Pollen performance before and during the autotrophic–heterotrophic transition of pollen tube growth

Andrew G. Stephenson^{1*}, Steven E. Travers¹, Jorge I. Mena-Ali¹
and James A. Winsor²

¹Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA

²Department of Biology, The Pennsylvania State University, Altoona, PA 16601, USA

For species with bicellular pollen, the attrition of pollen tubes is often greatest where the style narrows at the transition between stigmatic tissue and the transmitting tissue of the style. In this region, the tubes switch from predominantly autotrophic to predominantly heterotrophic growth, the generative cell divides, the first callose plugs are produced, and, in species with RNase-type self-incompatibility (SI), incompatible tubes are arrested. We review the literature and present new findings concerning the genetic, environmental and stylar influences on the performance of pollen before and during the autotrophic–heterotrophic transition of pollen tube growth. We found that the ability of the paternal sporophyte to provision its pollen during development significantly influences pollen performance during the autotrophic growth phase. Consequently, under conditions of pollen competition, pollen selection during the autotrophic phase is acting on the phenotype of the paternal sporophyte. In a field experiment, using *Cucurbita pepo*, we found broad-sense heritable variation for herbivore–pathogen resistance, and that the most resistant families produced larger and better performing pollen when the paternal sporophytes were not protected by insecticides, indicating that selection during the autotrophic phase can act on traits that are not expressed by the microgametophyte. In a study of a weedy SI species, *Solanum carolinense*, we found that the ability of the styles to arrest self-pollen tubes at the autotrophic–heterotrophic transition changes with floral age and the presence of developing fruits. These findings have important implications for selection at the level of the microgametophyte and the evolution of mating systems of plants.

Keywords: gametophytic self-incompatibility; microgametophyte; pollen competition; pollen performance; pollen tube growth; selection

1. INTRODUCTION

Although floral traits, inflorescence architecture and flowering patterns influence the export and deposition of pollen within and among plants (e.g. Stephenson 1982; Wyatt 1982; Waser 1983; Bell 1985; Thomson & Thomson 1992; Barrett & Harder 1996), there is still considerable variation in the size and genetic composition of the pollen loads that are deposited onto their stigmas. Observations of pollinator foraging behaviour, experimental studies of pollen carryover and paternity analyses of the seeds within fruit all indicate that pollen loads frequently consist of the pollen from multiple donors and often include a mixture of self and cross pollen (e.g. Ellstrand 1984; Campbell 1998; Rocha & Aguilar 2001; Campbell *et al.* 2002). Pollen addition studies and direct counts of both the number of pollen grains deposited per pollinator visit and the total number of pollen grains deposited onto stigmas indicate that the size of pollen loads frequently

exceeds the number of grains that is necessary to fertilize all of the ovules (see Stephenson *et al.* 1995; Winsor *et al.* 2000). Consequently, the pollen from various donors is placed into a competitive circumstance of great evolutionary importance—the winners get their genes into the next generation while the losers do not. The outcome of this competition is influenced by the paternal sporophyte, the genotype of the microgametophyte, myriad interactions between the pollen and the pistil (maternal sporophyte), and possibly by interactions among microgametophytes within the pistil and perhaps by interactions between the microgametophyte and the megagametophyte (see Stephenson *et al.* 1992; Hormaza & Herrero 1994; Marshall *et al.* 1996; Higashiyama *et al.* 2001).

In species that shed bicellular pollen, the growth of pollen tubes is biphasic. An initial period of slow growth (the autotrophic phase) is followed by more rapid growth (the heterotrophic phase) through the TT of the style (see Herrero & Dickinson 1980; Mulcahy & Mulcahy 1982; Cruzan 1986; Lubliner & Singh-Cundy 2003). The initial growth phase is often referred to as the autotrophic phase because pollen will germinate and grow *in vitro* in rather simple media (Brewbaker & Kwack 1963; de Graaf *et al.*

*Author for correspondence (as4@psu.edu).

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2001) and because energy and nutrient storage products within the pollen grain are depleted (see Stephenson *et al.* 1992, 1994; Hormaza & Herrero 1994; Delph *et al.* 1997). For example, phytic acid, a phosphorus storage compound found in many types of pollen, is metabolized upon germination and initial tube growth and is eventually incorporated into cell walls and membranes (Jackson *et al.* 1982; Helsper *et al.* 1984; Dickenson & Lin 1986).

It should be noted, however, that the initial period of tube growth is not truly autotrophic as the pollen tubes depend minimally upon the pistil for water, cations and, perhaps, sugars (see Taylor & Hepler 1997; de Graaf *et al.* 2001). Moreover, pollen tubes do not grow as rapidly *in vitro* as *in vivo* (Read *et al.* 1993*a,b*). Recently, Holden *et al.* (2003) have shown that ethylene production by the pistil shortly after pollen germination in *Petunia* accelerates tube growth during the autotrophic phase but not the heterotrophic phase, indicating that the initial phase of tube growth is influenced by the physiology of the pistil.

The transition between autotrophic and heterotrophic tube growth coincides with important cellular events within the pollen tube (the division of the generative cell into two gametes and the initial deposition of callose plugs) and with the transition from growth in the stigmatic tissue to growth in the TT of the style (see Brewbaker & Majumder 1961; Singh *et al.* 1992; de Graaf *et al.* 2001, and references therein). Furthermore, the stigmatic tissue and the stylar TT are structurally, developmentally and biochemically distinct (Herrero & Dickinson 1979; Wolters-Arts *et al.* 1996; de Graaf *et al.* 2001). In many species, this transition coincides with a physical constriction of the area through which the tubes must grow (i.e. it is the neck of the funnel-shaped path from stigma to ovary) (see Hormaza & Herrero 1994). Finally, for many species, this transition zone coincides with the greatest attrition of compatible pollen tubes and, in species with RNase-based GSI, it coincides with the arrest of self-pollen tubes (see Cruzan 1986; Singh *et al.* 1992; Winsor & Stephenson 1995; Herrero & Hormaza 1996; Hormaza & Herrero 1996). During the heterotrophic growth phase, pollen tubes are thought to be nutritionally dependent upon resources supplied by the TT of the style (see Taylor & Hepler 1997; de Graaf *et al.* 2001).

We examine the roles of the initial (autotrophic) phase of pollen tube growth in determining which of the population of pollen grains deposited onto a stigma actually achieve fertilization. Specifically, we review the literature and present new findings that indicate that pollen performance during the autotrophic growth phase is strongly influenced by the ability of the paternal sporophyte to provision its pollen grains during development, and that selection on the microgametophyte during the autotrophic growth phase is based, at least in part, on the phenotype of the paternal sporophyte. Next, we review the literature and present new data showing that there is plasticity (environmental and genotype by environment) in the performance of self pollen at the transition between autotrophic and heterotrophic growth, even in species with intact, functional RNase-type GSI. SI systems, we argue, should be viewed as a quantitative trait and a subtle regulator of the breeding system.

2. ROLE OF THE PATERNAL SPOROPHYTE DURING THE AUTOTROPHIC PHASE

The publication of the pollen competition hypothesis of Mulcahy (1979) focused attention on the role of the haploid genome of the microgametophyte in determining the growth rates of pollen tubes (e.g. Tanksley *et al.* 1981; Willing & Mascarenhas 1984). In particular, much attention focused on the ecological and evolutionary implications of selection acting on genes expressed by the microgametophyte, the effects of microgametophyte selection on the resulting seeds and the use of microgametophyte selection in plant breeding programmes (see Stephenson *et al.* 1992; Hormaza & Herrero 1994; Sari Gorla *et al.* 1994). Far less attention, however, has focused on the ecological and evolutionary implications of the influence of the paternal sporophyte on pollen performance (see Delph *et al.* 1997).

Pollen is completely dependent upon the paternal sporophyte for all of the resources necessary to complete development. These resources (or their precursors) are supplied by the tapetum and include structural materials (lipids, proteins, carbohydrates), non-structural proteins that are incorporated into the pollen coat, and nutrient and energy storage products (see Heslop-Harrison 1968; Stephenson *et al.* 1992; Taylor & Hepler 1997; de Graaf *et al.* 2001). Consequently, it is reasonable to expect that the growing conditions during pollen development can affect the ability of the paternal sporophyte to supply resources to the developing pollen grains that, in turn, could affect the size, chemical composition and performance of the pollen that is produced.

Environmental conditions during pollen development, such as temperature stress and soil fertility, are known to affect the size and chemical composition of pollen (see Stephenson *et al.* 1994, 1998). For example, temperature stress during pollen development affects the concentration and proportion of lipids and starches stored in the mature pollen grains of petunia plants (Baker & Baker 1979). Several recent studies (summarized in table 1) have shown that *in vitro* pollen tube growth, *in vivo* growth and the competitive ability of pollen tubes (ability to sire seeds when mixtures of pollen grains are deposited simultaneously onto the stigmas of flowers) are influenced by general soil fertility, specific soil nutrients, mycorrhizal infection levels, the temperature during development and simulated herbivory (table 1). Although these studies have been performed with only a few taxa, it is apparent that environmental conditions that affect resource availability during pollen development influence pollen production, pollen volume and/or the concentration of stored reserves within the pollen grain, and pollen performance (*in vitro* and/or *in vivo*). Moreover, it is reasonable to expect that resource provisioning by the paternal sporophyte would have its greatest impact on germination and the autotrophic phase of pollen tube growth. It should be noted that pollen tube growth *in vitro* most closely resembles the autotrophic growth phase, in that the pollen tubes do not undergo the cellular changes associated with the transition to heterotrophic growth and *in vitro* tube growth stops when the resources stored within the pollen grain are depleted (see de Graaf *et al.* 2001).

Table 1. Summary of studies demonstrating paternal environmental effects on pollen development and performance. (Symbols: ↓, significant decrease; ↔, no significant change; NA, not available.)

environmental condition	pollen volume/ stored energy and nutrients	<i>in vitro</i> pollen growth	<i>in vivo</i> pollen growth	pollen production	species	reference
soil nutrients	↔	NA	↓	↓	wild radish	Young & Stanton (1990)
soil nutrients	NA	NA	↓	↓	<i>Clarkia unguiculata</i>	Travers (1999)
soil nitrogen	↓	NA	↓	↓	zucchini	Lau & Stephenson (1993)
soil phosphorus	↓	NA	↓	↓	zucchini	Lau & Stephenson (1994)
temperature stress	↓	↓	↓	NA	zucchini, wild gourds	Jóhansson & Stephenson (1998)
temperature stress	↓	NA	↓	NA	lily	van Herpen (1986)
herbivory simulated	↓	↓	↓	↓	wild gourd	Quesada <i>et al.</i> (1995), Avila-Sakar <i>et al.</i> (2003)
herbivory simulated	NA	↓	↓	NA	<i>Lobelia siphilitica</i>	Mutikainen & Delph (1996), Delph <i>et al.</i> (1997)
mycorrhizal infection			NA		zucchini	Lau <i>et al.</i> (1995)
mycorrhizal infection					tomato	Poulton <i>et al.</i> (2001)
inbreeding	↓	↓	NA	↓	<i>Campanula rapunculoides</i>	Good-Avila (2000)
inbreeding hybridization	↓	↓	↓	↓	wild gourd	Jóhansson <i>et al.</i> (1998)
					wild gourd	Stephenson <i>et al.</i> (2001)

The reasons for the positive relationship between *in vitro* and *in vivo* growth is, perhaps, less obvious considering that the heterotrophic growth phase covers most of the total distance over which the tubes must grow. Moreover, it is likely that the heterotrophic growth phase places greater metabolic demands on pollen because growth is more rapid relative to the autotrophic phase and the tubes are nutritionally dependent on the TT. However, physical constriction at the autotrophic–heterotrophic transition may place a premium on early arrival at the transition, especially when pollen loads are large. Even if the number of tubes is not limited by space at the autotrophic–heterotrophic transition, the first tubes into the TT of the style would have access to all of the nutritional resources of the style (e.g. Cheung *et al.* 1995; Wu *et al.* 1995; Hormaza & Herrero 1996). If the resources available in the intercellular matrix of the TT are insufficient to support all of the tubes from large pollen loads, the first tubes into the TT would enjoy a nutritional advantage over the later tubes to enter the TT. It is curious that in *Cucurbita pepo* the growth rate of the first tubes into the TT accelerates significantly more than the growth rate of the mass of tubes that enter behind them (Winsor & Stephenson 1995).

These studies indicate that, under conditions of pollen competition, the pollen produced by the healthiest, most nutrient-rich plants outperforms the pollen from damaged and nutrient-poor plants. That is, the performance of pollen is influenced by the phenotype of the paternal sporophyte (the pollen-producing parent). The evolutionary significance of such differences in pollen performance depends upon heritable genetic variation for differences that affect the provisioning of pollen. Most of the studies in table 1 were designed solely to examine environmental (not genetic) effects on pollen performance. For example, Lau & Stephenson (1994) grew two inbred cultivars of zucchini (*C. pepo* ssp. *pepo*) in an agricultural field with and without fertilizer containing phosphorus. They found that the pollen produced by plants growing in high-P soil was larger, contained a greater concentration of P and fertilized more ovules under competitive conditions than the same inbred cultivar growing in low-P soil. Similarly, the other studies that varied soil fertility or simulated herbivory compared the size and performance of the pollen from stressed plants with that from non-stressed plants. While these studies clearly demonstrate non-random fertilization (or differences in tube growth *in vitro*), the differences in pollen performance are due entirely to environmentally based plasticity in pollen provisioning. The paternal sporophytes growing in the more benign conditions sired the most seeds.

Two recent studies have shown that inbreeding adversely affects pollen production, pollen size and pollen performance *in vitro* and/or *in vivo* (see table 1) as well as plant vigour and fruit and seed production. Pollen size and performance is, therefore, a manifestation of inbreeding depression. Genetically, inbreeding increases homozygosity, thereby exposing deleterious recessives to selection while simultaneously decreasing the number of overdominant (heterotic) loci. That is, the differences in performance of the pollen from inbred and outbred plants are due to non-additive genetic variation in the sporophyte. Inbreeding leads to an increase in homozygosity in the sporophyte but it does not alter allele frequencies (i.e. it

would not adversely affect the genotypes of the microgametophytes). Briefly, when a large pollen load, consisting of a mixture of pollen from inbred and outbred plants, is deposited onto a stigma, the pollen from the inbred plants sires fewer seed. Such non-random fertilization (based on the phenotype of the paternal sporophyte), however, would result in no evolutionary change because the selection is based on non-additive genetic variation in the sporophyte.

In natural populations, plants may differ with regard to a variety of traits (such as resistance to herbivores and pathogens, efficiency of nutrient uptake and utilization, and water use efficiency) that may affect their ability to provision their developing pollen grains. Because no studies have explicitly examined natural variation in traits related to pollen resource provisioning, we conducted a study in which 300 wild gourd plants (*C. pepo* ssp. *texana*) from five families were grown at the Pennsylvania State University Agricultural Experiment Station at Rock Springs, PA, in the summer of 2002 in three 0.4 ha plots. Half of the plants from each family were hand-sprayed weekly with an insecticide to control for herbivores (primarily aphids and diabroticite beetles that chew 1–2 cm holes in the leaves; see Quesada *et al.* (1995)). We recorded daily staminate and pistillate flower production, total fruit production, seed number per fruit and mean seed mass. At intervals of two weeks, we determined pollen production per flower, examined *in vitro* pollen tube growth, assessed beetle herbivory and aphid population sizes, and screened for a variety of bacterial and viral diseases that are transmitted by the herbivores (MacNab *et al.* 1994).

We found that the sprayed plants suffered significantly less beetle herbivory, harboured smaller populations of aphids and showed fewer disease symptoms than the non-sprayed plants. Moreover, the sprayed plants had significantly greater reproductive output through the female (fruit and seed) function, more total staminate flowers, more and larger pollen grains per staminate flower, and greater *in vitro* pollen tube growth rates than the non-sprayed plants. More importantly, we found that the five families differed significantly in their resistance to herbivores and susceptibility to disease, indicating that there is a broad-sense heritable genetic basis to these traits. Most importantly, we found significant interactions between family and spray treatment for pollen size and *in vitro* pollen performance. The families that were most resistant to the herbivores (and thus also exposed to fewer diseases) produced the largest and fastest growing pollen in the non-sprayed treatment.

If *in vitro* pollen tube growth rates correspond to *in vivo* pollen performance, as in the other studies of the effects of growing conditions on pollen performance, then pollen loads consisting of a mixture of pollen from the five families would result in non-random fertilization by the pollen from the most herbivore/pathogen-resistant families. In this case, the ability of the paternal sporophyte to provision its pollen is associated with a heritable genetic trait: herbivore/pathogen resistance. Even though the genes affecting herbivore/pathogen resistance may not be expressed in the microgametophyte, the frequency of alleles influencing resistance would increase in the next generation because selection is operating on the pheno-

type of the paternal sporophyte (the ability to well-provision pollen grains in an environment with herbivores and pathogens). Previous studies of microgametophyte selection, which have focused on selection operating on genes expressed by the microgametophyte, have overlooked this possibility for evolutionary change (e.g. Mulcahy 1979; Stephenson *et al.* 1992; Hormaza & Herrero 1994; Sari Gorla *et al.* 1994). If herbivores and pathogens are a consistent component of the environment, the pollen from resistant plants will continue to enjoy a performance advantage until the genetic variation for this trait is eroded. If, however, herbivore and pathogen populations fluctuate from year to year within a population or among populations that occasionally exchange genes, then variation for this trait will be maintained if there is a cost to resistance in the absence of herbivores/pathogens (Schlichting *et al.* 1990; Schlichting & Pigliucci 1998; Delph *et al.* 1997).

Together these studies indicate that the ability of the paternal sporophyte to provision its pollen during development affects the performance of the pollen during the autotrophic phase of tube growth that, in turn, affects the probability that its pollen will achieve fertilization when competing against pollen from conspecifics. Under stressful environmental conditions, plants make fewer flowers and fewer and smaller (less well provisioned) pollen grains (table 1; Stephenson *et al.* 1994). Presumably, it would be necessary to further decrease flower production and the number of pollen grains per flower under stressful conditions if pollen volume (pollen resource concentration) did not vary. This, in turn, is likely to decrease the probability that a plant's pollen would be deposited onto stigmas of conspecifics (e.g. Devlin *et al.* 1992).

From the perspective of the maternal sporophyte, pollen competition during the autotrophic phase of pollen tube growth increases the probability that her seeds will be fertilized by the pollen of the most vigorous (healthiest, most nutrient-rich) plants. When differences in pollen provisioning are due to heritable genetic variation among the paternal sporophytes (such as differences in the efficiency of nutrient uptake and utilization or differences in herbivore and pathogen resistance), then the resulting offspring are more likely to also possess these traits. In this regard, selection during the autotrophic phase of pollen tube growth is not unlike a female elephant seal (or red deer, or bighorn sheep) choosing to mate with the most vigorous and healthy male who has fared well in competition with other males. The female elephant seal chooses her mate based on the phenotype of the male (see Trivers 1985; Alcock 2001). It should be noted, however, that pollen competition during the autotrophic growth phase also involves genes expressed by the microgametophyte. Genes expressed by the vegetative nucleus are responsible for the metabolism of the stored reserves, for the growth of the pollen tube, and for many of the necessary pollen–stigma and pollen–style interactions (e.g. Tanksley *et al.* 1981; Willing & Mascarenhas 1984; Taylor & Hepler 1997; de Graaf *et al.* 2001). Consequently, differences among microgametophytes in the competence or efficiency of these gene products are also exposed to selection. This is a component of selection during the autotrophic growth phase of pollen tubes that is lacking in animal sexual selec-

tion, including sperm competition in animals (Smith 1984; Birkhead & Møller 1998).

3. BEHAVIOUR OF SELF-POLLEN TUBES AT THE AUTOTROPHIC–HETEROTROPHIC TRANSITION

In addition to selection based on the phenotype of the paternal sporophyte, the autotrophic–heterotrophic growth transition is also associated with the arrest of self-pollen tubes in many species with T2-type RNase-based GSI (Brewbaker & Majumder 1961; Singh *et al.* 1992; de Nettancourt 1997; Igic & Kohn 2001). RNase-based GSI is a multi-allelic (termed *S*-alleles) SI system found in the Solanaceae, Scrophulariaceae and Rosaceae in which pollen tubes bearing an *S*-allele in common with the pistil are recognized and rejected by RNases in the pistil (see de Nettancourt 1997; McCormick 1998). By preventing selfing (and mating with some close relatives), plants with SI avoid the deleterious effects of inbreeding. However, when population sizes are small or when pollinators are scarce, seed production in plants with SI may be limited by the availability of cross pollen. This would be especially problematic for weedy species, which must frequently colonize new habitats. Not surprisingly, few weedy species have SI systems (Baker 1955).

Because SI is regulated by *S*-genes, plant scientists have tended to think of SI as a qualitative trait (i.e. a species is either SI or self-compatible) and a binary regulator of the breeding system (SI species are obligate outcrossers). However, plant breeders have long noted that SI is often a phenotypically plastic trait that varies with environmental and physiological conditions (such as temperature and floral age) and field biologists studying natural populations have found that many species exhibit variable expression in the strength of SI among individuals (see Lloyd & Schoen 1992; Levin 1996; Stephenson *et al.* 2000 for reviews). A few studies have shown that there is a heritable component to the strength of SI (see Levin 1996; Good-Avila & Stephenson 2002). These variations in the strength of SI have many names in the literature (partial SI, pseudo-self fertility, leaky SI) and probably represent a collection of related phenomena involving subtle pollen–pistil interactions that affect the relative growth rates of self and cross tubes (see Lloyd & Schoen 1992; Lush & Clarke 1997; Sage *et al.* 1999; Stephenson *et al.* 2000). A few studies have begun to explore the evolutionary implications of partial SI (e.g. Lloyd 1992; Levin 1996; Stephenson *et al.* 2000), but the molecular and cellular bases of partial SI are poorly understood.

Recently, we have begun to investigate the behaviour of self-pollen tubes at the autotrophic–heterotrophic growth transition using the weedy species *Solanum carolinense* (Solanaceae). As is typical for species with GSI in the Solanaceae (Anderson *et al.* 1986), the pistils of *S. carolinense* produce an abundant, highly polymorphic RNase that is developmentally regulated, 32 kDa in size and has a highly basic isoelectric point (figure 1). Moreover, these proteins are known to co-segregate with the *S*-phenotype (Lee *et al.* 1994). Richman *et al.* (1995) cloned and sequenced *S*-alleles from plants in two populations (from North Carolina and Tennessee) and estimated that each population contained 12–14 *S*-alleles. We have cloned and sequenced the *S*-alleles from 15 plants in a population of

Figure 1. Isoelectric focusing gel of protein extracts from four tissues in *Solanum carolinense*: L, leaf; EB, early bud; LB, late bud; OF, open flower. The leftmost lane is a standard ladder of proteins with known pI. *S*-RNase proteins have an isoelectric point above a pI of 8.8.

Figure 2. Box plots of the number of seeds produced per self-pollination per plant for each of 15 different genets of *Solanum carolinense*. The plots indicate the 95th, 75th and 50th centiles as well as the mean (thick bar) of each distribution. Asterisks denote genets used in further experiments on floral age (see text).

S. carolinense from Cumberland, MD, and we estimate that this population contains 12 alleles of which nine are also present in the more southern populations that Richman *et al.* (1995) studied. More importantly, a preliminary greenhouse study using rhizome cuttings (clonal replicates) of 15 plants from the Cumberland, MD, population revealed: (i) that most individuals produce at least a few fruits/mature seeds when all of the flowers produced by a plant are self-pollinated; (ii) that individuals (genotypes) differ significantly in the proportion of selfed flowers that produce mature seeds; and (iii) that plants were less likely to produce selfed fruits/seeds if some flowers were already outcrossed (figure 2).

To examine the behaviour of self pollen at the autotrophic–heterotrophic growth transition, we grew clonal replicates of five genotypes (genets 1, 2, 8, 11 and 15 from

our preliminary study) that represented a range of SI strengths. *Solanum carolinense* is a herbaceous perennial with indeterminate growth and reproduction. Inflorescences consist of 1–20 flowers that mature acropetally. Each flower lasts for 7.7 ± 0.35 days (mean $\pm$ s.e.) under greenhouse conditions and new flowers open on an inflorescence every 1–2 days. Consequently, large inflorescences have approximately five or six flowers open at any given time. We self-pollinated flowers on inflorescences with five open flowers. All flowers on an inflorescence were pollinated at the same time so that the age of the flowers at the time of pollination ranged from 1 day old (flower node 1) to 8 days old (flower node 5). We replicated the pollination sequence on five inflorescences on each of the five genets. In addition, one sequence of five flowers was cross-pollinated on each genet using a mixture of cross pollen. After 48 h, the styles from each flower were harvested, the pollen tubes were stained with aniline blue, and pollen tube growth was examined with a microscope under ultraviolet light (Martin 1959). The progress of pollen tubes through the style was determined by counting the number of tubes at five equidistant points through the style: 20%, 40%, 60%, 80% and 100% of the distance from the stigmatic surface to the base of the style.

As with many species (see Hormaza & Herrero 1994; Hormaza & Herrero 1996), we found that the attrition of cross pollen tubes was greatest where the pollen tubes from the stigma funnelled into the TT of the style (between sections 1 and 2). Most of the pollen tubes that reached section 2 (40% of the distance between the stigmatic surface and the base of the style) successfully traversed the entire style (table 2).

A mixed-effect model analysis of variance with genet (specified as random), flower node (floral age) and stylar section (distance from stigma to base of style) and the two-way interactions revealed that all of the main effects and the two-way interactions had a significant influence on the growth of self-pollen tubes (table 3). Moreover, the model accounts for almost 95% of the variance in pollen tube growth! In general, self-pollen tubes are arrested at the autotrophic–heterotrophic transition in young flowers but some tubes grow beyond this point in older flowers. In addition, rhizome cuttings from the genotypes that set the most self seed in a prior greenhouse study permitted the most self-pollen tubes to traverse the length of the style in this study (compare table 3 with figure 2). Briefly, the growth of self-pollen tubes past the autotrophic–heterotrophic transition is significantly influenced by the genotype of the plant, the age of the flower, and their interaction. Studies are currently underway to determine if the differences among genotypes are due to specific *S*-alleles (and closely linked modifiers) or whether they are due to unlinked modifiers of SI.

This study, using a species with fully functional, intact RNase-type GSI, joins a growing list of studies that show plasticity (environmental and genotype by environmental) in the strength of SI and that the growth of self-pollen tubes in the styles of SI plants is perhaps best viewed as a quantitative, rather than a qualitative, trait (Levin 1996; Lush & Clarke 1997; Sage *et al.* 1999; Stephenson *et al.* 2000; Good-Avila & Stephenson 2002, and references therein). Moreover, the relative growth rate of self-pollen tubes in SI species appears to be a continuum ranging

Table 2. Pollen tube counts for outcross pollinations. (The number of days a flower has been open at the time of pollination increases with increasing flower node (floral age) number. Stylar section indicates the distance travelled by pollen tubes through the style (1 is 20%, 2 is 40%, 3 is 60%, 4 is 80% and 5 is 100% of the entire style length).)

genet	stylar section	younger ← flower node → older		
		1	3	5
1	1	118	158	319
	2	35	111	51
	3	35	111	51
	4	35	111	45
	5	31	72	35
8	1	159	204	88
	2	79	41	88
	3	79	19	88
	4	79	8	17
	5	79	8	6
2	1	41	85	143
	2	21	50	63
	3	21	50	56
	4	14	50	56
	5	10	50	56
11	1	212	192	279
	2	114	99	155
	3	118	93	94
	4	102	92	92
	5	62	52	53
15	1	118	99	151
	2	45	21	55
	3	45	21	40
	4	45	21	45
	5	32	21	37

from cryptic SI (in which self-pollination results in complete seed set but when mixtures of self and cross pollen are deposited onto stigmas the resulting seeds are sired by the cross pollen) through partial SI (sometimes called pseudo-self fertility, in which genotypic and environmental effects on self fertility are pronounced) to complete SI. From this perspective, plasticity in the strength of SI can be seen as a reproductive trait that strongly promotes outcrossing when cross pollen is available but does not preclude at least some selfing when cross pollen is scarce. For a few species, it is known that the strength of SI is heritable, indicating that the nature of the interactions between self pollen and the SI system of the pistil can be the object of selection (see Lloyd 1992; Levin 1996; Good-Avila & Stephenson 2002).

4. CONCLUSIONS

This paper has examined the literature and presented new data on the performance of pollen during the autotrophic growth phase, (i) to determine which of the pollen grains deposited onto stigmas are most likely to fertilize ovules, and (ii) to identify the consequences of pollen selection during the autotrophic phase on the resulting offspring. We have found that the ability of the paternal sporophyte to provision its developing pollen strongly influences pollen performance both *in vitro* and *in vivo*. Consequently, when large loads of cross pollen are

Table 3. Pollen tube counts for self-pollinations.

(The number of days a flower has been open at the time of pollination increases with increasing flower node (floral age) number. Stylar section indicates the distance travelled by pollen tubes through the style (1 is 20%, 2 is 40%, 3 is 60%, 4 is 80% and 5 is 100% of the entire style length). Each mean and standard error represent tube counts from five replicate flowers.)

genet	stylar section	younger ← flower node → older				
		1	2	3	4	5
		mean ± s.e.	mean ± s.e.	mean ± s.e.	mean ± s.e.	mean ± s.e.
1	1	86.00 ± 17.1	173.50 ± 47.6	200.75 ± 42.7	209.00 ± 31.6	175.75 ± 76.0
	2	0.75 ± 0.5	14.25 ± 10.8	21.00 ± 6.3	34.25 ± 6.0	31.50 ± 3.5
	3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	2.75 ± 2.1	2.50 ± 0.9
	4	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	2.25 ± 2.2	0.00 ± 0.0
	5	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	1.00 ± 1.0	0.00 ± 0.0
8	1	42.40 ± 10.9	83.80 ± 20.0	116.40 ± 27.1	69.00 ± 16.8	132.80 ± 28.2
	2	6.40 ± 2.8	10.60 ± 5.4	45.00 ± 18.1	18.20 ± 9.0	27.80 ± 11.0
	3	1.80 ± 1.0	0.60 ± 0.6	4.00 ± 0.9	1.20 ± 0.6	1.80 ± 1.8
	4	0.20 ± 0.2	0.00 ± 0.0	0.40 ± 0.2	0.00 ± 0.0	0.00 ± 0.0
	5	0.20 ± 0.2	0.00 ± 0.0	0.40 ± 0.2	0.00 ± 0.0	0.00 ± 0.0
2	1	117.20 ± 43.8	178.00 ± 46.3	196.00 ± 21.2	182.60 ± 38.7	250.20 ± 32.3
	2	6.20 ± 3.5	10.00 ± 2.9	10.67 ± 4.7	22.80 ± 9.2	75.80 ± 27.8
	3	0.20 ± 0.2	1.60 ± 0.8	2.33 ± 1.2	2.60 ± 1.4	19.20 ± 10.0
	4	0.20 ± 0.2	1.60 ± 0.8	1.33 ± 0.9	1.20 ± 0.6	7.60 ± 6.4
	5	0.20 ± 0.2	1.40 ± 0.7	0.67 ± 0.7	1.00 ± 0.5	3.40 ± 2.7
11	1	163.20 ± 53.9	247.00 ± 43.5	245.00 ± 48.2	253.33 ± 50.8	259.60 ± 42.4
	2	23.20 ± 6.1	54.50 ± 22.4	94.33 ± 19.1	121.00 ± 55.0	150.80 ± 30.0
	3	2.00 ± 0.8	4.00 ± 2.0	10.67 ± 2.2	26.67 ± 15.2	48.00 ± 16.3
	4	0.00 ± 0.0	1.25 ± 0.9	0.67 ± 0.7	5.67 ± 3.7	12.20 ± 5.9
	5	0.00 ± 0.0	0.50 ± 0.5	0.33 ± 0.3	1.67 ± 0.3	2.00 ± 0.6
15	1	121.25 ± 12.8	109.20 ± 9.8	147.25 ± 10.4	205.40 ± 29.1	143.00 ± 34.1
	2	13.75 ± 12.4	34.60 ± 14.2	41.25 ± 26.4	69.40 ± 28.4	68.25 ± 28.8
	3	0.75 ± 0.5	3.00 ± 1.1	11.00 ± 7.8	8.00 ± 3.0	21.00 ± 10.1
	4	0.25 ± 0.2	1.80 ± 0.7	3.50 ± 1.4	1.20 ± 0.6	4.00 ± 2.0
	5	0.25 ± 0.2	1.40 ± 0.7	3.00 ± 1.1	0.40 ± 0.2	3.50 ± 1.8

deposited onto stigmas, pollen selection is acting on the phenotype of the paternal sporophyte. To the extent that the ability of the paternal sporophyte to provision its pollen is determined by heritable genetic variation, the offspring will benefit from selection during the autotrophic phase of tube growth. When selection is based on the phenotype of the paternal sporophyte, it can act on traits/genes that are not expressed by the microgametophyte—a point that has been under-appreciated in previous discussions of pollen competition (but see Delph *et al.* 1997). If pollen competitive abilities were solely influenced by the phenotype of the paternal sporophyte, pollen competition would be directly analogous to sexual selection in animals—but, of course, the analogy breaks down when gene expression by the microgametophyte is also considered because there is nothing similar to this in animals.

In contrast to the autotrophic stage, pollen tube growth during the heterotrophic stage is dependent upon gene expression in the microgametophyte and on interactions between the genotypes of the pollen and pistil. There is no better example of pollen–pistil interactions with important fitness consequences than GSI. We have reviewed the literature and presented new data showing that there is more plasticity in the growth of self-pollen tubes than has been traditionally appreciated. When cross pollen is avail-

able, the growth of self-pollen tubes is typically arrested at the autotrophic–heterotrophic growth transition, thus permitting plants to avoid the deleterious effects of inbreeding. However, when cross pollen is scarce (i.e. when old flowers have not been cross-pollinated and/or when many flowers have failed to set fruit due to lack of pollination), the growth of self-pollen tubes (and hence the strength of SI) becomes a quantitative trait that varies among individuals. In this context, plasticity in SI systems can be viewed as mechanisms that strongly promote outcrossing but permit some self-fertilization when cross pollen is unavailable by modulating the intensity with which it handicaps the growth of self pollen.

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Discussion

S. C. H. Barrett (*Department of Botany, University of Toronto, Toronto, Canada*). What evidence is there that the mating system of individuals with partial or leaky SI is any different from individuals that have strong SI? Moreover, is there any evidence that the selfed seed produced by

plants with partial SI actually establish in the field? My prediction is that under most conditions the influence of leaky SI will be minimal and that plants are largely outcrossed. Of course under some circumstances, plants with leaky SI may have formed the basis of new populations and this could lead to selection for SC. The self-compatible species of *Solanum* may have arisen in this way.

A. G. Stephenson. Yes, I agree that most plants with leaky incompatibility (SI) are probably largely outcrossing in natural populations. We have not measured outcrossing rates in plants with leaky SI or looked at whether selfed offspring survive to reproduction. However, we have found one plant with homozygosity at the *S*-locus, indicating that some selfs must survive.

R. J. Scott (*Department of Biology and Biochemistry, University of Bath, Bath, UK*). Is there any correlation between breeding system (SI versus SC) and style length? An expectation would be that selfing species would have short styles because competition is limited, and self-compatible species have longer styles to provide an opportunity for competition.

A. G. Stephenson. When one compares closely related taxa, the selfing species have significantly shorter styles than the outcrossing species. In fact, all of the floral parts are reduced, as is pollen production per flower. Although the data are more scanty, it seems to me that within self-compatible species, the more outcrossing populations (as determined from electrophoretic data) tend to have styles that are exerted and longer than the more inbreeding populations. The longer styles would increase the distance over which pollen competition occurs ... but it is not clear that style length has been selected for pollen competition or whether it has been selected primarily for decreased self-pollination.

GLOSSARY

- GSI: gametophytic self-incompatibility
- SC: self-compatibility
- SI: self-incompatibility
- TT: transmitting tissue