Reproductive Differences and Their Mechanisms of Sagittaria trifolia in Two Habitats: Postprint

Authors: Luo Wenjie, Xiaofang Jin, Wang Zhengxiang, Dai Can

Date: 2018-05-29T00:00:00+00:00

Abstract

The growth environment of plants can not only directly affect the availability of resources and their reproductive allocation, but also indirectly cause reproductive differences by influencing the composition or behavior of the plant' s community and pollinators. However, the direction or intensity of direct and indirect effects have rarely been simultaneously considered, thereby limiting mechanistic understanding of how environment influences plant reproduction. Using Sagittaria trifolia L. as the study material, plants with identical genotypic composition were grown in two common gardens with distinct light conditions (shaded area and sunny area). During the peak flowering period, observations were made on the flowering quantity of S. trifolia and insect flower visitation, identifying the main flower-visiting insects, recording visitation behavior, and assessing the reproductive output level of S. trifolia. The study revealed that the number of daily open flowers and flowering plants in the sunny area was significantly higher than in the shaded area. The main flower visitors of S. trifolia in both areas fell into four categories: hoverflies, bees, butterflies, and flies; among these, the primary pollinators in the shaded area were hoverflies, whereas in the sunny area they were bees. The flower visitation frequency per unit time, the number of male flowers visited per foraging bout, total flower number, and inflorescence number by insects in the sunny area were all significantly higher than those in the shaded area. The fruit set rate of S. trifolia in the sunny area was significantly higher than that in the shaded area, while the seed number per fruit and seed area were comparable to those in the shaded area. Overall, the reproductive output of S. trifolia in the sunny area reached more than three times that in the shaded area, representing the result of superposition of environmental direct effects and environment-mediated pollination effects in the same direction, with the former being dominant. By focusing on the interactions among plants, physical environment, and biological factors, and quantifying the level of each reproductive stage, particularly the performance of

pollinating insects in different habitats, the fundamental causes of how environmental heterogeneity leads to plant reproductive differences were elucidated.

Full Text

Preamble

ACTA ECOLOGICA SINICA
ChinaXiv Partner Journal
Vol. 38, No. 10, May 2018
DOI: 10.5846/stxb201704100621

The Reproductive Difference in *Sagittaria trifolia* Under Two Contrasting Habitats: Direct and Indirect Effects

Luo Wenjie[1], Jin Xiaofang[2], Wang Zhengxiang[1], Dai Can[1],[3], ,*

[1]School of Resources and Environmental Science, Hubei University
[2]Institute of Ecology and Environmental Science, Nanchang Institute of Technology
[3]Hubei Collaborative Innovation Center for Green Transformation of Bio-Resources
Hubei Province Key Laboratory of Regional Development and Environmental Response, Wuhan 430062, China

Abstract: Plant habitats not only directly affect resource availability and allocation but also indirectly influence reproductive success by shaping community composition and pollinator behavior. The direction and relative strength of these direct and indirect effects are rarely examined simultaneously, limiting mechanistic understanding of environment-driven differences in plant reproduction. We planted genetically identical *Sagittaria trifolia* individuals in two common gardens with contrasting light conditions (understory vs. sun) and quantified flowering status, pollinator visitation, and reproductive output during peak anthesis. Plants in the sun habitat produced significantly more daily flowers and had more flowering individuals than those in the understory. The main pollinators differed between habitats: hoverflies dominated in the understory, while bees were primary in the sun habitat. Pollinator visitation frequency per unit time, total flowers visited, and inflorescences visited were all significantly higher in the sun habitat, which also showed significantly greater fruit set. Although seed number per fruit and seed size were comparable between habitats, total reproductive output in the sun was more than three times that in the understory. This resulted from the cumulative effects of direct environmental influences and environment-mediated pollination acting in the same direction, with the former being dominant. Our study highlights the importance of examining interactions among plants, physical environment, and biotic factors at each reproductive stage, particularly the distinct performance of pollinators across habitats.

Keywords: pollination; *Sagittaria trifolia* L.; foraging bout; sunlight; reproductive fitness

Introduction

Plant-environment interactions represent a central focus in ecology, with environmental conditions exerting profound direct effects on plant growth and reproduction. Essential resources such as light and nutrients inevitably vary across geographic scales, and such heterogeneity often induces changes in growth rates, reproductive modes, or life history strategies. For example, *Mimulus* species in the Scrophulariaceae family exhibit delayed flowering in high-light environments compared to shaded conditions, and similar phenological delays occur in alpine meadow communities in response to photoperiod. *Gentianopsis paludosa* shows significantly higher bud numbers in meadow habitats that receive more light than shrub-dominated habitats. Persistent environmental pressures can drive adaptive trait changes and, over evolutionary timescales, lead to population differentiation. *Campanulastrum americanum* is predominantly biennial in shaded understories but annual in open habitats, while *Geranium maculatum* exhibits sexual dimorphism mainly in dry environments but sexual monomorphism in moist conditions.

Physical environmental differences also alter biological communities, modifying interspecific relationships such as mutualism and herbivory. Pollinators play a crucial role in sexual reproduction of flowering plants, and changes in pollinator composition and behavior across environments inevitably affect reproductive success. In *Lavandula latifolia*, Herrera found that populations in different habitats were primarily pollinated by either bees or butterflies. Due to inherent differences in insect attributes and behaviors, bees visit flowers frequently but increase geitonogamy, whereas butterflies visit less often but promote outcrossing through longer flight distances. Previous studies have often emphasized environmental factors and corresponding reproductive outcomes—the start and end points of the process—without dissecting each potential stage of change or distinguishing the independent versus synergistic effects of biotic and abiotic factors.

Some research has simultaneously examined physical environment and pollinator effects. *Campanulastrum americanum* showed significant differences in flowering phenology, pollinator visitation, and pollen limitation between gap and understory habitats, with further analysis revealing that reproductive differences resulted from cumulative, same-direction effects of multiple direct and indirect influences rather than any single factor. Similar mechanisms caused individual reproductive variation in *Campanula persicifolia*. However, some studies find opposing directions of direct and indirect environmental effects; for example, low-altitude *Ranunculus acris* individuals had higher flower and ovule numbers than high-altitude plants, but experienced significant pollen limitation that reduced reproductive success.

These examples demonstrate that environment-mediated pollination effects cannot be ignored, as their omission hinders understanding the root causes of differential plant reproduction across habitats. Using a controlled comparative experiment, this study examines how two contrasting habitats—particularly light environments—affect plant reproductive fitness, emphasizing simultaneous consideration of direct environmental effects and indirect pollinator-mediated effects to elucidate the mechanisms underlying environment-driven reproductive differences. Using the perennial herb *Sagittaria trifolia* as our study system, we address four questions: (1) Does light environment affect pollinator composition and visitation behavior? (2) Does light environment influence *S. trifolia* flowering physiology? (3) How does reproductive output change across light environments? (4) Do direct and indirect environmental effects act in the same direction, and what is their relative magnitude?

1. Materials and Methods

1.1 Experimental Material

Sagittaria trifolia (Alismataceae) is a perennial monocot herb that grows in shallow water areas such as ponds and paddy fields. It has strong adaptability and is widely distributed across China, naturally occurring in various light environments. The species reproduces both sexually and asexually. In Hubei Province, the peak flowering period occurs from June to October. *S. trifolia* is a typical protogynous, monoecious species with unisexual flowers, usually three per whorl. Flowers open sequentially from bottom to top, with female flowers at the inflorescence base and male flowers at the top. Individual flowers last only one day, opening between 6:00 and 14:00. Both sexes have three white petals. Female flowers develop into mature spherical achenes three weeks after pollination. Various insects, including hoverflies and small bees, can serve as pollinators.

1.2 Habitat Selection

The experiment was conducted at the Wuhan Botanical Garden, Chinese Academy of Sciences, in 2015. We selected two experimental areas with different environmental conditions: (1) an understory habitat shaded by tall *Taxodium* and *Cinnamomum* trees with surrounding natural woody and shrub communities (hereafter "understory"), and (2) an open habitat with no tall plants or buildings, receiving continuous sunlight throughout the day (hereafter "sun"). Both areas contained potted *S. trifolia* plants derived from field-collected corm clones, ensuring identical genotypic composition. Light intensity was the primary factor considered in habitat selection to ensure it was the main source of environmental variation.

1.3 Light Intensity Measurement

To quantify light intensity differences between habitats, we placed identical quantum sensors (Apogee MQ-200) in both the understory and sun habitats. Sensors recorded photosynthetically active radiation (PAR) every 30 minutes from 8:00 to 19:30 daily, automatically calculating and storing average values. Each day, sensors were moved to new random positions within each habitat to capture spatial variation comprehensively.

1.4 Flowering Census

During the flowering census period (8:00–10:30 daily), we counted all open *S. trifolia* flowers in both habitats, recording male flower number, total flower number, and number of flowering plants. Female flowers opening on the same day were tagged for subsequent tracking.

1.5 Pollinator Observation

Pollinator observations were conducted during 6:00–14:00 on clear or cloudy days. We employed two approaches:

Approach 1: Flower-based observation
We selected approximately 20 flowers (half male, half female) in concentrated locations within each habitat. Observers recorded insect visits to these fixed flowers, with visits counted when pollinators remained on a flower for more than one second. Each habitat completed 15-minute observation sessions every hour, from which we calculated visitation frequency as: (total visits)/(number of observed flowers × 15 min).

Approach 2: Foraging bout-based observation
We recorded each pollinator' s complete foraging bout from entry to exit from the observation area. For each bout, we documented insect category, visitation duration, number of inflorescences visited, and number of male, female, and total flowers visited. We also calculated time spent per flower. Approximately 30 foraging bouts were observed per habitat. Insects were photographed and collected for identification.

1.6 Reproductive Output

Reproductive output was measured as fruit set, seed number per fruit, and mean seed area from all flowering plants. Approximately three weeks after flowering, all mature fruits were harvested and stored individually in paper bags. Fruit number and fruit set were calculated for each flowering plant in both habitats. Seeds were dried (50°C, 12 h) before measurement. For each plant, we randomly selected 10 fruits for seed counting. Seeds from each fruit were arranged on 6 cm × 10 cm paper, photographed with a fixed-position DSLR camera (D7000), and analyzed using ImageJ software to determine seed number and average area.

1.7 Statistical Analysis

We used ANOVA (proc glm, proc mixed) to compare PAR, pollinator visitation frequency, and per-bout visitation between habitats. In light intensity analysis, habitat was a fixed factor and date a random factor. In flowering analyses, habitat was fixed and date random. For visitation frequency analysis, habitat, time, and flower sex were fixed factors with date random; only significant results are reported. Pollinator composition differences between habitats were analyzed using Chi-square tests.

For per-bout visitation analysis, we used mixed models with habitat, insect type, and time as fixed factors and date as random. Response variables included visitation duration, time per flower, number of male/female/total flowers visited, and inflorescences visited. Total flower and inflorescence numbers were included as covariates to test whether bout-scale visitation was influenced by regional flowering status. Fruit set comparison used a generalized linear mixed model with binary response (proc glimmix, dist = binary). Seed number and area comparisons used ANOVA with habitat as fixed factor and plant as random factor. Seed area was square-root transformed to meet normality and homogeneity assumptions. Post-hoc Tukey tests were used for pairwise comparisons.

2. Results

2.1 Light Intensity and Flowering

Photosynthetically active radiation differed significantly between sun and understory habitats (F , = 13.67, P = 0.0002). The sun habitat averaged (751.6 ± 21.4) mol m^{2} s^{1}, while the understory averaged (248.3 ± 11.7) mol m^{2} s^{1}. Daily flower production in the sun habitat significantly exceeded that in the understory for male flowers, total flowers, and flowering plants (Table 1), indicating that high-light environments positively enhance sexual reproduction.

Table 1 Flowering status of *Sagittaria trifolia* under two habitats

Variables	Understory (Mean±SE)	Sun (Mean±SE)	F-value	P-value
No. of male flowers	112±11	203±16	30.30	<0.0001
No. of female flowers	71.16±11.75	91.66±18.57	8.85	0.014
Total flower number	245±17	557±85	18.57	0.0002
No. of flowering plants	18.5±1.2	28.6±1.4	1857.85	<0.0001

2.2 Pollinator Visitation Frequency

Pollinator visitation frequency was higher in the sun habitat than in the understory, approaching significance (F , = 6.58, P = 0.01). Visitation frequency showed a temporal trend, increasing from morning to afternoon (F , = 3.53, P = 0.06), but did not differ between male and female flowers (F , = 0.07, P = 0.79). These results suggest that high-light environments promote insect visitation to individual flowers.

Figure 1 [Figure 1: see original paper] Pollinator visitation frequency on *Sagittaria trifolia* under two habitats

2.3 Pollinator Composition

Pollinators of *S. trifolia* were categorized into five groups: hoverflies, bees, butterflies, flies, and others. The "others" category was taxonomically diverse but represented by few visits. The understory was dominated by hoverflies (e.g., *Eristalinus arvorum*, *E. aeneus*), while the sun habitat was dominated by bees (e.g., *Apis mellifera*). Butterfly visits were recorded in both habitats (*Parnara ganga*, *Tongeia filicaudis*, *Pseudozizeeria maha*). The proportional representation of pollinator types differed significantly between habitats (^{2} = 9.3, P = 0.026), indicating a generalized pollination system not reliant on a single taxon.

Table 2 Observed pollinator species for *Sagittaria trifolia*

Figure 2 [Figure 2: see original paper] Pollinator compositions of *Sagittaria trifolia* under two habitats

2.4 Per-Bout Visitation Patterns

Insects in the sun habitat visited more inflorescences per bout than those in the understory, with significantly higher numbers of total flowers and male flowers visited, though female flower visitation was similar. Notably, regional flowering status did not linearly affect any visitation metric, suggesting that behavioral differences were driven directly by environment rather than by variation in flower abundance. While per-bout duration did not differ significantly between habitats, time spent per flower tended to be higher in the understory (Table 3).

Table 3 Pollinators' visitation characters per foraging bout on *Sagittaria trifolia* under two habitats

Variables	Understory (Mean±SE)	Sun (Mean±SE)	F-value	P-value
Time per bout (s)	281.4±43.3	301.9±40.4	0.39	0.3999
Time per flower (s)	48.6±8.0	28.6±5.9	3.54	0.0604
Male flowers visited	4.9±0.6	10.6±1.0	7.35	0.0079
Female flowers visited	1.8±0.5	2.0±0.6	1.84	0.1771
Total flowers visited	6.9±0.7	12.6±1.4	6.35	0.0128
Inflorescences visited	2.6±0.3	6.6±0.5	18.57	<0.0001

At the insect group level, flies spent significantly longer per bout than bees (F , = 2.73, P = 0.04) and visited more female flowers than other groups (F , =

5.50, P = 0.002), showing a preference for female flowers and extended residence times.

Figure 3 [Figure 3: see original paper] Pollinators' visitation characters per foraging bout on *Sagittaria trifolia* for different pollinator categories

2.5 Reproductive Output

Total fruit production was 1,847 in the sun habitat versus 612 in the understory. Fruit set was significantly higher in the sun (F , = 17.1, P < 0.0001). However, seed number per fruit did not differ between habitats (F , = 0.16, P = 0.69), suggesting similar ovule numbers. Seed area also showed no significant difference (F , = 2.16, P = 0.14), indicating comparable seed quality.

Figure 4 [Figure 4: see original paper] Reproductive output of *Sagittaria trifolia* under two habitats

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3. Discussion

3.1 Direct Effects of Light Environment

Light is essential for plant growth. Our experimental design prioritized light intensity as the key differentiating factor between habitats. The sun and understory habitats showed a threefold difference in PAR, with sun habitat values of (751.6 ± 21.4) mol m^{2} s^{1} compared to understory values of (248.3 ± 11.7) mol m^{2} s^{1}. Although the two habitats were only ~800 m apart (suggesting similar macroclimatic conditions), microenvironmental temperature typically increases with light intensity, indicating our habitats differed in both light and temperature, both favoring growth and carbon gain in the sun habitat.

The flowering data demonstrate environmental dominance: daily mean male flower number, total flower number, and flowering plant number were all significantly higher in the sun habitat. Because genotypic composition was identical between habitats (via corm clones), these differences represent direct environmental responses, independent of pollination or fruiting stages. While we focused on flower-level differences, the lack of variation in seed number per fruit and seed area suggests ovule quantity and quality were comparable between habitats, consistent with reports that ovule number often shows little plasticity across environments, possibly due to developmental constraints.

3.2 Indirect Effects via Pollinators

Pollinator composition, relative importance, and behavior differed markedly between habitats. The sun habitat supported more diverse pollinators, likely due to surrounding plant communities rich in nectariferous species (Amaranthaceae, Asteraceae) that attracted numerous pollinators. The understory community consisted mainly of shade-tolerant species (Saururaceae, ferns) with lower pollinator demand. Butterflies, while present in both habitats, are less effective pollinators as they perch on corollas and use long proboscises to feed without contacting reproductive structures. Thus, effective pollinators comprised only bees and hoverflies.

The understory' s primary pollinators were hoverflies, while the sun habitat was dominated by bees, possibly reflecting community composition effects. Bee dominance in sun habitats may relate to thermotolerance; some *Apis mellifera* strains withstand high temperature and humidity. Bees showed significantly shorter per-flower residence times than hoverflies, suggesting more efficient pollen transfer. Hoverflies exhibited distinctive behavior, visiting more female flowers and staying longer per bout. Prolonged residence on single-sex flowers can reduce pollen transfer efficiency and potentially damage floral structures. Given that hoverflies occurred in both habitats (~21% of visits), they were unlikely primary drivers of reproductive differences.

Environment-mediated pollination effects were also evident in visitation frequency and per-bout flower numbers. Higher visitation frequency in the sun habitat delivers adequate pollen to promote fertilization. This likely reflects both environmental effects on insect behavior and enhanced floral display, as greater flower numbers in the sun habitat increased attractiveness. Per-bout visitation patterns showed sun habitat insects visited twice as many inflorescences and male flowers as understory insects, while female flower visitation was similar. This suggests sun habitat insects carried more and genetically more diverse pollen, increasing single-visit effectiveness. Importantly, these per-bout differences were independent of regional flower abundance, indicating that light environment directly modified pollinator foraging behavior.

3.3 Conclusion

The more than threefold difference in total reproductive output between habitats resulted from cumulative, same-direction effects of direct environmental influences and environment-mediated pollination, with direct effects dominating. Enhanced light in the sun habitat increased flower production, pollinator visitation frequency, and pollen quantity/quality per visit, ultimately yielding higher fruit set. While each reproductive stage was superior in the sun habitat, the direct effect on flower number was the primary driver, with indirect pollinator-mediated effects contributing secondarily. Although higher reproductive output in high-light environments is well-known, this study elucidates the mechanistic causes by quantifying performance at each stage, particularly pollinator responses across habitats. Our findings underscore the necessity of simultaneously considering plant-physical environment-biotic factor interactions to fundamentally understand how environmental heterogeneity influences plant growth and reproduction.

Acknowledgments

We thank Liang Xijian and Liao Minglin from Hubei University for experimental assistance and Li Lena for writing assistance.

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