



MULTI-YEAR SEASONAL SEX-ALLOCATION PATTERNS IN RED-WINGED BLACKBIRDS (*AGELAIUS PHOENICEUS*)

PATRICK J. WEATHERHEAD¹

Program in Ecology, Evolution and Conservation Biology, University of Illinois, Champaign, Illinois 61820, USA

ABSTRACT.—A previous study reported that climate-mediated increases in the length of the breeding season produced increasingly female-biased fledging sex ratios in Red-winged Blackbirds (*Agelaius phoeniceus*). Using those same data plus one additional year (11 years in total), I found that this phenomenon was not a result of greater production of females early and late in the season, contrary to what had been proposed. Instead, seasonal sex-allocation patterns interacted with season length. Early and midseason sex ratios became more female-biased as breeding seasons became longer, whereas late-season sex ratios tended to vary in the opposite manner, albeit weakly. Thus, the discrepancy between sex ratios late in the season and those earlier (early plus midseason) was a strong function of season length. Because fledging sex ratios did not vary with nestling mortality, these patterns appear to be a consequence of nonrandom sex-allocation rather than sex-biased survival. It is unclear whether these sex-allocation patterns are adaptive. Because the climatic factor (the North Atlantic Oscillation) associated with longer breeding seasons is also associated with higher winter mortality, however, it is possible that female Red-winged Blackbirds change how they allocate sex in response to changes in the breeding sex ratio. If climate change continues to alter these patterns, documenting how individuals and populations respond will be informative, from both basic and applied perspectives. *Received 5 February 2008, accepted 1 October 2008.*

Key words: *Agelaius phoeniceus*, breeding season, climate change, North Atlantic Oscillation, sex-allocation, sex ratio.

Patrons pluriannuels de l'allocation saisonnière des sexes chez *Agelaius phoeniceus*

RÉSUMÉ.—Une étude a rapporté que l'allongement de la saison de reproduction causé par les changements climatiques a amplifié le biais en faveur des femelles dans le rapport des sexes des jeunes à l'envol chez *Agelaius phoeniceus*. En utilisant ces données en plus de celles d'une année supplémentaire (11 ans au total), j'ai remarqué que ce phénomène n'était pas le résultat d'une plus grande production de femelles tôt et tard dans la saison, contrairement à ce qui avait été proposé. Au contraire, les patrons saisonniers d'allocation des sexes ont interagit avec la longueur de la saison. Les rapports des sexes hâtifs et de mi-saison sont devenus plus biaisés en faveur des femelles à mesure que s'est allongée la saison de reproduction, alors que les rapports des sexes en fin de saison avaient tendance à varier de façon inverse, quoique faiblement. En conséquent, la divergence entre les rapports des sexes en fin de saison et ceux plus tôt (hâtifs et en mi-saison) était fortement liée à la longueur de la saison. Puisque les rapports des sexes des jeunes à l'envol n'ont pas varié en fonction de la mortalité des jeunes au nid, ces patrons semblent être une conséquence d'une allocation des sexes non aléatoire plutôt qu'une survie biaisée selon le sexe. Il n'est pas certain si ces patrons d'allocation des sexes sont adaptatifs. Puisque le facteur climatique (l'Oscillation de l'Atlantique Nord) relié à des saisons de reproduction plus longues est également associé à un taux de mortalité hivernale plus élevé, il est possible que les femelles d'*A. phoeniceus* changent leur façon d'attribuer les sexes en réponse aux changements du rapport des sexes des individus reproducteurs. Si les changements climatiques continuent d'altérer ces patrons, le fait de documenter la manière dont les individus et les populations réagissent sera informatif, selon les perspectives fondamentales et appliquées.

MALE AND FEMALE offspring can differ in how much they contribute to parental fitness, which can create the potential for adaptive, nonrandom sex-allocation (Fisher 1930, Trivers and Willard 1973). There is now considerable evidence that birds vary offspring sex ratios in response to a variety of factors (Hasselquist and Kempenaers 2002, West and Sheldon 2002, Cassey et al. 2006), one

of which is the timing of egg laying during the breeding season. One way in which the time that an egg is laid can interact with offspring sex to affect fitness is through its effect on when the offspring will reach independence. If timing of independence affects an individual's prospects for recruiting into the breeding population and that effect differs between the sexes, mothers should

¹E-mail: pweather@uiuc.edu

allocate offspring sex over the season in a way that increases the recruitment prospects of their offspring. There is substantial evidence in support of this prediction (e.g., Dijkstra et al. 1990, Smallwood and Smallwood 1998, Cordero et al. 2001, Griggio et al. 2002, Husby et al. 2006). A second way in which offspring sex and laying time can interact to affect fitness, and the one relevant to the present study, involves differences in the costs of producing sons and daughters resulting from sexual size-dimorphism. If resources vary seasonally, females may benefit from producing more offspring of the expensive sex when resources are abundant and fewer when resources are scarce (Trivers and Willard 1973, Howe 1977). Sex-ratio variation in response to food availability has been reported in several species (see review by Hasselquist and Kempenaers 2002). Here, I explore whether a previously reported case of sex ratios varying with the length of the breeding season in Red-winged Blackbird (*Agelaius phoeniceus*; hereafter "blackbird") results from an underlying pattern of seasonal sex-allocation associated with sexual size-dimorphism, as I have proposed (Weatherhead 2005a).

An analysis of breeding data for blackbirds collected in Ontario over 25 years revealed a variety of climate-mediated patterns (Weatherhead 2005a, b). In association with changes in the North Atlantic Oscillation, breeding seasons became longer over the study, and longer breeding seasons were associated with the breeding population producing a more female-biased sex ratio at fledging. I hypothesized that female blackbirds may perceive the change in the length of the breeding season as primarily a change in the duration of the early and late stages of the season (Weatherhead 2005a). In a previous study of this population, I had found that more female offspring (the less costly sex) were produced early and late in the season, when resources were assumed to be less abundant (Weatherhead 1983). Thus, if nesting females perceived the tails of the season to be getting longer, that could account for the population producing more female offspring in longer seasons (Weatherhead 2005a).

Here, I test the hypothesis that the association between length of the breeding season and offspring sex ratio is a function of females producing more daughters early and late in the season, particularly in years with longer breeding seasons, as I proposed (Weatherhead 2005a). The test consists of two parts. The first is to determine whether the seasonal sex-ratio pattern (more females early and late, more males in between) reported from data collected in 1981 (Weatherhead 1983) occurred in subsequent years that were used in the long-term analysis. Single-year studies of sex-ratio patterns may not reliably reflect patterns found in longer studies (Johnson et al. 2005). The second part of the test is to determine whether the overall female bias in years with longer breeding seasons results primarily from greater production of females early and late in the season.

METHODS

Data presented here come from a series of studies of blackbirds conducted at the Queen's University Biological Station in eastern Ontario (45°37'N, 76°13'W). For detailed methods used in those studies, see the references provided in Weatherhead (2005a). Fledgling sex-ratio data were from the same years (1985–1993 and 1995) used in the analysis that identified the association between

sex ratio and length of the breeding season (Weatherhead 2005a). Note that other years with sex-ratio data (e.g., 1981) were not included in the previous analysis or here because sex ratios were determined at the end of incubation rather than at fledging. In addition, I collected new data in 2005. Data for all years came from the same group of cattail (*Typha latifolia*) marshes. Although every site was not used every year, all sites were used in multiple years and multiple sites were used each year.

Each year, every nest at a site was found and its fate determined. This included all re-nests and second nests. Because nesting females were not individually marked in all marshes or all years, first nests cannot be distinguished from re-nests and second nests, but most nests early in the season were likely to be first nests and most late nests were likely to be re-nests (nesting following a successful first nest is rare in this population). Nestlings were usually measured daily or on alternate days until day 8, after which nestlings were usually not handled to prevent premature fledging. Previously, I had used established criteria based on sexual size-dimorphism (Holcomb and Twiest 1970, Fiala 1981, Westneat et al. 1995, Weatherhead and Dufour 2000) to determine fledgling sex ratios (Weatherhead 2005a). For data collected in 2005, however, fledglings were also sexed using DNA-based methods (Kahn et al. 1998) using blood samples taken when nestlings were four to six days old (Weatherhead et al. 2007). These results were used to refine the morphological sexing methods, and these refinements were then applied retrospectively to recalculate the sex-ratio estimates for earlier years. For the new criteria, a nestling was considered to have hatched the day it was first measured (day 0) if it weighed ≤ 4.0 g. Respective weights used to assign females and males were ≤ 27 g and >30 g on day 6, ≤ 31 g and >32 g on day 7, and ≤ 31 g and >33 g on day 8. For last-hatched nestlings, however, respective female and male criteria were ≤ 26 g and >29 g on day 6, but thereafter were the same as for other nestlings.

The principal value of the refined sexing criteria was that they allowed more fledglings to be sexed when measurements close to fledging were lacking. However, the resulting sex ratios were highly correlated with estimates derived using the older method ($R^2 = 0.90$), and annual sex ratios estimated using the old and new methods were not significantly different (Weatherhead et al. 2007). Overall, slightly more than 90% of all fledglings from before 2005 were sexed using the new method (Weatherhead et al. 2007). The 2005 data, for which fledglings were sexed using both molecular and morphological methods, indicated that the latter tended to slightly underrepresent males (Weatherhead et al. 2007). For my present purpose, this bias in unsexed fledglings could potentially be problematic if the annual proportion of unsexed nestlings varied with either the length of the season or with the sex ratio of the fledglings that were sexed. There was no evidence of either relationship ($R^2 = 0.01$ and 0.08 , $P = 0.82$ and 0.38 , respectively).

The hypotheses tested here arose in previous studies that divided the breeding season into early, middle, and late periods. For consistency with those studies, I follow the same approach. Both studies defined "nest initiation" as the date the first egg was laid, but they used differing criteria for dividing the season. In the earlier study (Weatherhead 1983), I assigned all nests initiated on or before 10 May as "early," all those initiated on or after 7 June as

TABLE 1. Percentage of Red-winged Blackbird fledglings that were male in early-, mid-, and late-season nests. Early nests are the first 20% of nests initiated each year, and late nests are the last 20% initiated. Season length is the number of days between the mean initiation dates of early and of late nests. The number of young fledged in each period that were sexed is given as *n*. There was no sex-ratio estimate for the late-season period in 1995 because no late nests were successful.

Year	Season length (days)	Early		Middle		Late	
		<i>n</i>	Male (%)	<i>n</i>	Male (%)	<i>n</i>	Male (%)
1985	42	27	48.1	49	28.6	17	47.1
1986	36	41	61.0	53	35.8	23	43.5
1987	38	36	44.4	55	47.3	33	42.4
1988	29	73	56.2	80	73.7	55	45.5
1989	35	69	53.6	88	46.6	39	43.6
1990	44	83	42.2	106	43.4	71	49.3
1991	35	51	49.0	40	55.0	15	40.0
1992	34	27	40.7	38	52.6	28	60.7
1993	39	60	44.0	47	53.2	21	42.9
1995	35	22	72.2	37	51.4	0	—
2005	32	35	65.7	73	30.7	30	40.0

“late,” and all clutches in between as “midseason,” whereas later (Weatherhead 2005a) I adjusted dates to reflect differences in annual laying patterns. “Early” nests were the first 20% of all nests initiated in a year, “midseason” nests the central 60% of nests, and “late” nests the last 20% initiated. Because preliminary analyses using both approaches produced qualitatively similar results, here I present results using only the latter approach. Length of the nesting season was defined as the number of days between the mean initiation date of early nests and the mean initiation date of late nests (Weatherhead 2005a).

All sex-ratio analyses compare either total or seasonal percent male values among years, such that year is the unit of replication. Means are presented \pm SE.

RESULTS

Over the 11 years used here, data were obtained from 1,246 nests, of which 825 contributed to the total of 1,522 fledglings that were sexed, of which 785 (51.6%) were female and 737 (48.4%) were male. I found no evidence that female blackbirds consistently produced more sons in the midseason period and more daughters early and late in the season. That pattern occurred in only 4 of the 11 years (Table 1), and the overall mean percent males in midseason (47.9 ± 3.6) was intermediate in relation to early (52.5 ± 3.1) and late nests (45.5 ± 1.9), rather than being greater than both.

These results rule out the mechanism I proposed (Weatherhead 2005a) to explain the association between annual sex ratios and length of the breeding season (i.e., females were not simply perceiving that the early and late components increased disproportionately when breeding seasons were longer). However, this does not rule out the possibility that seasonal patterns are involved. The null hypothesis is that in longer breeding seasons the decrease in production of sons is evenly distributed across the season. The proportion of males decreased with season length

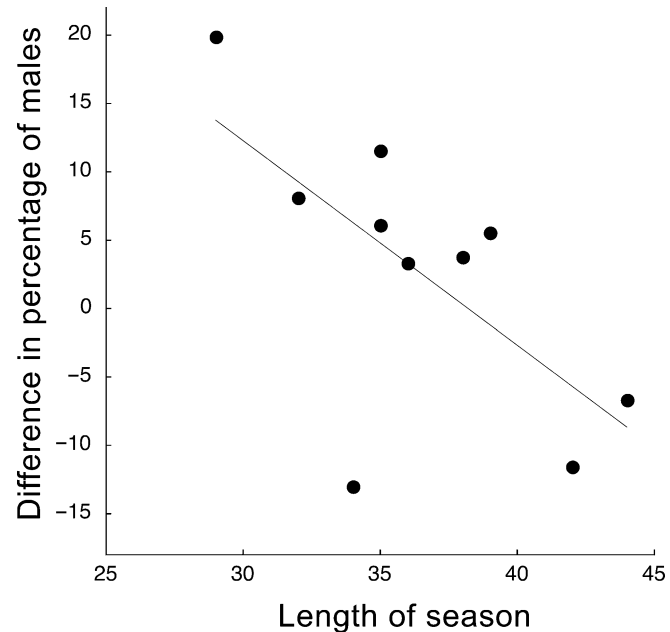


FIG. 1. Difference in percentages of male Red-winged Blackbird fledglings in the early and midseason periods versus the late-season period in relation to the length of the breeding season (in days).

in both the early and midseason periods ($r = -0.50$ and -0.60 , $P = 0.11$ and 0.05 , respectively; $n = 11$) but not in the late period ($r = 0.10$, $P = 0.78$, $n = 10$), where the (weak) trend was actually positive. Thus, the decrease in male fledglings with length of breeding season was not a proportionate response across the season. To capture this seasonal effect in a single analysis, I computed the difference between the sex ratio early in the season and in midseason and the sex ratio late in the season and regressed this value against breeding-season length. As breeding seasons got longer, the sex ratio became less male-biased in the early and middle parts of the season than late in the season ($r = -0.66$, $P = 0.04$; $n = 10$ because no late nests were successful in 1995; Fig. 1), because females produced an excess of daughters in the early and midseason periods.

Finally, the change in seasonal sex ratios with season length appears to have resulted from changes in the primary sex ratio rather than from sex-biased nestling mortality varying with the length of the season. Over the study years included here, the mean number of young fledged from successful nests (and, conversely, the number of young dying in the nest) each year was independent of season length ($r = -0.23$, $P = 0.50$, $n = 11$). Had sex-biased mortality been responsible for the association between sex ratio and length of the breeding season, starvation should have increased with season length. Furthermore, even though the number of young that fledged from successful nests increased through time over the years included here ($r = 0.73$, $P = 0.01$, $n = 11$), which is consistent with my earlier results (Weatherhead 2005a), the sex ratio did not change systematically across the study ($r = 0.02$, $P = 0.96$, $n = 11$), again indicating that the fledging sex ratio was not a function of how many nestlings starved.

DISCUSSION

My goal was to explore why fledging sex ratios in Red-winged Blackbirds become more female-biased in years with longer breeding seasons (Weatherhead 2005a). Contrary to my earlier hypothesis (Weatherhead 2005a), this pattern was not a result of female blackbirds always producing more sons in midseason and more daughters early and late, in conjunction with the early and late production increasing disproportionately in longer seasons. I have shown here that across years, there was no trend of always producing more of one sex during any particular stage (early, middle, or late) of the breeding season. However, I found that within-season sex ratios varied significantly with the length of the breeding season, which differs from the predicted pattern. As breeding seasons became longer, proportionately more daughters were produced in the early and middle parts of the season. This result explains, at least proximately, the overall sex-ratio pattern with season length and may also explain why the initial hypothesis was wrong.

The initial hypothesis was based on the results of a single-year study in which more male offspring were produced in the middle of the season and more female offspring were produced early and late in the season (Weatherhead 1983). Given the higher cost of producing sons in blackbirds (Fiala 1981), and assuming that food is most abundant in the middle of the season, this result was interpreted as being consistent with Trivers and Willard's (1973) general hypothesis of adaptive sex-allocation. Short-term studies can lead to incorrect interpretations for two reasons. First, results seen in a single year could be unrepresentative simply because of sampling error (Johnson et al. 2005). Second, if sex-allocation varies nonrandomly but over time-scales of multiple years, results from a single year would obviously not allow one to discern such a pattern. The latter scenario appears to apply here. The breeding season in 1981 was the third shortest in 19 years for which data are available (18 years included in Weatherhead [2005a], plus 2005). The analyses presented here show that in years with short breeding seasons, midseason sex ratios are male-biased. For example, the shortest season for years included here was 1988, when 73.7% of midseason fledglings were male. Thus, the data from 1981 were consistent with the long-term pattern, but in the absence of other years of data, there was no way to detect that pattern.

The results of the present study provide a proximate explanation of why sex ratios were more female-biased in years with longer breeding seasons. When breeding seasons were longer, proportionately more females were produced in the early and midseason periods, the latter being when the greatest number of total fledglings is produced. The question that remains, however, is why blackbirds allocate offspring sex differently depending on the length of the breeding season. If the pattern is part of an adaptive strategy, the answer to the question may involve some aspect of how the natural histories of male and female blackbirds differ. As mentioned above, sexual size-dimorphism makes male nestlings more expensive to produce. Perhaps in years with short breeding seasons, food is relatively abundant during the early and midseason periods, favoring overproduction of sons, whereas in long seasons food is less abundant in those periods.

Another possible explanation for why female blackbirds vary sex-allocation patterns with the length of the breeding season is that females are responding to some other factor associated with breeding-season length. Breeding seasons were longer in years with more positive winter values of the North Atlantic Oscillation (Weatherhead 2005a). In the winters preceding those breeding seasons, survival was lower (Weatherhead 2005b). Although survival was lower for both males and females, the effect was to lower only the number of breeding females, because in this socially polygynous species there is a surplus of males. Thus, declines in the population of territorial males appeared to be compensated for by nonterritorial floaters filling the vacancies (Weatherhead 2005b). It is possible that females responded to male-biased shifts in the breeding sex ratio by producing more daughters. This could account for the female-biased fledging sex ratios in years with longer breeding seasons. However, this hypothesis does not explain why the change in sex-allocation was restricted to the early and mid-season periods.

Investigating these or other hypotheses will be challenging. Multiple years are required, and key variables (e.g., breeding-season length) are not amenable to experimental manipulation. Furthermore, philopatry of <2.4% of fledglings in this study population (Weatherhead and Dufour 2000) makes it essentially impossible to quantify sex-specific recruitment rates to determine the fate of offspring produced at different times of the season and in years in which the lengths of the breeding seasons differ. Without knowing which, if any, of the proposed hypotheses is supported, there is no way of knowing whether this phenomenon is part of an adaptive sex-allocation strategy. In any case, if climate change causes further shifts in the breeding season, nonadaptive sex-allocation responses could result. Documenting how individuals and populations respond to these potential changes in sex ratios would be informative, from both basic and applied perspectives.

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