Cold Tolerance and Sex-Dependent Hypothermia May Explain Winter Sexual Segregation in a Farmland Bird

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ABSTRACT

Migration is an important event in the life cycle of many organisms, but considerable intraspecific variation may occur in its timing and/or destination, resulting in sexual segregation during wintering periods. In this study, we tested the body size hypothesis, or cold tolerance hypothesis, which predicts that body size dimorphism modulates metabolic costs associated with cold climate. Using the Eurasian skylark, we first investigated whether this species showed sexual differential migration. Then we explored the body size hypothesis by experimentally testing the effect of low ambient temperature (T_a) on both metabolic rate (MR) and body temperature (T_b). We tested for sex-related differences in metabolism and in energy-saving mechanism (hypothermia). We found clear differential migration by sex in skylark wintering populations, with a male-biased sex ratio decreasing toward southern latitudes. Measurements on captive birds at 20°, 6°, and −5°C demonstrated a significant increase in MR when T_a decreased, but there is no difference between sexes. While both males and females reduced their T_b overnight, T_b reduction was more pronounced in females exposed to the coldest temperature treatment. In addition, we found that individuals with the most reduced T_b lost less body weight during the night, suggesting that T_b reduction may help minimize energy expenditure when conditions become constraining. Our study suggests that functional mechanisms may be involved in latitudinal segregation between sexes and supports the hypothesis that sex-specific physiological strategies and thermal tolerance may explain segregation between sexes.

Introduction

The phenomenon of differential migration describes how, within a population, individuals of different sex or age classes differ in their migration with respect to timing, distance, or both (Terril and Abele 1988). When spatial segregation occurs in a population during the nonbreeding season, individuals occupying different nonbreeding sites may face contrasted food regimes, predation risk, competition level, or climate conditions, all of which may affect survivorship and, ultimately, population dynamics (Cristol et al. 1999). Differential migration and sexual habitat segregation during the nonbreeding season are widespread phenomena in birds (Myers 1981; Sherry and Holmes 1996; Cristol et al. 1999). For instance, 91% of 160 species from North America and Europe show differential migration (Cristol et al. 1999). Although differential migration is a widespread phenomenon, the underlying mechanisms are still poorly understood (Cristol et al. 1999; Berthold 2001; Jenkins and Cristol 2002) and the functional explanations are actively debated, as described below.

At least three different but not mutually exclusive hypotheses have been proposed to explain this pattern. First, the arrival time hypothesis posits that individuals of one sex (usually males) migrate earlier than the other sex and overwinter closer to the breeding grounds, thus conferring a reproductive advantage on the individuals that winter closer to the breeding grounds because they can secure high-quality territories earlier in the breeding season (Myers 1981). Second, the dominance hierarchy hypothesis predicts that the competition for winter resources leads the dominant sex to exclude the subordinate one (Gauthreaux 1978). Subordinate individuals are forced to move to areas farther away from the breeding grounds by dominant ones, which tend to move shorter distances or remain sedentary. Finally, the body size hypothesis, or cold tolerance hypothesis (Ketterson and Nolan 1976; Myers 1981), postulates that larger individuals are better suited to survive the colder and less predictable climates at higher latitudes. Under cold winter conditions, larger individuals are more likely to survive in comparison to small conspecifics, presumably because of several advantages including lower energetic cost of thermoregulation at low temperature (Piersma 1984; McNamara et al. 2004). By selecting

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winter habitats that minimize thermoregulatory costs, small individuals are expected to reduce their energy requirements for the benefit of other vital processes (Webb and Rogers 1988). Hence, energetic constraints are commonly invoked to explain the distributional patterns of small and large endotherms (Johnston and Bennett 1996). The variations observed in migration patterns between sexes are then often interpreted as a by-product of variations in body size (Catry et al. 2005). Still, disentangling the three functional hypotheses can prove difficult, because they are not mutually exclusive and based on similar predictions. For instance, social competition and cold tolerance hypotheses both predict that large individuals would take shorter migratory routes from breeding areas and therefore spend winter at higher latitudes. In this context, to clarify, the physiological response to variation in ambient temperature (T_a) can provide insights into cold environment adaptations.

When environmental temperature is low and/or food resources are scarce, small endotherms typically reduce their metabolic rate (MR) and body temperature (T_b) on a daily or seasonal basis, a phenomenon called facultative hypothermia, or torpor (Prinzinger et al. 1991). This physiological mechanism has evolved independently throughout the avian phylogeny, in species ranging in body mass (BM) from 3 g to 6.5 kg, and represents an important adjustment to balance energy budgets (McKechnie and Lovegrove 2002). Although many studies have investigated the link between species distribution and metabolism along a latitudinal gradient (Root 1988*a*, 1988*b*; Zuckerberg et al. 2011), few have investigated whether interindividual variation in cold tolerance within a species may reflect its spatial distribution, and the body size hypothesis remains largely untested (Guillemain et al. 2009). Importantly, the physiological response to variation in T_a can provide insights into how individuals are suited to different environments. In addition, it should be stressed that interindividual (i.e., size-related) variation in torpor or hypothermia may mask variation in MR. In this context, measurements of MR and T_b at low temperature are necessary to properly assess the relationship between energetic expenditure, T_a, and body size.

In this study we tested the cold tolerance hypothesis using the Eurasian skylark *Alauda arvensis* as model organism. This species is one of the most abundant and well-studied farmland birds in Europe (Donald et al. 2001). However, relatively little is known about the age/sex structure of wintering populations. The skylark, which is common in Western Europe, winters in west and south Europe in large numbers (Hegemann et al. 2010) and exhibits a slight sexual body size dimorphism, with males being larger than females (Cramp 1980; appendix). The skylark is thought to display differential migration (Senk et al. 1972; Cramp 1988), and among the different hypotheses evoked to explain differential migration in this species, only the arrival time hypothesis has received some attention (Hargues et al. 2007). Since differences in body size are often associated with differences in MR at low temperatures (Nagy 2005), differences in energy expenditure during winter may be expected to coincide with patterns of differential migration in this sexually dimorphic species.

After empirically confirming spatial segregation between sexes across France in winter, we experimentally exposed captive male and female skylarks at varying levels of T_a to test for sexual differences in MR and facultative hypothermia under different thermal treatments. We predicted that (i) females should overwinter at lower latitudes than males and (ii) females should face higher physiological costs than males at cold temperatures (i.e., higher MR) and should express a greater degree of T_b decrease than males at cold temperatures.

Material and Methods

Latitudinal Variation in Sex Ratio

The sex ratios of overwintering populations of skylarks were obtained by analyzing hunting bags from regional hunters along a latitudinal gradient in France. The sex of each bird was determined either by molecular analyses following Eraud et al. (2006) or by postmortem dissection. A total of 489 individuals (128 females and 361 males) were obtained during the winters of 2000–2001, 2009–2010, 2010–2011, and 2011–2012. To minimize the risk that samples included migrant birds, only the material collected from early December to late January was used. In addition, no sound or visual decoys were used by hunters, ensuring that sampling was random with regard to sex.

Bird Capture and Maintenance

For laboratory experiments, we caught skylarks in the western Atlantic flyway in France (region Poitou-Charentes) during the fall migration, between October and November 2011. Each bird was color ringed and weighed (± 0.1g), and the tarsus and maximum wing chord were measured. Thereafter, birds were randomly assigned to groups of 10–12 individuals and acclimatized for 2 mo in 4 × 3 × 2-m (l × w × h) outdoor aviaries at the Centre d'Etudes Biologiques de Chizé (CEBC-CNRS, western France). Birds were fed ad lib. with a commercial seed mixture, grit, green material (i.e., oilseed rape), and tap water. Birds showing any sign of sickness were removed from the experiment. All birds were released into the wild in the middle of March, during the prenuptial migration.

Metabolic Rates

We measured MRs of adult skylarks using multichannel open-circuit respirometry (Sables System, Las Vegas, NV). Measurements were performed on 10 males (BM = 38.51 $\pm$ 1.65 g) and 10 females (BM = 33.45 $\pm$ 1.09 g) during the first 2 wk of February 2012. Each individual was tested at three values of T_a ($-5°$, $6°$, and $20°C$) in a climatic chamber (400 DG LMS, Sevenoaks, Kent, UK). The last temperature ($20°C$) corresponds to the lower limit of this species' thermoneutral zone (Tieleman et al. 2002). The two other temperatures correspond either to challenging ($-5°C$) or mild ($6°C$) thermal conditions faced during winter. For trials conducted at $-5°$ and $6°C$, birds were first exposed and the chamber temperature was gradually decreased (within 2 h). The sequence in trial temperatures was randomized for each individual. Skylarks were taken from the outdoor aviaries in the late afternoon and then transported to the laboratory at

5:00 p.m. and acclimated to the temperature regime for 2 h. Birds were food deprived for 3 h before measurements. For a given session, seven individuals were weighed (± 0.1 g; i.e., initial BM) and then placed individually in an opaque test box (internal volume, 1,870 mL) in the climatic chamber set at target temperature. One test chamber was empty to provide continuous baseline sample. Birds were kept in total darkness to measure overnight oxygen consumption. Outside air was supplied by a Bioblock Scientific 551 air pump to a mass-flow controller (± 1 mL min^{-1}; FlowBar-8, Sable Systems) at a constant flow (415.5 $\pm$ 8.9 mL min^{-1}) to each chamber. The expelled air of each test chamber was then sequentially sampled with a gas flow switcher (RM-Multiplexer, Sable Systems). The air was stripped of water by passing it through Drierite (W. A. Hammond Drierite, Xenia, OH). Dried air was sent to the CO_2 analyzer (CA10A, Sable Systems) and then to the oxygen analyzer (FC10A, Sable Systems). Hardware outputs were simultaneously recorded with a specific interface (UI-2, Sable Systems). We used the following respirometric equations derived from Linghton (2008) and adapted to our setup to calculate skylark $\dot{V}_{O_2}$ (mL min^{-1} g^{-1}) and $\dot{V}_{CO_2}$ (mL min^{-1} g^{-1}):

$$\dot{V}_{O_2} = \text{flow rate} \times \frac{(FiO_2 - FeO_2) - FeO_2 \times (FeCO_2 - FiCO_2)}{1 - FeO_2},$$

$$\dot{V}_{CO_2} = \text{flow rate} \times \frac{(FeCO_2 \times FiCO_2) - FeCO_2 \times (FiO_2 - FeO_2)}{1 - FeCO_2},$$

where FeO_2 is the fractional proportion of O_2 entering the chamber, FiO_2 is the fractional proportion of O_2 exiting the chamber, $FeCO_2$ is the fractional proportion of CO_2 entering the chamber, and $FiCO_2$ is fractional proportion of CO_2 exiting the chamber.

Measurements were carried out overnight, and trial duration was 10 h (from 8:00 p.m. to 6:00 a.m. the following morning). Each test chamber was sequentially sampled for 300 s, leading to 10 replicates per individual throughout the night. Respiratory values were obtained by averaging replicates collected for each individual during stable resting periods. At the end of metabolic measurement, birds were weighed (i.e., final BM) and the mean BMs were obtained between initial BMs and final BMs.

Body Temperature

Our setup did not allow for simultaneous recording of MR and T_b. To test for changes in T_b with T_a, we recorded cloacal temperature (considered as a proxy of T_b; Hegemann et al. 2012) at two different values of T_a: $-5°$ and 20°C. Twenty skylarks (10 males and 10 females; different from those used in respirometry) were used in this experimental setup. All birds were captured from outdoor aviaries in late afternoon and transported to a nearby laboratory at 5:00 p.m. and then placed into a box (internal volume, 1,870 mL) in a temperature chamber. Birds were first exposed to thermoneutral T_a (20°C) for 3 h. At 8:00 p.m. the individual was removed from the box, its initial T_b was recorded, and the bird was placed back in the box in thermocontrolled chamber. One hour after the first measurements,

the light was turned off, and then the birds were kept in total darkness. For birds tested at low T_a, the T_a was gradually (within 2 h) reduced to $-5°$C, whereas the chamber was set at 20°C for the warm treatment. A second reading of T_b was taken during the night (2:00 a.m.). Finally, a third and last T_b recording was performed at 8:00 a.m. T_b was collected using a type T thermocouple inserted about 1.2 cm deep into the cloaca and maintained until a stable value was collected during 30 s (Nord et al. 2011; Hegemann et al. 2012). T_b was recorded within 60 s of handling using a 21X data logger (Campbell Scientific Instruments, Logan, UT). All measurements of T_b were taken by one person (T. Powolny). We are confident that repeated handlings during the night did not bias our results since previous studies have shown that potential disturbances inflicted by handling birds and measuring their T_b 4–5 h before a second T_b sampling does not modify the hypothermic response (Nord et al. 2011). All measurements were performed in two successive nights. The first night, 10 birds (sex ratio, 1 : 1) were exposed to 20°C and the remaining 10 were exposed to $-5°$C. The following night, temperature treatments were reversed for each group.

Statistical Analysis

We investigated the sex ratio variation at nine different locations from a total of 528 individuals according to latitude (expressed in decimal degrees WGS84), years, and longitude by fitting general linear models (GLMs; table 1). Following Wilson and Hardy (2002), GLMs were fitted with the proportion of females present at each site as the response variable using a binomial vector and a binomial error distribution. Quasi-likelihood estimations were used to estimate the scale parameter, and the significance of terms in the model was tested using F-tests (Wilson and Hardy 2002). We used general linear mixed models (GLMMs) to investigate whether oxygen consumption, BM changes, and T_b were affected by sex, BM, and T_a. T_a was included as a fixed factor into the models and bird identity as a random factor when repeated measures were used. We started from full models to obtain the minimally adequate model (Crawley 1993), following a backward stepwise model selection procedure where the nonsignificant terms at $P = 0.05$ were sequentially removed. Mass-corrected $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were log transformed to ensure normality and homoscedasticity assumptions. To compare T_b between groups, we used t-tests after a sequential Bonferroni correction in the level of significance had been made (Rice 1989). All analyses were performed using SPSS 17.0 software. Means are reported $\pm$ 1 standard error (SE).

Ethics Statement and Animal Welfare Information

This work was performed with governmental authorizations from the Préfecture des Deux-Sèvres (Niort, France, 10.79-219). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Research. The protocol was approved by the Committee on the Ethics of Animal Experiments of the National Conservation Authority (permit 79349). When

Table 1: Number of male and female skylarks collected in France during December and January in the eight localities

Region	No. females	No. males	Total	Proportion of females	Latitude	Longitude
Nord	0	8	8	.00	50.60	3.08
Oise	1	19	20	5.00	49.50	2.41
Indre-et-Loire	5	41	46	10.87	47.32	.79
Indre	1	17	18	5.56	47.21	3.18
Deux-Sèvres	45	140	185	24.32	46.23	−.38
Charente-Maritime	38	56	94	40.43	46.05	−.37
Lot-et-Garonne	3	3	6	50.00	44.37	.08
Tarn et Garonne	35	77	112	31.25	43.83	1.2

catching birds, animals showing any sign of sickness were removed before entering the experiment. Bird captures were performed under permit from the National Hunting and Wildlife Agency to T. Powolny (2009-014). All experiments were in accordance with guidelines for the treatments and use of animals in behavioral research and teaching as published by the Association for the Study of Animal Behaviour.

Results

Effect of Latitudinal Gradient on Skylarks' Sex Ratio

Overall, male skylarks predominated in our sample, with 361 males and 128 females (table 1). We found no evidence that sex ratio varied among years ($F_{1,50} = 0.89$, $P = 0.344$) or along a longitude gradient ($F_{1,49} = 4.03$, $P = 0.02$). Conversely, skylark sex ratio varied significantly with latitude ($F_{1,49} = 20.25$, $P < 0.0001$). Specifically, the probability of finding a female decreased from 0.35 in southern France to less than 0.1 at more northern latitudes (fig. 1).

Metabolic Rates

As expected, absolute oxygen consumption ($\dot{V}_{O_2}$) significantly increased as T_a decreased (see also table 2 for statistical results of the full model with T_a and sex as independent variables). Individuals tested under thermoneutrality ($T_a = 20°C$) had the lowest absolute $\dot{V}_{O_2}$ (mean $\pm$ SE, 0.042 ± 0.002 mL min^{-1} g^{-1}). Conversely, individuals tested under thermogenesis conditions ($T_a = −5°C$) presented the highest levels of $\dot{V}_{O_2}$ (mean $\pm$ SE, 0.071 ± 0.006 mL min^{-1} g^{-1}). We observed no effect of sex or interaction between sex and T_a on $\dot{V}_{O_2}$ (table 2). Respiratory quotient was significantly influenced by T_a ($F_{2,36} = 6.5$, $P = 0.004$) but was not different between sexes ($F_{2,36} = 2.4$, $P = 0.141$) or affected by interaction between T_a and sex ($F_{2,36} = 1.4$, $P = 0.264$).

Body Temperature Profile

T_b measured in early evening (8:00 p.m.) and the following morning (8:00 a.m.) was similarly independent of sex and temperature treatment (fig. 2). A global decrease in T_b was detected during the night (mean ΔT_b: $−3.2° \pm 0.3°C$, $n = 40$; fig. 2); ΔT_b was related to initial T_b and was more pronounced in individuals

with higher initial T_b values (table 2). Also, ΔT_b differed between temperature treatments and sexes (sex $\times$ T_a, $F_{1,18} = 9.94$, $P = 0.005$; table 2). At 20°C, ΔT_b was not different between males and females (respectively, $−2.6° \pm 1.6°C$ and $−3.03° \pm 1.7°C$; post hoc t-test, $t = 0.736$, df = 18, $P = 0.471$; fig. 2A). The ΔT_b was significantly higher in females than in males (respectively, $−4.4° \pm 0.5°C$ and $−2.9° \pm 0.3°C$; post hoc t-test, $t = 2.143$, df = 18, $P = 0.012$; fig. 2B), while for males, ΔT_b was similar between temperature treatments (paired t-test, $t = 0.816$, df = 18, $P = 0.408$; fig. 2).

Change in Body Mass

Initial BMs did not differ between temperature treatments, but males were heavier than females at the onset of the experiment. Specifically, at 20° and −5°C, initial masses were, respectively, 38.7 ± 0.8 and 39.0 ± 0.8 g for males and 33.6 ± 0.9 and 33.7 $\pm$
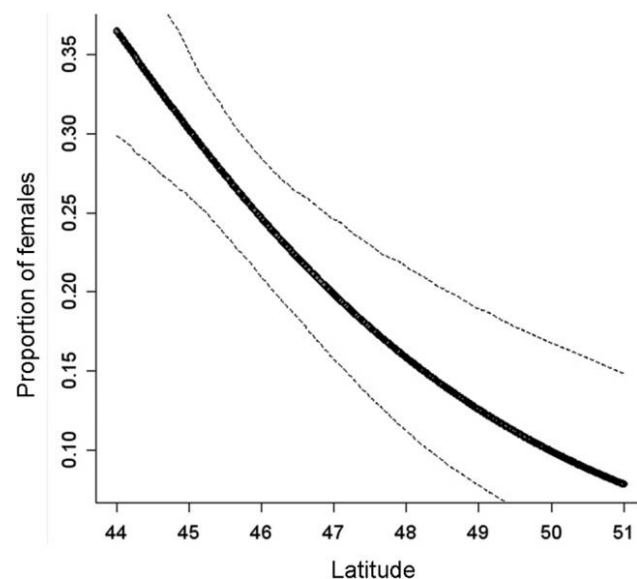

Figure 1. Proportion of females present at each site in relation to latitudinal gradient in France (at nine different locations, from a total of 489 individuals). General linear models were fitted with the proportion of females as the response variable using a binomial vector and a binomial error distribution. Lines represent the estimations obtained from the model and the 95% confidence interval.

Table 2: Results from generalized linear mixed models of oxygen consumption, body temperature (T_b), and body mass changes in skylarks

Dependent variable and fixed factor	Estimates	F	df	P
Oxygen consumption:				
Intercept	.304	.122	1, 18	.728
T_a ($-5°C$)	1.22	229.03	2, 18	**<.0001**
T_a ($6°C$)	.64	17.01	2, 18	**<.0001**
Sex (female)	$-.001$	1.768	1, 18	.092
Date	.277	.872	1, 18	.356
Mass	.058	2.021	1, 22	.164
T_a × sex	...	1.229	1, 18	.305
T_b:				
Intercept	37,711	868.6	1, 18	**<.0001**
Date	$-.255$	1.15	1, 33	.291
Initial T_b	$-.121$	1.24	1, 32	.273
Mass	.072	2.01	1, 22	.171
Sex (female)	.164	5.53	1, 18	**.03**
T_a ($-5°C$)	$-.314$	19.88	1, 18	**<.0001**
Sex × T_a	...	9.94	1, 18	**.005**
Sex × mass	...	3.92	1, 18	.056
ΔBody mass:				
Intercept	$-.202$	.041	1, 25	.842
Date	.255	3.99	1, 18	.061
Sex (female)	$-.179$	.482	1, 18	.496
T_a ($-5°C$)	.075	.284	1, 18	.6
Initial body mass	.093	13.156	1, 22	**.001**
ΔT_b	$-.133$	6.471	1, 37	**.015**
ΔT_b × sex	...	.975	1, 33	.331

Note. Ambient temperature (T_a) and sex were included as fixed factors in the models. Models were fitted with bird identity as a random factor. Results from the full models are provided. A backward selection procedure was used, with least significant variables being removed sequentially, until a minimum adequate model was reached in which all variables were retained at $P = 0.05$. Bold indicates significant P values.

0.6 g for females. All birds experienced BM loss overnight. This change in BM was not influenced by temperature treatment but was inversely related to initial BM in females (table 2). On average, males lost $3.1 ± 0.1$ g ($n = 20$), or $8.1\% ± 0.3\%$ of their initial BM. In females, these values were $2.4 ± 0.1$ g ($n = 20$), or $7.0\% ± 0.4\%$. The analysis further shows that mass loss was negatively related to ΔT_b overnight ($F_{1, 37} = 6.471$, $P = 0.015$; table 2). This relationship is well illustrated for birds exposed to a T_a of $-5°C$ (fig. 3; table 2; $F = 8.46$, $P = 0.006$).

Discussion

Skylark Latitudinal Distribution

We provide clear evidence for a winter spatial segregation between sexes at the scale of France, with a strongly biased male sex ratio at high latitudes and a more balanced sex ratio in the south. Probably due to the small range of longitude in our study, we observed no effect of longitudes on wintering skylark sex ratio. Assuming a balanced sex ratio on breeding grounds, females therefore migrate farther than males, supporting earlier reports of differential migration in skylarks in southern France (Hargues et al. 2007) and Italy (Scebba 2001). Despite an overlap

in wing length and BM between sexes (Hegemann et al. 2012), analyses of morphological measurements demonstrated that male skylarks are larger than females (Cramp 1980; appendix).

The body size hypothesis (Ketterson and Nolan 1976; Myers 1981) postulates that larger individuals are better suited to survive the colder and less predictable climates at higher latitudes. Cumulative effects of declining temperatures, increased energy costs of thermoregulation (Jackson et al. 2001), and declines in food availability may generate morphological, behavioral, or physiological adaptations that enhance winter survival. At high latitudes, birds typically face food limitation during winter imposed by a short photoperiod and obligate overnight fasting. Among major environmental factors impacting energy expenditure, temperature is a crucial parameter influencing metabolic level and energy requirements in endotherms (McNab 2002).

Metabolic Rate Does Not Differ between Males and Females

The body size hypothesis posits that under cold winter conditions, larger individuals have energetic cost of thermoregulation (Piersma 1984; McNamara et al. 2004). In small birds, wintering elevation of MR is often correlated with an improve-
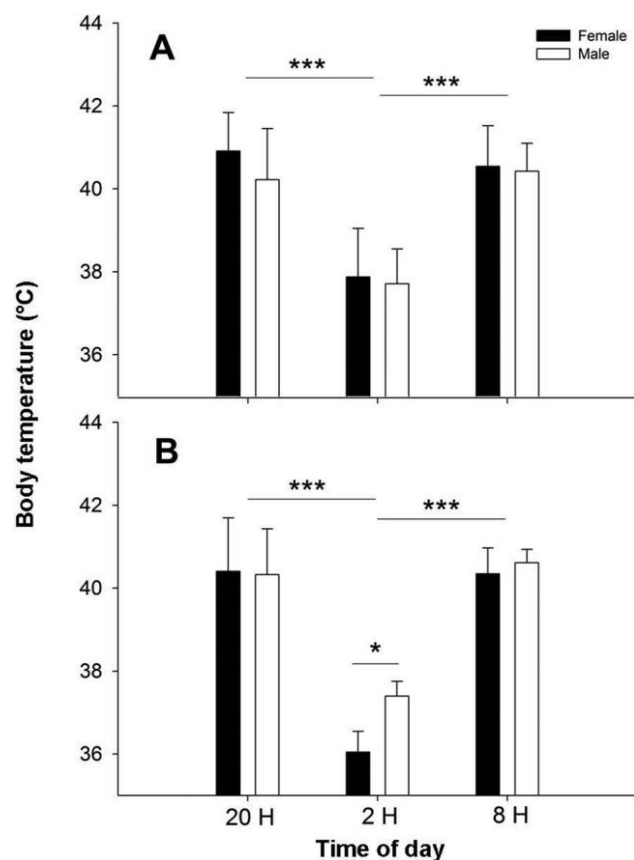
Figure 2. Change in body temperature of skylarks overnight for the various ambient temperatures (T_a). A, $T_a = 20°C$; B, $T_a = -5°C$. Means are presented $\pm$ SE. One asterisk, $P = 0.01$; three asterisks, $P = 0$.

ment in cold tolerance (Liknes et al. 2002; Liknes and Swanson 1996). However, using three different thermal treatments, we observed an increasing consumption rate of oxygen with a decreasing T_a. Males had a higher MR than females at all temperatures. However, MR was positively related to BM; hence, analyses based on mass-corrected MR revealed similar patterns of energetic expenditure between sexes (see Gillooly et al. 2001). These results suggest no additional energetic costs at low temperature for females, contradicting one of the main predictions derived from the body size hypothesis.

Sex Difference in Nightly Hypothermia

Under unfavorable environmental conditions, such as obligate overnight fasting, many bird species significantly decrease their rest-phase T_b below normothermic values. This state, depending on its depth, is called rest-phase hypothermia (T_b lowered by 3°–10°C) or torpor (T_b lowered by more than 10°C; McKechnie and Lovegrove 2002), though the distinction between rest-phase hypothermia and torpor remains controversial (Prinzinger et al. 1991). Contrary to hypothermia, in which only a decrease in T_b is described, torpor is characterized by a state of inactivity and a reduced responsiveness, usually characterized by

a reduced T_b and rate of metabolism. In this study, birds had a lower T_b in T_a of $-5°C$ (but still above 30°C), but their MR was higher and thus could not be considered in torpor but rather night hypothermia. Our results demonstrate that both male and female skylarks decreased their T_b during the night. Temperature decrease was more pronounced when T_a was lower, indicating a mechanism for reducing energy expenditure when thermoregulation imposes a high demand (Cooper and Gessaman 2005). Minimizing the difference between T_b and T_a also reduces the metabolic demand for heat production (McNab 2002). However, T_a alone may not fully explain the use of hypothermia (McKechnie and Lovegrove 2003) since this physiological adjustment is also dependent on the level of body reserves (see Hohtola et al. 1991). Importantly, we detected a significant sex difference in night hypothermia at $-5°C$: females decreased their T_b by more than 4° at 2:00 a.m., in contrast to males, which maintained a T_b reduction close to that observed at 20°C (fig. 2B). This adjustment suggests that energy constraints imposed by the low T_a are greater for females than for males. Such sexual difference may result from various processes. First, differences between sexes in plumage insulation could lead to differences in heat loss and energy budgets, resulting in different energy management strategies. Feather molt in autumn allows birds to increase body insulation before winter (Swanson 1991), but individual variation in winter plumage insulation is presumably associated with differences in perception of cold between individuals for a given set of ambient conditions (Vézina et al. 2009). Individuals experiencing greater heat loss for a given T_a could consequently rely more on hypothermia as a means for balancing their energy budget than individuals expressing high levels of thermal insulation. Second, individuals may differ in their use of thermal microhabitats, which may be dependent on their sex, age, and/or position in the social hierarchy. Skylarks flock in winter (Powolny et al. 2012), and differences in social dominance could result in differences in monopolizing the
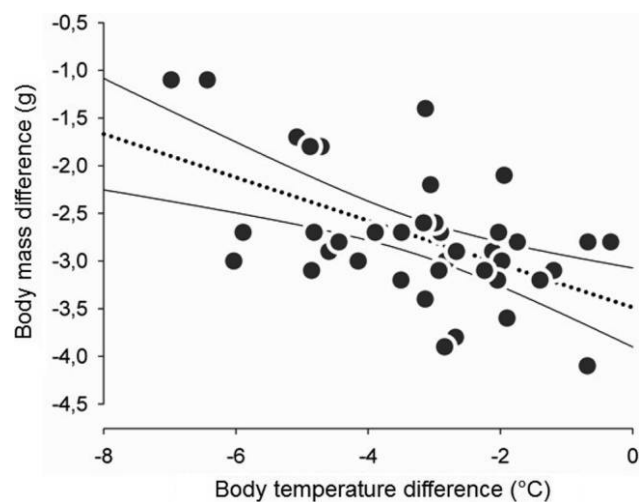

Figure 3. Relationship between body temperature decrease and body mass change for captive skylarks exposed to $-5°C$ overnight. Lines represent the least square regression slope and 95% confidence interval ($F = 15.81$, $P = 0.0003$, $r^2 = 0.29$).

best food sources (Piper 1990) or nocturnal roosts (Koivula et al. 1995). Thus, dominant individuals (males) could take advantage of shelters or microhabitats where heat gain might reduce their thermoregulation costs, which in turn would be reflected in individual variation in hypothermia.

We also found a negative relationship between the magnitude of body temperature decrease (ΔT_b) and BM loss (ΔT_m), suggesting that decreasing T_b allowed the maintenance of body condition when the environment becomes thermally costly. Therefore, female skylarks showed physiological compensation to low temperatures, in response to thermal environmental changes, while males did not. Although T_b was not measured during MR tests here, we suggest that the apparent similarity in mass-specific MRs was related to variation in T_b during overnight measurements. In particular we predict that when the temperature becomes challenging, females use more pronounced hypothermia to maintain their energetic balance.

Passive Hypothermia and Segregation

If females modulate their T_b to reduce their energetic expenditure to a level similar to males, why do females winter at more southern latitudes than males? While hypothermia is widespread in endotherms to minimize energy expenditure and spare body reserves, the relative decrease in T_b associated with low T_a may also incur costs (Boyles et al. 2007; Nord et al. 2009). For example, the decrease in internal temperature may compromise physiological fitness-related processes that are temperature dependent, such as immune function (Canale and Henry 2011) or somatic repair (Larkin and Heller 1999). Moreover, such physiological costs may be further amplified by ecological costs, such as increased predation risk (Laurila and Hohtola 2005) or failure to access food resources (Palchykova et al. 2006). Such constraints are widely viewed as one of the major disadvantages of hypothermia (Pavey and Geiser 2008). Consequently, energetic benefits from hypothermia could be directly dependant on the costs of rewarming. Rewarming to the normothermic T_b can involve two not mutually exclusive mechanisms: a passive rewarming or a metabolic rewarming (Pavey and Geiser 2008). Previous studies demonstrated that passive rewarming either by the increase in T_a or by basking in the sun appears to be common in heterothermic mammals and birds (Geiser and Drury 2003) and provides a clear benefit to reduce energy expenditure during rewarming (Geiser et al. 2004). The solar energy reaching the ground in France during winter increases southward, and it is thus possible that female distribution during winter reflects the potential passive rewarming or basking due to increase in T_a in the morning (Geiser and Pavey 2007), thus decreasing the cost of hypothermia. In this way, female skylarks may reduce the metabolic costs of rewarming by benefiting from sun basking and optimize the benefits of hypothermia by wintering at southern latitudes in France.

Overall, our study on the skylark demonstrates (i) clear spatial segregation, (ii) dynamic physiological response to low temperatures, and (iii) sex differences in physiological compensation, with females reducing their T_b more than males. Size-related differences in thermal tolerance and thermoregulatory costs may well provide explanation for the observed winter distribution pattern. Yet, the understanding of sexual segregation may involve multiple causes, and disentangling competing hypotheses remains difficult. Notably, variation in body size may affect multiple ecological (i.e., competition for food and breeding territories) and physiological (thermal tolerance, risk of starvation, MR) aspects. Studying systems where sexes are of equal size or exhibit reversed sexual size dimorphism may provide significant insights in this matter.
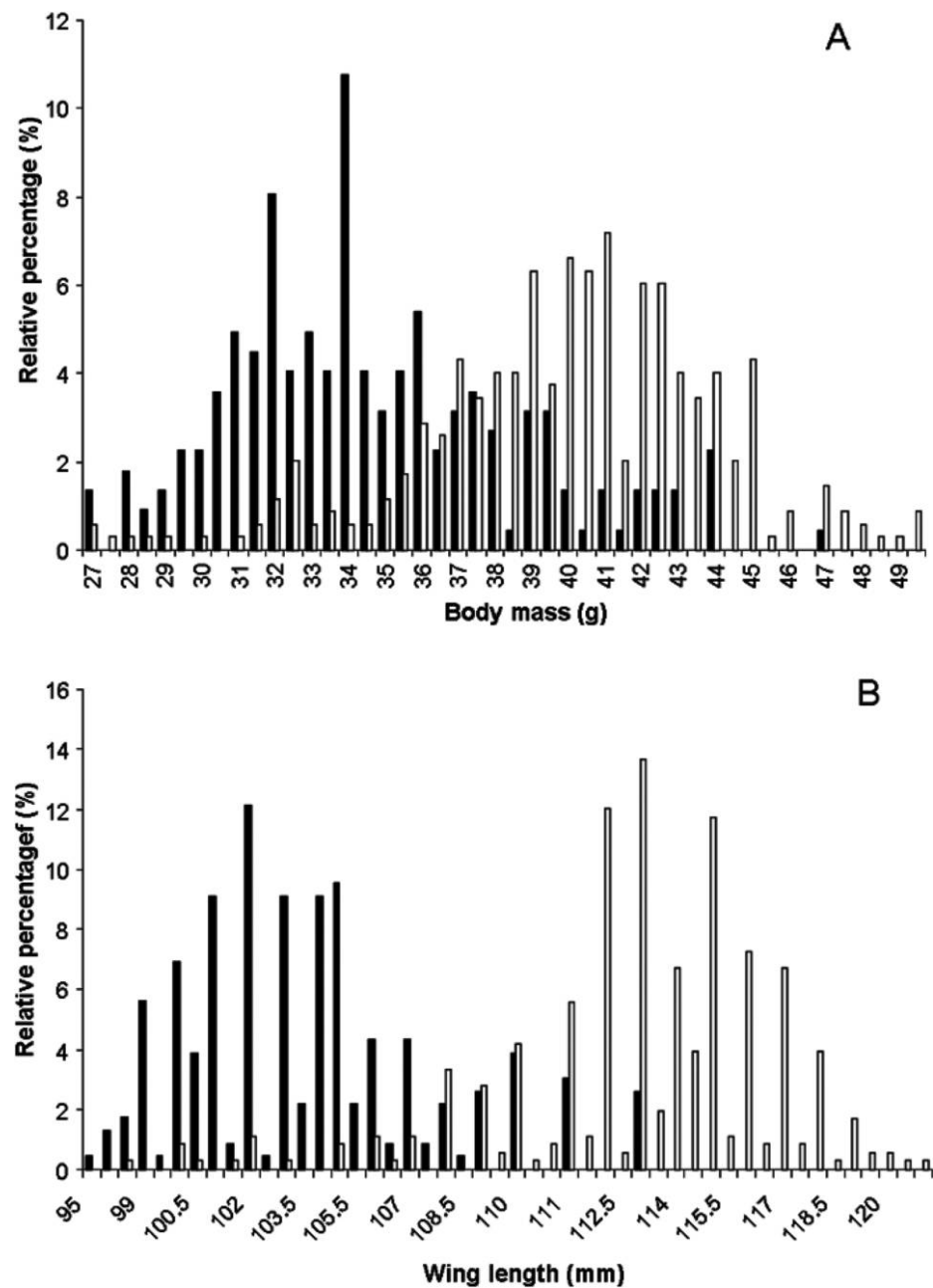
Acknowledgments

APPENDIX

Figure A1. Relative percentage of male (open bars) and female (filled bars) skylarks ($n = 584$) in function of two morphological body measures: body mass (g; *A*) and wing length (mm; *B*). Mean comparisons confirmed sexual differences for body mass (*t*-test, $t = -17.07$, df = 584, $P < 0.0001$) and wing length ($t = -30.33$, df = 584, $P < 0.0001$).

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