Regal Fritillary (*Argynnis idalia*) Monitoring Techniques, Movement, and Habitat Use

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Abstract–The Regal Fritillary (*Argynnis idalia*) butterfly is endemic to high-quality prairies in North America. Declines in population sizes and distribution across much of its range, primarily due to habitat loss, has led to a review by the United States Fish and Wildlife Service for potential protection under the federal Endangered Species Act. Management and restoration of this species will benefit from effective methods to assess population sizes as well as an improved understanding of habitat use and dispersal ability. Population sizes have been relatively consistent in west-central Missouri, USA. We found a significant correlation ($r \geq 0.73$) among population size estimates (spatially explicit capture-recapture model, POPAN in Program MARK, Jolly-Seber) and population indices (number captured, maximum daily count, Pollard Index). The similar effectiveness among monitoring approaches suggests that the less time intensive transect counts can be used to track trends across time. Surveys yielded varying densities of Regal Fritillary adults depending on the location within each preserve; however, habitat covariates did not differ between areas of high and low density. It is likely that we sampled habitats with minimal variation in quality relative to the potential variation across all potential habitat types. Fewer adult butterflies were observed during the first flight season following a prescribed fire, but increased the following year. In addition to allowing for population size estimates, marking of butterflies resulted in documented male and female dispersal among remnant prairie fragments. The largest minimum distance traveled was 7649 m, and 3866 m for a male and a female, respectively. Missouri grasslands require management in the form of a disturbance such as fire, mowing, or grazing, and until fire-induced mortality is better understood return interval and potential refugia should be carefully considered.

Introduction

The *Argynnis idalia* (Drury 1773; Regal Fritillary) is a butterfly endemic to North American prairies, but has declined in population size and distribution across much of its range, primarily due to habitat loss (Brock and Kaufman 2003, Chazal et al. 2010, Debinski and Kelly 1998, Ferster and Vulinec 2010, Glassberg 1999, Opler and Krizek 1984). The Regal Fritillary is considered an indicator of high-quality prairie in North America and sensitive to habitat degradation (Hammond and McCorkle 1983). Prairie ecosystems are one of the most endangered ecosystems in the world (Noss and Peters 1995, Samson and Knopf 1994), and their loss is jeopardizing the long-term viability of this butterfly. In response to the declining status of the Regal Fritillary, the United States Fish and Wildlife Service is currently conducting a status review (USFWS 2015). The conservation of most species, including the Regal Fritillary, depend on monitoring populations to prioritize and indicate when management intervention is required (Carwardine et al. 2012, Martin et al. 2018), habitat to support a sufficient number of individuals (Lande 1987), and connectivity among their disturbance prone habitat patches (Aviron et al. 2007).

Monitoring for changes in population size is important to natural resource management and conservation efforts (Bibby and Alder 2003, Martin et al. 2007, Yoccoz et al. 2001). Ef-

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fective and efficient protocols are desired in monitoring schemes (Montgomery et al. 2021) and have been a topic of interest with butterflies (Arnold 1987, Gall 1985, Pollard 1977, Pollard et al. 1995, Roy et al. 2001, van Strien et al. 1997). Previous monitoring of Regal Fritillary adult populations has included transect counts (Swengel 1998, Fester and Vulinec 2010), distance sampling (Powell et al. 2007), and marking studies (Debinski and Kelly 1998, Fester and Vulinec 2010, Kelly and Debinski 1998, Marschalek 2020).

The long-term viability of the Regal Fritillary depends on management and protection of high-quality prairies. Prairies are a disturbance-dependent ecosystem that require a disturbance such as fire, grazing, mowing, and/or haying; however, there is disagreement about best management practices. Prairie-specialist butterflies also generally require some sort of management (Swengel 1998), but not all management actions result in greater butterfly species richness or densities. For example, burning has been suggested to be detrimental to grassland butterfly species including Regal Fritillaries (Swengel 1998, Swengel et al. 2011) but Regal Fritillary densities increased two years after fire suggesting refugia (in both time and space) are required (Moranz et al. 2014). This finding was supported by Swengel (1998) and Swengel et al. (2011) where occasional fires (rather than frequent rotational burning) and habitat with non-fire refugia were more favorable to prairie-specialist butterflies. There is evidence that Regal Fritillary larvae can survive a fire, based on larval observations in a recently burned area (McCullough et al. 2017). In addition, McCullough et al. (2019) found that a moderate fire-return interval (three to five years) resulted in greater densities of Regal Fritillary adults.

Currently, eastern Kansas and western Missouri are thought to represent the last robust and stable populations of Regal Fritillary (Powell et al. 2007, Swengel 1998). However, there is limited information available regarding the effects of interactions of habitat use, management, and landscape context on Regal Fritillary abundances. Study sites for McCullough et al. (2019) were in the Flint Hills of Kansas, where there is less precipitation and invasion of woody plants compared to Missouri. In addition to differing in climate and associated vegetation responses, prairies in Kansas are more contiguous compared to more isolated remnant prairies of varying sizes and distances in Missouri. In Missouri, Moranz et al. (2014) compared Regal Fritillary numbers in patch-burn-grazing systems with rotational grazing at four relatively large grasslands. Characteristics (floral resources, grazing, fire) correlated with greater Regal Fritillary population sizes varied across June and July within the two years of the study. It also appears that data from all four sites were pooled, not assessing the common idiosyncratic nature of sites (Billick and Price 2010, Tabi et al. 2020). Preliminary work in Missouri has shown that Regal Fritillary densities vary substantially within and among prairies (Marschalek 2020), suggesting that habitat quality varies.

Dispersal among habitat patches can determine where a species persists (Gadgil 1971, Hanski 1991), metapopulation dynamics (Leibold et al. 2004), and minimize inbreeding (Frankham 2005). Considering that many landscapes are fragmented, understanding movement patterns is important for management and conservation of native species (Swift and Hannon 2010, Driscoll et al. 2013). Restoring connectivity to reverse, in part, the effects of fragmentation and climate change is becoming more common (Driscoll et al. 2014, Kerr 2020).

In Missouri, ~0.5% of the historic prairies and savannas remain (Missouri Department of Conservation 2015). Remnants of tallgrass prairie south of Sedalia, Missouri, are thought to have relatively large and stable Regal Fritillary populations (Marschalek 2020). We quantified population sizes, habitat use, and dispersal patterns of the Regal Fritillary at these remnant prairies. This information is important for large-scale conservation planning and local

management. Our specific objectives were to: 1) quantify Regal Fritillary population sizes and densities, including a comparison of sampling approaches; 2) correlate habitat covariates (vegetation structure, floral resources) and time since fire with Regal Fritillary butterfly densities; and 3) assess male and female movement patterns by conducting a marking study.

Materials and Methods

Surveys and marking study of the Regal Fritillary were conducted at remnant prairies south of Sedalia, Missouri in 2020–2022 (map available in Marschalek 2020). These surveys included sites that historically provided consistent detections of this species. Study objectives differed among the three years, focusing on calculating population size estimates/densities and male dispersal in 2020, quantifying female dispersal and habitat use in 2021, and further assessing the numbers of Regal Fritillary adults in recently burned and unburned areas in 2022. All of the surveys were conducted during the earlier male flight season, except for four surveys in 2021 when specifically targeting Regal Fritillary females. Both males and females are active during the summer (late May to late July), but females also have a later flight season in late summer-early fall following a diapause (Kopper et al. 2001, McCullough et al. 2021).

Population sizes/densities

In 2020, surveys for Regal Fritillary adults occurred in remnant prairies at Friendly Prairie Conservation Area (Friendly Prairie, 16 ha, 38.55°, −93.29°), Drover's Prairie Conservation Area (Drover's Prairie, 32 ha, 38.53°, −93.29°), Paint Brush Prairie Conservation Area (Paint Brush Prairie, 91 ha of the 128 ha preserve, 38.54°, −93.26°) and privately owned Marker's Prairie (13 ha, 38.54°, −93.25°). Surveys were conducted roughly every 3–4 days, depending on weather, during 1 June–28 July. The entire area at each remnant prairie was systematically searched by surveyors walking parallel transects about 50 m apart so that an active Regal Fritillary butterfly anywhere in the survey area would be detected. When a Fritillary was observed, it was pursued until it was caught or left the preserve. After capture and documentation, the surveyor returned to the transect where the pursuit started.

Locations of all Regal Fritillary butterfly observations were recorded on a handheld Garmin eTrex® 20x or 32x GPS and the plant species was recorded if the butterfly was feeding. Captured butterflies were uniquely marked with a felt-tipped marker to create different patterns of colored dots for each individual. Surveys were conducted during suitable weather for butterfly activity, 24° C or warmer and at least filtered sunlight penetrating the clouds, if clouds were present.

Marking data obtained in 2020 were used to calculate the following population size estimates: spatially explicit capture-recapture model (SECR), POPAN in Program MARK, and Jolly-Seber (JS) method for an open population. These population size estimates were compared with the following population size indices: number of unique individuals captured, maximum daily count (Max Count), and Pollard Index.

A SECR model estimates the density of organisms using recapture data. One advantage of SECR over non-spatial models is that it overcomes issues with determining the effective sampling area, which is problematic in traditional capture-recapture estimates (Efford and Fewster 2013). Spatial movements by recaptured individuals are used in SECR to estimate activity centers for each individual. Because density is a latent variable within the SECR model, there is no need to estimate effective sampling area when converting estimated population size to density. The *secr* package (version 4.3.0; Efford 2023) was used for the

analysis within R (version 4.0.2; R Core Team 2021). Using POPAN in Program MARK, a number of models that considered time-dependent and sex-dependent survival, capture, and emigration/immigration rates were used to determine the best fit model. Model comparison and selection were based on weighted AIC_C. The third estimate was the Jolly-Seber (JS) method (Jolly 1965; Seber 1965), calculated using equations from Krebs (1999), which is appropriate for an open population and involves relatively simple calculations. Regal Fritillary populations were considered open because of 1) the staggered emergence of individuals, including males typically emerging before females, and 2) individuals are capable of moving among preserves during the annual survey period.

For the population indices, the total number of unique individuals captured represented the minimum known population size. This value is expected to be an underestimate of the actual population size because some individuals are expected to escape detection and/or capture. The Max Count represents the maximum number of Regal Fritillary adults counted in a single day at each site, avoiding double-counted individuals (based on previous marks). The Pollard Index is a sum of all counts during one adult flight period, with surveys conducted on an even and similar schedule, and counting butterflies in a defined area (Pollard 1977). This index is widely used for national butterfly surveying schemes (e.g., Pollard et al. 1995, Roy et al. 2001, van Strien et al. 1997). Pollard walks have been modified to include varying widths of survey area along the transect (Swengel 1996; Kral-O'brien et al. 2021). We counted any Regal Fritillary observed, up to about 25 m from the transect, when flying or perched high on a flower. Due to low recapture rates of females, we did not attempt to calculate a sex-specific population size using marking data.

Habitat use

In 2021, surveys for Regal Fritillary adults occurred at the same four sites as in 2020, as well as Lordi Marker Prairie (32 ha, 38.54°, −93.29°). Survey areas were expanded at Paint Brush Prairie (107 ha of the 128 ha preserve) and Marker's Prairie (the entire 76 ha prairie including remnant prairie and areas in the process of restoration). Portions of Friendly Prairie, Paint Brush Prairie, and Marker's Prairie were involved in prescribed burns within one year of butterfly surveys. Drover's Prairie was not burned between the 2020 and 2021 flight seasons, and Lordi Marker Prairie had been hayed for at least several years prior to surveys. The 2020 protocols were followed in 2021 except each site was surveyed only two times during the male flight season (either 15 or 16 June and either 1 or 2 July), and only females were captured/marked for purposes of assessing female movement patters (see below). Results from 2020 surveys indicated that areas tend to have high densities of Regal Fritillary adults throughout the adult flight season (or no to low densities throughout the flight season), so two surveys were sufficient to define areas of low and high density within a site. An additional survey was conducted at Friendly Prairie on 17 June 2022 to record locations of each Regal Fritillary to further assess response to prescribed fires. Nearly all management units within state owned prairies in Missouri are attempted to be burned every three years or less to limit encroachment of woody vegetation.

Habitat characteristics were recorded in areas of high and low densities of Regal Fritillary butterflies based on the 2021 Regal Fritillary observations. The Kernel Density tool in ArcGIS Pro 3.1 was used to create heat maps for a visual representation of Regal Fritillary densities. Configurations included output cell size of 1, planar method for distance measurements, and the processing extent set to the boundary of the area searched. Individual heat maps were created for each prairie and helped guide habitat sampling.

Habitat covariate data were collected in areas where Regal Fritillaries were com-

monly observed (comparatively higher densities) and areas where Regal Fritillaries were not observed (absent or low densities) 21 June–19 July, 2021. These "used" and "unused" areas were identified in prairie sections that were both burned and unburned since the 2020 flight season. Efforts were made to have equal habitat sampling locations in used and unused areas and burned and unburned areas. It should be noted that imperfect detection was likely, making it difficult to determine that no Regal Fritillaries ever used certain portions of these prairies.

Sampling, similar to Marschalek et al. (2017), occurred in areas (referred to as plots) of the prairie where Regal Fritillary adults were observed (used) and areas within the same prairie where they were not observed (unused). A set of four 25-m transects extended from a central point in the four cardinal directions. Vegetation structure was measured by placing a 1-m^2 quadrat every meter positioned 1–5, 11–15, and 21–25 m from the center of the plot along each of the four transects (n = 60 quadrats total per used or unused area). This design allowed for testing of habitat associations at different scales (0.008, 0.069, and 0.198 ha). Covariates included percent cover of bare ground, litter, grasses/sedges, forbs, and woody vegetation for a rapid assessment of vegetation composition; number of inflorescences for each plant species; and vegetation structure using a Robel pole. We recorded the height on the pole where it was no longer visible in the vegetation from four meters away and at one meter height in the four cardinal directions. The relative Robel pole measurement was later calculated by subtracting the site mean Robel pole height from the sampling plot mean (60 quadrats). The number of inflorescences for each plant species were recorded both earlier (June) and later (July) at 10 of the 30 sampling areas.

The number of Regal Fritillary observations within 25 m of each plot's center point were calculated in ArcGIS Pro 3.1 and associated with the habitat covariate data from the same 25-m radius. Butterfly counts for June, July, and the sum of June and July were used. Habitat variables (percent cover for each category and inflorescence counts for each plant species) were log(x+1) transformed prior to parametric analysis as there was a pronounced right skew to these data. Habitat data relationships were visualized with non-metric multidimensional scaling (NMDS), assessed using principal coordinate analysis (PCO) and a type III Bray-Curtis similarity permutational multivariate analysis of variance (PERMANOVA) test with 9999 permutations. Pairwise comparisons followed the PERMANOVA to determine significance between sites and burn status (prescribed fire since the 2020 flight season or not) in PRIMER 7 (PRIMER-e, Quest Research Limited). A two-sample t-test for unequal variance was conducted in SYSTAT 13.1 (SYSTAT Software, Inc.) to compare habitat covariates between burned and unburned areas. A BEST test, was used to determine which flowering plant species were most influential in differentiating the sites and burn status, was performed using PRIMER 7 (PRIMER-e, Quest Research Limited). A forward stepwise logistic regression was used to assess relationships between Regal Fritillary presence/absence and habitat data in SYSTAT 13.1 (SYSTAT Software, Inc.). A p = 0.15 cutoff was used to avoid failing to include potentially important variables (Bendel and Afifi 1977).

Movement patterns

Dispersal of Regal Fritillary adults was assessed with individuals marked in 2020 and females marked in 2021 during the summer (see above for survey details). Since females have a later flight season in late summer to early fall following a diapause (Kopper et al. 2001, McCullough et al. 2021), four surveys on 24 and 31 August, and 7 and 14 September were conducted to focus on capturing and marking females. Due to time constraints, these later surveys were restricted to areas with the largest Regal Fritillary densities in June and

July. Movement from one preserve to another was the primary focus, rather than intrapatch movements. The minimum distance traveled between sightings was measured using ArcGIS Pro 3.1 for individuals that dispersed from one preserve to another.

Results

Population sizes/densities

In 2020, 12–16 surveys were conducted at each of the four prairies, resulting in the capture of 542 individual Regal Fritillary butterflies and 889 total captures (including recaptures; Table 1). Two individuals, accidently marked with the same color pattern, were excluded from further analysis. The majority (87.5%) of captured individuals were males, which experienced a greater recapture rate (males: 42.6%; females: 15.7%). All recaptured females were captured only two times (including the initial capture and marking), while 18.7% of males were captured at least three times and one male was captured eight times.

During the first two to three weeks of the flight season, males were relatively easy to capture, most commonly observed flying low over shorter vegetation and frequently dropping down in the vegetation for one to two seconds at a time. In late June, male behavior changed as they engaged in large circling flight paths more often than the low flight observed earlier. From late June and into July, males and females were observed feeding from flowers of 11 plant species and a turtle carcass (Table 2).

Table 1. Total marked unique Regal Fritillary butterfly individuals in 2020, representing the minimum known population size.

Site	Males	Females	Total
Drover's	30	0	30
Friendly	113	16	129
Marker's	97	18	115
Paint Brush	232	36	268
Totals	472	70	542

Table 2. Number of Regal Fritillary adults observed feeding from flowers of each plant species during 2020 surveys at Drover's, Friendly, Marker's, and Paint Brush prairies.

Plant Species*	Number of Observations
Pale purple coneflower (*Echinacea pallida*)	21
Mountain mint (*Pycnanthemum* sp.)	11
Prairie blazing star (*Liatris pycnostachya*)	10
Butterfly weed (*Asclepias tuberosa*)	7
Wild bergamot (*Monarda fistulosa*)	5
Prairie coreopsis (*Coreopsis palmata*)	4
Daisy fleebane (*Erigeron strigosus*)	1
Heal-all (*Prunela vulgaris*)	1
Purple milkweed (*Asclepias purpurascens*)	1

*Four butterflies were also observed feeding from a *Chelydra serpentina* (L.) (Common Snapping Turtle) carcass.

For all survey sites, the Program Mark model that incorporated a constant death rate, sex-specific capture rate, and time-dependent and sex-dependent birth/immigration rate [Program Mark notation: phi(.)p(sex*.)pent(sex*t)] was the most highly selected model based on weighted AIC_C. Population size estimates were generated by POPAN and JS methods with an undefined spatial extent due to the movement of individuals in and out of the sampled area. For this reason, comparisons to the SECR density are less straightforward. These three population size estimators, derived from mark-recapture data, were consistent in ranking populations from low to high (Table 3). The POPAN estimator produced mean estimates that were larger than the SECR or JS estimators. Both POPAN and SECR produced similar levels of precision (i.e., similar 95% CI spread), but POPAN precision tended to decrease as size of the study area increased. Interestingly, the 95% CI overlapped for most estimators within sites; however, the 95% CI for SECR estimates at the Friendly Prairie and Paint Brush Prairie were lower than the POPAN interval. The three population size indices were also consistent in ranking populations from low to high (Table 3). Max count had the lowest value, number of unique individuals was intermediate, and Pollard Index had the highest value for each site. In general, all estimates and indices were highly correlated (Table 4).

Table 3. Comparison of population size estimates (95% CI) and indices for each prairie in 2020. Since SECR generates a density, the area of each preserve was used to calculate a population size estimate.

Population abundance estimates

Site	SECR	POPAN	Jolly-Seber (JS)
Drover's	63.5 (15.9–256.3)	56.4 (40.2–80.2)	72.0 (8.8–2691.6)
Friendly	72.7 (49.7–106.4)	234.8 (195.5–284.7)	110.4 (49.0–507.7)
Marker's	232.2 (116.5–462.7)	342.6 (177.6–1046.3)	144.0 (31.2–2334.6)
Paint Brush	318.4 (270.8–375.0)	620.3 (531.6–727.1)	178.9 (94.7–512.6)

Population abundance indices

Site	# Captured	Max Count	Pollard Index
Drover's	30	13	36
Friendly	129	50	232
Marker's	115	31	169
Paint Brush	268	103	425

Table 4. Pearson correlation coefficients for pairwise comparisons among all population size estimates/indices derived from 2020 surveys at Drover's, Friendly, Marker's, and Paint Brush prairies.

	SECR	POPAN	JS	# Captured	Max Count	Pollard
SECR	—					
POPAN	0.932	—				
JS	0.948	0.983	—			
# Captured	0.820	0.969	0.919	—		
Max Count	0.730	0.915	0.836	0.984	—	
Pollard	0.755	0.940	0.886	0.994	0.987	—

Habitat use

We recorded 689 Regal Fritillary observations (range of 12–356 at a site) among the five sites during the first sampling period and 627 observations (range of 34–270 at a site) during the second sampling period in 2021. Data from these surveys were used to identify used and unused areas. The spatial distribution of observations changed slightly between the mid-June and early-July sampling periods (Fig. 1). Areas with high densities of Regal Fritillary adults were quite evident throughout the flight season. A common difference temporally was that Regal Fritillary adults tended to be more clumped in mid-June and slightly more spread out across the prairies by early July.

We sampled 31 plots for habitat covariates (Friendly n = 3/4 burned/unburned; Paint Brush n = 4/4; Marker's n = 5/2; Drover's n = 0/4; Lordi Marker n = 0/5; Drover's was managed by fire but was not burned in the year prior to sampling, Lordi Marker was hayed). Vegetation structure differed among sites ($F_{4,24} = 2.84$, $p = 0.007$) and burn status ($F_{1,24} = 9.72$, $p = 0.001$). Pairwise comparisons identified Lordi Marker Prairie (only hayed site) and Marker's Prairie (only site with extensive restoration efforts) as having different vegetation structure compared to the other sites (Table 5). There were no statistical differences detected among the three fully remnant prairies (Friendly, Paint Brush, and Drovers). Areas that were burned since the 2020 flight season tended to have more bare ground compared to areas that were not burned ($t_{17.8} = 6.101$, $p < 0.001$; Fig. 2). The percent cover of grass compared to bare ground and forbs explained greater than half (56.4%) of the variation represented across the sites.

As expected, the composition and relative abundance of flowers (inflorescences) varied through the season (from June to July for this study). In total, 54 plant species were observed flowering and included in the analysis (any fleabane, *Erigeron* L., was treated as a single taxon). There was a difference in the assemblage of flowering plants among sites ($F_{4,10} = 1.56$, $p = 0.045$) and burn status ($F_{1,10} = 2.22$, $p = 0.034$) for the June sampling period, but only burn status ($F_{1,14} = 2.28$, $p = 0.017$) for the July sampling period (sites $F_{4,14} = 4.14$, $p = 0.287$; Fig. 3). The flowering plant species that were most influential in generating these community patterns were *Pycnanthemum tenuifolium* Schrad. (Slender

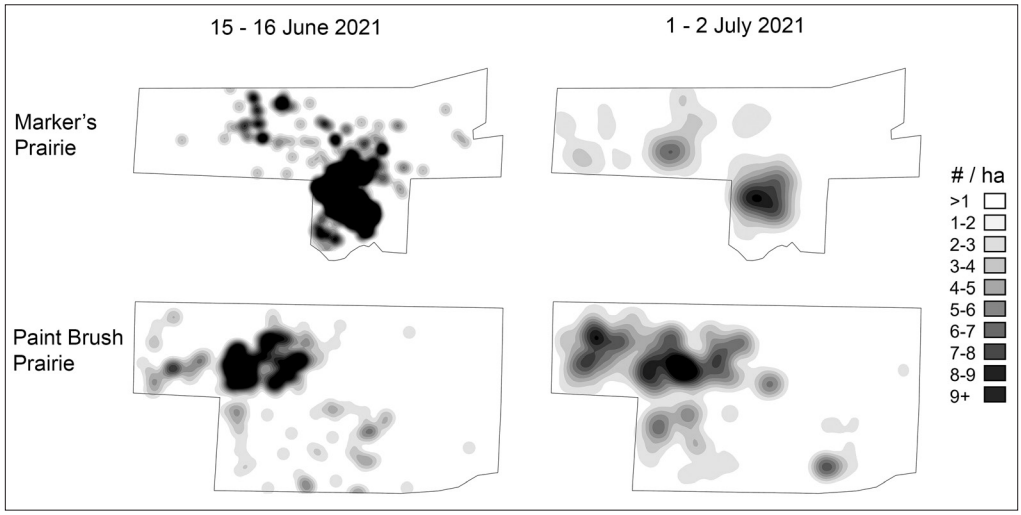

Figure 1. Kernel density heat maps illustrating areas of higher Regal Fritillary adult densities (darker gray) and lower densities (lighter gray), based on surveys conducted in mid-June or early July 2021. Thin outline (black) represents the survey area.

Mountain Mint) and *Erigeron* L. (Fleabane) species. Fleabane was more common in recently burned areas in both June and July, and *Eryngium yuccifolium* Michx. (Rattlesnake Master) was also more common in burned areas in July (Fig. 3).

There were no habitat covariates consistent with predicting the presence or absence of Regal Fritillary adults (used vs. unused areas; Table 6). Forward stepwise logistic regression identified that Regal Fritillaries were more likely to occupy unburned areas in early July ($p = 0.057$) and shorter relative vegetation using combined June and July butterfly counts ($p = 0.030$) within the 25-m radius sampling area.

Table 5. A pairwise comparison (PERMANOVA) of habitat covariates (percent cover bare ground, forbs, grasses, litter, shrubs) among all sites from sampling in 2021

Sites	t-statistic	df	p-value
Friendly – Paint Brush	0.706	11	0.714
Friendly – Marker's	1.854	11	0.019
Friendly – Lordi Marker	2.317	9	0.007
Friendly – Drover's	1.048	8	0.396
Paint Brush – Marker's	1.887	12	0.020
Paint Brush – Lordi Marker	1.855	10	0.018
Paint Brush – Drover's	0.665	9	0.737
Marker's – Lordi Marker	0.694	10	0.656
Marker's – Drover's	1.321	9	0.174
Lordi Marker – Drover's	2.972	7	0.006

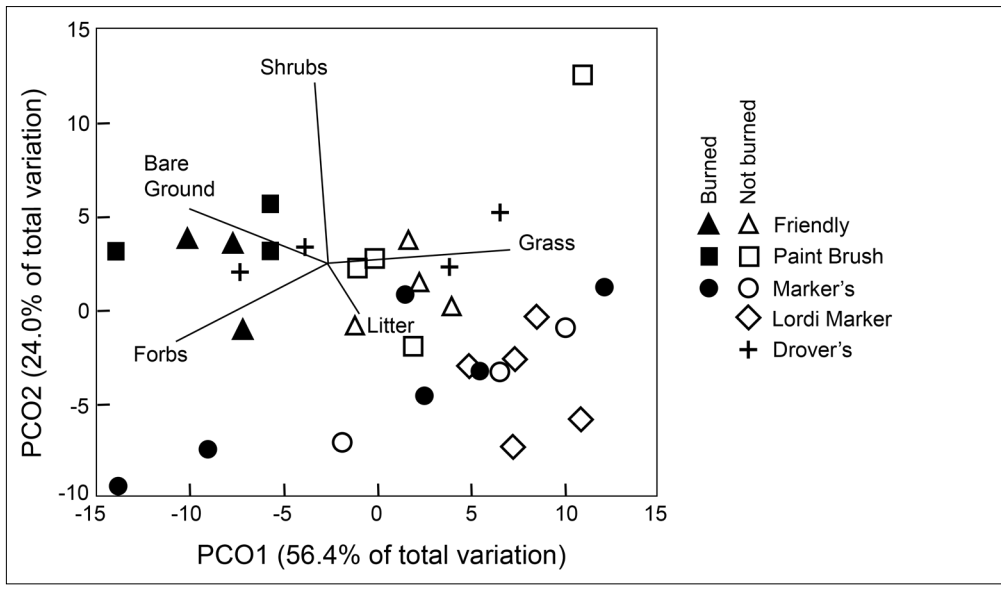

Figure 2. PCO using average percent cover for each habitat covariate (percent cover bare ground, forbs, grasses, litter, shrubs) for each sampling plot. Vectors for each of the habitat covariates are presented to interpret relative composition of each sampling plot. Symbol shape and color represent different prairies (sites) and burn condition, respectively.

Floral resources differed among some combinations of burn status and Regal Fritillary occupancy. The floral resources differed between all pairwise comparisons of burn status and occupancy ($p \leq 0.037$), occupied and unoccupied areas within unburned areas ($p = 0.007$), but not the occupied and unoccupied areas that were recently burned ($p = 0.855$). The experimental design aimed to balance the number of used and unused areas in both burned and unburned sections of prairies. This provided an ability for an initial assessment of how habitat characteristics (vegetation structure, flowering plants) and management practices influence Regal Fritillary adult numbers and distribution. Friendly Prairie provided an opportunity to assess the effect of prescribed burns on Regal Fritillary counts for two reasons. First, we surveyed the entire preserve although it should be noted that grasslands and native prairie are adjacent to this preserve, so it is not an isolated habitat patch. Second, half of the prairie was burned in each year of the study (2020: western half, 2021: eastern half, 2022: western half), alternating burned and unburned each year. Although there were different sampling protocols (Marschalek 2020), the unburned half of Friendly Prairie had higher counts of Regal Fritillary adults than the burned half in all three years: 191 versus 38, 172 versus 57, and 32 versus 4, respectively (Fig. 4).

Movement patterns

In 2020, most individuals (185, 87.7%) were recaptured in the same preserve as the initial capture, with detection of a total of 26 movements from one preserve to another. The median minimum distance traveled for these same individuals was 2017 m (range 1168 m to 7649 m). Only one of these 36 movements involved a female (from Marker's to Paint Brush). Capturing Regal Fritillaries at Drover's was difficult due to a relatively

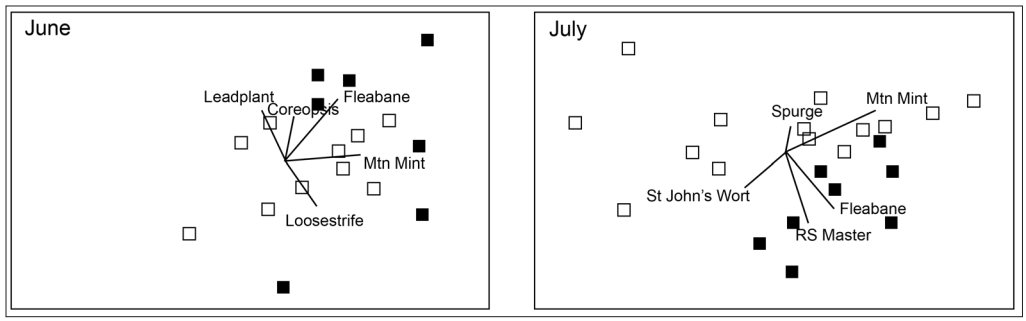

Figure 3. NMDS using the average count of inflorescences for each flowering plant species in each sampling plot sampled in June (left, stress = 0.16) and sampled in July (right, stress = 0.12). Dark squares represent sampling areas that were burned within one year of sampling and open squares were not burned in the previous year.

Table 6. Average (with standard deviation) percent cover estimates for habitat covariates in plots that were burned within one year and plots that were burned more than one year prior to the 2021 sampling, and areas that had higher densities of Regal Fritillary adult (used) and areas with low densities (unused).

Site Category	Bare Ground	Forbs	Grasses	Litter	Shrubs
Burned	51.6% (13.9)	61.8% (9.2)	63.9% (11.7)	35.1% (5.1)	17.5% (9.1)
Unburned	33.1% (6.2)	54.7% (8.8)	79.0% (8.0)	36.1% (5.6)	20.4% (10.1)
Used	39.8% (12.7)	55.7% (9.6)	75.2% (9.4)	35.1% (6.0)	19.8% (9.3)
Unused	41.1% (14.9)	60.6% (8.9)	69.4% (15.5)	36.9% (3.9)	18.3% (9.3)

consistent behavior of flying across large areas and/or leaving the preserve. Of the individuals originally marked at Drover's and later recaptured, most of the Regal Fritillaries were recaptured at a different site.

We captured and marked 101 individual Regal Fritillary females in 2021. Of these, four individuals were recaptured (three recaptured one time and one individual recaptured two times) for a total of 106 captures. One of the recaptured individuals was initially marked at Paint Brush Prairie Conservation Area and later captured at Friendly Prairie Conservation Area, a Euclidean distance of 3866 m. The other three recaptures occurred in the same prairie as the original capture.

Discussion

As expected, Regal Fritillaries were detected at all five survey sites and densities varied within and among sites. The population size estimates and indices were, in general, highly correlated and in agreement in ranking prairie sites by relative abundance. Despite the often stark contrast between areas with high and low use by Regal Fritillaries (primarily males), we were unable to detect statistically significant differences in vegetation composition and structure between the two areas. Areas burned within a year of sampling demonstrated different vegetation structure and flowering plants and approached significantly fewer Regal Fritillary adults compared to those areas with greater than one year since burning. Dispersal among the preserves, which facilitates locating resources and recolonizing following prescribed fires (if extirpated), was documented for both males and females.

Our assessment of population size estimates and indices suggest that a relatively simple transect count could be sufficient to compare across populations and assess trends over time. This assumes that the detection probability remains constant, which is likely considering the large size of the Regal Fritillary and the relatively open vegetation communities it inhabits. Specifically for the Regal Fritillary, generating a population index such as the Pollard Index (Pollard 1977) would require about six weekly surveys during June and July (based on Missouri phenology). Annual maximum count would require only one well timed survey during late June but would benefit from three surveys to confirm peak numbers were recorded. The Pollard Index is used in Europe for national monitoring schemes (e.g. van Swaay et al. 2008), and the annual maximum count is similar to the 4th of July Counts in the United States (e.g. Swengel 1990; note the 4th of July Counts require only one survey per year) in

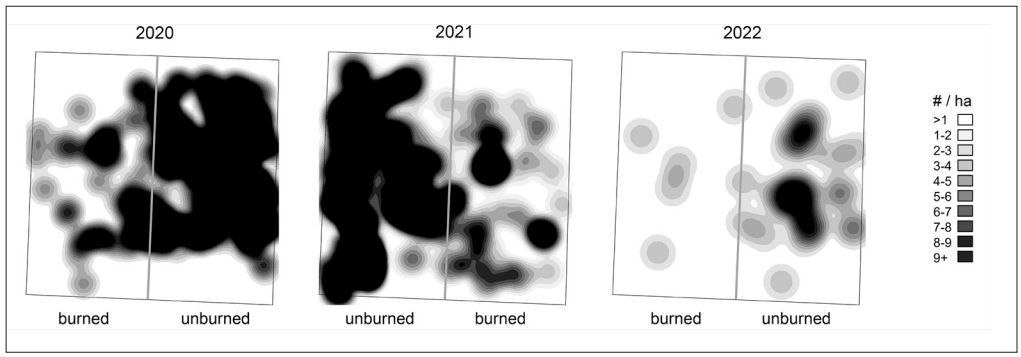

Figure 4. Kernal density heat maps illustrating areas of higher Regal Fritillary adult densities (darker gray) and lower densities (lighter gray) at Friendly Prairie in 2020–2022. Thin black outline represents the survey area, and the central gray line represents the fire break. Number of surveys varied across years so comparisons should be made between burned and unburned areas within each year, not across years.

that a single data point per site is used. However, there may be times when a time-intensive marking study is needed for a more precise population size estimate or obtaining movement data to better understand connectivity of habitat patches.

Management at the preserve level is also important for conservation efforts related to the Regal Fritillary. Consideration should be given to meet a target vegetation community and condition, as well as the effect of management actions implemented to meet these targets. This study was unable to refine the target vegetation community for Regal Fritillaries in Missouri due to being unable to detect differences in vegetation structure among areas of high- and low-density Regal Fritillaries within each prairie. This could be an artifact of the balance study design of equal representation of used and unused areas in both burned and unburned sections (i.e. not proportional). Considering most of the survey area consisted of remnant prairie and with Regal Fritillary adults present, it is likely that most sampled areas were suitable grasslands.

Grasslands require regular disturbances to limit the growth and encroachment of woody vegetation, either natural or initiated by preserve managers. The use of fire for prairie management has been the topic of much research (e.g., Hovick et al. 2014, Scholtz et al. 2018), including butterflies (Moffat and McPhillips 1993, Opler 1981, Panzer 1988, Vogel et al. 2007) and specifically Regal Fritillary butterflies (Moranz et al. 2014, Swengel et al. 2011, Swengel and Swengel 2009).

When considering vegetation characteristics, fire, and grazing in relationship to Regal Fritillary densities in Missouri, Moranz et al. (2014) found few clear patterns emerge. Their most evident pattern demonstrated a preference for recently burned and ungrazed areas in late part of the flight season (late July). McCullough et al. (2019, 2021) found that a moderate fire return (three to five years) in the Flint Hills of Kansas resulted in the largest densities of Regal Fritillaries. Other management practices (i.e., grazing, haying, or mowing) did not influence density (McCullough et al. 2019, 2021). On average, Missouri receives more precipitation than Kansas, facilitating woody vegetation growth. For this reason, most of the areas included in our study were burned every one to three years. Although not specifically stated, it appears that adult numbers are lower immediately after fire (0–2 years in Kansas and less than a year in this study). We did not observe many Regal Fritillaries using recently unburned areas in July if there was limited use in June. Due to management objectives at our study sites, fire intervals of three years or more and grazing were not investigated.

Fire has been suggested to cause high rates of mortality among larvae because of fewer adults in areas recently burned (Swengel and Swengel 2007, Wagner et al. 1997). The lower number of adults in burned areas could be due to fire-induced mortality, or the higher number of adults in unburned areas could be due to resource selection. To better understand and quantify either process, individual Regal Fritillaries would need to be tracked from early larval stages through the adult stage. However, observed male behavior adds to the speculation that fire induced mortality is relatively high. Early in the flight season, males were observed almost exclusively flying low over the vegetation and in a frequently probing manner, presumably searching for females emerging from pupae. More males were observed in areas burned more than a year before sampling, a pattern most easily seen with an alternating burn regime at Friendly Prairie. The few areas with a higher number of adults that experienced fire within a year of sampling had low vegetation and exposed rock, possibly resulting in a less intense fire. Although not a significant difference in the floral resource analysis, evidence for resource selection was supported by many adults feeding on a few *Asclepias tuberosa* L. (Butterfly Weed) plants, influencing the July 2021 density. We suggest that there is some mortality due to fire and some

resource selection by adults, especially later in the flight season, but these are likely context specific and require further investigation. Regardless, the relatively large and stable population at the study sites in the current landscape, have persisted with predominately prescribed fire management. Furthermore, Regal Fritillary adults (and possibly larvae) are often observed in high numbers by the second flight season after a fire.

To more accurately describe habitat use, grassland patches representing a wider range of characteristics (e.g. vegetation communities, patch size, patch isolation) are required. McCullough et al. (2019) suggests that landscape composition and fragmentation could influence Regal Fritillary densities. To disentangle landscape influences, a large number of sites in different landscapes would be required. Additionally, including the larval food plants (violets, *Viola* sp.) in habitat sampling could help explain the differences between more heavily used areas and those with few butterflies. While resources for Regal Fritillary adults are important for conservation, adults are more mobile than immature stages. For this reason, larvae and their larval food plants should be incorporated into future work. It is possible that the adults are remaining in close proximity to the violets, but it is difficult to make the connection when conducting adult surveys when the vegetation is relatively dense, and the violets are past flowering and much less obvious.

Both male and female Regal Fritillaries moved among grassland patches in the landscape of this study, suggesting a metapopulation structure. It is not expected that local populations at individual prairies/preserves will be extirpated some years and return in others. Instead, this system likely resembles the patchy metapopulation model where all habitat patches are occupied and connected with some dispersal (Stith et al. 1996). Williams et al. (2003) found increased genetic differentiation associated with habitat fragmentation within the Regal Fritillary range. As with any species, this dispersal provides geneflow and reduces potential negative effects of inbreeding (Crnokrak and Roff 1999, Frankham 2005). Within butterflies, inbreeding depression was found to be more common in smaller and fragmented Glanville Fritillary (*Melitaea cinxia*) populations, resulting in the extirpation of several populations (Saccheri et al. 1998).

Dispersal also demonstrates that habitat fragmentation is not restricting access to resources. Considering Fahrig's (2013) Habitat Amount Hypothesis, having access to more resources allows for a larger population. For these three reasons, the Regal Fritillary population south of Sedalia, Missouri, is more likely to persist. Connectivity among remnant prairies is likely the reason that these local populations are apparently relatively large and stable, while it appears Regal Fritillaries have been lost from the surrounding areas of Missouri.

While this study was relatively restricted in geographic scope, it provides important information for management and further studies. The use of fire can be effective for management of Regal Fritillary habitat, as demonstrated by the continued persistence of relatively large populations at the prairies just south of Sedalia, Missouri. However, until fire-induced mortality is better understood, return interval and potential refugia should be carefully considered. The inclusion of a wide range of grasslands with and without Regal Fritillaries will provide a clearer description of habitat and management needs of this butterfly, which is likely important with a species with such a large (historic) range.

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