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Research Article

Observations of Resource Use by the Threatened Diana Fritillary Butterfly (*Speyeria diana*) in the Southern Appalachian Mountains, USA

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We present four summers (2006–2009) of field observations of the Diana fritillary, *Speyeria diana* (Cramer, 1777), throughout the Southern Appalachian Mountains, USA, in the eastern portion of its distribution. We describe our observations of resource use by *S. diana* in sites located in Georgia, Tennessee, South Carolina, North Carolina, and Virginia. Butterflies imbibed nectar from five genera (>11 species) of flowering plants and also imbibed liquid from dirt roads and horse manure. The majority of butterflies (57%) were observed feeding on milkweed, *Asclepias* spp., a high-quality nectar-producing plant which is known to be an important resource for many Lepidoptera. We documented 14 species of *Viola* spp., the larval host plant used by *Speyeria*, in our survey sites. All butterflies were marked to observe their movement. Recapture rates ranged from 17% to 56%, suggesting that dispersal of *S. diana* out of suitable habitat was somewhat limited.

1. Introduction

The Diana fritillary, *Speyeria diana* (Cramer, 1777), is an endemic butterfly species in the southeastern USA that has experienced a severe range collapse over the past century [1]. *Speyeria diana* was once distributed more widely across the southeastern US than it is at the present, ranging from coastal Virginia across the Ohio River Valley to Arkansas and Missouri. This species now persists in two geographically separated population groupings across the Interior Highlands of Arkansas and Oklahoma in the west, and throughout the Southern Appalachian Mountains in the east [1]. Due to its rapid decline over the past century, *S. diana* is considered to be a species of federal concern in North Carolina by the Fish and Wildlife Service and is included on the North Carolina Animal Watch List published by the North Carolina Natural Heritage Program [2]. *Speyeria diana* is also included on the Red List of Pollinator Insects of North America, compiled by the Xerces Society for Invertebrate Conservation [3] and is listed by NatureServe as a G3 species (rare across its distribution) [4]. Despite regional listings, there is no federal protection in place for this species under the US Endangered Species Act(16 U.S.C. 1531-1544, 87 Stat. 884).

A number of studies have established the importance of violets, from the genus *Viola*, to *Speyeria* larvae [5–7]. While a number of violet species have been used to successfully rear *S. diana* in laboratory settings [8–11], to our knowledge there are no documented reports of *Viola* usage by *S. diana* from natural habitats. Evidence does suggest that *S. diana* may specialize in its nectar use, preferring high-quality nectar resources when available [12–14]. However, these studies have focused predominately on the western portion of the species' range, largely ignoring the eastern populations. Here, we present our notes on resource use by the Diana fritillary in the Southern Appalachian Mountains, USA, based on four years (2006–2009) of field observations.

2. Materials and Methods

2.1. Study Sites. We surveyed six field sites in the states of Georgia (GA), North Carolina (NC-1, NC-2), South Carolina

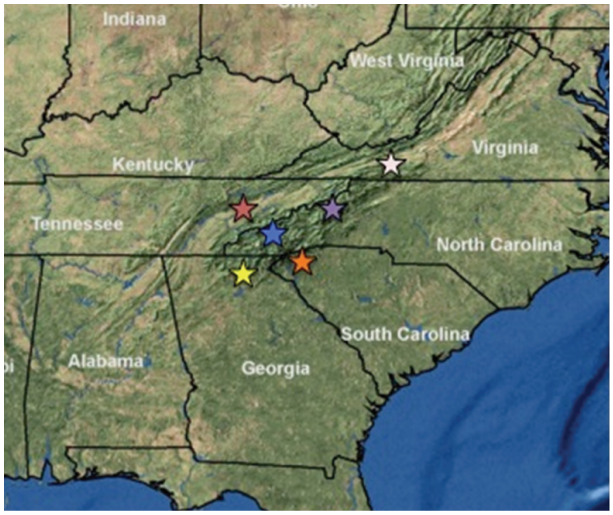

Figure 1: Southern Appalachian field sites sampled for *Speyeria diana* summers 2006–2009. From south to north, yellow: GA, orange: SC, blue: NC-1, red: TN, purple: NC-2, and pink: VA.

(SC), Tennessee (TN), and Virginia (VA) from late May to early October during the summers of 2006–2009 (Figure 1). Survey sites were 0.1 ha, and they were distributed from the southernmost boundary of the species' eastern range in north Georgia (34.76°N, −84.06°W) to the northernmost boundary of the species' range in Virginia (37.23°N, −80.42°W).

2.2. Surveys. Surveys were conducted between 1000 and 1700 hrs EST on days with temperatures ranging from 19° to 36°C. We spent a total of 1,100 hours searching the six field sites for *S. diana* using a combination of slow driving in a motor vehicle (5 mi per hr) along dirt roadsides, and hiking into the more remote habitats. For each *S. diana* encountered, we recorded the sex of the butterfly and any nectar source on which the butterfly fed. Horse manure and dirt roads were included because butterflies were observed actively imbibing from these sources. We recorded all violet *Viola* (Violacea) present in each field site. Plant resources were photographed for later identification when necessary, and all voucher specimens were housed in the Clemson University Arthropod Collection, in Clemson, SC.

We surveyed each site every 14–28 days during the flight period of *S. diana* (May through October, 2006 to 2009), attempting to capture each *S. diana* encountered in the field with a hand held net. We marked each captured butterfly with a unique number using a sharpie permanent marker, and removed a single leg for genetic studies being conducted by the lead author. Butterflies were handled as gently as possible to minimize wing damage and were released back to the location from which they came. When marked butterflies were recaptured, the date and location of these individuals were noted. Recaptures occurring on the same day of marking the individual were not included, so as not to influence butterfly behavior [15, 16].

3. Results

3.1. Adult Nectar Plants. We observed *S. diana* ($N = 203$) feeding on five plant genera, including common milkweed (*Asclepias syriaca* L.), butterfly milkweed (*A. tuberosa* L.), thistle (*Cirsium* spp.), iron weed (*Buddleja* spp.), goldenrod (*Solidago* spp.), purple cone flower (*Echinacea purpurea* L.), butterfly bush (*Vernonia* spp.), mint (*Pycnanthemum*), spreading dogbane (*Apocynum androsaemifolium* L.), blazing star (*Liatris* spp.), and wild burgemont (*Monarda fistulosa* L.) (Table 1). Fifty-seven percent of all feeding observations occurred on milkweed, *Asclepias* spp., followed by thistle, *Cirsium* spp. (11%), and *Buddleja* spp (9%). Horse manure (6%) and dirt roads (2%) were also found to be used by *S. diana*.

3.2. Larval Host Plants. We recorded fourteen *Viola* spp. across the six field sites (Table 2). The common blue violet, *V. sororia* Willd., was recorded in every field site except South Carolina (Table 2). The second most frequently recorded violet was bird's foot violet, *V. pedata*, which was recorded in four field sites (NC-1, SC, TN, and VA) (Table 2). The Georgia field site appeared to have eight violet species, while the NC-2 site had three species. *Viola sagittata* and *V. rotundifolia* were unique to the GA site, while *V. appalachiensis* was unique to NC-1 site.

3.3. Recaptures. Of the 203 *S. diana* butterflies we observed feeding, we were able to capture and mark 155 (76%) of them (Table 3). The highest percent of recapture was from the SC site (56%), followed by GA (32%), VA (25%), TN (21%), NC-2 (21%), and NC-1 (17%). The maximum number of times a single individual was recaptured was four (a male from NC-2), and this individual displayed the longest time between the first and last captures (13 days). Males were more likely to be recaptured than females ($\chi^2 = 80.4$, d.f. = 1, $P < 0.001$). Daily recaptures were too low to reliably estimate population sizes; however, this study was designed primarily to examine butterfly resource use and track movement within sites.

4. Discussion

The availability of nectar resources plays an important role in determining the distribution of *S. diana*. This has been previously established in western *S. diana* populations [17–19] and is supported by our survey results from the Southern Appalachian Mountains. While our study is purely observational, our results suggest that *S. diana* feeds preferentially on high-quality nectar plants in its eastern distribution, especially milkweed *Asclepias* spp. High-quality nectar availability is known to be an important resource in other endangered fritillaries as well [20, 21]. It has been suggested that phytochemicals present in the nectar of certain flowers, such as *Asclepias* spp. and *Echinacea* spp., may help protect long-lived *S. diana* females as well as overwintering *S. diana* larvae, from microbes, fungus, and disease [13]. It has recently been shown that amino acids from nectar can enhance female butterfly reproduction [19, 20], as well as male butterfly reproduction [21, 22]. Amino acids and electrolytes present in horse dung,

Table 1: Feeding observations ($N = 203$) of male/female *Speyeria diana* (2006–2009) in six field sites located across the Southern Appalachian Mountains, and the percentage of total use of each resource. Horse manure and dirt roads were included, as butterflies were observed actively imbibing from these sources.

Nectar source	GA	NC-1	NC-2	SC	TN	VA	Percentage of total
Asclepias syriaca L.	5/0	6/0			42/1	30/2	43%
Asclepias tuberosa L.	9/1			5/1	7/0	5/0	14%
Cirsium spp	0/5	0/9		3/2	1/3		11%
Buddleja L. spp			10/4	4/0			9%
Solidago spp		5/1		3/1		2/0	6%
Echinacea purpurea L.	2/0	4/1		1/0			4%
Vernonia spp		3/0	2/1				3%
Pycnanthemum Michx.						2/0	1%
Apocynum androsaemifolium L.				0/1	1/0		1%
Liatris spp	1/0					0/1	1%
Monarda fistulosa L.		1/0					0.5%
Horse manure	4/0	4/0			5/0		6%
Dirt road		1/0			1/0		2%

Table 2: Violet (*Viola* spp.) larval host plants observed at each of the Southern Appalachian field sites (2006–2009).

	GA	NC-1	NC-2	SC	TN	VA
Viola appalachiensis Henry		X				
V. bicolor Pursh.				X	X	
Viola blanda Willd.		X		X	X	
V. canadensis L.	X				X	X
V. cucullata Aiton	X	X		X		
V. hirsutula Brainerd				X		X
V. lanceolata L.		X				
V. papilionacea Pursh.	X		X			X
V. pedata L.		X		X	X	X
V. pubescens Aiton	X		X		X	
V. rotundifolia Michx.	X					
V. sagittata Aiton	X					X
V. sororia Willd.	X	X	X		X	X
V. tricolor L.	X					X

Table 3: Total number of feeding observations of *Speyeria diana* at each field site and the total number of butterflies captured and recaptured (2006–2009).

Field site	Number of feeding observations ($N = 203$)	Number of marked butterflies ($N = 155$)	Number of recaptures ($N = 41$)	Percent recapture
GA	27	19	6	32%
NC-1	35	18	3	17%
NC-2	17	14	3	21%
SC	21	16	9	56%
TN	61	52	11	21%
VA	42	36	9	25%

and some soils, are also known to provide butterflies with a rich source of nutrients [23].

The presence of particular *Viola* species did not appear to influence the activity of butterflies in our field sites. This result is consistent with laboratory rearing trials, which have not found any evidence of *Viola* preference in reproducing *S. diana* females, or developing larvae [11]. All of the violet species observed in this study are widely distributed in southeastern US forests [24].

Mark-release-recapture (MRR) techniques are used widely to estimate the population parameters of vagile species [25]. Our recapture rate of *S. diana* in the field was larger than expected; however, we want to emphasize that our mark-release-recapture methods were not intended to estimate *S. diana* population size in the field. We report our mark-release-recapture data for several reasons. First, we believe that our high recapture rate supports the hypothesis that *S. diana* is a highly localized species that does not disperse widely from a local habitat once it becomes established. We recaptured a large number of both males and females in all field sites, sometimes just meters from the butterfly's original location. Some recaptures occurred weeks after marking, further supporting the limited dispersal of *S. diana* over time as an adult. Second, the high recapture rate of *S. diana* also indicates that our nonlethal method of removing a single leg for genetic sampling did not negatively affect individual butterflies. The fact that a large number of butterflies were resampled several months after their initial capture suggests that these butterflies stayed in the local vicinity of the sampling site for the duration of their adult life without suffering demise resulting from the removal of a single leg.

Despite the importance of nectar resources in butterfly habitat, nectar plant use remains unstudied for many species on a small enough spatial scale [26]. Our observations of *S. diana* in the Southern Appalachian Mountains support the idea that this species is a specialist in its nectar use, preferring some sources of high-quality nectar over other sources. Our observations provide a preliminary list of nectar resources that can be targeted for preservation in *S. diana*, and other

fritillary habitat. Our observations also confirm the importance of *Asclepias* spp. to butterfly communities, a nectar plant that is required by the long-distance migrant, the monarch butterfly, *Danaus plexippus* [27]. Increased planting of high-quality nectar plants, like milkweed and Echinacea, in butterfly gardens and urban areas may benefit a large number of butterfly species, especially those that are long-lived like *D. plexippus* and *S. diana*.

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