Barriers to Gene Flow across a Light Gradient in *Cardamine cordifolia*

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Abstract

Ecotypic variation can be driven by a disproportionate balance between selective pressures and gene flow. If the strength of selection is weak relative to the level of gene flow, genetic variation will likely be homogenized among populations. However, in the absence or severe limitation of gene flow, divergent selection pressures are more likely to result in adaptive evolution to local environmental conditions. Both biotic and abiotic factors can limit gene flow, and these barriers can highly influence the exhibited traits in plants along a habitat gradient. At the Rocky Mountain Biological Laboratory, a native mustard *Cardamine cordifolia* has exhibited habitat specific differentiation across a light quality gradient. Flowering phenology was examined in *Cardamine cordifolia* in open sun, intermediate shade and deep shade habitats to determine if a separation of first, peak and last flowering time within the different environments was a mechanism for pre-zygotic isolation. I hypothesized that *C. cordifolia* found in open sun habitats will have an earlier flowering phenology in comparison to *C. cordifolia* found in deep shade habitats. Using two sites, a highly significant difference in first, peak and last flowering time was observed in the open sun and deep shade habitats. This gap in flowering phenology demonstrates a limited ability for open sun and deep shade plants to outcross in the short alpine growing season. Without gene flow the extremes of the light gradient are able to adapt locally in response to unique, divergent selective pressures.

Keywords: Gene Flow, Light Gradient, Cardamine, Brassicaceae, Mustard, Ecotypic Differentiation, Flowering Phenology

Introduction

Most species exhibit wide geographical variation in both genotype and phenotype. The magnitude of this variation is determined by a tug of war between evolutionary forces that promote local adaptation and forces that constrain it. Mechanisms that can promote evolutionary change include natural selection favoring adaptation to local environments, mutation, and genetic drift (Hendrick, 2007.) One mechanism that constrains genetic differentiation is the movement of gametes, individuals, or populations from one spatial unit to another, collectively known as gene flow (Slatkin, 1987.)

The quantification of gene flow between sub-populations is important for determining the potential for local adaptation. Without gene flow, sub-populations with divergent selective pressures are more likely to adapt locally to increase overall fitness. In large, freely-interbreeding populations there is typically ample genetic variation but the overall frequency of alleles reaches close to equilibrium due to gene flow, inhibiting evolution under static conditions. In smaller, reproductively isolated populations, alleles conferring higher local fitness are more likely to become fixed without the influence of a homogenizing effect (Wright, 1931.) The purpose of this research is to examine barriers to gene flow in *Cardamine cordifolia*, a native mustard found along alpine streams and ponds.

Although *C. cordifolia* is found in a variety of micro-habitats along a light gradient, early studies showed that there was a greater abundance of plants under willow shade when compared to open meadow sites (Louda and Rodman, 1983; Louda, 1988.) A reciprocal transplant common garden experiment was conducted to test the hypothesis that chronic herbivory accounts for the observed distribution of *C. cordifolia* under willow shade (Louda and Rodman, 1996.) In this experiment, sun-exposed plants showed higher leaf area damage from herbivory than shaded plants and sun-exposed plants that were treated with insecticide survived, grew, and reproduced at the same rate as shaded plants. Louda and Rodman concluded that chronic herbivory accounts for the observed distribution of *C. cordifolia* and rejected the notion that ecotypic variation or physiological adaptation to shade account for the distribution of *C. cordifolia* under willow shade.

Similar reciprocal transplant common garden experiments conducted by the Whiteman Lab from 2010-2014 at the Rocky Mountain Biological Laboratory (RMBL) (Humphrey et al., manuscript) showed that sun source plants showed increased petiole elongation in response to shading as compared to shade source plants and increased resistance to herbivory (larval mass) when in shade gardens. Contrary to Louda and Rodman's findings, this provides evidence for evolutionary divergence between sun source *C. cordifolia* and shade source *C. cordifolia*. This conflict between studies on the potential for ecotypic differentiation can be explained by the collection of shade source plants. While Louda and Rodman selected shade plants under willow trees, Humphrey et al. collected samples from the deep shade under evergreen forest canopies. It remains possible that evolutionary divergence is occurring at the extremes of the light gradient distribution (sun-exposed versus evergreen shade) but not in the middle (sun-exposed versus willow shade.)

One potential obstacle to gene flow between high light and low light habitats is the flowering phenology of *C. cordifolia*. In a previous experiment conducted at RMBL, increased herbivory on *C. cordifolia* in the sun as compared to willow shade was, at least in part, due to earlier plant development and flowering time of *C. cordifolia* found in the sun (Collinge and Louda, 1989). A more complete characterization of flowering phenology in *C. cordifolia* could show a limited extent of overlap between flowering times across a light gradient. This result would demonstrate that pre-zygotic isolation could facilitate evolutionary divergence at the extremes of the light gradient.

In summary, the results from past experiments indicate the possibility that *C. cordifolia* is ecotypically differential at the extremes of the light gradient distribution. There is also evidence that suggests there may be variation in the flowering phenology of *C. cordifolia* along a light gradient. The main objectives of my independent research are to answer the research question, does flowering asynchrony between sun and shade *C. cordifolia* reduce pollen flow between habitats? I hypothesized that *C. cordifolia* found in the sun will have an earlier flowering phenology in comparison to *C. cordifolia* found in the shade. This flowering asynchrony will act as a barrier to gene flow along a light gradient.

By examining flowering phenology of *C. cordifolia* across an ecotone, I was able to make conclusions on the restrictive forces on pollen flow between *C. cordifolia* across a light gradient. If there is indeed ecotypic differentiation between *C. cordifolia* found in high light and low light habitats, then the obstacles to gene flow between ecotypes is of great interest in determining the potential for evolutionary divergence. Examining these questions will contribute a unique perspective in regards to the adaptive divergence in *C. cordifolia*.

Methods

Study System and Study Site

At the Rocky Mountain Biological Laboratory (RMBL), *Cardamine cordifolia* (Heartleaf Bittercress) is a heavily studied native mustard (Brassicaceae) found along riparian corridors and in close proximity to other sources of water throughout the Rocky Mountains between 2150 and 3550 m in elevation (Louda and Rodman, 1983.) *C. cordifolia* is an herbaceous perennial that grows from short, slender rhizomes, with shoots 30-75 cm high (Louda and

Rodman, 1996.) Flowering occurs from late June through July, and small fruiting pods called siliques occur in August and early September (Louda and Rodman, 1983; Harte, 2003.) *C. cordifolia* exhibits alternate heart-shaped leaves, four-petaled white flowers, and dense growth patches connected by the underground rhizome system (Chew, 1977; Pappani, 2014.) *C. cordifolia* is capable of clonal reproduction as well as sexual reproduction using pollinators as a vector to deliver pollen and is able to be selfed but requires a pollen vector to do so (Louda and Rodman, 1983.)

Two sites were used to study flowering phenology of *C. cordifolia*. Both sites have been used by the Whiteman Lab in the past and have a stable population of *C. cordifolia* across a light gradient. The first site is along Copper Creek and the second is located along the U.S. Forest service trail 401 (Figure 1.) For the purpose of this paper the sites will be referred to as Copper Creek and 401.

Experimental Setup

Six transects were created at Copper Creek and 401 at locations along a suitable light gradient and each transect was divided into four, equally-spaced sample points. At each sample point, ramets of at least 25 *C. cordifolia* were counted and assigned a number. A random number table was used to select the numbers for five focal ramets and these plants were marked with a numbered identification tag. The total sample size of focal plants was 120 plants for each site. A densiometer was used to quantify canopy cover above each patch and each focal plant within a sample patch was assumed to have the same percent canopy cover. Each focal plant was measured twice a week for number of flowers, number of developing fruits, and, when flowering had ceased, number of mature fruits. Each patch was classified as sun (0-40% canopy cover), intermediate shade (40-80% canopy cover), and deep shade (80-100% canopy cover) based on natural cutoffs for percent canopy cover within each sample.

Statistical Analysis

First, peak and last flowering time was determined for each focal plant by looking at the first instance flowering occurred, the date the maximum flowers were observed, and the last day flowers were observed. Julian days after the first date of measurement at each site was used as the temporal scale for flowering phenology. If a focal plant had already completed flowering and exhibited developing or mature fruits by the first day of data

collection the plant was assigned 0 Julian days as if it had flowered on the first day of data collection. If a flower had not yet begun flowering by the last day of data collection it was assigned the maximum number of Julian days as if it had flowered on the last day of data collection. Using these values for the extremes of the flowering distribution allow a conservative estimation of flowering variation. An analysis of variance was used to determine the difference of means between sun, intermediate and deep shade first, peak and last flowering times. If the analysis of variance was significant, a post hoc Tukey-Kramer test was run to see which difference in means were significant and which were not.

Results

There was a significant difference in first flowering time between sun and shade habitats (P=0.0057) and sun and intermediate habitats (P=0.0395) at Copper Creek (Table 1, Figure 2) and sun and shade habitats (P=<0.0001) and intermediate and shade habitats (P=0.0233) at 401 (Table 1, Figure 3.) The difference in peak flowering time was significant between sun and shade habitats (P=0.0002) and sun and intermediate habitats (P=0.0051) at Copper Creek (Table 1, Figure 4) and sun and shade habitats (P=<0.0001) and sun and intermediate habitats (P=0.0216) at 401 (Table 1, Figure 5.) There was a significant difference observed between sun and shade habitats (<P=0.0001) and sun and intermediate habitats (P=<0.0001) at Copper Creek (Table 1, Figure 6) and sun and shade habitats (P=<0.0001) and sun and intermediate habitats (P=0.0379) at 401 (Table 1, Figure 3.)

Discussion

The results of this research support the hypothesis that flowering asynchrony along a light gradient exists in *C. cordifolia* and that the asynchrony is acting as a barrier to gene flow between sub-populations. Both the 401 and Copper Creek sites exhibited significant differences between sun and shade habitats in first, peak and last flowering time. At both sites the mean last flowering time in the sun habitat was earlier than the mean first flowering time in the shade. Although flowering time was highly variable in all treatments, this represents a strong barrier to gene flow that could inhibit the ability for a population to homogenize along a light gradient.

The displayed ecotypic variation in shade avoidance response and herbivory resistance in sun and deep shade *C. cordifolia* (Humphrey, manuscript) indicates that divergent selective pressures are strong relative to gene flow along a light gradient. Increased herbivory resistance in sun source plants is an adaptive response to chronic herbivory in the sun. Increased shade avoidance would not be an advantageous use of energy for a deed shade source plant that has been under a hundred year old spruce for its entire existence. However, increased shade avoidance in a sun source plant could result in dramatically different light availability if their competition for shade is other herbaceous plants. Under the migration-selection balance theory, geographic variation in the distribution of traits could reflect a disproportionate balance of selective pressures over gene flow in sub-populations (Chevillon et al., 1998.)

The results of this research show that gene flow in *C. cordifolia* is highly limited along a light gradient due to phonological gaps in flowering. If divergent selective pressures are relatively strong, then advantageous alleles are more likely to become fixed in the genetically isolated ecotypes. Deep shade and open sun habitats in the Rocky Mountains have vastly different selective pressures. *C. cordifolia* in the sun has increased light availability but suffers from much higher insect herbivory. *C. cordifolia* in the shade has much less light availability but has much less insect herbivory due to the colder conditions in the shade. These divergent selective pressures are likely strong enough relative to gene flow to shift the migration-selection balance towards habitat specific differentiation.

Intermediate shade plants exhibited first, peak and last flowering times between the flowering times of sun and deep shade plants. In terms of flowering phenology, intermediate shade plants behaved more like deep shade plants than sun source plants. Intermediate shade plants were significantly different than sun source plants in peak and last flowering time at both sites and significantly different than sun source plants in first flowering time at one site. A cline in traits may be observed in the intermediate sections of the light gradient as divergent traits are favored at the extremes (Haldane, 1948.) Moderate selection pressures as compared to the open sun and deep shade habitats could lead to variety of viable phenotypes in the intermediate shade. In this regard, plants in the willow shade may be the most phenotypically plastic out of all sub-populations along the light gradient.

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Appendices

Figure 1: Field sites for flowering phenology research. 401 site (north) consisted of two adjacent locations and Copper Creek site (west) consisted of just one location.

P-values for flowering time by site and habitat classification combination	First Flowering		Peak Flowering		Last Flowering	
	401	Copper Creek	401	Copper Creek	401	Copper Creek
Sun-Shade	<0.0001*	0.0057*	<0.0001*	0.0002*	<0.0001*	<0.0001*
Sun-Intermediate	0.0628	0.0395*	0.0216*	0.0051*	0.0379*	<0.0001*
Intermediate-Shade	0.0233*	0.7432	0.0587	0.6123	0.2139	0.3687

Table 1: P-values for flowering time by site and habitat classification combination. P-values were determined by a post-hoc Tukey Kramer test after an ANOVA test indicated significant differences in flowering time based on habitat classifications.

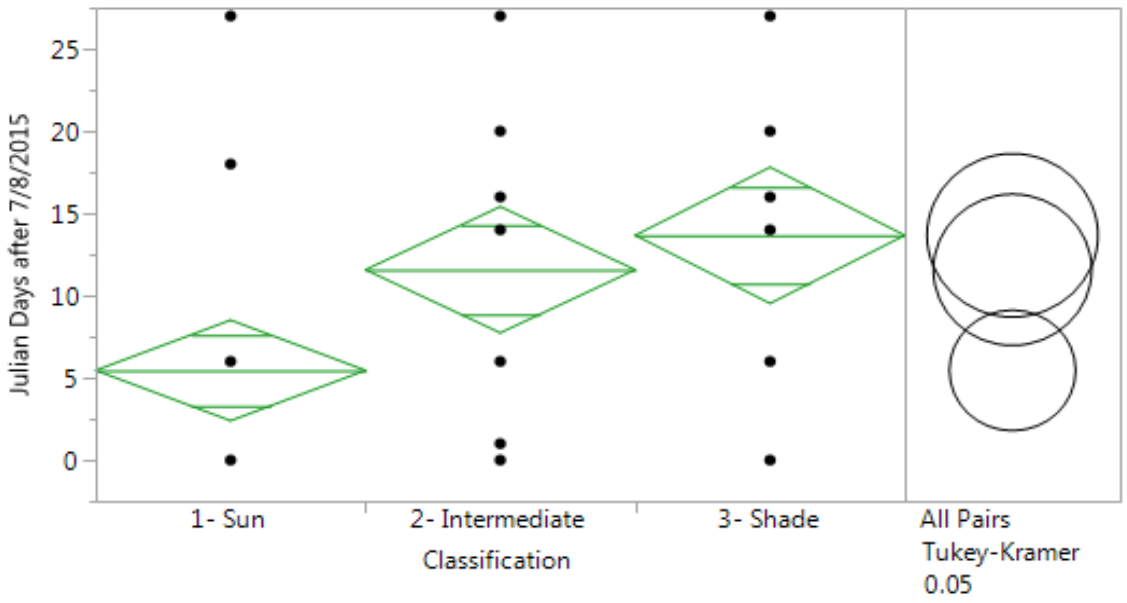

Figure 2: Mean day of first flowering by canopy cover classification for *C. cordifolia* at Copper Creek. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=0.0057) and Sun and Intermediate (P=0.0395). Diamond plots indicate means and 95% confidence intervals.

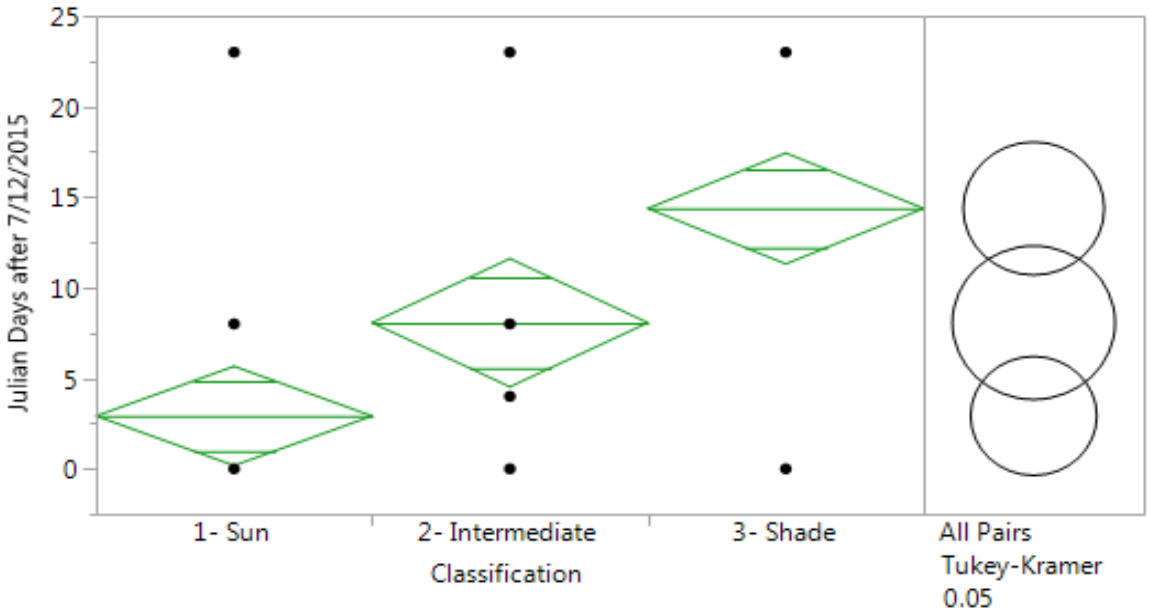

Figure 3: Mean day of first flowering by canopy cover classification for *C. cordifolia* at 401. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=<0.0001) and Intermediate and Shade (P=0.0233). Diamond plots indicate means and 95% confidence intervals.

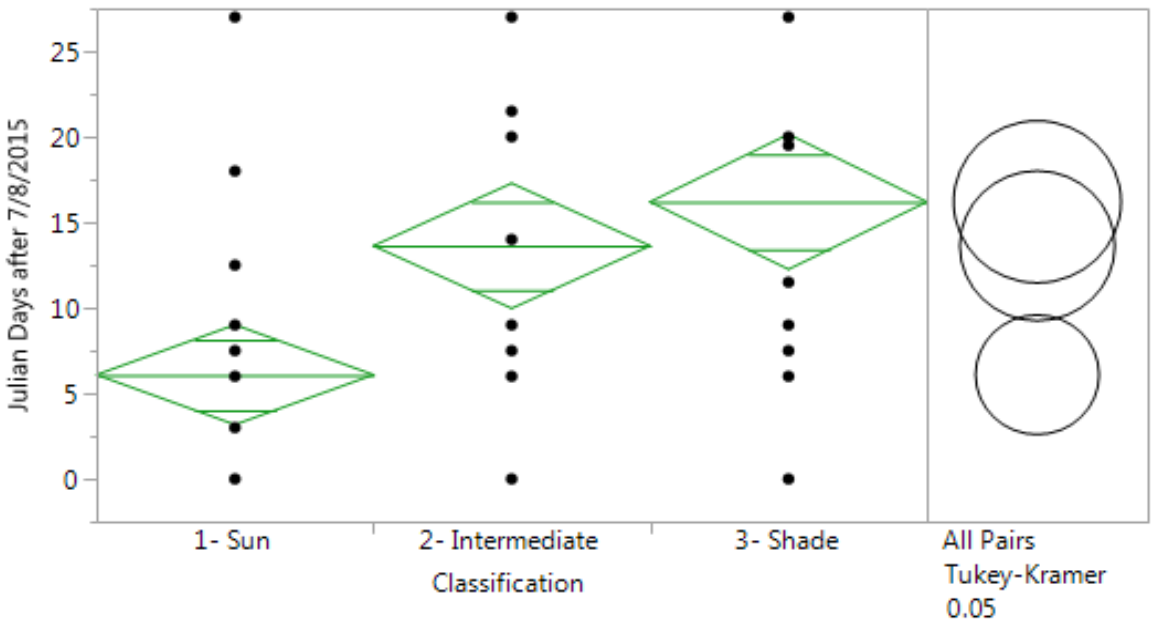

Figure 4: Mean day of peak flowering by canopy cover classification for *C. cordifolia* at Copper Creek. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=0.0002) and Sun and Intermediate (P=0.0051). Diamond plots indicate means and 95% confidence intervals.

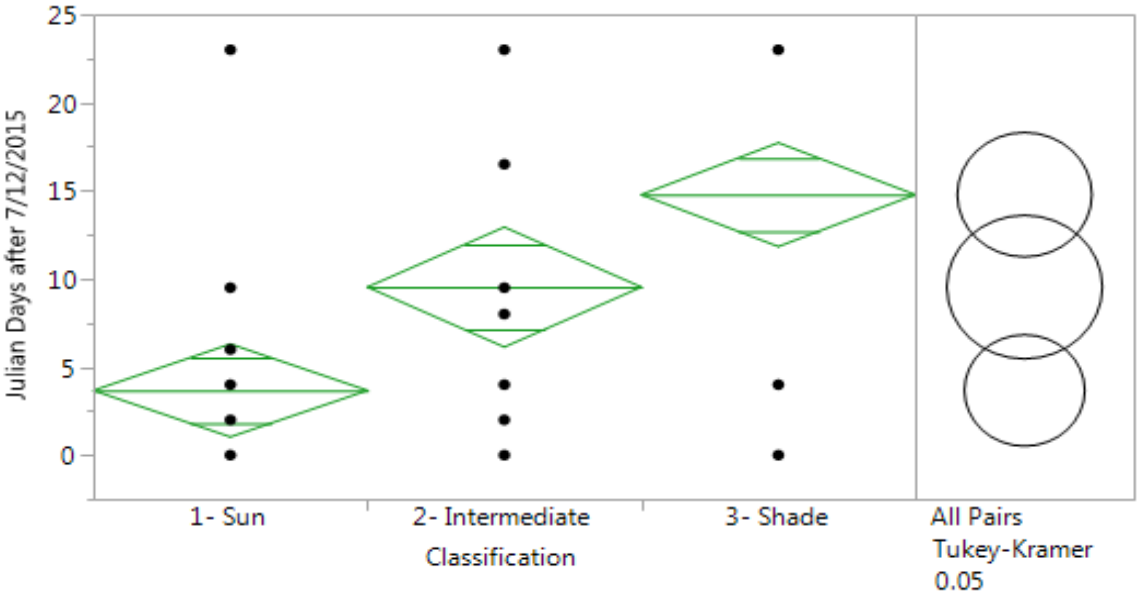

Figure 5: Mean day of peak flowering by canopy cover classification for *C. cordifolia* at 401. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=<0.0001) and Sun and Intermediate (P=0.0216). Diamond plots indicate means and 95% confidence intervals.

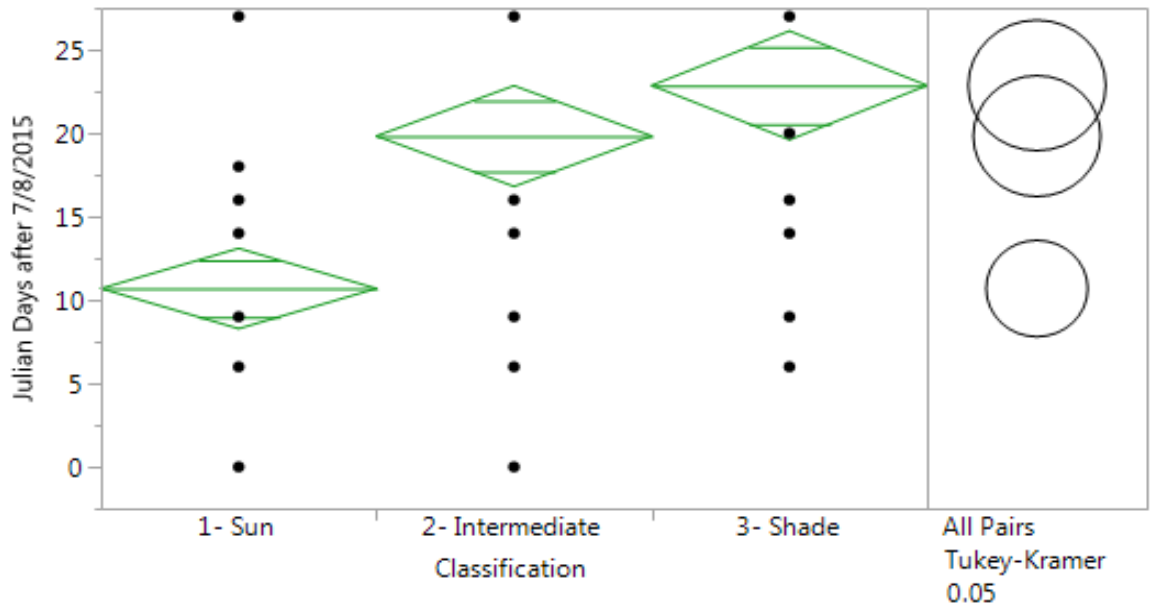

Figure 6: Mean day of last flowering by canopy cover classification for *C. cordifolia* at Copper Creek. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=<0.0001) and Sun and Intermediate (P=<0.0001). Diamond plots indicate means and 95% confidence intervals.

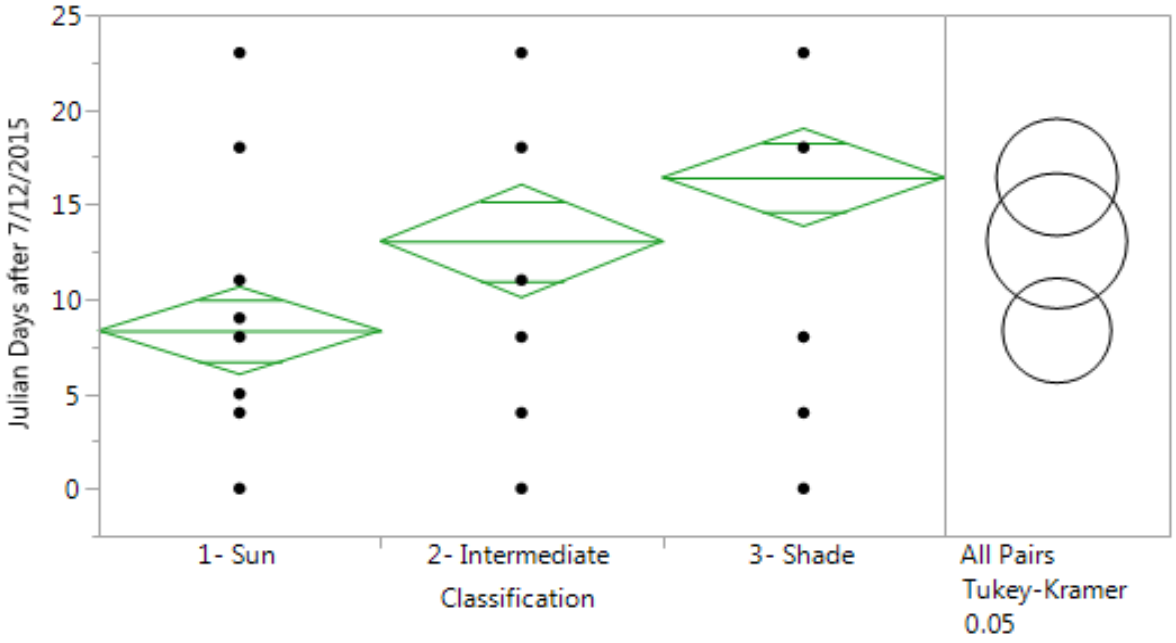

Figure 7: Mean day of last flowering by canopy cover classification for *C. cordifolia* at 401. A post-hoc Tukey-Kramer ordered differences report showed a significant difference in first flowering time between Sun and Shade (P=<0.0001) and Sun and Intermediate (P=0.0379). Diamond plots indicate means and 95% confidence intervals.

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