INFLUENCES OF TIMING AND POPULATION DENSITY ON THE SEX RATIO OF THE REGAL FRITILLARY, *SPEYERIA IDALIA*
(LEPIDOPTERA: NYMPHALIDAE)

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ABSTRACT.– In summer during 1988-99, we conducted 735 hrs and 1416 km of transect butterfly surveys at 116 grasslands in the range of the regal fritillary, *Speyeria idalia* (Drury), grouped into four subregions: (1) Wisconsin, (2) northwestern Iowa and adjacent Minnesota, (3) northwestern Minnesota and adjacent North Dakota, and (4) western Missouri. We recorded 22,685 regal fritillaries at 84 sites; 92% of the 20,968 sexed individuals were males. First female emergence typically followed first male emergence by about 1-3 weeks in subregion 1. In each subregion, male density (individuals/hr) showed a single mid-flight peak, while females increased gradually and linearly. The strongest and most consistent correlation for percent unsexed individuals was negative with year. During most of the flight period, the observed "sex ratio" (percent males of sexed individuals) was strongly male-biased. Sex ratio correlated significantly and negatively with date, consistent with eclosure by males earlier than females. But in all subregions, the strongest correlation for sex ratio was negative with female density, both in the entire survey period and prime (main) flight, which by definition had positive male and female densities that did not vary significantly by date. In most subregions, sex ratio covaried significantly with male density, both over the entire survey period and during prime flight. Observed sex ratios relate to relative densities of males and females actually present but also suggest a tendency for females to become less detectable at higher male densities. Since the observed range of densities was much greater for males than females in all subregions, females appeared to adjust their detectability (i.e., behavior) based on male density but not vice versa. In addition, more females may *actually be present* in a site earlier in the flight than when highest *observed* female densities occur.

KEY WORDS: aestivation, behavior, conservation, density dependence, detectability, endangered species, flight period, Iowa, Midwest, Minnesota, Missouri, Nearctic, Nebraska, North America, North Dakota, phenology, prairie, protandry, seasonality, sex ratio, *Speyeria*, sociality, USA, Wisconsin.

Like other large fritillaries (*Speyeria* Scudder) (Lepidoptera: Nymphalidae) in North America, adult regal fritillaries (*Speyeria idalia* (Drury 1773)) occur in single annual generations broadly spanning summer. Male *Speyeria* adults are usually first observed one to several weeks before females, and aestivation may occur during midsummer, at least in hotter or drier climates (Opler and Krizek, 1984; Sedman and Hess, 1985; Scott, 1986; Heitzman and Heitzman, 1987; Royer, 1988; Nagel *et al.*, 1991; Iftner *et al.*, 1992; Allen, 1997).

In this paper, we present analyses of the variation in sex ratio (percent males of sexed individuals) and proportion of unsexed individuals observed on transect surveys of adult regal fritillaries, in relation to seasonal and annual timing and density (individuals observed per hour) of total, male, and female regal fritillaries. These results are useful for interpreting survey results and understanding the behavior of this butterfly. Given the vast destruction of its prairie habitat in the past two centuries primarily for conversion to agriculture, the regal fritillary has experienced widespread decline and marked range contraction (Hammond and McCorkle, 1984; Johnson, 1986; Swengel, 1993; Allen, 1997; Debinski and Kelly, 1998; Layberry *et al.*, 1998; Schlicht and Orwig, 1998). Historically, the regal fritillary was distributed as far east as southern New Brunswick, but the remaining eastern populations are now scattered and restricted to just a few areas, as in parts of Pennsylvania and western Maryland. As a result of these conservation problems, much recent survey work has been conducted to assess this species' status and trend (e.g., Royer and Marrone, 1992; Debinski and Kelly, 1998; Kelly and Debinski, 1998; Schlicht and Orwig, 1998). Greater insight in the interpretation of regal fritillary observations should be useful for implementing effective monitoring and conservation programs on its behalf.

Furthermore, regal fritillaries are particularly amenable to investigations of sex ratio because they are readily sexed in the field by wing pattern. Given their relatively large size, distinctiveness from a distance, and the widely unobstructed field of view in their grassland habitat, regal fritillaries are also readily observed in the field. Thus, these results may be useful for applying to other butterfly species which are less readily sexed and/or detected in the field.

METHODS

Study Sites and Surveys

The 116 grassland study sites were deliberately selected because they were dedicated to conservation and/or likely to have conservation value for prairie species. All but 13 of the sites were conservation lands in private or government ownership, because of their public accessibility and importance for conserving rare species. In each subregion, the study sites varied greatly in size (1.2-2024 ha of prairie vegetation), degree of degradation, soil moisture type (from wet to dry), and management (primarily rotational burning, haying, and/or grazing, or idling), as described in Swengel (1996, 1997, 1998). Thirteen grassland reserves in Wisconsin consisted primarily of degraded grassland or old field (49-1336 ha of grassland vegetation), not original (untilled) prairie like all other sites.

The sites were categorized into four subregions (Table 1, Fig. 1): (1) Wisconsin, (2) northwestern Iowa and adjacent southeastern Minnesota, (3) northwestern Minnesota and adjacent southeastern North Dakota, and (4) western Missouri. Subregional boundaries were designed to maintain an adequate sample of regal fritillary individuals while reducing biogeographical and climatic variation within subregion. As a result, some study areas in northern Illinois and northeastern Iowa included in previous analyses (Swengel, 1996, 1997, 1998), where small numbers of regal fritillaries were observed, were excluded from this analysis. All sites could not be visited each year during the regal fritillary flight period, but most were visited more than once during that time both within and among
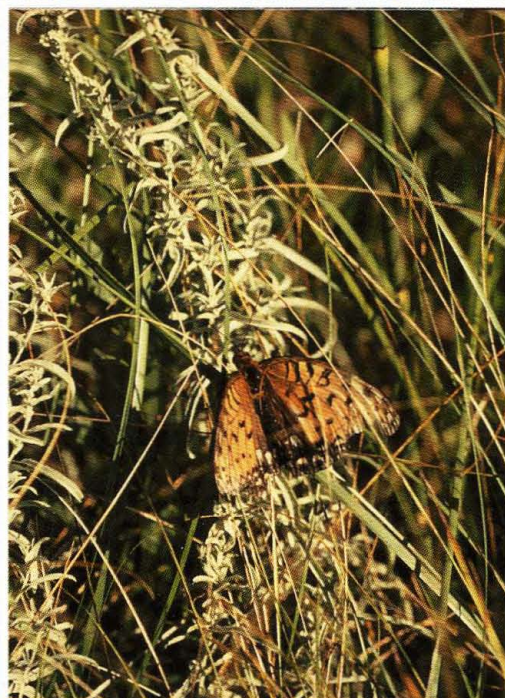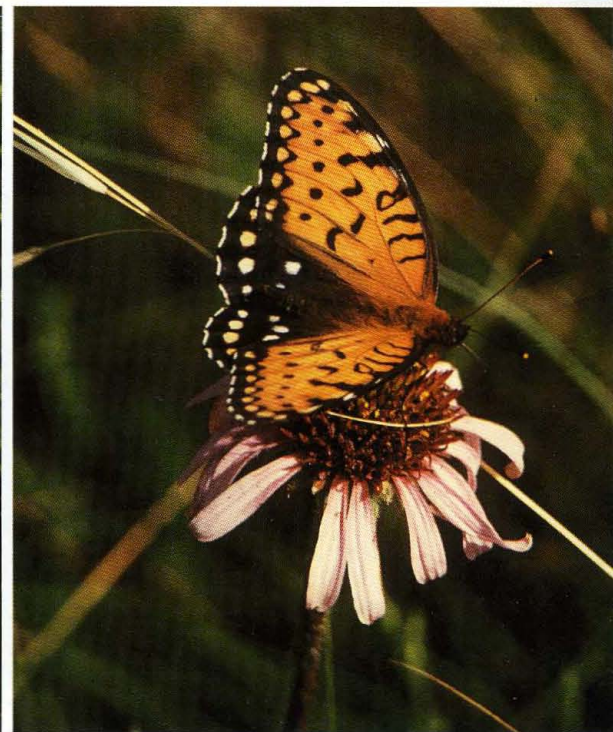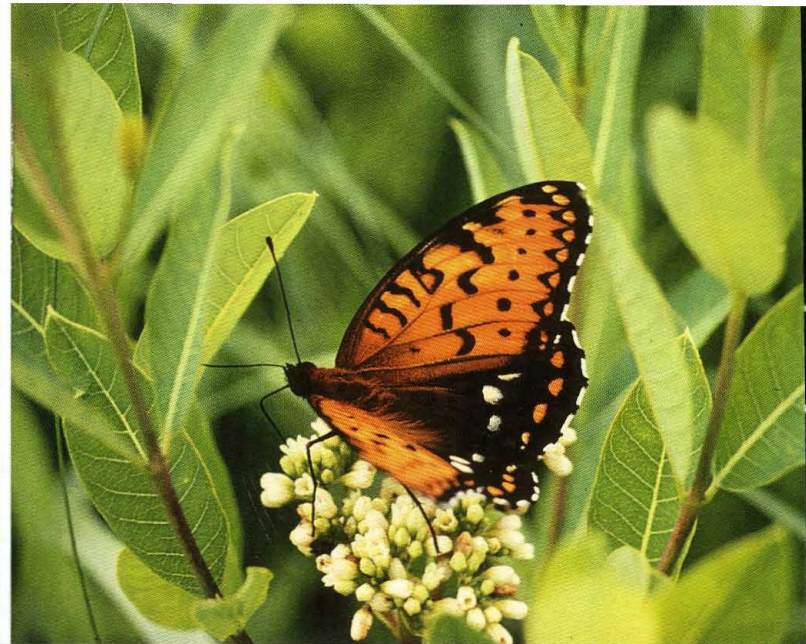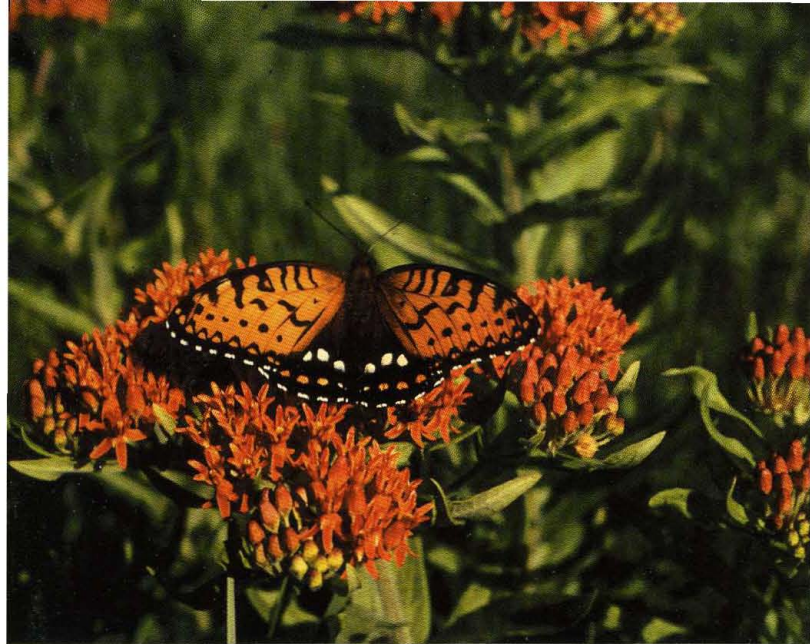
Fig. 1. Map of study subregions: 1 (Wisconsin), 2 (northwestern Iowa and adjacent southeastern Minnesota), 3 (northwestern Minnesota and adjacent southeastern North Dakota), and 4 (western Missouri).

years. A few Minnesota and Wisconsin sites were visited in 1988-89, but most surveys occurred during 1990-99. The most revisiting (2-8 times per year in 1990-99) and fullest coverage of the flight period occurred in subregion 1. In subregions 2-3, most sites were visited twice per year at an interval of about 3-6 weeks in 1990-93, but at most once per year in 1994-97. In subregion 4, sites were surveyed once in mid-June each year in 1992-99, except for a few sites resurveyed on 12 July 1994. The site contributing the most unit surveys comprised only 8-32% of each subregion's sample.

During 1988-99, the two authors conducted transect butterfly surveys along similar routes each visit. Walking at a slow pace (1.5-2 km/hr) on parallel routes 5-10 m apart, we counted all adult butterflies observed ahead and to the sides, to the limit of species identification (possibly with binoculars after detection) and our ability to track individuals. A new sampling unit was designated whenever the habitat along the fixed route changed markedly in vegetation type, degree of degradation, or management. Routes crossed rather than followed ecotones and management boundaries to reduce edge effects, and were designed to maximize sampling per unit but minimize number of unit changes during the site survey, while sampling representative areas of the site. For each unit survey, temperature and time spent surveying were recorded, and wind speed, percent time the sun was shining, percent cloud cover, and route distance were estimated. Surveys occurred during a wide range of weather conditions and times of day. Occasionally surveys occurred in intermittent light drizzle, so long as butterfly activity was apparent, but not in continuous rain. Data were kept separate by unit survey.

Sexing Individuals

During the surveys, regal fritillary individuals were sexed, if possible. Initially, this was based entirely on the color of the outer-most spot-row of the dorsal hind wing (orange for males, white for

females) (Plates 1-2). However, the outer spot-row can be hard to observe clearly when the individual is distant or in rapid/evasive flight. The adjacent inner spot-row – white in both sexes – may be difficult to distinguish from, or seem more prominent than, the outer spot-row. Furthermore, a faded male spot-row (pale orange) may resemble a female spot-row (white) (Plate 1), and it is our impression that we noted faded males much more often than faded females.

For several years, we field-tested sexing based on the dorsal front wing apex. Males have a narrow even width of dark margin along the entire outer margin; in females, this margin widens into a triangle in the apex and encloses several white spots (Plates 1-2). During this field-testing, we recorded an individual's sex only if both the dorsal front wing apex and hind wing spot-row were adequately observed. The two methods always agreed, but the front wing apex was usually easier to spot in distant and flying individuals and remained distinct even in very faded individuals. For the remainder of the study, sexing was based on either or both characteristics – whatever was easiest to observe and definitively established the gender fastest. Gender differences in the ventral front wing apex seemed more subtle (Plates 2-3) and more difficult to observe in flight, and this feature was sometimes obscured too much by the hind wing in perched individuals. Specimens of both sexes are illustrated in color photographs in Opler and Krizek (1984), Heitzman and Heitzman (1987), Glassberg (1999), and Nielsen (1999) (dorsal only), and Shull (1987) and Allen (1997) (dorsal and ventral).

It was essential to sex individuals reliably based on phenotype independent of seasonal timing and behavior. Although males seem typically more active (i.e., more often in flight) and females more sedentary (i.e., more often perched in obscuring vegetation), females can fly quickly and evasively and males may rest in the vegetation during peak hours of this species' activity. While males often engage

Plate 1. Dorsal views of male regal fritillaries in Minnesota (MN) and Missouri (MO), with fresh individuals in top two rows, worn individuals in bottom row. Top row, left: Taberville Prairie (St. Clair Co., MO), 18 Jun 1997; right: Ripgut Prairie (Bates Co., MO), 15 Jun 1992. Middle row, left: Taberville Prairie (St. Clair Co., MO), 18 Jun 1997; right: Bicentennial Prairie (Clay Co., MN), 19 Jul 1990. Bottom row, left: Taberville Prairie (St. Clair Co., MO), 18 Jun 1997; middle: Bicentennial Prairie (Clay Co., MN), 8 Aug 1991; right: Bicentennial Prairie (Clay Co., MN), 8 Aug 1991.
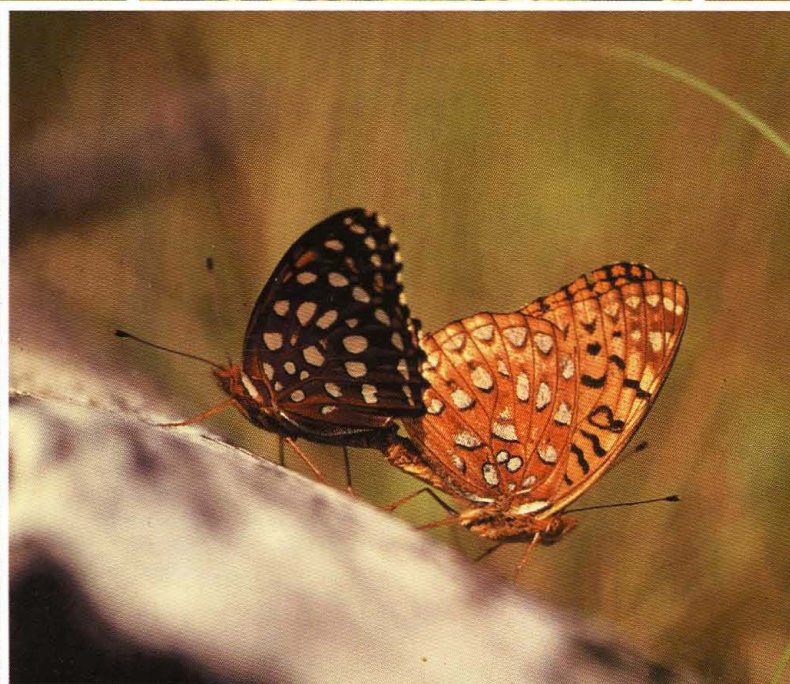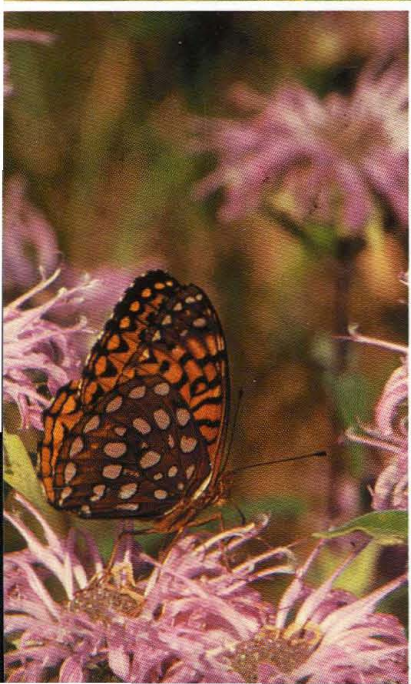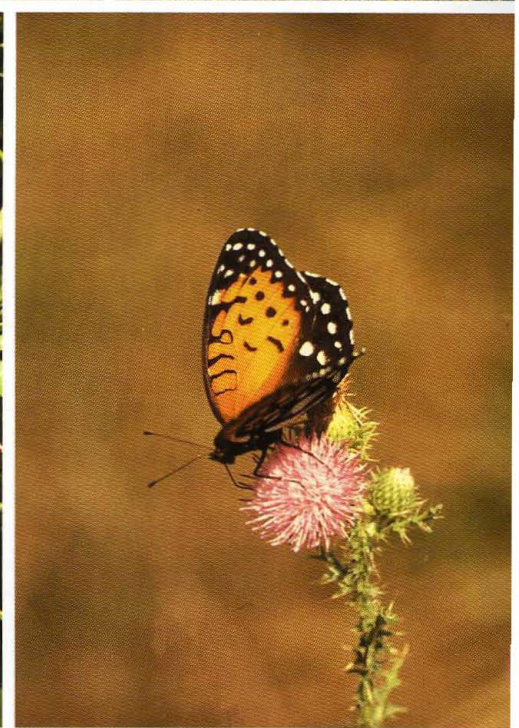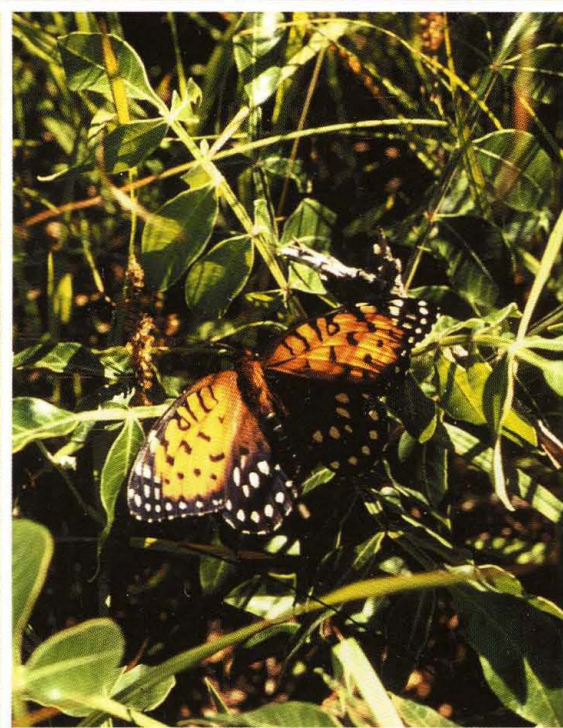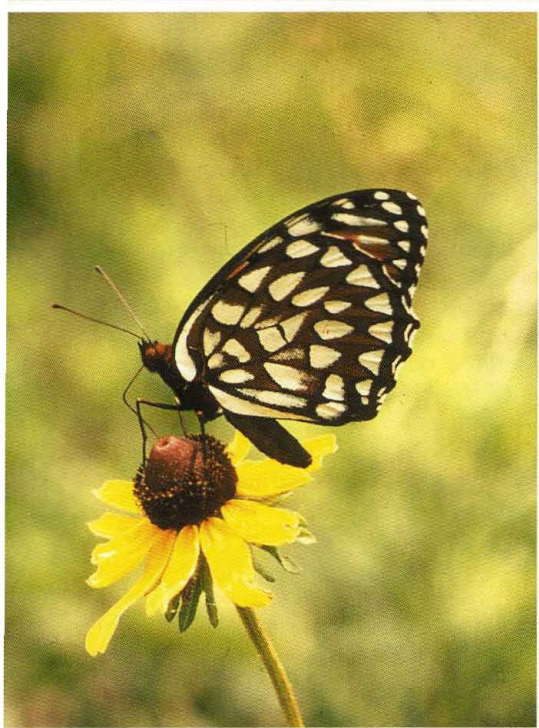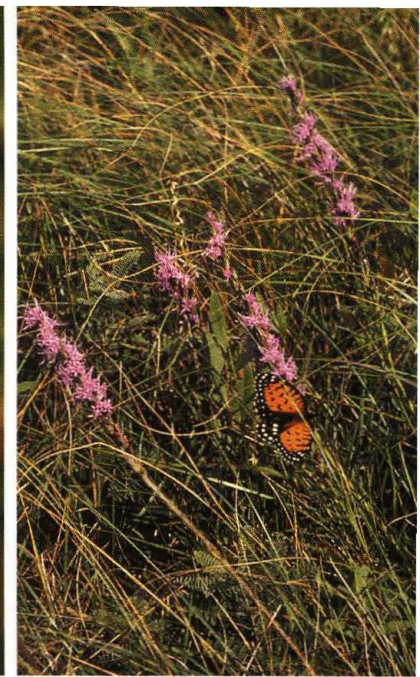
Table 1. Summary statistics on surveys held during June-September, by subregion (Fig. 1).

	Subregion 1: Wisconsin	Subregion 2: Iowa-Minnesota	Subregion 3: Minnesota-N. Dakota	Subregion 4: Missouri
Years of surveys	1988-99	1988, 1990-97	1990-97	1992-99
Total survey time (hr)	313.15	114.37	135.86	171.96
Total survey distance (km)	638.85	196.93	247.31	332.72
N sites	36	12	23	45
N sites with any regal fritillaries	12	11	21	40
N male regal fritillaries	2382	1308	978	14952
N female regal fritillaries	229	195	153	771
N regal fritillaries, sexed or not	2746	2275	1618	16046
Percent unsexed individuals	5%	34%	30%	2%

in rapid, horizontally zig-zagging flight over the vegetation, they also hover slowly with repeated and sometimes protracted dips down into the vegetation, which can suggest the stereotypical appearance of female butterflies searching for oviposition sites.

Phenological Adjustments

Before analysis, we adjusted the survey dates among years to similar seasonal timing by calculating phenological dates that moved each year's survey dates ahead or behind based on our informal assessment of plant development. The summer survey dates in the northern subregions (1-3) were adjusted as follows: 1990 -4; 1991 +14; 1992 -14; 1993 -14 (June-July), -7 (August-September); 1994 0; 1995 -9; 1996 -14; 1997 0; 1998 +18, 1999 +11. The survey dates in subregion 4 were adjusted only in 1993 (-4).

A more formal method to standardize phenology among years based on weather data would be desirable, but was beyond the scope of this study. This would require not only the compilation of a tremendous amount of data from numerous weather stations, but also extensive analysis to determine which weather measures related to the species' flight period, how (linear, threshold, etc.), and whether this varied by subregion.

The method of phenological dating used here, while informal, achieved the goal of improving the superimposition of flight periods from different years, compared to the marked dissociation of flight onset and peak based on calendar date – for example, in subregion 1 (Fig. 2). During 1990-99, we resurveyed more frequently each year there than in the other subregions, so that peak date in our surveys most closely approximates actual peak in that subregion (during 1992-99, regal fritillaries were observed both before and after peak date). The average peak dates (as calculated for flight period graphs, described below) were quite similar between calendar date (20 July) and phenological date (19 July), but the range of these dates was smaller for phenological date (6 July to 6 August, a span of 31 days) than calendar date (1 July to 8 August, 38 days). Thus, the phenological adjustment did not shift the long-term average of flight timing, but did more closely superimpose the different years' flight periods than calendar date did.

Flight Period Graphs

For each subregion, we constructed a graph of the relative density (by gender and for all individuals) and sex ratio of regal fritillaries during the flight p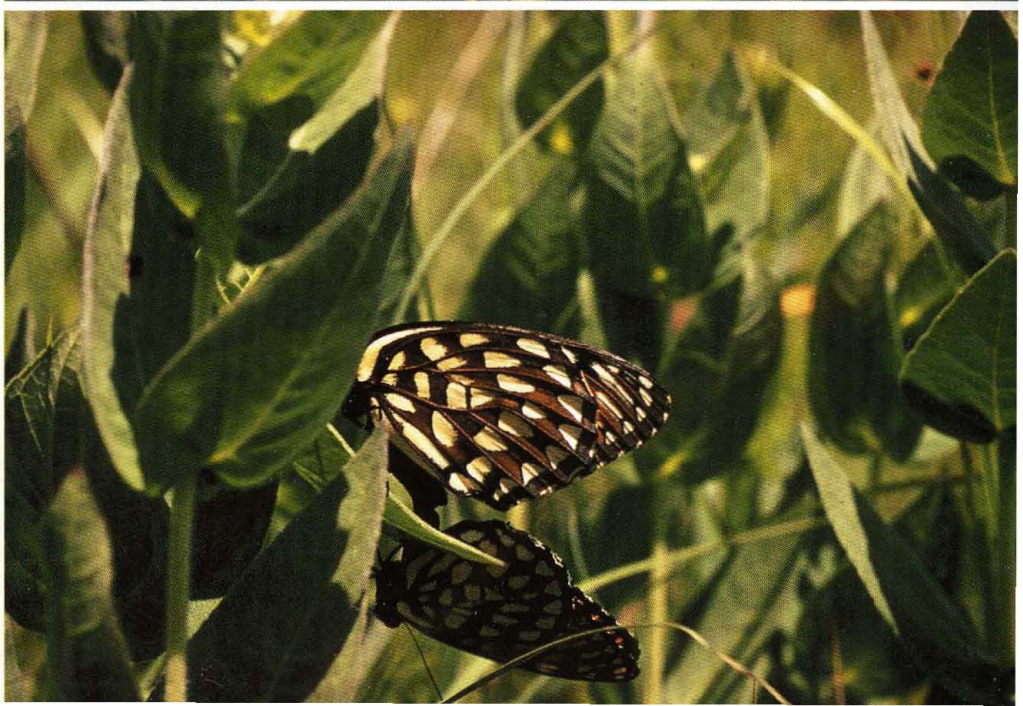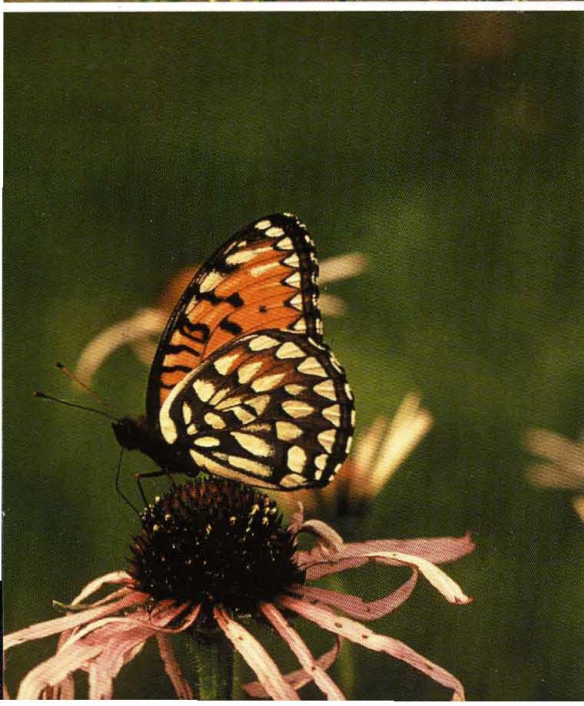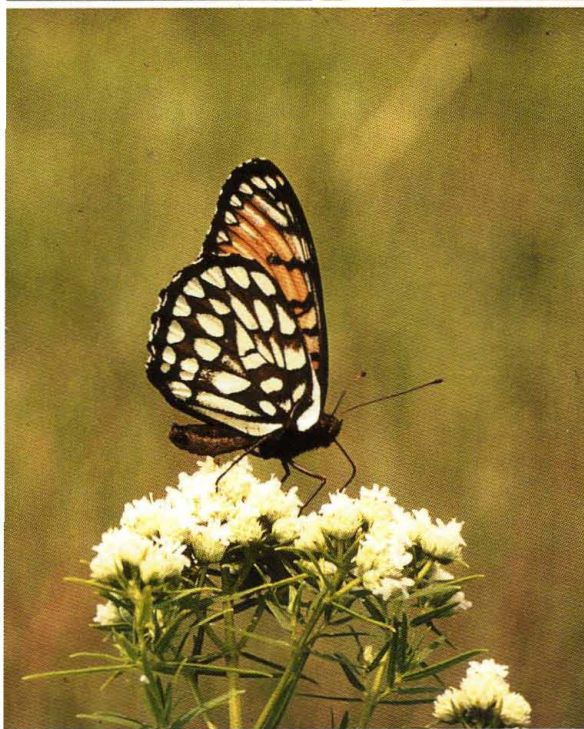eriod, pooled for all years. For each phenological date, the number of male, female, and total regal fritillaries observed and hours of formal survey effort were summed at sites where we had ever recorded regal fritillaries during the study. For each phenological date, we calculated the percent males of sexed individuals (sex ratio) if >2 individuals were sexed, and individuals per hour (relative density) of all, male, and female individuals if ≥0.5 survey hours occurred that day or if any regal fritillaries (sexed or not) were observed. We then calculated running means for the sex ratio and relative densities by averaging the value for that date, the nearest previous date, and nearest subsequent date. For the first and last dates, that date was averaged with the next later or earlier date. For subregion 4, the values from the one July survey date were not included in the running means (i.e., the values for that July date were graphed unaveraged), and the values from this July date were not connected by line to the other values, all from mid-June. This served to maintain the distinction between these two survey periods, as the results on the July date differed strikingly from the results in mid-June. In the other subregions, the line connecting sex ratio values was disconnected to indicate a date with a value missing for sex ratio but not densities.

Even with the pooling of observations from all sites and years on a given phenological date, and the use of three-date running means, these plots were still rather erratic. Besides the imprecision of the phenological adjustment, annual fluctuations in abundance and differences in population size among sites surveyed on different dates likely contributed to this. Increasing the number of dates in the running means would likely smooth the plots more, but would reduce the resolution by date and also assume such plots should be smoother than we actually observed.

Data Analysis

The Spearman rank correlation was used to test for co-occurrence of total density with male and female densities, by subregion. Densities were calculated as all (sexed or not), female, and male individuals observed per hour in each unit survey. The Spearman rank correlation was also used to test for correlation of sex ratio (percent males of sexed individuals) and percent unsexed (of all individuals) in each unit survey with timing (year, calendar and phenological dates) and density (all, male, and female density), by subregion. Unit surveys were analyzed only if during the regal fritillary flight period in that subregion that year at sites where the species was ever recorded during this study. Furthermore, >1 sexed
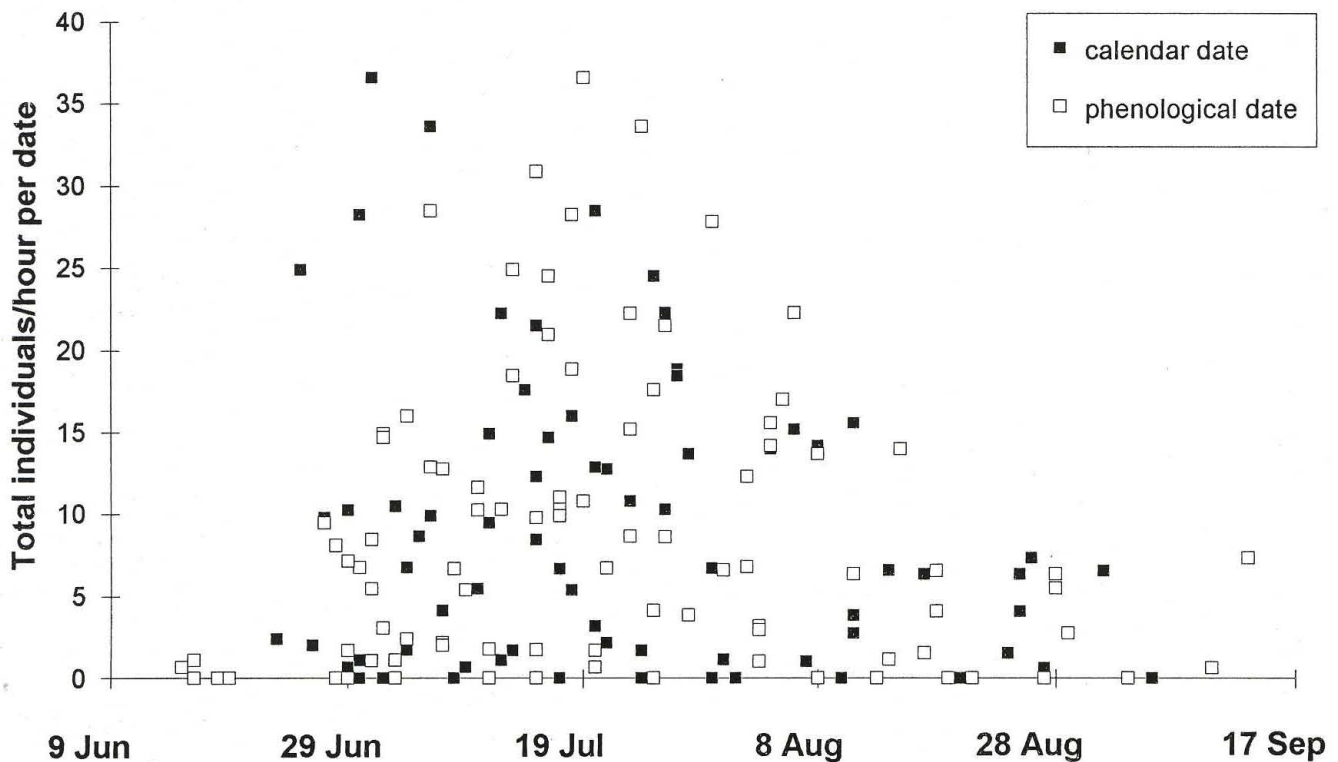
Fig. 2. Relative density (individuals per hour) of all regal fritillaries (sexed or not) on surveys at sites ever recording the species in subregion 1 (Wisconsin), by both calendar and phenological date. N=91 dates during 1990-99 with ≥0.5 survey hours or any regal fritillaries (sexed or not) observed.

individual was required for unit surveys in analyses of sex ratio, and >1 individual (sexed or not) for analyses of percent unsexed individuals. Analyses were performed at the scale of the unit (subsite), rather than by site, to allow for finer spatial resolution of density variables, which could vary greatly among units within the same site based on habitat characteristics (Swengel, 1997).

To control for date within flight period, the correlations of sex ratio and percent unsexed individuals were redone with samples limited to the broadest possible time period – termed "prime flight" – when male, female, and total densities were positive but did not correlate significantly (i.e., vary linearly) by phenological date. This was intended to approximate a broad period of peak flight. Prior to analysis, densities and sex ratio were expected to vary greatly by date within flight period, but not percent unsexed individuals.

Analysis was done with ABstat 7.20 software (1994, Anderson-Bell Corp., Parker, Colorado), with statistical significance set at $P < 0.05$. Since significant results occurred overall much more frequently than expected spuriously due to Type I statistical error, we did not lower the P value further, as many more Type II errors (biologically meaningful but statistically non-significant results) would then be created than Type I errors eliminated.

RESULTS

We recorded 22,685 regal fritillaries at 84 of 116 study sites (Table 1). Of 20,968 sexed individuals, 92% were males. Females did not appear to occur in concentrations away from areas occupied by males. Females that were flushed out of the vegetation, feeding, mating, and ovipositing occurred widely throughout the areas where males occurred.

In subregion 1, the frequencies of survey dates around first male and female appearances were adequate in four years (1992, 1995, 1997, 1999) to calculate fairly precise intervals (15, 3, 14, and 19 days, respectively) between first observed male and female (Fig. 3) – on the scale of the subregion, not site. In 1993, 1994, 1996, and 1998, the frequencies of survey dates were sufficient only to infer intervals > 22, 19, 11, and 5 days respectively, but likely less than the 43, 28, and 29 days that we actually recorded in 1993, 1994, and 1996. Our observations are insufficient even for rough inferences in 1990 and 1991.

Total Male and Female Densities

Male and total densities covaried very strongly (Table 2) and corresponded closely in flight period graphs (Fig. 4). In the three northern subregions (1-3), total and male densities roughly showed a single peak period with a more rapid rise prior than decline afterward. In the southern subregion (4), where we only surveyed early in the flight period, a strong drop in total and male (but not female) densities occurred between the many mid-June surveys and 12 July 1994, which had favorable weather (32-33° C, 80-100% sunshine, 15-40% clouds, wind 8-16 km/hr).

Female density increased gradually and linearly during most of the flight period, and peaked lower and later than male and total densities (Fig. 4). Thus, correlations between male and female densities were weaker, although mostly positive and often significant (Table 2). While never as strongly as male density did, female density always covaried significantly with total density (Table 2). The patterns of these correlations were similar when restricted to prime flight (Table 2).

Plate 3. Ventral views of male regal fritillaries in left column; regal fritillary mating pairs (male on left or bottom) in right column, in Minnesota (MN) and Missouri (MO). Left column, top: Treaty Line Prairie (Barton Co., MO), 15 Jun 1993; middle: Osage Prairie (Vernon Co., MO), 20 Jun 1994; bottom: Pennsylvania Prairie (Dade Co., MO), 19 Jun 1996. Right column, top: Hole-in-the-Mountain (Lincoln Co., MN), 16 Jul 1990; middle: Monegaw Prairie (Cedar Co., MO), 18 Jun 1992; bottom: Osage Prairie (Vernon Co., MO), 16 Jun 1997.
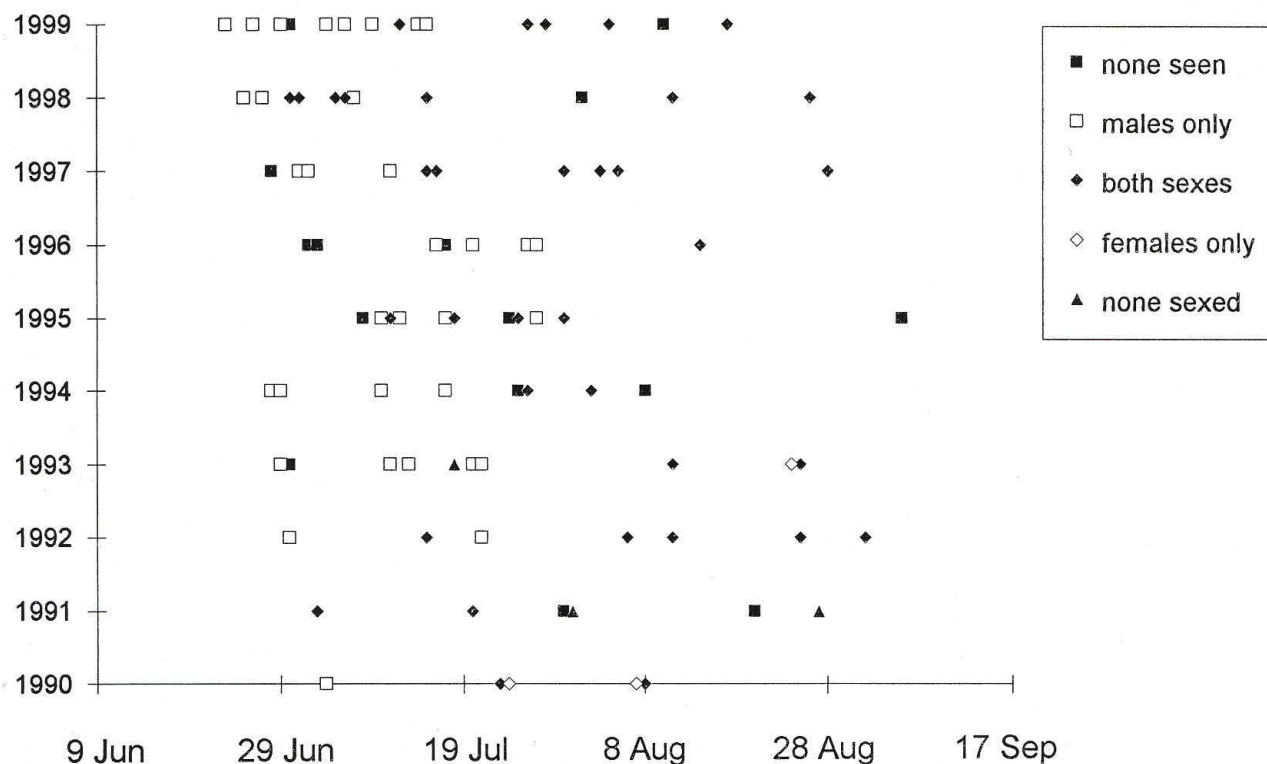
Fig. 3. Results of surveys at sites every recording regal fritillary in subregion 1 (Wisconsin), by calendar date during 1990-99: no individuals, males only, both sexes, females only, or no sexed individuals recorded. In 1995, we recorded 2-3 individuals (all males) in an informal visit to a study site two days before the first individuals were recorded on a formal visit.

Percent Unsexed Individuals

Unsexed individuals were a negligible proportion of the total observed in subregions 1 and 4 but a considerable minority in subregions 2-3 (Table 1). Percent unsexed individuals consistently correlated strongly and negatively with year (Table 3). Significant correlations with date variables were fewer but usually positive. Three negatively significant correlations occurred with male density (subregions 2-3, entire study region), two positively significant correlations with total density (subregions 1-2), and none with female density.

Observed Sex Ratio

The running means of sex ratio (percent males of sexed individuals) were >50% males except late in the flight in subregion 2 (Fig. 4). In calculations for these means, only two August dates in each of the northern subregions (1-3) and the last survey date (12 July) in the southern subregion (4) had sex ratios of 50% or fewer males, ranging as low as 12.5% males. In all subregions, prime flight had means of >75% males (Table 4). In subregion 1, where sites were resurveyed most frequently each year over the broadest span of the flight period, peak could be defined most precisely. There during 1990-99, on the date of peak total density, the sex ratio averaged 93.2% males (range 72-100%).

For the entire survey period, the strongest correlation for sex ratio in each subregion was negative with female density (Table 5). In the three northern subregions (1-3), the next strongest density pattern was positive with male density. These appear logical in that if few females, or many males, are recorded, then most observed individuals will be male. However, the correlations with female and male densities were not simply mirror images of each other, given the great disparity in strength between these coefficients in each subregion. By contrast, in subregion 4, where the greatest range of male densities but shortest span of survey dates occurred, the

correlation with male density was negative. This may relate at least in part to the lack of any unit surveys in analysis there with 100% females (i.e., 0% males and a male density of 0, but female and total densities >0). All other subregions had a few such surveys, which would increase the likelihood of positive correlation with male density because of the dichotomy between 0% males at 0 male density and >0% males (no matter how variable the sex ratio) at >0 male densities. Correlations of sex ratio with female and male densities were much weaker for the entire study region, but still significant and opposite. Significant correlations with total density were fewer but always negative – in this case, male and total densities did not have similar results (Table 5) despite their strong covariance with each other (Table 2).

Second in strength only to the correlations with female density in subregions 1-3 were the strongly negative correlations of sex ratio with date (Table 5). For the entire study region, these were the strongest correlations. Phenological date always correlated more strongly than calendar date, as would be expected if phenological dating made surveys more comparable in seasonal timing among years. Results were erratic for year: non-significant in two subregions, significantly positive in another subregion and the entire study region, significantly negative in the final subregion.

Density-dependent patterns were similar during prime flight (Table 6). The strongest pattern remained the negative correlation with female density, with a notable increase in strength for the entire study region. Nearly all correlations with male density were significant and positive, while the one significant correlation with total density remained negative.

Even though by definition prime flight had total, male, and female densities that were positive but not significantly changing by date, sex ratio still correlated significantly and negatively with date in several subregions and the entire study region (Table 6). A minor (non-significant) decrease in male density and especially increase in

Table 2. Spearman rank coefficients and significance (* P < 0.05, ** P < 0.01) of pairwise correlations of total, male, and female densities (individuals/hr, per unit survey), if any individuals (sexed or not) were observed, by subregion. N = 458, 203, 169, and 568 unit surveys for all surveys for each subregion, respectively; N = 173, 110, 71, and 407 unit surveys for each subregion, respectively, in prime flight (defined in Table 6).

	Subregion 1: Wisconsin		Subregion 2: Iowa-Minnesota		Subregion 3: Minnesota-North Dakota		Subregion 4: Missouri		All subregions	
Densities on all surveys										
Total and male	+0.895	**	+0.782	**	+0.780	**	+0.991	**	+0.933	**
Total and female	+0.108	*	+0.363	**	+0.464	**	+0.468	**	+0.375	**
Male and female	-0.150	**	+0.109		+0.088		+0.391	**	+0.210	**
Densities in prime flight										
Total and male	+0.885	**	+0.860	**	+0.855	**	+0.991	**	+0.963	**
Total and female	+0.155	*	+0.352	**	+0.426	**	+0.508	**	+0.416	**
Male and female	-0.156	*	+0.080		+0.055		+0.436	**	+0.269	**

Table 3. Spearman rank coefficients and significance (* P < 0.05, ** P < 0.01) of correlations of percent unsexed individuals with timing factors and density (total, male, female individuals/hr, per unit survey), by subregion. N = 295, 168, 121, and 514 unit surveys with >1 individual (sexed or not) for each subregion, respectively.

	Subregion 1: Wisconsin		Subregion 2: Iowa-Minnesota		Subregion 3: Minnesota-North Dakota		Subregion 4: Missouri		All subregions	
Timing										
Year	-0.327	**	-0.661	**	-0.720	**	-0.264	**	-0.518	**
Date, phenological	-0.045		+0.007		+0.252	**	-0.010		+0.216	**
Date, calendar	+0.001		-0.052		-0.243	**	-0.016		+0.199	**
Density										
Total	+0.153	**	+0.145	*	+0.090		+0.031		-0.034	
Male	-0.027		-0.279	**	-0.279	**	+0.027		-0.220	**
Female	+0.067		-0.038		-0.060		+0.083		+0.052	

Table 4. Mean sex ratio (percent males of sexed individuals), for all survey dates and during prime flight (defined in Table 6), by date and by unit survey, if >2 individuals sexed on that date or unit survey.

		By date				By unit survey			
		All dates		Prime flight		All unit surveys		Prime flight	
		N	sex ratio	N	sex ratio	N	sex ratio	N	sex ratio
1:	Wisconsin	39	87.9	14	85.6	217	90.2	85	89.8
2:	Iowa-Minnesota	18	83.3	8	83.7	119	83.5	80	79.4
3:	Minnesota-North Dakota	26	85.7	9	78.9	88	81.5	43	76.1
4:	Missouri	16	91.8	7	94.1	468	93.6	343	93.0

Table 5. Spearman rank coefficients and significance (* P < 0.05, ** P < 0.01) of correlations of sex ratio (percent males of sexed individuals) with timing factors and density (total, male, female individuals/hr, per unit survey), by subregion. N = 289, 150, 107, and 511 unit surveys with >1 sexed individual for each subregion, respectively.

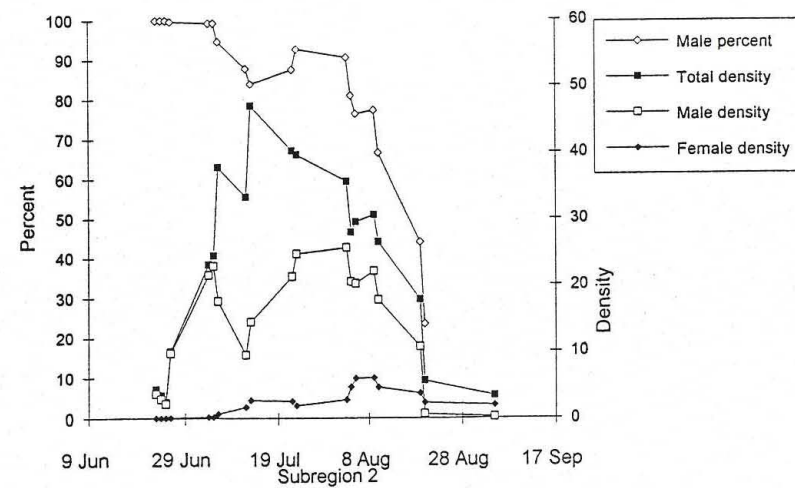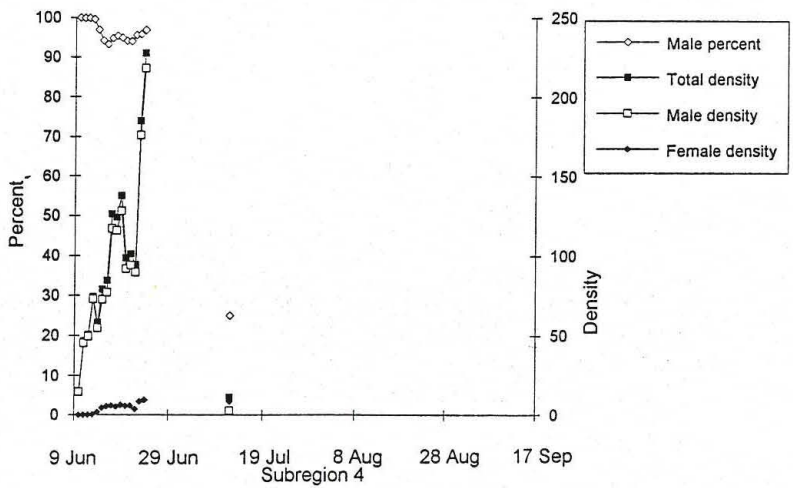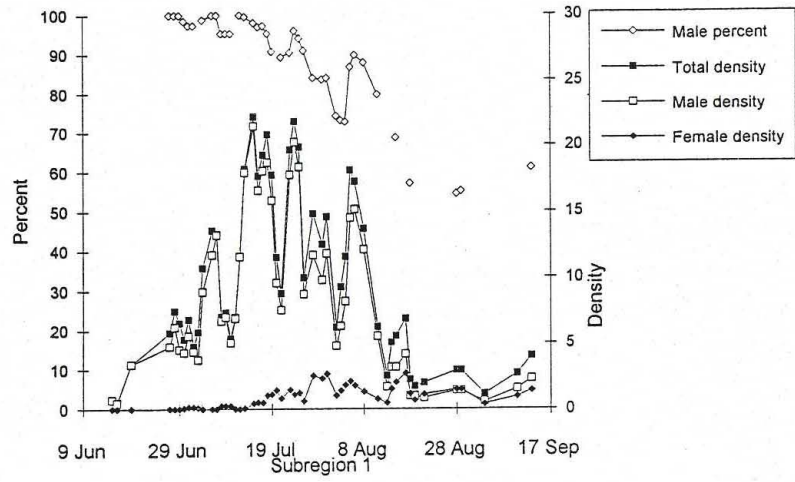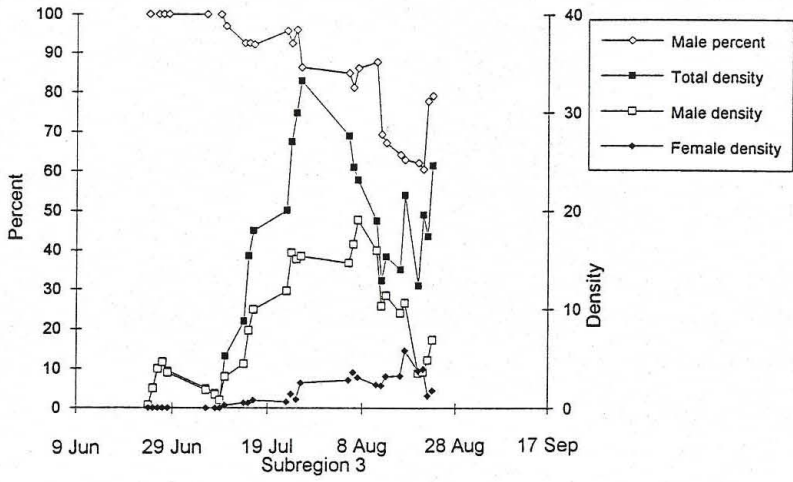|	Subregion 1: Wisconsin		Subregion 2: Iowa-Minnesota		Subregion 3: Minnesota-North Dakota		Subregion 4: Missouri		All subregions	
Timing										
Year	+0.081		+0.251	**	-0.008		-0.104	*	+0.096	**
Date, phenological	-0.496	**	-0.551	**	-0.510	**	-0.213	**	-0.250	**
Date, calendar	-0.460	**	-0.479	**	-0.324	**	-0.149	**	-0.220	**
Density										
Total	+0.104		-0.090		-0.235	*	-0.227	**	-0.098	**
Male	+0.331	**	+0.262	**	+0.262	**	-0.137	**	+0.084	**
Female	-0.967	**	-0.943	**	-0.938	**	-0.929	**	-0.098	**

Table 6. Spearman rank correlation coefficients and significance (* $P < 0.05$, ** $P < 0.01$) of correlations of sex ratio (percent males of sexed individuals) with timing factors and density (total, male, female individuals/hr, per unit survey), by subregion in prime flight (when male, female, and total density were positive but did not correlate significantly by phenological date). N = 110, 95, 53, and 368 unit surveys with >1 sexed individual for each subregion, respectively.

	Subregion 1: Wisconsin		Subregion 2: Iowa-Minnesota		Subregion 3: Minnesota-North Dakota		Subregion 4: Missouri		All subregions	
Span of dates	20.Jul-8.Aug		13.Jul-10.Aug		26.Jul-17.Aug		15.Jun-22.Jun			
Span of years	1990, 1992-99		1990-93		1991-94		1992, 1994-99			
Timing										
Date, phenological	-0.222	*	-0.143		-0.250		-0.011		-0.187	**
Date, calendar	-0.356	**	-0.264	**	+0.237		-0.011		-0.197	**
Year	+0.385	**	-0.083		-0.334	*	+0.005		+0.177	**
Density										
Total	+0.095		+0.013		+0.104		-0.187	**	-0.026	
Male	+0.341	**	+0.320	**	+0.490	**	-0.101		+0.125	**
Female	-0.925	**	-0.908	**	-0.847	**	-0.888	**	-0.864	**

female density resulted in relatively greater and sometimes significant decreases in sex ratio (Fig. 4). But in contrast to the analysis of the entire period sampled (Table 5), phenological date had weaker correlations than calendar date in this analysis during prime flight (Table 6), which would be expected if phenological dating succeeded in making surveys more comparable in seasonal timing among years. Nonetheless, correlations of sex ratio with male density and especially female density were typically stronger than with date. In prime flight, patterns of correlation with year continued to be erratic.

Combining all subregions, the graph of sex ratio against female density during prime flight showed a wide scatter (Fig. 5a). At the lowest females densities, the range of sex ratios was narrow and very strongly male biased, a pattern reinforced by the 283 unit surveys (45% of the 626 analyzed) where the sex ratio was 100% males and consequently female density was 0. For the remainder of observed female densities, the range of sex ratios broadened considerably but still included highly male-biased sex ratios, although necessarily <100% males. Within subregion, the more female-biased sex ratios tended to occur at higher female densities, more so in subregions 1-3 than subregion 4, which had the highest female densities yet the narrowest range of sex ratios with the most male bias.

Within each subregion and for the entire study region, the graph of sex ratio against male density in prime flight showed a consistent pattern of widely varying sex ratios at low male densities but strong male bias at the highest male densities (Fig. 5b). A sex ratio of 0% males (and consequently a male density of 0) occurred on only 12 unit surveys in subregions 1-3. Subregion 4 had the highest male and female densities (Fig. 5a-b), but the difference in peak density between subregion 4 and subregions 1-3 was much greater for males than females. In the northern subregions, the highest observed male density on a unit survey exceeded that for females by about a two-to fivefold difference, but by 17-fold in subregion 4.

The graph of sex ratio against total density was similar to that against male density, but with somewhat more variable sex ratios over more of the range of total densities (Fig. 5c). One discrepancy

in sign (slope) occurred in one subregion (4) between a significant correlation with total density (Table 6) and a corresponding regression line in the graphs (Fig. 5c). This was apparently due to differences in weighting between these two kinds of statistics. The relatively few values at the highest densities, which occurred only in subregion 4, were weighted more strongly in the linear regression lines than in the nonparametric correlations.

DISCUSSION

In the years with adequate data (1992-99) in the one analyzable subregion (1), males were always found earlier in the flight period than females (Fig. 3). In 1993, we recorded a span of only three days between first observed male and female, likely an underestimate of the true interval, since that year we surveyed low-density populations through 10 July and then high-density populations on 11 July. The other spans ranged from >5 days to >22 days, or about 1-3 weeks. This is consistent with the literature on this genus (see Introduction; McCorkle and Hammond, 1988; and Nagel *et al.*, 1991) and the protandry observed for many butterfly species (Opler and Krizek, 1984; Scott, 1986; Brakefield and Shreeve, 1992; Carvalho *et al.*, 1998).

Total, Male, and Female Densities

The close correspondence of total and male densities (Table 2, Fig. 4) is a logical consequence of total observations skewing strongly to males (Table 1). In the three northern subregions (1-3), total and male densities roughly showed a single peak period in mid-summer, with no apparent aestivation (i.e., midsummer inactivity and lower detectability). The flight graph in Iftner *et al.* (1992) is similar. In the southern subregion (4), where we only surveyed early in the flight period, total and male densities dropped strongly between mid-June and mid-July. This is consistent with Nagel *et al.*'s (1991) observation of aestivation by regal fritillaries in Nebraska. In this same subregion, female density was highest in July, which suggests that males but not females become relatively less findable at this time. In all subregions, peak female density was

Fig. 4. Three-date running means of relative density (individuals per hour, by gender and for all individuals) and sex ratio (percent males of sexed individuals) for regal fritillaries in each subregion (mapped in Fig. 1), by phenological date during the flight period, pooled for all years. In subregions 1 and 3, the line connecting sex ratio values was disconnected to indicate a date with a value missing for sex ratio but not densities. In subregion 4, the values from the one July survey date were not included in running means.
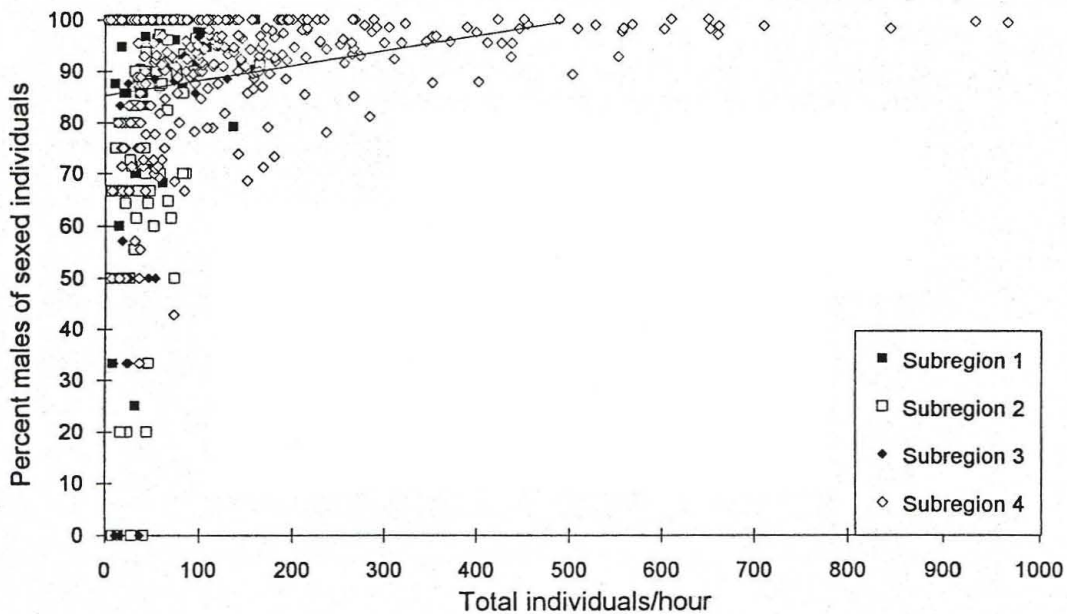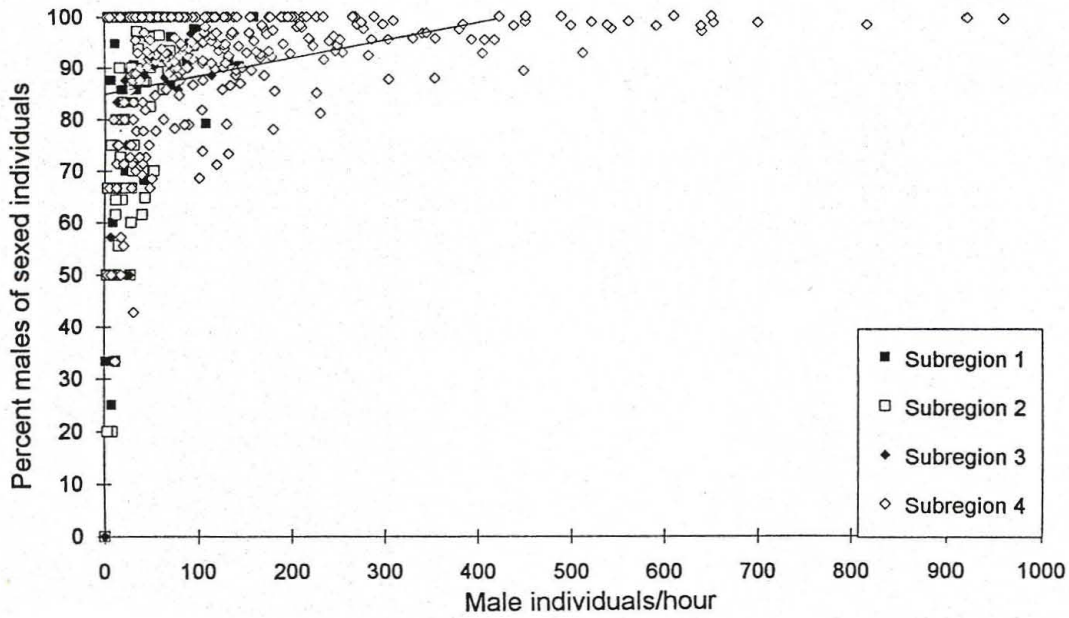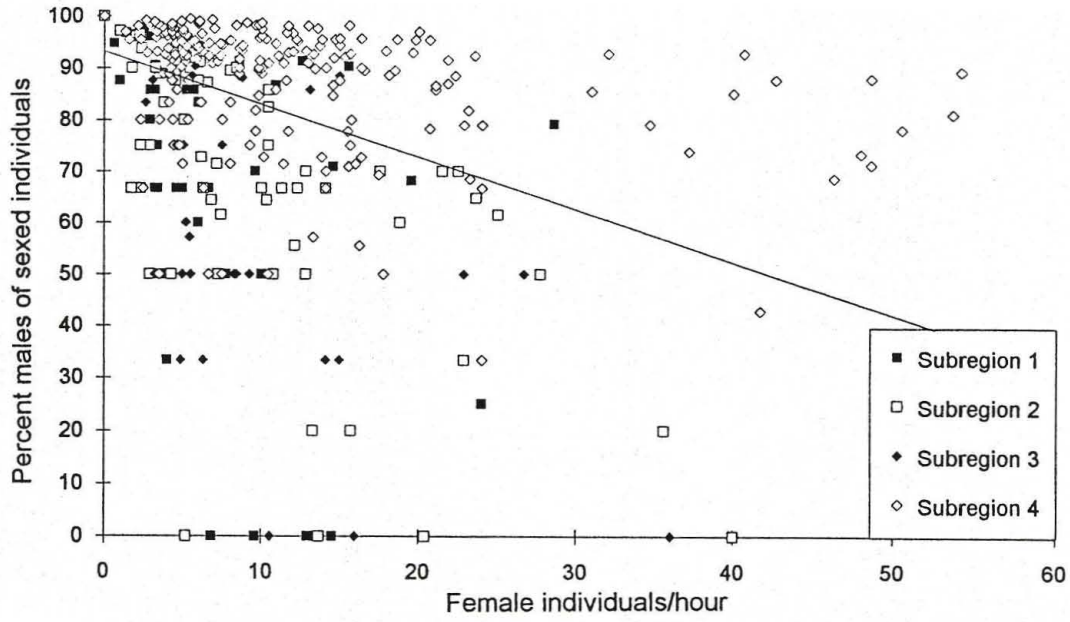
consistently lower and later than peak male density (Fig. 4), as in others' observations of the same species (Nagel *et al.*, 1991), a congener (Launer *et al.*, 1992), and a blue (Lycaenidae) in England (Pollard and Yates, 1993: 114-115). In contrast to the sharp peak in male density of regal fritillaries, female density generally increased gradually and linearly, as also reported by Nagel *et al.* (1991). Nonetheless, correlations between male and female densities, although weaker, were still usually positive and often significant (Table 2). The rates of increase and levels of peak density are somewhat displaced between the sexes but of course cannot be entirely dissociated.

Percent Unsexed Individuals

Although unsexed individuals were a negligible proportion of total individuals in several subregions (Table 1), percent unsexed individuals correlated strongly and negatively in all subregions with year (Table 3). Our increasing ability to sex a higher proportion of individuals in later years of this study may result simply from increased experience, but is also likely due to our greater reliance in more recent years on the front wing apex for sexing, rather than dorsal hind wing marginal spot-row (see Methods).

Significant relationships were relatively few and somewhat inconsistent between percent unsexed individuals and date (Table 3). This implies that the influence of seasonal timing on sexing individuals was variable and/or minor – in contrast to the strong and consistent effect of seasonal timing on observed sex ratio (Table 5). It did not appear that sexing was consistently easier earlier in the flight period when individuals would likely be less worn – especially males, which seemed to experience more wear.

Proportions of unsexed individuals might be expected to increase at higher densities, which is consistent with the significant positive correlation of percent unsexed individuals with total density in subregions 1-2 (Table 3). However, no density-related correlations were significant in subregion 4, where the highest (and widest range of) total, male, and female densities occurred (Fig. 5). Since we began research last in this subregion, we were more experienced in sexing regal fritillaries at the start of research there than in the other subregions and had the lowest proportion of unsexed individuals there (Table 1). Despite the very high densities observed in subregion 4, the coefficient with year was weakest there, suggesting relatively little change in our ability to sex individuals during the study years there. This implies that experienced observers can be effective at sexing regal fritillaries at high-density sites. The coefficient with year was also relatively low in subregion 1, where the lowest densities were observed (Fig. 4). This suggests the value of mastering field sexing in low-density sites. Percent unsexed individuals correlated significantly and negatively with male density in two northern subregions (2-3) and the entire study region, but never with female density (Table 3). Regardless of the underlying mechanisms for these negative correlations, density-dependent relationships were relatively fewer and weaker for percent unsexed individuals than for sex ratio (Tables 5-6), and the strongest, most consistent influence on proportion of unsexed individuals in this study was year.

Observed Sex Ratio

The observed sex ratio (percent males of sexed individuals) during most of the flight period was strongly male-biased (Table 4, Fig. 4), as in Nagel *et al.* (1991) and Kelly and Debinski (1998),

although the latter reported proportionately more females late in the flight than we observed. Similar patterns have been observed in many other butterfly species, often attributed to greater frequencies of more active or overt behaviors, and therefore greater detectability, by males (Rutowski, 1984, 1991; Gall, 1985; Clayton and Petr, 1992; Garroway *et al.*, 1993; Pollard and Yates, 1993: 114-115; Vojnits and Ács, 1995; Swengel and Swengel, 1996; Scriber *et al.*, 1998). Even in hotter parts of the range, where the regal fritillary's flight period is protracted, males are observed toward the end (Nagel *et al.*, 1991).

During the entire flight period surveyed, negative correlations of sex ratio with date were significant in all tests (Table 5), consistent with observations by Nagel *et al.* (1991) and Kelly and Debinski (1998) and as expected due to protandry (see earlier discussion). Sex ratio showed relatively weaker and inconsistent relationships with year, indicating that this factor was variable and/or minor in effect — in contrast to its strong consistent influence on percent unsexed individuals (Table 3).

Throughout the survey period in all subregions, the strongest correlations for sex ratio were negative with female density (Table 5). Given the mid-flight peak in male density but linear increase in female density (Fig. 4), highest *observed* female density would occur when male density was past peak, so that males would also be proportionately lower in the sex ratio. In the three northern subregions (1-3), the next strongest density-dependent relationship was positive with male density (Table 5) — the converse of the relationship with female density. However, these correlations with male density were much weaker, perhaps because the time when 100% males occurred (at the start of flight period) was not when highest male densities occurred (peak flight) — at peak male densities, females were also observed. The Karner blue (*Lycaeides melissa samuelis* Nabokov) (Lycaenidae) showed the same pattern (same signs and relative strengths of significant correlations) of sex ratio with male and female densities (Swengel and Swengel, 1998).

These density-dependent correlations might seem to mirror the relationships with seasonality, except that the coefficients were much higher with female density than date (Table 5). Furthermore, the strong correlations with female density persisted even in prime flight, when total, male, and female densities were statistically "constant" relative to date (Table 6).

This density dependence during prime flight suggests a behavioral component to observed sex ratio. Female butterflies can become more cryptic or evasive at higher male densities, presumably to avoid increased harassment by courting males (Grossmueller and Lederhouse, 1987; Odendaal *et al.*, 1989; Clayton and Petr, 1992). A "ceiling" on female density, even as male density continues to increase, is apparent by comparing Fig. 5a-b. Subregion 4 had the highest male and female densities, but the highest male densities exceeded those in the other subregions by far more than the highest female densities did. It appears likely that the highest-density populations in subregion 4 had relatively more females than observed, compared to lower-density populations there. This is likely the case in all subregions, as the observed range of densities was much greater for males than females in all subregions. Thus, female behavior appears much more density-dependent than male behavior — females adjusted their behavior (such that detectability is affected) based on male densities but not vice versa. In addition, more females may *actually be present* in a site earlier in the flight period than when the highest *observed* female densities occur.

Fig. 5. Sex ratio (percent males of sexed individuals) during prime flight (defined in Table 6) by densities (individuals/hour, per unit survey) of (a) females, (b) males, and (c) all individuals (sexed or not), in each subregion (mapped in Fig. 1). The least-squares regression lines are significant ($P < 0.00005$ for each), with $r = -0.468$ for female density, $r = +0.224$ for male density, and $r = +0.189$ for total density. For each graph, N = 110, 95, 53, and 368 unit surveys with >1 sexed individual for each subregion, respectively.

In contrast to the northern subregions, the correlation of sex ratio with male density in subregion 4 was negative (but also weaker) during the entire survey period (Table 5) and non-significant in prime flight (Table 6). Nonetheless, results from this subregion, where the highest (and widest range of) male densities occurred, graphed consistently with those from the other subregions (Fig. 5b). The non-significant correlation between sex ratio and male density in subregion 4 during prime flight may relate to the very high male bias in the sex ratio; the number of observed females might be too low to generate adequate statistical power for significance.

By contrast, sex ratio related significantly to total density only once (in subregion 4) in prime flight (Table 6), and negatively as in the entire survey period (Table 5). Combining all subregions, sex ratio varied greatly at most densities, except for a strong male bias at the very highest densities, which occurred only in subregion 4 after the female flight period had begun (Fig. 5c). These weaker patterns further suggest that density-dependent behavior is strongly gender dependent. Females did not markedly adjust their detectability (i.e., behavior) in response to total density, which at many given values include a wide range of proportions of males and females. Rather, females adjusted their behavior (i.e., their detectability) to male density.

ACKNOWLEDGEMENTS

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