

Selection for Delayed Flowering Time in Response to Long Photoperiod to Increase Vegetative Growth and Multiple Harvests in Spider Plant (*Cleome gynandra*)

Martin Zorde¹, David R. Byrnes¹, Fekadu F. Dinssa², Steve Weller³ and James E. Simon*¹

¹New Use Agriculture and Natural Plant Products Program, Department of Plant Biology and the Center for Agricultural Food Ecosystems, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901

²World Vegetable Center, Eastern and Southern Africa, P.O. Box 10 Duluti, Arusha, Tanzania

³Department of Horticulture & Landscape Architecture, Purdue University, West Lafayette, In, SA

*Corresponding author: jimsimon@rutgers.edu

Manuscript received: April 21, 2020

Key words: African Indigenous Vegetables, non photoperiodic, slow bolting

ABSTRACT

Spider plant (*Cleome gynandra*), an herbaceous annual commonly used as a leaf vegetable across Sub-Saharan Africa is valuable as a rich source of vitamins, minerals, and protein. However, little work has been done to improve vegetative yield despite its nutritional value. Spider plant is considered a facultative long-day plant, and this study sought to screen for plants that do not flower during a long photoperiod, to improve vegetative yield. A total of 4536 spider plants from nine different advanced lines were evaluated in a greenhouse under 14-hour day/24 hr cycle daily for six months. From this initial screening, seeds were collected from isolated inflorescences of seven individuals selected for early flowering, six for intermediate flowering, and four for delayed flowering. These seeds were used to evaluate flowering cycles at the AVRDC - The World Vegetable Center, Eastern and Southern Africa research station, located in Arusha, Tanzania. Results indicated that among the selections compared, cultivar SP7-1 expressed significantly longer vegetative duration than SP1-1. Results from this study showed that flowering response

in specific spider plant accessions was heritable and that slow bolting is a legitimate breeding objective to improve vegetative yield.

INTRODUCTION

Spider plant (*Cleome gynandra*) is an annual herb used primarily as a leafy vegetable in India, Thailand, and several sub-Saharan African countries. Spider plant has been reported to be an important source of vitamins, minerals, and protein, particularly in countries like Ghana, Kenya, Zimbabwe, Namibia, South Africa and Benin (Chweya and Eyzaguirre 1999; Abukutsa-Onyango 2007; Dansi et al., 2012; Redhead, 1990) and Zambia (Weller et al., 2015). Spider plant also has important ethnopharmacological properties such as repelling ticks and alleviating fevers (Kwarteng et al., 2018). Despite its importance as a food source and pharmacological properties, little work has been done to evaluate germplasm for optimized production systems. The modest development status of this vegetable is disproportionate given how important the crop is as a food source in these regions (Kwarteng et al., 2018; Onyango et al., 2013).

Very few countries where spider plant is

grown wild or cultivated maintain a collection of spider plant germplasm (Kwarteng et al., 2018). The National Gene Bank of Kenya has been described as holding 45 accessions (Kemei et al., 1997). The spider plant germplasm held by the National Gene Bank of Kenya and in South Africa are reportedly lacking documentation as well as morphological and agronomic characterization (Wasonga et al., 2015). The AVRDC World Vegetable Center research station in Arusha, Tanzania, holds about 90 spider plant accessions collected from different countries in Africa, and is actively characterizing the germplasm held in its Genetic Resources Unit for field performance and production (Dinssa et al., 2015).

Spider plant produces perfect flowers on a terminal inflorescence that are both self- and outcrossing-compatible and has been observed to be protogynous, which would facilitate greater rates of outcrossing (Chweya & Mnzava, 1997; Raju & Rani, 2016). Spider plant has been reported to be a majority-outcrossing crop when pollinators are present (Raju & Rani, 2016). This likely means that wild populations have higher genetic variability (Wright, Ness, Foxe, & Barrett, 2008), while still having the ability to produce pure lines as spider plant is autogamous (Chweya & Mnzava, 1997).

Vegetative yield has been shown to have low heritability in spider plants due to yield being a quantitative trait with environmental effects as contributing factors (Chweya & Mnzava, 1997). Deflowering, the physical removal of flower buds, is often predicted to extend vegetative production, indirectly increasing leaf yield in crops with production limited by bolting. Deflowering has had variable results in the limited studies available in the literature (Chweya, 1995; Wangolo et al., 2015). Development of genetic resources capable of continuous vegetative production would obviate intensive labor requirements for deflowering, extend harvest season, and save significant time and costs to growers in replanting/resowing. As such, it is widely accepted as a reliable method for vegetative yield improvement (Kim et al., 2007; Morales et al., 2006; Wallace et al., 1993). Late flowering has been observed to have relatively high

heritability (Chweya & Mnzava, 1997; Omondi & Ayeicho, 1992).

Flowering time is a widely studied trait given the impact on optimal yield performance and conditional adaptability of cultivars. For instance, in maize (*Zea mays*), an outcrossing crop, flowering time is regulated by small additive QTLs with little association with genetic or environmental interactions (Buckler et al., 2009). In contrast, self-pollinating crops, such as *Arabidopsis* and rice (*Oryza sativa*), have been described as having relatively fewer genes with more substantial interaction effects (Izawa et al., 2003; Yano & Izawa, 2007).

Spider plant is considered a facultative long-day plant; therefore, selection for delayed bolting has potential for improving yield (Koevenig, 1973). However, little screening for such a trait has been done in spider plant (Wasonga et al., 2015). One of the most informative studies available in the literature characterizing multiple accessions of spider plant observed 26 accessions for traits including days to flowering (Onyango et al., 2016). They observed a range of flowering of between 30 and 42 days from when planted; demonstrating that there is significant variation in flower time across populations and concluded that later flowering is more advantageous for vegetable production in spider plant (Onyango et al., 2016). This study was initiated to identify genetic resources of spider plant with delayed flowering time for introduction as breeding lines or advanced cultivars for the improvement of vegetable production.

MATERIALS AND METHODS

Plant materials. Entries for this study included five advanced lines from WorldVeg: UG-SF-23 (SP3), ML-SF-17 (SP4), PS (SP5), UG-SF-15 (SP6), ML-SF-29 (SP7) and two separate batches of seeds from Simlaw seeds (SP1 and SP2) (www.simlaw.co.ke). The WorldVeg lines were originally developed by mass selection from germplasm collections. Five plants from each line were grown with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) in 3L plastic containers under 14 h

day, high-pressure sodium lights at the Rutgers University New Jersey Agriculture Experiment Station (NJAES) Greenhouses in New Brunswick, NJ from September 5th, 2016 and allowed to openly pollinate to produce seed. Seed was collected from individuals from each accession with two from SP3 (SP3-1 and SP3-3) and two from SP5 (SP5-1 and SP5-2) as they showed distinct phenotypes. This seed was used in the photoperiodism evaluation.

Photoperiodism evaluation. The progeny of nine lines were evaluated based on their response to a 14-hour a day photoperiod with 504 individuals from each line. The nine lines were SP1-2, SP2-2, SP3-1, SP3-3, SP4-1, SP5-1, SP5-2, SP6-1, and SP7-1. Seeds from each of the nine lines were sown in 72-cell trays with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) and germinated at the NJAES Research Greenhouses in New Brunswick, NJ. One seed was planted in each cell with 7 trays per line, for a total of 4,536 seeds planted. Plants were hand-watered and were observed daily over the course of six months with respect to emergence and days to flowering. Each plant was recorded individually. Seeds were collected from isolated inflorescences of selected individuals and used for the field study. The plant codes of the selected individuals from the photoperiodism evaluation reflects the parent line of the selected plant (SP1-2 parent line of SP1-1 through SP1-11; SP2-2 parent line of SP2-2 and SP2-3; SP3-1 Parent line of SP3-6; SP3-3 parent line of SP3-4, SP3-5, and SP3-7; SP4-1 parent line of SP4-1; SP5-1 parent line of SP5-2 and SP5-3; ML-SF 29-SP7 parent line of SP&-1) The photoperiodism evaluation identified seven individual plants selected for early flowering (SP1-9, SP1-10, SP1-11, SP2-3, SP3-6, SP3-7, SP5-3, and SP6-1. Note SP6-1 did not have viable seed for field study), seven for average flowering (SP1-5, SP1-6, SP-7, SP-8, SP2-2, SP4-1, and SP5-2), and four for delayed flowering (SP1-1, SP3-4, SP3-5, and SP7-1).

Field evaluation. The progeny of greenhouse-selected individuals were evaluated at the WorldVeg field station, Arusha, Tanzania, eastern

and southern Africa (lat. 3.4°S, long. 36.8°E, 1290 m elevation) to observe time to flowering. Seedlings were grown in 72-cell trays with sterilized media composed of forest soil/compost, manure, sand, and rice husks in a ratio of 3:2:1:1 by mass. Progeny were transplanted on April 21, 2017 and individually evaluated for a number of days to flowering from date of transplant. The site was characterized by well-drained clay loam soil with pH 6.4. Furrow irrigation was applied as needed.

Statistics. Data were analyzed from the greenhouse trial and field trial separately, using PROC UNIVARIATE MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to create histograms to demonstrate distributions from observed days to flowering across all observations on individual plants (Figures 1 and 2). PROC ANOVA in SAS 9.4 was used to conduct mean separation analysis with Fisher's Least Significant Difference (LSD) (Table 1), results from field trial observation of days to flowering (Table 2) to compare plots was used to create box plot visualization (Figure 3).

RESULTS AND DISCUSSION

The distribution of days to flowering across individuals included in the greenhouse trial was found to follow a normal distribution excluding a group with highly delayed flowering (Figure 1). The mean days to flowering was 52.7, the median days to flowering was 50. After 173 days, twelve individuals had not yet flowered and were moved to a growth chamber under 10h light. Those that did not bolt under reduced daylength were moved back to the main greenhouse room under 14h light. Four individuals with delayed flowering produced seed, which was collected from inflorescences isolated with glassine bags. Inflorescences were isolated for seed collection from seven "intermediate" time to flowering individuals, and eight "early" time to flowering individuals. Seed was transferred to WorldVeg in Arusha, Tanzania, for field evaluation.

The time to flowering response of individuals in the greenhouse trial had a wide range from a minimum of two days to a maximum of >182 with a standard deviation of 27.9. The median in the

greenhouse trial at 50 days was not widely inconsistent from the mean, approximately 53 days (Figure 1). The outlier group of plants recorded as taking longer than the approximately six-month period of data collection caused the frequency distribution of responses observed in this trial to be considerably non-normal.

The median days to flowering observed in the field trial in Arusha was 38 days, the mean was about 36 days with a SD of 7.3, and the maximum was 57 days (Table 2). The frequency distribution of days to flowering in the field trial was considered to fit a normal distribution (Figure 2). This distribution of results is more similar to the results found by Onyango et al. (2016) in Kenya. However, the maximum days to flowering in that study was observed to be 42, while the mean days to flowering across entries in this study was about 47. This comparison of results may represent the beneficial effect of purposefully selecting for delayed flowering time; however, the results of these studies are not directly comparable, having been conducted in different trials and locations (Table 1 and Figure 3).

There was a wide discrepancy between the observed days to flowering in the greenhouse trial and field trial in Arusha (Figures 1 and 2). A comparison of these distributions demonstrates days to flowering were more frequently lower in the field trial than in the greenhouse-trial, indicating substantial variation due to environment. One possible environmental difference could be day length and light intensity, as the plants were grown under daylight conditions between 11:52 hours to 12:17 hours of daylight (Solartopo.com), as opposed to 14 hours for the greenhouse study. The exact light hours required to induce flowering in spider plant would help to explain the differences between the field and greenhouse. Additionally, light intensity may have played a role as differences in light intensity have been shown to impact flowering time and yield (Kang, Krishnakumar, Atulba, Jeong, & Hwang, 2013) and the field evaluation likely received more intense light due to the latitude of the research station (Stunda-Zujeva, Zuteris, & Rugele, 2018).

Field performance of the progeny collected after greenhouse evaluation was found to be most inconsistent with the initial selection observations of the individual parents. The progeny of SP1-1 had delayed flowering in the greenhouse trial but had the lowest mean days to flowering in the field trial. The individual SP 3-4 was also observed to have delayed flowering in the greenhouse, yet the mean days to flowering were only narrowly within the lower threshold of the least significant difference value difference from the overall field trial mean. The progeny of SP3-5 were observed to be within the least significant difference value from the overall field trial mean.

The progeny of SP7-1 were the only plants selected for delayed flower time greater than the least significant difference value from the overall field trial mean. The average days to flowering of the progeny of SP7-1 was found not to be significantly greater than the mean days to flowering for the progeny of SP5-2 and SP4-1, individuals selected from the greenhouse trial as having intermediate days to flowering. The mean days to flowering for the progeny of SP7-1 was substantially higher than all other plots with a difference of approximately six days greater than the plot with the nearest mean observed days to flowering.

The flowering behavior of parents and their progenies were not consistent, indicating that genetic control of the phenomenon was weak or inconsistent. These plots were all found to be within the least significant difference value of the overall field trial intermediate days to flowering and can be considered to have average days to flowering in the field trial.

There are multiple potential explanations for the delayed flowering of SP7-1 progeny. If spider plant follows a simple additive model like that of Maize, SP7-1 may have the greatest homogeneity in loci associated with delayed flowering. Alternatively, SP7-1 may lack expression of genes associated with early flowering time. If spider plant follows a genetic flowering archetype like rice or *Arabidopsis*, it may be the case that SP7-1 lacks interaction effects otherwise found in the

individuals in this study selected for delayed flowering. The potential genetic factors could be further explored if the flowering times of selfed SP7-1 were compared to that of a cross between SP7-1 and an early flowering line (possibly SP1-1). This would likely help to explain some of the possible genetic factors involved with late flowering. This yields a better understanding of the inheritance of this trait.

Early flowering of spider plant limits the total marketable yield and number of vegetative leaf harvests growers in Africa can expect in a growing cycle, thus limiting their profits and maximizing expenses in replantings (Chweya & Mnzava, 1997). Results from this study indicate that slow-bolting can be a trait for which plant breeders can select. Yet, the results also show the importance that the environment plays on the expression and selection process, confirming the importance of final selections and reconfirmation of the slow bolting trait in the field under target production environment where the trait is needed to be expressed.

Progeny from SP1-1 were observed to exhibit below-average for days to flowering; having been selected for delayed flowering in the greenhouse, these observations would support an interaction mechanism with the environment, or associated with poor seed quality that might come from late harvest in the production season – this is suggested from low number of seeds germinated or plants established from this line. Despite the limited number of progeny observed and a large number of potential genes underlying this trait, the distinct contrast between the greenhouse and field

performance supports the hypothesis that there are significant genotype x environment interactions that affect flowering time in spider plant.

The variation among progeny in SP3-4 and SP3-5 would indicate a distribution consistent with a quantitative trait. Having been derived from individuals observed to flower beyond 180 days in the greenhouse, an interaction effect of environment on the genetic mechanisms in these entries may exist. Alternatively, a genetic distinction between entries from progeny and parent generations observed may be responsible for the variation of performances.

Given the uncontrolled open-pollination origin of seed evaluated in this trial, further evaluation would be appropriate to be confirmed consistent performance with additional trials. Seed from the individuals observed in the field trial in Arusha, Tanzania has been collected for further evaluation. The apparent genetic improvement of SP7-1 represents the longest vegetative production of a spider plant accession described in the limited observations currently available on this crop. Further work is needed to verify the pattern of inheritance of late bolting under long day conditions, and will likely be done by observing the bolting times of F_2 's and the backcrosses of F_1 hybrids from early x late bolting crosses. Understanding of the patterns of inheritance would provide a strong foundation to be able to develop a series of non-bolting/non-photoperiodic spider plant that have good field performance and high nutritional composition.

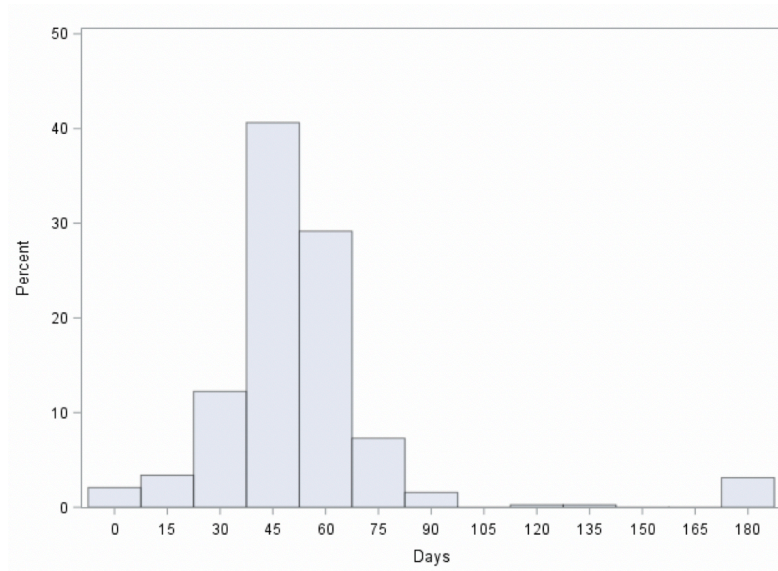


Figure 1. Histogram of time (days) to flowering from individuals observed in initial screens at the New Jersey Agricultural Experiment Station, research greenhouses, Rutgers University, New Brunswick, New Jersey. Screened population had the following measures of central tendency in relation to days to flower: mean 52.7 days, median 50.0 days, minimum 10.0, max 182 days, standard deviation 27.9 days.

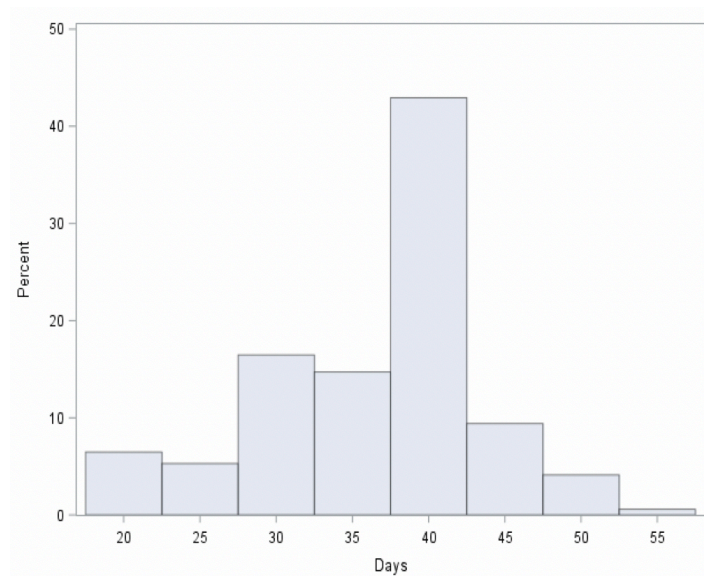


Figure 2. Histogram of days to flowering for field trial at World Vegetable Center field research station, Arusha, Tanzania. Screened population had the following measures of central tendency in relation to days to flower: mean 36.8 days, median 38.0 days, minimum 20.0 days, maximum 57.0 days, standard deviation 7.3days.

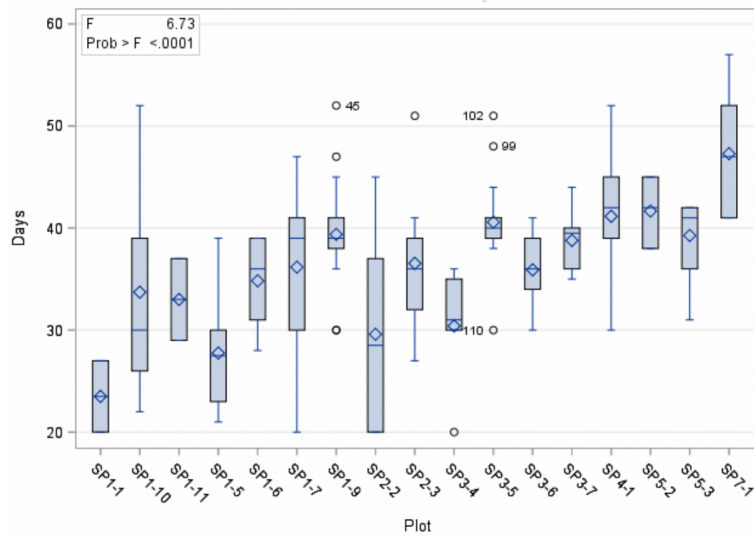


Figure 3. Box plot of field trial results for days to flowering for different advanced Spider plant lines at WorldVeg Center, Arusha, Tanzania.

Table 1. Field trial results with mean separation of mean days to flower for progeny plots in Arusha, Tanzania. Mean separation performed with Fisher’s least significant difference analysis $\alpha=0.05$, error degrees of freedom 153, error mean square 34.94171, critical value of t 1.97559, least significant difference value 6.6.

Entry	n (number of entries in progeny plot)	Mean days to flowering	T-test grouping	Greenhouse characterization of parent entry
SP7-1	7	47.3	A*	Delayed
SP5-2	3	41.7	AB	Intermediate
SP4-1	13	41.2	ABC	Intermediate
SP3-5	19	40.6	BC	Delayed
SP1-9	13	39.4	BCD	Early
SP5-3	11	39.3	BCD	Early
SP3-7	14	38.8	BCD	Early
SP2-3	13	36.5	BCDE	Early
SP1-7	23	36.2	BCDEF	Intermediate
SP3-6	10	35.9	BCDEF	Early
SP1-6	6	34.8	CDEF	Intermediate
SP1-10	7	33.7	DEFG	Early
SP1-11	2	33.0	DEFG	Early
SP3-4	5	30.4	EFG	Delayed
SP2-2	10	29.6	FGH	Intermediate
SP1-5	12	27.8	GH	Intermediate
SP1-1	2	23.5	H	Delayed

*Means with the same letter are not significantly different

Table 2. Spider plant lines selected for late flowering at Rutgers University and later field-grown at WorldVeg, Arusha for further evaluation, 2017. Table shows days to flowering of the 20 progeny (P1-P20) from each of the 18 selected lines*.

Line ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	Mean
SP1-1	27	20																			24
SP1-5	21	25	25	21	30	27	21	30	37	28	39	29									28
SP1-6			35	39	37	31	39	28													35
SP1-7	39	37	32	30	30	41	20														33
SP1-8	39	38		39	41	36	41	41	26	39		47	40		29	46	45		34	22	38
SP1-9	47	52	30	39	39	38	39		30	36		38	41	45		38					39
SP1-10	52	29	30	38	22	39	26														34
SP1-11	29	37																			33
SP2-2	45	31	30	20	20	20	39	37	27	27											30
SP2-3		51	36	29	32	38	36	36	41	32	40		27	39	38						37
SP3-4	35	20		30	36	31															30
SP3-5	44	40	41	38	39	48	41	40	51	41	41	39	39	39	40	42	30	40	38		41
SP3-6	39	37	35	41	34			39		30	39	30	35								36
SP3-7	39		41	40	38	36	38	40	36	40	40	40	44	35	36						39
SP4-1	45		52	44	39	42	46	42		30	45	42	41	36		31					41
SP5-2	42	45	38																		42
SP5-3			35		42		36		42	42	41	38	42		31	42	41				39
SP6-1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SP7-1	41	41								57	46				52	47	47				47
Mean																					36
Min																					24
Max																					47

*, Line SP6-1 provides original selections with significant delayed flowering, but not sufficient viable seeds were available for the follow-up field trial and thus not included in this field study.

ACKNOWLEDGMENTS

This research was supported by the Horticulture Innovation Lab with funding from the U.S. Agency for International Development (USAID EPA-A-00-09- 00004), as part of the U.S. Government's global hunger and food security initiative, Feed the Future, for project titled "Improving nutrition with African indigenous vegetables" in eastern Africa. Funding also was provided by the New Jersey Agricultural Experiment Station Hatch Project 12131, and the Rutgers IFNH Center for Food Systems Sustainability. We thank the World Vegetable Center and its long term strategic donors: Republic of China (Taiwan), UK aid from the UK government, US Agency for International Development, Australian Centre for International Agricultural Research, Germany, Thailand, Philippines, Korea, and Japan for also contributing resources and logistical support. We thank John Bowman, USAID- Washington, D.C., Beth Mitcham, UC-Davis and Marcos Wopereis, WorldVeg Center for their support. This work was conducted as part of D. Byrnes dissertation studies.

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