MEASURING THE EFFECTS OF SELECTION AND PLEIOTROPY ON SHORT STAMEN NUMBER IN *ARABIDOPSIS THALIANA*

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ABSTRACT

Loss of function of adaptive traits, resulting in trait loss, is a common area of study in evolution. Traits with lost function are frequently lost but may also be reduced or completely maintained. The fate of traits with lost or decreased function rely on the interplay of three factors – selection, pleiotropy, and genetic drift. In most systems, the exact mechanisms of trait loss are unknown, including in the loss of short stamen in *Arabidopsis thaliana* on latitudinal and altitudinal clines. Previous observation has shown there to be more short stamens at higher latitudes and altitudes and fewer at lower latitudes and altitudes. This study seeks to investigate two possible hypotheses for the cause of these clines. First, that there is directional selection for more short stamens at higher latitudes and altitudes or for fewer short stamens at lower altitudes and latitudes. Second, that pleiotropy between short stamen number and another trait that varies on the same clines may be leading to indirect selection. To address these hypotheses, we measured a variety of traits on latitudinal and altitudinal clines, tested if they varied on those clines, examined correlations between these traits and short stamen number, and finally performed a fitness regression analysis. Overall, we find little evidence for pleiotropic correlations between short stamen number and any of the measured traits, as well as evidence of weak selection for fewer short stamens.

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INTRODUCTION

Loss of function of adaptive traits is a common occurrence in evolution, usually caused by changing selective pressures on those traits (Wiens 2001). Traits with lost function are expected to experience loss with no selection acting to maintain them (Yoshizawa 2012), as all traits require a cost to produce. If a trait is no longer providing enough adaptive benefit to offset the cost of production, selection is expected to act against the maintenance of the trait in favor of allocating those resources elsewhere (Sicard and Lenhard 2011; Charlesworth and Charlesworth 2008).

There are many examples of trait loss due to loss of function, such as the classic example of cavefish going blind and losing pigmentation after moving into a dark cave environment, as there is no longer selection acting to maintain these traits in the dark (Lahti et al. 2009). However, there are also examples of traits with seemingly lost function being maintained, such as continued ability to recognize foreign eggs in village weaverbirds even after moving into habitats without egg-mimicking parasites (Lahti 2006). Additionally, partial loss or reduction of traits is seen, as in *Ceratandra* orchids, which show a complete loss or reduction of oil glands as they shift from pollination by oil-collecting bees to pollination by beetles (Steiner 1998).

As seen in these examples, traits with supposed lost function might not always be lost, instead they may be totally or partially maintained. This difference in responses to lost function is likely due to the interaction of three factors influencing the loss/maintenance of traits - selection, pleiotropy, and genetic drift. These forces of evolution may act independently or as a collective, interacting to completely lose or retain traits with lost function, or even creating clines within that trait (Lahti et al. 2009). In most systems, it is not fully known which of these forces are at play, nor exactly to what extent. Generally, the impact of selection is straightforward. As mentioned above, traits that are costly or impact fitness in some other way will experience selection against them, while

traits that minimal effects of fitness may be maintained. Pleiotropy, which is the influence of one gene on multiple traits, can impact trait loss via correlated response to selection. If one trait is experiencing strong selection and it is pleiotropically connected to another trait, the second trait will also change in frequency with the selected-upon trait. Finally, genetic drift can fluctuate trait frequencies to the point of loss, especially in populations with low variation or small population sizes.

Tetradynamy is the presence of four long medial stamens and two short lateral stamens. Many plants, including most of the 3000+ species in the family Brassicaceae (Al-Shehbaz et al. 2006) for which the trait is diagnostic (Endress 1992; Cronquist 1981), exhibit tetradynamy. Despite the prevalence of this trait, short stamen numbers may fluctuate, such as the loss or fusion of stamens in *Lepidium* species (Bowman and Smyth 1998) or loss of short stamens in *Cardamine hirsuta* (Matsuhashi et al. 2012). This loss of short stamens has also been observed in the model plant *Arabidopsis thaliana* (Goodman et al. 1995; Royer et al. 2016)

It is uncertain exactly why short stamens are being lost in *A. thaliana*, but it could be because they have potentially lost their function (Royer et al. 2016) during the plant's transition to self-pollination (referred to as selfing). Short stamens are considered adaptive for outcrossing, by constricting the pollen removal by per visit or allowing pollen removal from different types of pollinators (Darwin 1897; Conner et al. 2003; Kudo 2003). Additionally, there is evidence of selection maintaining tetradynamy in outcrossing plants (Conner et al. 2003). As *A. thaliana* made the transition to being 97 percent selfing (Abbott and Gomes 1989), it is possible that the short stamens are no longer beneficial. Additionally, Royer et al. (2016) found that short stamens do not contribute significantly to selfed seed set, likely due to their distance from the stigma, which may relax selection maintaining them.

What makes this loss of function of short stamens in *A. thaliana* so interesting is that there has not been a complete loss of the structure in any population (Royer et al. 2016). Flowers may have as many as two short stamens, but as few as zero, and this number varies between flowers on a single plant as well as between plants and between populations. This incomplete loss may be due to the somewhat recent evolution of selfing within the last one million years (Tang et al. 2007; Shimizu et al. 2011), and so loss may still be in progress, or it may represent an equilibrium point of the three factors influencing trait loss. This incomplete loss has resulted in parallel clines; the number of short stamens varies between populations, with more short stamens per flower at higher latitudes (Royer et al. 2016) and altitudes (Buysse et al. 2024, in prep.) and fewer at lower latitudes and altitudes.

While selection on short stamen number is relatively weak (Royer et al. 2016), it is possible that direct selection is acting on this trait to cause the clines. There might be some force of selection acting to maintain short stamen at higher altitudes and latitudes, to lose them at the lower altitudes and latitudes, or perhaps both at once. Growing these structures, if they had no purpose, might be selected against if there is a cost incurred to do so. If there were a greater cost at lower latitude and altitude populations, that could help to explain the clines. Additionally, it is possible that there is an unknown fitness benefit short stamens provide at higher altitudes and latitudes, resulting in selection to keep them there.

Selection could also act on a pleiotropically linked trait, and so affect short stamen number through correlated selection. There are a number of traits that vary along the same clines as short stamen number, and if any of those are influenced by the same genes, selection on those traits could affect the number of short stamens.

Even without selection acting on short stamens, populations could still lose short stamens due to genetic drift. Selfing results in a low effective population size (N_e), making these populations

more susceptible to genetic drift which could affect the frequency of short stamen. Additionally, genetic drift could cause these clines by constraining the complete loss or complete retention of short stamen. Montesinos et al. (2009) and Beck et al. (2007) found that higher altitude and latitude populations have decreased genetic diversity, potentially due to founder effects after postglacial recolonization. This lack of genetic variance could dampen the effects of selection acting on short stamen number.

This study aims to investigate the selection and pleiotropy hypotheses by examining the relationships between short stamen number, fitness, and other traits that could potentially vary on the same clines. First, we will investigate what traits vary on latitudinal and altitudinal clines. Next, we will look at correlations between these traits and short stamen number. If a trait both varies on the same clines as short stamen number and is correlated with it, then that trait could be a candidate for having pleiotropic correlations with short stamen number. If one or both of those are not true, then it is unlikely that there is pleiotropy linking that trait with short stamen number. Finally, we will look for selection on short stamen number and the other measured traits. These three questions will help us to further uncover the mechanism behind short stamen loss.

METHODS

Seeds were sourced from the 1001 Genomes Project, representing a broad distribution across Eurasia and the United States of America (1001 Genomes Consortium 2016). The subset used in this study includes 253 of these lines representing 25 countries, altitudes of -24 to 1804 meters, and latitudes of 31.48 to 63.32 degrees. Plants were grown in growth chambers at Michigan State University's Kellogg Biological Station. One plant from each line was grown in each of four blocks that were separated temporally with two or more weeks between each block as well as spatially with each block grown in a separate growth chamber. Within each chamber, pots were randomly placed within trays, which were moved within the chamber and rotated end-to-end twice a week to reduce position effects. Plants were grown in Pro-Mix FLX soil in (POT SIZE) pots, and each was fertilized with 1/32nd teaspoon of Osmocote Plus fertilizer. Watering was done daily as needed. Seeds were stratified at 5°C for one week, then conditions were set to provide light and temperature cycles that mimicked natural conditions. The light intensity and temperature settings, as well as time frame, are detailed in Table 1. These temperatures and their durations were chosen to be an approximate average of those at our latitudinal extremes, Sweden and Italy (The Weather Company 2024, Ågren and Schemske 2012).

Trait Measurements

A variety of phenological, vegetative, and reproductive traits were measured, some of which have already been shown to vary with latitude and/or altitude.

To monitor germination success, four seeds were planted per pot and the number of successful emergents was recorded. This excess planting also increased the likelihood that there would be a successful germinant in each pot. Emergence was recorded daily. Four weeks after planting, germinants were thinned to leave only the oldest surviving plant in each pot. If multiple

germinants emerged on the same day, or if the oldest plant was unknown, the largest or healthiest looking was kept. 57 (5.6% of the total planted) pots were removed from chambers to dry cycle due to not germinating within six weeks of stratification. Only one pot successfully germinated after this dry cycle, and so was introduced back into the fourth block and grown as normal but was excluded in the days to emergence trait analysis.

The date of bolting was recorded for each plant, and the number of leaves in the rosette was counted on or shortly following this day. The date on which the first flower opened on each plant was recorded, and the height of that flower was measured from the soil to where the pedicel met the main stem.

Three collections of three flowers each were made. To ensure that collections were taken across stages of growth, the eighth, ninth, and tenth flowers were taken for the first collection, the 18th, 19th, and 20th flowers were taken for the second, and 28th, 29th, and 30th were taken for the third. These numbers were not exactly met for every plant, but collections were done as close to these as possible. Short stamen number was recorded for all nine flowers collected, and one flower from each plant was dissected to count ovule number. One additional flower from each plant was photographed under a stereo microscope for later measurement of floral traits using ImageJ. Pistil length, petal tube and limb lengths, long and short stamen anther and filament lengths, and short stamen herkogamy, that is, the distance from the top of the short stamen anther to the stigma, were measured using these flower photos.

When flowering stopped and siliques matured, plants were removed from the growth chamber and allowed to dry out. Once dry, the number of fruits on the main stalk and side stalks were counted and recorded, as well as the total number of side stalks.

In summary, the traits used in analysis include days to emergence, germination success, days to bolting (Montesinos-Navarro et al. 2011; Stinchcombe et al. 2004), rosette leaf number (Luo et al. 2015; Montesinos-Navarro et al. 2011), days to first flower (Debieu et al. 2013; Montesinos-Navarro et al. 2011; Stinchcombe et al. 2004) , first flower height (Singh et al. 2015; Hämälä et al. 2018), ovule number (Royer et al. 2016), number of side stalks, petal limb and tube lengths, long and short stamen anther and filament lengths, pistil length, and short stamen herkogamy. Fruit production, a fitness estimator, was also measured.

Analysis

For the analyses, days to emergence was counted from planting, days to bolting was counted from the emergence date kept during thinning, and days to first flower was counted from bolting to allow these traits to be independent from one another (Caicedo et al. 2004). Total time to first flower was added as a separate trait, measuring the complete time from planting to flowering.

All analyses were done using RStudio with R version 4.3.1. Line means across the four replicates were used to avoid pseudoreplication; means were calculated using the dplyr package.

To test the hypothesis that short stamen loss may be due to a pleiotropic genetic correlation with another trait, each measured trait was regressed separately on latitude and altitude. This determined whether each trait varies on the same clines, as would be expected if a pleiotropic genetic correlation was present. Next, a correlation matrix among all traits was generated using the packages ggplot2 and GGally. If any of the measured traits varied on the same clines and showed a correlation with short stamen number in the matrix, this could present a case for the pleiotropy hypothesis.

Finally, to test the hypothesis that selection may be acting to maintain short stamens at high latitudes and altitudes or lose them at low latitudes and altitudes, selection gradients were estimated

by regressing the fitness estimate on all traits together. To generate selection gradients, fruit number was standardized to relative fitness (w) by dividing each line value by the value of the mean fitness. The traits were also standardized by subtracting the mean of each trait from each line's value and dividing by the standard deviation. The traits are then in standard deviation units, allowing them to be compared. If relative fitness changed with the number of short stamens, this could suggest that selection is acting on short stamen number.

RESULTS

Variation of Traits Along Latitudinal and Altitudinal Clines

The first step to determine if there is a pleiotropic genetic correlation causing the clines seen in short stamen number is to examine if there are similar clines in other traits. Of the 18 measured traits, five showed a significant relationship with latitude. Days from emergence to bolting, rosette leaf number at bolting, days from planting to first flower, and short stamen number all increase with latitude (Figure 1A, 1B, 1C, 1E), with the latter by far the strongest relationship (Table 2). Pistil length has a negative relationship with latitude (Figure 1D). In contrast, none of the measured traits showed significant altitudinal clines (Table 3).

Trait Correlations

Next, we checked to see if short stamen number shows a correlation with any of the measured traits. Short stamen number was only weakly correlated with the other measured traits, with all correlations less than 0.25 and all but three less than 0.15. (Figure 2). Days to bolting and days from planting to first flower showed an extremely strong ($r = 0.92$, $p = 4.4e\text{-}103$) correlation, likely because most of the variance in the total flowering time is due to the days to bolting. Days to bolting and rosette leaf number at bolting showed a very strong correlation ($r = 0.66$, $p = 1.7e\text{-}32$), as did days to first flower and first flower height ($r = 0.39$, $p = 1.3e\text{-}10$). These make intuitive sense, as taking longer to bolt and flower would give the plant time to get larger, producing more leaves and taller inflorescences.

Germination success showed a strong negative correlation with days to emergence. This correlation between days to emergence and germination success may reflect that germination rate is an important part of seed vigor (Finch-Savage et al. 2010), and so it is likely that seeds that germinate slower may be less viable (Reed et al. 2022; Nguyen et al. 2012) and a smaller percentage

will successfully emerge. It is also possible that the slow-germinating seeds could be dormant, which would line up with the lack of germination.

Many of the floral traits showed significant intercorrelations. Pistil length and short stamen filament length were correlated (r = 0.48 and -0.45, respectively) with short stamen herkogamy, because herkogamy is estimated from these two measurements. Pistil length also had positive correlations (between 0.45 and 0.67) with tube length, limb length, long and short stamen filament lengths, and ovule number.

Fitness Regressions

Fitness regressions can give insight as to how short stamen number may be impacting fitness. Fruit production decreased with increasing short stamen number, as well as increasing days from emergence to bolting and long stamen filament length (Figure 3A, C, D, Table 4). Conversely, fruit production increased with increasing rosette leaf number (Figure 3B, Table 4).

DISCUSSION

The motivation of this study was to investigate why clines in short stamen number exist in *A. thaliana*. There are three primary hypotheses, including selection on short stamen number, pleiotropic correlations between short stamen number and other traits on the same clines, and genetic drift. To explore these hypotheses, this study has looked at three aspects of traits that vary on latitudinal and altitudinal clines – testing clines, correlations between traits, and fitness effects on trait differentiation among populations. These investigations and the intersections between them can provide insight into the mechanisms of short stamen loss in *Arabidopsis thaliana,* focusing on the role of selection and pleiotropy.

Pleiotropic Genetic Correlation Hypothesis

If the cline in short stamen number is caused by a pleiotropic genetic correlation with another trait, it's likely that that trait would vary on the same clines as short stamen number and also show a correlation with short stamen number.

Latitudinal clines were observed for days from emergence to bolting, rosette leaf number at bolting, days from planting to first flower, pistil length, and short stamen number. This aligns with what has previously been observed for days from planting to first flower (Debieu et al. 2013), days from emergence to bolting (Montesinos-Navarro et al. 2011), and short stamen number (Royer et al. 2016). Few studies have examined rosette leaf number varying on a latitudinal cline, and Samis (2008) only found a cline in one haplotype of the *PHYC* gene. Similarly, Love et al. (2024) found no significant latitudinal cline with pistil length and latitude, but a significant negative cline was found here. A negative relationship has been found previously between ovule count and latitude (Royer et al. 2016), but that cline is not observed here.

The latitudinal cline with flowering time is well established (Stinchcombe et al. 2004) and is well known, but here we observe that the cline with short stamen number is much stronger than that with days to first flower. Latitude accounts for 23.1% of the variance in short stamen number, while only accounting for 5.2% in days to first flower. Stinchcombe et al. (2004) did observe this cline in the field rather than in growth chambers, as were used in this study, but both were common gardens and took place outside of the native habitat.

The absence of any altitudinal clines contradicts what has been previously shown for rosette leaf number at bolting (Luo et al. 2015; Montesinos-Navarro et al. 2010), days to bolting from emergence (Montesinos-Navarro et al. 2010), days to first flower (Debieu et al. 2013; Montesinos-Navarro et al. 2010), and first flower height (Singh et al. 2015; Hämälä et al. 2018). Buysse et al. (2024, in prep.) found that short stamen number increases with altitude, but that is not observed here.

While this contradiction between expected and observed clines may support a case against previously recorded clines, another possible explanation is that the lack of significant altitudinal clines may be due to a limited distribution of elevations. The lines used in this study represent elevations of -24 to 1804 meters above sea level, while the total range of *Arabidopsis thaliana* reaches higher altitudes (Singh and Roy 2017). It's possible that this data doesn't reflect clines because it lacks the full range necessary. This aligns with the fact that most predicted latitudinal clines were demonstrated in lines used here, which represent a fuller portion of the range for latitudes (Hoffmann 2002). However, Buysse et al. (2024, in prep.) found that the altitudinal short stamen number cline levelled out between 1000 and 1500m, which suggests that the lines used here should have enough range to demonstrate the cline. Most of the altitudes for the lines we used fall within the lower range of latitudes (31 to 45 degrees), and there is a strong negative correlation between altitude and latitude in this dataset (r = -0.54, p = 1.4E-20). It is possible that isolating altitude from

latitude, perhaps by looking at these traits in a range of altitudes across a smaller latitude, like within a single mountain range, that the altitudinal cline might be observed.

Looking only at the latitudinal cline, there are four traits that vary along the cline and so could be candidates for the pleiotropic genetic correlation hypothesis. These traits include days from emergence to bolting, rosette leaf number at bolting, days from planting to first flower, and pistil length.

Within the correlations matrix (Figure 2), five traits showed a significant correlation with short stamen number, including days from emergence to bolting, days from planting to first flower, first flower height, ovule number, and long stamen anther length. It is worth noting that these correlations haven't been corrected for multiple comparisons, and so it's likely that not all of these traits are actually correlated with short stamen number.

The best candidates for pleiotropic genetic correlations with short stamen number are days from emergence to bolting and days from planting to first flower, as both of these traits vary on the latitudinal cline and show a significant correlation with short stamen number. However, due to the weak significance of the correlation and lack of shared variance with short stamen number (R^2 = 0.0225 at most), along with the weakness of the latitudinal cline compared to that of short stamen number, it is unlikely that either of these traits are contributing substantially to the clines in short stamen number via pleiotropy.

Selection Hypothesis

There is a decrease in fitness as days from emergence to bolting increases. If a plant flowers later, it may have less time in the growing season to produce flowers and fruits before it dies. This means that plants that flower earlier could be at a selective advantage, as they can produce more fruits before drought or frost comes, although that didn't happen to these plants as they were grown

in growth chambers. However, flowering earlier also means that they might be smaller and have less photosynthetic capability and so fewer resources to produce fruits, which aligns with the increase in fitness seen with increasing rosette leaf number.

The decrease in fruit production caused by increasing long stamen filament length might be a bit more straightforward, as it could be due to resource allocation. Longer long stamen filaments, which are correlated with more ovules, longer pistils, tubes, limbs, and short stamen filaments, represent a larger overall flower. Producing larger flowers likely requires more resources, and so these plants may produce fewer flowers (and so, fewer fruit).

Previous work has found that short stamens don't contribute significantly to selfed seed set (Royer et al. 2016), and so may not experience strong selection. That same pattern is seen here, as selection for fewer short stamens is weak but significant ($p = 0.008$). This selection may be due to some cost incurred by the plant for spending resources on a vestigial structure. Preliminary data (Conner, unpub.) has found direct selection for increased short stamen number at high latitudes, contrasting what is seen here. It is possible that there is direct selection acting both for more short stamens at higher latitudes and for fewer short stamens at lower latitudes.

CONCLUSION

In conclusion, many latitudinal clines were confirmed while no altitudinal clines were observed, notably short stamen number for both. There were quite a few correlations between short stamen number and the other traits, though they were barely significant and had very little shared variance. Days from emergence to bolting would be the most likely candidates for future investigation into pleiotropic genetic correlations, but due to weak correlations and the strength of their clines with latitude, this is not very likely. Lastly, weak but significant selection for fewer short stamens was observed. These results find no evidence for pleiotropy causing the clines in short stamen number, at least with the traits measured here. There is support for the hypothesis that direct selection acting on short stamen number may be responsible for the clines.

There is still much unknown about the mechanisms behind short stamen loss. Future studies should once again measure the selection on short stamens in the field, ideally in high and low altitude and latitude sites. The cost to produce short stamens should also be measured. Additionally, further investigation into the altitudinal clines should be done, ideally with a large range of altitudes within a limited range of latitudes. Alternative candidates for pleiotropic genetic correlations should be further screened, potentially in field environments and with a wider range of lines. Finally, more should be done to investigate the role of genetic drift in short stamen loss.

TABLES AND FIGURES

Table 1. Chamber settings for the duration of plant growth. *During vernalization, day light conditions cycled from 111, 110, 100, 100, 110, 111 over the course of six weeks, changing once per week.

Program	Program Length	Setpoint 1	Setpoint 2	Setpoint 3	Setpoint 4
Seed Stratification	1 week	5°C, lights 000			
Fall 1	4 weeks	**2:30am**: 11°C, lights 000	**8am**: 14.5°C, lights 111	**2:30pm**: 20°C, lights 111	**9pm**: 16.5°C, lights 000
Fall 2	2 weeks	**1:30am**: 5.4°C, lights 000	**8am**: 8.8°C, lights 111	**1:30pm**: 14.3°C, lights 111	**7pm**: 10.8°C, lights 000
Winter Vernalization	6 weeks	**10am**: 4°C, lights *		**5pm**: 4°C, lights 000	
Spring 1	2 weeks	**2:45am**: 4°C, lights 000	**8am**: 6.8°C, lights 111	**2:45pm**: 11.6°C, lights 111	**9:30pm**: 8.8°C, lights 000
Spring 2	2 weeks	**2:15am**: 4°C, lights 000	**7am**: 7.9°C, lights 111	**2:15pm**: 13.8°C, lights 111	**9:30pm**: 9.9°C, lights 000
Spring 3	2 weeks	**1:45am**: 4.9°C, lights 000	**6am**: 9.3°C, lights 111	**1:45pm**: 15.8°C, lights 111	**9:30pm**: 11.3°C, lights 000
Summer 1	2 weeks	**2am**: 6.6°C, lights 000	**6am**: 11°C, lights 111	**2pm**: 17.4°C, lights 111	**10pm**: 13°C, lights 000
Summer 2	2 weeks	**2:15am**: 8.2°C, lights 000	**6am**: 12.3°C, lights 111	**2:15pm**: 18.4°C, lights 111	**10:30pm**: 14.3°C, lights 000
Summer 3	2 weeks	**2:15am**: 9.4°C, lights 000	**6am**: 13.7°C, lights 111	**2:15pm**: 20.1°C, lights 111	**10:30pm**: 15.7°C, lights 000
Summer 4	2 weeks	**2:15am**: 10.4°C, lights 000	**6am**: 14.8°C, lights 111	**2:15pm**: 21.2°C, lights 111	**10:30pm**: 16.8°C, lights 000

Table 2. Model outputs from linear models regressing the measured traits on latitude. Values include the estimate of the slope of the relationship, standard error, *t*-value, *p*-value, and R^2.

Trait	Slope	Standard Error	*t*-value	*p*-value	R^2
Days to Emergence	0.0163	0.0237	0.685	0.494	-0.00212
Days to Bolting	0.308	0.09995	3.081	0.00229 **	0.0327
Rosette Leaf Number at Bolting	0.289	0.136	2.122	0.0349 *	0.0138
Days to First Flower	-0.0283	0.0327	-0.867	0.387	-0.000994
Total Days to First Flower	0.312	0.0815	3.833	0.00016 ***	0.0517
First Flower Height	-0.329	0.216	-1.526	0.128	0.00526
Short Stamen Number	0.036	0.00412	8.737	3.52E-16 ***	0.231
Ovule Number	0.00779	0.0564	0.138	0.89	-0.00392
Pistil Length (mm)	-0.00564	0.0026	-2.17	0.031 *	0.0146
Tube Length (mm)	0.00257	0.00187	1.377	0.17	0.00355
Limb Length (mm)	0.00282	0.00228	1.237	0.217	0.00211
Long Stamen Anther Length (mm)	0.000214	0.000388	0.55	0.583	-0.00279
Long Stamen Filament Length (mm)	-0.000986	0.0023	-0.429	0.688	-0.00326
Short Stamen Anther Length (mm)	-0.000115	0.000584	-0.197	0.844	-0.00402
Short Stamen Filament Length (mm)	-0.001997	0.00298	-0.671	0.503	-0.0023
Short Stamen Herkogamy	-0.00564	0.0026	-2.17	0.031 *	0.0146
Number of Side Stalks	0.00325	0.0284	0.114	0.909	-0.00395

Table 3. Model outputs from linear models regressing the measured traits on altitude. Values include the estimate of the slope of the relationship, standard error, t-value, p-value, and R2.

Trait	Slope	Standard Error	*t*-value	*p*-value	R^2
Days to Emergence	-0.000173	0.000415	-0.416	0.678	-0.00331
Days to Bolting	0.00168	0.00178	0.945	0.346	-0.000429
Rosette Leaf Number at Bolting	-0.00642	0.00241	-0.267	0.79	-0.00371
Days to First Flower	-0.0000504	0.0000574	-0.088	0.93	-0.00397
Total Days to First Flower	0.00139	0.00147	0.948	0.344	-4.07E-04
First Flower Height	-0.00144	0.0038	-0.38	0.705	-0.00342
Short Stamen Number	-0.000101	0.0000821	-1.227	0.221	0.00201
Ovule Number	-0.00127	0.000984	-1.29	0.198	0.00264
Pistil Length (mm)	0.0000706	0.0000457	1.546	0.123	0.0055
Tube Length (mm)	0.0000127	0.0000328	0.385	0.7	-0.0034
Limb Length (mm)	-0.0000252	0.00000401	-0.629	0.53	-0.00241
Long Stamen Anther Length (mm)	0.00000667	0.00000679	0.983	0.327	-1.38E-04
Long Stamen Filament Length (mm)	0.0000389	0.0000402	0.97	0.333	-2.38E-04
Short Stamen Anther Length (mm)	0.0000106	0.0000102	1.047	0.296	3.99E-04
Short Stamen Filament Length (mm)	-0.00000323	0.0000519	-0.062	0.95	-0.00417
Short Stamen Herkogamy	0.0000706	0.0000457	1.546	0.123	0.0055
Number of Side Stalks	-0.000603	0.000496	-1.217	0.225	0.00191

Table 4. Outputs from the linear model regressing fitness (estimated by fruit count) on the measured traits. Values include estimate representing the slope of the relationship, standard error, *t*-value, and *p*-value. Asterisks represent significance.

Trait	Estimate	Standard Error	*t*-value	*p*-value
Days to Emergence	-0.0239	0.0169	-1.417	0.158
Days to Bolting	-0.096	0.0274	-3.505	0.0005 ***
Rosette Leaf Number	0.0849	0.0226	3.757	0.0002 ***
Days to First Flower	0.0274	0.0245	1.12	0.264
First Flower Height	0.0229	0.02	1.141	0.255
Short Stamen Number	-0.0475	0.0178	-2.667	0.008 **
Ovule Number	0.0115	0.0178	0.649	0.517
Pistil Length	0.00478	0.0482	0.099	0.921
Tube Length	-0.00179	0.023	-0.078	0.938
Limb Length	0.0377	0.0193	1.951	0.0523
Long Stamen Anther Length	-0.0177	0.0203	-0.876	0.382
Long Stamen Filament Length	-0.0774	0.0289	-2.675	0.008 **
Short Stamen Anther Length	-0.0126	0.0255	-0.495	0.621
Short Stamen Filament Length	-0.00382	0.0464	-0.082	0.934
Short Stamen Herkogamy	-0.0188	0.0426	-0.441	0.66

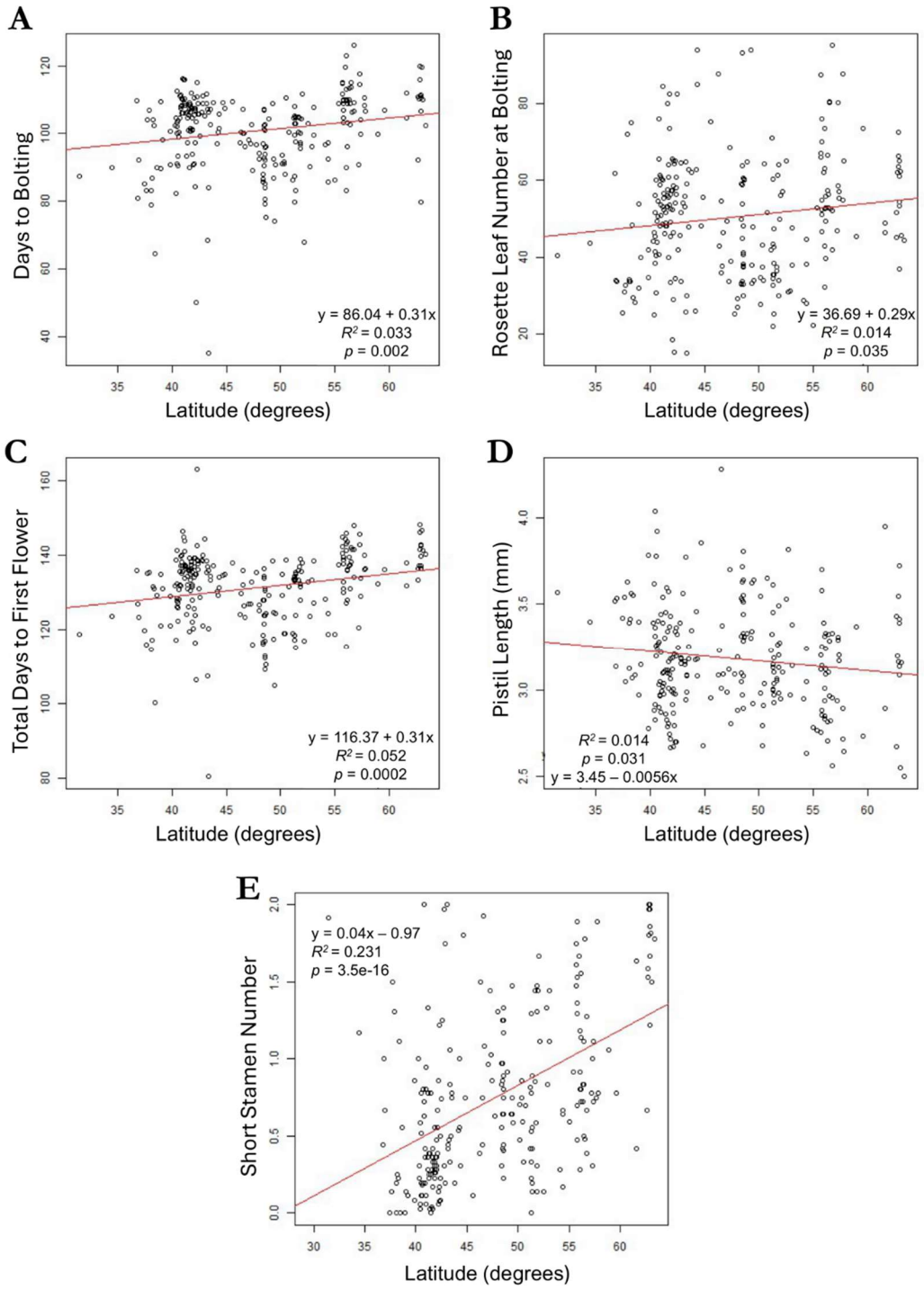

Figure 1. Regression plots for traits that show a significant ($p < 0.05$) cline with latitude. Lines representing this regression are graphed over the plots, as well as the equation of the line, R^2, and p-values. Days to bolting (A) is measured from emergence to bolting and total days to first flower (C) is measured from planting to first flower. N= 252 lines.

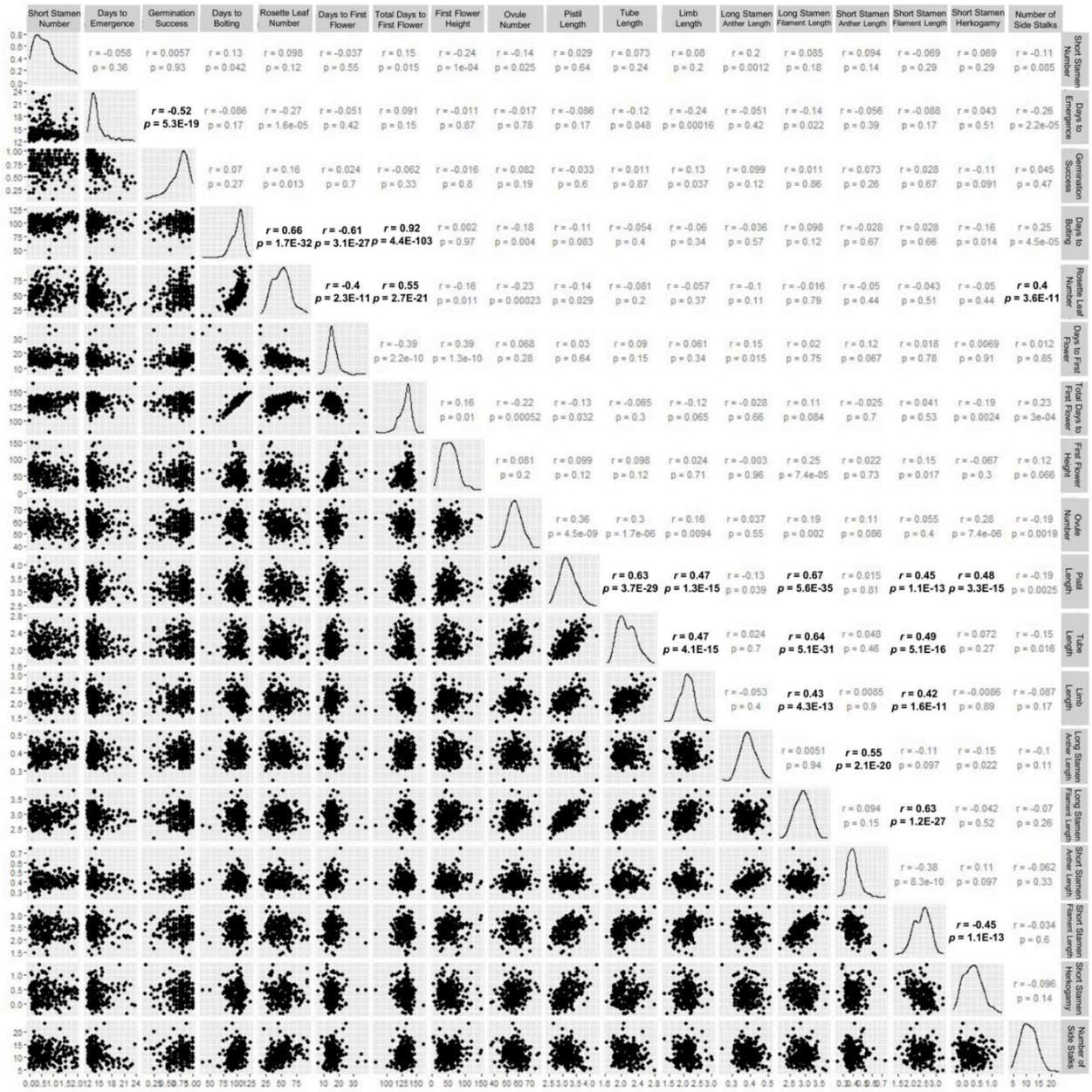

Figure 2. Correlations matrix for all measured traits, fitness components, and geographic information. The upper right corner shows Pearson correlation coefficients (r) and p-values for significance of the relationship (N = 253 lines). Significant relationships with *r* values over |0.4| are bolded. The diagonal plots show distributions of each trait. The lower left corner shows a scatterplot of the two intersecting traits.

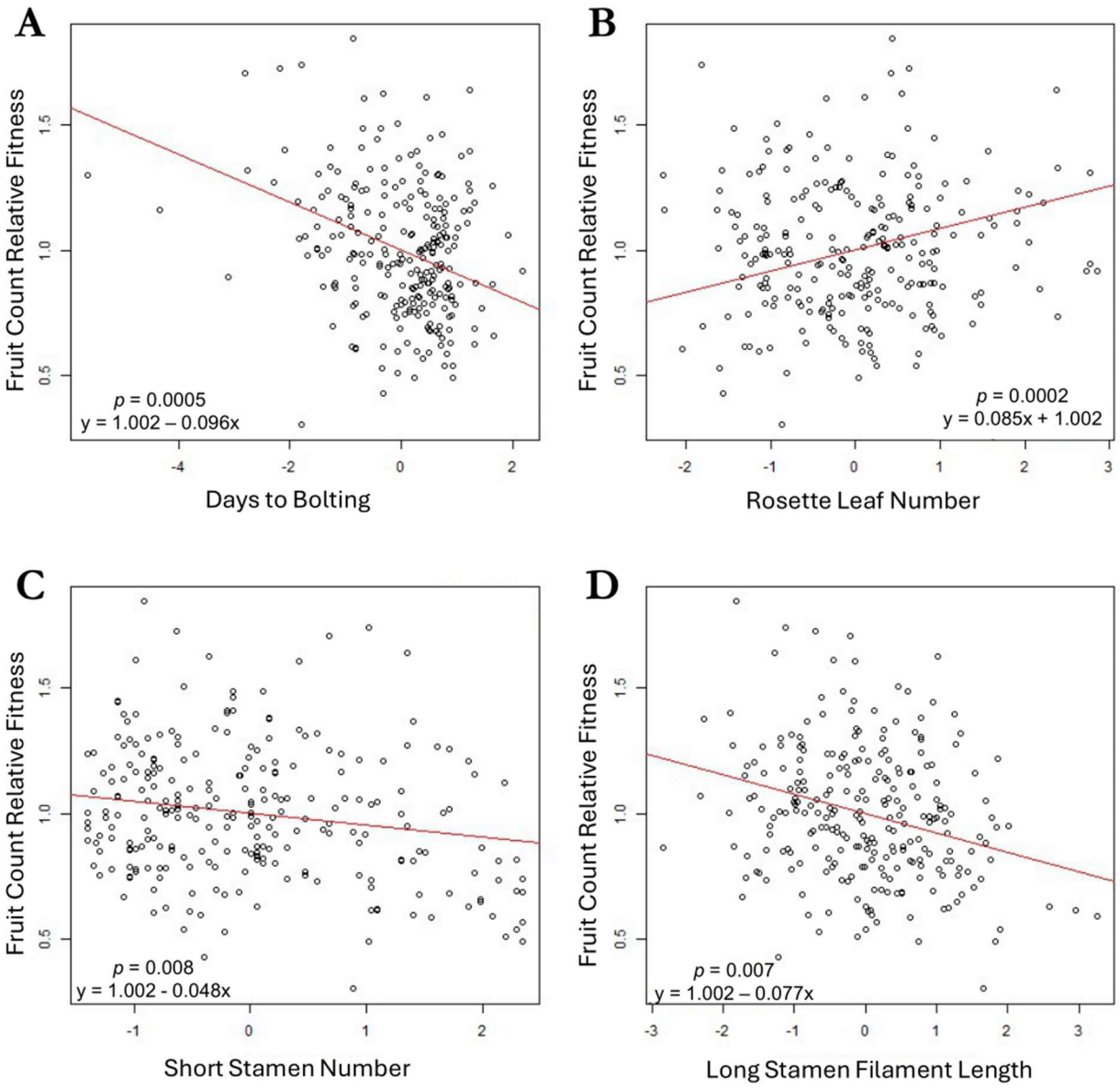

Figure 3. Selection gradients of relative fitness for fruit number regressed on the standardized measured traits. Only traits with a significant ($p < 0.05$) effect on fruit count are plotted. The equation for each relationship and the *p*-value indicating significance of that interaction is plotted in the lower corners.

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