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PHENOTYPIC VARIATION OF LARKS ALONG AN ARIDITY GRADIENT: ARE DESERT BIRDS MORE FLEXIBLE?

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Abstract. We investigated interindividual variation and intra-individual phenotypic flexibility in basal metabolic rate (BMR), total evaporative water loss (TEWL), body temperature (T_b), the minimum dry heat transfer coefficient (*h*), and organ and muscle size of five species of larks geographically distributed along an aridity gradient. We exposed all species to constant environments of 15°C or 35°C, and examined to what extent interspecific differences in physiology can be attributed to acclimation. We tested the hypothesis that birds from deserts display larger intra-individual phenotypic flexibility and smaller inter-individual variation than species from mesic areas.

Larks from arid areas had lower BMR, TEWL, and *h,* but did not have internal organ sizes different from birds from mesic habitats. BMR of 15°C-acclimated birds was 18.0%, 29.1%, 12.2%, 25.3%, and 4.7% higher than of 35°C-acclimated Hoopoe Larks, Dunn's Larks, Spike-heeled Larks, Skylarks, and Woodlarks, respectively. TEWL of 15°C-acclimated Hoopoe Larks exceeded values for 35°C-acclimated individuals by 23% but did not differ between 15°C- and 35°C-acclimated individuals in the other species. The dry heat transfer coefficient was increased in 15°C-acclimated individuals of Skylarks and Dunn's Larks, but not in the other species. Body temperature was on average 0.4°C ± 0.15°C (mean ± 1 SEM) lower in 15°C-acclimated individuals of all species. Increased food intake in 15°C-acclimated birds stimulated enlargement of intestine (26.9–38.6%), kidneys (9.8–24.4%), liver (16.5–27.2%), and stomach (22.0–31.6%). The pectoral muscle increased in 15°C-acclimated Spike-heeled Larks and Skylarks, remained unchanged in Hoopoe Larks, and decreased in 15°C-acclimated Woodlarks and Dunn's Larks. We conclude that the degree of intra-individual flexibility varied between physiological traits and among species, but that acclimation does not account for interspecific differences in BMR, TEWL, and *h* in larks. We found no general support for the hypothesis that species from desert environments display larger intra-individual phenotypic flexibility than those from mesic areas.

The coefficient of variation of larks acclimated to their natural environment was smaller in species from arid areas than in species from mesic areas for mass-corrected BMR and surface-specific *h,* but not for mass-corrected TEWL. The high repeatabilities of BMR, TEWL, and *h* in several species indicated a within-individual consistency on which natural selection could operate.

Key words: Alaudidae*; aridity, basal metabolic rate; dry heat transfer coefficient; larks; phenotypic flexibility; phenotypic plasticity; total evaporative water loss.*

Introduction

Efforts to understand physiological diversity have traditionally concentrated on explaining variation among species from different environments whereas few studies have focused on intraspecific variation in physiological phenotypes, either between or within individuals. Because the geographical distribution of most species includes different environments, it is unlikely that a single phenotype has high fitness in all conditions. Phenotypic plasticity, a concept that includes changes in adult phenotypes depending on the environment (acclimatization or acclimation) and differences among phenotypes resulting from developmental conditions (ontogenetic plasticity), can be a solution to the problem of adaptation to spatially or temporally heterogeneous environments (Via et al. 1995, Schlichting and Pigliucci 1998). Variation in physiological phenotypes among species (interspecific variation) or among individuals within a species (interindividual variation) may involve a combination of genotypic diversity that is potentially influenced by natural selection and irreversible and/or reversible phenotypic adjustments. Reversible changes in individual phenotypes that reflect flexible responses to

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changing tasks have been termed phenotypic flexibility (Piersma and Lindström 1997). Although this intra-individual phenotypic flexibility is not mediated by heritable change, the capacity to change could be under the influence of natural selection.

Why some species are more phenotypically plastic and/or genetically diverse than others has been strongly debated (Parsons 1987, 1996, Via et al. 1995, Schlichting and Pigliucci 1998). One hypothesis predicts that intra-individual phenotypic flexibility will be large in species from temporally heterogeneous environments where ecological situations vary in the course of an individual's life (Schlichting and Pigliucci 1998). The maintenance of interindividual variation among phenotypes appears dependent on the frequency of environmental change and on the spatial or temporal nature of heterogeneity of the environment. Spatial heterogeneity can maintain genetic variation and temporal heterogeneity favors phenotypic plasticity, both potentially resulting in phenotypic variation (Hedrick 1986, Schlichting and Pigliucci 1998).

Despite a lack of water, scarce food resources, and high ambient temperatures (T_a), deserts harbor a number of bird species. Interspecific comparisons have shown that in these species, basal and field metabolic rates are on average 17% and 49% lower, respectively, and total evaporative water loss rates (TEWL) are ~35% lower than in birds from mesic areas (Williams 1996, Tieleman and Williams 2000). These findings are consistent with the idea that birds in deserts have adjusted their physiology to the environment (Bartholomew and Cade 1963, Dawson and Schmidt-Nielsen 1964, Serventy 1971, Dawson 1984, Withers and Williams 1990). The extent to which the differences in basal metabolic rate (BMR) and TEWL between desert and non-desert species can be attributed to genetic adaptations or phenotypic plasticity remains obscure.

Some birds adjust BMR in response to season (Kendeigh 1969, Pohl and West 1973, Cooper and Swanson 1994, Piersma et al. 1995) or to varying temperatures during acclimation experiments (Gelineo 1964, Williams and Tieleman 2000), whereas others do not change BMR in the field or in the laboratory (Hudson and Kimzey 1966, O'Connor 1995). Not only metabolic rates, but also organs, show flexibility in some birds, usually in response to alterations in diet or environment (Karasov 1996, Piersma and Lindström 1997). The flexibility of other components of an individual's physiology such as TEWL, body temperature (T_b), and the dry heat transfer coefficient (h) has received less attention (Williams and Tieleman 2000). Total evaporative water loss can be reduced in small granivorous birds in response to water deprivation (Cade et al. 1965, Dawson et al. 1979), but this feature is usually not considered in the context of acclimation to T_a.

We investigated intra-individual phenotypic flexibility and interindividual variation in BMR, TEWL, h, T_b, and organ sizes of five species of larks that are distributed over an aridity gradient. When aridity increases, decreasing water and food availability and increasing T_as could exert stronger selection on the rates of metabolism and water loss in birds. Hoopoe Larks (*Alaemon alaudipes*) and Dunn's Larks (*Eremalauda dunni*) occur in arid deserts, Spike-heeled Larks (*Chersomanes albofasciata*) in semi-arid regions, and Woodlarks (*Lullula arborea*) and Skylarks (*Alauda arvensis*) live in mesic temperate habitats (Cramp 1988, Pätzold 1994). We examined the extent to which acclimation to T_a contributes to interspecific differences in physiology. In addition, we tested the hypothesis that with increasing aridity, when selection pressures on the energy and water balance might be stronger and the temporal heterogeneity of the environment larger, birds display more intra-individual flexibility and less interindividual variation in their physiology than do species from more moderate climates.

Methods

Animals

Skylarks (*Alauda arvensis*) and Woodlarks (*Lullula arborea*) were mist-netted in the late spring of 2000 in the northern part of the Netherlands (52°52′ N, 06°20′ E), and housed in outdoor aviaries at the Zoological Laboratory of the University of Groningen. We captured Hoopoe Larks (*Alaemon alaudipes*) and Dunn's Larks (*Eremalauda dunni*) during June 2001 in Mahazat as-Sayd, a reserve in the west-central Arabian Desert (22°15′ N, 41°50′ E) and transported them to the National Wildlife Research Center, near Taif, Saudi Arabia (see Plate 1). Spike-heeled Larks (*Chersomanes albofasciata*) were captured in May and October of 2001 on Benfontein game farm, Northern Cape, South Africa (28°50′ S, 24°50′ E) and transferred to Rhodes University, Grahamstown. All birds spent 3–6 wk in captivity prior to experimentation. Studies were carried out under license DEC 2425 from the Animal Experimentation Committee of the University of Groningen.

Protocol

We measured BMR and TEWL of all birds before assigning them to one of two groups, each with equal numbers of males and females; birds were similar in body mass in both assemblages for all species. Before pre-acclimation measurements, birds were kept in outdoor aviaries under natural day length and climate conditions. One group of each species was then housed in a constant T_a room at 15 ± 2°C (12L:12D), a T_a below the thermoneutral zone of all species and close to the average T_a experienced by larks in the Netherlands during the breeding season. We placed the other group in a room with a T_a of 35 ± 2°C (12L:12D) to mimic environmental temperatures of the Arabian Desert during spring. Birds were housed in cages of 1 × 1 × 2 m. Absolute humidities were not controlled, but mea-

PLATE. 1. The Hoopoe Lark (left) lives in Mahazat as-Sayd (right), the arid extreme of the gradient.

sured to be 5–7 g H_2O/m^3 in the 15°C rooms in all locations, and 9–12 g H_2O/m^3 in the 35°C rooms in the Netherlands and Saudi Arabia, and 32 g H_2O/m^3 in South Africa. We fed birds a mixture of seeds, insects, raw beef heart, and boiled eggs.

Metabolism and evaporative water loss

We measured basal rates of oxygen consumption and TEWL for postabsorptive birds during their nocturnal phase using standard flow-through respirometry and hygrometry methods (Gessaman 1987, Williams and Tieleman 2000). Measurements of BMR were made at T_a values previously established to be within the thermoneutral zone of all species: 25–30°C for Skylarks (n = 14), 30°C for Woodlarks (n = 14), and 35°C for Dunn's Larks (n = 16), Hoopoe Larks (n = 14), and Spike-heeled Larks (n = 20). The initial and final values for TEWL, the minimum h, and T_b were based on measurements at 25°C for all species. For the Spike-heeled Lark, we combined data for BMR of the experiments in May (cold + warm: n = 10) and in October (cold + warm: n = 10). For TEWL, h, and T_b for this same species we used data from October only, because we did not measure TEWL and T_b at 25°C in May.

Details of our laboratory setup and measurement protocol in Saudi Arabia and the Netherlands and calculations of oxygen consumption and evaporative water loss are given elsewhere (Williams and Tieleman 2000, Tieleman et al. 2002). In brief, birds were fasted for 3 h prior to the start of our metabolism trials. They were then placed in a metabolic chamber on a wire-mesh platform over a layer of mineral oil that trapped feces, thus excluding feces as a source of water in the measurements. Air coursed through Drierite (W. A. Hammond Drierite Company, Xenia, Ohio, USA), soda lime, and Drierite, the chamber, a dew-point hygrometer (model M4-DP, General Eastern, Wilmington, Massachusetts, USA), and again through Drierite, soda lime, and Drierite, before passing through the mass flow controller (model 5850E, Brooks Instruments, Hatfield, Pennsylvania, USA), a diaphragm pump, and into an overflow from which the O_2-analyzer sampled air (in Saudi Arabia: model S-3A/II, Applied Electrochemistry, Pittsburgh, Pennsylvania, USA; in the Netherlands: model 4100, Servomex Xentra, Tulsa, Oklahoma, USA). After a 2–3-h equilibration period, we recorded the oxygen concentration and dew point of inlet and outlet air, the T_a of the dew-point hygrometer and the chamber, using a data logger (model 21X or CR23X, Campbell Scientific, Logan, Utah, USA). Outlet air had a relative humidity that was always below 25% (Lasiewski et al. 1966) and an oxygen concentration between 20.55% and 20.85%. When, during the third hour of measurements, the traces for oxygen consumption and dew point were stable for at least 10 min, we noted these times and used these data for calculations.

In South Africa, Spike-heeled larks were fasted for 3 h, weighed to ±0.1 g, and then placed in a perspex metabolic chamber (29 × 18 × 18 cm) with an airtight lid and on a wire mesh platform over a layer of mineral oil to trap feces. The chamber was placed inside a darkened, constant-temperature cabinet. A thermocouple was inserted into the chamber through a rubber stopper to measure chamber T_a, and a passive infrared sensor mounted inside the chamber detected activity. Air, drawn from outside the laboratory, passed through columns of Drierite, Ascarite, and Drierite to remove CO_2 and water vapor. Air then passed through a side-trak mass flow controller (Sierra Instruments, Monterey, California, USA) set at 700 mL/min before entering the chamber. Air exiting the chamber passed again through columns of Drierite, Ascarite, and Drierite before a subsample was drawn through an O_2-analyzer (model FC-1B, Sable Systems, Las Vegas, Nevada, USA). After a 60-min equilibration period, readings of percentage O_2, flow rate, chamber temperature,

and bird activity were recorded at 20-s intervals for 2 h using DATACAN V data acquisition software (Sable Systems International 1996). Percentage O_2 of inlet air, assumed to be 20.95%, was measured before and after each experimental run using the Sable Systems computer-controlled baselining system. Calculations of oxygen consumption were carried out with the DATACAN V analysis program using Equation 4a in Withers (1977), and were converted to heat production assuming 20.08 J/mL O_2 (Schmidt-Nielsen 1997). BMR was calculated from the lowest, stable 10 min of oxygen consumption.

For Spike-heeled Larks, we calculated TEWL by measuring the difference in the amount of water vapor in the air immediately before entering and after leaving the chamber using a relative humidity probe (model MCS 174, MC Systems, Cape Town, South Africa). Measurements of RH and temperature were recorded at 1-min intervals onto an MC-120E data logger (MC Systems). The amount of water vapor in the inlet and outlet air (milligrams per minute) was subsequently calculated from measurement of RH and T_a (Smithsonian Tables in Lide and Frederikse 1997). TEWL was determined as the difference between the amount of water vapor entering and leaving the chamber, and averaged over the last 30 min of measurement.

After metabolism measurements, we immediately measured cloacal T_b in all larks with a thermometer (OMEGA Engineering, Stamford, Connecticut, USA, or Sensortek, Costa Mesa, California, USA) and an Omega copper-constantan thermocouple (30 gauge). Because we did not have continuous recordings of T_b, we calculated the dry heat transfer coefficient (h) as $h = M - E/(T_b - T_a)$, and assumed that the change in T_b during our measurements was zero (Tieleman and Williams 1999). In this equation, M equals metabolic heat production (kilojoules per day), and E is evaporative heat loss (kilojoules per day).

To establish if a three-week period was sufficiently long for birds to complete any adjustments to their metabolic rate (MR) and TEWL, we measured these variables at 25°C in Woodlarks and Skylarks after two weeks and after three weeks. We calculated the change in body mass, MR, TEWL, and h, and tested for differences in change between 35°C and 15°C treatments with an ANOVA, but found no significant differences (mass: treatment $F_{1,25} = 1.05$, $P = 0.32$, species $F_{1,25} = 1.5$, $P = 0.71$; MR: treatment $F_{1,25} = 0.34$, $P = 0.57$, species $F_{1,25} = 0.98$, $P = 0.33$: TEWL, treatment $F_{1,25} = 2.24$, $P = 0.15$, species $F_{1,25} = 0.34$, $P = 0.57$; h: treatment $F_{1,25} = 0.02$, $P = 0.88$, species $F_{1,25} = 2.18$, $P = 0.15$). We then pooled the data for 35°C- and 15°C-acclimated groups and tested if the changes in mass, MR, TEWL and h differed significantly from zero, but found no statistical support (mass: $t = 0.05$, df = 27, $P = 0.96$; MR: $t = 1.08$, df = 27, $P = 0.29$; TEWL: $t = 1.79$, df = 27, $P = 0.09$; h: $t = 0.47$, df = 27, $P = 0.64$). We concluded that three weeks of acclimation was ample time for birds to adjust their physiology to these environments. For Woodlarks and Skylarks, we used the average values after two and three weeks as finals for TEWL, h, and T_b.

Food intake

We measured food intake of Woodlarks, Skylarks, Hoopoe Larks, and Dunn's Larks in the cold and warm rooms during week 3 of the acclimation period by isolating individual birds in small cages (50 × 30 × 30 cm) for 24 h. We fed Skylarks and Woodlarks a diet of mealworms and dry insects, Dunn's Larks mealworms and seeds, and Hoopoe Larks mealworms only. Food was weighed to ±0.1 g before and after the 24-h trial period. We also placed a weighed amount of food outside the cage and reweighed it after the 24-h trial period to account for desiccation of the food in our calculations. Birds maintained mass during the food intake measurements.

Body composition

At the end of the 3-wk acclimation period, we sacrificed the birds and dissected out their organs and muscles of the pectoral region on the left side of the body. Organs and muscles were dried to constant mass for 2 d at 75°C and weighed on a Mettler analytical balance (Mettler, Greifensee, Switzerland) to ±0.1 mg.

Repeatability

Repeatability (r) is a measure of within-individual consistency of a character, estimated from multiple measurements of the same individual, and sets an upper limit to heritability (Lessells and Boag 1987, Boake 1989, Falconer and Mackay 1996). The repeatability is defined as $r = (V_G + V_{Eg})/V_P$, where $V_P = V_G + V_{Eg} + V_{Es}$ is the total phenotypic variance, V_G is the genotypic variance, V_{Eg} is the general environmental variance common to all repeated measurements of the same individual due to permanent effects, and V_{Es} is the special environmental variance within individuals due to temporary factors (Falconer and Mackay 1996). Repeatabilities can be calculated as: $r = S_A^2/(S^2 + S_A^2)$, where S_A^2 is the among-individual variance and S^2 the within-individual variance (Falconer and Mackay 1996). The variance components were derived from mean squares in a one-way analysis of variance with BMR, TEWL, or h as the dependent variables and individual and treatment as fixed factors: $S^2 = MS_W$ and $S_A^2 = (MS_A - MS_W)/n_0$, where MS_W is the error mean square, MS_A the mean square among individuals, and n_0 a coefficient related to the sample size per individual (Lessells and Boag 1987). Incorporating treatment as fixed factor in the analyses accounted for the effect of acclimation. Standard errors were calculated following Becker (1984).

Statistical analyses

Statistical analyses were performed using SPSS 10.0 (SPSS 1999). Values are presented as means ± 1 SD

TABLE 1. Body masses of 35°C and 15°C treatment groups before and after three weeks of acclimation for Skylark, Woodlark, Spike-heeled Lark, Dunn's Lark, and Hoopoe Lark.

Species	Treatment	*n*	Initial mass (mean ± 1 SD)	*P*	Final mass (mean ± 1 SD)	*P*	Difference (mean ± 1 SD)	*P*
Skylark	35°C	7	31.4 ± 3.31	0.84	32.2 ± 3.59	0.23	0.8 ± 1.72	0.11
	15°C	7	31.7 ± 2.69		34.9 ± 4.46		3.2 ± 3.31*	
Woodlark	35°C	7	25.8 ± 1.39	0.36	27.0 ± 1.24	0.03	1.2 ± 1.08*	0.006
	15°C	7	25.3 ± 0.48		28.7 ± 1.44		3.5 ± 1.49*	
Spike-heeled Lark	35°C	10	23.9 ± 3.67	0.32	25.5 ± 3.26	0.31	1.7 ± 1.23*	0.96
	15°C	10	25.4 ± 3.04		27.1 ± 3.23		1.6 ± 2.37	
Dunn's Lark	35°C	8	20.7 ± 1.47	0.66	22.2 ± 1.65	0.53	1.5 ± 0.92*	0.64
	15°C	8	20.3 ± 2.13		21.6 ± 2.26		1.3 ± 1.02*	
Hoopoe Lark	35°C	7	37.1 ± 3.76	0.56	39.0 ± 3.73	0.90	1.9 ± 1.92*	0.28
	15°C	7	35.9 ± 3.78		38.8 ± 4.18		2.9 ± 1.15*	

Note: Significance of differences between the 35°C and 15°C groups are indicated with *P* values based on *t* tests.
* Differences significantly different from zero ($P < 0.05$).

unless noted otherwise. We used analysis of covariance (ANCOVA) with BMR, TEWL, or *h* as the dependent variable, treatment as fixed factor, and mass as a covariate. Although we always tested the interaction between covariate and fixed factor, we do not always report the results of insignificant interactions. Proportional data were arcsine square-root transformed before performing parametric statistics (Zar 1996).

RESULTS

Body mass

Before acclimation, average body mass did not differ between the 15°C and 35°C groups in any of the species, but during the acclimation period, individuals in all groups gained mass, except for those in the 35°C group of the Skylarks and in the 15°C group of the Spike-heeled Larks (Table 1). Woodlarks acclimated to 15°C gained significantly more mass and were heavier after three weeks than 35°C-acclimated conspecifics, but the change in mass during acclimation did not significantly differ between treatments in the other four species (Table 1). When we combined all five species in an ANOVA with change in mass as the dependent variable and species and treatment as fixed factors, we found a significant effect of treatment ($F_{1,72} = 5.45$, $P = 0.022$), but no significant effects of species ($F_{4,72} = 0.87$, $P = 0.48$) or of the interaction term (species × treatment $F_{4,68} = 1.95$, $P = 0.11$). We concluded that the overall effect of the acclimation period for all species was a larger increase in mass in the 15°C group than in the 35°C group, even though species-specific differences were only evident between groups of the Woodlarks.

Basal metabolic rate

Prior to acclimation, BMR did not differ between groups in any of the species, except for the Woodlark (Fig. 1), where random assignment of individuals to the two groups resulted, accidentally, in a significant effect of the interaction between mass and group (ANCOVA $F_{1,10} = 6.11$, $P = 0.033$). After three weeks of acclimation, the two groups of Woodlarks did not differ significantly in BMR ($F_{1,11} = 1.37$, $P = 0.27$), although the 15°C-acclimated birds tended to have a higher BMR than the 35°C-acclimated individuals. Skylarks, Spike-heeled Larks, Dunn's Larks, and Hoopoe Larks in the 15°C groups had significantly higher BMR than birds in the 35°C groups (Skylark: $F_{1,11} = 26.96$, $P < 0.001$; Spike-heeled Lark: $F_{1,17} = 4.66$, $P = 0.045$; Dunn's Lark: $F_{1,13} = 42.71$, $P < 0.001$; Hoopoe Lark: $F_{1,11} = 11.72$, $P = 0.006$) (Fig. 1). Corrected for body mass differences between treatments and expressed as a percentage of BMR of the 35°C group, BMR in the 15°C group was increased by 4.7% in the Woodlark, 25.3% in the Skylark, 12.2% in the Spike-heeled Lark, 29.1% in the Dunn's Lark, and 18.0% in the Hoopoe Lark.

To facilitate comparisons among species, we calculated residuals of BMR based on an allometric equation for 12 species of larks: log BMR = 0.225 + 0.901 log mass, with BMR measured in kilojoules per day, and mass measured in grams (Tieleman et al. 2003) (Fig. 2A). Woodlark and Skylark had higher residual BMR values than the other three species (Fig. 2A). We used univariate ANOVA with the residual BMR of all initials, or of the finals after acclimation to 15°C and 35°C as dependent variables to test for differences between species. Species had a significant effect on residual BMR (initial values: $F_{4,79} = 57.26$, $P < 0.0001$; 15°C: $F_{4,34} = 94.52$, $P < 0.0001$; 35°C: $F_{4,34} = 61.00$, $P < 0.0001$), and subsequent post hoc tests indicated that BMR was below predictions in the Hoopoe Larks, near predictions in Dunn's Larks and Spike-heeled Larks, and exceeded predictions in Skylarks and Woodlarks (Fig. 2A).

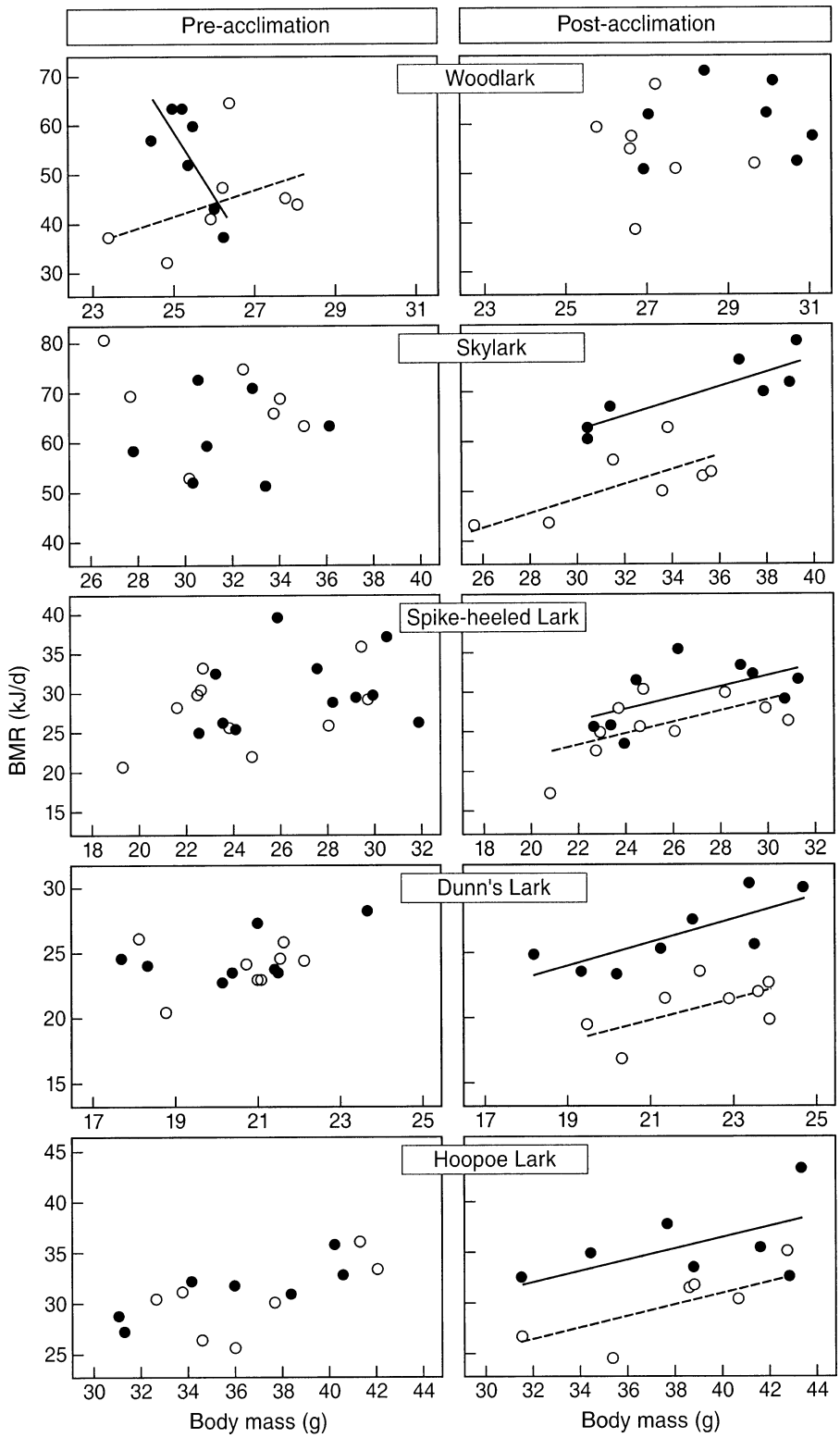

FIG. 1. Basal metabolic rate (BMR) as a function of body mass of birds assigned to acclimation at 35°C (open symbols) and 15°C (solid symbols) for Woodlark, Skylark, Spike-heeled Lark, Dunn's Lark, and Hoopoe Lark when acclimated to their natural environment (pre-acclimation) and after acclimation to 35°C and 15°C (post-acclimation). Lines indicate significant differences between groups acclimated to 15°C and 35°C.

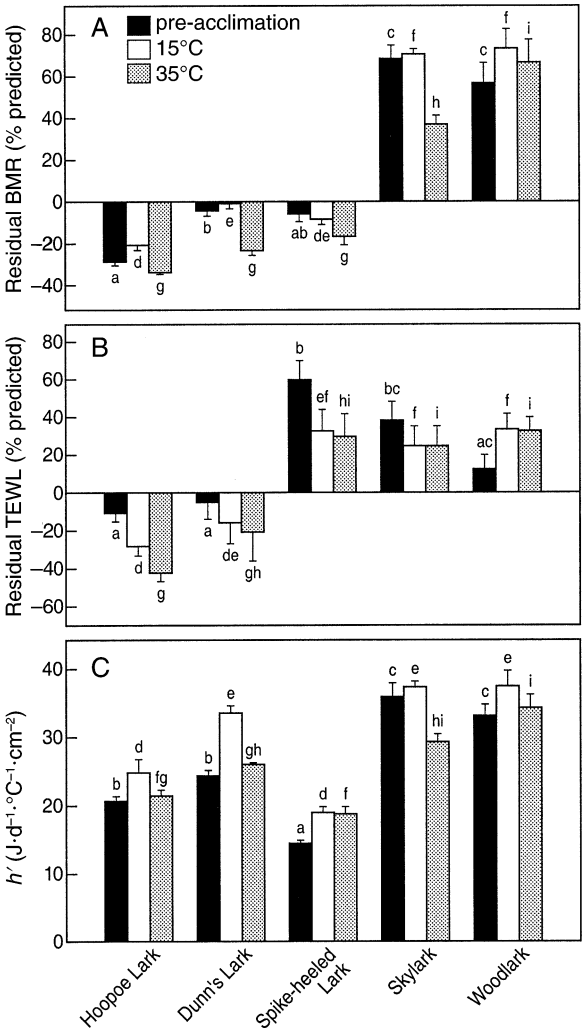

FIG. 2. (A) Residuals of basal metabolic rate (BMR, mean ± 1 SEM) of Hoopoe Lark, Dunn's Lark, Spike-heeled Lark, Skylark and Woodlark when acclimated to the outside environment (pre-acclimation) and after acclimation to 15°C or 35°C. Common letters indicate no statistically significant difference between species when analyzed with separate Tukey tests for pre-acclimation values, and for post-acclimation values for 15°C and 35°C groups (critical $P = 0.05$). (B) Residuals of total evaporative water loss (TEWL, mean ± 1 SEM) of Hoopoe Lark, Dunn's Lark, Spike-heeled Lark, Skylark, and Woodlark when acclimated to the outside environment (pre-acclimation) and after acclimation to 15°C or 35°C. (C) Surface-specific minimum dry heat transfer coefficient (h', mean + 1 SEM) of Hoopoe Lark, Dunn's Lark, Spike-heeled Lark, Skylark, and Woodlark when acclimated to the outside environment (pre-acclimation) and after acclimation to 15°C or 35°C.

Total evaporative water loss

Initial rates of TEWL at 25°C did not differ between birds assigned to the 35°C and 15°C groups in Woodlark, Spike-heeled Lark, Dunn's Lark, and Hoopoe Lark ($P > 0.05$). In Skylarks, TEWL was significantly higher in the 15°C group than in the 35°C group, despite random assignment of individuals ($F_{1,11} = 13.02$, $P = 0.004$) (Fig. 3). Final rates of TEWL did not differ between 15°C- and 35°C-acclimated groups in Woodlarks, Spike-heeled Larks, and Dunn's Larks (Woodlark: $F_{1,11} = 0.74$, $P = 0.41$; Spike-heeled Lark: $F_{1,7} = 0.24$, $P = 0.64$; Dunn's Lark: $F_{1,13} = 0.04$, $P = 0.85$), but were significantly higher in the 15°C-acclimated groups of Hoopoe Lark and Skylark (Hoopoe Lark: $F_{1,11} = 7.85$, $P = 0.017$, Skylark: $F_{1,11} = 5.80$, $P = 0.035$) (Fig. 3). Hoopoe Larks had TEWL rates 23% higher in 15°C-acclimated birds. Because initial values of Skylarks differed between birds assigned to the 15°C and 35°C group, we calculated the difference in TEWL between pre- and post-acclimation TEWL for each individual and tested if these differences were the same for the 15°C-acclimated and the 35°C-acclimated group. The average difference between pre- and post-acclimation TEWL in the 15°C group was 0.05 ± 0.85 g/day and in the 35°C group was −0.28 ± 0.53 g/day, with values not significantly different ($t = 0.89$, df = 12, $P = 0.38$). We concluded that Skylarks did not change their TEWL in response to a three-week acclimation period at either 15°C or 35°C.

We calculated residuals of TEWL based on the allometric equation for 12 species of larks: log TEWL = −0.814 + 0.816 log mass, with TEWL measured in grams per day and mass measured in grams (Tieleman et al. 2003) (Fig. 2B). TEWL for Dunn's Lark and Hoopoe Lark were below predictions, whereas TEWL for the other three species exceeded predictions (Fig. 2B). We used ANOVA with pre- or post-acclimated TEWL as the dependent variable to test for differences between species. Because TEWL of Skylarks differed between 15°C and 35°C room birds, but did not change in response to acclimation, we combined data of 15°C-acclimated and 35°C-acclimated Skylarks as final values in the analyses. Species had a significant effect on residual TEWL (initial values: $F_{4,63} = 11.40$, $P < 0.0001$; 15°C: $F_{4,36} = 6.98$, $P < 0.0001$; 35°C: $F_{4,36} = 8.73$, $P < 0.0001$), and subsequent post hoc tests indicated that residuals of TEWL did not differ between Dunn's Larks and Hoopoe Larks, or between Skylarks and Woodlarks (Fig. 2B).

Dry heat transfer coefficient

The initial values of the minimum dry heat transfer coefficient (h) did not differ between the 35°C and 15°C groups of Dunn's Larks, Hoopoe Larks, or Spike-heeled Larks (Dunn's Lark: $F_{1,13} = 0.36$, $P = 0.56$; Hoopoe Lark: $F_{1,11} = 0.82$, $P = 0.39$; Spike-heeled Lark: $F_{1,7} = 3.73$, $P = 0.10$), but were different between the 35°C and 15°C groups of Skylarks and Woodlarks (Fig. 4). The interaction between mass and treatment had a significant effect on h in Woodlarks (Woodlark: $F_{1,9} = 12.18$, $P = 0.007$), whereas Skylarks in the 35°C group had a significantly higher h than those in the 15°C group ($F_{1,11} = 5.62$, $P = 0.04$). Post-acclimation h did not differ between treatments in the Woodlarks ($F_{1,11} =$

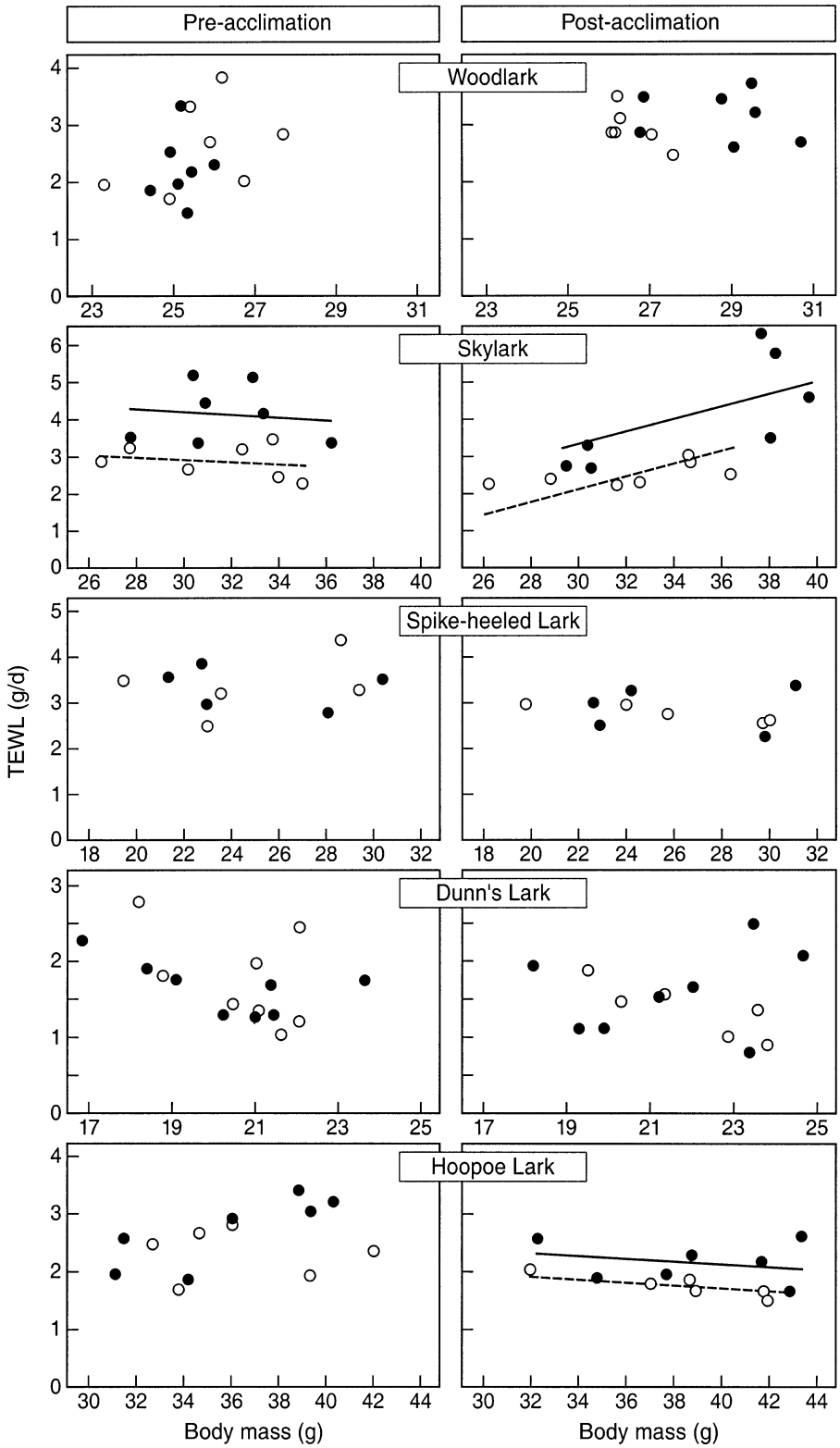

FIG. 3. Total evaporative water loss (TEWL) as a function of body mass of birds assigned to acclimation at 35°C (open symbols) and 15°C (solid symbols) for Woodlarks, Skylarks, Spike-heeled Larks, Dunn's Larks, and Hoopoe Larks when acclimated to their natural environment (pre-acclimation) and after acclimation to 35°C and 15°C (post-acclimation). Lines indicate significant differences between groups acclimated to 15°C and 35°C.

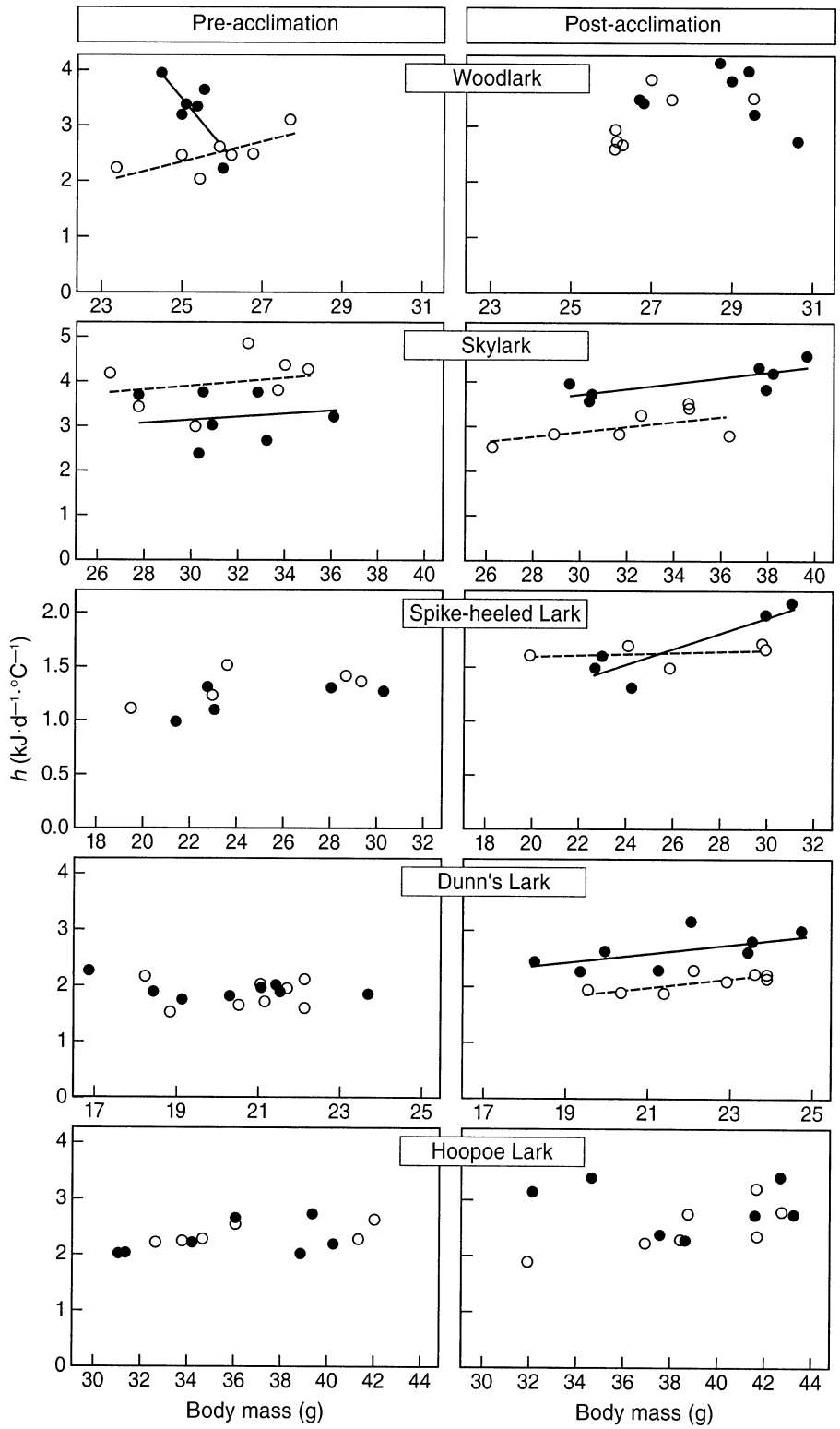

FIG. 4. Minimum dry heat transfer coefficient (h) as a function of body mass of birds assigned to acclimation at 35°C (open symbols) and 15°C (solid symbols) for Woodlarks, Skylarks, Spike-heeled Larks, Dunn's Larks, and Hoopoe Larks when acclimated to their natural environment (pre-acclimation) and after acclimation to 35°C and 15°C (post-acclimation). Lines indicate significant differences between groups acclimated to 15°C and 35°C.

TABLE 2. Body temperatures (T_b) of 15°C and 35°C treatment groups before and after three weeks of acclimation for Skylark, Woodlark, Spike-heeled Lark, Dunn's Lark, and Hoopoe Lark.

Species	Treatment	*n*	Mean ± 1 SD		
			Initial T_b (°C)	Final T_b (°C)	Difference (°C)
Skylark	35°C	7	40.6 ± 1.70	42.1 ± 0.80	1.5 ± 1.21
	15°C	7	41.3 ± 1.45	42.7 ± 0.60	1.3 ± 1.44
Woodlark	35°C	7	41.4 ± 0.90	42.7 ± 0.48	1.2 ± 0.99
	15°C	7	41.6 ± 1.28	43.1 ± 0.39	1.5 ± 1.17
Spike-heeled Lark	35°C	5	⋯	41.0 ± 0.39	⋯
	15°C	5	⋯	41.2 ± 0.69	⋯
Dunn's Lark	35°C	8	40.5 ± 0.26	39.5 ± 0.77	−1.0 ± 0.66
	15°C	8	40.4 ± 0.49	39.8 ± 0.64	−0.6 ± 0.45
Hoopoe Lark	35°C	7	40.8 ± 0.69	40.4 ± 0.69	−0.4 ± 0.95
	15°C	7	40.7 ± 0.35	40.8 ± 0.44	−0.1 ± 0.59

1.24, $P = 0.29$) and the Hoopoe Larks ($F_{1,11} = 2.48$, $P = 0.14$), but was higher in the 15°C-acclimated Skylarks and Dunn's Larks than in their 35°C-acclimated conspecifics (Skylark: $F_{1,11} = 28.53$, $P < 0.001$, Dunn's: $F_{1,13} = 36.44$, $P < 0.001$). In the Spike-heeled Lark, we found a significant interaction between mass and treatment ($F_{1,6} = 7.94$, $P = 0.030$), but average values for *h* did not differ between treatments ($t = 0.36$, df = 8, $P = 0.73$).

To account for body mass differences in interspecific comparisons, we calculated surface-specific h' by dividing *h* by surface area calculated from Meeh's equation (Walsberg and King 1978) (Fig. 2C). Skylarks and Woodlarks had higher h' than the semi-arid and arid species (Fig. 2C). We tested pre-acclimation, and 15°C and 35°C post-acclimation h' for differences between species. In all three analyses, species had a significant effect on h' (initial values: $F_{4,62} = 38.05$, $P < 0.0001$; 15°C: $F_{4,29} = 24.95$, $P < 0.001$; 35°C: $F_{4,29} = 24.30$, $P < 0.0001$). Subsequent post hoc tests showed no differences in h' between Hoopoe Lark and Dunn's Lark and between Skylark and Woodlark (Fig. 2C).

Body temperature

Pre- and post-acclimation T_b values varied between species and between treatments (Table 2). Pre-acclimation T_b did not differ between birds assigned to the 35°C and 15°C groups ($F_{1,53} = 0.57$, $P = 0.45$), but differed significantly between species ($F_{3,53} = 3.15$, $P = 0.03$). Post hoc analysis revealed that T_b of Woodlarks was significantly higher than that of Dunn's Lark (Tukey, 1.1 ± 0.36°C [mean difference ± 1 SEM], $P = 0.02$), but that other species did not differ significantly from each other (Tukey, $P < 0.05$). Post-acclimation T_b differed among species ($F_{4,62} = 72.63$, $P < 0.001$) and between treatments, and was 0.4 ± 0.15°C (mean ± 1 SEM) lower in the 35°C-acclimated birds than in the 15°C-acclimated birds for all species combined ($F_{1,62} = 7.22$, $P = 0.01$). Post hoc analysis showed that T_b of Dunn's Lark was lower than that of any of the other four species (all Tukey $P < 0.001$), and that T_b did not differ between Hoopoe Lark and Spike-heeled Lark (0.5 ± 0.25°C [mean difference ± 1 SEM], Tukey $P = 0.33$) or between Woodlark and Skylark (0.5 ± 0.23°C, Tukey $P = 0.21$). All other pairwise combinations of species indicated significant differences in T_b (Tukey, all $P < 0.001$).

Food intake

Individuals of all species in the 15°C room ate more food than conspecifics in the 35°C room. Skylarks and Woodlarks consumed 78% and 71% more food, respectively, when exposed to 15°C than to 35°C (Table 3). Dunn's Larks at 15°C ate 163% more seeds and 101% more mealworms than conspecifics at 35°C, and

TABLE 3. Food intake (mean ± 1 SD) of 15°C and 35°C treatment groups during week 3 of the acclimation period for Skylark, Woodlark, Dunn's Lark, and Hoopoe Lark.

Species	15°C group		35°C group	
	Food intake (g/d)	*n*	Food intake (g/d)	*n*
Skylark	15.1 ± 2.7	7	8.5 ± 3.1	7
Woodlark	9.7 ± 2.1	7	5.7 ± 0.6	7
Dunn's Lark (seeds)	2.3 ± 1.1	5	0.9 ± 0.9	5
Dunn's Lark (mealworms)	6.7 ± 2.2	5	3.3 ± 1.3	5
Hoopoe Lark	11.8 ± 3.6	7	6.3 ± 2.0	7

TABLE 4. Dry mass and relative dry mass (as percentage of dry body mass) of organs and muscle of larks after three weeks at 15°C or 35°C.

Species and organ	Dry mass (mg, mean ± 1 SD)		Change (%)	*P*	Mass (%, mean ± 1 SD)	
	15°C	35°C			15°C	35°C
Woodlark						
Brain	171.2 ± 9.5	170.3 ± 10.7	0.5	0.870	1.60 ± 0.12	1.69 ± 0.13
Heart	109.5 ± 14.8	96.7 ± 12.1	13.2	0.102	1.02 ± 0.11	0.96 ± 0.11
Intestine	238.4 ± 28.6	187.9 ± 33.3	26.9	0.010	2.23 ± 0.26	1.86 ± 0.32
Kidney	87.9 ± 7.2	75.3 ± 12.1	16.7	0.039	0.82 ± 0.05	0.74 ± 0.11
Liver	360.4 ± 40.2	292.4 ± 43.9	23.3	0.011	3.37 ± 0.38	2.89 ± 0.40
Lungs	70.2 ± 8.4	71.6 ± 10.9	−2.0	0.795	0.66 ± 0.06	0.71 ± 0.09
Pectoral muscle	543.4 ± 64.4	855.3 ± 62.9	−36.5	0.000	8.42 ± 0.49	8.45 ± 0.46
Stomach	243.6 ± 13.3	192.0 ± 13.3	26.9	0.000	2.28 ± 0.12	1.90 ± 0.13
Skylark						
Brain	197.0 ± 11.4	189.4 ± 14.7	4.0	0.300	1.51 ± 0.17	1.60 ± 0.10
Heart	132.9 ± 21.5	124.3 ± 25.3	6.9	0.508	1.01 ± 0.09	1.04 ± 0.10
Intestine	346.3 ± 54.2	265.2 ± 46.1	30.6	0.011	2.64 ± 0.36	2.23 ± 0.27
Kidney	102.1 ± 12.2	93.0 ± 11.5	9.8	0.175	0.78 ± 0.06	0.79 ± 0.12
Liver	402.6 ± 68.6	322.9 ± 44.1	24.7	0.027	3.07 ± 0.46	2.73 ± 0.32
Lung	102.6 ± 19.0	102.3 ± 13.3	0.3	0.168	0.79 ± 0.17	0.87 ± 0.12
Pectoral muscle	1014.3 ± 137.1	901.1 ± 72.4	12.6	0.078	7.72 ± 0.76	8.45 ± 0.83
Stomach	271.1 ± 52.1	212.2 ± 32.6	27.8	0.026	2.06 ± 0.29	1.78 ± 0.10
Spike-heeled Lark						
Brain	167.6 ± 25.17	159.2 ± 29.9	5.3	0.088	1.76 ± 0.13	1.86 ± 0.26
Hear	91.9 ± 16.4	83.3 ± 18.3	10.3	0.075	0.96 ± 0.09	0.97 ± 0.15
Intestine	342.2 ± 74.6	263.7 ± 54.9	29.8	0.003	3.64 ± 0.95	3.07 ± 0.43
Kidney	61.0 ± 19.4	49.3 ± 14.2	23.7	0.047	0.64 ± 0.17	0.58 ± 0.17
Liver	270.2 ± 34.4	231.9 ± 57.5	16.5	0.420	2.87 ± 0.44	2.69 ± 0.51
Lung	…	…	…	…	…	…
Pectoral muscle	623.8 ± 82.4	590.0 ± 90.5	5.7	0.078	6.89 ± 0.58	6.56 ± 0.48
Stomach	195.4 ± 86.8	177.0 ± 52.4	10.4	0.009	2.03 ± 0.84	2.06 ± 0.55
Dunn's Lark						
Brain	159.2 ± 14.4	156.8 ± 16.6	1.5	0.759	2.02 ± 0.14	1.95 ± 0.17
Heart	70.1 ± 5.7	70.0 ± 13.4	0.1	0.983	0.89 ± 0.09	0.87 ± 0.15
Intestine	210.9 ± 42.2	166.0 ± 24.9	27.0	0.024	2.67 ± 0.62	2.06 ± 0.30
Kidney	66.9 ± 11.0	57.8 ± 10.5	15.7	0.109	0.85 ± 0.18	0.72 ± 0.11
Liver	217.2 ± 43.9	182.5 ± 37.6	19.0	0.111	2.76 ± 0.63	2.26 ± 0.37
Lung	43.0 ± 7.5	43.9 ± 6.0	−2.1	0.811	0.55 ± 0.11	0.55 ± 0.07
Pectoral muscle	574.3 ± 94.3	1028.0 ± 161.9	−44.1	0.000	7.24 ± 0.79	6.96 ± 0.80
Stomach	166.5 ± 22.4	126.5 ± 6.8	31.6	0.001	2.12 ± 0.38	1.57 ± 0.07
Hoopoe Lark						
Brain	209.9 ± 28.2	193.4 ± 13.6	8.5	0.187	1.48 ± 0.20	1.39 ± 0.15
Heart	122.5 ± 19.44	131.7 ± 21.4	−7.0	0.420	0.86 ± 0.10	0.94 ± 0.14
Intestine	354.0 ± 63.9	255.4 ± 37.1	38.6	0.006	2.49 ± 0.41	1.83 ± 0.29
Kidney	108.0 ± 13.4	86.8 ± 8.4	24.4	0.004	0.76 ± 0.05	0.62 ± 0.06
Liver	358.0 ± 54.1	281.4 ± 38.8	27.2	0.010	2.51 ± 0.20	2.03 ± 0.38
Lung	84.9 ± 16.5	84.0 ± 14.9	1.1	0.916	0.59 ± 0.06	0.60 ± 0.07
Pectoral muscle	962.4 ± 204.6	947.4 ± 19.6	1.6	0.891	6.71 ± 1.01	7.23 ± 0.61
Stomach	207.9 ± 29.9	170.4 ± 27.1	22.0	0.030	1.47 ± 0.20	1.21 ± 0.11

Notes: We assumed a body water content of 65% to calculate dry body mass. Sample sizes in each group are: Woodlark, $n = 7$; Skylark, $n = 7$; Spike-heeled Lark, $n = 10$; Dunn's Lark, $n = 8$; Hoopoe Lark, $n = 7$.

Hoopoe Larks ate 89% more mealworms in the 15°C room than in the 35°C room (Table 3).

Body composition

After three weeks of acclimation, larks in the 15°C and 35°C environments had developed differences in the size of several organs (Table 4). In all species the dry masses of organs involved in digestion or catabolism of food, such as intestine, kidney, liver, and stomach, were larger in the 15°C groups than in the 35°C groups. The magnitude of the difference ranged from 26.9–38.6% for the intestine, 9.8–24.4% for the kidney, 16.5–27.2% for the liver, and 22.0–31.6% for the stomach (Table 4). Using ANOVA with treatment and species as fixed factors, we tested if organ dry mass differed between treatments, and found significant effects of species and significant increases in 15°C compared with 35°C-acclimated birds for intestine (species $F_{4,72} = 21.00$, $P < 0.001$, treatment $F_{1,72} = 39.80$, $P < 0.001$), kidney (species $F_{4,72} = 39.09$, $P < 0.001$, treatment $F_{1,72} = 19.32$, $P < 0.001$), liver (species $F_{4,72} = 29.97$, $P < 0.001$, treatment $F_{1,72} = 28.92$, $P < 0.001$), and

TABLE 5. Results of comparisons of relative organ size between five species of larks and between groups within each species that have been acclimated for three weeks to 15°C or 35°C.

Organ	Treatment†		Species		Tukey test results‡				
	$F_{1,72}$	P	$F_{4,72}$	P	Dunn's Lark	Hoopoe Lark	Spike-heeled Lark	Skylark	Woodlark
Brain	0.45	0.506	24.56	<0.001	a	b	d	bc	cd
Heart	0.02	0.887	3.96	0.006	a	ab	ab	b	ab
Intestine	26.87	<0.001	21.87	<0.001	a	a	b	a	a
Kidney	7.88	0.006	7.77	<0.001	a	ab	b	a	a
Liver	16.20	<0.001	9.30	<0.001	ab	a	bc	bc	c
Lungs§	1.67	0.202	24.47	<0.001	a	ab		c	b
Pectoral muscle	2.65	0.108	17.29	<0.001	a	a	a	b	b
Stomach	8.57	0.005	9.28	<0.001	a	b	a	a	a

Notes: ANOVAs for each organ are based on arcsine square-root transformed data in Table 4. Post hoc test results were obtained with Tukey tests.

† Significant differences indicate smaller organs in the 35°C-acclimated birds compared with the 15°C-acclimated birds.

‡ Insignificant differences between species are indicated with the same letter. Criterion for significance: $P < 0.05$.

§ Treatment $F_{1,53}$; Species $F_{3,53}$.

stomach (species $F_{4,72} = 10.32$, $P < 0.001$, treatment $F_{1,72} = 16.24$, $P < 0.001$). The interaction between species and treatment was not significant in any of these analyses. Organs involved in the respiratory system did not show a consistent difference between 15°C- and 35°C-acclimated birds in the five species (Table 4). The differences in dry heart mass ranged from −7.0% to +13.2% and in mass of dry lung from −2.0% to +1.1%. Although heart and lung mass differed significantly between species (heart: $F_{4,72} = 32.29$, $P < 0.001$; lungs: $F_{3,53} = 61.96$, $P < 0.001$), differences between the 15°C and 35°C groups were not significant (heart: $F_{1,72} = 1.26$, $P = 0.27$; lungs: $F_{1,53} = 0.006$, $P = 0.94$). Brain mass differed significantly between species, but not between treatments (species $F_{4,72} = 14.83$, $P < 0.001$, treatment $F_{1,72} = 2.68$, $P = 0.11$). Pectoral muscle dry mass did not differ between 15°C and 35°C groups in Hoopoe Larks, was 5.7% larger and 12.6% larger in 15°C-acclimated compared with 35°C-acclimated Spike-heeled Larks and Skylarks, respectively, and was smaller in the 15°C-acclimated individuals of Dunn's Lark and Woodlark by 44.1% and 36.5%, respectively (Table 4). The significant interaction between species and treatment in our ANOVA indicates that the response of the pectoral muscle to acclimation differed between species ($F_{4,68} = 15.25$, $P < 0.001$).

To take into account differences in body mass between species, we expressed organ size as a percentage of dry body mass, assuming a total body water content of 65% of wet mass (Williams 1985, 1999), and tested if relative organ size differed between treatments and species (Tables 4 and 5). When expressed as proportion of total body mass, intestine, kidney, liver, and stomach were larger in the 15°C-acclimated than in the 35°C-acclimated birds (Table 5). The remaining organs and the pectoral muscle did not differ significantly between 15°C- and 35°C-acclimated birds (Table 5). Relative size of all organs and the pectoral muscle differed among species, but the post hoc tests did not reveal systematic differences between the arid, semi-arid, and mesic larks (Table 5). The only distinctive difference between Hoopoe Lark, Dunn's Lark, and Spike-heeled Lark on the one hand and Skylark and Woodlark on the other was the relatively smaller pectoral muscle in the arid-zone species.

Interindividual variation and repeatability of BMR, TEWL, and h

The interindividual variation in phenotypes of birds acclimated to their natural climatic conditions was ~50% less in the Hoopoe Lark and the Dunn's Lark compared with the Skylark and Woodlark for mass-adjusted BMR and h', but not related to environment for mass-adjusted TEWL (Table 6). Interindividual variation in mass-adjusted BMR of the Spike-heeled Lark was similar to that of the two mesic species, whereas variation in h' resembled values for the two arid-zone species (Table 6).

Repeatability estimates for BMR varied between 0.48 and 0.66 in the larks from semi-arid and arid areas, but were not significantly different from zero in the two mesic species (Table 6). Repeatabilities of h showed the reverse pattern with higher values for the mesic species than for the Dunn's Lark and the Hoopoe Lark. Repeatabilities of TEWL were 0.73 and 0.50 in the Skylark and the Dunn's Lark, respectively, 0.22 in the Woodlark, and zero in the Spike-heeled Lark and the Hoopoe Lark.

DISCUSSION

Individuals of five closely related species of larks showed considerable short-term phenotypic flexibility of physiological and morphological characters when acclimated to 15°C or 35°C. The interspecific variation among the five larks was consistent with results of a previous study that showed that among 12 species of larks increasing aridity correlated with decreasing BMR and TEWL (Tieleman et al. 2003). Phenotype-

TABLE 6. Coefficients of variation (CV) and repeatability estimates (r) for basal metabolic rate (BMR), total evaporative water loss (TEWL), and the minimum dry heat transfer coefficient (h) for five species of larks.

Species	BMR						TEWL	
	CV (%)	$r \pm 1$ SE	n	n_0	F	P	CV (%)	$r \pm 1$ SE
Skylark	18.2	-0.17 ± 0.184	14	2.42	$0.64_{13,17}$	0.788	26.8	0.73 ± 0.104
Woodlark	23.0	0.17 ± 0.265	14	2	$1.40_{13,12}$	0.285	28.0	0.22 ± 0.178
Spike-heeled Lark	16.7	0.66 ± 0.128	20	2	$4.88_{19,18}$	0.001	19.1	-0.04 ± 0.324
Dunn's Lark	9.0	0.48 ± 0.196	16	2	$2.84_{15,14}$	0.029	33.9	0.50 ± 0.192
Hoopoe Lark	8.0	0.57 ± 0.184	14	2	$3.63_{13,12}$	0.016	19.7	-0.10 ± 0.270

Notes: Coefficients of variation are based on mass-adjusted values of BMR and TEWL, and surface-specific h before the acclimation period. Repeatabilities are based on pre- and post-acclimation whole-animal data. Abbreviations are: n, number of individuals; n_0, coefficient related to the sample size per group in the ANOVA (Lessells and Boag 1987).

by-environment correlations based on interspecific comparisons have been criticized because of the difficulty in distinguishing the effects of phylogenetic inertia, genetic adaptation as a result of natural selection, and phenotypic adjustment to the environment (Leroi 1994, Westoby et al. 1995, Hansen 1997). In a previous study, we constructed a phylogeny of the larks and excluded phylogenetic relatedness as a factor explaining the decrease in BMR and TEWL with increasing aridity (Tieleman et al. 2003). This study shows that the effects of acclimation were insufficient to explain the interspecific differences in physiology among five species of larks. Therefore, the reductions in BMR and TEWL in larks from arid environments are likely to have a genetic component, although we cannot rule out that developmental conditions play a role. The magnitude of intra-individual flexibility varied between physiological traits and depended largely on species, but was not correlated with aridity. In addition, the interindividual variation in physiological phenotypes and the repeatability of physiological traits differed between species and appeared correlated with environment for BMR and h, but not for TEWL.

Differences between species in BMR have been attributed to the size of internal organs, especially heart, liver, and kidneys, that have relatively high tissue-specific metabolic rates (Krebs 1950, Martin and Fuhrman 1955, Kersten and Piersma 1987, Daan et al. 1990, Williams and Tieleman 2000). The reduced mass-corrected BMR in arid-zone larks prompted us to ask if these differences could be explained by smaller organs or muscles. With the exception of the relative size of the pectoral muscle, which is smaller in Dunn's Lark, Hoopoe Lark, and Spike-heeled Lark compared with Skylark and Woodlark, we found no evidence for systematic differences in body composition between larks from different environments (Tables 4 and 5). The pectoral muscle accounted for on average 13.9% of the total body mass of the former three species and 16.5% of the Skylarks and Woodlarks. Therefore, the relative size of the pectoral muscle is 16% smaller in the arid-zone birds, a reduction unlikely to explain the 50% reduction in BMR. We hypothesize that in larks not only the size of internal organs, but also the intensity of the tissue-specific metabolic rates of various organs may influence BMR.

Individuals of all five species showed large phenotypic flexibility of the organs of the digestive system in response to exposure to 15°C and 35°C (Table 4). When acclimated to 15°C, birds consumed more food, which apparently stimulated hypertrophy of intestine, kidney, liver, and stomach compared with birds acclimated to 35°C. The increase in size of the digestive organs was paralleled by a significant increase in BMR in the 15°C-acclimated birds of all species, except the Woodlark. These results support data from a previous study that reported larger liver, intestine, kidney, and possibly stomach, correlated with increased BMR in cold-acclimated compared with warm-acclimated Hoopoe Larks (Williams and Tieleman 2000). The pectoral muscle responded to acclimation by an increase in size in Spike-heeled Larks and Skylarks from the 15°C room, a decrease in size by Dunn's Larks and Woodlarks in the 15°C room, and no change in the Hoopoe Lark. The increase in pectoral muscle mass may have resulted from increased thermoregulatory demands that required shivering thermogenesis in the 15°C-acclimated Spike-heeled Larks and Skylarks. The opposite finding in the Dunn's Larks and Woodlarks might be attributed to different activity levels; individuals of these species at 35°C appeared more active than conspecifics at 15°C. The combination of a decreased pectoral muscle mass and increased digestive organ sizes in the Woodlark may have resulted in no net difference in BMR between treatments. Organs and muscles may have not only changed in size, but also in structure. Capillary density, capillary surface area, and mitochondrial volume density in aerobic fibers increased in the pectoral muscles of cold-acclimated Rock Doves (*Columba livia*) to meet the increased energetic demands of shivering (Mathieu-Costello et al. 1998). If a parallel change occurred in larks, the increase in BMR in 15°C-acclimated birds may be partially attributable to higher metabolic rates of the muscle tissue. Similar studies in mammals have found larger internal organs together with increased BMR in cold-acclimated mice (Toloza et al. 1991, Konarzewski and Diamond 1995). The magnitude of the changes in organ size fell within

TABLE 6. Extended.

TEWL				h					
n	n_0	F	P	CV (%)	$r \pm 1$ SE	n	n_0	F	P
14	3	$9.24_{13,24}$	<0.0001	19.4	0.27 ± 0.178	14	3	$2.10_{13,24}$	0.056
14	3	$1.87_{13,24}$	0.090	21.7	0.49 ± 0.162	14	2.93	$3.78_{13,23}$	0.003
10	2	$0.92_{9,8}$	0.555	11.2	0.52 ± 0.236	10	2	$3.20_{9,8}$	0.058
16	2	$2.96_{15,14}$	0.025	14.2	−0.07 ± 0.253	16	2	$0.87_{15,14}$	0.605
14	2	$0.82_{12,12}$	0.635	9.4	0.12 ± 0.269	14	2	$1.26_{13,12}$	0.348

the range for other birds in response to dietary changes or in preparation for migration (Karasov 1996, Piersma and Lindström 1997). In general, the phenotypic flexibility of the size of digestive organs and pectoral muscle within individuals was as large as the variation in organ mass between species, when corrected for body mass differences.

Interspecific differences in TEWL cannot be explained by acclimation to temperature. Examination of the role of developmental plasticity is necessary to further support a genetic basis for the variation in TEWL among larks from different environments. Although acclimatory responses to humidity remain untested in birds, kangaroo rats (*Dipodomys merriami merriami*) from the Sonoran Desert, reared at different humidities but constant T_a and later acclimated to the opposite humidity regime, showed that developmental plasticity and acclimation accounted for all variation between individuals from different geographic areas (Tracy and Walsberg 2001).

Mechanisms responsible for the intra-individual flexibility of TEWL in Hoopoe Larks may lie in the variability of their cutaneous water loss (CWL), which accounts for more than two-thirds of TEWL in these larks (Tieleman and Williams 2002). Differences in the structure and lipid composition of the skin possibly correlate with differences in CWL between species and within individuals acclimated to different conditions. Zebra Finches (*Taeniopygia guttata*), for example, appear able to decrease CWL in response to water deprivation by increasing the deposition of multigranular bodies in the epidermal stratum corneum (Menon et al. 1989). In contrast, water-deprived Rock Doves (*Columba livia*) do not alter CWL at moderate T_a, although at high T_a, they do have lower CWL than hydrated birds (Arad et al. 1987). Cold-acclimated Rock Doves have a lamellar, extracellular water barrier in the epidermis that minimizes evaporation through the skin, whereas heat-acclimation leads to the formation of structurally heterogeneous skin that facilitates CWL (Peltonen et al. 2000).

Maintenance of a constant T_b when metabolic heat production is increased requires either an adjustment in evaporative heat loss or in dry heat loss. In contrast to Hoopoe Larks that adjusted their TEWL when acclimated to cold and warm environments, Skylarks and Dunn's Larks elevated their minimum dry heat transfer coefficient during the three-week period of exposure to 15°C compared with 35°C. The intra-individual flexibility in these species was insufficient to explain the interspecific differences in h' between arid- and mesic-zone birds by effects of acclimation only (Fig. 2C). The lower dry heat loss in arid-zone species compared with mesic-zone birds might contribute to minimizing their energy requirements, but the mechanisms that explain this difference remain elusive.

Accurate statements about the selective value of a physiological trait and about its inheritance can only be made if one understands how variable that trait is both between and within individuals. The hypothesis that species in deserts experience stronger selection for a frugal energy and water balance, and therefore show less interindividual variation in physiological phenotypes, was supported by the coefficients of variation (CV) of BMR and h', but not TEWL (Table 6). The CV for TEWL was lower in the Hoopoe Lark than in both mesic species, but higher in the Dunn's Lark. These results do not comply with the idea that phenotypic variation is larger in species from harsh environments (Parsons 1987, 1996). The high repeatabilities of BMR (0.48–0.66) for Hoopoe Larks, Dunn's Larks, and Spike-heeled Larks, of TEWL (0.50–0.73) for Skylarks and Dunn's Larks, and of h (0.49–0.52) for Spike-heeled Larks and Woodlarks indicate a within-individual consistency on which natural selection could operate, although we do not know if the variation among individuals has a genetic basis and therefore could respond to selection. At the level of intra-individual flexibility of physiological traits, all species appear flexible in at least one of the studied traits, and we found no general support for the hypothesis that species from the temporally more heterogeneous arid environments display a larger intra-individual plasticity (Parsons 1996).

The intraspecific heterogeneity in phenotypes and the intra-individual phenotypic flexibility of desert larks allow some optimism in view of the prediction of an increase in annual T_a of 5°C over the next 100 years in Saudi Arabia (Mitchell and Hulme 2000). Studying the capacity for phenotypic change of physiological traits will benefit from a broad approach in which genetic and environmental influences on phenotypes of different species are distinguished, and species are no longer viewed as genetically fixed entities.

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