen Transfer (IPT) in a
Specialist and a Generalist Flower**

Christopher D. Muir - REU 2005

*College of William and Mary, Williamsburg, Virginia 23186 USA
and Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224 USA*

A major question in pollination biology is the evolution of specialization to a specific animal pollinator. Past studies have found that increased male fitness may increase male fitness by excluding inefficient, generalist pollinators. This study examines whether specialization may occur in order to enhance female fitness. Specifically, interspecific pollen transfer by generalist pollinators may cause reductions in pollen quantity and quality at the stigma. We simulated inconstant pollinator foraging behavior on a specialist and generalist plant by applying pollen from conspecific and heterospecific donors. Data collection was not finished at the time of this writing, but we discuss anticipated results.

Keywords: *Ipomopsis aggregata*, *Linum lewisii*, interspecific pollen transfer, specialization

Introduction

The evolution of specialization and generalization in flowering plants is a long-standing question among biologists. Traditionally, pollination biologists believed that plant-pollinator interactions were characterized by a ‘lock and key’ pairwise interaction (e.g. Darwin 1862). Pollinators exert selective pressure on a several traits, such as color, tube length, and nectar, to increase pollination efficiency. A suite of morphological features, called pollination syndromes, evolves and can be used to infer the “appropriate” animal pollinator across many ecosystems as a result of convergent evolution. Under this paradigm, specialization toward a particular pollinator, a pollination syndrome, was thought to be the typical outcome of evolution and could lead to speciation (Grant and Grant 1965; Stebbins 1970).

In contrast, generalization is defined as pollination of a single species of plant by many species of animals. Waser et al. (1996) challenged the assumption that specialization is the rule and argued that both theory and empirical evidence support that generalization is common.

To further inform this debate, it is useful to understand under what ecological circumstances specialization might be favored. The present study therefore examines whether specialization is an adaptation against the deleterious effects of interspecific pollen transfer (IPT). IPT is caused by inconstant foraging behavior of pollinators, often generalist bees and flies.

Theoretical considerations suggest that IPT may facilitate competition through loss of pollen to competitors, a reduction in male fitness, or from direct effects of heterospecific pollen on the stigma, a reduction in female fitness (Waser 1978, 1983; Rathcke 1983). Specialist flowers that can promote pollinator constancy should thus have increased fitness (Waser 1986). For example, previous studies have found that some floral adaptations in the hummingbird syndrome flower, *Penstamen babatus*, are “anti-bee” rather than “pro-bird” (Castellanos et al. 2004) and have evolved to increase male fitness (Castellanos et al. 2003).

Specialization may also evolve to increase female fitness by reducing IPT. IPT is common in nature among multiple families (e.g. McLernon et al. 1996) and varies widely from 4% (Campbell and Motten 1985) to >50% (Brown and Kodric-Brown 1979; Neiland and Wilcock 1999) where it has been measured. Several studies have examined the effect of mixed (conspecific and heterospecific) pollen loads on female fitness, though none have specifically addressed the evolution of specialization. Simultaneous

application of heterospecific and conspecific pollen has been shown to impair seed set via occlusion (Galen and Gregory 1989; Randall and Hilu 1990), allelopathy (Sukhada and Jayachandra 1980; Thomson et al. 1981; Murphy and Aarssen 1995a, b, c, d), and indeterminate means (Neiland and Wilcock 1999; Brown and Mitchell 2001).

Sequential application, (heterospecific pollen prior to conspecific pollen), can cause premature stigma closure (Waser and Fugate 1986) or otherwise inhibit seed set (Caruso and Alfaro 2000) in *Ipomopsis aggregata*. Researchers have also failed to find any effect of heterospecific pollen among co-flowering species (Campbell and Motten 1985; Kwak and Jennersten 1991). If IPT commonly reduces female fitness, we might expect to find that traits which enhance pollinator constancy, specialization, to evolve through natural selection. Conversely, generalist flowers that experience high levels of IPT might have evolved other mechanisms than a specialized morphology. Though the deleterious effects of IPT have been documented, it is unclear whether any adaptive pattern exists between specialist and generalist flowers for resistance to its deleterious effects.

The present study addresses the effect of IPT on female fitness effects of two plant species, *Ipomopsis aggregata*, a specialist, and *Linum lewisii*, a generalist, (for definitions of specialist and generalist, see “Methods and Materials”), by simulating inconstant foraging behavior of pollinators. Specifically, we measured seed set in response to applying mixed loads of pollen onto stigmas.

We tested several test hypotheses against the null hypotheses that mixed pollen loads have no effect of female fitness. Mixed pollen loads might decrease seed set either through a reduction in pollen quantity or pollen quality. A reduction in pollen quantity occurs when, even though there is a sufficient amount of conspecific pollen on the stigma

for fertilization, dilution by heterospecific pollen interferes through occlusion or other means. Reduced quantity of pollen may occur in the presence of a single heterospecific pollen species, or it may take more severe dilution to observe an effect. A reduction in pollen quality occurs when even a small amount of heterospecific pollen induces reduced fitness, presumably due to allelopathy or some other means.

Mixed pollen loads can have (1) a greater effect on female fitness in *I. aggregata* than in *L. lewisii*, or (2) similarly effect female fitness of both species. The former result would suggest that there is a deleterious effect of IPT and that *I. aggregata* may have evolved specialization to avoid the costs of IPT. The latter result would suggest that a specialization in *I. aggregata* is not the result of evolution to avoid the costs of IPT, and that *I. aggregata* may be more generalized than previously thought, congruent with ideas proposed in Waser et al. (1996).

Materials and Methods

Study System

We carried out this project near the Rocky Mountain Biological Laboratory (RMBL; elevation 2,900m), a high altitude field station located amid the Elk Mountain Range in Gothic, Colorado. The field site was located in a meadow along Copper Creek, in the Maroon Bells-Snowmass Wilderness.

We studied *I. aggregata* and *L. lewisii*, two co-flowering, sympatric plant species from sub-alpine meadows located in the vicinity of the RMBL. *I. aggregata* is monocarpic, producing only one seed set after 2-10 years as a rosette, meaning that the reproductive bout we measured represents lifetime female fitness. *I. aggregata* conforms well to the hummingbird pollination syndrome, as flowers are red, tubular, and produce

large volumes of dilute nectar. Two species of hummingbirds, broad-tailed (*Selasphorus platycercus* Swainson) and rufous (*S. rufus* Gmelin), perform most of the pollination in *I. aggregata* (Campbell et al. 1991).

A prototypical generalist morphology can be described as any flower that is radially symmetrical, upright, and has accessible reproductive organs (Fenster et al. 2004). We used *L. lewisii* in this study because it conforms well to a generalist morphology, is pollinated mostly by flies and bees, and is sympatric and co-flowering with *I. aggregata*.

Plant and Pollen collection

Within the site, we haphazardly bagged thirty plants in bud stage of each species to prevent natural pollination. Thirty specimens allowed for adequate statistical power, even if some plants were accidentally killed or damaged due to weather, herbivores, or livestock. Our study did not detrimentally affect local population sizes of either species because both are highly abundant at the site and in surrounding areas.

We hand pollinated all flowers between July 7-27, 2005, with a toothpick when stigmas were receptive. A reflexed stigma indicated receptivity in *I. aggregata* and stigmas were always receptive in *L. lewisii* since flowers only last one day. I haphazardly assigned four flowers, as they emerged, from each plant to one of four pollen treatments:

- (1) C - mixture of conspecific pollen from multiple flowers to minimize a potential father effect
- (2) M1 – mixture of conspecific pollen and one type of heterospecific pollen
- (3) M3 – mixture of conspecific pollen and three types of heterospecific pollen
- (4) M5 – mixture of conspecific pollen and five types of heterospecific pollen

For M1, M3, and M5, we have chose heterospecific pollen donors that grow sympatrically and co-flower with *I. aggregata* and *L. lewisii*. We also purposefully chose plants with generalist morphologies in order to more accurately simulate what an

inconstant, generalist pollinator might deposit on the stigmas of target species. Table 1 provides specific details on treatment mixtures. We measured the average anther weight for each species and used that information to approximate stoichiometric relationships between each species. Using our stoichiometric relationships, we thoroughly mixed approximately equal weights of pollen were applied them to stigmas. Donor pollen was collected the same day as treatment from freshly dehisced anthers (< 1 d old). Recipient flowers received similar amounts of pollen across all treatments. Therefore, stigmas used in multiple species treatments received a lower proportion of conspecific pollen. After treatments were finished, we pollinated five stigmas of each species per treatment and counted the number of conspecific pollen grains to assess the actual amount of pollen stigmas were receiving.

I measured a component of female fitness, seed set and seed weight. Seed weight is an important factor to measure because the effect could be on seed quality rather than seed quantity (Neiland and Wilcock 1999). I used the donor plants from the same locations for all treatments to remove potential father effects.

Heterospecific Pollen Survey

One assumption of my hypotheses is that flowers evolve a specialized, more complicated morphology to avoid IPT through promoting constant pollinator behavior. If complex, specialist flowers actually receive comparable amounts of interspecific pollen transfer as simple, generalist flowers then it is illogical to assume that specialization occurs as a result of adaptation against deleterious effects of heterospecific pollen. To test this, we counted the number of heterospecific pollen grains and number of species on

three stigmas from 25 plants of naturally pollinated *I. aggregata* adjacent to the field site. We gathered similar data for *L. lewisii* from Kearns (1990).

Data Analysis

We used a randomized block ANOVA, treating each plant as a block. We took the average seed set of flowers of a treatment within each plant. We used *a priori* contrasts to test my hypotheses that female fitness are significantly ($\alpha = 0.05$) reduced in the presence of heterospecific pollen ($C > M1 > M3 > M5$). We applied a profile analysis to see if the relationship between heterospecific pollen and female fitness is similar between specialist and generalist plant species. All statistical analysis were done using R Statistical Software.

Hypothetical Results

Since no data has yet been analyzed, we have developed some hypothetical predictions. First, there may be no effect of treatments at all. However, if we see an effect, it will either be due to a reduction in pollen quantity or pollen quality. A reduction in pollen quantity can lead to (1) a linear decline in female fitness or (2) a decline in female fitness only after a certain threshold in the number of heterospecific pollen species have been applied (Fig 1a,b). We would interpret such results as an effect of occlusion or something else related to pollen dilution. If there is a reduction in pollen quality, due to allelopathy or other means, we would expect to a quadratic decline in female fitness, as only a few heterospecific pollen grains need be present (Fig. 1a,b). For any of the above results, the effect may be greater in *I. aggregata* (Fig. 1a), indicating that *I. aggregata* may have specialized to reduce IPT, while *L. lewisii* has developed other mechanisms than specialization to cope with IPT. If both plants react similarly (Fig. 1b), then we

would conclude that both have evolved or not evolved similarly against the effects of IPT.

Acknowledgements

I would like to thank my advisor George Aldridge for help with the entire process. Diane Campbell provided laboratory space. George Gilchrist and Jenny Reithel provided useful comments on the proposal. This project was funded through the NSF-REU program at the RMBL.

Literature Cited

- Alarcon, R. 2004. The structure of plant-pollinator interactions in montane meadow environments. PhD Dissertation, University of California, USA.
- Brown, B.J., and R.J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set in a native congener. *Oecologia* **129**:43-49.
- Brown, J.H., and A. Kodric-Brown. 1979. Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated wildflowers. *Ecology* **60**:263-272.
- Campbell, D.R., and A.F. Motten. 1985. The mechanism of competition for pollination between two forest herbs. *Ecology* **74**:1043-1051.
- Campbell, D.R., N.M. Waser, and M.V. Price, E.A. Lynch, and R.J. Mitchell. 1991. Components of phenotypic selection – pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution* **45**:1458-1467.
- Caruso, C.M., and M. Alfaro. 2000. Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. *Canadian Journal of Botany* **78**:600-606.
- Castellanos, M.C., P. Wilson, and J.D. Thomson. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**:2742-2752.
- Castellanos, M.C., P. Wilson, and J.D. Thomson. 2004. ‘Anti-bee’ and ‘pro-bird’ changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* **17**:876-885.

- Fenster, C.B., W.S. Armbruster, P. Wilson, M.R. Dudash, and J.D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* **35**:375-403.
- Galen, C. and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia*, **81**:120-123.
- Grant, V., and K.A. Grant. 1965. Flower pollination in the phlox family. Columbia University Press, New York, New York, USA.
- Kohn, J.R., and N.M. Waser. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *American Journal of Botany* **72**:1144-1148.
- Kwak, M.M., and O. Jennersten. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: heterospecific pollen and pollen limitation. *Oecologia* **86**:99-104.
- Mayfield, M.M., N.M. Waser, and M.V. Price. 2001. Exploring the 'most effective pollinator principle' with complex flowers: Bumblebees and *Ipomopsis aggregata*. *Annals of Botany* **88**:591-596.
- McLernon, S.M., S.D. Murphy, and L.W. Aarssen. 1996. Heterospecific pollen transfer between sympatric species in a midsuccessional old-field community. *American Journal of Botany* **83**:1168-1174.
- Murphy S.D., L.D. Aarssen. 1995a. Reduced seed set in *Elytrigia repens* caused by allelopathic pollen from *Phleum pratense*. *Canadian Journal of Botany* **73**:1417-1422.
- Murphy S.D., L.D. Aarssen. 1995b. Allelopathic pollen extract from *Phleum pratense* L. (Poaceae) reduces seed set in sympatric species. *International Journal of Plant Science* **156**:435-444.
- Murphy S.D., L.D. Aarssen. 1995c. Allelopathic pollen extract from *Phleum pratense* L. (Poaceae) reduces germination, in vitro, of pollen of sympatric species. *International Journal of Plant Science* **156**:425-434.
- Murphy S.D., L.D. Aarssen. 1995d. In vitro allelopathic effects of pollen from three *Hieracium* species (Asteraceae) and pollen transfer to sympatric Fabaceae. *American Journal of Botany* **82**:37-45.
- Neiland, M.R.M., and C.C. Wilcock. 1999. The presence of heterospecific pollen on stigmas of nectariferous and nectarless orchids and its consequences for their reproductive success. *Protoplasma* **208**:65-75.

- Rathcke, N.M. 1983. Competition and facilitation among plants for pollination. Pages 305-327 in L.A. Real, editor. Pollination biology. Academic Press, New York, New York, USA.
- Stebbins, G.L., 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: Pollination mechanisms. Annual Review of Ecology and Systematics **1**:307-326.
- Sukhada, K., and Jayachandra. 1980. Pollen allelopathy – a new phenomenon. New Phytologist **84**:739-746.
- Thomson, J.D., B.J. Andrews, and R.C. Plowright. 1981. The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). New Phytologist **90**:777-783.
- Waser, N.M. 1978. Interspecific pollen transfer and competition between co-occurring plant species. Oecologia **39**:107-121.
- Waser, N.M. 1983. The adaptive nature of floral traits: ideas and evidence. Pages 241-285 in L.A. Real, editor. Pollination biology. Academic Press, New York, New York, USA.
- Waser, N.M. 1986 Flower constancy: definition, cause, and measurement. The American Naturalist **127**:593-603.
- Waser, N.M., L. Chittka, M.V. Price, N.M. Williams, J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology **77**:1043-1060.
- Waser, N.M., and M.L. Fugate. 1986. Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. Oecologia **70**:573-577.

Figures and Tables

Table 1: Treatments and Pollen Donors

Treatment	Pollen Donors for <i>I. aggregata</i>	Pollen Donors for <i>L. lewisii</i>
C	<i>Ipomopsis aggregata</i>	<i>Linum lewisii</i>
M1	<i>Ipomopsis aggregata</i> <i>Linum lewisii</i>	<i>Linum lewisii</i> <i>Ipomopsis aggregata</i>
M3	<i>Ipomopsis aggregata</i> <i>Linum lewisii</i> <i>Frasera speciosa</i> <i>Aquilegia caerulea</i>	<i>Linum lewisii</i> <i>Ipomopsis aggregata</i> <i>Frasera speciosa</i> <i>Aquilegia caerulea</i>
M5	<i>Ipomopsis aggregata</i> <i>Linum lewisii</i> <i>Frasera speciosa</i> <i>Geranium richardsonii</i> <i>Aquilegia caerulea</i> <i>Potentilla pulcherrima</i>	<i>Linum lewisii</i> <i>Ipomopsis aggregata</i> <i>Frasera speciosa</i> <i>Geranium richardsonii</i> <i>Aquilegia caerulea</i> <i>Potentilla pulcherrima</i>

Fig 1: Hypothetical results for the effect of mixed pollen loads (“Treatments”) on *L. lewisii* (solid line; circles) and *I. aggregata* (dashed line; triangles). A. *L. lewisii* suffers no reduction in female fitness due to treatments, while *I. aggregata* is effected. B. Both *L. lewisii* *I. aggregata* show similar reactions to treatments.

