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Influence of plant on distribution of an endangered butterfly, *Leptalina unicolor* (Bremer & Grey, 1853), in restored riverside areas along the Geum River

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Abstract

Background: The dramatic worldwide decline in the butterfly species *Leptalina unicolor* (Bremer & Grey) is largely the result of continuous habitat decline and disturbance by humans. The discovery of a narrow habitat in riverside wetlands utilized by *L. unicolor* raises the hope that such restricted key areas could be rather easily protected.

Results: Here, we explain the environmental variables and habitat characteristics that primarily influence the distribution of *L. unicolor* discovered at the riverside areas along the Geum River. *L. unicolor* larvae were found at 9 of 13 study sites, and their abundance was strongly positively correlated with plant biomass. Our investigation showed that among four plant species (*Miscanthus sinensis*, *Spodiopogon cotulifer*, *Setaria viridis*, and *Imperata cylindrica*), *L. unicolor* larvae were the most abundant on the leaves of *M. sinensis*. They were not abundant on the leaves of *S. cotulifer*, *S. viridis*, or *I. cylindrica*. Interestingly, the number of *L. unicolor* larvae was positively correlated with the coverage area (m^2) of *M. sinensis* ($F = 41.7$, $r^2 = 0.74$, $P < 0.0001$).

Conclusions: It appears that water (e.g., wetlands, ponds, and watersides) located along the riverside areas along the Geum River is important for the constant maintenance and conservation of *L. unicolor*. This is based on the habitat characteristics (water preference) of *M. sinensis*, which is used as a habitat by *L. unicolor* larvae. However, the waterside is dry and terrestrialization is in progress owing to the decreased water levels and water supply caused by an opened weir. Hereafter, this area will likely require management to secure a stable habitat for *L. unicolor*.

Keywords: Wetland, *Miscanthus sinensis*, Habitat, Food source, Restoration, Four Rivers Project

Background

The dramatic worldwide decline in butterfly species *Leptalina unicolor* (Bremer & Grey) is largely the result of continuous habitat decline and disturbance by humans. *L. unicolor* is distributed across a narrow area of Korea, China, Russia, and Japan (Tsukiyama et al. 1997). It favors wetlands where *Miscanthus sinensis* is abundant and therefore is locally distributed. In Korea, small populations have been identified in areas such as Miryang-si (Jeyak Mountain) and Inje-gun (Simjeok wetland) (Hong et al. 2016), and in Japan, it is mainly observed in the Hokkaido and Honshu regions. *L. unicolor* lives in waterside areas such as riverbanks and surrounding wetlands (Fukuda et al. 1984). Therefore, the increasing desiccation of environments might be contributing to its decline.

Kim et al. (2012) reported that the habitat range of southern family species is gradually expanding north, while that of northern butterfly species is also moving northward, but their populations are decreasing. Accordingly, the Korean habitat range of *L. unicolor* is likely to decrease further owing to environmental changes. *L. unicolor* is recognized as an endangered species in Korea and is listed as a near-threatened species on the National Red List (IUCN 2007; Mano and Fujii 2009). It is crucial to obtain data in order to restore *L. unicolor*, as populations are decreasing across all the countries in its range.

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However, few studies have been conducted on the habitat characteristics and distribution of *L. unicolor* in Korea, and there have been only fragmentary reports on its habitat range and wintering patterns in Japan.

The discovery of a narrow habitat in riverside wetlands utilized by *L. unicolor* raises the hope that such restricted key areas could rather easily be protected. In this study, we investigated the distribution patterns and habitat characteristics of *L. unicolor* in a riverside wetland located midstream in the Geum River. We hypothesized that *L. unicolor* might prefer wetland around microhabitats in which emergent plants dominate and might utilize plant leaves and stems as its food source. Based on these data, the distribution and habitat characteristics of *L. unicolor* are discussed. We predicted that the abundance of *L. unicolor* would change based on plant species coverage in riverside wetlands.

Results and discussion

Distribution characteristics of *L. unicolor*

Plant community species composition differed among study sites. *Phragmites communis* and *M. sinensis* dominated most of the study sites, but a total of 13 plant species were found [*Salix koreensis* Andersson, *Lespedeza cuneata* G. Don, *Typha orientalis* C. Presl, *Typha angustifolia* L. sensu lato, *Paspalum distichum* var. *indutum* Shinners, *Paspalum distichum* L. Syst. Nat., *Nelumbo nucifera* Gaertner, *Zizania latifolia* (Griseb.) Turcz. ex Stapf, *Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla, *Pueraria lobata* (Willd.) Ohwi, *Festuca arundinacea* Schreb., *Dendranthema zawadskii* var. latilobum, and *Artemisia princeps* Pampanini].

L. unicolor was observed at 9 of the 13 sites (Table 1). In these observations, we found larvae and imagines of *L. unicolor* in wetlands where aquatic plants were abundant (Fig. 1 and Table 1). However, the species showed low density in a dryland where there was very little water. Larvae of *L. unicolor* were abundant in plant communities where *M. sinensis* dominated. Larvae of *L. unicolor* were observed on leaves of emergent plants, living in the rolled-up end of each leaf. By contrast, larval densities of *L. unicolor* were low in areas covered mainly by plant species (*P. communis*) other than *M. sinensis*. These characteristics suggest that the spatial distribution of *L. unicolor* larvae is closely related to plant cover. The larvae of most butterflies and insects, including *L. unicolor*, choose plants as their food source and habitat (Kitahara 2004). Thus, plant species coverage in a region is used as a major indicator of insect species richness (Kitahara 2004). Preferred food plants differ depending on the insect species. *L. unicolor* larvae prefer leaves of plants such as *M. sinensis* and *Spodiopogon cotulifer* Hack as food sources (Inoue 2012; Kim 2002; Shin and Paek 2014). In this study, *L. unicolor* larvae were found mainly in plant communities with high coverage rates of *M. sinensis*, and low densities were found in areas where *Lespedeza cuneate* and *P. communis* were dominant. *L. unicolor* larvae are found mainly in the vicinity of wetlands because their preferred food plant (*M. sinensis*) inhabits waterfronts. Its leaves are not only easy for *L. unicolor* larvae to feed on, but also allow them to conceal themselves from predators by rolling the leaves. Therefore, in Korea, *L. unicolor* larvae are found mainly in the vicinity of watersides and wetlands such as Sajapyeong Alpine Wetland at Jeyak Mountain in Miryang and Simjeok Wetland in Inje-gun.

L. unicolor larvae were more abundant in summer, the first study period, than in other seasons. They were observed only at five survey sites (sites 1, 2, 3, 4, and 5) dominated by *M. sinensis* in September, which was the second survey period, and rarely observed at sites other than survey site 3 during the third period (October). The decrease in the frequency of *L. unicolor* larvae after the summer can be explained by various factors, including the effects of predation. The larvae of other butterfly species similar to *L. unicolor* are also prey for predators such as birds and spiders.

There were few differences in environmental characteristics among the study sites (Table 2). However, the environmental characteristics differed among the three study periods. Although some study sites had exceptionally high or low values, the coefficients of variation (CV; standard deviation/mean × 100%) were lower than 100%, except for rainfall. Rainfall had the highest CV, at approximately 123.7%. However, we did not investigate rainy days (at the end of August).

Influence of *L. unicolor* on plant species

L. unicolor was found on the leaf surfaces of a total of four (*M. sinensis*, *Spodiopogon cotulifer*, *Setaria viridis*, and *Imperata cylindrica*) of the 13 plant species found. Densities of *L. unicolor* differed among the plant species (Fig. 2). The highest density was found on the surface of *M. sinensis* (mean ± SD, 28 ± 5.4 ind/m^2), followed by *I. cylindrica* (mean ± SD, 6 ± 3.2 ind/m^2). In contrast, *S. cotulifer* and *S. viridis* supported relatively low densities of *L. unicolor*.

Emergent plants are known to be appropriate refuges for avoiding predators as well as food sources for butterflies such as *L. unicolor* (Gilbert 1980, Cassidy et al. 2003). The plant habitat of *L. unicolor* is determined by the shape and structure of the plant species. *M. sinensis* is relatively easy to eat owing to the thinness of its leaf veins and thickness compared to other plants. Therefore, among the various plant species, *M. sinensis* is most likely to be selected by *L. unicolor*. The higher the percentage of coverage of *M. sinensis* in the plant community, the lower the rate of selection by *L. unicolor* of other plants,

Table 1 Abundance and habitat characteristics of endangered *Leptalina unicolor* in the riverside area located at Geum River. MS *Miscanthus sinensis*, PC *Phragmites communis*

Num.	Coordinates		Dominant plant species	Plant area (m^2)	Abundance of *Leptalina unicolor* (ind./m^2, larva (adult))		
	Latitude	Longitude			1st	2nd	3rd
1	N 36° 14′56.05″	E 126° 57′7.34″	MS	168,156	82	43	–
2	N 36° 14′48.05″	E 126° 56′13.43″	MS	103,234	10	5	–
3	N 36° 14′11.82″	E 126° 57′34.35″	MS	183.860	32	11	3
4	N 36° 11′50.95″	E 126° 58′56.56″	MS	259,880	114	98	–
5	N 36° 11′22.22″	E 126° 59′29.43″	MS	395,922	124(2)	38	–
6	N 36° 10′45.06″	E 127° 0′4.98″	LC	261,381	–	–	–
7	N 36° 8′59.96″	E 126° 58′59.64″	LC	138,415	–	–	–
8	N 36° 9′30.52″	E 127° 0′22.83″	LC	108,361	–	–	–
9	N 36° 10′30.43″	E 126° 59′37.70″	MS	215,684	13	–	–
10	N 36° 9′21.36″	E 126° 58′14.68″	PC	86,278	2	–	–
11	N 36° 9′21.36″	E 126° 58′14.68″	PC	46,381	4	–	–
12	N 36° 8′8.73″	E 126° 55′37.14″	PC	96,471	4	–	–
13	N 36° 8′7.98″	E 126° 53′32.30″	PC	167,426	–	–	–

Fig. 1 a, **b** Pictures of *Leptalina unicolor* in the riverside area located at Geum River

including *I. cylindrical*, as habitats. However, when other plants including *I. cylindrical* dominate, *L. unicolor* selects other plant leaves as habitats but stays for a relatively short time. Therefore, plant leaves other than *M. sinensis* are not suitable as long-term habitats for *L. unicolor*, which rolls up and shelters inside the leaves. We found many empty leaves of three plant species (*S. cotulifer*, *S. viridis*, and *I. cylindrica*) where there were no *L. unicolor* larvae. On the other hand, the ratio of empty houses was lower in most *M. sinensis* leaves, and *L. unicolor* larvae were found in most of the leaves.

Interestingly, *L. unicolor* larvae were positively correlated with the rate of coverage of *M. sinensis* (m^2) ($F = 41.7$, $r^2 = 0.74$, $P < 0.0001$, see Fig. 3). No matter how large the area of the plant community in which *L. unicolor* was found, its abundance was low in areas where the coverage rate of *M. sinensis* was low. Although *L. unicolor* can select leaves of plants other than *M. sinensis* as a habitat, those plants are not suitable in the long-term; thus, they appear to have little impact on *L. unicolor* distribution. However, the settlement and development of *M. sinensis* are very closely related to the abundance of *L. unicolor*, and *M. sinensis* provides a suitable habitat for increasing the *L. unicolor* population size.

Conservation strategy for *Leptalina unicolor* habitat

The discovery of *L. unicolor* along the riversides of the Geum River is an unusual case in that this endangered species was restored naturally at an artificial waterside park. The riversides of four major rivers, including the Geum River, were reorganized thanks to the Four Major Rivers Project, which was conducted from 2009 to 2012. In this process, the existing green houses and farmland

Table 2 Environmental parameters measured at the study sites where endangered *Leptalina unicolor* were found

Variable	Units	Max	Min	Mean ± SD	CV (%)
Temperature	°C	30.3	9.4	20.3 ± 4.9	24.0
Rainfall	mm	88	0.1	21.8 ± 27.0	123.7
Wind	m/s	2.7	0.6	1.4 ± 0.6	38.9
Humidity	%	96.9	48.6	76.4 ± 10.5	13.7
Sunshine hours	Hr	12.3	0	5.8 ± 4.3	74.3
Cloud cover	%	10	0	5.4 ± 2.9	54.8

were removed, and various plants such as *M. sinensis* and *I. cylindrica* were planted. In this study, *L. unicolor* was found in an area where farmland and green houses had been distributed over a large scale, but most of the space had become a park during the refurbishment. Compared with the riversides of the Nakdong River, Han River, and other areas, the area where *L. unicolor* was discovered had a high flooding frequency and thus was highly affected by rainfall or inundation. In addition, unlike in other regions, this area has a high ratio of water space, including tributary streams, waterways, and wetlands, which are factors likely to lead to the settlement and development of *M. sinensis* (especially *Miscanthus sinensis* Andersson) rather than other plants. In Japan, as well as in Korea, *L. unicolor* was found to be highly associated with the distribution of *M. sinensis* (Inoue, 2012). The existing *L. unicolor* habitats discovered in Korea, such as Jeyak Mountain in Miryang and Daeam Mountain in Cheorwon, Gangwon-do, have a unique area and a small range of *M. sinensis*. However, the midstream and downstream areas of the Geum River are wide and have a high rate of coverage of *M. sinensis*, making them easy for *L. unicolor* to inhabit.

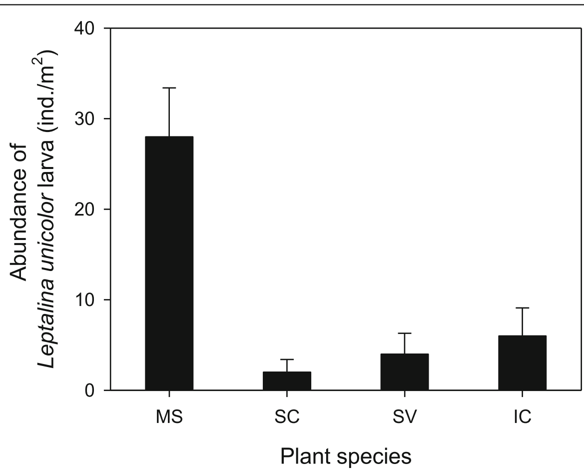

Fig. 2 Abundance of *Leptalina unicolor* larva from each plant species. Bars represent an average of replicates (± SE). *MS Miscanthus sinensis*, *SC Spodiopogon cotulifer*, *SV Setaria viridis*, *IC Imperata cylindrical*

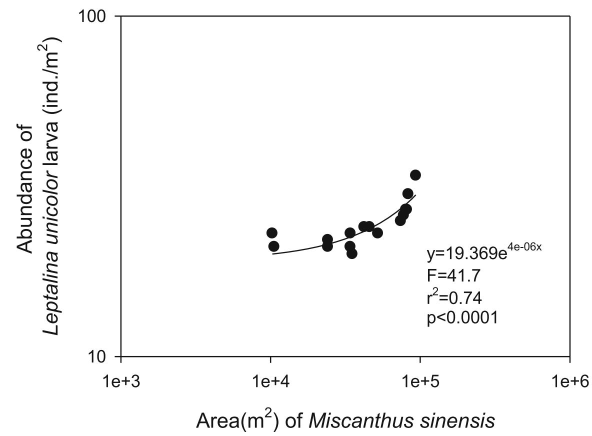

Fig. 3 The relationship between the area of *Miscanthus sinensis* and abundance of *Leptalina unicolor* larva (n = 16, regression df = 1, residual df = 14)

There is an urgent need to obtain research data and manage habitat for the restoration of *L. unicolor*, as it is endangered and its population size has decreased not only in Korea but also in other countries such as Japan and Russia. However, there have been few studies on the life cycle and ecology of *L. unicolor* in Korea (Hong et al. 2016), and only fragmentary reports on its habitat range and wintering patterns in Japan (Inoue, 2012). The spring and summer types of *L. unicolor* are familiar, but in this study, a fall type, as well as the existing two types, appeared. This might be because the midstream and downstream regions of the Geum River located in the lowlands are warmer than those located at higher altitudes, considering that this habitat is an alpine wetland. In addition, continuous human intervention might limit the predation activities of birds and spiders toward *L. unicolor*, making it easier for *L. unicolor* to inhabit this area as compared to the desolate alpine regions.

However, habitats of *L. unicolor*, which are located in the midstream and downstream of the Geum River, are periodically threatened by damage and disturbance. This is because its habitats are located near parks used by people, where municipalities regularly remove *M. sinensis* communities that invade trails, bicycle lanes, and plazas for management purposes. In addition, *M. sinensis* inhabits wetlands and watersides, periodically affecting human access to these areas for fishing and dock use. This represents a conflict between conservation and use. As *L. unicolor* is an endangered species, it is necessary to preserve its habitat. However, unfortunately, its habitat surrounds frequently used trails and bicycle lanes in the park. Thus, it is necessary to establish management measures that can satisfy both conservation and utilization needs.

Considering the habitat requirements of *L. unicolor*, which is heavily influenced by hydrophytes such as *M. sinensis*, the creation of wetlands and watersides seems necessary to preserve the species. In this study, *L. unicolor* was distributed along the riverside where *M. sinensis* communities were developed and in an area where a large-scale wetland was created. However, these wetlands and watersides are currently undergoing a serious dry stream phenomenon, and many regions have shown plant succession from aquatic to terrestrial plants. The causes of this include the deterioration of the wetland inlet and outlet and the continuous erosion of the revetment slope. However, the main cause is the high step caused by the decreased main river water level owing to the opening of the weir. Therefore, to secure the habitat of *L. unicolor*, it is necessary to continuously maintain the watersides, including the wetlands. To do so, it is also necessary to prepare measures such as securing instream flows and preventing erosion to avoid terrestrialization.

Conclusions

L. unicolor distribution along the riverside of the Geum River is heavily influenced by plant communities. In this study, *L. unicolor* appeared in 9 of 13 survey sites, and in most cases, it showed high densities in plant communities where *M. sinensis* was dominant. The larval density per area (m^2) results from four plant communities (*M. sinensis*, *S. cotulifer*, *S. viridis*, and *I. cylindrica*), showed that *L. unicolor* was found at low densities in *S. cotulifer*, *S. viridis*, and *I. cylindrica*. *L. unicolor* larvae were largely dependent on the rate of coverage rate of *M. sinensis*: the higher the rate of coverage by *M. sinensis*, the more larvae were present. Considering the habitat characteristics of the *L. unicolor* larvae that inhabit *M. sinensis*, it is clear that the creation of wetlands and riversides is important for the continuous maintenance and conservation of this species. However, the wetland and watersides constructed along the Geum River waterfront are experiencing a serious dry phenomenon owing to decreased water levels following the opening of a weir and a lack of water sources. Some areas are even undergoing terrestrialization. Therefore, continuous management and maintenance of these wetlands and watersides are necessary to secure a stable habitat for *L. unicolor*.

Materials and methods

Study sites

South Korea is located in East Asia and has a temperate climate. Four distinct seasons lead to the dynamic succession of biological communities in the freshwater ecosystems of South Korea. Annual mean rainfall is ~ 1150 mm, more than 60% of which occurs from June to early September (Choi et al. 2011; Jeong et al. 2007). Our study sites were located in western South Korea, along the middle part of the Geum River. Historically, there have been numerous riverine wetlands and areas prone to flooding by summer rainfall on the riversides of the Geum River. The riversides were reorganized by the River Refurbishment Project in 2012, and large areas of wetland have vanished owing to the expansion of human society. The manmade wetlands were constructed as an alternative to wetlands that had disappeared. A total of 147 created wetlands were built in four river basins (Ministry of Land, Transport, and Maritime Affairs, 2014).

The research area of this study encompassed the areas surrounding riversides or wetlands located in the middle of the Geum River inhabited by various plants including *M. sinensis*. To compare the dynamics of *L. unicolor* at each study site, we obtained environmental data (temperature, rainfall, wind, humidity, sunshine hours, and cloud cover) from the Korea Meteorological Administration (KMA, http://www.kma.go.kr). We investigated the abundance of *L. unicolor* and environmental factors in 13 plant communities.

Monitoring strategy

The target species, *L. unicolor*, is known to prefer hydrophytes such as *M. sinensis* (Hong et al. 2016) and is prevalent in temperate zones during summer and fall (O'Neill and Farr, 1996). Based on this information, we selected 13 study sites dominated by hydrophytes in summer and fall with relatively high temperatures. We investigated environmental parameters and the abundance of *L. unicolor* larvae during three time periods (August, September, and October) in 13 riverine wetlands and areas prone to flooding located on the riversides of the Geum River. We established six sampling points (quadrat, 1 × 1 m) at each study site to collect *L. unicolor.* The sampling points were randomly selected based on virtual grids constructed over maps of the area. We counted all *L. unicolor* larvae found within each quadrat, handling them carefully to prevent the larvae from accidentally detaching. *L. unicolor* abundance was expressed as the number of individuals per quadrat (1 m^2). The area (m^2) of plant community coverage and the dominant plant species were investigated in study sites where *L. unicolor* was found. We considered these factors to be closely related to the distribution of *L. unicolor.*

To compare the dynamics of *L. unicolor* in wetlands, we obtained environmental parameter data (temperature, rainfall, wind, humidity, and sunshine hours) from the Korea Meteorological Administration (KMA, http://www.kma.go.kr). The closest gauging station to the studied wetland (Buyeo Station) was selected.

Data analysis

We implemented a regression analysis to determine the trend of *L. unicolor* abundance with an area of *M. sinensis* coverage. All statistical tests were conducted using the statistical package SPSS Statistics ver. 20 (IBM, New York, USA). We tested for linear, exponential, inverse, power, and logistic functions to determine an equation generating the curve of best fit. Among the regression results, the curve-fitting equation that returned the highest determination coefficient was selected to explain the observed relationships.

Abbreviation

Ind: Individuals

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Authors' contributions

JYC and SKK participated in the design of the study, field survey, and data analyses and wrote the manuscript draft. YHB and JAJ participated in the field survey and data analyses and edited the manuscript draft. JCK and JHY conceived the study, participated in the design of the study, edited the manuscript draft, and secured the funding. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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