

# Nest-site preference and maternal effects on offspring growth

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Maternal preferences for oviposition sites are assumed to be adaptive, but offspring fitness is not always higher at preferred sites and, thus, further study of the selection pressures that influence oviposition behavior is warranted. Among birds, predation is regarded as the primary agent of selection on nest-site microhabitat preferences, but alternatives are rarely considered. We tested the hypothesis that avian nest-site preferences are an adaptive response to fitness costs imposed by variation in nest-site microclimate. We documented that Chestnut-collared Longspurs (*Calcarius ornatus*) strongly preferred to orient nests towards the southeast and showed that this preference influenced microclimate: nests facing southeast had the highest midday temperatures. Yet, preferences were not adaptive because nestlings in nests with the preferred orientation gained mass at a slower rate, had retarded skeletal growth, and reached a smaller final size. We experimentally tested this result by altering orientation of nests and confirmed, for the first time, that variation in nestling growth was causally linked to variation in nest microclimate arising from nest-orientation preferences. Adults responded to the high temperatures at preferred southeast-facing nests by spending more time shading young from the sun, apparently attempting to ameliorate heat costs. This response, however, resulted in parents spending less time feeding young, potentially explaining slower growth in these nests. Direct effects of higher temperatures may also play a role in slower growth. Although we lack an explanation for this apparently maladaptive preference, these results demonstrate that nest-site choices of birds can yield fitness costs imposed by variation in nest microclimate. *Key words:* *Calcarius ornatus*, grassland bird, maternal effects, nest microclimate, nest-site selection, nestling growth. [*Behav Ecol* 15:816–823 (2004)]

Non-genetic maternal effects on offspring phenotype appear to be widespread and often of profound importance (Agrawal, 2001; Etges, 1998; Kirkpatrick and Lande, 1989). Most attention has focused on maternal allocation decisions; far less is known about how maternal behavior influences offspring phenotype and fitness (Bernardo, 1996). However, for species with extensive parental care, maternal behavior may be an important source of maternal effects (Price, 1998; Reinhold, 2002). Oviposition behavior may be a particularly important source of maternal effects because the choice of an oviposition site can have profound effects on survival rate and development of offspring (e.g., Bernardo, 1993; Boag, 1987; Fox et al., 1994; Mousseau and Fox, 1998; Roitberg, 1998; Sinervo and Doughty, 1996). Most organisms have few opportunities to breed during a lifetime, and thus maternal preference for appropriate oviposition habitat is assumed to be under strong natural selection such that site preference is correlated with suitability for offspring development (Jaenike, 1978; Jaenike and Holt, 1991; Martin, 1998). Although some studies support the preference-performance hypothesis (Fox et al., 1994; Kolbe and Janzen, 2002; Qualls and Shine, 1998; Shine and Harlow, 1996), in many other cases juvenile performance is lower or unchanged at preferred sites compared to non-preferred sites (e.g., Courtney and Kibota, 1990; Mayhew, 1997; Thompson, 1988), which has led to the suggestion that oviposition decisions need not be in the best interest of offspring (Mayhew, 1997, 2001; Roitberg, 1998; Scheirs and De Bruyn, 2002). Further tests are needed to understand the adaptive value of oviposition preferences and the selection pressures that shape them.

Preferences for sites for raising offspring (i.e., nest sites) have been widely studied in birds, but almost exclusively in the context of predator avoidance (Clark and Shutler, 1999; Martin, 1998). Yet a wide variety of environmental factors may exert selection on preferences (Werner et al., 1983; Lima and Dill, 1990; Martin, 2001). One important alternative to predation is nest microclimate; embryos and nestlings are sensitive to thermal and hydric conditions at the nest (Davis et al., 1984; Webb and King, 1983) and thus, as in other taxa, microclimate may impose significant constraints on nest-site choice in birds (Conway and Martin, 2000; Haftorn, 1988; Kolbe and Janzen, 2002; Madsen and Shine, 1999; Shine and Harlow, 1996; With and Webb, 1993).

Avian nest-site preferences often appear to be influenced by microclimatic conditions (Gloutney and Clark 1997; Walsberg, 1981). In addition, nest-site features that are assumed to influence microclimate are thought to influence offspring fitness (Austin, 1974; Yanes et al., 1996). Clear tests of the hypothesis that nest-site preferences are an adaptive response to selection pressures imposed by microclimate are lacking, however. Testing this hypothesis by demonstrating that preferred nest-site features influence microclimate, and that microclimate influences offspring fitness, is a critical first step in demonstrating adaptive nest-site preferences and understanding the evolution of nest-site choice.

Here, we first document nest-site preferences in Chestnut-collared Longspur (*Calcarius ornatus*), a songbird of the northern Great Plains of North America, and then show how nest-site preferences affect nest microclimate. We focus on the choice of a nest orientation in measuring nest-site preferences because the directionality of the nest opening has a clear, causal link to nest microclimate: nest orientation determines when, and for how long, the nest is exposed to direct insolation and thus largely determines temperature within the nest (Walsberg, 1981; Walsberg and King, 1978). Next, we relate variation in nest microclimate arising from variation in nest-site preference to a measure of fitness. We focus on nestling growth because it is an important component of

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fitness (Gebhardt-Henrich and Richner, 1998) that is likely to be sensitive to variation in nest microclimate (Bryant, 1975; Ernst et al., 1984; Konarzewski and Taylor, 1989; McCarty and Winkler, 1999; Petersen et al., 1986). Temperature in particular can have a major influence on growth through its effect on energy and water budgets (Ernst et al., 1984; Sullivan and Weathers, 1992; Wolf and Walsberg, 1996). Through the use of video observations, we then provide a behavioral mechanism linking microclimate to fitness. Finally, we conduct an experiment to causally link variation in microclimate to variation in fitness.

## METHODS

### Study site

Work was conducted at Medicine Lake National Wildlife Refuge (48.5° N, 104.5° W), which is located on the glaciated plains north of the Missouri River in eastern Montana. For a description of the study site, see Lloyd (2003). Data for the descriptive portion of this study were gathered between 2000–2002 and our experiment was conducted in 2002.

### Study organism and nesting biology

Chestnut-collared Longspurs (hereafter, longspurs) build open-cup nests on the ground. The female appears to choose the site and performs nearly all of the construction (J. Lloyd, personal observation; Hill and Gould, 1993). Construction begins with the female digging a 4–5 cm deep hole in the ground, which is subsequently lined with grasses such that the rim of the nest is approximately level with the surface of the ground (Lloyd J, personal observation; Hill and Gould, 1993). Nests are usually placed next to a clump of grass but have little overhead cover; in 4 years of study at this site the percentage of the nest obscured from overhead by vegetation averaged only 27% ( $n = 161$ ). However, lateral vegetation cover is nearly complete except for a single opening that is used by adults to access the nest (of 315 nests monitored in this study, only two had >1 lateral opening and both of these nests were excluded from this analysis). Adults rarely fly directly to the nest and instead generally land  $\geq 0.5$  m away and then approach the nest on foot (J. Lloyd, personal observation).

### Nest-site selection and nest temperature

We located nests from April to July of each year using systematic searches and behavioral observations of adult longspurs. During systematic searches, we flushed adults from nests by dragging a weighted rope across the ground. After a nest was located, we marked its position with a small piece of flagging. Whether flags attract predators remains controversial (Gotmark, 1992; Hein and Hein, 1996); however, the use of flags was necessary to allow us to relocate nests.

To examine patterns of nest-site selection, we measured the following variables immediately after the termination of a nesting attempt: orientation, side cover, and the volume of vegetation immediately surrounding the nest. We measured nest orientation by first locating the opening in the vegetation used by adults to access the nest. Nest orientation was then recorded, relative to magnetic north, as the azimuth bisecting the nest opening. We used video observations of adult behavior (see below) as a check on our assumption that the orientation as recorded in the field was actually used by adults to access the nest. This assumption was valid in all cases. We assessed side cover by placing a 5 cm radius cardboard disc in the nest and then estimating the percentage of the disc that

was occluded when viewed from 1 m away in each of the cardinal directions. At four points, 1 cm outside the edge of the nest in each cardinal direction, we estimated the volume of vegetation by measuring the visual obstruction of vegetation against a wooden pole marked in 2.5 cm increments (Robel et al., 1970). We measured vegetation volume in the same fashion at a random point within the same territory, allowing us to determine the importance of this feature in nest-site selection. Directional patterns in side cover and the height and density of vegetation surrounding the nest determine the orientation of the nest, and thus we included these measures to shed light on how females shape the exposure of their nest to the sun.

We quantified nest microclimate at each nest by measuring temperature within the nest cup continuously for 24 h as soon as the nesting attempt ended. By measuring temperature immediately following the end of a nesting attempt, before any structural changes could occur to the nest-site vegetation (e.g., due to trampling, senescence, or new vegetative growth) that might affect microclimate, we believe our measures of nest-site microclimate provide an unbiased index of conditions experienced by eggs, nestlings, and adults. Given that we are interested primarily in comparisons of microclimate among nest orientations, an unbiased index of nest temperature relative to nest orientation is more important than a complete elucidation of nest temperature throughout the nesting cycle. Furthermore, our video observations confirmed that our measure of nest microclimate accurately reflected conditions experienced by nestlings (see Results, Effect of microclimate on parental behavior). At the same time that we measured nest-site temperature, we also measured ambient air temperature at a point 5 m from the nest in order to control for variation in ambient temperature that could have influenced our estimate of nest temperature. For both temperature measures we used Stow-Away data loggers (Onset Computer Corporation, Bourne, MA) equipped with external, black-bulb thermistors. For nest temperature measurements, we used a piece of wire to position the sensor approximately 1 cm above the center of the base of the nest, such that the sensor was suspended horizontally above and parallel to the base of the nest. Nest temperatures obtained from our sensors are not the same as the operative environmental temperature experienced by birds at the nest (Walsberg and Weathers, 1986), but they do provide an unbiased way of characterizing thermal conditions at the nest (Stoutjesdijk, 2002). To measure ambient air temperature we positioned the sensor approximately 5 cm above the ground and shielded the thermistor from direct sunlight with a plastic shade.

We determined if longspurs preferred to orient their nests in particular directions using a one-sample Kolmogorov-Smirnov test, in which the observed distribution of directions was compared against the null hypothesis that the distribution of nest orientations was uniform (Bergin, 1991). MANOVA was used to compare vegetation volume (square-root transformed for normality) at nest sites and random, non-nest sites. Because side cover is a product of the actual construction of the nest, it can not be meaningfully measured at random points and therefore we were unable to determine whether longspurs select nest sites with more or less cover than is generally available in the environment. Thus, we only present descriptive statistics for side cover. We used repeated-measures ANCOVA to analyze the relationship between nest orientation and nest temperature. Because we measured nest temperatures over the length of the breeding season, ambient air temperature varied and thus was included as a covariate. Orientation has little effect on temperature when the sun is down or low in the sky, so to achieve reasonable power in our repeated-measures test we limited our comparison of nest temperatures to the hours of 1000–1600.

For comparisons among unmanipulated nests, we treated orientation as a categorical variable with four levels: northeast ( $0^{\circ}$ – $90^{\circ}$ ), southeast ( $91^{\circ}$ – $180^{\circ}$ ), southwest ( $181^{\circ}$ – $270^{\circ}$ ), and northwest ( $271^{\circ}$ – $360^{\circ}$ ). These four categories generally reflect natural breaks in the distribution of nest orientations. For example, longspurs preferred southeast orientations; 54% of the nest orientations recorded in this study fell within the arc from  $91^{\circ}$ – $180^{\circ}$ . Categories based on this arc, and the three complimentary arcs, therefore seem reasonable. Furthermore, our categories reflected four distinct temperature regimes imposed by the azimuth angle of the sun: northeast orientations receive direct sun only immediately after sunrise, when ambient temperatures are low; southeast orientations receive insolation during the morning as temperatures climb rapidly; southwest orientations receive afternoon sun as air temperature declines; and northwest orientations receive sun only immediately prior to sunset. Thus, we feel that our categories reflected biologically relevant physical conditions that birds may use in deciding upon a nest orientation.

### Nestling growth and survival

To determine the fate of nestlings we returned every 2 days to inspect the contents except when fledging was expected, at which point we visited daily. If the nest was empty prior to the expected fledging date, we searched the territory for adults to determine if they were feeding fledglings. We assumed that predation had occurred if we were unable to locate adults feeding fledglings. To estimate nestling growth rate, we individually marked nestlings as they hatched using a felt-tipped pen, and returned every 2 days to measure body mass, total length of the outermost primary on each wing (shaft, and feather when applicable), and length of both tarsi. For analysis, we used the mean of the right and left measurements for tarsus and primary length. Mass was estimated to the nearest 0.1 g using a portable electronic balance, and primary and tarsus length were both measured to the nearest 0.1 mm using calipers.

We estimated growth rates of all nestling traits by using nonlinear regression to fit a logistic growth curve to the entire data set for each trait (Remes and Martin, 2002; Ricklefs, 1983). The logistic curve provided an excellent fit for all measured traits (mass  $r^2 = .85$ , tarsus  $r^2 = .81$ , primary feather  $r^2 = .86$ ; all  $p < .001$ ). To avoid artificially inflating error degrees of freedom, residuals from the growth curve were pooled among nestlings within a nest before analysis (Ricklefs, 1983). Thus, for each nest, growth rate was reduced to a single residual value that reflected the average growth of nestlings in a nest relative to all other nests in the sample. Because this approach does not rely on applying separate regressions to each individual, it allowed us to include all measured individuals in the analysis; had we attempted to estimate growth parameters separately for each individual or each nest we would have been forced to exclude samples with fewer than three measurements. To compare growth among orientations, we analyzed pooled residuals from the nonlinear regression using MANCOVA with brood size and hatching date as covariates (Ricklefs, 1983). We used MANCOVA because growth rates for different traits were not independent, and we applied Bonferroni corrections to all subsequent univariate comparisons. For successful nests, we compared the final mass of nestlings directly using ANCOVA on mass as measured the day prior to fledging, with the same covariates as above. We also used ANOVA to compare treatment effects on survival, expressed as the percentage of eggs that fledged young (arcsin transformed), the number of young fledged, and the length of the nestling period.

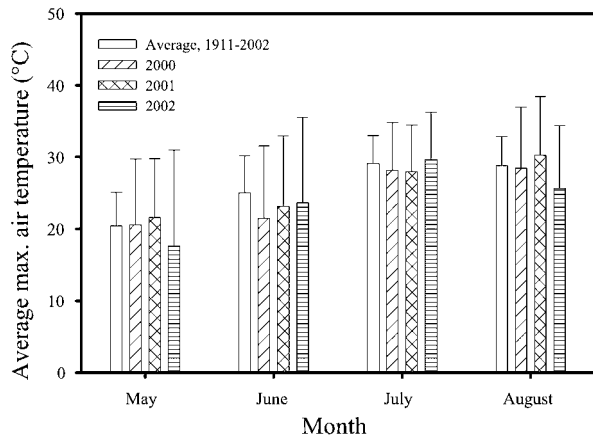
### Effect of microclimate on parental behavior

Because microclimate effects on nestling growth may be mediated through indirect effects on parental behavior we used video cameras to examine how two elements of parental behavior, feeding rate and time spent brooding young, varied in response to microclimate. On day three of the nestling period (day of hatching = 0) we placed a Hi-8 video camera at each nest and recorded activity from 0700–1400 h. By taping all nests at the same developmental stage, we were able to control for natural variation in feeding and brooding rates that occur as nestlings age. To control for differences among nests in weather conditions at the time of taping, we also recorded ambient air temperature during videotaping. Ambient air temperature was recorded with a shaded temperature probe attached to a data logger and placed 5 m from the nest. To examine how parents respond to changes in microclimate, we compared percentage of time spent brooding (arcsin transformed) and the number of feeds per hour (natural log transformed) among treatments using ANCOVA, with ambient temperature and brood size as covariates. In all cases, post hoc comparisons among treatments were adjusted for multiple comparisons using the Bonferroni method. Unless otherwise noted, variables were normally distributed and thus not transformed for analysis.

### Experimental manipulation of microclimate

Fitness costs of microclimate may be confounded with individual quality if low-quality individuals tend to choose non-preferred orientations. To counter this problem, we experimentally shifted the orientation of nests to examine the effect on nestling growth. By randomly re-assigning a new orientation to a nest, we were able to examine the causal relationship between maternal preference for nest orientation and offspring phenotype and fitness. In order to manipulate the radiative environment experienced by nestling and adult longspurs, we experimentally altered nest orientations by removing vegetation and adding artificial shade. We manipulated nests to create three experimental orientations: northeast ( $40^{\circ}$ – $50^{\circ}$ ), southeast ( $130^{\circ}$ – $140^{\circ}$ ), and southwest ( $220^{\circ}$ – $230^{\circ}$ ). We chose these orientations to represent the three general radiative conditions a nest might experience: no direct sun, morning sun, and afternoon sun. We applied the treatment with the constraint that the experimental orientation must be in a different quadrant than the natural orientation; for example, nests that faced between  $0^{\circ}$ – $90^{\circ}$  were assigned to either a southeast or southwest orientation but never a northeast orientation. Otherwise, treatments were assigned randomly and nests not selected were left unmanipulated. All treatments were applied on the day of hatching.

We manipulated nest orientation by clipping vegetation to expose the nest in the desired direction while simultaneously using an artificial shade to eliminate the natural nest opening. We standardized the size of the artificial nest openings by clipping vegetation until approximately 90% of the disc used to measure concealment was left completely unobscured by vegetation when viewed from eye-level 1 m away at the desired orientation. Among unmanipulated nests measured in the same way, median concealment was 12.5% and thus our treatments were within the range of variation occurring naturally. Artificial shade was created by placing a  $15 \times 15$  cm square piece of fine-mesh (1 mm) metal window screen, stretched between two metal pins, in front of the nest opening. We placed the screens immediately adjacent to the nest and at a slight angle such that the top of the screen was in the same plane as the edge of the nest cup. Thus, by shading the natural opening and clipping vegetation to create a new opening, we were able to artificially manipulate realized nest orientation.



**Figure 1**

Average maximum air temperature (+1 SD) at Medicine Lake, Montana by month during the years included in this study in relation to long-term (1911–2002) averages.

After clipping and shading a nest, an observer hid 150 m from the nest and recorded the time that elapsed until parents returned either to feed the nestlings or to brood them. Using ANOVA, time to return at experimental nests was compared with data gathered in a similar fashion at unmanipulated nests of the same age that were visited for routine nest checks (i.e., parents flushed off of the nest and contents recorded). Comparing return times allowed us to assess the extent to which the application of our treatments disrupted normal behavioral patterns.

## RESULTS

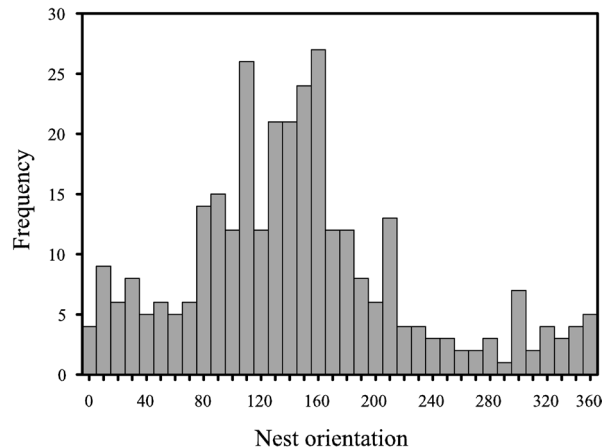
### Climate at the study site

Average maximum air temperatures during the course of our study were similar to the long-term average (Lloyd, 2003; Figure 1). During 2002, when we conducted our experiment, temperatures in May, June, and August were slightly cooler than the long-term average and nearly identical to the long-term average during July. Weather station data from site, averaged across the breeding season, showed that maximum daily temperatures are generally achieved between 1400–1700 h and that prevailing winds come from the east-northeast and the west-southwest (Lloyd, 2003).

### Nest-site selection and nest temperature

Longspurs preferred to orient their nests towards the southeast ( $n = 313$ , Kolmogorov-Smirnov  $Z = 4.41$ ,  $p < .001$ ; Figure 2). Preference for southeast-facing nests did not change seasonally and the distribution of nest orientation was similar when comparing early (initiated before 1 June,  $n = 155$ , median =  $141^\circ$ ) and late (initiated after 1 June,  $n = 121$ , median =  $145^\circ$ ) breeding attempts (Kolmogorov-Smirnov  $Z = 0.88$ ,  $p = .48$ ). The distribution of vegetation around nest sites differed from random points ( $F_{4,204} = 2.28$ ,  $p = .05$ ); nest sites had lower volume of vegetation on the south ( $F_{1,207} = 4.96$ ,  $p = .03$ ) and east ( $F_{1,207} = 4.91$ ,  $p = .03$ ) sides. Nest orientation was not related to the risk of predation ( $n = 130$  depredated nests and 116 successful nests; Kolmogorov-Smirnov  $Z = 0.93$ ,  $p = .35$ ).

Midday nest temperatures varied among orientations (ambient temperature:  $F_{1,49} = 116.0$ ,  $p < .001$ ; nest orientation:  $F_{3,49} = 5.89$ ,  $p = .002$ ; Figure 3a). Nests with the preferred southeast orientation were hotter than nests oriented towards



**Figure 2**

Nest orientations of Chestnut-collared Longspur nests monitored during 2000–2002.

either the northeast (mean difference =  $8.7^\circ\text{C}$ ,  $p = .04$ ) or the northwest (mean difference =  $10.3^\circ\text{C}$ ,  $p = .007$ ). Temperatures at nests facing southeast were not different than at nests facing southwest (mean difference =  $3.6^\circ\text{C}$ ,  $p = .64$ ).

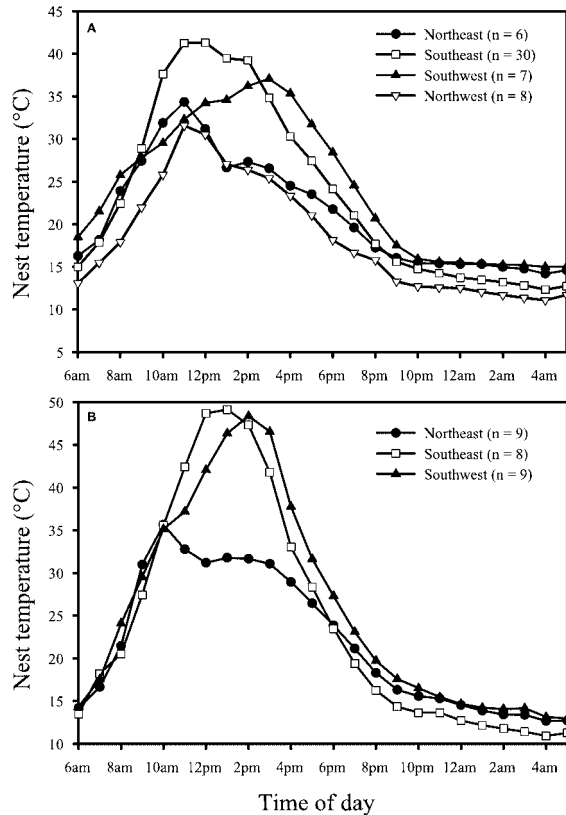
Midday nest temperatures also differed among manipulated orientations (ambient temperature:  $F_{1,22} = 16.34$ ,  $p = .001$ ; treatment:  $F_{2,22} = 20.33$ ,  $p < .001$ ) and in the same pattern as at unmanipulated nests (Figure 3b); experimental nests with the preferred southeast orientation ( $n = 17$ ) experienced higher midday temperatures than did experimental nests with a northeast orientation ( $n = 22$ ; mean difference =  $10.2^\circ\text{C}$ ,  $p < .001$ ). Temperatures at nests facing southwest ( $n = 17$ ) were not different than nests facing southwest ( $n = 22$ ; mean difference =  $2.2^\circ\text{C}$ ,  $p = .70$ ). Thus, our experimental nests were successful in recreating the radiative environment experienced at unmanipulated nests.

The disturbance associated with applying treatments did not affect the return time of adults (mean return time: experimental nests = 15.1 min; unmanipulated nests = 12.3 min;  $F_{1,23} = 0.93$ ,  $p = 0.41$ ), and no nest was abandoned following treatment.

### Nestling growth and survival

Among unmanipulated nests, orientation affected nestling growth (orientation:  $F_{3,48} = 5.6$ ,  $p = .02$ ; hatching date:  $F_{3,46} = 4.2$ ,  $p = .01$ ; brood size:  $F_{3,46} = 5.4$ ,  $p = .03$ ). Subsequent univariate tests revealed that the significant effect of orientation on nestling growth was a result of differences among orientations in the rate of mass gain ( $p = .05$ ); however, contrary to adaptive predictions, growth was slowest among nests facing the preferred direction (Figure 4a). Rate of mass gain was significantly less for nestlings in southeast-facing nests than in nests with a northeast orientation ( $p = .05$ ), but none of the other means differed significantly. Tarsus growth, feather growth, length of the nestling period, survival, and number of nestlings fledged were similar among orientations (all  $p > .20$ ).

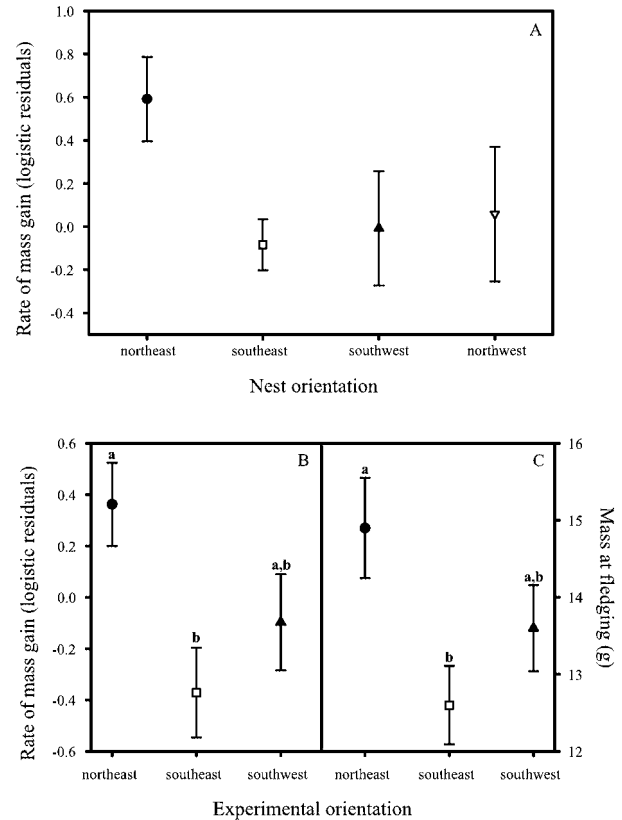
Similar results were obtained at experimentally altered nest orientations and in no case did nestlings perform better in nests that had been shifted to face the preferred direction. Experimentally changing the orientation of a nest had a significant effect on nestling growth (orientation:  $F_{3,53} = 3.8$ ,  $p = 0.015$ ; hatching date:  $F_{3,52} = 4.9$ ,  $p = 0.005$ ; brood size:  $F_{3,52} = 2.3$ ,  $p = 0.09$ ). The rate of mass gain varied among orientations ( $p = 0.01$ ; Figure 4b) and, as in natural nests,



**Figure 3**  
(A) Hourly marginal means (controlling for ambient air temperature at the time nest temperature was recorded) of operative environmental temperature at Chestnut-collared Longspur nests as a function of nest orientation. (B) Hourly marginal means (controlling for ambient air temperature at the time nest temperature was recorded) of operative environmental temperature recorded at experimentally re-oriented Chestnut-collared Longspur nests.

nestlings in southeast-facing nests grew significantly slower than nestlings in northeast-facing nests ( $p = 0.01$ ) and at a similar rate to nestlings in southwest-facing nests ( $p = 0.91$ ). Variation in rate of mass gain led to differences among treatments in mass at fledging (orientation:  $F_{2,26} = 3.86$ ,  $p = 0.03$ ; hatching date:  $F_{1,26} = 2.77$ ,  $p = 0.11$ ; brood size:  $F_{1,26} = 2.89$ ,  $p = 0.10$ ; Figure 4c). Nestling mass at fledging was lower in nests with the preferred southeast orientation than in nests with a northeast orientation ( $p = 0.03$ ). As with growth rate, fledging mass was intermediate in nests modified to face southwest (both  $p > .30$ ). In contrast to the observational data, our experimental data revealed that nest orientation also affected tarsus growth ( $p = .01$ ; Figure 5). Tarsus growth, as with mass gain, was lower in southeast-facing nests than in northeast-facing nests ( $p = .01$ ) and similar between southeast- and southwest-facing nests ( $p = 1.0$ ). Feather growth was similar among treatments ( $p = .22$ ). The length of the nestling period was also similar among treatments (northeast: 8.4 days; southeast: 8.9 days; southwest: 8.2 days;  $F_{2,23} = 0.88$ ,  $p = .43$ ) and similar to that of unmanipulated nests (over the course of 4 years at this site, mean length of nestling period = 9.1 days;  $n = 112$ ).

The percentage of young that survived to fledge did not vary among treatments (northeast: 29%; southeast: 22%; southwest: 24%; orientation:  $F_{2,58} = 0.22$ ,  $p = 0.81$ ; hatching date:  $F_{1,28} = 0.64$ ,  $p = .02$ ) and mean percent survival at experimental nests (25.3%) was similar to that at unmanipulated nests (24.9%). As is typical of passerine birds, nearly all mortality was the

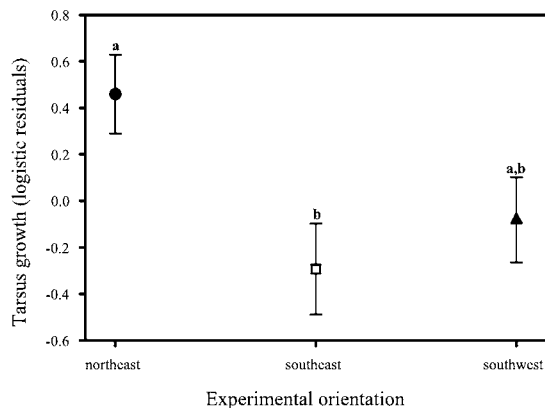


**Figure 4**  
(A) Growth rate of nestling Chestnut-collared Longspurs as a function of nest orientation. Each point represents the marginal mean ( $\pm 1$  SE) of residuals from a logistic curve fit to the entire data set, averaged among nestlings within a nest and among nests within each directional quadrant. Data were collected from 2000–2002. (B) Growth rate and (C) mass at fledging of nestling Chestnut-collared Longspurs as a function of experimental shift in nest orientation. For growth, each point is as described in (A). Mass at fledging is the average mass of all nestlings in a nest on the day before fledging. For all figures, means with different letters are significantly different (see text for  $p$ -values).

result of predation, and starvation was rare. Partial brood losses, whether by predation or starvation, were distributed equally among treatments: the number of young fledged from successful nests (e.g., at least one young fledged) did not vary among treatments (orientation:  $F_{2,32} = 0.82$ ,  $p = .45$ ; hatching date:  $F_{1,32} = 0.009$ ,  $p = .93$ ).

#### Effect of microclimate on parental behavior

The percentage of time adults spent brooding varied dramatically among treatments (orientation:  $F_{2,10} = 10.38$ ,  $p = .004$ ; ambient air temperature:  $F_{1,10} = 0.82$ ,  $p = .39$ ; brood size:  $F_{1,10} = 0.62$ ,  $p = .45$ ; Figure 6a). At the cooler, northeast-facing nests, adults spent less time brooding than at hot southeast-facing nests ( $p = .003$ ); percentage of time brooding was intermediate at southwest-facing nests and did not differ significantly from other orientations (northeast,  $p = .18$ ; southwest,  $p = .36$ ). The rate at which adults fed young also varied among orientations (orientation:  $F_{2,22} = 4.19$ ,  $p = .03$ ; ambient air temperature:  $F_{1,22} = 0.81$ ,  $p = .38$ ; brood size:  $F_{1,22} = 0.35$ ,  $p = .56$ ; Figure 6b). Adults at hot, southeast-facing nests fed young significantly less often than adults at northeast-facing nests ( $p = 0.03$ ); none of the other comparisons differed (all  $p > .45$ ).

**Figure 5**

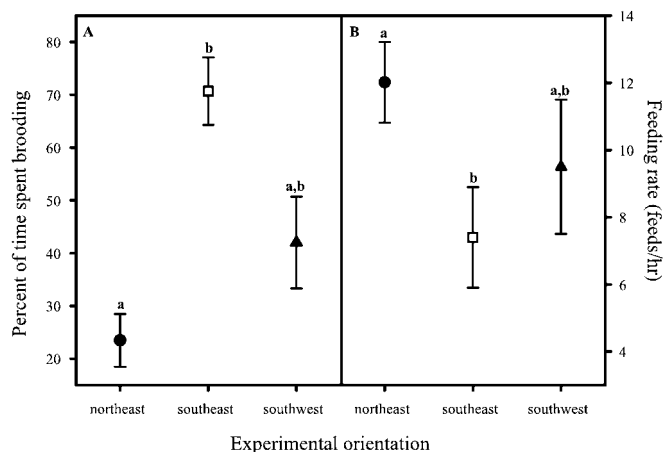
Tarsus growth rate of nestling Chestnut-collared Longspurs as a function of experimental shift in nest orientation. Each point represents the marginal mean ( $\pm 1$  SE) of residuals from a logistic curve fit to the entire data set, averaged among nestlings within a nest and among nests within each directional quadrant. Means with different letters are significantly different (see text for  $p$ -values).

## DISCUSSION

Fitness consequences of oviposition or nest-site choices should yield selection on site preferences, assuming a genetic basis for habitat selection (Martin, 1998). In this study, we showed that nest-orientation preferences cause variation in nest microclimate that results in behaviorally mediated fitness costs for nesting birds. Furthermore, by experimentally manipulating nest microclimate, we demonstrated a causal link between microclimate and components of fitness. Thus, nest-orientation preferences should evolve in response to selection by microclimate. At equilibrium, nest-site preferences should be adaptive such that preferred nest sites confer the most amenable microclimate for adults, nestlings, and eggs.

In our system, longspurs exhibited a strong preference for nest sites with a southeast orientation. However, this preference was not adaptive in terms of offspring performance: rate of mass gain, rate of tarsus growth, and mass at fledging were all significantly lower among nestlings in nests with a southeast orientation. Fledglings from southeast-facing nests were more than 2 g lighter than fledglings at nests with a northeast orientation; this 18% reduction in fledging mass likely has a strong negative effect on future survival prospects (Magrath, 1991; Martin, 1987).

Growth differences among orientations appear to be a complex result of the effect that orientation has on the radiative environment experienced by nestlings, as well as on parental behavior. Although altricial nestlings, such as longspurs, thermoregulate proficiently at high temperatures, even above 40°C (Olson, 1991; Visser, 1998), they can only deal with relatively short periods of heat stress without becoming dehydrated (Visser, 1998). Growth performance was worst in nests with the preferred southeast orientation, which had the highest midday temperatures. Conversely, growth rates were significantly faster in the relatively cool nests that faced northeast. Southwest-facing nests were intermediate both in temperature and in nestling growth. The behavioral response of adults to microclimate likely contributed to observed growth rate patterns. Adults at nests with a southeast orientation spent more than 70% of the time they were under observation shading their young, a 3-fold increase over those with northeast-facing nests (Figure 4a). This time spent sitting on, or immediately above, the nestlings appears to have constrained the amount of time adults could spend foraging

**Figure 6**

Percentage of time adult Chestnut-collared Longspurs spent brooding nestlings (A) and hourly rate at which adults fed nestlings (B) as a function of experimental shift in nest orientation. Each point represents the marginal mean ( $\pm 1$  SE) as estimated from 6–7 h of video observation conducted on day 3 of the nestling period. Means with different letters are significantly different (see text for  $p$ -values).

and consequently nestlings at southeast-facing nests were fed less often (Figure 4b). Thus, growth differences among treatments may arise from either or both the direct physiological cost of elevated temperature and the indirect cost of reduced feeding rates by adults.

Although we suggest that variation in growth is a result of the effect of orientation on the radiative environment, we cannot rule out a role of convective heat transfer. However, wind generally has much less of an effect on heat balance than does solar radiation (Wolf et al., 2000; Wolf and Walsberg, 2000). Furthermore, for ground nesters such as longspurs, most wind reduction is accomplished by virtue of being within the boundary layer of the ground (With and Webb, 1993), and thus nest orientation may have a minimal effect on the convective environment at the nest. Nonetheless, the cooling effect of prevailing west-southwest winds may help explain the similar growth rate of nestlings in northeast- and southwest-facing nests.

The reason why longspurs in this system prefer to orient nests towards the southeast, when doing so incurs measurable costs, is unclear. Other species breeding in similar habitat prefer to orient nests towards the north (With and Webb, 1993), and in other areas of their range Chestnut-collared Longspurs prefer northwest orientations (Hill and Gould, 1993). Predation can shape nest-site preferences (Clark and Shutler, 1999; Martin, 1988, 1993) but did not appear to constrain nest orientation preferences. Preference for a southeast orientation may reflect a trade-off with an unmeasured variable, such as adult survival or incubation efficiency. In this population, females may begin incubating as early as the beginning of May, when temperatures can drop below 0°C, and thus a southerly orientation may help reduce energetic demands for the adults. However, why females should continue to prefer southeast orientations as summer progresses and ambient temperatures rise remains unclear. Another possibility is that we somehow underestimated the benefit of a southeast orientation, perhaps because benefits only accrue when ambient temperatures are low. However, temperatures during our study were slightly cooler than the long-term average, and thus our results are not an artifact of conducting the study during abnormally warm years.

Infrequent severe weather that caused significant mortality might also favor a southeast orientation, although weather-related mortality during our study was fairly common and affected nests without apparent regard to orientation.

Given that longspurs in other parts of the geographic range orient their nests northward, including nearby populations in southern Canada, a lack of genetic variation for this trait seems unlikely. Perhaps individuals at our site were physically or mechanically constrained in their ability to construct a nest with a northerly orientation. Dead vegetation from growth in previous years tends to fall to the south as a consequence of winter storms coming out of the north. Longspurs often build nests among mats of dead vegetation, and thus nest orientation may be constrained by the direction in which standing dead vegetation becomes matted. This hypothesis could be tested directly by comparing nest orientation in areas with differing prevailing winter winds, or in areas in which natural disturbance regimes prevent the accumulation of dead vegetation.

Although we do not have a tested explanation for the apparently maladaptive nest-site preferences exhibited by longspurs at our site, our results demonstrate that variation in nest-site selection, in particular the choice of a nest orientation, leads to variation in nest microclimate that has consequences for fitness. Thus, our results are significant because they show that nest microclimate can exert selective pressure upon nest-site preferences, and thus it can play a role in the evolution of avian nest-site preferences. Our results also suggest that similar evolutionary forces act upon nest-site preferences in birds and reptiles, the other taxon in which nest-site preferences have been extensively studied (Kolbe and Janzen, 2002; Madsen and Shine, 1999; Qualls and Shine, 1998; Shine and Harlow, 1996). Predation and microclimate are important in both taxa, although studies of avian nest-site selection tend to ignore findings from reptilian systems, and vice versa. Future work on the adaptive value of nest-site preferences will benefit by recognizing that nest-site selection is a multifarious process, responding to multiple agents of selection, and that much broader similarities exist among nest-building taxa than is commonly recognized.

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