Mechanisms and Genetic Control of Interspecific Crossing Barriers in *Lycopersicon*

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The goal of this program is to use *L. esculentum* and *L. pennellii* as a model system to study the interspecific reproductive barriers unilateral incongruity (UI), hybrid breakdown and interspecific aberrant ratio syndrome (IARS). The specific experiments proposed and progress to date in each category are as follows:

A. Unilateral incongruity:

1) Determine the functional basis of UI including the timing of the failure of incongruous crosses, the developmental step(s) interrupted by UI, the tissue and genomes involved in UI.

The work for this section is largely completed. Manual self and cross pollinations were made on emasculated buds, and flowers were harvested at different periods of time post pollination, fixed, and stained using fluorescent aniline blue to observe pollen tube growth and fertilization. The crosses included all possible congruous and incongruous crosses using *L. esculentum*, and both self compatible (SC) and self incompatible (SI) accessions of *L. pennellii*. In congruous crosses between species and compatible crosses within species, pollen tubes form normally and transverse the entire style within 24 h after pollination. The time of UI expression in incongruous crosses is early in pollen tube growth; the pollen tubes form normally but transverse only the top fourth of the style by 24 h, stopping at this point. There is no additional pollen tube elongation in an additional 24 h. Since UI occurs early in pollen tube growth, we cannot, and need not, do work planned on early vs late rates of pollen tube growth and time of fertilization in incongruous crosses - the late growth phase of the pollen tubes and fertilization have been eliminated in incongruous crosses.

UI is observed in crosses of *L. pennellii* x *L. esculentum* regardless of whether SC or SI accessions of *L. pennellii* are used in the crosses. The nature of the UI response is strikingly different from the SI response observed by selfing SI accessions of *L. pennellii*. In SI self pollinations, pollen tubes form normally but only transverse the top half of the style by 24 h. The pollen tubes will continue to grow slowly, transverse the lower half of the style within the next 24 h. This information regarding UI and SI indicate that they are distinct in time nature and location of expression.

All possible manual self and cross pollinations were also made using the interspecific layer chimeras prepared using *L. esculentum* and a SC accession of *L. pennellii* (PEE, PPE, F₁EE, F₁F₁E) as well as *L. esculentum*, and *L. pennellii*. The styles were observed at 24 and 48 h to determine whether a UI response occurred in the pollination. The results indicate that the incongruity response involves the interaction of layer 1 and 2 of the female tissue (stigma/style) and layer 2 of the male (pollen grain/tube).

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The sole part of the work in this segment yet to be done is an examination of second mitotic division in congruous versus incongruous crosses. Since both second mitotic division and UI occur early in tube growth, the question as to whether second mitotic division occurs in incongruous crosses, and/or whether second mitotic division is involved in the incongruous response is an important one. Initially, there were difficulties in getting a protocol in place for examining second mitotic division. Several protocols had to be tested and modified before an adequate clearing technique/stain protocol was identified. The work can now proceed, and should be completed within the next year.

2) Study male/female and direct or indirect male/male interaction in mixed pollen pollinations using pollen from plants homozygous for a pollen expressed GUS transformants, and determine whether effects or male/female interaction may be associated with UI.

In order to do this work, we first had to determine an acceptable histochemical technique that would permit clearing of style for viewing pollen tubes without obscuring the GUS reaction. The current status of this work is that we now have a technique that gives good results, we have the necessary plants, and are ready to proceed with the experiment. Since the UI response occurs relatively early in pollen tube growth, this experiment will be simpler than initially devised. There is no need to examine later stages in pollen tube growth, as these are eliminated in incongruous crosses.

3) Study the genetic control of UI.

Initially we were going to eliminate the work in this section due to the reduced level of funding. However, we have started this experiment, since the information derived from it is critical to the program as a whole. The crosses were made following a scheme similar to McGuire and Rick (1954) using the F₂ plants also being used for a RFLP mapping study within the lab. The crosses, *L. esculentum* x F₂ and F₂ x *L. pennellii*, are congruous and were made to determine whether the F₂ plants are male and female fertile. Nearly all the pollen producing F₂ plants tested were able to set seed when used as a pollinator of *L. esculentum*, indicating that the pollen produced was viable and capable of affecting fertilization. The results of the F₂ x *L. pennellii* crosses also generally set seed, showing that the F₂ plants were capable of producing seed. The incongruous pollinations, F₂ X *L. esculentum* and *L. pennellii* X F₂ were made to compare with the congruous crosses to determine whether the F₂ plants were exhibiting the standard UI response. Data from these crosses show that there is observable segregation for UI in the F₂, but data from more plants is needed before a complete analysis of the data can be done.

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B. Hybrid Breakdown:

1) determine the functional basis of non-fecundity, including the stage in reproduction affected, the role of pollen tube growth in non-fecundity, and whether female sterility or a non-reciprocal barrier such as UI is associated with non-fecundity,

Nearly all the F₂ plants tested produce seed if pollinated with *L. pennellii* pollen, so non-fecundity observed in self pollinations is not due to female sterility. Pollen tube growth *in vitro* was comparable for plants with similar levels of stainable pollen from the F₂^{Le} and F₂^{LP}. Pollen tube growth *in vivo* was normal and occurred at normal rates following self pollination of F₂^{Le} and F₂^{LP}, regardless of the fecundity observed for the plant after self pollinations. Therefore, the mechanism causing non-fecundity occurs either immediately before, at, or immediately after the double fertilization event. We plan finer cytological examination of ovules during this narrow window in development to determine the cause of the non-fecundity.

2) determine the effects of cytoplasm on the reproductive parameters affected by hybrid breakdown, and whether these effects are direct or indirect, by its affect on IARS.

The two populations, F₂^{Le} and F₂^{LP}, used to examine hybrid breakdown were produced using isocytoplasmic lines. The data collected include: flower development, pollen development, pollen stainability, *in vitro* and *in vivo* pollen germination, fruit set, seed set, and seed germination. Comparing reproductive parameters measured for F₂^{Le} and F₂^{LP} demonstrates the striking effects of cytoplasm substitution on reproduction (Fig. 1). In the F₂^{Le} populations, 9.4% of the plants never flowered, and 5.7% of the plants had a premeiotic block on pollen development. In addition, the production of plump stainable pollen was reduced, so that the F₂^{Le} mean was 38%. In contrast, the F₂^{LP} populations did not contain either plants which failed to flower or plants with a premeiotic block on pollen development, and average percent stainable pollen for F₂^{LP} was nearly doubled that of the F₂^{Le}. For comparison, all of the control plants, including New Yorker, New Yorker^{LP}, *L. pennellii* and the two interspecific F₁s produced on average 95 to 98% stainable pollen. Therefore, the presence of *L. pennellii* cytoplasm in the F₂^{LP} reduced but did not eliminate the reduction in percent stainable pollen.

The fecund F₂^{Le} plants produced very few seeds per fruit, and generally showed low percent seed germination. In contrast, the fecund F₂^{LP} plants produced on average doubled the number of seeds per fruit of the F₂^{Le}, and generally showed much higher percent seed germination. In overview, *L. pennellii* cytoplasm substitution in the F₂ population reduced the effect of hybrid breakdown on flower production, pollen development, including both the number of plants with premeiotic block and percent stainable pollen, fruit set, seed set, and seed germination.

The second part of the questions posed in this section involves whether the effects of the cytoplasm on reproductive parameters are direct or indirect, by the effect of cytoplasm on IARS. The answer to this question requires the completion creation of 20cM maps using the twin populations, F_2^{Le} and F_2^{LP} . To date, 78 markers have been placed on these maps. Analysis by Mapmaker showed that there were still several regions on one or both maps for which there are gaps of more than 20cM between markers, so several more probes are being added to the maps. This work should be completed within 2 months, allowing data analysis to proceed.

3) determine the number and location of chromosomal regions associated with reduction in male fertility and non-fecundity

Like 2) above, this question depends on the completion creation of 20cM maps using the twin populations, F_2^{Le} and F_2^{LP} , and since that work should be completed within 2 months, this work can proceed after that time.

C. Interspecific Aberrant Ratio Syndrome (IARS):

1) determine the functional basis of IARS including the stage of reproductive development affected by IARS, what layers of the female sporophyte are involved, whether both male and female gametes are affected and whether gamete competition/selection is involved in IARS,

Several interspecific F_1 hybrids heterozygous for GUS constructs controlled by pollen expressed promoters have been created and are currently flowering. Examination of GUS expression is proceeding in pollen, pollen tubes, and ovules pre and post fertilization to determine timing and location of IARS.

2) study the effect of cytoplasm on IARS, does exchange of cytoplasm affect IARS at chromosomal regions other than those near PGI-1 and PGM-2, and do the effects at these regions involve reversal of direction or elimination of IARS.

As in section B2 and 3 above, this work depends on RFLP data from populations the F_2^{Le} and F_2^{LP} . However, the full 20 cM map was not needed for data analysis could answer this question. Segregation was compared for the 78 RFPL markers which are spaced at least 20cM apart over the 12 pairs of chromosomes (Figure 2). Observed segregation for each marker was tested for fit to Mendelian expectations using G^2 analysis. In G^2 analysis, the 1:2:1 test shows whether skewness exists for genotypic frequencies, the 1:1 test detects skew in allele frequency and the allele based genotypic test shows genotypic skew based upon the observed allele frequencies. The G^2 measured by the allele test and the allele based genotypic test adds up to that of the genotypic test. Analysis of the 78 RFLP markers placed to date reveal that cytoplasm has several affects on IARS. Summarizing the results of all the probes (Figure 2), there are differences in the number, direction and location of regions with skew in allelic frequencies. In F_2^{Le} , there are 17 regions of allelic skew, of which 16 favoring the *L*.

pennellii allele. In F_2^{LP} , there are only 7 regions of allelic skewing, of which only 2 favoring the *L. pennellii* allele. In F_2^{Le} , there are 15 regions with 1:2:1 genotypic skew, 9 of which are due to an allelic skew favoring *L. pennellii*, and 1 of which is due to an allelic skew favoring *L. esculentum*. The results obtained from F_2^{LP} contrast to that of F_2^{Le} ; there are 10 regions with genotypic skew, 2 of which are due to an allelic skew favoring *L. pennellii*, and 3 of which is due to an allelic skew favoring *L. esculentum*. In addition to effects on the number of regions skewed and the direction of skew, cytoplasm substitution also affected the location of the regions of skew.

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