The Places Pollen Will Go: Spatial and Temporal Genetic Structures of *Brassica rapa*

Initial results for the workshop in Toronto

Prepared by Laura Leventhal

Brief background

Proximity, timing, and pollinator presence influence mating patterns in plants (e.g. Fenster, 1991; Greenleaf et al., 2007; Mitchell et al., 2009; Schmitt, 1983). Outcrossing plants receive pollen from another plant in the population via wind or animal pollination. Plants disperse pollen nearby in a <mark>leptokurtic distribution</mark> (Levin and Kerster, 1974). When pollen movement is restricted between populations by distance, the individuals are Isolated by Distance (IBD) (Wright, 1943). Over generations, this nonrandom mating pattern can result in the formation of a spatial genetic structure.

In addition, plants that flower more synchronous are more likely to mate than plants with little flowering overlap (Ison et al. 2014). Since, perfect flowering synchrony rarely occurs in phenological assortative mating, or a non-random mating pattern where early flowering plants mate with other early flowering plants, appears common in many plant populations (Fox 2003, Weis et al. 2014, Ison and Weis 2017). Flowering time of plants in natural populations is affected by biotic and abiotic conditions (reviewed by Elzinga et al., 2007; Rathcke and Lacey, 1985), however many studies have also found a strong genetic component to flowering time (reviewed in Geber and Griffen, 2003, Weis and Kossler 2004, Franks et al. 2007 and many others).

When there is variation in flowering time, pollen enters the mating pool at different times. Thus, early flowering alleles seldom cross with late flowering alleles (Hendry and Day, 2005; Weis and Kossler, 2004). Further, more alleles associated with early flowering will be

present at the beginning of the season and more alleles associated with late flowering will be present at the end of the season, shifting the mean breeding value throughout the flowering season (Hendry and Day, 2005; Weis and Kossler, 2004). When flowering time is heritable and there is a consistent pattern of phenological assortative mating, populations can become Isolated By Time (IBT) and temporal genetic structures can form (Hendry and Day, 2005).

In this study, we determine the spatial and phenological movement of pollen in several different plots of an annual plant *Brassica rapa* (Brassicaceae). *Brassica rapa* has perfect flowers, it is self-incompatible, and is pollinated generalist bees and flies. We reconstructed the paternity of 2,064 *B. rapa* offspring, from 287 maternal *B. rapa*, with 860 potential paternal plants using ten *Brassica* specific microsatellite markers. We examined spatial and temporal parameters using the full-probability reconstruction method. Using the pedigrees, we assessed how traits such as number of flowers, duration of flowering, and flowering synchrony affected siring success of the paternal plants. Our study uses this retrospective approach to directly measure pollen movement both spatially and temporally in four naturalized plant populations. We also fully characterize the interaction of spatial and temporal pollen movement, while previous studies have only examined a trait such as flowering time (Ison and Weis 2017) or did not have sufficient sampling to elucidate the interaction (e.g. Ison et al. 2014, Kitamoto et al. 2006).

Our study also assesses male fitness through siring success, while most studies on plants only address female fitness. A recent meta-analysis found a widespread pattern of selection for early flowering in many plant species, while flowering synchrony appears to not be under selection (Munguía-Rosas et al., 2011). However, the vast majority of these studies only examine female fitness, in fact only five of the 87 studies examined male fitness. Thus, selection on

flowering time through male fitness is largely unknown (but see Austen and Weis 2016). A previous study designed to assess female fitness that directly measured pollen movement in *Echinacea* using genetic markers yielded compelling evidence that flowering synchrony, not flowering time, was likely more important for male fitness (Ison et al. 2014).

This study directly builds on a previous study on this system where Ison and Weis (2017) evaluated and quantified the presence of temporal genetic structure in flowering time. They found temporal genetic structure in seven of their eight fields, suggesting that temporal genetic structures are common in natural populations of plants. They compared prospective and retrospective estimates of phenological assortative mating in *B. rapa,* and found that the retrospective and prospective estimates were similar and that the retrospective estimate was 35% larger than the prospective estimate. Therefore, temporal genetic structures may be larger in natural populations than expected under random mating conditions. However, they found no difference in temporal genetic structure between plots with different local flowering densities.

Pedigree reconstruction also can retrospectively estimate the strength of phenological assortative mating. Parentage analysis creates a pedigree between an F_1 and F_2 generation in three steps (Hadfield et al., 2006; Thompson, 1976). First, the number of pedigrees based on demographic, sexual, and behavioral data are restricted. Second, the relative likelihood of each pedigrees using genetic data is calculated. Third, the parameter of interest using the new pedigree information is estimated (Hadfield et al., 2006; Thompson, 1976). Categorical and fractional allocation are the two methods proposed for estimating the parameter of interest (Hadfield et al., 2006). In the categorical model, the three steps are completed sequentially and the program selects the pedigree with the most support. The categorical method disregards uncertainty with the analysis and inflates type 1 error (Hadfield et al., 2006; Thomas, 2005). The fractional

allocation model uses the full probability distribution of the proposed pedigrees, taking into account uncertainty and reducing bias. However, both models assume a random mating pattern by completing the three steps sequentially and fail to account for prior information that shift the population to a non-random mating pattern. Parentage Bayseian analysis use the fractional allocation model, but also estimate the parentage assignment (step 2) and parameter of interest (step 3) simultaneously (Hadfield et al., 2006; Neff et al., 2001). The prior information that is supplied form step 3 is therefore used to inform pedigree assignment in step 2 (Hadfield et al., 2006).

Research questions:

1. How does the local spatial density of *B. rapa* affect the strength of phenological assortative mating?

2. To what extent does the distance that pollen moves change over the season?

3. To what extent does the phenological distance (as measured by pair-wise flowering synchrony) pollen moves change over the flowering season?

4. Is there an interaction between spatial and phenological distance on siring success?

5. How does the siring success of *B. rapa* plants vary based on phenological traits (e.g. duration of flowering, total number of flowers, start and end date of flowering)?

Initial results

In 2011, researchers planted *B. rapa* in four different experimental plots, with clumped and even spatial structure and with natural and amplified temporal structures (please read Ison and Weis for detailed information on the methods); Ison and Weis 2017 figure 2 illustrates the plot layouts). They obtained 2064 offspring from this experiment. Researchers extracted the DNA from the offspring and parents and identified them at ten microsatellite markers. They took

other data from the parents such as flowering time, duration of flowering, and total number of
flowers. From here, we reconstructed the paternity of the offspring using the MasterBayes
package in R.

All of the plots from the Ison and Weis (2017) field experiment had a decay in siring
success as distance between mates increased (Figure 1). The two even plots had a faster decay in
siring success as distance between mates increased compared to the two clumped (Figure 1). The
spatial distance pollen moved changed over the course of the flowering season (Figure 2). In the
beginning and end of the season in the even and amplified plot, pollen moved further distance
than at peak flowering time (as illustrated by the steeper decay in the middle batches; Figure 2C),
while there is no clear pattern in the even natural plot (Figure 2A). In both clumps there is less
variation in pollen movement distances in the clumped plots over the course of the flowering
season (Figure 2B and 2D).
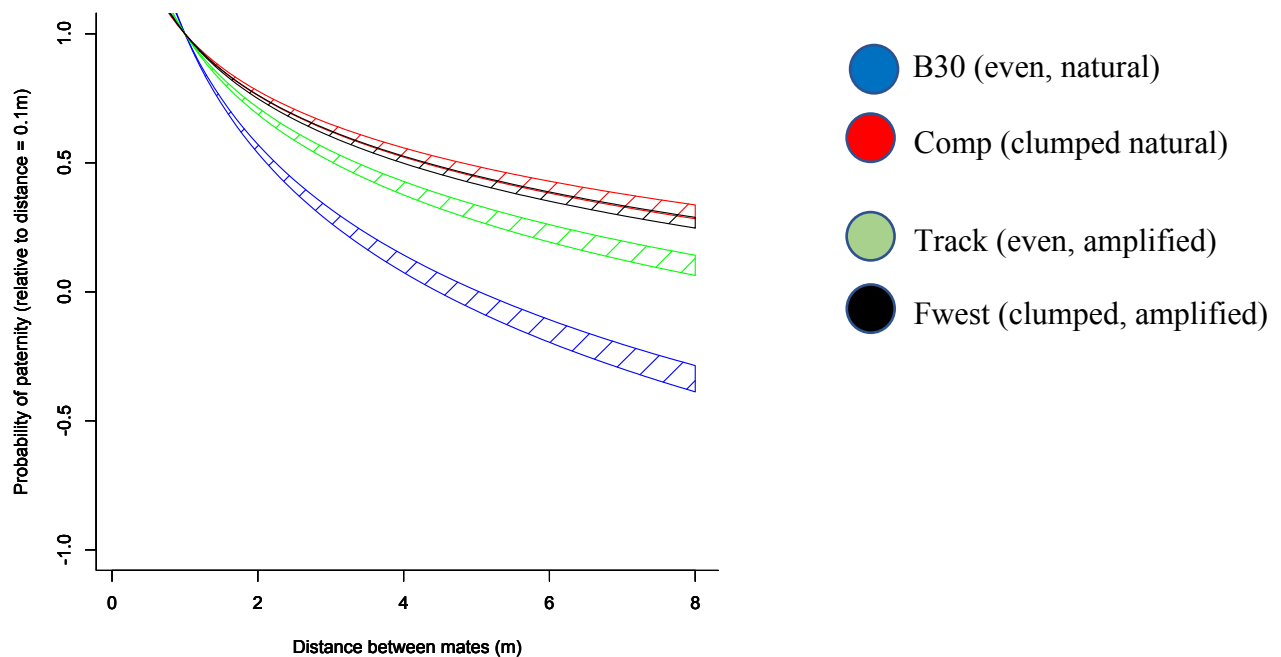
Figure 1. Probability of paternity with increasing distance between mates in four open pollinated
experimental plots. B30 has even spatial structure and a natural temporal structure. Comp has a
clumped spatial structure and a natural temporal structure. Track has an even spatial structure

and an amplified temporal structure. Fwest has a clumped spatial structure and an amplified temporal structure.
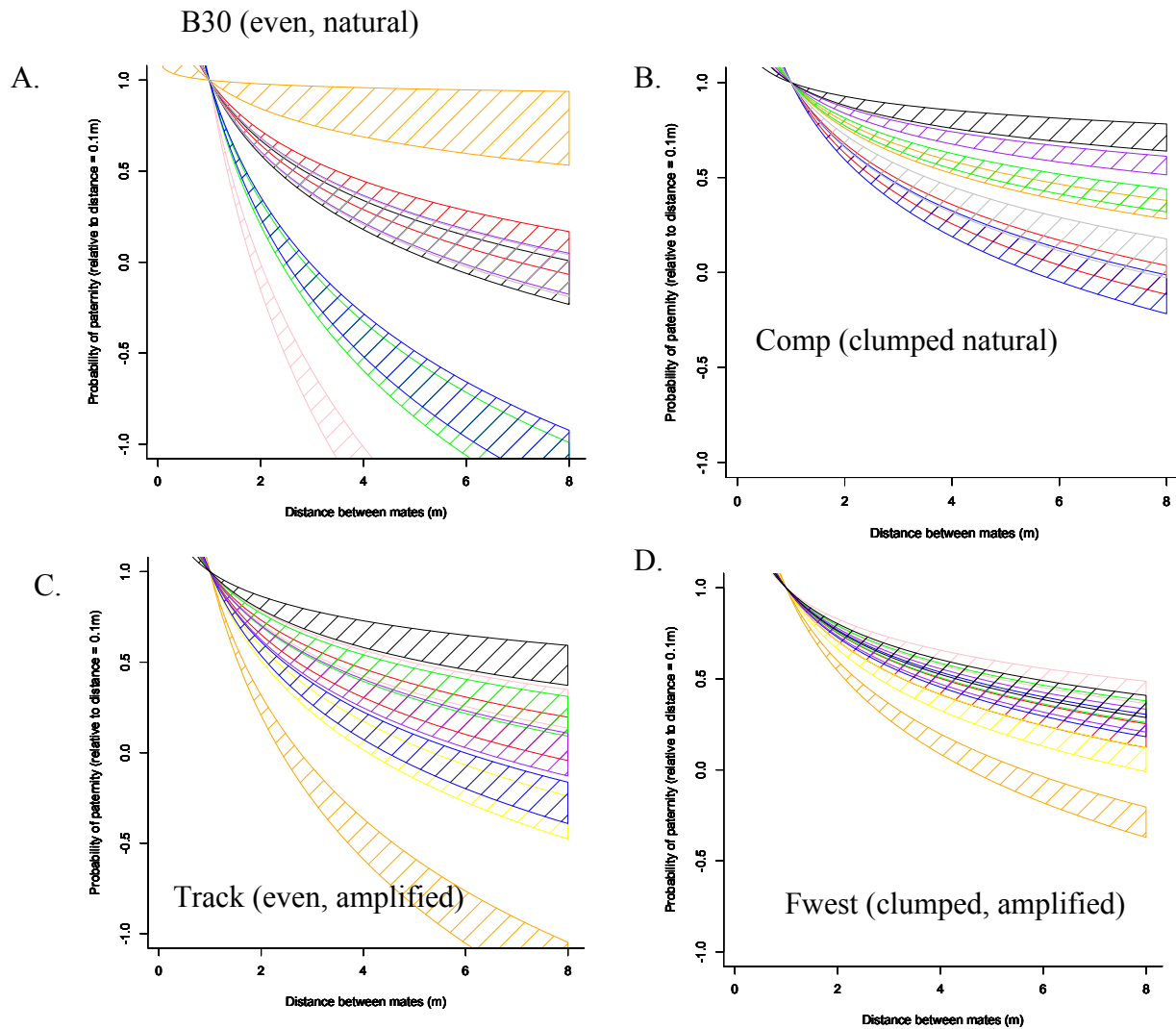

Figure 2. Probability of paternity with increasing distance between mates in four open pollinated experimental plots over the course of the flowering season. Each color is a different 'gene trap' (i.e. maternal) batch (see Ison and Weis 2017). The 'gene traps' sampled pollen over the course of the flowering season. The earliest 'gene trap' batch is pink, followed by red, orange, green, blue, purple, black, and then latest, gray. B30 (A) is an even spatial structure and a natural temporal structure. Comp (B) is a clumped spatial structure and a natural temporal structure. Track (C) is an even spatial structure and an amplified temporal structure. Fwest (D) is a clumped spatial structure and an amplified temporal structure.

We measure pair-wise synchrony between paternal plants as the proportion of days that a given paternal plant was flowering while a 'gene trap' (i.e. maternal) plant was in the plots. For example, if a paternal plant was flowering every day a gene trap batch was in the plot, their pair-wise synchrony would be 1. If a paternal plant was flowering for two of the four days a gene trap was in the plot, their pair-wise synchrony would be 0.5. One even and one clumped plot show a trend where siring success is increased with increased synchrony at the beginning and end of the season (with the exception of last batch in Comp, which needs to be revisited) (Figure 3).
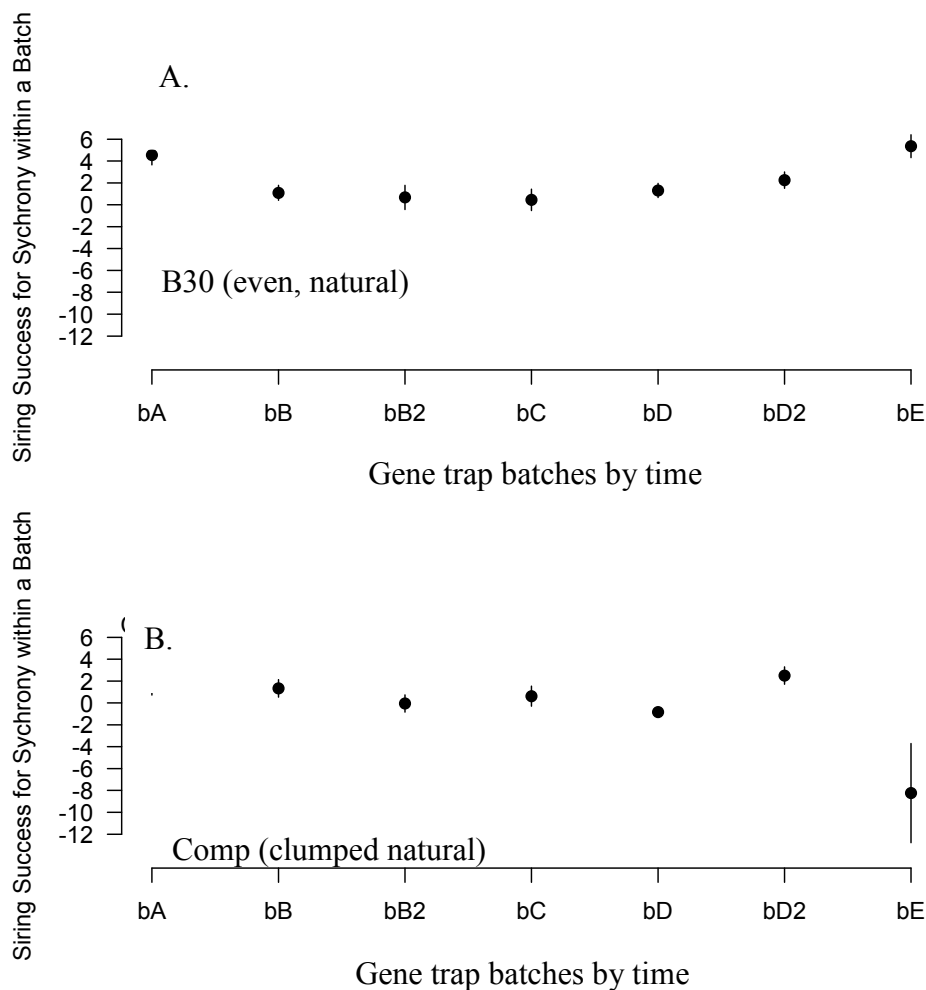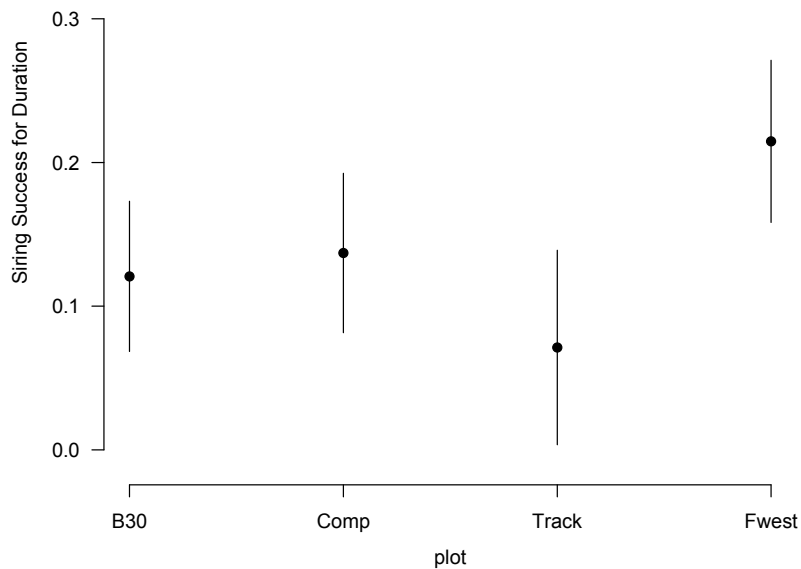

Figure 3. Siring success by pair-wise synchrony in two plots throughout time. B30 (A) is an even spatial structure and a natural temporal structure. Comp (B)I s a clumped spatial structure and a natural temporal structure. The 'gene trap' batches are arranged from beginning (bA) to the end (bE) of the flowering season.

In both of the even plots, siring success increased if a paternal plant had more total flowers, while total flower production did not impact siring success in the clumped plots (Figure 4). Siring success increased when a paternal plant had longer flowering duration all plots and was strongest in Fwest, the clumped plot with amplified temporal structure (Figure 5).
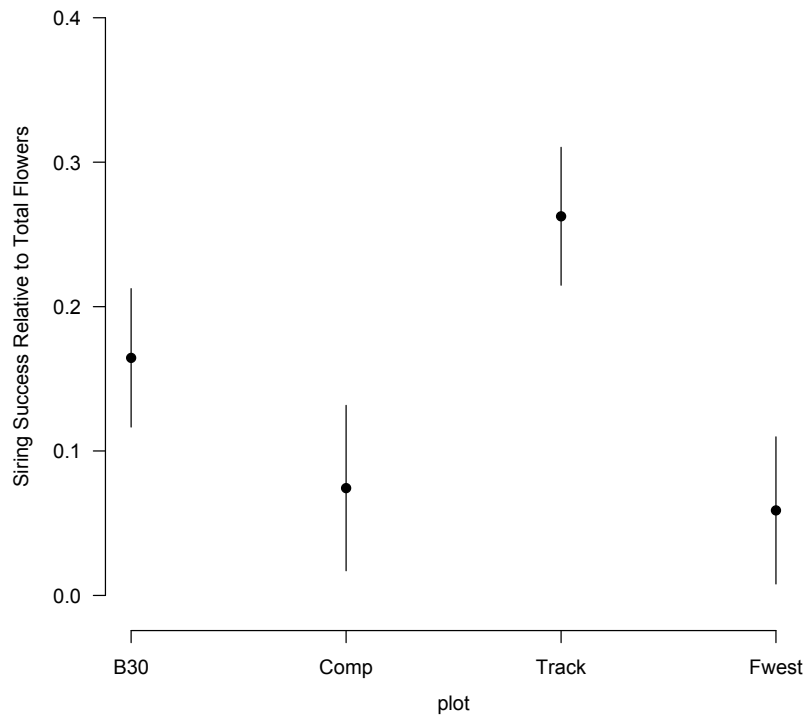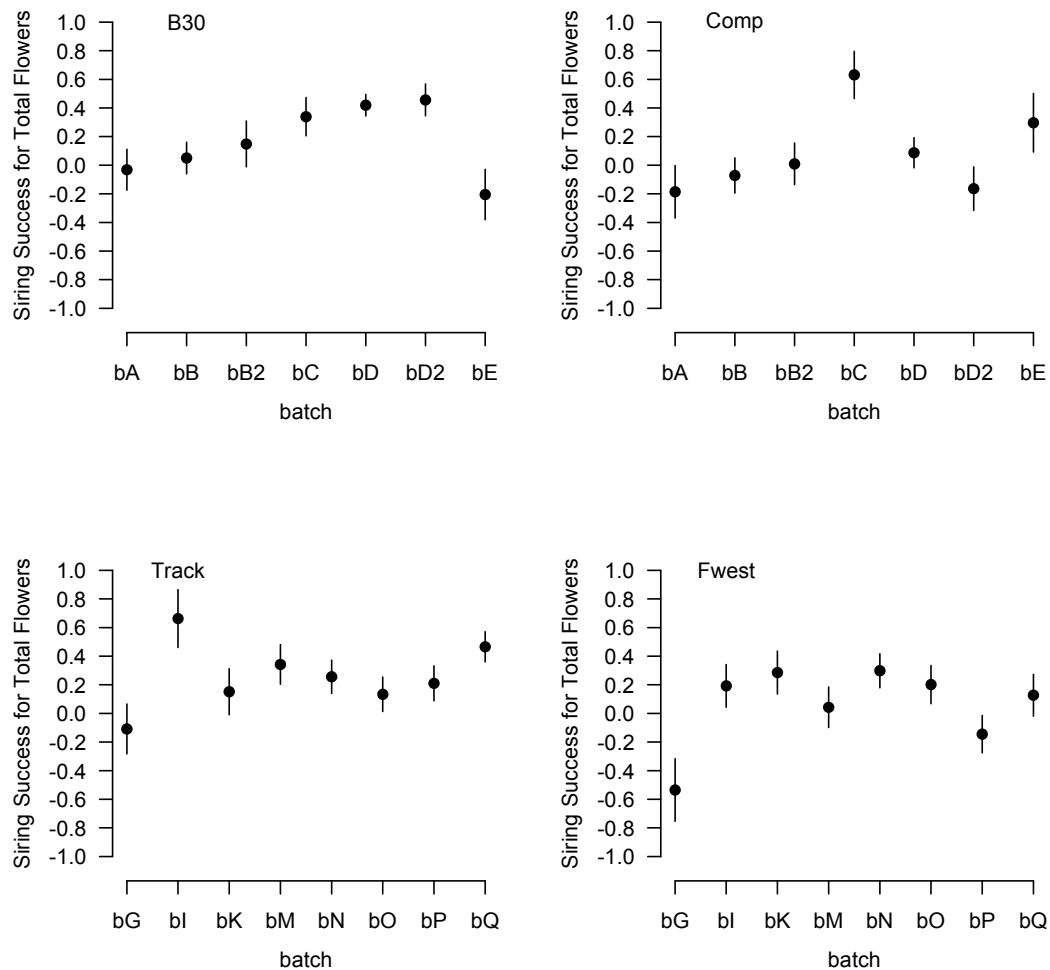

Figure 4. Siring success's relationship to total flowers in four plots. B30 is an even spatial structure and a natural temporal structure. Comp is a clumped spatial structure and a natural temporal structure. Track is an even spatial structure and an amplified temporal structure. Fwest is a clumped spatial structure and an amplified temporal structure.
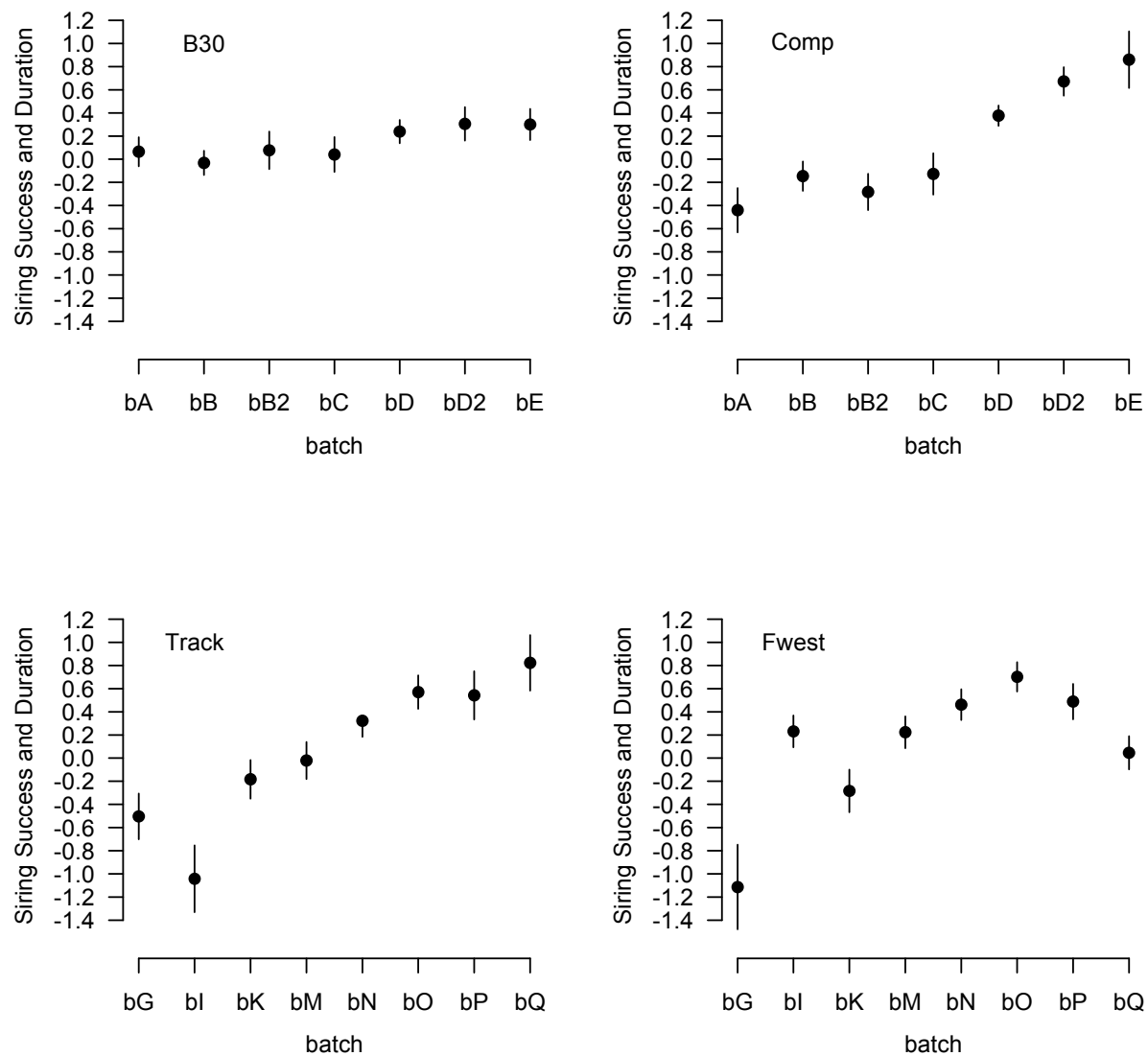
Figure 5. Siring success's relationship to flowering duration in the four experimental open pollinated plots. B30 is an even spatial structure and a natural temporal structure. Comp is a clumped spatial structure and a natural temporal structure. Track is an even spatial structure and an amplified temporal structure. Fwest is a clumped spatial structure and an amplified temporal structure.

We also examined siring success for total flower number and total flowering duration by 'gene trap' batch. In all four plots a paternal plant's siring success increased with more total flowers over the course of the flowering season except for the last batch in B30 (Figure 6). Siring success based on total flowering duration also generally increased over the course of the flowering season all four plots (Figure 7). Please note, we are still thinking about these results and are not sure of their biological interpretation.

Figure 6. Siring success's relationship to total flowers in four plots throughout time. B30 is an even spatial structure and a natural temporal structure. Comp is a clumped spatial structure and a natural temporal structure. Track is an even spatial structure and an amplified temporal structure. Fwest is a clumped spatial structure and an amplified temporal structure.

Figure 7. Siring success's relationship to duration flowering in four plots throughout time. B30 is an even spatial structure and a natural temporal structure. Comp is a clumped spatial structure and a natural temporal structure. Track is an even spatial structure and an amplified temporal structure. Fwest is a clumped spatial structure and an amplified temporal structure.

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