THESIS

INTERSPECIFIC REPRODUCTIVE BARRIERS IN THE TOMATO CLADE

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2011

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ABSTRACT

INTERSPECIFIC REPRODUCTIVE BARRIERS IN THE TOMATO CLADE

Interspecific Reproductive Barriers (IRBs) preserve species identity by preventing interspecific hybridization, an essential facet of the biological species concept. Wild tomato species (*Solanum* sect. *lycopersicum*) are useful for studying interspecific reproductive barriers. Within the tomato clade there are 13 closely related species possessing diverse mating systems and complex IRBs. IRBs can be divided into two types: those occurring before mating (premating barriers) and those operating after mating (postmating barriers). Premating barriers include a variety of floral morphological characters correlated with a diversity of mating systems. Postmating barriers can be subdivided into prezygotic, those acting after mating but before fertilization, and postzygotic, those acting after fertilization. In the tomato clade, regulation of pollen tube growth in pistils constitutes postmating prezygotic barriers that are known to be important for preventing hybridization. Unilateral incongruity/incompatibility (UI), which prevents hybridization in one direction of an interspecific cross by inhibiting pollen tube growth in the pistil, is common in the tomato clade. Postzygotic barriers are also important as genetic isolating mechanisms resulting in failure of fruit or viable seed production in cases where prezygotic barriers are absent.

In this study, I first examined the hypothesis of positive correlation between pollen grain size and style length among nine species in the tomato clade, because differences between species in pollen size and style length have been proposed to be a potentially

important isolating mechanism between species, since larger pollen grains (containing more stored nutrients) may be needed to traverse longer styles. However, I found no correlation between pollen grain size and style length in the tomato clade, and therefore did not find this to be a likely isolating mechanism among the species in this study. Second, I examined UI barriers between species of domesticated tomato (self-compatible, SC) and three wild red-fruited SC species as pollen donors onto pistils of eight green-fruited species. Pistils of (self-incompatible) SI green-fruited species rejected pollen from all SC red-fruited species. However, pollen rejection and/or pollen tube growth of the three wild SC red-fruited species varied in pistils of green fruited SC species and SC populations of SI species. Finally, three types of IRBs including stigma exsertion, UI, and postzygotic barriers were investigated in 10 sympatric pairs of wild species. In these sympatric pairs, prezygotic and postzygotic barriers were found to prevent interspecific hybridization. This research will help elucidate the nature of reproductive barriers in wild populations. Studies of IRBs in tomato, a major food crop, also have potential for understanding reproductive barriers as they pertain to agronomic improvement.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Bedinger, for her patience support and time spent mentoring me. I also want to thank my committee members who have supported me and were willing to share their knowledge and experience during the course of my studies. Finally, I thank to my family for their support.

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CHAPTER 1:

INTRODUCTION

Understanding the nature of interspecific reproductive barriers (IRB) among closely related taxa can provide insight into how new lineages arise, and how they are maintained as discrete biological units in the face of interspecific gene flow. Thus, identifying which isolating mechanisms currently exist between close relatives may tell us how species maintain their integrity when plants grow sympatrically (co-occur) in the wild.

Classification of Isolating Mechanisms

Numerous reproductive isolating mechanisms prevent or reduce hybridization and gene exchange between species. Isolating mechanisms have been classified into two broad categories: those that occur prior to mating and those that occur post-mating. Pre-mating barriers include geographic and ecological barriers (e.g., ecogeographic) that greatly reduce or prevent contact of two lineages, and thus reduce the opportunity for gene flow. In addition, behavioral and morphological traits reduce the probability of mating even when lineages co-occur (Grant 1981; Levin 1971).

In plants, premating barriers involve complex interactions between flowers and pollinators, because most plants rely on external pollen vectors such as generalist insect pollinators (Bertin and Peters 1992; Grant 1994). Premating isolation mechanisms include differences in sizes, colors, outlines and fragrances of flowers which influence on pollinator visits (Darwin 1876; Levin 1971). Pollinator specificity or floral constancy is advantageous for

plant species because it improves the reliability of pollination and prevents hybridization (Grant 1994).

Post-mating barriers can be classified into two broad categories: post-mating prezygotic and post-mating post-zygotic. Post-mating prezygotic barriers occur after mating but before fertilization. In plants, post-mating prezygotic barriers include pollen-pistil interactions. Incompatibility in pollen-pistil interactions is the central isolating barrier addressed in this study. In compatible crosses, when a pollen grain reaches the stigma of the female structure it germinates a pollen tube, which grows through the style and into the ovary (Cheung 1996). However, in incompatible crosses pollen tubes are prevented from reaching the ovary. In species with self-incompatibility (SI), self-pollen is rejected, which is thought to be a mechanism to prevent inbreeding and promote outcrossing (Levin 1971; Grant 1981; Hogeboom 1984). Plant species also have mechanisms to limit fertilization by distantly related lineages, which I will refer to as post-mating interspecific reproductive barriers. Interspecific Reproductive Barriers (IRBs) help to maintain species integrity by preventing hybridization, which is critically important to the biological species concept (Lewis and Crowe 1958). In some species, the post-mating mechanism that prevents self-fertilization appears to be related to the mechanism that prevents fertilization by distant relatives. For example, some self-compatible female species will hybridize with related self-incompatible pollen species while the reciprocal cross inhibits pollen tube growth in the style. This phenomenon is known as unilateral incompatibility/incongruity (UI) (Levin 1971; Lewis and Crowe 1958; Martin 1964; de Nettancourt 1978; Hogenboom 1975).

Postzygotic barriers which are also important isolating mechanisms come in two types; intrinsic, independent of environment, and extrinsic, environmentally dependent (Coyne

1992). Intrinsic postzygotic isolation includes hybrid inviability and sterility. Hybrid inviability decreases hybrid survival rates (e.g. embryo dies before birth), while hybrid sterility results in hybrid progeny that fail to produce viable gametes (Coyne 1992; Coyne and Orr 2004). Conversely, extrinsic postzygotic isolation, includes ecological and behavioral sterility, and arises whenever hybrid progeny experience lower environmental fitness because they express an intermediate phenotype which is not well suited for either parental environment (Coyne and Orr 2004).

Here I examined IRBs in wild tomato species (*Solanum* Sect. *Lycopersicon*).

The tomato clade

Wild tomato species (*Solanum* sec. *Lycopersicum*) are useful for studies of Interspecific Reproductive Barriers (IRBs) (Bedinger et al. 2010). Wild tomatoes display significant differences in morphology, mating systems, and habitat preferences. There are 12 wild species related to the domesticated tomato according to recent taxonomic studies (Fig. 1.2; Peralta et al. 2008; Rodriguez et al. 2009). These wild species are endemic to South America and range from central Ecuador through Peru to northern Chile on the western Andean Slope (Fig. 1.1 and 2; Rick 1973; Peralta et al. 2008; Peralta and Spooner 2005; Moyle 2008; Darwin et al. 2003).

All of species of *Solanum* have the same chromosome number and are diploid (2n=24). There are no major differences in chromosome structure among the wild tomato species, and they share a high degree of genomic synteny (Chetelat and Ji 2007), although some chromosomes have been detected structural changes such as mismatched kinetochores or inversion loops in F_1 hybrids (Anderson et al. 2010). In addition to its diploid genome,

there are many genetic resources that make the tomato clade a good study system. These include genomic resources, extensive collections of wild species, collections of expressed sequenced tags, and mutants (Moyle 2008; Bedinger et al. 2010).

Species in the tomato clade exhibit three types of mating systems. Autogamous self-compatible species that accept self-pollen include *S. lycopersicum*, *S. galapagense*, *S. cheesmaniae*, *S. pimpinellifolium*, and *S. neorickii*. Facultative self-compatible species such as *S. chmielewskii* self fertile but has floral morphology characters to promote outcrossing. Allogamous self-incompatible species reject self-pollen, which forces outcrossing. Species that are mostly SI but have some SC populations include *S. arcanum*, *S. habrochaites*, and *S. pennellii* (Rick et al. 1978; Peralta and Spooner, 2005; Moyle 2008; Bedinger 2010). These mating systems are correlated with floral morphology characters. Self-compatible species in the tomato clade, including the domesticated tomato, *S. lycopersicum* have smaller flower size and less stigma exsertion. In contrast, self-incompatible species have larger flower size and longer exserted stigmas both of which promote outcrossing.

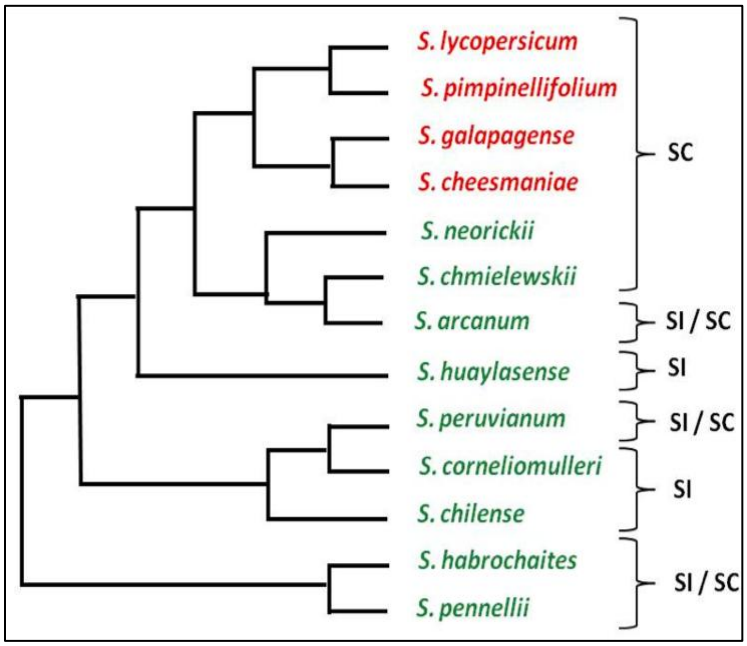


Figure 1.1 Phylogenetic tree of tomato species. Red colored species: red-fruited species, green colored species: green-fruited species. SC= self-compatible, SI=self-incompatible, SI/SC= both mating system exhibit (modified from Bedinger et al. 2010).

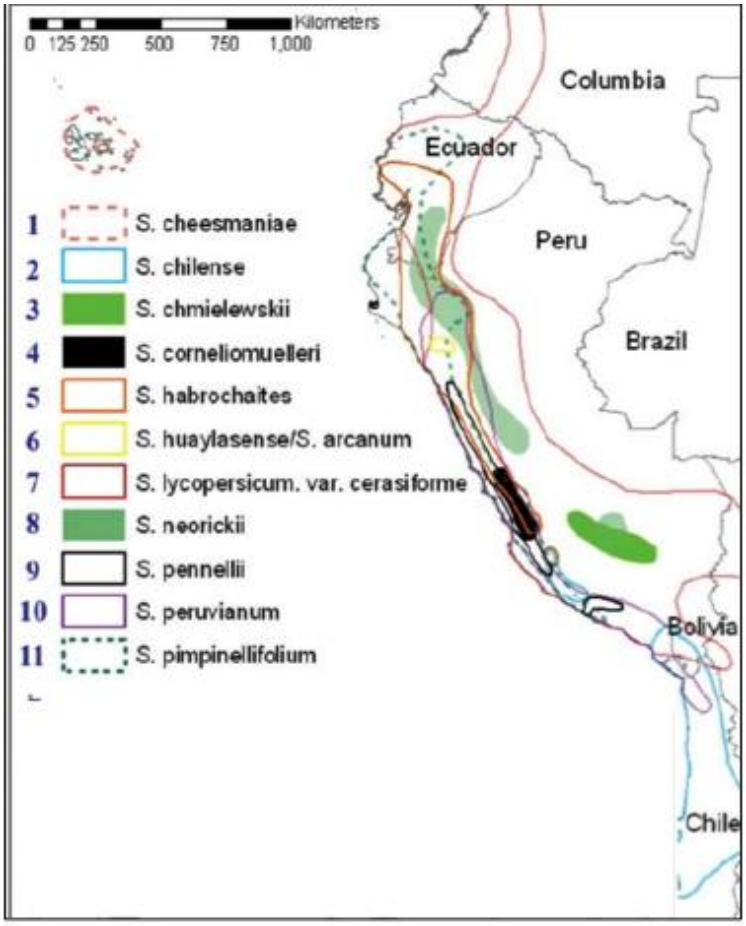


Figure 1.2 Wild tomato species geographical distribution in South America. Inset: Environmental variation of wetness based on distribution of populations' location. (from Moyle, 2008)

Reproductive barrier mechanisms in *Solanum* sect Lycopersicon

Premating: *Floral structure in the tomato clade*

Outcrossing is an important factor to preserve genetic variability in sexually reproducing populations (Barrett 2002). Cross-pollination (allogamy) can be ensured by SI. Variation in flower morphologies can be associated with variation in mating system (Peralta and Spooner 2005). The placement of the female stigma, either beyond (exserted) or below the anther cone (inserted), is one such polymorphism associated with mating system changes. Evolutionarily, changes in flower morphology including reduction in flower size and more inserted stigma placement represent a trend from SI to self-compatibility (SC). Both of these changes make self-pollination (autogamy) more likely than cross-pollination (allogamy), since smaller flowers attract fewer pollinators and inserted stigmas receive less non-self-pollen (Rick et al. 1978; Peralta and Spooner 2005; Georgiady 2002; Chen et al. 2007).

Stigma exsertion is quantitatively inherited and controlled by a few genes. Several quantitative trait locus (QTL) mapping studies for stigma exsertion in tomato have been reported. Chen et al. (2004) mapped the genes associated with stigma exsertion using introgression lines between the wild SI species *S. pennellii* and cultivated tomato (SC). The authors identified a single QTL on chromosome 2, *stigma exsertion 2.1* or *se2.1,* in the same region of five loci important for stigma exsertion in autogamous flowers. Of the five tightly linked loci, one controls style length, three control stamen length, and one affects anther dehiscence. The locus controlling style length (*Style 2.1*) has the greatest impact on stigma exsertion (Chen et al. 2004). It is likely that mutations at this locus have contributed to the evolution from allogamy to autogamy the red-fruited tomato species including the domesticated tomato. A striking example of this is *S. pimpinellifolium,* an SC species with a

varying degree of outcrossing success that is dependent on floral morphology (Rick 1978; Georgiady 2002; Chen et al. 2007).

Intraspecific Postmating-prezygotic barriers: Self-Incompatibility in the tomato clade

Self-incompatibility (SI), a genetically controlled character in which female pistils can recognize and stop self-pollen tube growth, is a widespread intraspecific reproductive barrier in angiosperms. SI prevents self-fertilization and promotes outcrossing with genetically different individuals of the same species (de Nettancourt 1997; Mau et al. 1991).

SI is well studied at the molecular level. There are two systems of self-incompatibility, sporophytic and gametophytic, which evolved independently. Both types of SI have male and female recognition proteins that are encoded at single multiallelic locus known as the *S-locus.* In the sporophytic system found in at least 10 plant families, the diploid *S* genotype of pollen parent plants (Igic et al. 2008) and the diploid *S*-genotype of the pistil determine whether pollen will be rejected as "self" or accepted as "non-self." The gametophytic system is found in more than 60 plant families. In this system, the haploid pollen *S*-genotype and the diploid pistil S-genotype determine whether pollen will be accepted or rejected (Hua 2008).

Gametophytic SI (GSI) is found in the Solanaceae and is one of the best-understood pollen rejection mechanisms (de Nattancourt 1997; McClure 1989; Zhang et al. 2009). GSI is controlled by the polymorphic *S*-locus. Pollen rejection occurs when the haploid *S*-allele of pollen tube matches with either of S-alleles in the diploid style. In the style, the products of the *S*-locus are secreted stylar specific ribonucleases, called S-RNases. RNase activity is necessary to reject pollen tubes because the RNases act as *S*-allele-specific cytotoxins that

degrade RNA of pollen tubes in the SI reaction (Kao and Tsukamoto 2004; Qiao et al 2004; McClure 2004).

F-box proteins encoded at the *S*-locus (SLF) have been identified as the male determinant (Lai et al. 2002; Kubo 2010). Most F-box proteins are involved in ubiquitin-mediated protein degradation as components of a type of E3 ubiquitin ligase complex, named SCF (for Skp1, Cullin, F-box) responsible for transfering E1 uniquitin-activiating enzyme and E2 unbiquitin-conjugating enzyme to target a protein for degradation. It is thought that SLF proteins recognize and interact with non-self S-RNases and mediate their degradation (Entani 2003; Lai, et al. 2002; Entani et al. 2003; Sijacic et al. 2004)

Other non-*S* factors are also required for SI. McClure *et al*. (1999) identified the HT protein, which is a pistil specific protein, as an SIß factor. HT proteins are small, roughly 100 amino acid residues, asparagine-rich proteins. They are expressed late in style development and are likely secreted into the transmitting tract of the style (McClure *et al*. 1999). Another study in *Solanum. chacoense* (O'Brien 2002) found two gene paralogs, *HT-A* and *HT-B.* Mapping experiments of *HT*-A and *HT*-B showed that they are tightly linked from 1.57 kb apart in *S. lycopersicum* to 4.5 kb apart in *S. habrochaites* and are located on chromosome 12 (Covey et al. 2010)

Kondo *et al*. (2002) examined domesticated tomato, *S. lycopersicum* (SC), and discovered point mutations in the open reading frames (ORF) of *HT-A* and *HT-B*, rendering the genes nonfunctional. All SC species were shown to have low HT-B expression in the style, implying that HT-B genes are more important in the SI reaction (Kondo *et al*. 2002). However, Covey *et al.* (2010) found null mutations in *HT-B* in all *S. habrochaites*

populations, SC and SI. It implies that *HT-B* is not required for SI responses in this species, and it still remains a possibility that *HT-A* might be important in SI (Covey et al. 2010).

Interspecific Postmating prezygotic barriers: Unilateral Incompatibility or incongruity

Intraspecific reproductive barriers e.g. SI forces outcrossing and helps to maintain genetic diversity within a species, while interspecific barriers help prevent hybridization between species (de Nattancourt 1997). One kind of IRB, called unilateral incompatibility or incongruity (UI), is present in *Solanaceae* (Mutschler and Liedl 1994). In this study, UI refers to the case when successful pollen tube growth in crosses between two species occurs only in one direction of a cross.

It has been thought that SI and UI might be related because when UI is observed in crosses between SI and SC species, UI often follows the 'SI X SC' rule (Lewis and Crowe 1958; Martin 1967; Hogenboom 1973). For example, the wild tomato *S. pennellii* (SI) rejects pollen from domesticated tomato (SC), while domesticated tomato accepts pollen from *S. pennellii* (Liedl 1996). Further support for an overlap of mechanisms is that a UI QTL was mapped to the *S*-locus in *S. habrochaites* (Bernacchi and Tanksley 1997).

Two modes of UI pollen rejection, early and late, have been observed in crosses between S. *lycopersicum* and wild tomato species (Covey et al. 2010). Liedl *et. al* (1996) observed that UI pollen rejection occurs in the upper part of the style in both SI and SC populations in *S. pennellii* when crossed by *S. lycopersicum* pollen. Covey *et. al* (2010) tested timing of *S. lycopersicum* pollen rejection by SI and SC species of wild tomato. SI accessions of *S. habrochaites* shows early pollen rejection, about 10-14% of the style, while

late pollen rejection occurs 63~74% of the way down the style in crosses using the northern SC accessions of *S. habrochaites* and *S. chmielewskii* as female.

Genetic mechanisms that prevent interspecific hybridization are less well understood than SI. The role of S-RNase expression is not required for either the early or late mode of UI pollen rejection. SI accessions of *S. habrochaites* and *S. pennellii* show high level of S-RNase, while SC accessions of both species, able to reject interspecific pollen, have low level of S-RNase activity (Covey et al. 2010). This suggests that there may be non *S*-RNase UI mechanisms in the tomato clade.

HT-A and *HT-B* have been associated with UI mechanisms. As mentioned before, null mutations of *HT-A* and *HT-B* were found in *S. lycopersicum* (cultivated tomato) which cannot reject self or interspecific pollen. *HT-B* is mutated in all *S. habrochaites* accessions, SI and SC, while *HT-A* genes were detected and expressed in all species of *S. habrochaites*. *HT-A* and *HT-B* mapped to a UI QTL on chromosome 12, *ui12.1* QTL (Bernacchi and Tanksley 1997; Covey et al. 2010). The mapping of these genes to a UI QTL suggests that *HT* genes may be involved in the UI mechanism. It implies that *HT-A* might function in both UI and SI.

Postzygotic barriers

Species that do not have functional premating barriers or postmating prezygotic barriers still have a chance for hybridization to occur between species. In these cases, postzygotic barriers can to contribute to preventing hybridization, especially in sympatric species (Bedinger 2010). Postzygotic barriers have been used to map chromosomal regions in the tomato clade. For example, about 10 QTL were detected for pollen sterility and 4 QTL

for failure of hybrid seeds when introgression lines of *S. pennellii* were assessed for these traits (Moyle and Nakazato 2009).

My thesis includes three chapters on how interspecific reproductive barriers in wild tomato species prevent hybridization.

Chapter 2: Do pollen grain size play a role in reproductive barriers

Correlation between pollen grain size and style length has been proposed by Delpino (1867), Torres (2000) and Aguilar (2002) based on pollen grain provisioning; i.e. pollen grains of different sizes contain sufficient nutrients to grow through respective styles of different lengths. However, Darwin rejected the hypothesis because he observed species with a single size of pollen grain but variable style length. In Chapter 2, I examine a correlation between pollen grain size and style length in wild tomato species.

Chapter 3: Assessment of postmating prezygotic reproductive barriers in the tomato clade

In Chapter 3, I address how prevalent prezygotic barriers are in interspecific crosses in wild tomatoes by analyzing pollen tube growth. As mentioned before, most studies of prezygotic UI barriers have used the cultivated tomato but this species is not found in natural populations. I examined pollen tube growth in crosses using all members of the tomato clade with pollen from domesticated tomato and wild red-fruited species.

Chapter 4: Reproductive barriers between sympatric populations in the tomato clade

In Chapter 4, I examine interspecific barriers in 10 sympatric pairs of wild tomato species to investigate how they maintain their species integrity in the wild. I examine these features: premating barrier (exserted stigma length), postmating prezygotic barriers (pollen-

pistil interactions) and postzygotic barriers. Since there are SC and SI species in several sympatric pairs, I compare stigma exsertion between SI and SC. Also, I examine pollen-pistil interactions to see whether or not UI barriers act in between sympatric pairs. In cases where pollen rejection is not seen, I assess fruit development and seed set to see whether postzygotic barriers act.

CHAPTER 2:

DO POLLEN GRAIN SIZE PLAY A ROLE IN REPRODUCTIVE BARRIERS

Introduction

In higher plants, pollen tubes transport sperm cells from pollen grains on the stigma surface to the ovule by growing through the style of the pistil. Mature pollen contains essential nutrients such as carbohydrates, lipids, enzymes, membranes, and amino acids that can be utilized for pollen tube growth through styles. However, it is thought that pollen grains do not contain sufficient nutrients for their entire journey, and that pollen tubes may absorb nutrients such as polysaccharides and amino acids from the style as they grow (Vasil 1974). Pollen grain size varies widely in plants and determines the amount of resources in the pollen grains (Baker and Baker 1979).

Amici (1830) first observed that pollen tubes grew through the transmitting tissue of the style into the ovary. He also reasoned that pollen tubes obtain resources from the transmission tissue of the style, because pollen grains do not contain enough nourishment to support their growth along the entire style. However, Delpino (1867) proposed that pollen grains need to contain sufficient nutrients to sustain pollen tube growth through pistils. Thus, he suggested that larger pollen grains would be found within species with longer styles.

Darwin (1884) rejected Delpino's suggestion because there were many exceptions to Delpino's tenet, especially in heterostyle species, which produce a single size of pollen grain that can traverse variable style lengths. For example, in heterostylus *Linum*, pistils of the two

stylar forms vary in length by two-fold but the pollen produced in each form is the same size. Darwin suggested that nutrients stored in pollen grains support early pollen tube growth through the style, then the transmitting tissue of pistil provide nourishment to pollen tubes for further growth. He therefore suggested that there should be a positive relationship between stigma depth and pollen grain size.

More recently, Plitmann and Levin (1983) proposed the idea of differential "pollen provisioning" wherein pollen size limits pollen tube growth in pistils. The hypothesis, like Delpino's, predicts that species with longer style length should have larger pollen grains than species with shorter styles. Based on this assumption, there should be a positive correlation between pollen grain size and style length.

If pollen size limits the extent of pollen growth, style length can act as a reproductive barrier between species. Buchholz et al. (1935) found that the short-styled of *Datura* species yielded the largest number of hybrids in interspecific pollinations between ten different species. A similar pattern was found in *Nicotiana* section *Alatae* (Lee et al. 2008). This result can be explained if pollen from a short styled species cannot reach the ovule in long style species.

The objective in this chapter is to assess whether there is a relationship between pollen size and style length among species in the tomato clade Solanum, and if so, if this could contribute to reproductive isolation among species with styles that differ in length.

Materials and methods

We determined the relationship between pollen grain size and style length in nine wild tomato species; *S. lycopersicum* cultivars VF36 and M82, *S. pimpinellifolium* (self-

compatible (SC), accessions LA3798, LA1610, LA2149, LA3798), *S. neorickii* (SC, accession LA4023), *S. arcanum* (self-incompatible (SI) accession LA2150, and SC, accession LA2157), *S. peruvianum* (SI, accession LA3799), *S. corneliomullei* (SI, accession LA1609), *S. chilense* (SI, accession LA2884), *S. habrochaites* (SI accessions LA1777 and LA1353, SC accession LA0407), and *S. pennellii* (SI accessions LA1340 and LA2560, SC accessions LA0716).

Mature freshly opened flowers (stage=1) were collected and buzzed with an electric tooth polisher to collect pollen grains into centrifuge tubes. Pollen was transferred to microscope slides and 5µl of pollen germination medium (40% polyethylene glycol 4000, 0.1% Boric Acid, 40% Sucrose, 0.5M HEPES buffer pH6.0, 0.1M $Ca(NO_3)_2 \bullet 4H_20$, 2% $MgSO_4 \bullet 7H_20$, 0.1M KNO_3, H_2O), was dropped onto the pollen grains. After placing a cover slip on the slides, images were immediately collected using a Leica DM5500 B microscope (Leica Microsystems, http://www.leica.com/) with IPlab version 4 software (BD biosciences, http://www.bdbiosciences.com/home.jsp) coupled with a Hamamastu C4742095 camera (http://www.hamamastu.com). Images were captured at 40x magnification using a BF filter. For pollen volume measurements, the diameter of 50 pollen grains was measured using Image J 1.33 (http://rsb.info.nih.giv/ij//). Using this measure, we calculated the mean volume ($^4/_3\pi r^3$for) of 50 pollen grains for each accession of each species.

For style length measurements, 15 flowers of each accession at anthesis were collected and emasculated on one side. Images were taken using a Nikon SMZ1500 (http://www.nikon.com/) dissecting microscope with Image_pro_Plus software (http://www.mediacy.com/index.aspx?page=IPP) coupled with a Nikon Digital camera DMX1200 (http://www.microscopyu.com/). Style lengths were measured from the top of the

stigmatic area to the base of style (not including ovary area) using Image J 1.33. In order to determine whether or not pollen size correlates with style length in section Lycopersicon, we calculated a mean for pollen and style length for each accessions of each species. We then looked for a significant correlation between style length and pollen size among the nine species using the program of Pearson correlation coefficients in *Statistical Analysis System* (SAS) Version. 92. (http://www.sas.com/technologies/analytics/statistics/).

Results

Pollen grain volume varied from 4419.06 μm^3 in *S. arcanum* accession LA2157 (SC) to 13388.27μm^3 in *S. pennellii* accession LA1340 (Fig. 2.3 a), which is an almost a three-fold difference. Previously reported data on pollen size in the tomato clade is consistent with our findings. Garcia (2007) determined pollen volume in 11 wild tomato species (*Solanum* Sect. *Lycopersicon*) and two close relatives (*S. lycopersicoides* and *S. sitiens*) to assess its correlation with pollen grain starch content. These studies showed found no correlation between starch content and pollen grain size. Among wild tomato species, they found the smallest pollen size in *S. arcanum* followed by *S. neorickii*, and the largest pollen size in *S. pennellii*, consistent with our results. Chetelat et al. (2009) examined pollen grain size and other reproductive traits in six different wild tomato species as well as related species. Pollen grain sizes of each species from this study are also consistent with our measurements. For example, the diameter of pollen grain in *S. peruvianum* is about 21.9 µm in both studies. There was some minor variation in some measurements, for example, the diameter of pollen

grain radius in accession LA0716 of *S. pennellii* is about 29.1 µm in the Chetelat study, and about 28.3 µm per our data.

Style length ranged from 5.35 mm in *S. neorickii* to 11.76 mm in *S. habrochaites* accession LA1353. Therefore the longest style length was more than two times longer than the shortest style (Fig. 2.1). Among nine species, we did not find a positive correlation between style length and pollen volume. For example *S. habrochaites* styles are the longest, but this species had smallest pollen grain size. As shown Fig 2.2, we did not find a significant correlation between style length and pollen grain volume among the 9 species examined ($r = -0.0954$; $p=0.7158$; $n=17$) There also was no correlation between pollen grain diameter and style length ($r = -0.148$; $p=0.5705$, $n=17$).

We qualitatively examined the relationship between style length and pollen grain size within a species. Because we used three accessions each of *S. pimpinellifolium, S. habrochaites,* and *S. pennellii*, we were able to compare pollen volumes and style lengths within these three species (Fig. 2.1) although statistical analysis is not meaningful due to sample size. In *S. pimpinellifolium*, LA1589 has the longest style and the largest pollen grain, whereas LA3798 has the shortest style and the second largest pollen grain. In *S. habrochaites,* the style of LA1353 is the longest in the species, but has the smallest pollen grain size. In *S. pennellii*, LA1340 has the longest style and the second largest pollen grain size while LA0716 has the shortest style and the largest pollen grain size. Thus, we found that the predicted relationship between pollen grain size and style length was also not present within species.

Discussion

We used 9 species within the tomato clade to test Delpino's hypothesis that species with longer styles would have larger pollen grains. Delpino (1867) proposed that pollen grains store sufficient nutrients to grow through their respective styles. If this were the case, species with longer styles would have to store more nutrients in their pollen, which suggests a positive correlation between pollen grain size and style length (Delpino 1867). This correlation between pollen grain size and style length has been found in several plant families including the Asteracea (Torress 2000), the Polemoniaceae (Plitmann and Levin 1983), starchy pollen species of the Argentinian Nyctaginaceae (Lopez et al. 2006), the Orobanchaceae (Yang and Guo 2004), the Onagraceae (Baker and Baker 1979), *Brassica rapa L*. (Sarkissian and Harder 2001), and the Actinidiaceae (Gonzalez 1999). Aguilar (2002) evaluated an association between pollen grain volume and pistil length in tribe-*Lycieae* (subfam. Solanoideae) and found a strong positive correlation between pollen grain size and pistil length. Three variables were measured in this study: style length, pollen volume, and pollen diameter. Our data show no correlation either between style length and pollen volume or style length and pollen diameter among nine species. Therefore, our study does not support the predictions of Delphino's hypotheses.

Other factors may explain differences in pollen grain size between closely related species. For example, variation in genome size may be associated with variation in pollen size (Bennett 1972). A study comparing DNA content among populations of *Armeria maritime* found that variation in pollen size variation was due to differences in DNA content (Vekemans et al. 1996). For species in the tomato clade, we used DNA content data from three studies (Bennett and Smith 1976; Arumuganatha and Earle 1991; Stack personal

communication) to determine whether DNA content was related to pollen grain size. However, in our study, DNA content is unlikely to play a role. For example, the nuclear DNA content in *S. pimpinellifolium*, with intermediate pollen grain size, is 0.85pg/1C, which is a smaller than the amount of DNA found in *S. habrochaites* (0.93pg/1C), which has the smallest pollen grain size. Also, *S. peruvianum* has a smaller pollen grain size than some populations of *S. pimpinellifolium*, but a larger DNA content (1.135pg/1C). In another study, Stack (personal communication) found that genome sizes from the same accessions used in our study of pollen grain size and style length; e.g. LA2157 *S. arcanum* (SC) had a larger DNA content (1.24pg/1C) than LA1589 *S. pimpinellifolium* (1.145pg/1C) which has a larger pollen grain size, as shown Table 1. Therefore, there is not a correlation between pollen grain size and the DNA content in the tomato clade. Genome size varies from 0.85pg/1C in *S. pimpinellifolium* to 1.23pg/1C in *S. pennelli*, a 45% difference (Bennett and Smith, 1976; Arumuganathan and Earle, 1991).There were differences in measurements of genome sizes among Bennett and Smith (1976), Arumuganathan and Earle (1991) and Stack (personal communication) because the estimates were done in different labs using different standards. Determining absolute amounts is problematic, but relative genome sizes are most useful when taken from a single lab that analyzed most all of the samples. For species in the tomato clade, we compared pollen grain sizes with DNA content measured in three different studies.

Darwin's observation that heterostyled species produce styles of different lengths but produced pollen grains of equal size led him to propose that pollen grains obtain resources from female tissue. He postulated that pollen tubes initially utilize storage substances within the pollen grain for autotrophic growth through the stigma to reach the stylar transmitting tissue. At this point pollen tubes begin to grow heterotrophically by using pistil-derived

nutrients to support their growth to the ovary. Therefore, he proposed a positive correlation between pollen grain size and stigma depth (Darwin 1884). More recent studies have shown that to date, there are no pollen size-style length correlations observed in the legume tribe Trifolieae (Small 1988), the Umbelliferae, the Brassicaceae (Cruden and Lyon 1985). Cruden and Lyon (1985) also found no correlation between pollen grain size and pistil length in six species of *Solanum*; *S. dulacamara* L., *S. nigrum* L., *S. psudo-capsicum* L., *S.* sp., *S. carolinense* L., *S. crinitum* Lam, but found a significant positive correlation between stigma depth and pollen grain volume. Cruden (2009) examined pollen grain size, style length, and stigma depth to see if there was any correlation among the three variables that would support either Darwin's or Delpino's hypothesis. No correlation was found between pollen grain size and style length for 15 species in the Fabaceae and 20 species in the Proteaceae, but there was a strong positive correlation between pollen grain size and stigma depth.

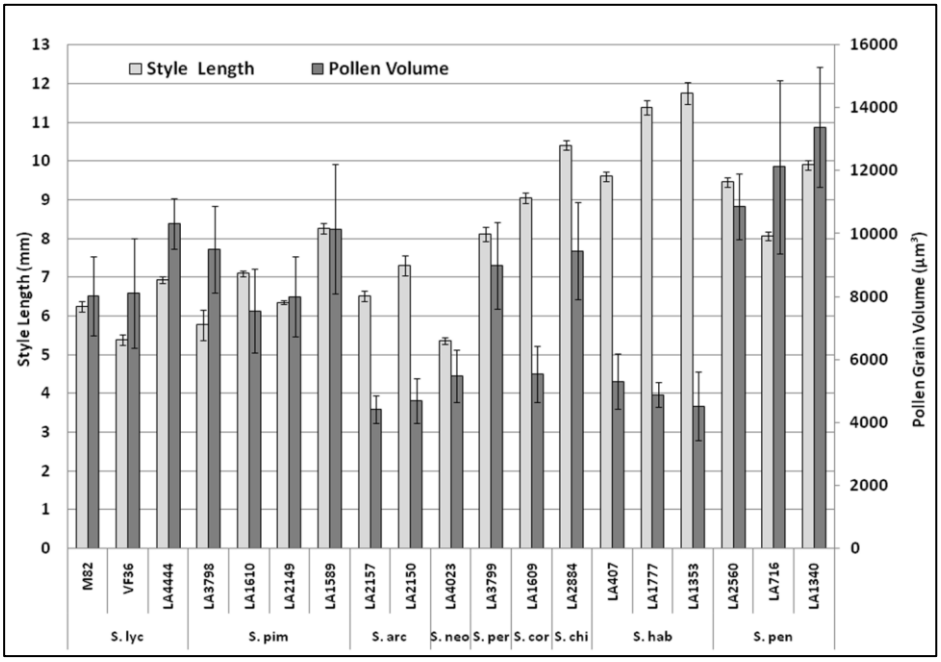
Pollen tubes obtain resources, a variety of molecules including sugar, polysacchrides, nucleic acids, amino acids, and proteins from the extracellular matrix (ECM) of the stylar transmission tissue in many species (Wu et al. 1996; Campbell and Ascher 1975; Gawlik 1984; Cheung 1996).

We examined the stigmas of several members of the tomato clade to see whether there was variation in size or structure (Bedinger et al. 2010). The stigma and style region of cultivated tomato has been described as having lipid-rich intercellular material between transmitting tract cells that are continuous between the stigma and style. *S. habrochaites* (Fig. 2.4 d ,e , h) and *S. arcanum* (not shown) which have small pollen grains and small stigmas with a similar/same type of transmitting tissue continuum. A much larger stigma/style interface was observed in *S. pennellii*, one that lacked lipid-rich intercellular material in the

stigma region (Fig. 2.4 c, f, i). These results are consistent with a positive correlation between stigma architecture and pollen grain size postulated by Darwin and more recently by Cruden (2009).

In summary, in *Solanum* sect. *Lycopersicon*, there is no correlation between pollen grain size and style length among nine species tested in this study. However, pollen grain size might be correlated with other floral traits such stigma architecture. It should be noted that species with both small and large stigma have the ability to reject interspecific pollen rapidly. Therefore, although pollen grain size, stigma depth and style length are important factors that influence reproduction, they do not appear to act as reproductive barriers between species in the tomato clade.

a)



b)

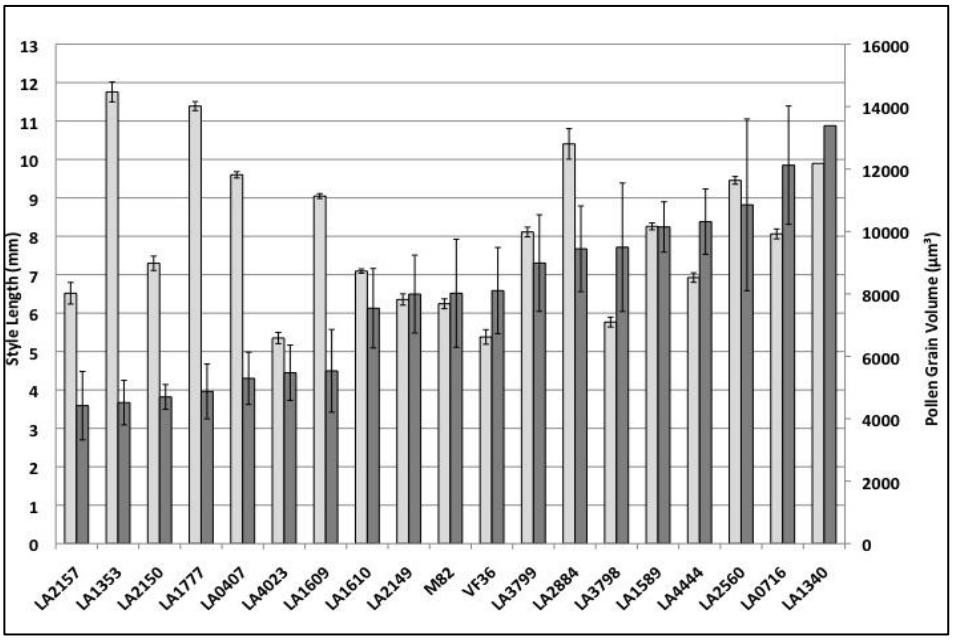


Figure 2.1 Measurements of pollen grain sizes and style lengths in nine species of tomato clade (a). Measurements of pollen grain sizes and style length arranged in order of increasing pollen grain volume (b).

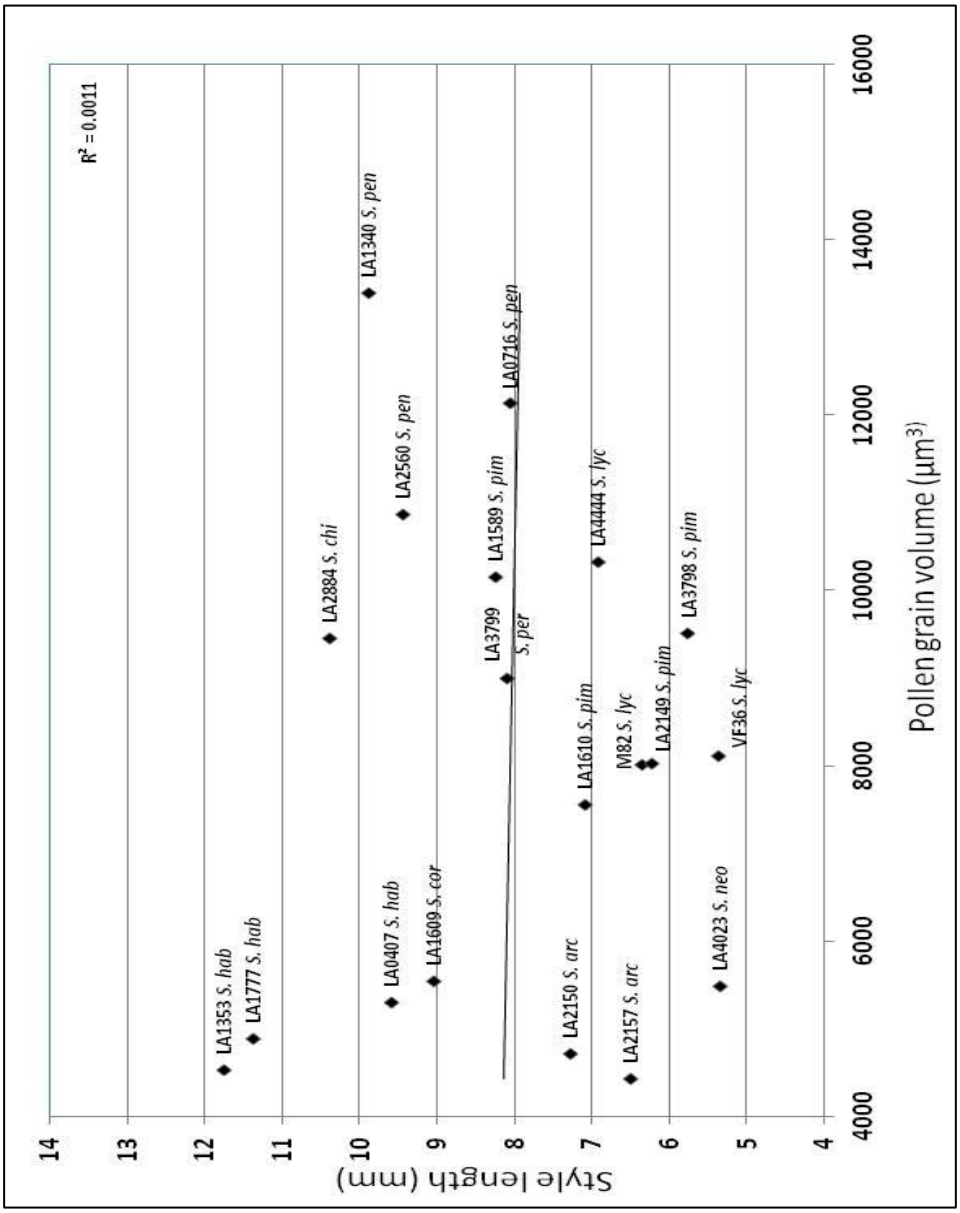


Figure 2.2 Graph of the correlation between pollen grain size (volume) and style length (n=13).

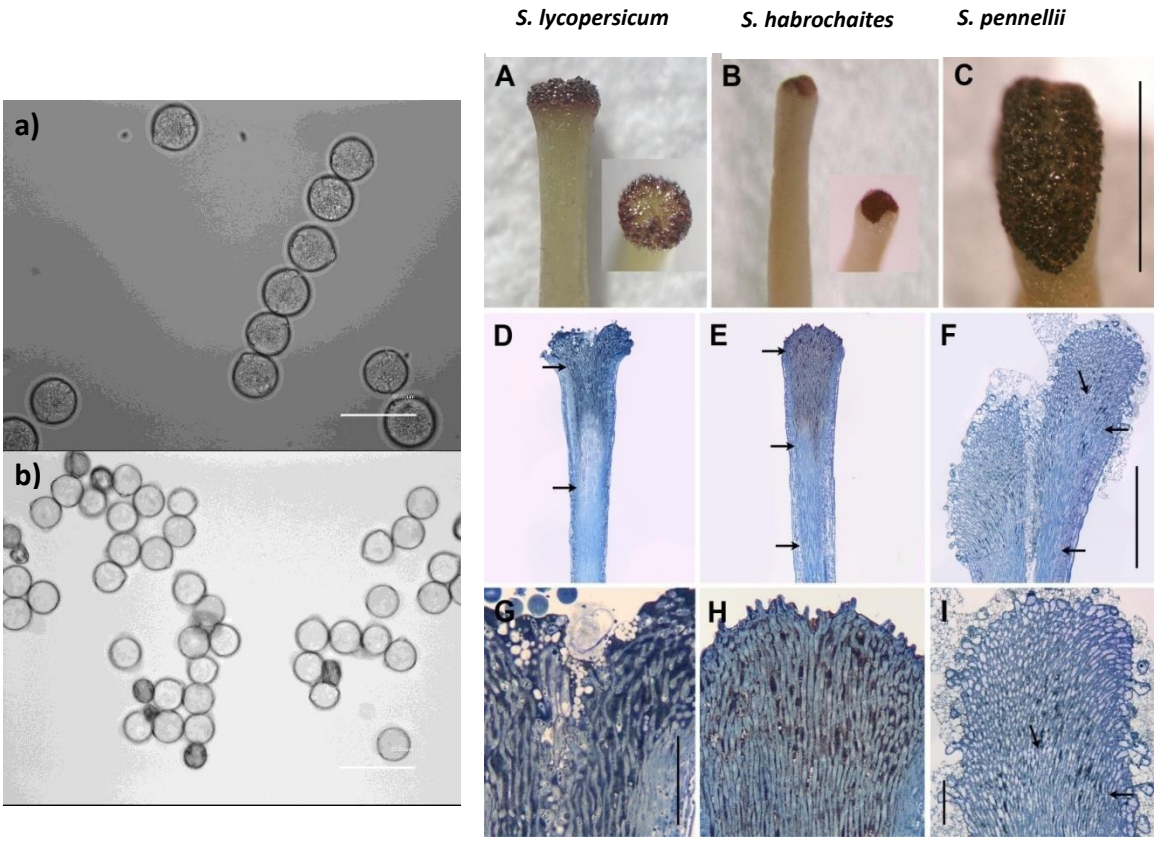


Figure 2.3 (Left) Pollen grains from *S. pennellii* accession LA1340 (a), and *S. habrochaites* accession LA1353(b). Bar is 50μm.**Figure 2.4** (Right) Stigma and style interface of tomato clade, pistils were stained with 0.05% Toluidine blue; LA4444 in *lycopersicum* (cherry tomato) (a,d,g); LA1777 in *S. habrochaites* (b,e,h); LA2560 in *S. pennellii* (c,f,i). Arrows indicates transmission tract tissue. Arrowhead indicates the tip of the vascular bundle**. A~C:** The whole mounts of stigma surface, and 1mm bar was used. **D~I:** lipidic material in stigma/styles is stained in dark. 0.5mm bar in f. 0.1mm in g, h, and i. Photographs by Suzanne Royer

Table 2.1. Summary of pollen grain sizes, style lengths, and genome sizes from accession with the smallest pollen grain size to the largest. Genome size data: A = Stack personal communication; B = Bennett and Smith 1976; C = Arumuganathan and Earle 1991

Species	Accessions	Pollen grain volume (μm³)	Style length (μm)	Genome size (pg/1C)		
				A	B	C
S. arcanum	LA2157	4419.057	6.517	1.24		
S. habrochaites	LA1353	4517.351	11.758			0.93
S. arcanum	LA2150	4698.586	7.296			
S. habrochaites	LA1777	4875.715	11.389	1.24		
S. habrochaites	LA0407	5294.904	9.6	1.195		
S. neorickii	LA4023	5475.26	5.351	1.23		
S. corneliomulleri	LA1609	5534.701	9.047			
S. pimpinellifolium	LA1610	7540.711	7.093	1.095		0.85
S. pimpinellifolium	LA2149	7994.196	6.354			
S. lycopersicum	M82	8021.368	6.243	1.185		0.95
S. lycopersicum	VF36	8104.695	5.379	1.105		
S. peruvianum	LA3799	8987.745	8.112	1.225	1.13	
S. chilense	LA2884	9443.829	10.404	1.295		
S. pimpinellifolium	LA1589	10144.253	8.258	1.145		0.85
S. lycopersicum	LA4444	10314.836	6.925			
S. pennellii	LA2560	10853.217	9.455	1.26	1.23	
S. pennellii	LA0716	12123.517	8.061	1.39		
S. pennellii	LA1340	13388.273	9.897	1.44		

CHAPTER 3:

ASSESSMENT OF POSTMATING PREZYGOTIC REPRODUCTIVE BARRIERS IN THE TOMATO CLADE

Introduction

Studies of reproductive barriers between species in the tomato clade have previously measured the success of seed production (Muschler and Liedl 1994). Most studies that examine post-mating prezygotic barriers, specifically unilateral incongruity/incompatibility (UI), use pollen from the domesticated species, *S. lycopersicum*, in interspecific crosses with wild species. In crosses between cultivated tomato and wild species, e.g. *S. pennellii* (Liedl et al. 1996) and *S. habrochaites* (Covey et al. 2010), pistils of the cultivated tomato act as a "universal acceptor," that fails to reject pollen from other species (Mutschler and Liedl 1994). In the reciprocal cross, pollen of the cultivated species is most often rejected by pistils of wild species. However, the domesticated species does not reside sympatrically with wild species whereas the wild red-fruited species *S. pimpinellifolium* can frequently be found in sympatry with other wild species (see Chapter 4).

In this chapter, I present data on whether interspecific postmating prezygotic reproductive barrier between species in the tomato clade act during pollen-pistil interactions. The completed part of this study focuses on using red-fruited tomato species as pollen donors to examine pollen tube growth in pistils of different wild tomatoes. Pollen tube growth

between domesticated tomato, *S. lycopersicum*, and wild red-fruited tomato species in pistils of wild tomatospecies is compared.

In addition to the domesticated red-fruited tomato species, *S. lycopersicum*, there are three wild red-fruited species and nine wild green-fruited species in section Lycopersicon (Figure 2.1). The tomatoes have been divided into two subgenera by Muller (1940) and Luckwill (1943): 1) *Eulycopersicon* species with fruits colored red to orange and exhibiting self compatibility (autogamous) and hereafter referred to as "red-fruited" species, and 2) *Eriopersiocn* and species with either self compatible or self-incompatible (allogamous) mating systems and fruits that range from greenish to yellowish to purple tinged in color and frequently having dark green, purple, or lavender stripe, hereafter referred to as "green-fruited" species (Fig. 4-2).

The tomato clade contains four red-fruited self-compatible species; *S. lycopersicum* (*S. lyc*), *S. pimpinellifolium* (*S. pim), S. galapagense* (*S. gal*), and *S. cheesmaniae* (*S. che*). All four of the red-fruited species lack interspecific reproductive barriers (IRBs), i.e. they accept pollen from all other species in this clade (Rick 1963; Mutschler and Liedl 1994). All of the red-fruited species are closely related to *S. lycopersicum* based on phylogenetic analyses of DNA, though they have quite different morphological characters; for example *S. lycopersicum* produces large flowers, while *S. pimpinellifolium* produces small flowers (Peralta and Spooner 2005). Cultivated tomato (*S. lycopersicum)* is most closely related to the wild species *S. pimpinellifolium*, which is very likely the direct ancestor of today's cultivated tomato, despite the considerable variation between the two species in several morphological characteristics, particularly flower and fruit size and growth habit (Rick 1978; Nesbitt and and Tanksley 2002). The lab group at Cold Spring Harbor Laboratory (P.I Dreen

Ware) sequenced the wild tomato species, *S. pimpinellifolium*. Over 50% of the *S. pimpinellifolium* contigs have been aligned to the domesticated tomato, suggesting they are closely related each other. Unlike *S. lycopersicum*, *S. pimpinellifolium* is frequently observed to grow sympatrically with other green-fruited species in the wild. Thus, this analysis will give insight into whether prezygotic UI barriers actually are exhibited in the wild.

The other two "red-fruited" species, *S. galapagense* and *S. cheesmaniae*, are native in the Gálapagos Islands which are located about 1000 km off the west coast of South America. *S. pimpinellifolium* is the closest relative to these Gálapagos tomatoes (Darwin 2003). Since Gálapagos tomato species are geographically isolated from other wild tomatoes, the opportunity to compare pollen tube growth during crosses with wild "green-fruited" species between Gálapagos tomatoes and the other two "red-fruited" tomato species should prove to be interesting, since the two species have not been exposed to other wild tomato species on mainland South America for thousands of years.

In addition to a completed set of reciprocal crosses studies between the four red-fruited species and other wild tomatoes, I have made significant process toward completing a comprehensive study of post-mating prezygotic barriers between all of the species within the tomato clade. These results will also be presented.

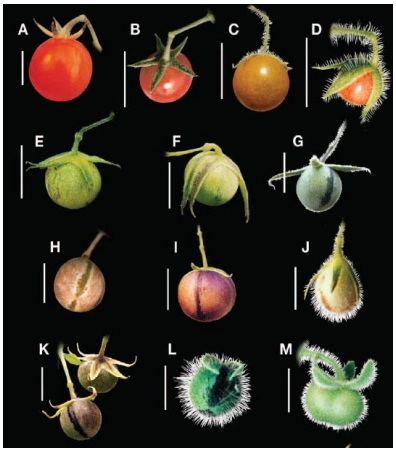


Figure 3.1 Fruits in tomato clade;
a) *S. lycopersicum.* b) *S. pimpinellifolium*.
c) *S. galapagense.* d) *S. cheesmaniae.*
e) *S. neorickii.* f) *S. chmielewskii.*
g) *S. arcanum.* h) *S. huaylasense.*
i) *S. peruvianum*. j) *S. corneliomulleri.*
k) *S. chilense*. l) *S. habrochaites.*
m) *S. pennellii* (Peralta et al. 2008)

Materials and Methods

Plant materials:

Twelve tomato species were used in this study (Table 3.1), germplasm of which was obtained from the Tomato Genetic Resource Center at UC Davis. Four red-fruited SC species were used as females and males in this study: *S. lycopersicum, S. galapagense, S. cheesmaniae,* and *S. pimpinellifolium.* Eight green-fruited species were used only as females in this study. Two green-fruited SC species, *S. neorickii* and *S. chmielewskii,* were used. Three green-fruited SI species were used: *S. peruvianum, S. corneliomulleri,* and *S. chilense.* Three green-fruited SI species which also have SC populations were used: *S. arcanum, S. habrochaites,* and *S. pennellii.* Collections from different locations have different accession numbers (e.g., LA0317), and may represent different populations.

Table 3.1 Summary of 12 species used in this study.

Species	Mating system	Accessions
S. lycopersicum	SC	Cultivars: M82 VF36
S. galapagense	SC	LA0317 LA1408 LA0483
S. cheesmaniae	SC	LA0522 LA0166 LA0421
S. pimpinellifolium	SC	LA1589 LA1610 LA2149 LA3798 LA1590 LA1383
S. neorickii	SC	LA4023 LA2403 LA1321
S. chmielewskii	SC	LA1316 LA3653 LA3656 LA1325
S. arcanum	SC	LA2157
	SI	LA2150 LA1708
S. peruvianum	SI	LA0445 LA1949 LA3799
S. corneliomulleri	SI	LA1609 LA1694
S. chilense	SI	LA2884 LA2773 LA3153 LA4330
S. habrochaites	SC	LA0407
	SI	LA1777 LA1353
S. pennellii	SC	LA0716
	SI	LA1340 LA2560

Pollination and Pistil Staining

Seed collections of wild tomatoes were acquired from the TGRC and grown in the greenhouse. Plants were grown in ProMix-BX soil in greenhouse conditions (12 hours light at 24 °C and 12 hours dark at 21 °C). Genetic crosses were performed by emasculating flowers of the female parent one day before bud break or anthesis. Emasculated buds were left for 24 hours, after which stigmas were dipped in collected pollen. To obtain pollen, mature flowers of male parents were vibrated over gelatin capsules using a tooth polisher as a means of releasing pollen from antehers. In some cases, crosses were performed by R. Chetelat or at UC. Davis.

After pollination, pollen tubes were given another 24 to 48 hr to grow through the style. The entire pistil, stigma/style plus ovary, was collected after another 24 hr (48 to 72 hr after pollination) and placed in fixative solution (3:1 95% ethanol:glacial acetic acid) for 24 hr. After fixative solution was removed, 10 M NaOH softening solution was used for 24 hr. After 24hr, softening solution was removed and styles were rinsed three times with ddH_2O. After rinsing, 0.2 mL ABF (Aniline Blue Fluorochrome) in 0.1 M K_2HPO_4 buffer, pH 10, was added (1/20 dilution for 4 hr or 1/100 dilution for 24 hr staining). Samples were left in stain for 24 hr in the dark to stain pollen tube. Pistils were then mounted on glass microscope slides with a drop of 50% glycerin, covered with a cover slip, and imaged using a Leica DM5500 B florescence microscope (Leica Microsystems, http://www.leica.com/) with IPlab version 4 software (BD biosciences, http://www.bdbiosciences.com/home.jsp) coupled with a Hamamastu C4742095 camera (http://www.hamamastu.com/). Fluorescence microscopy UV excitation of ABF using DAPI filter cubes allows the visualization of fluorescent signals, particularly from callose in pollen tubes. 10-20 images were taken to capture an entire style's

length from stigma to ovary. Whole-style images were composited with Adobe Photoshop, and pollen tube lengths were measured using the 'segmented line' tool of Image J 1.33 (http://rsb.info.nih.gov/ij/). From the top of the stigma, then along the two vascular bundles to the style-ovary junction were measured to obtain an average style length (note: vascular bundles do no go all the way to the stigma). Finally, pollen tubes were measured for the longest and average of 10-15 of the longest pollen tubes.

Data analysis

For each cross, at least three replicates were performed. From measurements in mm from the top of the stigma of 15 pollen tubes in each image, the average and longest pollen tube were noted in mm and in some cases the lengths were calculated as a percent of the style length. Bar graphs were then created to show the box-and-whisker plot of pollen tube growth inset over the style length (Grey box; mean $\pm$ SEM; Fig. 3.1). The white box represents 50% of the pollen tube lengths spanning 25th to 75th percentiles, the middle line in the box represents the median of averaged pollen tube length across all replicates, and the two whisker bars represent the data range. I used t-test and ANOVA in Microsoft Excel (2007) to compare average pollen tube lengths when different populations were used.

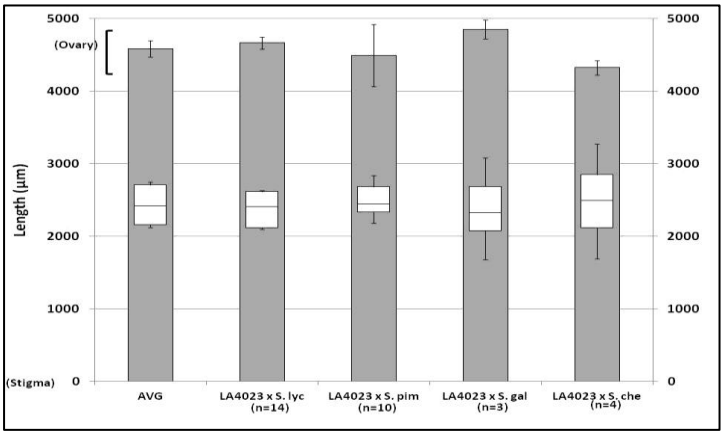


Figure 3.1 An example of bar graphs. X-plot labels crosses and number of replicates and y-plot shows length of styles and pollen tubes. 0 on y-plot marks the top of the stigma whereas the top of the gray box marks the style-ovary boundary.

Results

A summary of the results in this chapter:

- Pollen from all of red-fruited species is accepted by pistils of red-fruited SC.
- Pollen from all red-fruited species is rejected by pistils of all SI green-fruited species.
- Pollen from *S. lycopersicum* (cultivated tomato) is rejected by pistils of SC green-fruited species and SC populations of SI species.
- Pollen from three wild red-fruited species varies in rejection by pistils of green-fruited SC species.
- Pollen from three wild red-fruited varies in rejection by pistils of some SC populations of some SI green-fruited species.

Table 3.2 Pollen tube growth in crosses using pollen of red-fruited tomato species on pistils of members of the tomato clade. SC = self-compatible mating system, SI = self-incompatible mating system. Seed = seed set occurs, A= pollen acceptance occurs, R = pollen rejection occurs, A/R = variability in pollen rejection.

Male → / Female ↓	*S. lyc* (SC)	*S. pim* (SC)	*S. gal* (SC)	*S. che* (SC)
S. lyc (SC)	SC	Seed	Seed	Seed
S. pim (SC)	Seed	SC	Seed	Seed
S. gal (SC)	Seed	Seed	SC	Seed
S. che (SC)	Seed	Seed	Seed	SC
S. neo (SC)	R	A/R	A/R	A/R
S. chm (SC)	R	A/R	A/R	A/R
S. arc (SI)	R	R	R	R
S. arc (SC)	R	A/R	A/R	A/R
S. per (SI)	R	R	R	R
S. cor (SI)	R	R	R	R
S. chi (SI)	R	R	R	R
S. hab (SI)	R	R	R	R
S. hab (SC)	R	A/R	A/R	A/R
S. pen (SI)	R	R	R	R
S. pen (SC)	R	R	R	R

Four types of variability in pollen tube growth and/or pollen tube rejection were observed in this study (summarized in Table 3.3). First, in some cases there was variability in the average length of pollen tubes due to factors on the male or female side (type a) in interspecific crosses. This was seen when pollen from different accessions grew differently in pistils of the same accessions or vice versa. In other cases, there was variability in the extent of pollen tube growth within a single cross producing a wide range of pollen tube lengths (type b). In type c variability, differences were seen in rejection of pollen from the same accession by pistils of different accessions. Finally, in some crosses, differences in pollen rejection were observed, even when both the male and female accessions in crosses were identical (type d). This puzzling kind of variability was sometimes even seen within a single individual. Both consistent and variable results are described in more detail below.

Table 3. 3 Observation of variability of pollen tube growth or rejection in crosses.

	Variability of Pollen tube growth		Variability in pollen rejection/acceptance	
Type	**a.** Variation in average pollen tube length	**b.** Wide range of pollen tube lengths within single cross	**c.** Variation on Female accessions	**d.** Variation among crosses using the same male and female accessions
Examples	*S. per* x *S. lyc*	SC *S. pen* LA0716 x LA1589	*S. neo* x *S. pim*	SC *S. arc* LA2157 x *S. gal*
	S. per x *S. pim*	SC *S. pen* LA0716 x *S. gal*	*S. neo* x *S. gal*	SC S. arc LA2157 x S. che
	S. cor x *S. pim*	*S. chm* x *S. lyc*	*S. neo* x *S. che*	SC *S. hab* LA0407x *S. pim*
	SI *S. pen* x *S. pim*	SC *S. arc* LA2157 x *S. lyc*	*S. chm* x *S. pim*	SC *S. hab* LA0407x *S. gal*
	SC *S. pen* LA0716 x *S. pim*	SC *S. hab* LA0407 x *S. lyc*	*S. chm* x *S. gal*	SC *S. hab* LA0407x *S. che*
		S. neo x *S. gal*	*S. chm* x *S. che*	
		S. neo x *S. che*		
		S. chm x *S. gal*		

1) Red-fruited SC (cultivated and wild) pollen is accepted by pistils of SC red-fruited species.

Crosses performed within the red-fruited species group produced viable seed, consistent with findings from Rick (1963) and Darwin et al. (2003) and confirmed that red-fruited species are fully inter-compatible. Pollen tube growth to the ovaries was consistently observed in reciprocal crosses.

2) Pollen from all of red-fruited species is rejected by pistils of green-fruited SI

Pollen from all red-fruited species is rejected by all SI green-fruited species and SC populations of *S. pennellii* (Table 3.2; Table 3.4). The average range of pollen tube growth is 0.81 to 2.78 mm in the pistils (Table 3.3). The types of variability observed in these crosses is type a and b, in which rejection always occurs but the average range of pollen tube lengths is wide or different average pollen tube lengths are observed on either the male or the female side (Table 3.4). Details of pollen tube growth in each cross are explained below in order of the female species.

Table 3.4 Length of pollen tubes in mm on average ± SE from red-fruited species in the pistils of SI green-fruited populations and species.

Male	*S. lyc*	*S. pim*	*S. gal*	*S. che*
Female	Pollen tube length (mm) on avg			
S. arcanum (SI)	1.69 ± 0.23 (11)	1.73 ± 0.22 (7)	1.82 ± 0.2 (3)	1.4 ± 0.06 (3)
S. habrochaites (SI)	0.95 ± 0.08 (18)	1.31 ± 0.04 (6)	1.25 ± 0.08 (7)	1.08 ± 0.23 (8)
S. peruvianum (SI)	1.29 ± 0.29 (7) #	1.16 ± 0.28 (12) *	1.17 ± 0.08 (4)	0.9 ± 0.04 (3)
S. corneliomuelleri (SI)	1.61 ± 0.11 (10)	1.69 ± 0.23 (19) *	1.37 ± 0.12 (5)	1.29 ± 0.27 (5)
S. chilense (SI)	1.37 ± 0.19 (4)	1.49 ± 0.26 (15)	2.28 ± 0.21 (5)	1.31 ± 0.07 (6)
S. pennellii (SI)	1.01 ± 0.28 (19)	1.32 ± 0.4 (17) *	1.05 ± 0.04 (4)	0.81 ± 0.23 (3)
S. pennellii (SC)	1.27 ± 0.2 (13)	2.54 ± 0.4 (20) Δ*	1.59 ± 0.3 (7) Δ	2.78 ± 0.4 (9) Δ

(n)= numbers of replications, #= different rate of pollen tube length depends on female accessions, *= different pollen tube length among different male accessions. Δ= variability of pollen tube lengths in a cross

Table 3. 5 Variability in crosses with male side effects. Lengths of pollen tube in mm from *S. lycopersicum* and *S. pimpinellifolium* in the pistils of *S. peruvianum*, *S. corneliomuelleri,* and *S. pennellii*. (n)= number of replication.

		S. pimpinellifolium (pollen)			
		LA1589	LA1610	LA2149	LA3798
S. peruvianum	LA3799	**1.7 ± 0.5 (3)**		0.8 ± 0.1 (3)	1.0 ± 0.1 (6)
S. corneliomulleri	LA1609	**2.4 ± 0.4 (4)**	1.3 ± 0.2 (8)	1.5 ± 0.3 (4)	1.35 ± 0.1 (3)
S. pennellii (SI)	LA1340	**2.5 ± 0.4 (7)**	0.9 ± 0.3 (3)		1.1 ± 0.4 (2)
S. pennellii (SI)	LA0716	**3.4 ± 0.5 (9)**	2.1 ± 0.1 (4)	1.8 ± 0.2 (4)	2.5 ± 0.5 (3)

a) *S. arcanum* (SI)

Consistent rejection of pollen from all red-fruited species occurs in pistils of two accessions (LA1708, LA2150) of SI *S. arcanum* with average range of pollen tube growth from 1.4 mm to 1.82 mm (Table 3.4; Fig. 3.3, Fig. 3.4). Rejection of pollen from all accessions of each of these four species occurred at a very similar place in the upper part of style of both accessions of *S. arc* (SI).

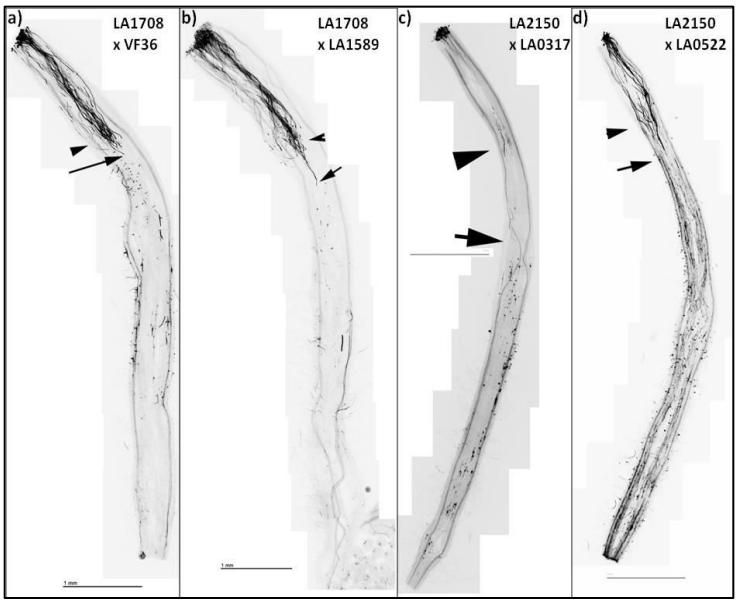


Figure 3.3 The red-fruited species pollen tube growth in the pistils of *S. arcanum* (SI). LA1708, LA2150= *S. arcanum* (SI).
a)*S. arc* (SI) x *S. lyc* VF36,
b)*S. arc* (SI) x *S. pim* LA1589,
c)*S. arc* (SI) x *S. gal* LA0317,
d)*S. arc* (SI) x *S. che* LA0522.
Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

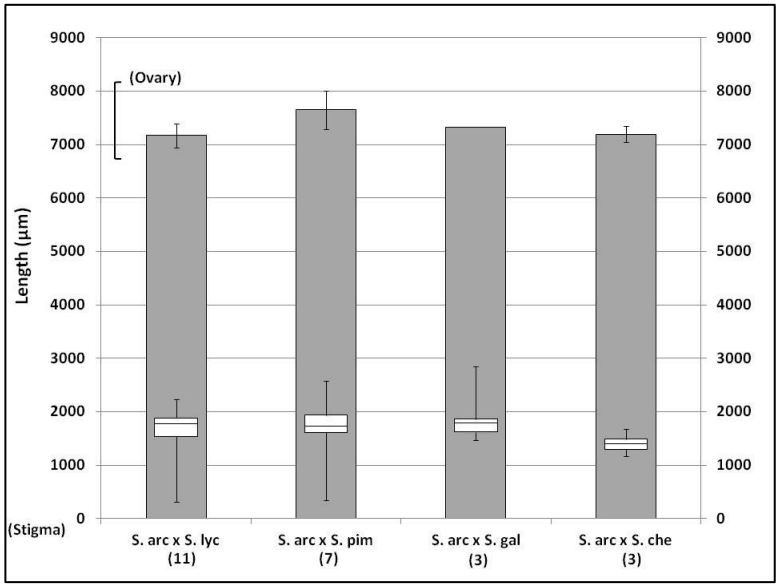


Figure 3.4 Comparison of pollen tube lengths among the red-fruited species in pistil of *S. arcanum* (SI).

b) *S. habrochaites* (SI)

Consistent rejection of pollen from all red-fruited species occurs in pistils of two accessions (LA1777, LA1353) of SI *S. habrochaites* with average range of pollen tube growth from 0.99 mm to 1.31 mm (Table 3.4; Fig. 3.5 and Fig. 3.6). Both accessions of SI *S. habrochaites* reject pollen from all accessions of the red-fruited pollen in the upper portion of the style.

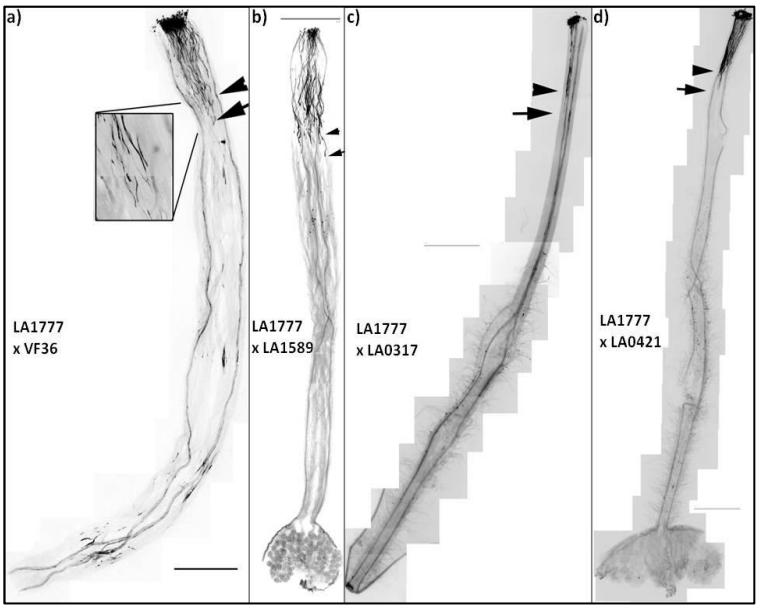


Figure 3.5 The red-fruited species pollen tube growth in the pistils of, SI *S. habrochaites* LA1777.
a)*S. hab* (SI) x *S. lyc* VF36,
b)*S. hab* (SI) x *S. pim* LA1589,
c) *S. hab* (SI) x *S. gal* LA0317,
d) *S. hab* (SI) x *S. che* LA0421.
Arrowhead represents average of pollen tubes in the style;
Arrow indicates the longest pollen tube in the style.

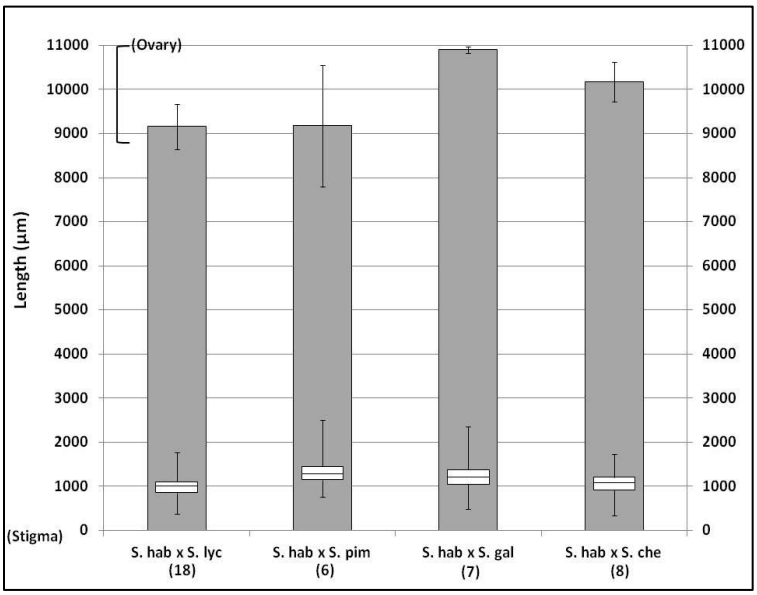


Figure 3.6 Comparison of pollen tube lengths among the red-fruited species in the pistil of *S. habrochaites* (SI).

c) *S. peruvianum*

Consistent rejection of pollen from all red-fruited species occurs in pistils of three accessions (LA3799, LA0445, LA1949) of SI *S. peruvianum* with average range of pollen tube growth from 0.99 mm to 1.3 mm (Table 3.4; Fig. 3.7; Fig. 3.8). Different female accessions of *S. peruvianum* show variability (type a) of pollen tube growth of *S. lycopersicum.* Pollen tubes from *S. lyc* fail to grow past 0.8 mm in the pistil of SI *S. per,* LA3799 which is somewhat more rapid rejection than is seen in other populations of SI *S. per* LA0445 (1.3mm) and LA1949 (1.8mm; Fig. 3.9). In this case, there were not enough replications to perform statistical analysis. Another variability of pollen tube growth was observed with the effect due to different male accessions of *S. pimpinellifolium* in that pollen tubes from LA 1589 grow farther than other *S. pim* accessions (Table 3.5; Fig. 3.7; Fig. 3.10). However, according ANOVA statistical analysis, it did not show significant different among different male populations (p-value=0.090312).

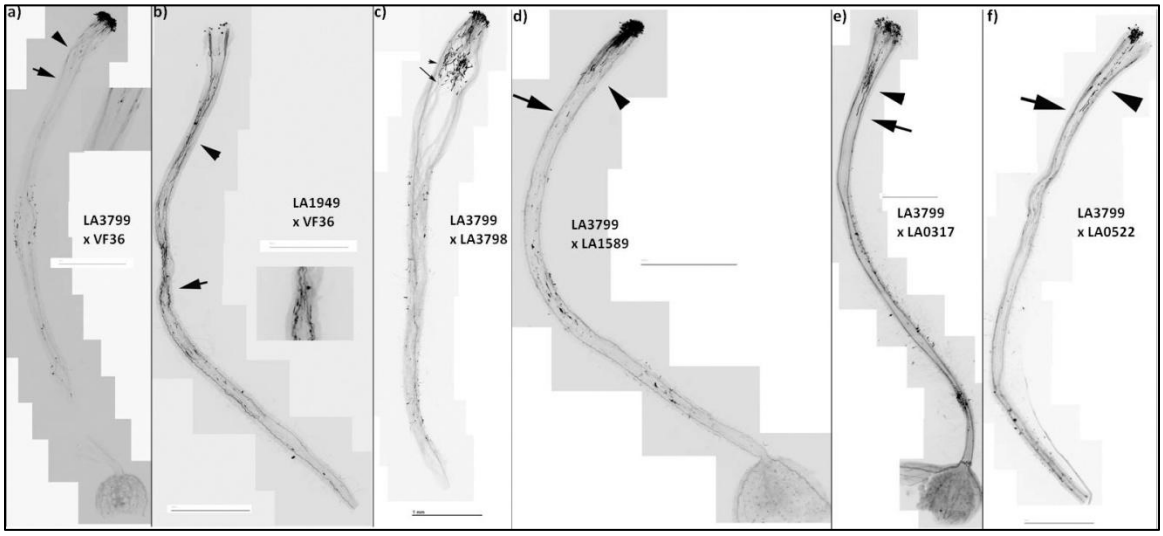


Figure 3.7 (Left) The red-fruited species pollen tube growth in the style of *S. peruvianum*. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style. **a**) *S. per* LA3799 x *S. lyc* VF36, **b**) *S. per* LA1949 x *S. lyc* VF36, **c**) *S. per* LA379) x *S. pim* LA3798, **d)** *S. per* LA379) x *S. pim* LA1589, **e)** *S. per* LA3799 x *S. gal* LA0317, **f)** *S. per* LA3799 x *S. che* LA0522

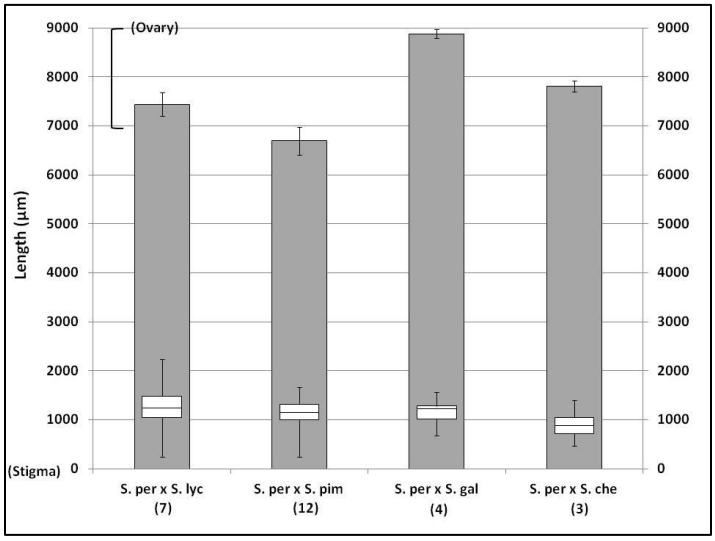


Figure 3.8 Comparison of pollen tube lengths among the red-fruited species in the pistil of *S. peruvianum.*

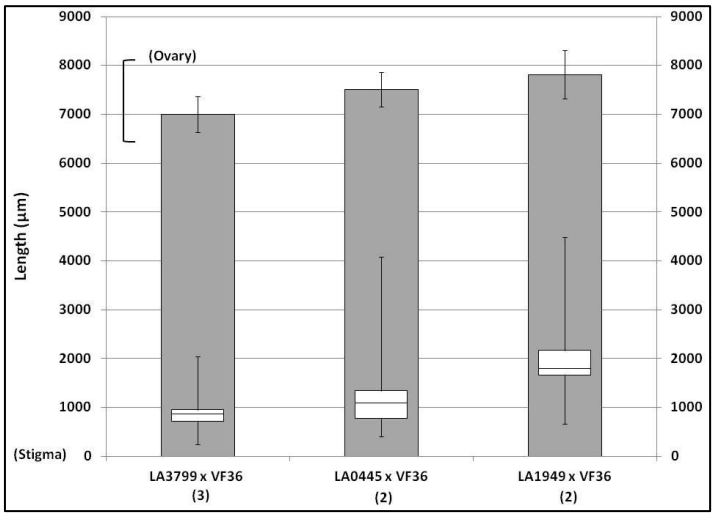


Figure 3.9 Comparison of pollen tube lengths of *S. lycopersicum* in the pistils of different female accessions of *S. peruvianum* LA3799, LA0445, LA1949

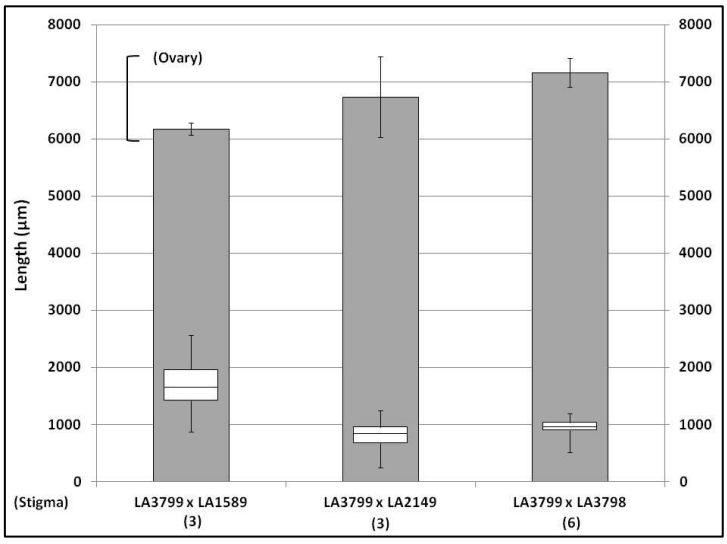


Figure 3.10 Comparison of pollen tube lengths among different male accessions of *S. pimpinellifolium* in the styles of *S. peruvianum* LA3799.

d) *S. corneliomulleri*

Consistent rejection of pollen from all red-fruited species occurs in pistils of two accessions (LA1609, LA1694) of SI *S. corneliomuelleri* with average range of pollen tube growth from 1.3 mm to 1.7 mm (Table 3.4; Fig. 3.11). *S. corneliomulleri* rejects pollen from all red-fruited species in the upper portion of the style (Fig. 3.11; Fig. 3.12). Variability (type a) was observed among different male accessions of *S. pimpinellifolium* in that pollen tubes from LA 1589 grow farther than other *S. pim* accessions (p-value=0.028347; Table 3.5; Fig. 3.12).

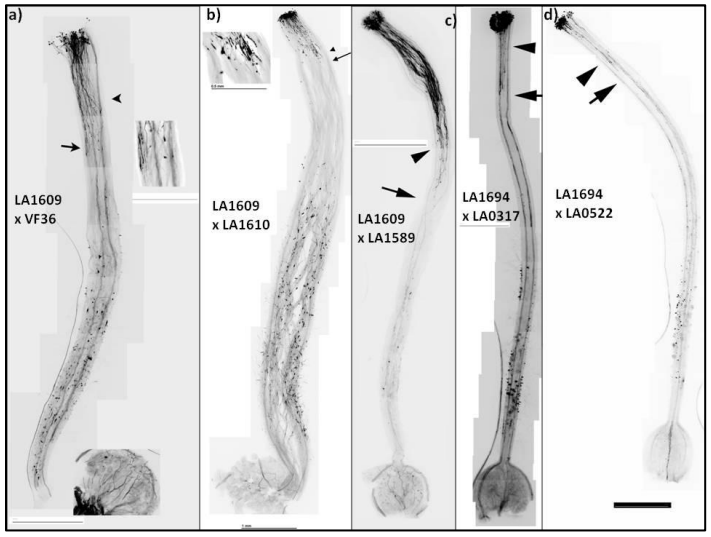


Figure. 3.11 The red-fruited species pollen tube growth in the pistils of SI *S. corneliomulleri* SI LA1609, LA1694 .
a)*S. cor* x *S. lyc* VF36,
b) *S. cor* x *S. pim* LA1610
b) *S. cor* x *S. pim* LA1589,
c) *S. cor* x *S. gal* LA0317,
d) *S. cor* x *S. che* LA0522.
Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

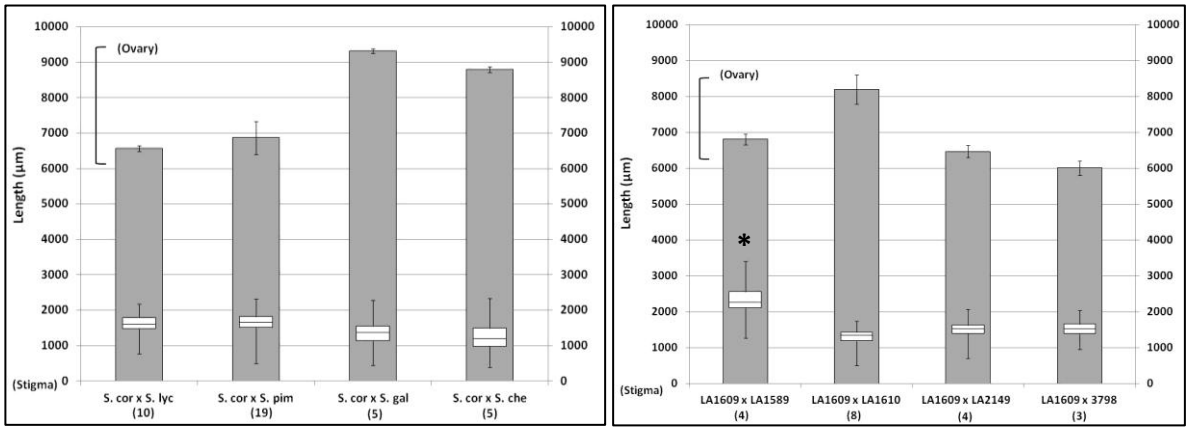


Figure 3.12 Comparison of pollen tube lengths among the red-fruited species in the style of *S. cor*. (Left) Comparison of pollen tube length among different male accessions (LA1589, LA1610, LA2149, LA3798) of *S. pim* in the styles of SI *S. cor* LA1609 (Rights). Asterisk indicates ssignificant different average pollen tube length than other crosses.

e) *S. chilense*

Consistent rejection of pollen from all red fruited species occurs in pistils of four accessions (LA2884, LA3153, LA2773, LA4330) of SI *S. chilense* with average range of pollen tube growth from 1.3 mm to 2.3 mm (Table 3.4; Fig. 3.13; Fig. 3.14). Crosses of all accessions of SI *S. chilense* by pollen of accessions of red-fruited species show pollen tube rejection at a similar place in the style.

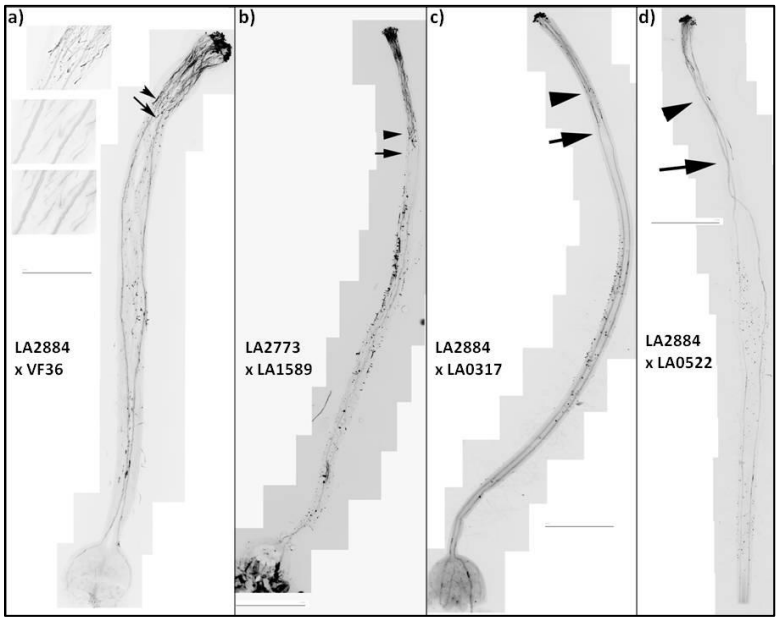


Figure 3.13 The red-fruited species pollen tube growth in the pistils of SI *S. chilense* LA2773, LA2884
a) *S. chi* x *S. lyc* VF36,
b) *S. chi* x *S. pim* LA1589,
c) *S. chi* x *S. gal* LA0317,
d) *S. chi* x *S. che* LA0522.
Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

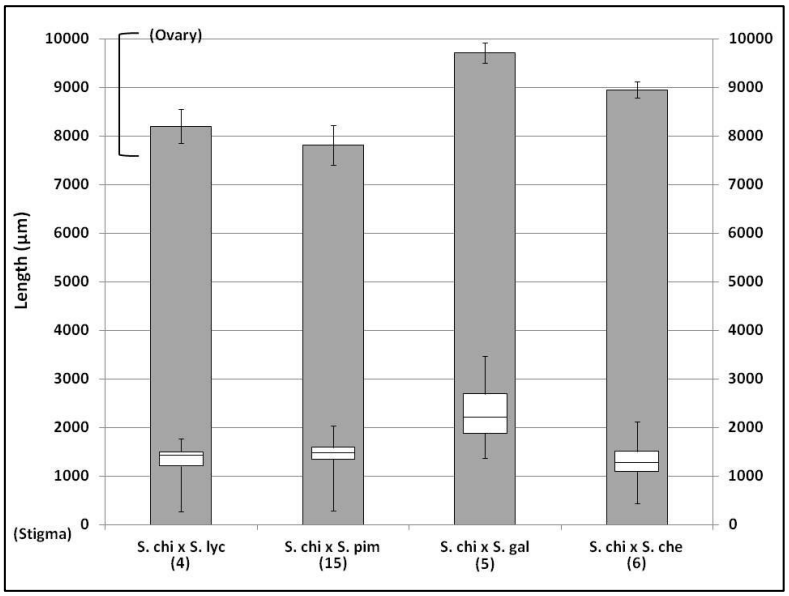


Figure 3.14 Comparison of pollen tube lengths among the red-fruited species in the style of *S. chi*.

f) *S. pennellii* (SI)

Consistent rejection of pollen from all red fruited species occurs in pistils of two accessions (LA1340 and LA2560) of SI *S. pennellii* with average range of pollen tube growth from 0.8 mm to 1.3 mm (Table 3.4; Fig. 3.15; Fig. 3.16). All pollen rejection occurs in the upper portion of the style. Different male accessions of *S. pimpinellifolium* show variability of pollen tube growth in the pistils, in that pollen tubes from *S. pim* accession LA1589 grow farther than other *S. pim* accessions (p-value=0.036257; Table 3.5).

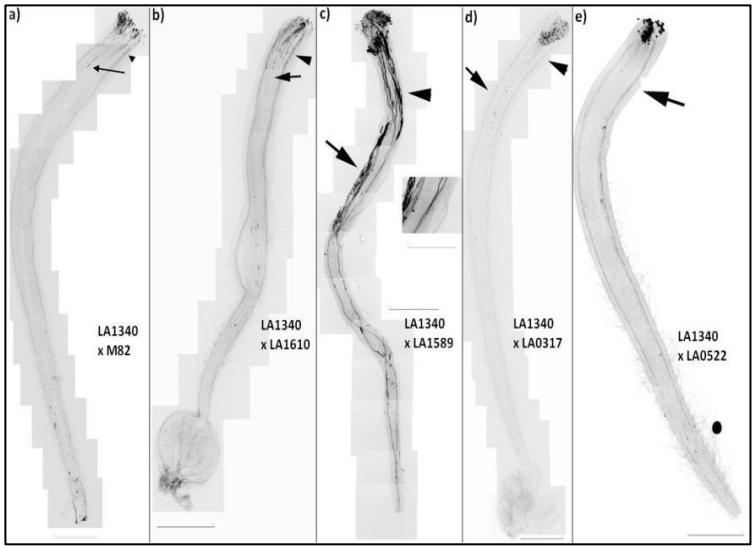


Figure 3.15 The red-fruited species pollen tube growth in the pistils of SI *S. pennellii* LA1340. a) *S. pen* (SI) x *S. lyc* VF36, b) *S. pen* (SI) x *S. pim* LA1610 c) *S. pen* (SI) x *S. pim* LA1589, d) *S. pen* (SI) x *S. gal* LA0317, e) *S. pen* (SI) x *S. che* LA0522. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

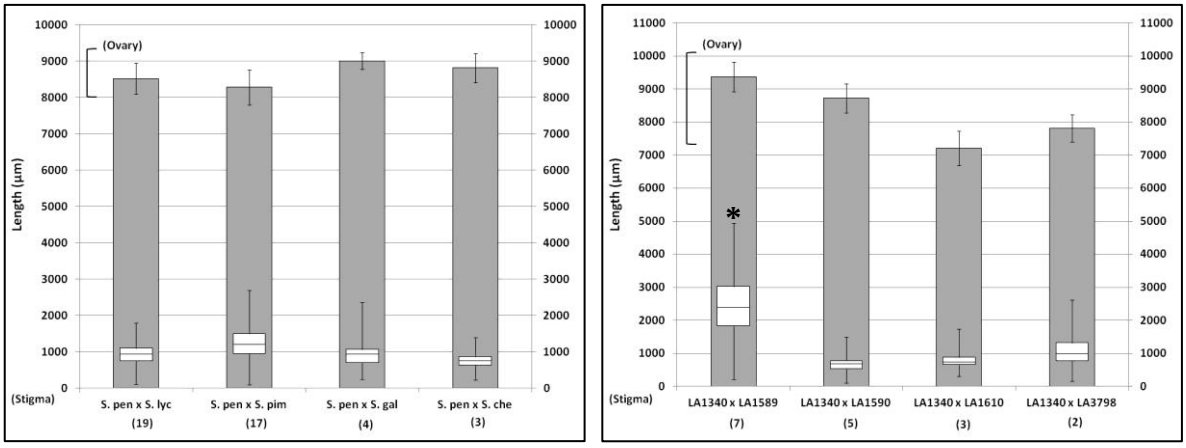


Figure 3.16 Comparison of pollen tube lengths among the red-fruited species in the style of *S. pen* (SI) (Left). Comparison of pollen tube length among different male accessions of *S. pim* (LA1589, LA1590, LA1610, LA3798) in the styles of SI *S. pen* LA1340 (Right). Asterisk indicates significant different average pollen tube length than other crosses.

g) *S. pennellii* (SC)

There is only one SC population of *S. pennellii*, LA0716. Consistent rejection of pollen from the red-fruited species is occurs in the pistils of SC *S. pen* LA0716 with the average range from 1.3mm to 2.8 mm in the style (Table 3.4; Fig. 3.17; Fig. 3.18). This rejection happens slightly later than that in pistils of SI populations with an average pollen tube length of 1.1 mm (Table 3.4).

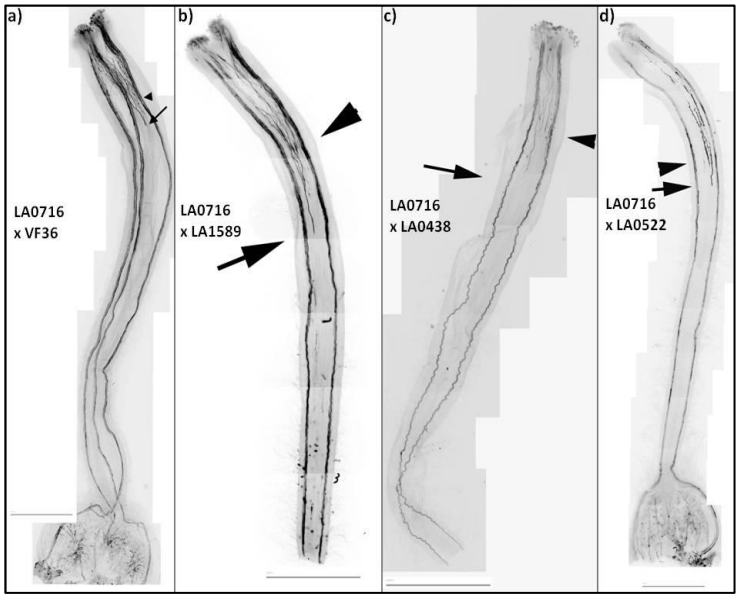


Figure 3.17 The red-fruited species pollen tube growth in the pistils of SC *S. pennellii* LA0716. a) *S. pen* (SC) x *S. lyc* VF36, b) *S. pen* (SC) x *S. pim* LA1589, c) *S. pen* (SC) x *S. gal* LA0438, d) *S. pen* (SC) x *S. che* LA0522. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

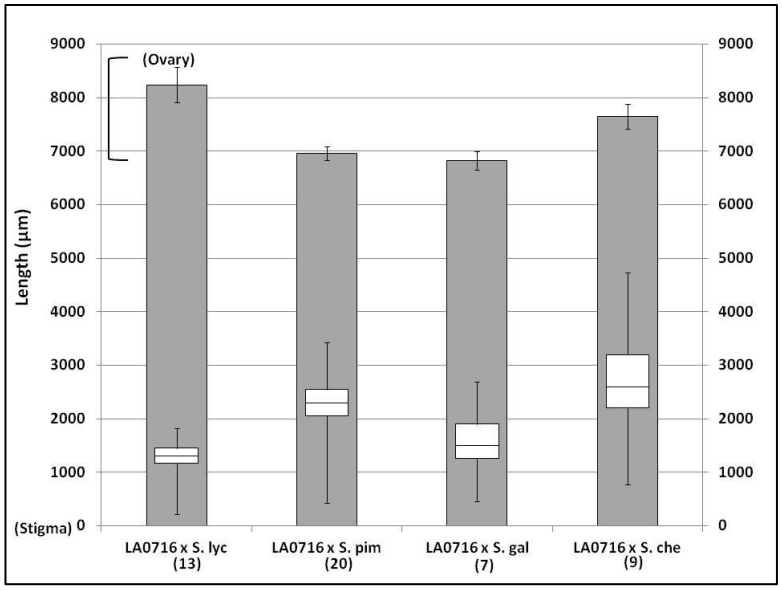
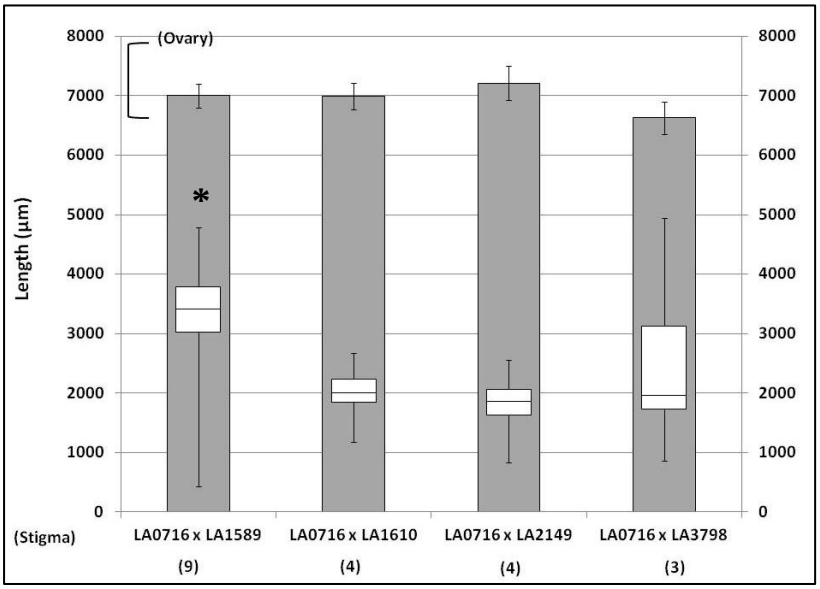


Figure 3.18 Comparison of average pollen tube length among the red-fruited species in the style of SC *S. pen* LA0716.

Variability in pollen tube growth of type a and b is observed in the pistils of LA0716 (*S. pen*, SC) with different male accessions of *S. pim* (LA1589, LA1610, LA2149, and LA3798) (Table 3.5; Fig. 3.19). Pollen tubes from LA1589 grow further than other *S. pim* (LA1610 p-value=0.026552; LA2149 p-value 0.014071; LA3798). There was no significant difference in average pollen tube lengths between LA1589 and LA3798 (p-value=0.0222769). Pollen from *S. gal* and *S. che* exhibits a wide range of pollen tube growth in the pistils of LA0716 (Table 3.5; Fig 3.20).



igure 3.19 Comparison of pollen tube length among different female accessions of *S. pimpinellifolium* in the style of LA0716, *S. pen* (SC). Asterisk indicates significant different average pollen tube length than other crosses.

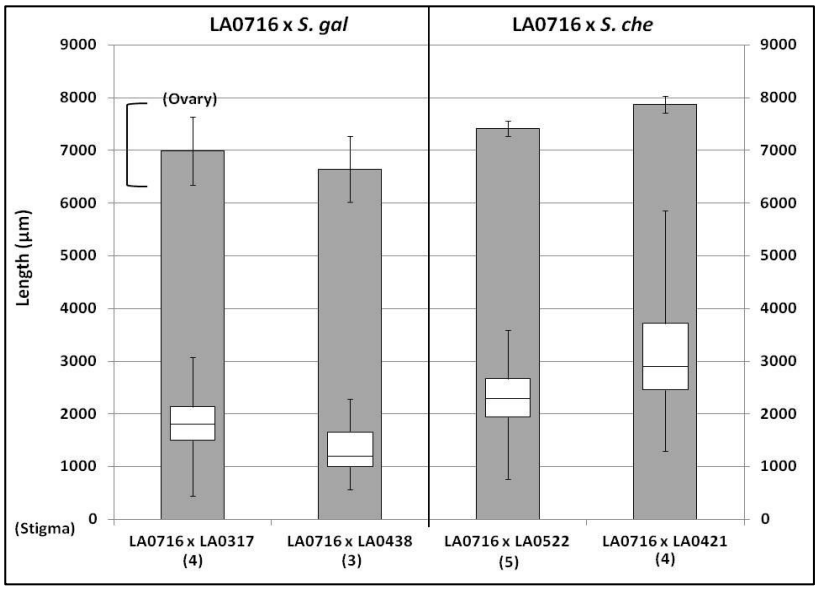


Figure 3. 20 Comparison of pollen tube length of Galapagos tomato species (*S. gal* and *S.che*) in the style of LA0716, *S. pen* (SC). LA0317and LA0438= *S. galapagense*, LA0522 and LA0421=*S. cheesmaniae.*

3) Pollen from *S. lycopersicum* (cultivated tomato) is rejected by pistils of green-fruited SC species and SC populations of *S. arcanum* and *S. habrochaites*

Table 3.6 Length of *S. lycopersicum* pollen tubes in mm in the pistils of green-fruited SC species and SC populations of SI species.

	Male
	S. lyc
Female	
S. neorickii	2.4 ± 0.2 (10)
S. chmielewskii	5.3 ± 0.3 (6)
S. arcanum (SC; LA2157)	3.3 ± 0.7 (5)
S. habrochaites(SC; LA0407)	5.5 ± 0.7 (11)

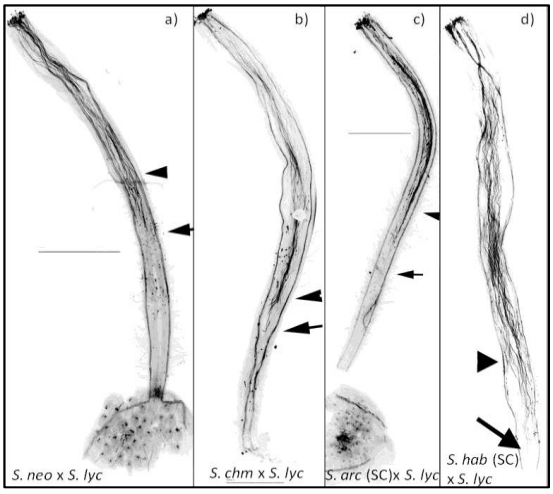


Figure 3.21 *S. lycopersicum* pollen tube growth in the pistils of green-fruited SC species and populations of *S. arcanum* and *S. habrochaites* a) SC *S. neo* x *S. lyc*, b) SC *S. chm* x *S. lyc*, c) SC *S. arc* LA2157 x *S. lyc*, d) SC *S. hab* LA0407 x *S. lyc*. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

Consistent rejections of *S. lycopersicum* pollen occurred in pistils of SC green-fruited species (*S. neorickii* and *S. chmielewskii*). Also consistent rejection of pollen from *S. lycopersicum* occurs in the pistils of SC green-fruited populations of *S. arcanum, S. habrochaites* (Table 3.6). Although rejection of *S. lyc* pollen consistently occurs in crosses of all SC green-fruited species and populations, rejection of pollen from *S. lyc* occurs at different locations in the different female species (Table 3.6; Fig. 3.21). It is worthy that pollen tubes from *S. lyc* grow very close to the ovary in pistils of SC *S. habrochaites* LA0407 and *S. chmielewskii.* I have never observed pollen tubes in the ovaries in these crosses. However, this summer crosses between another SC *S. hab* LA1223 and *S. lyc* show pollen tubes entering the ovary. In this case, a type C variability in pollen rejection with a change in pollen rejection/acceptance is seen.

4) Variable rejection of pollen from three wild red-fruited species varies in pistils of SC green-fruited species

Crosses using pollen from three wild red-fruited species onto the pistils of SC green-fruited species *S. neorickii* (three accessions), and *S. chmielewskii* (four accessions) showed variability of types a, b, and c. Details of pollen tube growth are described below in order of female species.

Table 3.7 Pollen tube growth in mm of three wild red-fruited species in the pistils of *S. neorickii* and *S. chmielewskii*. Percentage pollen tube length as a percentage of style length. (n)= number of replication. (Note: LA4023 crosses done in Colorado State University. LA2403 crosses done in U.C. Davis; All crosses of *S. chm* done in U.C. Davis).

		S. pim	*S. gal*	*S. che*
	Accessions	LA1589	LA0317/LA1408	LA0522/LA0421
S. neo	LA4023	2.4 ± 0.3 (59%) (7)	2.5 ± 0.5 (50%) (3)	2.5 ± 0.4 (57%) (4)
	LA2403		4.7 ± 0.1 (100%) (5)	4.9 ± 0.1 (100%) (4)
S. chm	LA1316	5.9 ± 0.3 (75%) (4)	7.1 ± 0.2 (82%) (4)	7.1 ± 0.3 (82%) (3)
	LA3643	5.9 ± 0.1 (100%) (3)		
	LA1325		7.4 ± 1.2 (100%) (5)	7.3 ± 0.1 (100%) (4)

a) *S. neorickii*

Three different SC accessions of *S. neorickii* (LA4023, LA1321, and LA2403) were used as female in this study. When SC *S. neo* LA4023 was used as female, pollen from four red-fruited species was rejected after average of 2.4mm with considerable variability in the range of pollen tube lengths in each cross (viability type b). In SC *S. neo* LA4023 styles, all of the crosses with pollen of red-fruited species show consistent pollen rejection (Table 3.7; Fig. 3.22; Fig. 3.23). Pollen tubes stop growth at approximately half of the style length with

one exceptional cross with pollen tube reaching the oavary that may have been mislabeled out of total 31 crosses of SC *S. neo* LA4023 with pollen from red-fruited species.

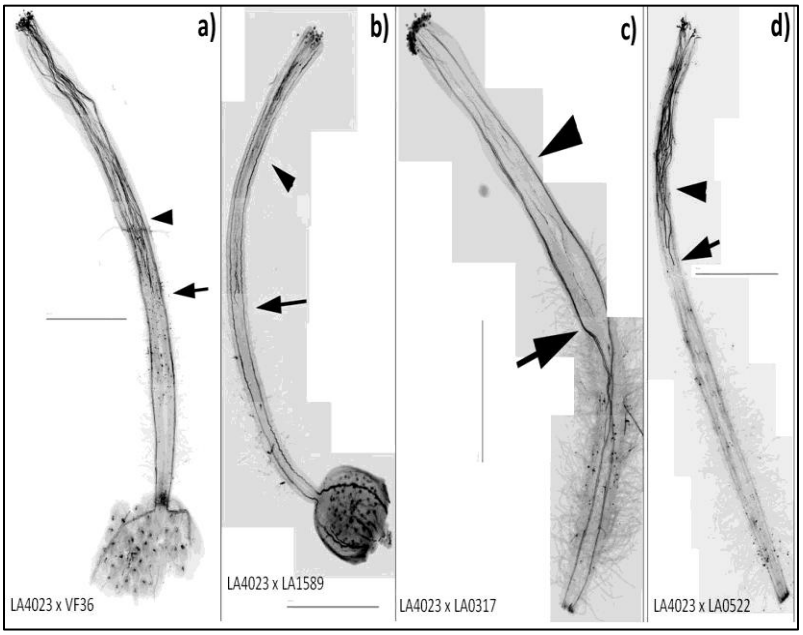


Figure 3.22 The red-fruited species pollen tube growth in the pistils of SC *S. neorickii*, LA4023.
a) LA4023 x *S. lyc* VF36,
b) LA4023 x *S. pim* LA1589,
c) LA4023 x *S. gal* LA0317,
d) LA4023 x *S. che* LA0522. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

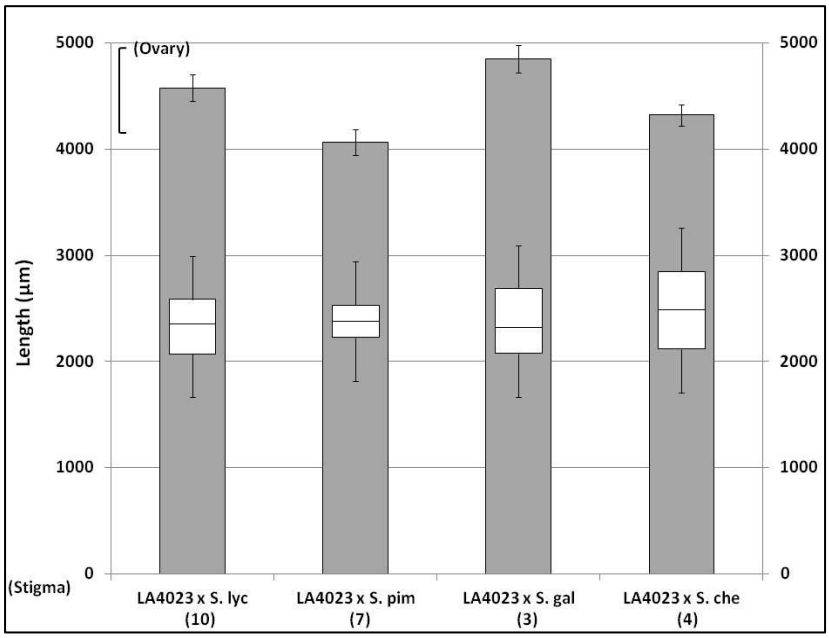


Figure 3. 23 Comparison of pollen tube lengths among the red-fruited species in the style of *S. neo* LA4023.

Other accessions of *S. neorickii* show variability (type c) in pollen rejection with pollen from the Galapagos red-fruited species (*S. galapagense, S. cheesmaniae*). As mentioned before, pollen tubes from Galapagos tomatoes fail to grow farther than 2.7 mm in

the styles from SC *S. neo* LA4023 on average, but the styles from SC *S. neo* LA2403 accept pollen of the two Galapagos tomato species (Table 3.7; Fig. 3.24; Fig. 3.25). Also, one out of three crosses of SC *S. neo* LA1321 x SC *S. pim* LA1383 do not show pollen tube rejection while the other two crosses show pollen rejection (data not shown). Thus, crosses between *S. neorickii* and the wild red-fruited species demonstrate variability (type c).

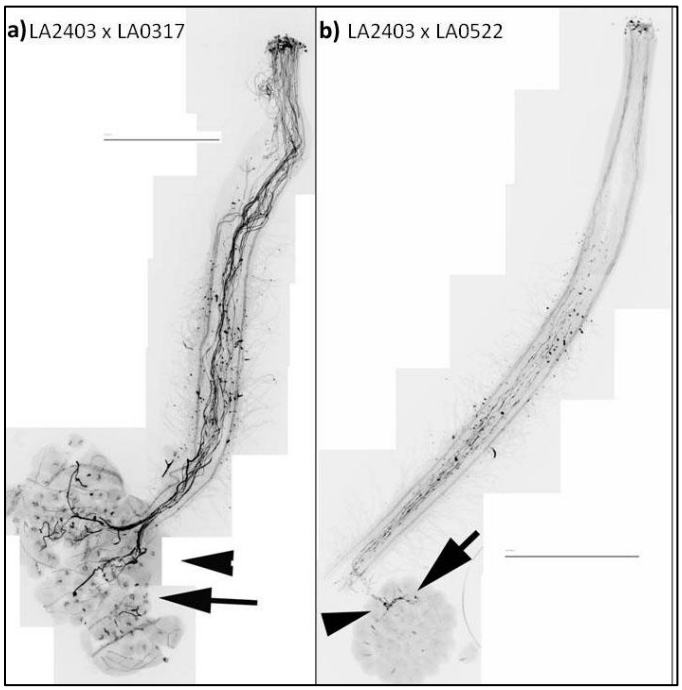


Figure 3.24 *S. gal* LA0317 and *S.che* LA0522 pollen tube growth of in the styles of SC *S. neorickii* LA2403. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

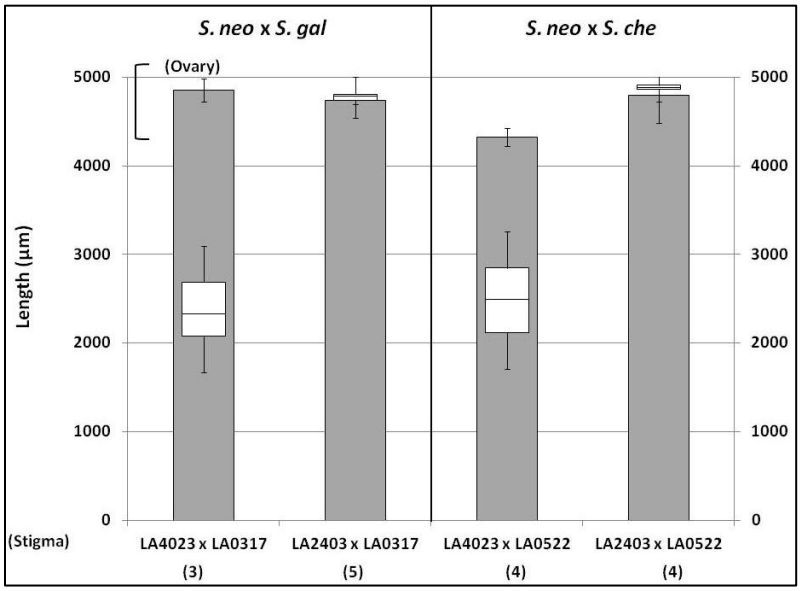


Figure 3.25 Comparison of pollen tube lengths of *S. gal* LA0317 and *S. che* LA0522 in the pistils of different accessions of *S. neorickii* LA4023 and LA2403.

b) *S. chmielewskii*

Three different accessions of SC *S. chmielewskii* were used as female (LA1316, LA3643, and LA1325) in crosses with the wild red-fruited species. When SC *S. chm* LA1316 was used as female, pollen from the red-fruited species did not grow further than average of 6.2 mm, in which tubes travel through most of the style (75~80%) before stopping(Table 3.7; Fig. 3.26).

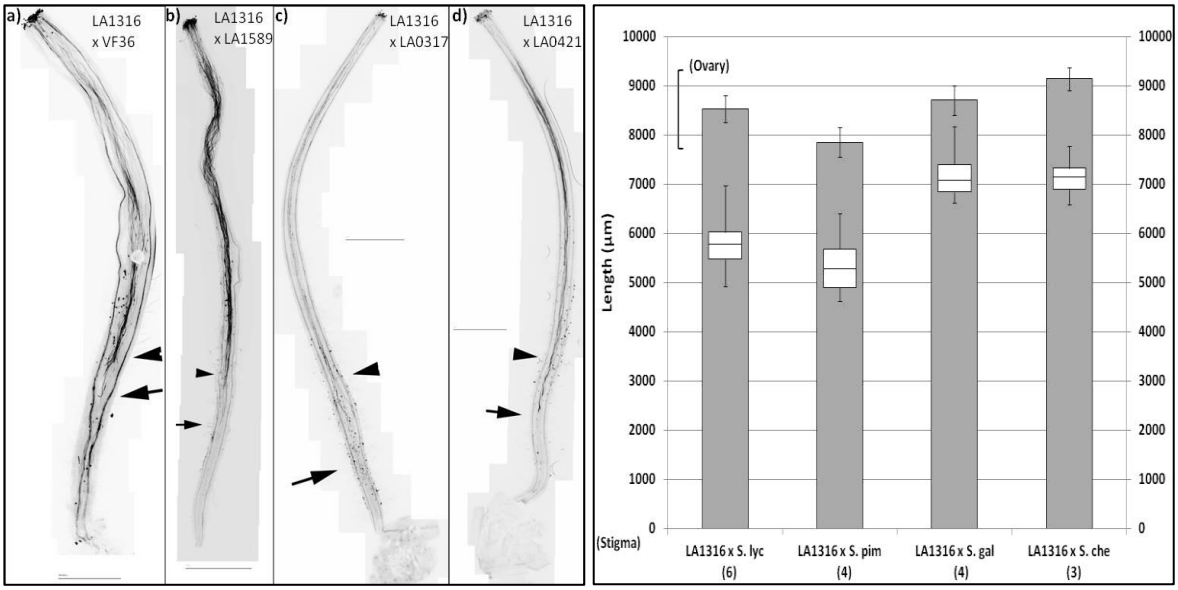


Figure 3.26 The red-fruited species pollen tube growth in the pistils of SC *S. chm*, LA1316. a) LA1316 x *S. lyc* VF36, b) LA1316 x *S. pim* LA1589, c) LA1316 x *S. gal* LA0317, d) LA1316 x *S. che* LA0421. Arrowhead represents average of pollen tubes in the style; Arrow indiciates the longest pollen tube in the style (Left). Pollen tube lengths among the red-fruited species in the pistils of SC *S. chm* LA1316 (Right).

However, in crosses of other accessions of *S. chm* with the wild red-fruited species, variability of Type C was observed. For example, SC *S. chm* LA3643 accepted pollen from *S. pim* (Table 3.7; Fig 3.27). It is possible that in the case of *S. chm* "rejection" or "not reaching ovary" may depend on style length. In all cases, *S. pim* pollen tubes grow about 5.5mm. Style lengths on average in *S. chm* are 8.5 mm for LA1316 and 5.9 mm for LA3643, as shown in Fig. 3.27. The same kind of variability due to different female accessions was

observed in crosses of *S. chm* x *S. gal* and *S. chm* x *S. che* (Table 3. 7; Fig. 3.27). *S. gal* and *S. che* pollen tubes fail to reach the ovary of SC *S. chm* LA1316 after the pollen tubes traverse 7.1 mm of the 8.5 mm style. However, pollen tubes of *S. gal* and *S. che* are able to traverse the entire style and reach the ovary of SC *S. chm* LA1325. As mentioned before, different female accessions of *S. chm* have different style lengths, 8.5 mm for LA1316 and 7.4 mm for LA1325, while the length of grown pollen tubes of *S. gal* and *S. che* in both these accessions is similarly 7.2 mm (Table 3.7; Fig. 3.27). Variability in pollen acceptance/rejection was observed among different female accessions.

Therefore, it is possible that pollen tubes from red-fruited species cannot reach the ovary of *S. chm* if the style exceeds a certain length. In other words, there may be a physical rather than genetic basis for the success or failure of these crosses, or probably not active rejection.

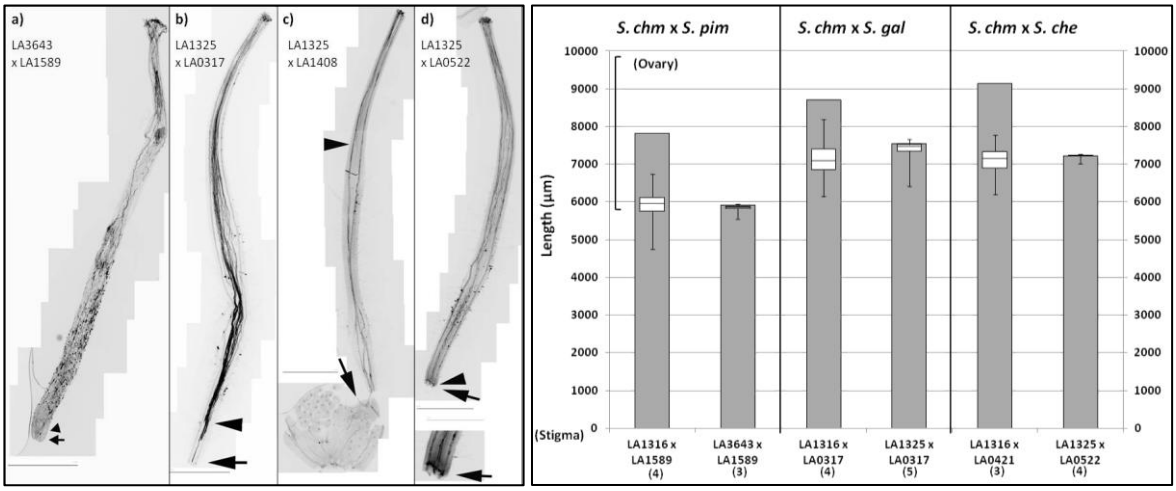


Figure 3.27 a) SC *S. pim* LA1589 pollen tube growth in the style of SC *S. chm* LA3643. b~d) the Galapagos species pollen tube growth in the styles of SC *S. chm* LA1325. Arrowhead represents average of pollen tubes in the style. Arrow indicates the longest pollen tube in the style (Left). Comparison of pollen tube lengths of *S. pim* LA1589 and the Galapagos species in the styles of different female accessions of SC *S. chm* LA1316, LA3643 and LA3656.

5) Variable rejection of pollen from three wild red-fruited in pistils of SC populations of *S. arcanum* and *S. habrochaites.*

Pollen from the wild red-fruited species shows variability in pollen rejection in the pistils of green-fruited SC populations of *S. arcanum* and *S. habrochaites* with different male accessions of *S. pimpinellifolium.* Only one accession of each SC *S. arcanum* and *S. habrochaites* is available to use as the female in these crosses. Variability of types c and d were seen in these crosses

Table. 3.8 Three wild red-fruited species pollen tube growth in mm in the pistils of SC populations of *S. arcanum*, and *S. habrochaites.* Pollen tube length as a percentage of style length is shown in parentheses. (n) = number of replications

		S. pimpinellifolium				*S. galapagense*	*S. cheesmaniae*
		LA3798	LA1589	LA1590	LA2149	LA0317	LA0522/LA0421
S. arc (SC)	LA2157	5.5 ± 0.5 (79%) (3)	5.1 ± 0.5 (100%) (3)	6.1 ± 0.5 (100%) (3)		5.9 ± 0.3 (100%) (3) 5.4 ± 0.5 (85%) (2)	6.0 ± 0.1 (100%) (3) 4.3 ± 0.3 (69%) (3)
S. hab (SC)	LA0407	8.6 ± 0.3 (99%)(2) 2.9 ± 0.8 (30%)(5)	4.4 ± 1.0 (58%) (6)		3.0 ± 0.5 (45%)(3)	8.3 ± 0.3 (100%) (4) 6.2 ± 0.4 (72%) (5)	7.6 ± 1.9 (100%) (2) 2.7 ± 1.1 (28%) (2)

a) *S. arcanum* (SC population)

LA2157 is the only known SC population of *S. arcanum*. Variability of type c was observed in pollen rejection using different male accessions of *S. pimpniellifolium* as male in the pistils of SC *S. arc* LA2157. Pollen from SC *S. pim* LA3798 was the only accession not reached the ovary, while other populations of *S. pim*, LA1383, LA1589, and LA1590, were accepted (Table 3.8; Fig 3.28). Lengths of pollen tubes among different male accessions of *S. pm* are similar between accepted and rejected pollen tubes. Pollen tubes

from *S. pim* LA3798 stop growth at 5.5 mm on average in the style, which is longer than the length of accepted pollen tubes (5 mm) of *S. pim* LA1589. The longest pollen tube from *S. pim* LA3798 was 5.8 mm, which is longer than styles used in crosses for *S. pim* LA1589 and almost similar to styles used in crosses for *S. pim* LA1383 (Fig. 3.28). Variability in pollen rejection/acceptance might be due to physical rather than genetic basis for the success or failure of these crosses, or probably not active rejection, as similar to observations in crosses with *S. chm*.

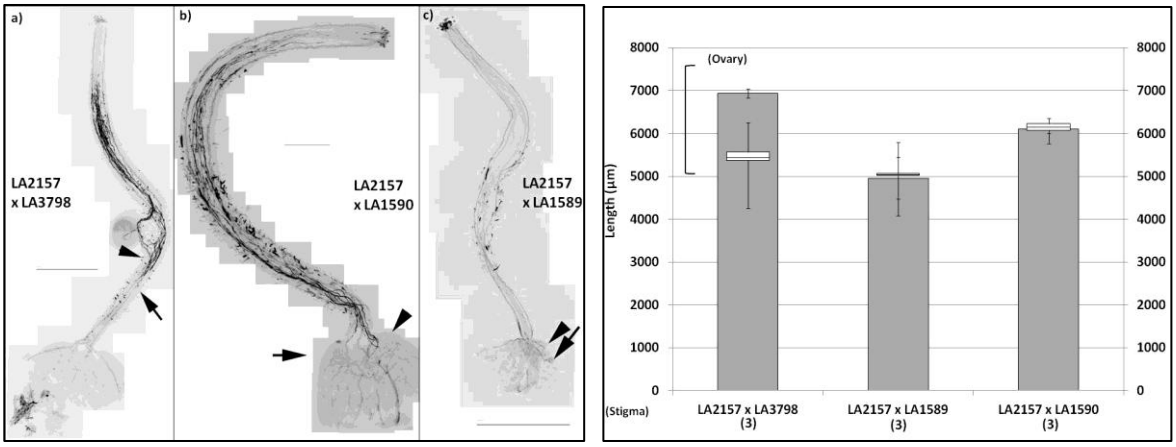


Figure 3.28 Pollen tube growth among different male accessions of *S. pimpinellifolium* in the pistils of SC *S. arcanum* LA2157. a) LA2157 x *S. lyc* VF36 and b-d) LA2157 x *S. pim* LA3798, LA1590, LA1589. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style (Left). Comparison of pollen tube length among different male accessions of *S. pim* LA3798, LA1589, LA1590, LA1383 in the pistils of SC *S. arc* LA2157.

The most puzzling type of variability (type d) is observed in crosses of SC *S. arc* LA2157 with pollen from *S. gal* and *S. che.* The same female pollinated with same pollen sample on the same day showed variability in whether tubes reached ovary seen at 48h and 72h (Note: normally pollen tubes reach ovary in 24h) (Table 3.8; Fig 3.29; Fig 3. 30). In 2/5 crosses of SC *S. arc* LA2157 x *S. gal*, pollen tubes from *S. gal* did not reach the ovary, while pistils of SC *S. arc* LA2157 accept pollen tubes from *S. gal* in 3/5 crosses. For

LA2157 x *S. che* crosses, rejection of pollen from *S. che* was seen in 3/6 crosses, while the other three crosses result in pollen tubes being accepted in the pistils of LA2157 (*S. arc,* SC) (Table 3.8. Fig. 3.29; Fig. 3.30).

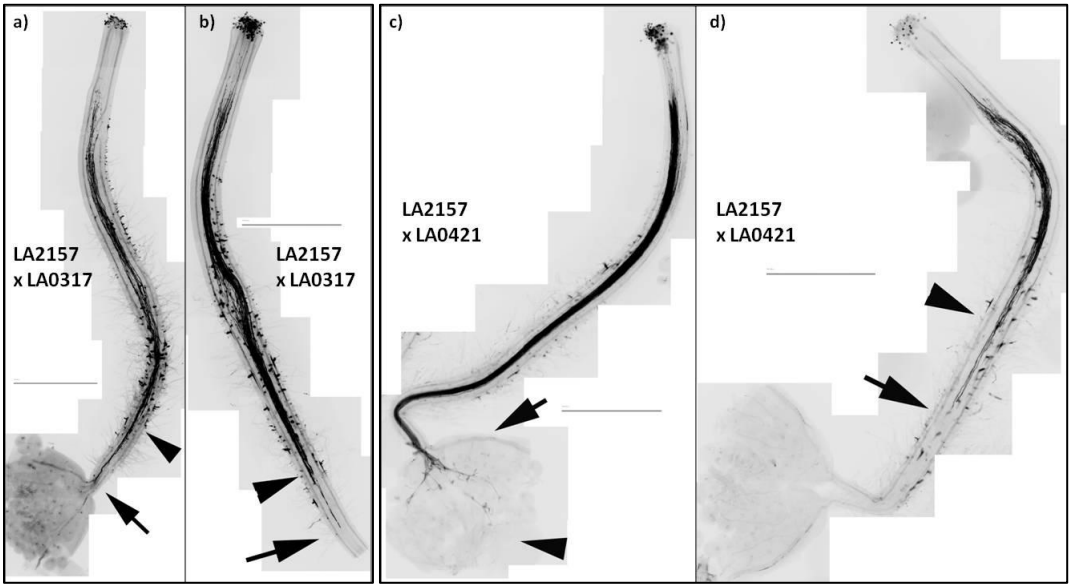


Figure 3.29 Galapagos tomato species (*S. gal* (LA0317), and *S. che* (LA0421) pollen tube growth in the pistils of SC *S. arc* LA2157. Arrowhead represents average of pollen tubes in the style; Arrow indicates the longest pollen tube in the style.

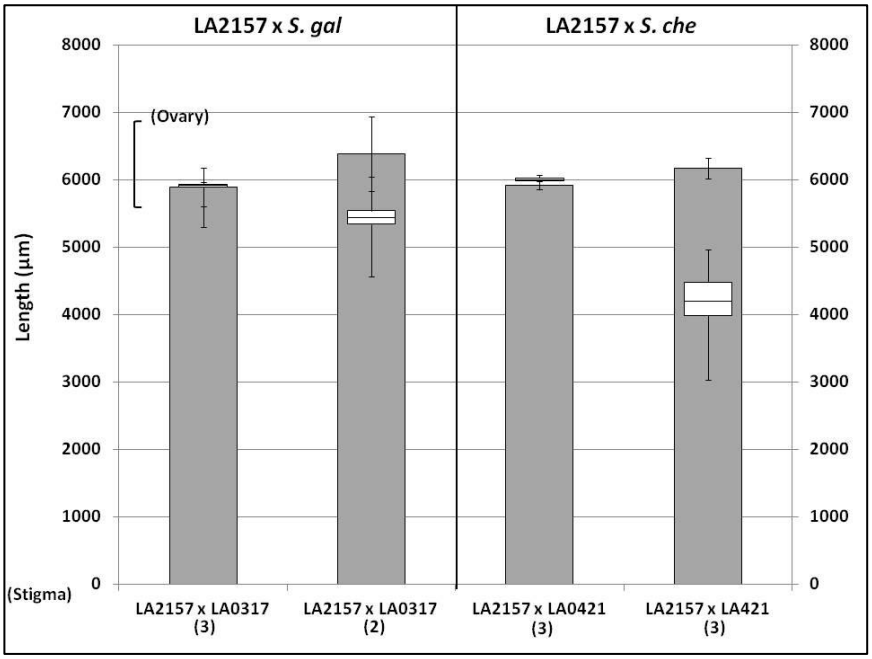


Figure 3.30 Comparison of pollen tube lengths of Galapagos species (*S. gal* LA0317, and *S. che* LA0421) in the pistils of SC *S. arc* LA2157.

b) *S. habrochaites* (SC population)

In this study, the northern *S. hab* SC LA0407 accession was used because it exhibits a "late" rejection of *S. lyc* pollen rather than "early" rejection seen in all other *S. hab* accessions (Covey et al. 2010). Pollen tube growth of different accessions of *S. pimpinellifolium* exhibits variability of type a, and d (Table 3.8), because variability in pollen tube length is exhibited between different male accessions and also in whether pollen is rejected as shown in Fig. 3.31 and Fig. 3.32. Pollen from SC *S. pim* LA1589, LA1617, and LA2149 is rejected at different points by the style of *S. hab* SC LA0407. *S. pim* LA3798 pollen can be either rejected or accepted due to the puzzling variability of type d. Another possibility is that females are segregating for pollen acceptance or rejection. Since I did not note which individuals were used in each cross, more studies are needed to fully understand the basis of this variability.

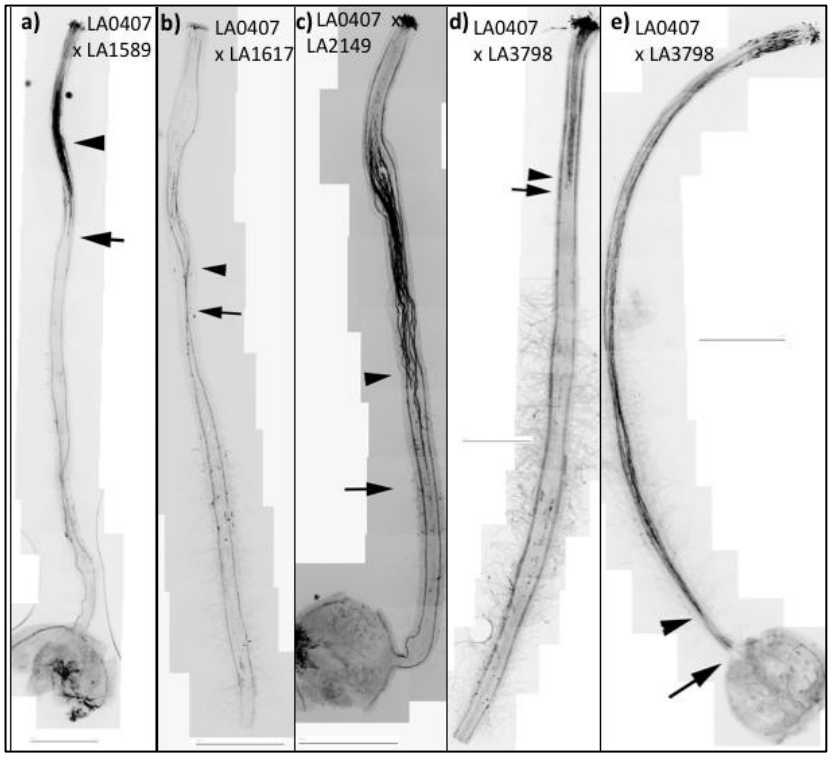


Figure 3. 31 Pollen tube growth among different accessions of *S. pim* LA1589, LA1617, LA3798 in the pistils of SC *S. hab* LA0407. Arrowhead represents average of pollen tube growth. Arrow indicates the longest pollen tube in the style.

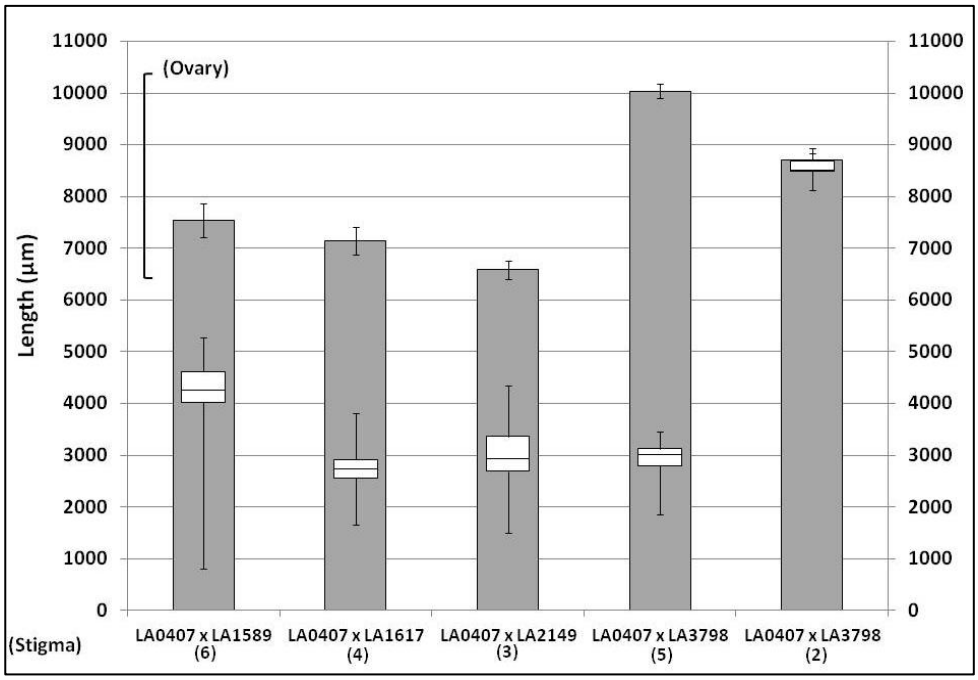


Figure 3. 32 Comparison of pollen tube lengths among different male accessions of *S. pim* LA1589, LA1617, LA2149, LA3798 in pistils of SC *S. hab* LA0407.

Variability in pollen tube growth and/or pollen rejection was also observed in crosses of SC *S. hab* LA0407 with pollen from S. *gal* and *S. che* (Table 3.7). Two accessions of *S. gal* were used as pollen donor, LA0317 and LA1408. With pollen from *S. gal* LA0317, 5/9 crosses show rejection and 4/9 do not. In two crosses of SC *S. hab* LA0407 with another *S. che* pollen donor, LA0522, a few tubes do reach the ovary (note: variability in LA0407 style length was observed).

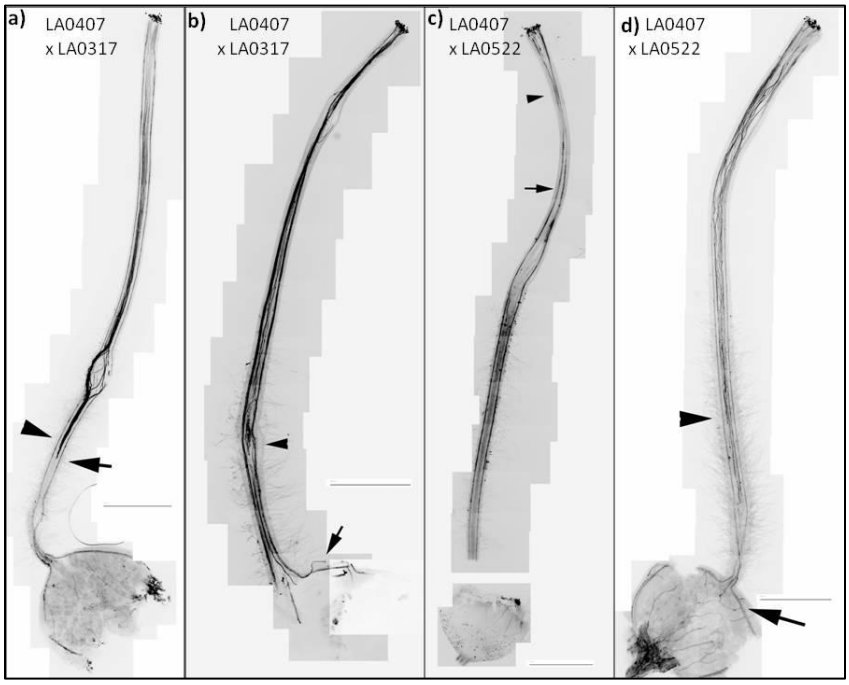


Figure 3.33 Pollen tube growth in the pistils of LA0407 (*S. habrochaites* SC) with Galapagose tomato species (*S. gal*; LA0317 and *S. che*; LA0522). Arrowhead represents average of pollen tubes in the style. Arrow indicates the longest pollen tube in the style.

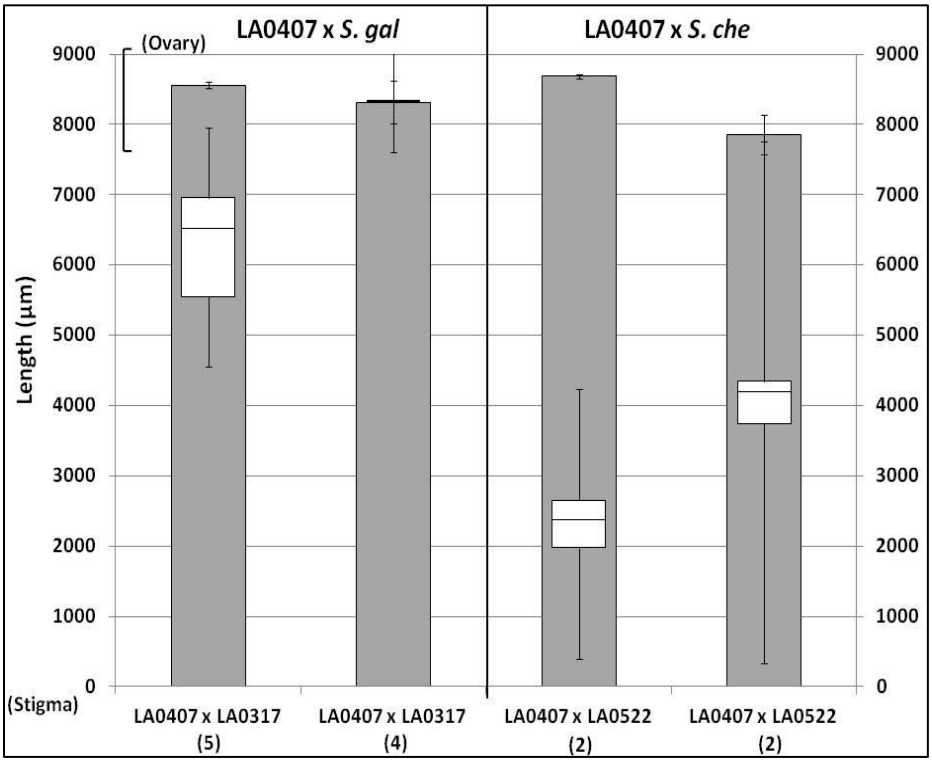


Figure 3.34 Comparison of pollen tube length of *S. gal* (LA0317, LA1408) and *S. che* (LA0421, LA0522) in the pistils of LA0407 (*S. hab* SC).

Discussion

In this study, I performed interspecific crosses using domesticated and wild red-fruited tomato species as pollen donors on pistils of green-fruited members of the tomato clade. Since UI as a reported prezygotic barrier has only previously been investigated only in *S. lycopersicum*, the domesticated tomato, I sought to understand whether UI would be found in the context of wild species. Since *S. pimpinellifolium* is often found growing sympatrically with other wild species (Chapter 4), these crosses are particularly relevant to natural populations.

Intercrosses within red-fruited species produced seeds as reported by Rick (1963). All the green-fruited species rejected pollen from *S. lycopersicum* as expected, although some variability in average pollen tube lengths was detected. Pollen from other wild red-fruited species was rejected only by green-fruited SI species whereas green-fruited SC species displayed variable pollen rejection in crosses with three wild red-fruited species. SC populations of *S. arc* and *S. hab* showed the greatest variability in pollen tube growth.

Mutschler and Liedl (1994) summarized interspecific crosses to investigate reproductive barriers by looking at seed set in *Lycopersicon*. UI can also be observed by examining pollen tube growth in crosses, and this UI often generally conforms to the "SI x SC rule" (Lewis and Crowe 1958).

Although in most cases I observed pollen rejection following the SI x SC rule, some cases of UI not following the "SI x SC rule" were also observed in this study. For example, SC *S. neorickii* and SC *S. chmielewskii* reject interspecific pollen of *S. lycopersicum,* so UI can be seen with an SC x SC cross. In addition, variability in rejection of pollen from the wild red-fruited species was observed in the pistils of SC *S. neorickii* and SC *S. chmielewskii.*

Liedl (1996) performed intercrosses between SC populations of *S. pennellii* and *S. lycopersicum* and found UI; another example of UI in SC x SC crosses. An additional example of UI in an SC x SC cross is seen when crossing populations of *S. habrochaites* with *S. lycopersicum* (Covey et al. 2010).

I have observed some differences with previous studies of interspecific crosses in the tomato clade (Mutschler and Liedl 1994). For example, *S. neorickii* was reported to accept *S. lycopersicum* while rejecting *S. pimpinellifolium* and *S. galapagense* (Mutschler and Liedl 1994). In this study, *S. neorickii* rejects *S. lycopersicum* pollen with variable pollen rejection of three wild red-fruited species, *S. pimpinellifolium, S. galapagense*, and *S. cheesmaniae* (depending on the female accession, there are cases in which *S. neorickii* accepts pollen of these wild red-fruited species). Previous studies reported *S. chmielewskii* as rejecting pollen of all the red-fruited species, but in my study of *S. chmielewskii* showed variability type c in pollen rejection in crosses with three wild red-fruited species, depending on female populations.

Variable pollen tube growth and rejection

I frequently observed variability in pollen tube growth and rejection (Table 3.3). Variability can depend on either the male or female genotype. In some cases, it should be noted that variability in crosses may involve style length (e.g. *S. chmielewskii* x *S. pimpinellifolium, S. galapagense,* and *S. cheesmaniae*). It should be noted that even with the

precaution of using a day before bud break (anthesis), self-pollen contamination when using SC green-fruited species as female in crosses cannot be ruled out.

When *S. pimpinellifolium* was used as a pollen donor in crosses, variability in average pollen tube length was observed (type a). *S. pimpinellifolium* has been known as a highly heterogeneous species (Rick et al. 1977). Since *S. pimpinellifolium* has a large amount of genetic variability between and within populations, these genetic factors may underlie this phenotypic variability.

The *ui6.1* gene is known pollen UI factor and may be involved in variable pollen tube growth. The *ui6.1* locus was mapped and found to contain a gene called Cullin1 (CUL1; Li et al. 2010; Li and Chetelat 2010). A deletion in *CUL1* was found in red-fruited species (*S. lyc, S. gal,* and *S. che),* while a full-length intron of *CUL1* was detected in green-fruited species (Li and Chetelat 2010). Interestingly, different populations of *S. pimpinellifolium* showed either the deletion or the full-length allele. *S. pim* population LA1589 which frequently grows further in interspecific styles than other *S. pim* accessions, contains the full-length allele and the deletion allele is detected in LA3798. However, recently, it has been suggested that *CUL1* found in LA1589 (*S. pim*) may be non-functional (Chetelat personal communication).

Several crosses show variable pollen rejection depending on different female populations. Two different populations of *S. neorickii* give different pollen rejection results when crossed with pollen of the Galapagos tomato species (*S. galapagense* and *S. cheesmaniae*). Pistils of SC *S. neo* LA4023 reject pollen from both species, while pistils of another accession; SC *S. neo* LA2403 accepts pollen from both. These differences between accessions are very interesting, because *S. neorickii* is an autogamous species with relatively

low inter-population genetic diversity. However, crosses that showed pollen rejection were done in Colorado State University, while crosses that showed pollen acceptance were done in UC. Davis. These crosses are needed to repeat with both accessions in both places.

Variability depending on female populations was observed in crosses of *S. chmielewskii*, a facultative autogamous species with high levels of heterozygosity. In crosses of *S. chmielewskii* with pollen from wild red-fruited species, three populations were used as female. One of these accessions, LA1316, has a longer style (average of 8.5 mm) compared to the other accessions (5.9 mm to 7.3 mm).

Lee et al. (2008) observed that the pollen donor's style length and interspecific seed set are positively correlated suggesting that pollen from short-style species cannot traverse in long styles due to limitations of pollen growth. Pollen tubes from a population of *S. pimpinellifolium*, LA1589 (*S. pim*), with a 8.2 mm style length at anthesis, traversed 6.5 mm on average in the 8.8 mm of LA1316 (*S. chm*) pistils, ultimately failing to reach the ovary. However, some pollen tubes from LA1589 (*S. pim*) were able to reach the ovary in *S. chm* LA3645, traversing on average 5.5 mm of the on average 5.7 mm styles. Therefore, in this case, style length may be an important factor in determining whether or not pollen tubes reach the ovary. Variability in SC *S. arc* LA2157 crosses also could be due to style length variability.

There could also be a genetic basis for the variable pollen tube growth or pollen rejection in crosses with red-fruited species. Variability in pollen rejection of red-fruited species was observed only in crosses with green-fruited SC species and SC populations of *S. arcanum* and *S. habrochaites* (Table 3.9). According to previous studies by Kondo et al. (2002) and Covey et al. (2010), the same green-fruited SC species and SC populations lack

two or more SI factors that might be involved in UI (i.e., *S*-RNase, HT-A, and HT-B proteins; Table 3.9). Green-fruited SC species and SC populations of *S. arcanum* and *S. habrochaites* show little or no activity of *S*-RNase (due to transcriptional depression, or decreased activity) and also lack HT-B proteins. However, all of these have functional HT-A proteins. It should be noted that all red-fruited species lack all three of these factors and consistently have lost the ability to reject self and interspecific pollen. All green fruited SI species contain S-RNases and at least one HT protein and consistently reject pollen from all red fruited species. However, green-fruited SC species (and SC populations of SI species), which only express HT-A, show variability in rejection of red-fruited species pollen. The SC population of *S. pennellii* (LA0716) is exceptional in that it lacks S-RNAse but consistently rejects the pollen of all red-fruited species. It has been proposed that there are multiple and redundant mechanisms for rejection of interspecific pollen (Covey et al. 2010), and the IRB functioning in SC *S. pen* LA0716 may be representative of this redundancy of mechanisms.

Table 3.9 Summary of factors involved in SI; *S*-RNase, HT-A, and HT-B in tomato species. Note; at the time of publication of this data, the two Galapagos species were a single species, and *S. arcanum* was thought to be *S. peruvianum.* (Kondo et a*l.* 2002; Covey et al. 2010)

	S-RNase activity	HT-A	HT-B	Pollen rejection/acceptance of red-fruited species
SC populations/ species				
Red-fruited				
S. lycopersicum	**No/ low**	**No**	**No**	**Accept**
S. pimpinellifolium	**No/ low**	**No**	**No**	**Accept**
S. cheesmaniae	**No/ low**	**No**	**No**	**Accept**
Green-fruited				
S. neorickii(SC)	**No/ low**	**Yes**	**No**	**Variability**
***S. chmielewskii* (SC)**	**No/ low**	**Yes**	**No**	**Variability**
***S. arcanum* population (SC)**	**No/ low**	**N/A**	**N/A**	**Variability**
***S. habrochaites* population(SC)**	**No/ low**	**Yes**	**No**	**Variability**
***S. pennellii* population (SC)**	**No/ low**	**Yes**	**Yes**	**Reject**
SI populations/ Species				
***S. arcanum* (SI)**	**High**	**Yes**	**Yes**	**Reject**
***S. chilense* (SI)**	**High**	**N/A**	**N/A**	**Reject**
***S. habrochaites* (SI)**	**High**	**Yes**	**No**	**Reject**
***S. pennellii* (SI)**	**High**	**Yes**	**Yes**	**Reject**

Summary

All red-fruited species in the tomato clade are closely related. However, I found that they act differently as pollen donors in the crosses I performed. Cultivated *S. lycopersicum* was rejected by all the green-fruited species, regardless of whether they were SI or SC. The three wild red-fruited species were rejected by green-fruited SI species, but showed variable pollen tube growth or rejection in pistils of green-fruited SC species or SC populations of SI species.

Future Work

This study of postmating prezygotic IRBs is being extended to study interspecific crosses in both directions among all members of the tomato clade. Most of these crosses have been performed as shown in Table 3.10. There is a pattern of interspecific crossing behavior in terms of pollen tube growth. In many cases, the UI barriers follow the "SI x SC Rule," i.e. all the SC species accept pollen from SI species while the SI species reject pollen from SC species except in two cases. One exception to this rule was that pollen tubes of SC *S. chmielewskii* traversed through the style of SI *S. corneliomulleri* to reach the ovary. In another possible exception, *S. habrochaites* failed to reject pollen from *S. neorickii* according to a previous study by Rick (1979). This is puzzling so I plan to repeat these crosses.

UI barriers were also found in crosses between SI *S. arcanum* and SI *S. chilense*. SI *S. arcanum* was rejected by SI *S. chilense* while the reciprocal crosses do not show pollen rejection. UI is also observed in crosses between SI *S. pennellii* and SI *S. habrochaites*;

pollen from *S. habrochaites* was rejected by *S. pennellii*, while the reciprocal cross was successful.

I plan to continue working on interspecific crosses which still need to be done. Only a few remain; *S. pim* by *S.chm*, *S. neo* by *S. per* and *S. chi*, and *S. arc* by *S. cor*. Also, in crosses where acceptance of pollen tubes has been demonstrated, I am analyzing seed set to understand whether postzygotic barriers could act to prevent interspecific hybridization in the wild. This study will contribute to a global understanding of interspecific reproductive barriers in the tomato clade.

Table3.10 Interspecific crossing behavior within the tomato clade

FEMALE	MALE											
	S. lyc (SC)	S. pim (SC)	S. gal (SC)	S. che (SC)	S. neo (SC)	S. chm (SC)	S. arc (SI)	S. per (SI)	S.cor (SI)	S. chi (SI)	S. hab (SI)	S. pen (SI)
S. lyc (SC)	SC	Seed	Seed	Seed	Seed	Seed	PZ	PZ	PZ	PZ	Seed	Seed
S. pim (SC)	Seed	SC	Seed	Seed	A	ING	A	A	A	A	Seed	Seed
S. gal (SC)	Seed	Seed	SC	Seed	Seed	Seed	PZ	PZ	PZ	PZ	Seed	Sed
S. che (SC)	Seed	Seed	Seed	SC	Seed	Seed	PZ	PZ	PZ	PZ	Seed	Seed
S. neo (SC)	R	R	A/R	A/R	SC	A	A	ING	A	ING	Seed	Seed
S. chm (SC)	R	A/R	A/R	A/R	Seed	SC	A	A	A	A	A	A
S. arc (SI)	R	R	R	R	R	R	SI	A	ING	A	A	A
S. per (SI)	R	R	R	R	R	R	A	SI	A	A	PZ	A
S. cor (SI)	R	R	R	R	R	A	R	A	SI	A	A	A
S. chi (SI)	R	R	R	R	R	R	R	A	A	SI	PZ	A
S. hab (SI)	R	R	R	R	Seed	R	A	PZ	A	A	SI	A
S. pen (SI)	R	R	R	R	R	R	A/R	A	A	A	R	SI

SC = self-incompatibility, SI = self-incompatibility, R = Pollen rejection occurs, Seed = seed set, PZ = postzygotic barrier, A = pollen tubes reach the ovary, A/R= Variability in pollen rejection, ING = crosses in progress. All of the crosses have been done in Bedinger lab except PZ crosses (Rick, 1979 and 1986; Rick et al. 1976).

CHAPTER 4:

REPRODUCTIVE BARRIERS BETWEEN SYMPATRIC POPULATIONS IN THE TOMATO CLADE

Introduction

A diversity of reproductive barriers prevents hybridization when plants grow sympatrically. Barriers can be classified as those that act either before fertilization (prezygotic) or after fertilization (postzygotic). Prezygotic barriers can be further divided into 1) pre-mating barriers that prevent pollen and ovules from coming into contact, and include ecogeographic isolation, flowering time, pollinator preference, and morphological differences between species, and 2) post-mating barriers that act during interaction of male and female prior to fertilization, including pollen-pistil interactions in higher plants. Postzygotic barriers include failure of seed/fruit production, low viability or fertility of hybrids (Levin 1971; Coyne and Orr 2004).

Floral morphology characters that can contribute to reproductive barriers include flower size, stigma exsertion, and flower color influence the frequency or effectiveness of pollinator visits or even change pollinator preferences. In the tomato clade, since they share pollinators, floral morphology characters play a role to prevent hybridization as premating reproductive barriers. It has been noted that decreases in flower size and insertion of stigmas both promote self-pollination (autogamy) over cross-pollination (allogamy), because smaller

flowers attract fewer pollinators and inserted stigmas receive less non-self-pollen (Rick et al. 1977; Georgiady 2002).

Flowers in the tomato clade rely on external pollinators such as bees. Compatible pollen is deposited on the stigmatic area and grows through the style into the ovary, while incompatible pollen tube growth is inhibited in the style. Thus, pollen-style interactions play a key role in determining the success of interspecific crosses in the tomato clade. Unilateral incompatibility or incongruity (UI) occurs between species to prevent hybridization. Liedl et al. (1996) studied UI between *S. pennellii* and *S. lycopersicum*. Pollen from *S. pennellii* grows through the styles of *S. lycopersicum* into ovary, while pollen of *S. lycopersicum* is rejected from *S. pennellii.*

Previous studies on interspecific barriers in the tomato clade have focused on seed production (Mutschler and Liedl 1994), using greenhouse-grown plants from the collection of wild germplasm collected throughout South America that is available through the Tomato Genetics Resource Center (http://tgrc.ucdavis.edu/). I wanted to test for reproductive barriers between populations of species that are known to be sympatric in natural settings. I expected to find these barriers, because hybrids have not been detected in the wild (R. Chetelat, personal communication).

Here, I examined three types of reproductive barriers that could act between 10 pairs of sympatric populations of wild tomato species. First, I focused on pre-mating isolation by comparing differences in stigma exsertion. I compared SI and SC species to track the evolutionary morphological trend from cross-pollinating (exserted stigma) to self-pollinating (inserted stigma), since we have three sympatric pairs with SI and SC members. I also examined pollen-pistil interactions between sympatric species pairs to assess postmating

prezygotic barriers. Finally, I assessed postzygotic isolation in terms of fruit and seed development in cases where prezygotic barriers did not seem to function between sympatric species.

Materials and methods

1. Plant Materials:

Germplasm of wild tomato species growing sympatrically at ten locations in Peru (shown in Fig. 3.1; Table 2.1) were obtained from the Tomato Genetic Resource Center (TGRC) at University of California, Davis (UC Davis). Plants were grown and pollinated in the greenhouse and in the field in Colorado and in some cases at UC Davis. One pair located at Tembladera was not available from TGRC. Five of these 10 pairs were confirmed to be growing sympatrically in Peru in 2009, but at three locations, Sisicaya, Surco and Asia-El Pinon, only one member of the pair was confirmed at the site. At one location, Rio-Pativilca, no wild tomatoes were found because the area was entirely planted with sugar cane. Since it was not possible to export seed from Peru in 2009, few crosses were performed at the sites. Then, crosses were imaged by International Potato Center (CIP), and analyzed at CSU. Therefore this study was limited to those accessions, representing sympatric pairs, available through the TGRC.

2. Measurement of Exserted Stigma

Stigma exsertion lengths were measured using 7 to 15 mature flowers at anthesis (+1 stage) from one individual. Mature flowers were collected and petals and sepals dissected away. Exserted stigma images were taken using a Nikon SMZ1500 (http://www.nikon.com/) dissecting microscope with Image-Pro_Plus software

(http://www.mediacy.com/index.aspx?page=IPP) coupled with a Nikon Digital camera DMX1200 (http://www.microscopyu.com/). From these images, visible exserted stigma lengths were measured using Image J 1.33 (http://rsb.info.nih.gov/ij/). In analyzing stigma exsertion between SI and SC populations, all measurements of stigma exsertion in each group were contrasted with each other using a t-test in Microsoft Excel.

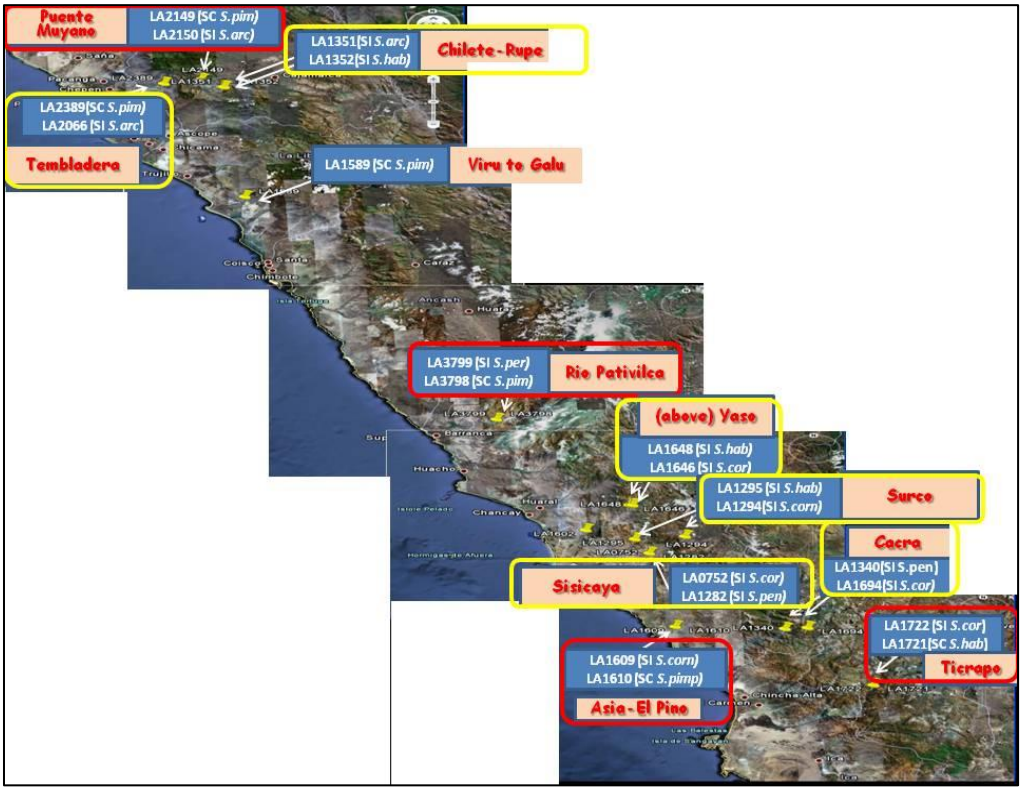


Figure 4.1 Map of sympatric pairs; red-circled groups: pairs consisting of SI and SC species; yellow-circled groups: pairs consisting of only SI species>

Table 4.1 Species at 10 sites including mating system, accession number, and corresponding collection number in Peru.

	Species	Mating system	Accession #	Collection # in Peru
Puento Muyano	*S. pimpinellifolium*	SC	LA2149	8044
	S. arcanum	SI	LA2150	8043
Chilete-Rupe	*S. arcanum*	SI	LA1351	8050
	S. habrochaites	SI	LA1352	8049
Tembladera	*S. pimpinellifolium*	SC	LA2389	8041
	S. arcanum (N/A)	SI	LA2066	8042
Rio Pativilca	*S. pimpinellifolium*	SC	LA3798	N/A
	S. peruvianum	SI	LA3799	N/A
Yaso	*S. corneliomulleri*	SI	LA1646	8031
	S. habrochaites	SI	LA1648	8029
	S. pimpinellifolium	SC	N/A	8030
Sisicaya	*S. corneliomulleri*	SI	LA0752	N/A
	S. pennellii	SI	LA1282	8024
Cacra	*S. pennellii*	SI	LA1340	8036
	S. corneliomulleri	SI	LA1694	8034
	S. pimpinellifolium	SC	N/A	8035
Asia-El Pino	*S. pimpinellifolium*	SC	LA1610	N/A
	S. corneliomulleri	SI	LA1609	N/A
Ticrapo	*S. habrochaites*	Facultative SC	LA1721	8039
	S. corneliomulleri	SI	LA1722	8040

3. Pollen-Pistil Interactions

Crosses between sympatric species were performed according to the Chapter 3 protocol. For analysis of pollen tube rejection or growth between sympatric pairs and allopatric populations, allopatric species are also listed in Table 3.1, Chapter 3. When I observed pollen rejection in both crosses between sympatric pairs and in crosses between allopatric populations, I compared pollen tube length. To compare pollen tube length, I used t-test and ANOVA in Microsoft Excel (2007).

4. *Postzygotic Barriers (Fruit Set)*

When pollen tubes successfully reached the ovary in crosses, I left pollinated crosses to produce fruit in the greenhouse. Fruits were collected as they were soft. Collected fruits were cut in half and seeds were squeezed out of the fruit compartment. Collected seeds were washed with ddH_2O and left in 12ml culture tubes with small amount of water, where they remained at room temperature for 1 week to ferment. After 1 week, seeds were washed and left to dry overnight on filter paper.

Results

1) Premating barrier= Stigma exsertion

Comparison of exserted stigma length between SI and SC species.

In the tomato clade, less stigma exsertion promotes self-pollination (SC) over cross-pollination, thus decreasing the likelihood of hybridization with other species. I measured stigma exsertion in sympatric species to compare SI and SC species to see whether less exserted stigmas could provide a barrier between SC species and their outcrossing (SI) sympatric partner species (exserted stigma) to SC (inserted stigma).

After measuring exserted stigma length for all sympatric populations, SI and SC groups were clustered together for statistical analysis. The SC group consists of three accessions of *S. pimpinellifolium*, while the SI group contains two accessions of *S. arcanum,* one accession of *S. peruvianum*, six accessions of *S. corneliomulleri,* three accessions of *S. habrochaites*, and one accession of *S. pennellii.* The average length of stigma exsertion in the SC group was 0.38 $\pm$ 0.09 mm and 0.99 $\pm$ 0.14 mm for the SI group (Fig. 4. 2). A T-test based between the two groups gave a p-value is 0.0025, which suggests there is a significant

difference between two groups. The finding that the SC group has shorter exserted stigma length than the SI group is consistent with largely selfing mating system. Therefore, shorter stigma exsertion length could act as premating barrier in some sympatric pairs that include both an SC and an SI species.

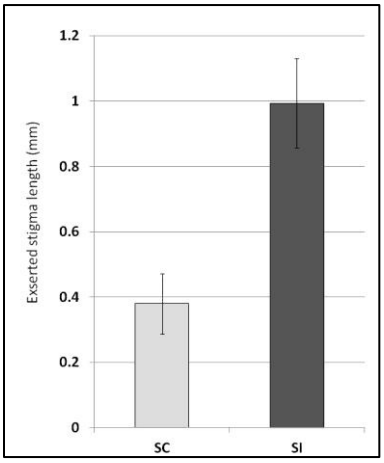


Figure 4. 2 Comparison of stigma exsertion between SC and SI groups.

Table 4. 2 Exserted stigma measurements in sympatric populations and allopatric populations. N/A= not available.

	Sympatric	Exserted stigma Length (mm)
S. pimpnielliofolium (SC)	LA2149	0.54 ± 0.14
	LA3798	0.21 ± 0.18
	LA1610	0.24 ± 0.09
S. arcanum (SI)	LA2150	0.83 ± 0.22
	LA1351	0.49 ± 0.19
S. peruvianum (SI)	LA3799	0.8 ± 0.18
S. corneliomuelleri (SI)	LA1609	1.03 ± 0.3
	LA1646	1.16 ± 0.22
	LA1694	0.83 ± 0.17
	LA1294	1.22 ± 0.2
	LA0752	1.25 ± 0.38
	LA1722	0.73 ± 0.18
S. habrochaites (SI)	LA1352	0.58 ± 0.28
	LA1648	0.89 ± 0.31
	LA1295	1.53 ± 0.26
(SI/SC)	LA1721	0.73 ± 0.18
S. pennellii (SI)	LA1340	2.22 ± 0.65
	LA1282	N/A

2) Pollen tube growth

Failure of pollen tube growth in pistil is one way to prevent hybridization in postmating prezygotic barriers in the tomato clade. Unilateral incompatibility/ incongruity prevents interspecific hybridization, as pollen tubes from a species are inhibited, while the reciprocal crosses do not show pollen rejection. UI usually is observed between SC and SI species, but may or may not occur between two SI species. I assessed pollen tube growth in reciprocal crosses between populations of species that grow sympatrically.

a) Interspecific barriers between sympatric species

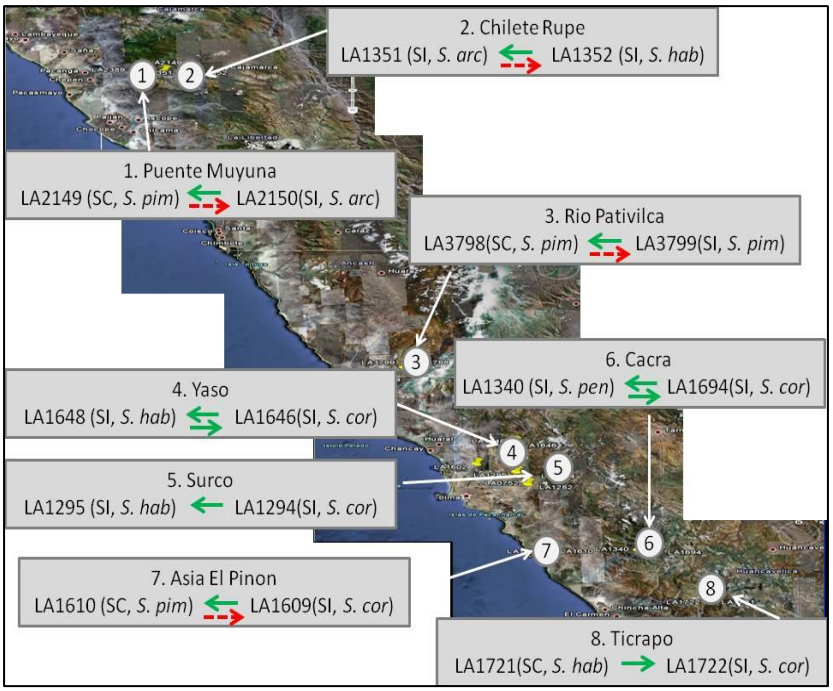


Figure 4. 3 Interspecific barriers between sympatric pairs at eight sites; arrows present pollen direction; green=accept; red=reject

1. Puente Muyano

This pair located at Puente Muyana consists of a population of SC *S. piminellifolium*, LA2149, and SI *S. arcanum*, LA2150. SC *S. pim* LA2149 accepted pollen from SI *S. arc* LA2150, whereas SI *S. arc* LA2150 rejected SC *S. pim* LA2149 pollen at 1.9 mm of the style

length on average. In some crosses of SC *S. pim* LA2149 by SI *S. arc* LA2150, pollen was unable to reach the ovary in 24 hr (6/10 crosses) but always reached the ovary in 48h. Thus, UI barriers were observed in 48h for this sympatric pair in the expected direction following the SI x SC rule.

In Peru, SI *S. arcanum* (8043) at the site was shown to be SI when a self-pollination was performed on site. Other crosses were not successful in Peru.

2. Chilete-Rupe

This pair consists of SI *S. arcanum* LA1351 and SI *S. habrochaites* LA1352. The styles of SI *S. hab* LA1352 rejected pollen from SI *S. arc* LA1351 at average of 8.9 mm which is 80% of the style. When SI *S. arc* LA1351was pollinated by pollen from SI *S. hab* LA1352, pollen tube growth differed when crosses were done in the field as opposed to in the greenhouse. 3/10 crosses done in the field showed that pollen tubes of SI *S. hab* LA1352 reached the ovaries of SI *S. arc* LA1351 in 48h, while the 7/10 crosses of this set showed that pollen tubes from SI *S. hab* LA1352 only grew 6.1 mm which is 73% of the style of SI *S. arc* LA1351. In crosses done in the greenhouse, pollen tubes consistently reached the ovary in 48h. Possibly unexpected UI barrier is observed between SI *S. hab* and SI *S. arc*.

Crosses on sites in Peru, pistils of SI *S. arcanum* (8050) and pollen from SI *S. habrochaites* (8049) revealed that *S. hab* pollen did not reach the ovary of SI *S. arcanum* 8050, instead growing halfway through the styles (4.6 mm).

3. Rio Pativilca

SC *S. pimpinlellifolium* LA3798, is paired with SI *S. peruvianum,* LA3799, at Rio Pativilca. The styles of SC *S. pim* LA3798 accepted pollen from *S. per* LA3799 while pollen

tubes of SC *S. pim* LA3798 were rejected at 1 mm in the style of *S. per* LA3799. Because no wild tomatoes were found at this site in 2009, no crosses were performed in Peru. As expected, UI barriers are found between SC *S. pim* and SI *S. per* and these follow SI x SC rule.

4. Yaso

The fourth pair, at Yaso, consists of SI *S. habrochaites,* LA1648 and SI *S. corneliomulleri* LA1646. Pollen tubes from SI *S. hab* LA1648 reach the ovary in the pistils of SI *S. cor* LA1646 and the reciprocal cross also shows acceptance of pollen. There was variation in pollen rejection between crosses done in the field and the greenhouse, when pollen tubes from SI *S. hab* LA1648 grew at 7.1mm which is 76% in the pistils of the SI *S. cor* LA1646 in the field, while no rejections occurred in crosses done in the greenhouse. Overall, in Colorado no prezygotic barrier was observed in reciprocal crosses between *S. hab* (SI) and *S. cor* (SI).

In addition, these crosses were performed on the site in Peru. SI *S. corneliomulleri* (8031) rejected pollen from SI *S. habrochaites* (8029) at 5.4 mm in the style length. There was another sympatric species, SC *S. pimpinellifolium* (8030), at this site in Peru (not used in Colorado studies). Pollinations of SC *S. pim* 8030 with two species were performed at the site of Peru as well. Pollen from SC *S. pim* 8030 was rejected at 1.4 mm in the style of SI *S. cor* 8031, while pollen from SI *S. cor* 8031 was accepted by pistils of SC *S. pim* 8030. Pistils of SC *S. pim* 8030 also accepted pollen from SI *S. hab* 8029. The crosses of SI *S. cor* with pollen from SI *S. hab* done in Peru are consistent with the results of the same crosses done in the field plots in Colorado.

5. Surco

This pair is composed of SI *S. habrochaites*, LA1295 and SI *S. corneliomulleri* LA1294 at Surco. In 2009, only *S. corneliomulleri* was found at this site. Crosses in one direction have been performed in Colorado; the styles of SI *S. hab* LA1295 accepted pollen from SI *S. cor* LA1294. Reciprocal cross will be done in the near future. As observed in a previous pair, the pistils of SI *S. hab* accept pollen from SI *S. cor*.

6. Cacra

The sixth sympatric pair at Cacra consists of SI *S. corneliomulleri* LA1694 and SI *S. pennellii* LA1340. Pollen from SI *S. cor* LA1694 was accepted by the pistils of SI *S. pen* LA1340 and LA1340 also accepts LA1694. Therefore, there were no postmating prezygotic barriers between SI *S. pen* and SI *S. cor*. There is another sympatric species, SC *S. pimpinellifolium* (8035), growing at the site of Peru but not available through TGRC. In crosses done in Peru, pistils of SI *S. cor* 8031 rejected pollen from SC *S. pim* 8035, while the reciprocal pollination did not show pollen rejection. Therefore, UI barriers are functioning between this SC and SI species.

7. Asia El Pinon

SC *S. pimpinellifolium*, LA1610 is paired with SI *S. corneliomulleri* LA1609 at Asia-El Pino. SC *S. pim* LA1610 accepted pollen from SI *S. cor* LA1609, while pollen tubes of SC *S. pim* LA1610 were arrested at 1.3 mm in the pistil of SI *S. cor* LA1609. Therefore, UI barrier between SC *S. pim* and SI *S. cor* were observed according to the SI x SC rule. Since no *S. pimpinellifolium* was found at this site in 2009, no crosses in Peru were performed.

8. Ticrapo

The last pair consists of SC *S. corneliomullei* LA1722 and SI *S. habrochaites* LA1721 at Ticrapo. Only one cross of pistils of SC *S. hab* LA1721 with pollen from SI *S. cor* LA1722 has been done so far, and pistils of SC *S. hab* LA1721 accepted pollen from SI *S. cor* LA1722. The reciprocal cross has not been performed yet.

The results of reciprocal crosses between sympatric pairs in terms of pollen tube growth are pistils are shown in Fig 4.3. As expected, all SC and SI crosses exhibited UI which followed SI x SC rule, i.e., pollen from SC species is rejected by pistils from SI species, while the reciprocal cross does not show pollen rejection. In crosses between sympatric populations of SI species, pollen tubes seem to be accepted in crosses in both directions, with one possible exception (Chilete-Rupe pair).

b) Comparison of pollen tube acceptance and rejection between sympatric pairs and allopatric populations.

Crosses were made using non-sympatric populations (= allopatric) to compare pollen rejection between sympatric and allopatric populations (Table 4.3). Because I did not have allopatric populations of *S. corneliomuelleri*, populations from different sympatric sites were used.

Only one difference in pollen tube acceptance was found. In sympatric population at the Chilete-Rupe sites, pollen tubes from SI *S. arc* LA1351 were rejected by pistils of SI *S. hab* LA1352. Pollen tubes of LA1351 grew very far in the style of LA1352, but most pollen tubes failed to reach the ovary (Note, few 1~3 pollen tubes grew almost the end of the style, but none of pollen tubes found in the ovary). In contrast, when allopatric populations of these

species were used, pollen from SI *S. arc* LA2163 was accepted by the pistils of SI *S. hab* LA1777.

Table 4. 3 Comparison of pollen rejection between sympatric pairs and allopatric populations; arrows represent pollen direction; green=accept; red=reject.

Sites	Symp/Allp	Accessions (species)	Pollen direction	Accessions (species)
1. Puente Muyuna	Sympatric	LA2149 (*S. pim*)		LA2150 (*S. arc*)
	Allopatric	LA1589 (*S. pim*)		LA1708 (*S. arc*)
2. Chilete Rupe	Sympatric	LA1351 (*S. arc*)		LA1352 (*S. hab*)
	Allopatric	LA2163 (S. arc)		LA1777 (*S. hab*)
3. Rio Pativilca	Sympatric	LA3798 (*S. pim*)		LA3799 (*S. per*)
	Allopatric	LA1589 (*S. pim*)		LA4317 (*S. per*)
	Allopatric	LA1581 (*S. pim*)		LA0371 (*S. per*)
4. Yaso	Sympatric	LA1646 (*S. cor*)		LA1648 (*S. hab*)
	Allopatric	LA1694 (*S. cor*)		LA1777 (*S. hab*)
5. Surco	Sympatric	LA1294 (*S. cor*)		LA1295 (*S. hab*)
	Allopatric	LA1694 (*S. cor*)		LA1777 (*S. hab*)
6. Cacra	Sympatric	LA1340 (*S. pen*)		LA1694 (*S. cor*)
	Allopatric	LA2560 (*S. pen*)		LA1609 (*S. cor*)
7. Asia El Pinon	Sympatric	LA1610 (*S. pim*)		LA1609 (S. cor)
	Allopatric	LA1589 (S. pim)		LA1694 (S. cor)
8. Ticrapo	Sympatric	LA1722 (*S. cor*)		LA1721 (*S. hab*)
	Allopatric	N/A		N/A

c) Comparison of pollen tube length between sympatric pairs and allopatric populations.

The extent of pollen tube growth was also compared in sympatric vs. allopatric crosses where UI is observed. In pairs at Puente Muyuna, Rio Pativilca, and Asia El Pinon, SI species (*S. arc*, *S. per*, and *S. cor*) rejected SC species (*S. pim*), and it was observed in allopatric crosses as well. Pollen tube growth was compared among four different crosses; 1) crosses in sympatric pairs, 2) crosses using allopatric female and sympatric pollen, 3) crosses

using sympatric female and allopatric pollen, 4) crosses between allopatric populations (Summarized in Table 4. 4).

In most cases, pollen tube growth in crosses between sympatric and allopatric species pairs did not display a significant difference in pairs at Puente Muyuna and Rio Pativilca. (1.4 ~1.9 mm, $p > 0.05$).

However, the pair at Asia El- Pino shows different pollen tube lengths of SC *S. pim* in four crosses ($p < 0.05$; Table 4.4). Pollen tubes of allopatric population of SC *S. pim* LA1589 grew slightly farther in the pistil of sympatric SI *S. cor* LA1609 than pollen from sympatric accession of SC *S. pim* LA1610. However, it should be noted that pollen from LA1589 tends to grow longer than pollen from other *S. pim* accessions in pistils of several different SI species (Chapter 3).

Table 4. 4 Comparison of pollen tube lengths in mm among crosses between sympatric and allopatric populations.

	Sympatric pair	Allopatric x sympatric	Sympatric x allopatric	allopatric x allopatric	P-value
Puente Muyuna	LA2150 x LA21549	LA2163 x LA2149	N/A	LA1708 x LA1589	0.467161
	1.9 ± 0.37	1.4 ± 0.14		1.9 ± 0.23	
Rio Pativilca	LA3799 x LA3798	LA4317 x LA3798	LA3799 x LA1589	LA0371 x LA1581	0.116468
	0.8 ± 0.08	1.4 ± 0.55	0.9 ± 0.12	0.9 ± 0.03	
Asia El Pinon	LA1609 x LA1610	LA1694 x LA1610	**LA1609 x LA1589**	LA1694 x LA1589	0.025175
	1.3 ± 0.03	1.6 ± 0.2	**2.3 ± 0.4**	1.5 ± 0.2	

3) Postzygotic barriers – Fruit set

In the sympatric crosses that do not show prezygotic barriers, postzygotic isolation in terms of fruit and seed development was assessed when possible. Due to the late flowering time of *S. habrochaites* outdoor in Colorado, and poor flowering of this species under

greenhouse conditions, fruit and seed development was not assessed when this species was the female in crosses.

As shown in table 4.5, five of these crosses successfully made fruits. Five flowers of SI *S. cor* LA1646 were pollinated, but none of them made fruits. In cases that did produce fruit, the shape and color of fruits were similar to those produced by sib pollination of designated female. However, seeds were much smaller than normal seeds from sib crosses. After the process of seed collection, seeds were not able to collect, since there was only seed coat like structures left. It was not determined if any viable plants could have been produced by embryo rescue.

Table 4. 5 Crosses have been used for fruit and seed development.

Sites	Female	Male	Success of Fruits	Seed
Puente Muyuna	LA2149 (*S. pim*)	LA2150 (*S. arc*)	Fruits	No viable
Rio Pativilca	LA3798 (*S. pim*)	LA3799 (*S. per*)	Fruits	No viable
Yaso	LA1646 (*S. cor*)	LA1648 (*S. hab*)	No-fruits	N/A
Cacra	LA1340 (*S.pen*)	LA1694 (*S. cor*)	Fruits	No viable
	LA1694 (*S. cor*)	LA1340 (*S. pen*)	Fruits	No viable
Asia El pinon	LA1610 (*S. pim*)	LA1609 (*S. cor*)	Fruits	No viable

Discussion

In this chapter, I examined reproductive barriers between species in sympatric pairs in three ways; 1) reduced stigma exsertion as a premating barrier, 2) reduced pollen tube growth in pistils as a postmating prezygotic barrier, and 3) lack of fruit or seed development as postzygotic barriers. In sympatric species, studying reproductive barriers is important for understanding how species maintain species identity.

1) **Premating (stigma exsertion)**

The extent of stigma exsertion influences the degree of outcrossing, since stigmas can serve as landing platforms for pollinators. Studies of *S. pimpinellifolium* done by Rick et al. (1977) showed that this species displays high variation in flower size and stigma exsertion, and that this variation correlates with outcrossing vs. self-crossing populations. A two-fold difference in stigma exsertion was observed between outcrossing and selfing in these studies. I compared stigma exsertion between SI and SC sympatric species to see whether less exserted stigmas could provide a barrier between SC species and their outcrossing (SI) sympatric partner species (Rick et al. 1997; Kalisz et al. 1999; Chen and Tanksley 2004).

I measured stigma exsertion in three populations of SC *S. pimpinellifolium* that grow sympatrically with SI species, and in five different SI species. Between SC and SI groups, I can conclude that there is a significant difference in the length of stigma exsertion was observed between sympatric SC and SI species (Fig. 4.2). Therefore, in some cases stigma exsertion could contribute to reproductive barriers between species.

2) Postmating prezygotic barriers (pollen-pistil interactions between sympatric species pairs)

Prezygotic barriers are known to be important to prevent hybridization in the tomato clade (McGuire and Rick 1954; Lewis and Crowe, 1958; Liedl et al 1996; Covey et al. 2010). Unilateral incompatibility or incongruity (UI) is often observed in SC x SI species crosses, where pollen rejection occurs in one direction, but the reciprocal cross is compatible. Studies of UI in the tomato clade have used pollen of *S. lycopersicum* with wild tomato species (Liedl et al 1996; Covey et al. 2010). In this study, I examined pollen-pistil

interactions in crosses made between sympatric pairs of wild tomato species to examine pollen tube growth in reciprocal crosses between co-occurring species.

In three sets of sympatric pairs consisting of SC and SI species, UI barriers were observed; SI species rejected SC species while in the reciprocal cross pollen tubes reach the ovary. This follows the SI x SC rule, as predicted. In addition, one sympatric pair in which both species are SI show UI barriers; *S. arc* was rejected by *S. hab*, but *S. arc* accepted pollen of *S. hab* (although this result needs further confirmation). However, two other pairs consisting of two SI species showed no pollen rejection in either direction (between *S. cor* and *S. hab*, and between *S. cor* and *S. pen*).

In some cases, plant growth conditions influenced whether pollen was rejected. For example in crosses of SI *S. arc* LA1351 x SI *S. hab* LA1352 (Chilete-Rupe pair) and crosses of SI *S. cor* LA1646 x SI *S. hab* LA1648 (Cacra pair) showed differences in pollen rejection between crosses done in the greenhouse and in the field. In some crosses that were done in the field in Colorado pollen did not reach the ovary, while crosses done in a greenhouse showed pollen tubes reach ovaries. I did not record the daily temperature for crosses done in field, but the crosses were done in late September 2010. The average range of late September in past years was from 25 to 7 °C. Temperature is known to affect to pollen tube growth and mating systems (Levin 1996), so temperature functions could have affected my results.

Another type of variability was observed in crosses of SC *S. pim* LA2149 x SI *S. arc* LA2150 (Puente Muyuna pair). I performed these crosses 10 times and found that SC *S. pim* LA2149 accepted pollen from SI *S. arc* LA2150 in 48h, but not always in 24h, whereas in self-pollinations of *S. pim* LA2149 pollen tubes reached the ovary in 24h. In other words, interspecific pollen tubes seemed to grow more slowly in this cross than self-pollen tubes.

Self-pollen tubes reached ovary in 24h. It should be noted that *S. pim* LA2149 has the longest exserted stigma among the three *S. pim* populations used in this study. Therefore, this population is more likely to receive pollen from nearby species and partial barriers may have evolved to give self-pollen a growth advantage over interspecific pollen.

3) **Postzygotic barriers (fruit and seed set)**

Even though prezygotic barriers play a key role in preventing hybridization in tomato clade, in some cases prezygotic barriers do not seem to function (Fig. 4.3). In these cases, postzygotic barriers are important to avoid gene flow between species. In this study, several crosses did not show pollen rejection even in cases where the female species have exserted stigma, yet no hybrid has been reported in the tomato clade in the wild. Therefore, I assessed postzygotic barriers to see whether these can prevent the formation of hybrids in the wild.

Six crosses which do not show postmating prezygotic barriers were tested for fruit set, and five out of six crosses resulted in normal appearing of fruits. However, fruits of these five crosses successfully made fruits did not contain viable seeds. These results are similar to those of Costa et al. (2007) who performed crosses between sympatric taxa in the *Chamaecrista desvauxii* complex, and suggested no prezygotic barriers were observed, while postyzygotic barriers resulted in fruit production with no seeds formed.

In sympatric species, reproductive traits may be shifted by selection against the production of or fitness of hybrids between species. There are several cases of reproductive trait shift in floral morphology in *Phlox* (Levin and Kerster 1967), or flowering timing in *Anthoxanthum* (Antonovics 1968). Here, I compared pollen rejection/acceptance in crosses between sympatric populations and crosses between allopatric populations. If these kinds of

differences are detected, it would suggest that reproductive character displacement (RCD) had occurred. RCD can result in increased post-mating prezygotic isolation when species in sympatry display a pattern of greater divergence of reproductive traits than in than allopatry. For example, in *Gilia*, sympatric species are isolated by incompatibility barriers, while allopatric species are able to produce hybrids (Grant 1965).

In this study, no major differences in pollen tube growth were observed when sympatric and allopatric populations were compared, with one possible exception. A difference in pollen rejection was observed in one case; in the Chilete-Rupe sympatric pair, SI *S. arc* LA1351 pollen does not reach the ovary in its sympatric partner SI *S. hab* LA1352, while in an allopatric pair cross, pollen tubes of SI *S. arc* LA2163 reached the ovary of SI *S. hab* LA1777. However, the sympatric crosses have been done only in the field while allopatric crosses were performed in both greenhouse and the field. I need to confirm that SI *S. hab* LA1352 rejects pollen from SI *S. arc* LA1351 in greenhouse crosses.

Summary

Reproductive barriers are an important mechanism for plants to prevent interspecific hybridization, an important facet of the biological species concept (Lewis and Crowe, 1958; Murfett et al. 1996). My work represents the first evaluation of reproductive barriers that function between sympatric populations of species of the tomato clade. I found that SC species in sympatric populations have significantly shorter stigma exsertion than their SI species pairs. However, since most SC species still have some degree of stigma exsertion, they can still receive interspecific pollen from sympatric species, because they overlap in

flowering time and share pollinators. SI species mostly prevent hybridization with SC species with postmating prezygotic barriers, i.e. pollen of SC species is rejected during pollen-pistil interactions. However, postzygotic barriers seem important in preventing hybridization in the SC members of a sympatric pair, because the SC species accept pollen from the SI member of the pair. In addition, in some crosses between sympatric SI species pairs where pollen tubes grow to the ovaries in reciprocal crosses, postzygotic barriers also seem important to prevent interspecific hybridization. Therefore, I conclude that wild tomato species which grow sympatrically in the wild prevent hybridization using both prezygotic and postzygotic barriers (Table 4.6).

Table 4. 6 Summary of reproductive barriers, stigma exsertion, prezygotic (pollen-pistil interaction), and postzygotic barriers in sympatric pairs. + = Presence of barriers, - = absence of barriers, N/D not done.

			Prezygotic	Postzygotic	Prevent hybridization?
1. Puente Muyuna	***S. pim* x *S. arc***	**SC x SI**	-	+	**Yes**
	S. arc* x *S. pim	**SI x SC**	+	-	**Yes**
2. Chilete Rupe	***S. arc* x *S. hab***	**SI x SI**	-	**N/D**	**N/D**
	S. hab* x *S. arc	**SI x SI**	+	-	**Yes**
3. Rio Pativilca	***S. pim* x *S. per***	**SC x SI**	-	+	**Yes**
	S. per* x *S. pim	**SI x SC**	+	-	**Yes**
4. Yaso	***S. hab* x *S. cor***	**SI x SI**	-	**N/D**	**N/D**
	S. cor* x *S. hab	**SI x SI**	-	+	**Yes**
5. Surco	***S. hab* x *S. cor***	**SI x SI**	-	**N/D**	**N/D**
	S. cor* x *S. hab	**SI x SI**	**N/D**	**N/D**	**N/D**
6. Cacra	***S. pen* x *S. cor***	**SI x SI**	-	+	**Yes**
	S. cor* x *S. pen	**SI x SI**	-	+	**Yes**
7. Asia El Pinon	***S. pim* x *S. cor***	**SC x SI**	-	+	**Yes**
	S. cor* x *S. pim	**SI x SC**	+	-	**Yes**
8. Ticrapo	***S. hab* x *S. cor***	**SI x SI**	-	**N/D**	**N/D**
	S. cor* x *S. hab	**SI x SI**	**N/D**	**N/D**	**N/D**

CHAPTER 5:

CONCLUSIONS

Interspecific reproductive barriers (IRBs) in the tomato clade are important for preventing hybridization in wild sympatric species. I studied IRBs in 13 tomato species (*Solanum* sect. *Lycopersicon*).

Chapter 2: Does pollen grain size play a role in reproductive barriers?

The positive correlation between pollen grain size and style length suggested by Delpino (1867) has been tested in the tomato clade. In chapter 2, I found no correlation between pollen grain size and style length in nine species in the tomato clade, and conclude that pollen grain size seem not involved in reproductive barriers.

Chapter 3: Assessment of postmating prezygotic reproductive barriers in the tomato clade

Unilateral incongruity/incompatibility (UI) reportedly following the "SI x SC" rule" has been previously tested using the domesticated species *S. lycopersicum*. Here, I used three wild red-fruited SC species as pollen donors. Pollen from all red-fruited SC species was rejected by pistils of green-fruited SI species, while pollen rejection and/or pollen tube growth of wild red-fruited SC species varies in pistils of green-fruited SC populations and species. Results from this study generally support the trend that UI follows the "SI x SC" rule, although several interesting exceptions to this rule were found.

Chapter 4. Reproductive barriers between sympatric populations in the tomato clade

IRBs were examined in three ways to determine how sympatric species avoid hybridization to maintain their genetic integrity in the wild. These include stigma exsertion (premating) between SI and SC populations of sympatric pairs, pollen-pistil interactions (postmating prezygotic), and fruit and seed development (postzygotic). Sympatric SC species have significantly less stigma exsertion than sympatric SI species. My results also suggest that sympatric species prevent interspecific hybridization using both postmating prezygotic and postzygotic reproductive barriers.

This project contributes to an understanding of reproductive barriers, crucial factors in maintaining species integrity, especially for sympatric organisms. These studies will also provide further information to tomato breeders who are interested in the transfer of desirable traits such as resistance to abiotic and biotic stressors into crops from wild species.

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