

NATURAL SELECTION BY POLLINATORS ON FLORAL ATTRACTIVE AND DEFENSIVE TRAITS DID NOT TRANSLATE INTO SELECTION VIA FRUITS IN COMMON MILKWEED

Yedra García^{1,2}, Benjamin S. Dow^{1,3}, Lucie Vézina^{1,4}, Amy L. Parachnowitsch^{1,*}

¹Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada

²Current address: Biodiversity Unit, Department of Biology, Lund University, Lund, Sweden

³Current address: Biology Department, Saint Mary's University, Halifax, Nova Scotia, Canada

⁴Current address: Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

Abstract—Considering both pollinator and herbivore pressures on plant reproductive and defensive traits is key to understanding patterns of selection for plants. However, phenotypic selection studies connecting floral traits and plant defenses with pollinator activity and herbivore damage remain rare. We used the common milkweed, *Asclepias syriaca* (Apocynaceae), to study phenotypic selection on attractive and defensive traits, and nectar rewards. We measured herbivore (leaf damage) and pollinator activity (pollinia movement) and quantified selection via female (pollinia insertions and fruit number) and male fitness (pollinia removals). We found selection to increase plant and inflorescence size and to decrease floral size (i.e. petal width) via female fitness. We also detected selection to increase floral but not leaf latex. The lack of selection on leaf latex was congruent with the low herbivory observed, however we also did not observe florivory in the population that would explain the advantage of more floral latex. Interestingly, we found selection on attractive traits differed via pollinia insertions and fruits initiated, suggesting that something other than pollinators was driving selection via fruit production. In contrast to female fitness, we did not find selection on any trait through male fitness, suggesting no sexual conflicting selection, at least through these proxies. Our findings reinforce the importance of the direct assessment of pollinator pressures in phenotypic selection studies before assuming pollinators as drivers of floral evolution by natural selection. Further work in southern populations closer to the centre of the species range, where herbivory and plant defense investment are higher, may help elucidate selection on attractive and defensive traits.

Keywords—*Asclepias syriaca*, herbivory, latex, nectar, male fitness, phenotypic selection

Journal of Pollination Ecology,
36(6), 2024, pp 73–84

DOI: [10.26786/1920-7603\(2024\)758](https://doi.org/10.26786/1920-7603(2024)758)

Received 1 June 2023,
accepted 24 February 2024

*Corresponding author:
aparachn@unb.ca

INTRODUCTION

Plant mutualists and antagonists such as pollinators and herbivores are important agents of selection on plant traits (Strauss 1997; Strauss & Whittall 2006; Caruso et al. 2019). Pollinator-mediated selection can favor floral traits that increase pollinator attraction such as large floral displays, bright colours, or particular compounds of the floral scent (Parachnowitsch & Kessler 2010; Sletvold et al. 2016; Caruso et al. 2019; Chapurlat et al. 2019). However, more apparent floral signals

may also attract antagonists such as plant herbivores. Attracting both your mutualists and antagonists can lead to patterns of conflicting selection on floral traits (e.g., Sletvold et al. 2014; Knauer & Schiestl 2017), although selection by pollinators and herbivores does not always lead to simple patterns of conflicting selection (e.g., Parachnowitsch & Caruso 2008; Sletvold et al. 2014; Egan et al. 2021). Plants that require pollinators for reproduction may also be limited in their defences due to shared repellence of both herbivores and pollinators (Adler et al. 2012;

Manson et al. 2012). Furthermore, herbivores' feeding, developing and/or mating on plant tissues can alter pollinator attraction through direct effects on the plant's appearance or via herbivore-induced defenses (Kessler et al. 2011; Burkle & Runyon 2016; Kessler & Chautá 2020), showing some of the possible complexities for these plant-animal interactions. Much like pollinators driving selection on floral traits, herbivores exert selective pressures on plant defenses (e.g., latex exudation, trichome density, secondary metabolites, Agrawal 2005, Lankau 2007); however, herbivores can also influence selection on plant reproductive traits (Parachnowitsch & Caruso 2008; Knauer & Schiestl 2017; Ramos & Schiestl 2019; Santangelo et al. 2019). Hence, considering pollinator and herbivore selection pressures on both defensive and reproductive traits is important to understand how phenotypic selection shapes plant evolution.

We used the common milkweed *Asclepias syriaca* L. (Apocynaceae) to study patterns of phenotypic selection on attractive and defensive traits and nectar rewards. *Asclepias syriaca* has a diverse community of pollinators and herbivores and has become a model system of chemical ecology and plant-insect coevolution (Agrawal 2017). Pollinator activity on milkweed flowers is easy to detect because of its specialized pollen packages called "pollinia" as seen in orchids. Pollinia are moved as a unit by pollinators and because of the specialised structures, removal or insertion in a flower can only happen by pollinator activity. Therefore, insertions and removals can be tracked and used as a proxy of female and male fitness components (e.g., Morgan & Schoen 1997; Caruso et al. 2005; Thompson et al. 2017). While pollinia movements are indirect measures of male and female fitness, studies in other milkweed species have found positive correlations between the number of removed pollinia and seeds sired, suggesting pollinia removal may be a reasonable estimate of male fitness in milkweeds (Broyles & Wyatt 1990; La Rosa 2015). In previous studies of *A. syriaca*, phenotypic selection via female and male fitness through pollinia movement has shown contrasting patterns (e.g., Morgan & Schoen 1997), highlighting the importance of examining both to understand the evolution of traits. Measuring selection via female and male fitness could be especially relevant given herbivores may have varying effects on patterns of

selection depending on the fitness estimate (Lehtilä & Strauss 1999; Thompson et al. 2017), however plant selection studies have mainly focused on female fitness due to the challenges of estimating male fitness for most species (Ashman & Morgan 2004).

Natural selection on floral characters in *A. syriaca* populations show some variation and consistency between studies. Selection for increased floral hood dimensions is common (Morgan & Schoen 1997; Caruso et al. 2005; La Rosa & Conner 2017) but in other populations, selection on floral dimensions has not been detected (Thompson et al. 2017). While *A. syriaca* inflorescences have many flowers that only set one to a few fruits (Caruso et al. 2005; La Rosa & Conner 2017), selection for bigger display sizes (more flowers) is also common via fruits and/or seeds (Caruso et al. 2005;) suggesting these flowers play a role in successful seed set despite many not developing into fruits. Floral nectar acts as a reward in these long-lived flowers (Southwick 1983; Wyatt et al. 1992) and also plays a role in pollen germination in *A. syriaca* (Kevan et al. 1989). Floral nectar is an important trait mediating plant ecological interactions and the primary reward for many pollinators (Parachnowitsch et al. 2019). Nectar characteristics, such as amount and sugar concentration, can influence pollinator behaviour and ultimately plant outcrossing rates (Cnaani et al. 2006; Heil 2011; Pyke 2016). Nectar can also change in response to herbivory (Adler et al. 2006; Bruinsma et al. 2014; Hoffmeister & Junker 2017). In *A. syriaca*, herbivory may alter the sugars in nectar (Gustafson et al. 2023). Despite its importance to plant-pollinator interactions, there are surprisingly few studies that measure selection on nectar traits (reviewed in Parachnowitsch et al. 2019) and none that connect selection on nectar to plant defences.

Milkweed deploys a battery of defensive traits including latex, trichomes, and toxic metabolite compounds (Agrawal 2005; Agrawal et al. 2014; Jones and Agrawal 2016). Latex is a sticky substance that acts as both a physical and chemical defense because it can clog the mouthparts of herbivores obstructing their feeding and contains secondary metabolites (Agrawal 2005; Agrawal et al. 2008), although latex production and the chemical defence cardenolides appear uncoupled

for the species (Agrawal et al. 2014). While latex is a constitutive defense produced without damage, herbivores can also induce latex exudation (Agrawal 2005; Rassman et al. 2009). Previous work detected selection to increase leaf latex and suggested herbivores as agents of selection on defensive traits (Agrawal 2005). Experimentally inducing defenses via jasmonic acid also led to selection for fewer flowers contrasting with selection for inflorescences with more flowers in control conditions (Thompson et al. 2017). The latter shows that antiherbivore defenses may also shape the evolution of flowers and reproductive traits through phenotypic selection, yet studies remain rare.

In addition to the vegetative parts, flowers can also have defensive traits including floral latex, defensive metabolite compounds such as cardenolides, and deterrent volatiles in nectar (Raguso 2004; Parachnowitsch et al. 2012; Villalona et al. 2020; Gustafson et al. 2023). While floral defenses may protect flowers from herbivores, nectar robbers, and less effective pollinators, they may also impose costs to plant fitness by decreasing pollinator visits (Jones & Agrawal 2016; Kessler & Chautá 2020). Attraction, nectar, and defense traits in flowers and leaves may be linked via physiological pathways, co-regulation, pleiotropy and/or linkage showing correlated responses to leaf damage by herbivores (e.g. Adler & Irwin 2005). However, relationships among leaf, floral latex, nectar and how these traits evolve in

response to selection by mutualists and antagonists has received little attention.

To address how selection acts on floral traits and defences, we studied a natural population of *A. syriaca* in New Brunswick (Canada). We measured nectar concentration, flower number and size, inflorescence size, and plant height as phenotypic traits involved in pollination, leaf and floral latex to estimate traits involved in plant defense, as well as herbivore damage. We estimated phenotypic selection via male (pollinia removal) and female fitness (pollinia insertion and fruits initiated) to compare these functions. We used pollinia movement to examine selection by pollinators and herbivore damage to understand the role of herbivores.

MATERIALS AND METHODS

STUDY SYSTEM

Common milkweed, *Asclepias syriaca*, is a perennial species found in disturbed sunny habitats native to North America. Milkweed has hermaphroditic flowers that bloom from late May to early August, producing an indeterminate number of self-incompatible flowers (Pleasants 1991). The flowers are highly modified and composed of five petals that unfold to release a showy corona with five pink/white coloured hoods separated by stigmatic slits (Fig. 1, Thompson et al., 2017; Wyatt & Broyles, 1994). Common milkweed produces nectar in the



Figure 1. (Left) The study population of common milkweed (*Asclepias syriaca*), Fredericton, New Brunswick. (Right) Detail of an inflorescence of buds and open flowers with five hoods separated by stigmatic slits; inset shows floral measurements of petal width, hood width and hood length (photographs by Yedra García).

stigmatic slits which fills into the hoods (Galil & Zeroni 1965). Reproduction occurs both vegetatively via rhizomes and sexually by fertilization via pollinia (male function), that are inserted into stigmatic slits of another flower (female function) by insect pollinators (Morgan & Schoen, 1997; Wyatt & Broyles, 1994). Diurnal and nocturnal pollinators include butterflies, bees, beetles and moths (Jennersten & Morse 1991; La Rosa & Conner 2017; Gustafson et al. 2023), although diurnal pollinators can lead to higher fitness (Morse & Fritz 1983) and some pollinators are less effective at moving pollinia to other individuals in this self-incompatible species (e.g., introduced honeybees, Howard & Barrows 2014). However, pollinia limitation has been observed (Morse & Fritz 1983), suggesting pollinators have an opportunity to drive selection on traits. There is a limited number of host-specific herbivores for milkweed and some examples include the well-known monarch butterfly (*Danaus plexippus*, Lepidoptera) and the common milkweed bug (*Lygaeus kalmii*, Hemiptera) (Agrawal 2005; Birnbaum & Abbot 2018).

FIELD SITE

We conducted our study in a population of *A. syriaca* in Fredericton, New Brunswick, Canada (45°57'48.0"N, 66°36'43.3"W, Fig. 1). The site is within city limits in a semi-naturalized area between a walking/biking trail and the Nashwaak River. In July 2020, we ran transects separated by 5 m across the population and tagged 120 *A. syriaca* flowering stems at least 1 m apart along the transects to ensure that they were independent individuals. Phenology varied among plants, but most had early-stage inflorescences with floral buds when selected.

TRAIT MEASUREMENTS

We chose nine phenotypic traits we hypothesized may experience natural selection in our population through interactions with pollinators and herbivores, as well as assessing herbivore damage. To estimate plant size, we measured plant height and counted the number of leaves for each plant coinciding with nectar collection measurements (between July 10-22). Because plant size can be an important predictor of fitness, especially for seed production, including it in selection estimates can better allow for detection of the targets of selection. Reproductive effort was

estimated as the number of inflorescences and total number of flowers (estimated by counting flowers and buds at peak flowering).

For individual flowers, we measured flower size and rewards. We estimated hood length and width, and petal width as Thompson et al. (2017) for five flowers per plant and used the mean value of flower measures for each plant. To measure floral nectar, we covered plants with mesh bags the evening before nectar collection to exclude floral visitors. The following morning, we collected nectar with microcapillary tubes (Drummond Scientific) and estimated sugar concentration (%) with a hand-held refractometer (Palm Abbe Misco). We were unable to accurately estimate nectar volume per flower because many had very little nectar. Therefore, nectar concentration was estimated from several flowers per inflorescence once sufficient volume was achieved in the tube (~4-5 μ L). Nectar collection took place across seven sampling days between 09:00 to 11:00 because *A. syriaca* nectar traits can vary across the day (Wyatt et al. 1992). Experience in the field and preliminary analyses found nectar concentration was sensitive to sampling day ($F_{6,108} = 35.66$, $P < 0.001$) and humidity varied across the sampling dates. We used the humidity recorded by Environment Canada (<https://climate.weather.gc.ca/>) at 10:00 to represent the period of nectar measurements (9:00 to 11:00) and found a general pattern of lower concentrations at higher humidity ($R^2 = 0.51$, $F_{1,101} = 107.37$, β humidity = -0.80, $P < 0.001$). Therefore, we used the residuals from the model with humidity as a predictor of nectar concentration, as our nectar variable in the selection analyses to control for the sampling date effect (see below).

To assess plant defences, we measured floral and leaf latex. We followed the established protocol for leaf latex measurements in *A. syriaca* (e.g. Bingham & Agrawal 2010): cutting the leaf tip (5mm) of a newly expanded undamaged leaf at the top of each plant and collecting extruded latex on pre-weighted filter paper. Each latex sample was stored individually and weighed the same afternoon as collecting to quantify latex wet weight, which approximates the physical defence of latex flowing to a wound. Samples were then dried and re-weighed for latex dry weight, which is an estimate of the chemical constituents in latex. We assessed floral latex for two flowers by cutting

the floral stem and collecting latex as for the leaves (similar to Parachnowitsch et al. 2012). We used the mean in analyses. While we cannot exclude that our manipulation may have had some effect on pollinator visitation, we expect it to be minor as we only damaged one leaf (leaves: mean = 20.6 ± 4.10) and two flowers (flowers: mean = 122.8 ± 54.69) per individual. Induced responses to herbivory can have a range of effects in *A. syriaca* (e.g. Van Zandt & Agrawal 2004; Thompson et al. 2017), however, the small physical damage alone is unlikely to fully induce a response and all plants received the same damage so effects should be comparable. To assess herbivory, we counted the number of leaves per plant with damage and estimated the extent of leaf damage using photographs. The amount of leaf area (cm^2) consumed by herbivores was estimated using LeafByte (Getman-Pickering et al 2020). We also calculated a damage index as Agrawal (2005), dividing the number of damaged leaves by the total leaves on the plant.

To examine pollinia movements, we collected five flowers from two inflorescences per plant at peak flowering. Flowers were stored in 80% ethanol until assessment with a microscope; five of these were also measured for flower size (above). For male fitness, we counted pollinia removals by verifying if the pollinia were present in the pollinium chamber. Female fitness was measured by probing all stigmatic slits to count pollinia insertions. We used the total number of removals and insertions from ten flowers per plant as fitness estimates in the selection analyses. We also estimated female fitness by counting initiated fruits per plant after flowering was finished (as in Caruso et al. 2005). Initiated and mature fruits in *A. syriaca* are correlated (Willson & Rathcke 1974), suggesting it is a reasonable estimate of fruit number.

STATISTICAL ANALYSES

We estimated phenotypic selection using multiple regression to calculate selection gradients and determine the targets of selection (Lande & Arnold 1983). We first determined which traits to include in our model by calculating Pearson's correlation coefficients (Table S1) and confirmed multicollinearity was not a problem in the final model by calculating variance inflation factors (VIF) with the 'car' package in R. The final model

included inflorescence number and size, hood length and width, petal width, nectar concentration, floral and leaf latex, plant height and the damage index. Because the wet and dry measures of leaf latex were highly correlated (Table S1), we ran separate models with the wet and dry measures. However, all models with dry latex showed no evidence for selection on latex (Table S2) so for simplicity we present the models with wet latex measures. We calculated variance standardized selection gradients by standardizing to a mean of 0 and variance of 1. We also calculated mean standardized selection gradients to better understand the strength of selection on traits (Matsumura et al. 2012; Opedal 2021); the rank order of traits was similar for both models (Table 1). We estimated selection via female fitness for both inserted pollinia and initiated fruits and selection through male fitness by pollinia removal. We used relative fitness in our models by dividing by the population mean. We did not have the statistical power to detect non-linear selection and exclude these analyses.

RESULTS

We observed pollinators visiting the study population such as honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp., likely *B. impatiens* and *B. ternarius* based on field identification), as well as other insects including butterflies and flies. We did not visit the population during the night to observe nocturnal pollinators. There was evidence for pollinia removal and insertion on one or more of the 10 flowers sampled for each plant (removals: mean = 1.0 ± 0.68 per flower; insertions: mean = 0.2 ± 0.25 per flower, $N = 119$), suggesting that pollinators were active in the population. Pollinia insertion and removal was positively correlated ($r = 0.44$, $P < 0.001$) but neither was correlated with fruit initiation (insertion: $r = -0.12$, removal: $r = 0.12$). Evidence of insect damage was minimal which was reflected in the damage index (Table S1), although one *D. plexippus* larva and a few adults were observed in the population. Only 34% of our plants had any evidence of damaged leaves ($N = 35$ plants) and generally there was little leaf area consumed ($< 3\%$), although two plants had approximately 27% and one had 40% tissue consumed. The phenotypic traits measured generally varied among the 103 individuals in our final dataset (Table S1). Excluded individuals were

Table 1. Variance-standardized ($\beta \pm SE$) and mean-standardized ($\beta_{\mu} \pm SE$) linear selection gradients on ten plant traits of *Asclepias syriaca* ($N = 103$) via fruits initiated, pollinia inserted and pollinia removed. Significant selection gradients are in bold. * $P < 0.05$, ** $P < 0.01$.

Trait	Female fitness – fruit		Female fitness – inserted pollinia		Male fitness – pollinia removal	
	$\beta \pm SE$	$\beta_{\mu} \pm SE$	$\beta \pm SE$	$\beta_{\mu} \pm SE$	$\beta \pm SE$	$\beta_{\mu} \pm SE$
Inflorescence number	0.464 \pm 0.137**	1.183 \pm 0.349**	-0.016 \pm 0.11	-0.040 \pm 0.281	-0.013 \pm 0.08	-0.033 \pm 0.204
Inflorescence size	0.291 \pm 0.133*	0.955 \pm 0.436*	-0.001 \pm 0.107	-0.002 \pm 0.351	0.052 \pm 0.078	0.171 \pm 0.255
Hood length	-0.164 \pm 0.138	-1.630 \pm 1.368	-0.03 \pm 0.111	-0.299 \pm 1.010	0.049 \pm 0.081	0.491 \pm 0.80
Hood width	0.116 \pm 0.138	1.831 \pm 2.174	0.044 \pm 0.111	0.688 \pm 1.75	-0.048 \pm 0.081	-0.756 \pm 1.272
Petal width	0.07 \pm 0.144	0.976 \pm 1.993	-0.235 \pm 0.116*	-3.265 \pm 1.604*	-0.053 \pm 0.084	-0.731 \pm 1.66
Nectar concentration	-0.082 \pm .0126	0.001 \pm 0.002	0.048 \pm 0.101	-0.001 \pm 0.001	-0.039 \pm 0.074	0.001 \pm 0.001
Floral wet latex	0.056 \pm 0.141	0.072 \pm 0.182	0.326 \pm 0.114**	0.421 \pm 0.147*	0.123 \pm 0.083	0.159 \pm 0.107
Leaf wet latex	0.038 \pm 0.140	0.044 \pm 0.160	-0.027 \pm 0.112	-0.031 \pm 0.129	-0.047 \pm 0.082	-0.054 \pm 0.094
Plant height	0.217 \pm 0.145	1.601 \pm 1.074	0.132 \pm 0.117	0.975 \pm 0.864	-0.002 \pm 0.085	-0.014 \pm 0.628
Damage index	0.118 \pm 0.128	0.059 \pm 0.064	0.009 \pm 0.103	0.005 \pm 0.051	-0.01 \pm 0.075	-0.005 \pm 0.037

missing data for one or more traits. Taller plants Our flower size measurements were positively correlated but interestingly, hood and petal widths were also positively correlated with flower latex (Table S1). We found that plants with higher leaf latex also produced more latex at the flowers and generally more latex was exuded from the flower stem than the leaf tips. Correlations with other traits were not always consistent for the two latex measures. Plants with more concentrated nectar had lower leaf latex production, suggesting water may constrain both leaf latex and nectar production.

We found significant patterns of phenotypic selection on floral display, floral size, and latex production via female fitness in *A. syriaca* (Table 1). Interestingly, selection varied between our two measurements of female fitness (i.e., pollinia inserted and fruits initiated). While we found selection for smaller petals and higher floral latex production via pollinia inserted (Fig. 2 A, B), we found selection to increase plant size (i.e., number of inflorescences) and inflorescence size (i.e., number of flowers) via fruits initiated (Fig. 2 C, D). In addition, selection for increasing number of inflorescences was stronger than selection on inflorescence size (Table 1). In contrast to female fitness, we did not detect significant patterns of

phenotypic selection acting through male fitness (i.e., pollinia removed) on any of the study traits (Table 1). We also found no evidence of selection against herbivory via the damage index through any of our fitness estimates.

DISCUSSION

Few traits were under selection in our population of *A. syriaca*, despite including a wide range of traits related to pollinator attraction, mechanical fit of pollinators in the flowers, rewards, and herbivore defense. Pollinator-mediated selection estimated via pollinia movement was only detected for pollinia insertions, not removals, and only for smaller petals and more floral latex. Pollinator-mediated selection on floral traits is common and often stronger than other biotic factors (Caruso et al. 2019), as was seen here with the lack of effect by herbivores. Given that herbivory on *A. syriaca* is generally low in New Brunswick (Woods et al. 2012) and milkweed defences are also generally lower towards the northern range edge (Rasmann & Agrawal 2011; Woods et al. 2012; Agrawal et al. 2022), it was not surprising that we found little herbivory (i.e. less than 35% of study plants showed any signs of foliar damage) and no selection to reduce herbivory as seen in Agrawal

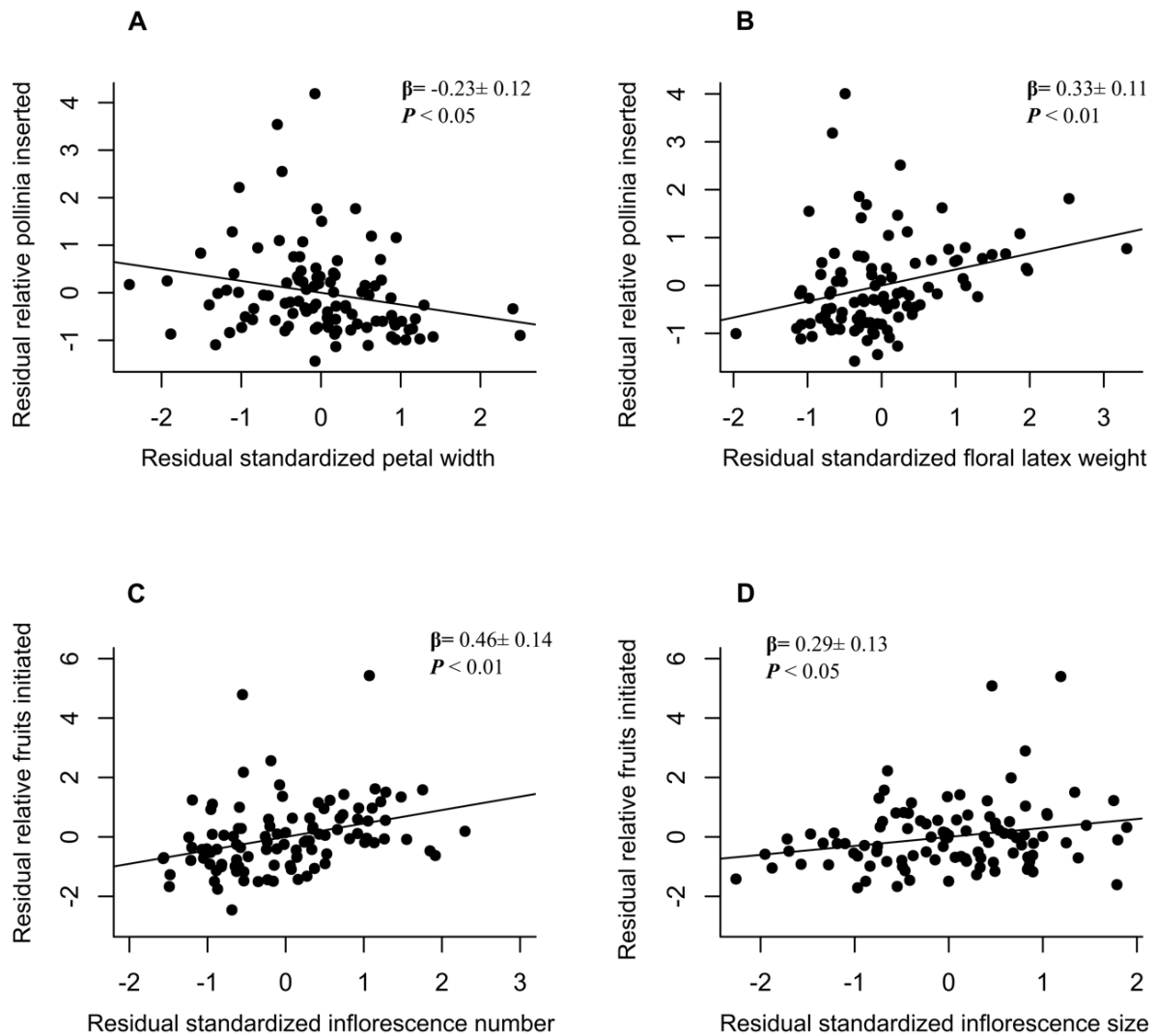


Figure 2. Variance-standardized linear phenotypic selection gradients ($\beta \pm SE$) for (A) petal width, (B) floral wet latex weight, (C) inflorescence number and (D) inflorescence size. Selection on petal width and floral wet latex acted via pollinia inserted and on inflorescence number and size via fruits initiated. Selection gradients are represented with added-variable plots.

(2005). Considering the geographic variation in ecological interactions across *A. syriaca*'s range, we might expect geographic differences in selection on this diverse set of traits, especially in populations with higher herbivory that may drive adaptation in defence traits such as latex (Agrawal 2005). Indeed, defensive traits such as cardenolide concentration in seeds of *A. syriaca* show a geographical cline with increasing levels towards the centre of the species range (Agrawal et al. 2022). Geographic variation in natural selection can be driven by differences in selective pressures including both from herbivores and pollinators and can be important for local adaptation (Siepielski et al. 2013, 2017), therefore it would be interesting to compare selection on floral and leaf

latex across the species range to see if pollinator-mediated selection on floral latex is common.

We found selection on floral but not leaf latex via pollinia insertion in our population of *A. syriaca*. Few studies have examined latex in flowers or flowing to flowers to allow comparisons. In *Lobelia siphilitica*, latex to floral structures likely help protect the developing fruits from attack by their predispersal seed predator which attacks early flowering plants (Parachnowitsch & Caruso 2008). Latex production is related to flowering time in this species (Parachnowitsch et al. 2012), suggesting a relationship between the likelihood of herbivory and the production of latex defence. In *A. syriaca*, florivory by floral specialists and

generalist herbivores suggests there could also be a defensive function of floral latex (Matter et al. 1999; Baker & Potter 2018). Florivory can also be costly, for example, floral damage by the Japanese beetle *Popillia japonica*, which preferentially feeds on the nectaries and ovaries, can reduce seed set by 90% (Matter et al. 1999). Furthermore, plant defenses in the milkweeds, such as cardenolide content and concentration, can vary among tissues in response to herbivores specialized on different plant parts (Manson et al. 2012; López-Goldar et al. 2022). While this suggests that selection on latex in *A. syriaca* might vary between flowers and leaves in response to different herbivory pressures, information on patterns of latex variation between floral and foliar tissues is scarce. We cannot attribute the selection on floral latex to leaf herbivores and although not measured, we also did not observe evidence of florivory during field sampling. Thus, the drivers of selection on floral latex and not leaf latex, despite their positive correlation in the population, warrants further study. Furthermore, the connection between floral latex and pollinia insertion needs investigation to understand the mechanisms because pollinators are unlikely to be directly affected by the latex. Underlying relationships such as chemical defenses in nectar and latex may explain these patterns (Manson et al. 2012) but need further work to confirm.

Nectar concentration was highly variable between individuals, but generally not correlated with other traits and was not a target of selection in this population. Non-significant selection for nectar traits is common (Parachnowitsch et al. 2019), although there are too few estimates to compare with selection on other well-studied floral traits (Harder & Johnson 2009; Caruso et al. 2019). Nectar production may be costly for *A. syriaca* (Southwick 1984) and likely influences a mix of floral visitors (Willson & Bertin 1979; Fritz & Morse 1981; Gustafson et al. 2023). While insect pollinators often prefer more concentrated nectars, nectar rewards are likely shaped by the plants interests as much as, or more so, than the pollinators (Pyke 2016; Parachnowitsch et al. 2019). Despite the challenges of quantifying nectar traits due to individual and environmental influences on the trait (e.g. Southwick & Southwick 1983; Wyatt et al. 1992), further study of selection on nectar traits in *A. syriaca* will allow more comparisons to

its better studied plant defences and floral characters.

Unlike Morgan and Schoen (1997), we saw no evidence for conflicting selection on *A. syriaca* traits between male and female fitness estimated by pollinia removals and insertions in our population. Interestingly, Thompson et al. 2017 also did not observe selection on any *A. syriaca* traits via pollinia removal and Caruso et al. (2005) only found significant selection via pollinia removal to increase *A. syriaca* hood length in one of their three experimental conditions (resource addition). While selection via male fitness remains an important goal in understanding evolution in hermaphroditic plants (Conner et al. 1996; Ashman & Morgan 2004; Austen & Weis 2016; Briscoe Runquist et al. 2017; Christopher et al. 2020) and sexual conflict has also been observed in other species (e.g. Maad & Alexandersson 2004), the difference between flower number and fruits in milkweed inflorescences may influence how strongly sexual conflict translates into evolution of floral traits. Pollinia movement therefore likely reflects pollinator preferences and behaviour on the flowers, but ultimately might be a weak proxy for fitness and pollinator visitation may better predict insertions than removals for *A. syriaca* (La Rosa & Conner 2017). Unlike comparing pollinia movements, we found the two stages of female fitness did show differences, in line with previous work on *A. syriaca* (Caruso et al. 2005; La Rosa & Conner 2017). Different mechanisms including resource limitation in fruits produced, incompatible self-pollinia and/or ineffective pollinia deposition may have weakened the link between inserted pollinia and number of fruits, ultimately resulting in lack of consistency in selection estimates from the two female fitness components (Caruso et al. 2005; La Rosa & Conner 2017). In addition, selection for higher resource acquisition or by non-pollinator selective agents might have also contributed to selection for increased attractive traits at the plant level such as inflorescence number and size through fruits initiated but not through pollinia inserted in our population (Sletvold et al. 2010; reviewed by Caruso et al. 2019). Overall, the different targets of selection between the pollinia and fruits highlights the importance of considering fitness components involved in different levels of the pollination process and the continued need to assess the role

of pollinators on natural selection via direct measurements rather than assumptions that pollinators are responsible for floral evolution (Ashman & Morgan 2004; Caruso et al. 2019; Sletvold 2019).

ACKNOWLEDGEMENTS

Our research population and university are located on unsundered and unceded traditional Wolastoqey land that is governed by Peace and Friendship Treaties with the Wabanaki people. Thank you to the City of Fredericton and specifically Mike Glynn for allowing access to the population when other research plans were disrupted due to COVID-19. We thank the anonymous reviewer and Maria Clara Castellanos for helpful comments that improved the manuscript. The project was made possible by funding from the Vetenskapsrådet (Swedish Research Council) and an NSERC Discovery Grant.

AUTHOR CONTRIBUTION

YG and ALP designed the study with input on data collection from LV. YG, LV, and BSD collected the data. YG and ALP analysed the data. ALP, YG, and BSD wrote first drafts of sections of the paper, all contributed to the final manuscript.

DISCLOSURE STATEMENT

The authors have no conflicts to declare. Author ALP is an Associate Editor of the Journal of Pollination Ecology. Thus, the peer-review process for this article was handled independently by another member of the editorial board.

DATA AVAILABILITY STATEMENT

The data used to write this article are available as Appendix, see below.

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I.

Table S1: summary statistics of traits measurements and correlations.

Table S2: linear selection gradients on ten plant traits of *Asclepias syriaca*. For latex measurements dry weights were considered in the models.

Appendix II. Data from *Asclepias syriaca* included in phenotypic selection analyses.

REFERENCES

- Adler LS, Irwin RE (2005) Ecological costs and benefits of defenses in nectar. *Ecology* 86:2968-2978. <https://doi.org/10.1890/05-0118>
- Adler LS, Seifert MG, Wink M, Morse GE (2012) Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology Letters* 15:1140-1148. <https://doi.org/10.1111/j.1461-0248.2012.01838.x>
- Adler LS, Wink M, Distl M, Lentz AJ (2006) Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* 9:960-967. <https://doi.org/10.1111/j.1461-0248.2006.00944.x>
- Agrawal AA (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research* 7:651-667.
- Agrawal AA (2017) Monarchs and milkweed: a migrating butterfly, a poisonous plant, and their remarkable story of coevolution. In: *Monarchs and Milkweed*. Princeton University Press <https://doi.org/10.1515/9781400884766>
- Agrawal AA, Espinosa del Alba L, López-Goldar X, Hastings AP, White RA, Halitschke R, Dobler S, Petschenka G, Duplais C (2022) Functional evidence supports adaptive plant chemical defense along a geographical cline. *Proceedings of the National Academy of Sciences* 119:e2205073119. <https://doi.org/10.1073/pnas.2205073119>
- Agrawal AA, Lajeunesse MJ, Fishbein M (2008) Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata* 128:126-138. <https://doi.org/10.1111/j.1570-7458.2008.00690.x>
- Agrawal AA, Patrick ET, Hastings AP (2014) Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere* 5:126. <https://doi.org/10.1890/ES14-00161.1>
- Ashman TL, Morgan MT (2004) Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context? *Proceedings of the Royal Society of London Series B: Biological Sciences* 271:553-559. <https://doi.org/10.1098/rspb.2003.2642>
- Austen EJ, Weis AE (2016) The causes of selection on flowering time through male fitness in a hermaphroditic annual plant. *Evolution* 70:111-125. <https://doi.org/10.1111/evo.12823>
- Baker AM, Potter DA (2018) Japanese beetles' feeding on milkweed flowers may compromise efforts to restore monarch butterfly habitat. *Scientific Reports* 8:12139. <https://doi.org/10.1038/s41598-018-30731-z>

- Bingham RA, Agrawal AA (2010) Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. *Journal of Ecology* 98:1014-1022. <https://doi.org/10.1111/j.1365-2745.2010.01681.x>
- Birbauma SSL, Abbot P (2018) Insect adaptations toward plant toxins in milkweed-herbivores systems - a review. *Entomologia Experimentalis et Applicata* 166:357-366. <https://doi.org/10.1111/eea.12659>
- Briscoe Runquist RD, Geber MA, Pickett-Leonard M, Moeller DA (2017) Mating system evolution under strong pollen limitation: Evidence of disruptive selection through male and female fitness in *Clarkia xantiana*. *The American Naturalist* 189:549-563. <https://doi.org/10.1086/691192>
- Broyles SB, Wyatt R (1990) Paternity analysis in a natural population of *Ascepias exaltata*: multiple paternity, functional gender, and the "pollen-donation hypothesis." *Evolution* 44:1454-1468. <https://doi.org/10.1111/j.1558-5646.1990.tb03838.x>
- Bruinsma M, Lucas-Barbosa D, ten Broeke CJM, van Dam NM, van Beek TA, Dicke M, van Loon JJA (2014) Folivory affects composition of nectar, floral odor and modifies pollinator behavior. *Journal of Chemical Ecology* 40:39-49. <https://doi.org/10.1007/s10886-013-0369-x>
- Burkle LA, Runyon JB (2016) Drought and leaf herbivory influence floral volatiles and pollinator attraction. *Global Change Biology* 22:1644-1654. <https://doi.org/10.1111/gcb.13149>
- Caruso CM, Eisen KE, Martin RA, Sletvold N (2019) A meta-analysis of the agents of selection on floral traits. *Evolution* 73:4-14. <https://doi.org/10.1111/evo.13639>
- Caruso CM, Remington DLD, Ostergren KE (2005) Variation in resource limitation of plant reproduction influences natural selection on floral traits of *Asclepias syriaca*. *Oecologia* 146:68-76. <https://doi.org/10.1007/s00442-005-0183-4>
- Chapurlat E, Ågren J, Anderson J, Friberg M, Sletvold N (2019) Conflicting selection on floral scent emission in the orchid *Gymnadenia conopsea*. *New Phytologist* 222:2009-2022. <https://doi.org/10.1111/nph.15747>
- Christopher DA, Mitchell RJ, Karron JD (2020) Pollination intensity and paternity in flowering plants. *Annals of Botany* 125:1-9. <https://doi.org/10.1093/aob/mcz159>
- Conner JK, Rush S, Kercher S, Jennetten P (1996) Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*) II. Selection through lifetime male and total fitness. *Evolution* 50:1137-1146. <https://doi.org/10.1111/j.1558-5646.1996.tb02354.x>
- Egan PA, Muola A, Parachnowitsch AL, Stenberg JA (2021) Pollinators and herbivores interactively shape selection on strawberry defence and attraction. *Evolution Letters* 5:636-643. <https://doi.org/10.1002/evl3.262>
- Fritz RS, Morse DH (1981) Nectar parasitism of *Asclepias syriaca* by ants: effect on nectar levels, pollinia insertion, pollinaria removal and pod production. *Oecologia* 50:316-319. <https://doi.org/10.1007/BF00344969>
- Galil J, Zeroni M (1965) Nectar system of *Asclepias curassavica*. *Botanical Gazette* 126:144-148. <https://doi.org/10.1086/336310>
- Gustafson NW, Couture JJ, Dagleish HJ (2023) Herbivory, plant traits and nectar chemistry interact to affect the community of insect visitors and pollination in common milkweed, *Asclepias syriaca*. *Oecologia* 201:91-105. <https://doi.org/10.1007/s00442-022-05290-w>
- Harder LD, Johnson SD (2009) Darwin's beautiful contrivances: Evolutionary and functional evidence for floral adaptation. *New Phytologist* 183:530-545. <https://doi.org/10.1111/j.1469-8137.2009.02914.x>
- Hoffmeister M, Junker RR (2017) Herbivory-induced changes in the olfactory and visual display of flowers and extrafloral nectaries affect pollinator behavior. *Evolutionary Ecology* 31:269-284. <https://doi.org/10.1007/s10682-016-9875-y>
- Howard AF, Barrows EM (2014) Self-pollination rate and floral-display size in *Asclepias syriaca* (common milkweed) with regard to floral-visitor taxa. *BMC Evolutionary Biology* 14:144. <https://doi.org/10.1186/1471-2148-14-144>
- Jennersten O, Morse DH (1991) The quality of pollination by diurnal and nocturnal insects visiting common milkweed, *Asclepias syriaca*. *The American Midland Naturalist* 125:18-28. <https://doi.org/10.2307/2426365>
- Jones PL, Agrawal AA (2016) Consequences of toxic secondary compounds in nectar for mutualist bees and antagonist butterflies. *Ecology* 97:2570-2579. <https://doi.org/10.1002/ecy.1483>
- Kessler A, Chautá A (2020) The ecological consequences of herbivore-induced plant responses on plant-pollinator interactions. *Emerging Topics in Life Sciences* 4:33-43. <https://doi.org/10.1042/ETLS20190121>
- Kessler A, Halitschke R, Poveda K (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* 92:1769-1780. <https://doi.org/10.1890/10-1945.1>
- Kevan PG, Eisikowitch D, Rathwell B (1989) The role of nectar in the germination of pollen in *Asclepias syriaca* L. *Botanical Gazette* 150:266-270. <https://doi.org/10.1086/337771>
- Knauer AC, Schiestl FP (2017) The effect of pollinators and herbivores on selection for floral signals: a case

- study in *Brassica rapa*. *Evolutionary Ecology* 31:285-304. <https://doi.org/10.1007/s10682-016-9878-8>
- La Rosa RJ (2015) Floral evolution in milkweeds: evidence for selection past and present. Ph.D., Michigan State University, United States -- Michigan.
- La Rosa RJ, Conner JK (2017) Floral function: effects of traits on pollinators, male and female pollination success, and female fitness across three species of milkweeds (*Asclepias*). *American Journal of Botany* 104:150-160. <https://doi.org/10.3732/ajb.1600328>
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210. <https://doi.org/10.2307/2408842>
- Lehtilä K, Strauss SY (1999) Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* 80:116-124. [https://doi.org/10.1890/0012-9658\(1999\)080\[0116:EOFHOM\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0116:EOFHOM]2.0.CO;2)
- López-Goldar X, Hastings A, Züst T, Agrawal AA (2022) Evidence for tissue-specific defence-offence interactions between milkweed and its community of specialized herbivores. *Molecular Ecology* 31:3254-3265. <https://doi.org/10.1111/mec.16450>
- Maad J, Alexandersson R (2004) Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *Journal of Evolutionary Biology* 17:642-650. <https://doi.org/10.1111/j.1420-9101.2004.00703.x>
- Manson JS, Rasmann S, Halitschke R, Thomson JD, Agrawal AA (2012) Cardenolides in nectar may be more than a consequence of allocation to other plant parts: a phylogenetic study of *Asclepias*. *Functional Ecology* 26:1100-1110. <https://doi.org/10.1111/j.1365-2435.2012.02039.x>
- Matsumura S, Arlinghaus R, Dieckmann U (2012) Standardizing selection strengths to study selection in the wild: A critical comparison and suggestions for the future. *BioScience* 62:1039-1054. <https://doi.org/10.1525/bio.2012.62.12.6>
- Matter SF, Landry JB, Greco AM, LaCourse CD (1999) Importance of floral phenology and florivory for *Tetraopes tetraophthalmus* (Coleoptera: Cerambycidae): tests at the population and Individual level. *Environmental Entomology* 28:1044-1051. <https://doi.org/10.1093/ee/28.6.1044>
- Morgan MT, Schoen DJ (1997) Selection on reproductive characters: floral morphology in *Asclepias syriaca*. *Heredity* 79:433-441. <https://doi.org/10.1038/hdy.1997.178>
- Morse DH, Fritz RS (1983) Contributions of diurnal and nocturnal insects to the pollination of common milkweed (*Asclepias syriaca* L.) in a pollen-limited system. *Oecologia* 60:190-197. <https://doi.org/10.1007/BF00379521>
- Opedal ØH (2021) A functional view reveals substantial predictability of pollinator-mediated selection. *Journal of Pollination Ecology* 30:273-288. [https://doi.org/10.26786/1920-7603\(2021\)673](https://doi.org/10.26786/1920-7603(2021)673)
- Parachnowitsch AL, Caruso CM (2008) Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. *Ecology* 89:1802-1810. <https://doi.org/10.1890/07-0555.1>
- Parachnowitsch AL, Caruso CM, Campbell SA, Kessler A (2012) *Lobelia siphilitica* plants that escape herbivory in time also have reduced latex production. *PLoS ONE* 7:e37745. <https://doi.org/10.1371/journal.pone.0037745>
- Parachnowitsch AL, Kessler A (2010) Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist* 188:393-402. <https://doi.org/10.1111/j.1469-8137.2010.03410.x>
- Parachnowitsch AL, Manson JS, Sletvold N (2019) Evolutionary ecology of nectar. *Annals of Botany* 123:247-261. <https://doi.org/10.1093/aob/mcy132>
- Pleasants JM (1991) Evidence for short-distance dispersal of pollinia in *Asclepias syriaca* L. *Functional Ecology* 5:75-82. <https://doi.org/10.2307/2389557>
- Pyke GH (2016) Floral Nectar: Pollinator attraction or manipulation? *Trends in Ecology & Evolution* 31:339-341. <https://doi.org/10.1016/j.tree.2016.02.013>
- Raguso RA (2004) Why are some floral nectars scented? *Ecology* 85:1486-1494. <https://doi.org/10.1890/03-0410>
- Ramos SE, Schiestl FP (2019) Rapid plant evolution driven by the interaction of pollination and herbivory. *Science* 364:193-196. <https://doi.org/10.1126/science.aav6962>
- Rasmann S, Agrawal AA (2011) Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters* 14:476-483. <https://doi.org/10.1111/j.1461-0248.2011.01609.x>
- Santangelo JS, Thompson KA, Johnson MTJ (2019) Herbivores and plant defences affect selection on plant reproductive traits more strongly than pollinators. *Journal of Evolutionary Biology* 32:4-18. <https://doi.org/10.1111/jeb.13392>
- Siepielski AM, Gotanda KM, Morrissey MB, Diamond SE, DiBattista JD, Carlson SM (2013) The spatial patterns of directional phenotypic selection. *Ecology Letters* 16:1382-1392. <https://doi.org/10.1111/ele.12174>
- Siepielski AM, Morrissey MB, Buoro M, Carlson SM, Caruso CM, Clegg SM, Coulson T, DiBattista J, Gotanda KM, Francis CD, Hereford J, Kingsolver JG, Augustine KE, Kruuk LEB, Martin RA, Sheldon BC, Sletvold N, Svensson EI, Wade MJ, MacColl ADC (2017) Precipitation drives global variation in natural selection. *Science* 355:959-962. <https://doi.org/10.1126/science.aag2773>

- Sletvold N (2019) The context dependence of pollinator-mediated selection in natural populations. *International Journal of Plant Sciences* 180:934-943. <https://doi.org/10.1086/705584>
- Sletvold N, Grindeland JM, Ågren J (2010) Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist* 188: 385-392 <https://doi.org/10.1111/j.1469-8137.2010.03296.x>
- Sletvold N, Moritz KK, Ågren J (2014) Additive effects of pollinators and herbivores result in both conflicting and reinforcing selection on floral traits. *Ecology* 96:214-221. <https://doi.org/10.1890/14-0119.1>
- Sletvold N, Trunschke J, Smit M, Verbeek J, Ågren J (2016) Strong pollinator-mediated selection for increased flower brightness and contrast in a deceptive orchid. *Evolution* 70:716-724. <https://doi.org/10.1111/evo.12881>
- Southwick EE (1983) Nectar biology and nectar feeders of common milkweed, *Asclepias syriaca* L. *Bulletin of the Torrey Botanical Club* 110:324. <https://doi.org/10.2307/2996186>
- Southwick EE (1984) Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology* 65:1775-1779. <https://doi.org/10.2307/1937773>
- Southwick AK, Southwick EE (1983) Aging effect on nectar production in two clones of *Asclepias syriaca*. *Oecologia* 56:121-125. <https://doi.org/10.1007/BF00378227>
- Strauss SY (1997) Floral characters link herbivores, pollinators, and plant fitness. *Ecology* 78:1640-1645. [https://doi.org/10.1890/0012-9658\(1997\)078\[1640:FCLHPA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1640:FCLHPA]2.0.CO;2)
- Strauss SY, Whittall JB (2006) Non-pollinator agents of selection on floral traits. In: Harder LD, Barrett SCH (eds) *Ecology and evolution of flowers*. Oxford University Press, Oxford, UK, pp 120-138. <https://doi.org/10.1093/oso/9780198570851.003.0007>
- Thompson KA, Cory KA, Johnson MTJ (2017) Induced defences alter the strength and direction of natural selection on reproductive traits in common milkweed. *Journal of Evolutionary Biology* 30:1219-1228. <https://doi.org/10.1111/jeb.13045>
- Van Zandt PA, Agrawal AA (2004) Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85:2616-2629. <https://doi.org/10.1890/03-0622>
- Villalona E, Ezray BD, Laveaga E, Agrawal AA, Ali JG, Hines HM (2020) The role of toxic nectar secondary compounds in driving differential bumble bee preferences for milkweed flowers. *Oecologia* 193:619-630. <https://doi.org/10.1007/s00442-020-04701-0>
- Willson MF, Bertin RI (1979) Flower-visitors, nectar production, and inflorescence size of *Asclepias syriaca*. *Canadian Journal of Botany* 57:1380-1388. <https://doi.org/10.1139/b79-172>
- Willson MF, Rathcke BJ (1974) Adaptive design of the floral display in *Asclepias syriaca* L. *The American Midland Naturalist* 92:47-57. <https://doi.org/10.2307/2424201>
- Woods EC, Hastings AP, Turley NE, Heard SB, Agrawal AA (2012) Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs* 82:149-168. <https://doi.org/10.1890/11-1446.1>
- Wyatt R, Broyles SB, Derda GS (1992) Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *American Journal of Botany* 79:636-642. <https://doi.org/10.1002/j.1537-2197.1992.tb14605.x>