

# Adaptation of metabolism and evaporative water loss along an aridity gradient

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Broad-scale comparisons of birds indicate the possibility of adaptive modification of basal metabolic rate (BMR) and total evaporative water loss (TEWL) in species from desert environments, but these might be confounded by phylogeny or phenotypic plasticity. This study relates variation in avian BMR and TEWL to a continuously varying measure of environment, aridity. We test the hypotheses that BMR and TEWL are reduced along an aridity gradient within the lark family (Alaudidae), and investigate the role of phylogenetic inertia. For 12 species of lark, BMR and TEWL decreased along a gradient of increasing aridity, a finding consistent with our proposals. We constructed a phylogeny for 22 species of lark based on sequences of two mitochondrial genes, and investigated whether phylogenetic affinity played a part in the correlation of phenotype and environment. A test for serial independence of the data for mass-corrected TEWL and aridity showed no influence of phylogeny on our findings. However, we did discover a significant phylogenetic effect in mass-corrected data for BMR, a result attributable to common phylogenetic history or to common ecological factors. A test of the relationship between BMR and aridity using phylogenetic independent contrasts was consistent with our previous analysis: BMR decreased with increasing aridity.

**Keywords:** basal metabolic rate; total evaporative water loss; aridity; larks; Alaudidae; phylogeny

## 1. INTRODUCTION

Terrestrial vertebrates continuously expend energy to carry out chemical processes necessary to sustain life, and constantly lose water through respiration, cutaneous evaporation and excretion. Rates of energy expenditure and water loss vary considerably among and within vertebrate taxa even under standard laboratory conditions. The reasons underlying this variation have been the focus of intensive research and are still only partially understood (Kleiber 1961; Bartholomew & Cade 1963; Crawford & Lasiewski 1968; Aschoff & Pohl 1970; McNab 1986; Nagy 1987; Bennett 1988; Williams 1996; Mueller & Diamond 2001). Basal metabolic rate (BMR), the minimum energy expenditure of a fasted endotherm in its rest phase at thermoneutral temperatures (King 1974), integrates all catabolic energy transformations required for bodily maintenance. BMR correlates with the energy expenditure of free-living animals (Nagy 1987; Daan *et al.* 1990; Ricklefs *et al.* 1996) and with life-history attributes, such as growth and reproduction (Kleiber 1961; Bennett 1988; Trevelyan *et al.* 1990; Harvey *et al.* 1991; Hulbert & Else 2000). Total evaporative water loss (TEWL), the sum of respiratory and cutaneous water losses, constitutes a significant proportion of an animal's total water loss, up to 70–80% in small birds when measured at 25 °C (Lee & Schmidt-Nielsen 1971; Bartholomew 1972; Dawson 1982; Williams 1996). Both BMR and TEWL appear, in part, to be genetically determined, suggesting that natural selection

could operate on these variables (Furuyama & Ohara 1993; Konarzewski & Diamond 1995).

After correcting for body mass and higher level taxonomic affiliations, a large variation in metabolism and TEWL remains (Kleiber 1961; Crawford & Lasiewski 1968; Dawson 1982; McNab 1988; Williams 1996). Differences in BMR and TEWL have been reported between species or populations differing in diet, altitude, latitude, temperature and aridity (Dawson & Bennett 1973; Weathers 1979; Nagy 1987; McNab 1988; Nagy *et al.* 1999; Williams & Tieleman 2001), but a unifying environmental characteristic that determines the metabolic physiology of animals has not been identified. Mueller & Diamond (2001) reported that primary productivity explains 88% of the variation in BMR between five species of *Peromyscus* mice, and suggested that food availability, a correlate of primary productivity, might be a unifying explanatory variable for metabolic physiology. One might also predict that water availability is a major factor explaining variation in TEWL.

Previous work on BMR and TEWL has compared species from disparate environments, such as the tropics versus temperate or desert versus non-desert (Scholander *et al.* 1950; Bartholomew & Cade 1963; Dawson & Schmidt-Nielsen 1964; Dawson & Bennett 1973; Weathers 1979; Dawson 1984; Williams 1996; Tieleman & Williams 2000). However, the use of a dichotomous categorization of environments might obscure useful biological information. Climatologists have long recognized that environments form a continuum with respect to meteorological parameters, and have emphasized that, for example, the environment of a given desert depends on the interaction

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of a number of variables, including temperature, amount and timing of rainfall, relative humidity and wind (Thornthwaite 1948; Meigs 1953). To the best of our knowledge, our study is the first to relate variation in avian physiological variables to a continuously varying measure of environment, aridity. Aridity is directly related to primary productivity (Emberger 1955) and provides a proxy for the selection pressures that animals experience with increasing aridity, including decreasing water and food availability and increasing air temperatures.

The challenge in understanding interspecific correlations between phenotype and environment is to distinguish the contributions of the various processes that underlie the pattern, including genetic adaptation, phylogenetic inertia (Gould & Lewontin 1979; Westoby *et al.* 1995; Hansen 1997) and phenotypic responses (acclimatization). Our limited understanding of the time-scale at which natural selection and other evolutionary processes operate has resulted in two main approaches to questions in evolutionary biology, the phylogenetic comparative method and the optimality approach. Comparative methods that take into account potential effects of phylogeny emphasize the historical component of adaptation (Gould & Vrba 1982) and implicitly rule out the possibility that a trait is maintained as an adaptation by stabilizing selection (Frumhoff & Reeve 1994; Westoby *et al.* 1995; Hansen 1997). By contrast, optimality studies assume that traits with ample genetic variation, such as most quantitative characters (Houle 1992; Lynch 1988), are probably maintained by stabilizing selection (Hansen 1997). A comparative approach with an optimality viewpoint of adaptation would assume that each species is at an optimum (Hansen 1997). One way to reduce problems relating to differences in phylogenetic history is to compare traits among closely related species (Coddington 1988; Bennett 1988; Price 1991; Leroi 1994).

The family of larks (Alaudidae) occurs over a wide range of environments and continents, with representatives in habitats ranging from hyperarid deserts to mesic grasslands (Cramp 1988; Pätzold 1994). Because all larks eat similar foods, a mixture of insects and seeds, diet is not a confounding variable in our analyses. This family provides a model to investigate physiological adaptation to environment.

This study tests the proposal that lower BMRs and TEWLs in birds are correlated with increasing aridity, and investigates the role of phylogenetic inertia in shaping the relationship between physiology and environment. We constructed a phylogeny of larks based on two mitochondrial genes, and investigated whether phylogeny could explain the variation in BMR and TEWL. We used conventional and, when appropriate, phylogenetically corrected analyses to examine the relationships between aridity, BMR and TEWL.

## 2. MATERIAL AND METHODS

### (a) *Animals*

We mist-netted hoopoe larks (*Alaemon alaudipes*), Dunn's larks (*Eremalauda dunni*), desert larks (*Ammomanes deserti*), black-crowned finchlarks (*Eremopterix nigriceps*) and crested larks (*Galerida cristata*) at various sites in the west-central Arabian Desert (table 1), and housed them in outdoor aviaries at the

National Wildlife Research Center (NWRC), near Taif, Saudi Arabia. Calandra larks (*Melanocorypha calandra*) from Iran were transported to Saudi Arabia and kept at the NWRC. We caught skylarks (*Alauda arvensis*) and woodlarks (*Lullula arborea*) in the province of Drenthe, The Netherlands, and kept them in outdoor aviaries at the Zoological Laboratory of the University of Groningen. We measured BMR and TEWL of all birds in Saudi Arabia and The Netherlands between June and August of 1998–2001. Birds were measured in the post-absorptive state during their nocturnal phase by standard flow-through respirometry and hygrometry methods (Williams & Tieleman 2000; Tieleman *et al.* 2002). In addition, we used data for spike-heeled larks (*Chersomanes albofasciata*) from Kimberley, South Africa (C. Brown, unpublished data), for short-toed larks (*Calandrella brachydactyla*) and lesser short-toed larks (*Calandrella rufescens*) from the North Caspian region, Russian Federation (Shishkin 1980), for Stark's lark (*Eremalauda starki*) and grey-backed finchlarks (*Eremopterix verticalis*) from the Namib Desert (Willoughby 1968) and for horned larks (*Eremophila alpestris*) from North America (Trost 1972). For all these species, birds were measured during spring or summer, in the post-absorptive state and during their rest phase.

### (b) *Environmental aridity*

We calculated an aridity index as  $Q = P / ((T_{\max} + T_{\min})(T_{\max} - T_{\min})) \times 1000$ , where  $P$  is the average annual precipitation (mm),  $T_{\max}$  is the mean maximum temperature of the hottest month (°C) and  $T_{\min}$  is the mean minimum temperature of the coldest month (°C) (Emberger 1955). Although not perhaps intuitively straightforward, this index was empirically derived to describe primary productivity in arid and semi-arid areas (Emberger 1955). The index is low in hot dry deserts and high in cool wet areas. We collected climatic data from local or national meteorological institutes, from the literature (Walter & Lieth 1967; Williams 2001) and from <http://www.worldclimate.com/> and <http://www.onlineweather.com/> (table 1). Because  $Q$  increases rapidly when environments become more mesic, we avoided unequal weighing of data for mesic species by using  $\log Q$  in our analyses (table 1).

### (c) *Phylogeny of larks*

The geographical origins of the DNA samples of all larks in our phylogeny are available on request from the authors. DNA was extracted from blood or tissue samples using standard protocols (50 mM of Tris, pH 7.6; 100 mM of NaCl; 1 mM of ethylenediamine tetra-acetic acid (EDTA), pH 8.0; 0.5% sodium dodecyl sulphate (SDS), 1 mg ml<sup>-1</sup> of proteinase K and 0.1 mg of Rnase A). Impurities were removed by phenol-chloroform extraction, and total genomic DNA was ethanol-precipitated and eluted in sterile distilled water.

Polymerase chain reaction (PCR) amplification (Saiki *et al.* 1988) followed standard protocols (Kocher *et al.* 1989). The cytochrome *b* gene was amplified using two primer sets: L14990 (shortened primer L14841 of Kocher *et al.* (1989)) and H15696 (primer H15547 of Edwards *et al.* (1991)); and L15245 (modified primer CB4a-L of Palumbi *et al.* (1991)) and H16064 (located in the tRNA<sub>thr</sub>). A portion of the 16S rRNA gene was amplified using primers L2313 and H4015 (Lee *et al.* 1997). Successful amplicons were purified using a High Pure PCR Purification kit (Roche Diagnostics).

We sequenced DNA using the four PCR primers for the cytochrome *b* gene, and H4015 and an internal primer, L2925, designed for passerines in our laboratory (5' AGCCATCAA-

Table 1. Geographical origin and environmental aridity for 14 species of lark. The environmental aridity index ( $Q$ ) was calculated following Emberger (1955), based on precipitation ( $P$ ), maximum temperature ( $T_{\max}$ ) and minimum temperature ( $T_{\min}$ ).

species	latitude	longitude	log $Q$	$P$ (mm)	$T_{\max}$ (°C)	$T_{\min}$ (°C)
hoopoe lark <sup>a</sup>	22°15' N	41°50' E	1.78	89.6	40.2	10.7
Dunn's lark <sup>a</sup>	22°15' N	41°50' E	1.78	89.6	40.2	10.7
desert lark <sup>a</sup>	22°55' N	41°09' E	1.78	89.6	40.2	10.7
Stark's lark <sup>b</sup>	23°34' S	15°03' E	1.76	57.2	33.0	10.0
grey-backed finchlark <sup>b</sup>	23°34' S	15°03' E	1.76	57.2	33.0	10.0
crested lark <sup>a</sup>	21°15' N	41°42' E	2.24	209.1	35.7	8.1
black-crowned finchlark <sup>a</sup>	21°15' N	41°42' E	2.24	209.1	35.7	8.1
calandra lark <sup>c,d</sup>	35°00' N	51°00' E	2.26	250.0	37.0	-2.4
horned lark <sup>e</sup>	33°17' N/ 34°35' N	116°40' W/ 118°06' W	2.41	309.9	34.8	-1.2
short-toed lark <sup>c,f</sup>	49°25' N	46°51' E	2.59	281.0	30.2	-13.7
lesser short-toed lark <sup>c,f</sup>	49°25' N	46°51' E	2.59	281.0	30.2	-13.7
spike-heeled lark <sup>c,f</sup>	28°48' S	24°42' E	2.60	420.4	32.6	2.7
woodlark <sup>g</sup>	52°52' N	52°52' E	3.20	750.0	21.7	-0.7
skylark <sup>g</sup>	52°52' N	52°52' E	3.20	750.0	21.7	-0.7

Climate data sources: <sup>a</sup> National Wildlife Research Center, Taif, Saudi Arabia; <sup>b</sup> Williams (2001), includes fog precipitation; <sup>c</sup> Walter & Lieth (1967); <sup>d</sup> <http://www.onlineweather.com>; <sup>e</sup> US National Weather Service; <sup>f</sup> <http://www.worldclimate.com>; and <sup>g</sup> Koninklijk Nederlands Meteorologisch Instituut.

CAAGAGTGC 3') for the 16S rRNA gene. Sequences of the light and heavy strands were determined using dye-terminator cycle sequencing (Big Dye DNA sequencing kit, Applied Biosystems) and an ABI377 or ABI3100 automated DNA sequencer (Applied Biosystems). Sequences for each taxon were proofread in Sequence Navigator, and complete sequences were aligned in CLUSTAL X (Thompson *et al.* 1997).

We analysed the aligned sequences with PAUP 4.0 using multiple heuristic searches with default settings (Swofford 1998). We assessed the resolution of internal nodes using 1000 bootstrap replicates with random replacement (Felsenstein 1985a). Phylogenetic signal was determined by evaluating the tree-length distribution of 1000 randomly generated trees (Hillis & Huelssenbeck 1992). In addition to using unweighted parsimony analysis, we attempted to reduce homoplasy by downweighting characters based on their consistency indices (Farris 1969). Pairwise estimates of nucleotide sequence divergence were calculated using the HKY85 model (Hasegawa *et al.* 1985). Gene sequences are deposited in GenBank (accession numbers AY165123–AY165147 (16S rRNA) and AY165148–AY165172 (cyt b)).

#### (d) Phylogenetic effect

Correlations between species may be statistically biased if sister taxa tend to be similar to one another as a result of common ancestry (Felsenstein 1985b; Cheverud *et al.* 1985; Harvey & Pagel 1991). To evaluate whether a phylogenetic effect (*sensu* Grafen (1989) and Harvey & Pagel (1991)) exists among the larks in this study, we used tests for serial independence to determine whether there was a significant positive autocorrelation for mass-corrected BMR, mass-corrected TEWL or aridity (Abouheif 1999; Reeve & Abouheif 1999). In each simulation the topology was randomly rotated 2000 times per iteration and the original data were shuffled 2000 times to provide the null hypothesis sampling distribution (Reeve & Abouheif 1999). We calculated mass-corrected BMR and mass-corrected TEWL by dividing BMR and TEWL, respectively, by mass<sup>x</sup>, where  $x$  is the slope of the allometric equations relating log BMR and log TEWL to log body mass in 12 lark species (see § 3). The test

for serial independence is more suitable than other phylogenetic autocorrelation methods for small datasets (Cheverud *et al.* 1985; Gittleman & Kot 1990; Martins & Hansen 1996; Abouheif 1999). If no phylogenetic effect exists, then incorporating phylogeny in statistical methods is unnecessary (Gittleman & Kot 1990; Björklund 1997; Abouheif 1999). If a phylogenetic effect does exist, it may be attributable to phylogenetic constraint or to ecological factors, and corrections for phylogenetic relationships may or may not be appropriate (Westoby *et al.* 1995).

#### (e) Statistics

We performed analyses of variance and regression analyses with SPSS 10.0, and calculated phylogenetic independent contrasts (Felsenstein 1985b) with the PDI module in the computer program PDAP (Garland *et al.* 1992). We calculated the degrees of freedom as  $N - P_u$ , where  $N$  is the number of independent contrasts and  $P_u$  is the number of unresolved polytomies (Purvis & Garland 1993).

### 3. RESULTS

#### (a) Physiology and environment

For 12 species of lark, BMR was related to body mass as  $\log \text{BMR (kJ day}^{-1}\text{)} = 0.225 + 0.901 \log \text{mass (g)}$  ( $r^2 = 0.53$ , d.f. = 11,  $\text{s.e.}_{\text{slope}} = 0.269$ ,  $p = 0.007$ ; table 2). A multiple regression analysis with log BMR as the dependent variable and log mass and log  $Q$  (aridity) as independent variables showed that both body mass ( $t = 6.17$ ,  $p < 0.0001$ ) and aridity ( $t = 6.08$ ,  $p < 0.0001$ ) had a significant effect on BMR:  $\log \text{BMR} = -0.194 + 0.845 \log \text{mass} + 0.208 \text{aridity}$  ( $r^2 = 0.91$ , d.f. = 11,  $p < 0.0001$ ). The BMR of larks increased as the environment became more mesic (figure 1a).

TEWL among larks was related to body mass as  $\log \text{TEWL (g day}^{-1}\text{)} = -0.814 + 0.816 \log \text{mass (g)}$  ( $r^2 = 0.72$ , d.f. = 11,  $\text{s.e.}_{\text{slope}} = 0.162$ ,  $p = 0.001$ ; table 2). We used multiple regression analysis to assess the effect of aridity on TEWL:  $\log \text{TEWL} = -0.903 + 0.684 \log$

Table 2. Basal metabolic rate (BMR) and total evaporative water loss (TEWL) for 14 species of larks.

species	mass (g)	BMR (kJ day <sup>-1</sup> )	s.d. BMR (kJ day <sup>-1</sup> )	n	mass (g)	TEWL (g day <sup>-1</sup> )	s.d. TEWL (g day <sup>-1</sup> )	n
grey-backed finchlark <sup>a</sup>	—	—	—	—	15.1	1.31 <sup>e</sup>	—	—
Stark's lark <sup>a</sup>	—	—	—	—	15.6	1.31 <sup>e</sup>	—	—
desert lark	21.5	20.1	2.46	6	21.5	1.60	0.26	6
Dunn's lark	20.9	24.7	2.61	22	20.5	1.69	0.49	16
hoopoe lark	36.9	32.8	4.45	21	36.9	2.59	0.62	21
black-crowned finchlark	15.2	16.5	1.10	6	15.2	1.34	0.36	6
crested lark	31.2	32.2	2.30	6	31.2	2.44	0.24	6
calandra lark	50.6	49.5	1.07	2	50.6	3.03	0.57	2
horned lark <sup>b</sup>	26.0	28.6	—	—	26.0	2.08	—	—
lesser short-toed lark <sup>c</sup>	23.6	31.6	3.08	27	—	—	—	—
short-toed lark <sup>c</sup>	24.0	35.6	6.73	8	—	—	—	—
spike-heeled lark <sup>d</sup>	25.7	29.1	4.98	20	25.0	3.33	0.53	10
skylark	32.0	62.4	8.43	29	31.7	3.47	0.88	15
woodlark	25.6	49.4	9.96	20	25.5	2.41	0.70	14

Data sources: <sup>a</sup> Willoughby (1968); <sup>b</sup> Trost (1972); <sup>c</sup> Shishkin (1980); <sup>d</sup> C. Brown (unpublished data).

<sup>e</sup> Day-time values.

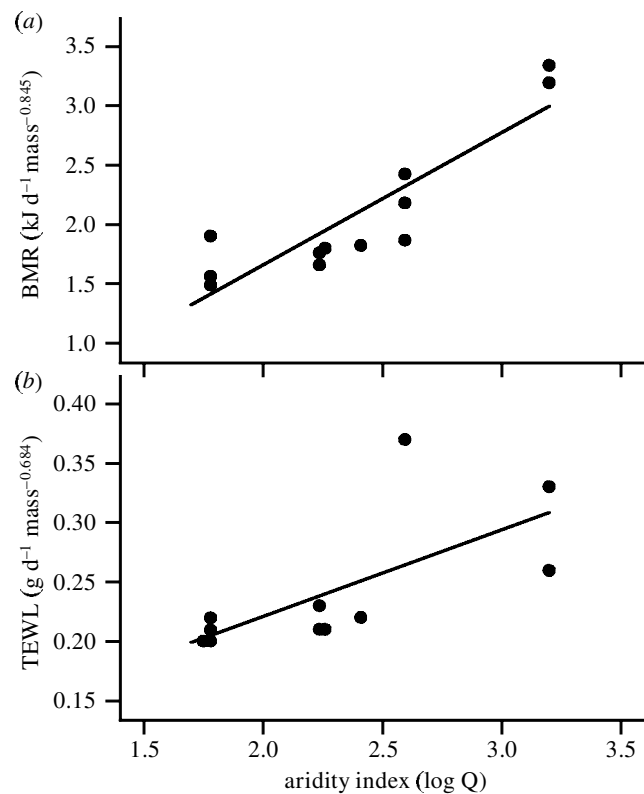


Figure 1. Mass-adjusted (a) BMR and (b) TEWL of 12 species of lark as a function of environmental aridity. Symbols are species averages.

mass + 0.121 aridity ( $r^2 = 0.87$ , d.f. = 11,  $p < 0.0001$ ). Mass and aridity both had a significant effect on TEWL (log mass:  $t = 5.50$ ,  $p < 0.0001$ ; aridity:  $t = 3.12$ ,  $p = 0.011$ ), a finding consistent with the hypothesis that larks have a lower TEWL with increasing aridity (figure 1b).

Because aridity was correlated with latitude (table 1;  $r = 0.84$ ,  $n = 9$ ,  $p = 0.005$ ), we performed stepwise multiple regression analyses with log BMR or log TEWL as the dependent variable and log mass, latitude and aridity

as independent variables. The models with the best fit to the data (see § 3a) included log mass and aridity, but not latitude (BMR:  $t = 1.85$ ,  $p = 0.10$ ; TEWL:  $t = 1.17$ ,  $p = 0.28$ ). Hence, aridity explained more of the variation in BMR and TEWL than did latitude after body mass was taken into account.

#### (b) *Phylogeny of larks*

Cytochrome *b* (975 bases) and 16S rRNA (566 bp) sequences were generated for 22 species. In addition to the species for which ecophysiological data were available, we included other African species in an attempt to improve the resolution of the phylogenetic placement of our focal taxa. A heuristic search of the combined dataset yielded two equally most-parsimonious trees (length 1424 steps, CI = 0.407, RI = 0.492,  $gI = -1.018$ ,  $p < 0.001$ ). One round of reweighting (using a base weight of 1) yielded a single tree of 579.27 steps (figure 2; CI = 0.490, RI = 0.559). Out of the 378 parsimony-informative characters, 41 had a weight of 1 and 337 had a weight of less than 1, reflecting homoplasy in the dataset. We performed bootstrap analysis with 1000 iterations using the hoopoe lark as an outgroup (figure 2). Several highly supported clades (more than 70% bootstrap support) were consistently retrieved (also in separate analyses of the two genes, trees not shown): basal placement of spike-heeled lark clade; finchlarks/desert larks clade; singing bush lark/monotonous lark clade; lesser short-toed/Athi short-toed lark clade; and skylark/woodlark clade. Several currently recognized genera appear to be polyphyletic (e.g. *Ammomanes*, *Certhilauda*, *Mirafr*). A lack of resolution of some of the terminal nodes may be caused by the relatively short internal branch lengths compared with the long terminal branch lengths. The divergence among these lark clades is also indicated by genetic distances ranging from 7 to 19% based on the cytochrome *b* gene and between 2.5 and 8% based on the 16S rRNA fragment.

We did not have DNA sequence information for the calandra lark, horned lark and short-toed lark. To apply the test for serial independence to all species for which we

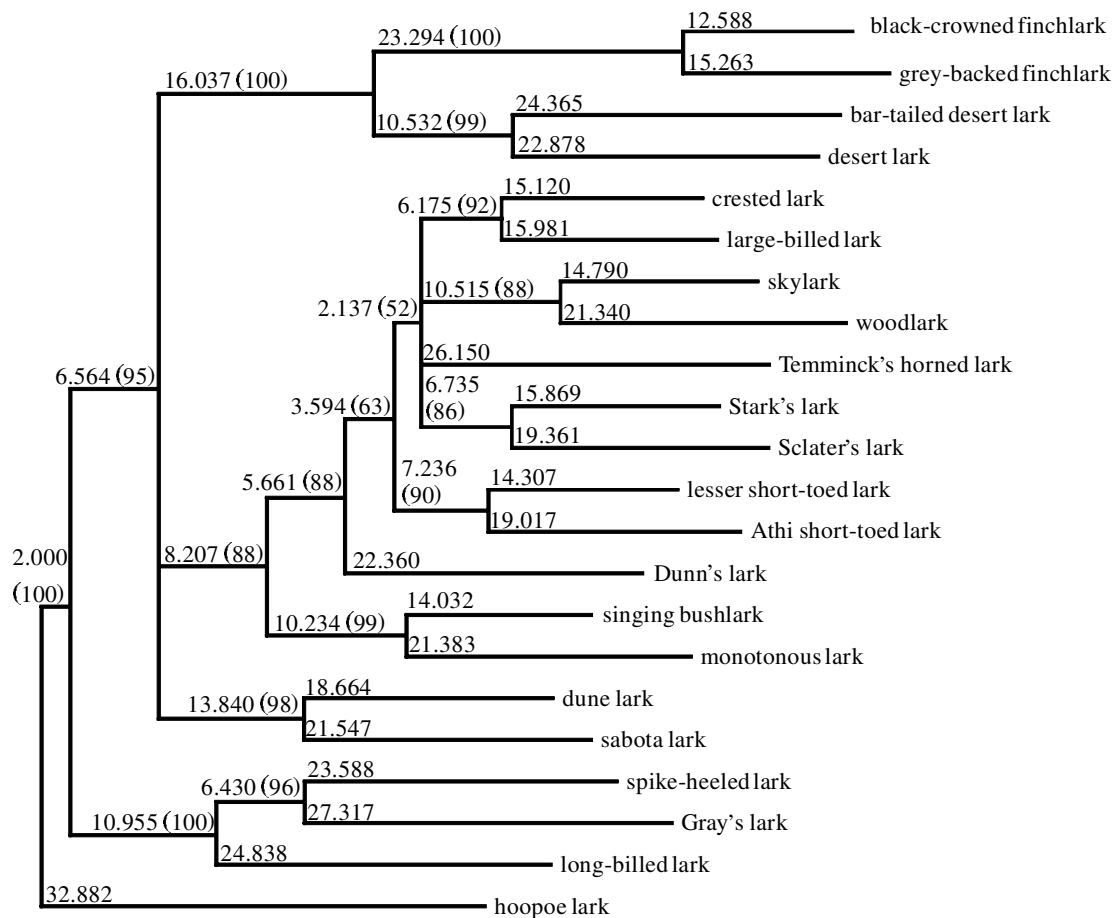


Figure 2. Phylogenetic tree of 22 species of lark based on cytochrome *b* and 16S rRNA sequences, and analysed using maximum parsimony criteria. Numbers above the branches indicate reweighted branch lengths and, in parentheses, per cent bootstrap recovery in 1000 replications.

had ecophysiological data, we placed the calandra lark in the polytomy with dune lark/sabota lark, the finchlark/desert lark clade and a third clade containing *Galerida*, *Lullula*, *Calandrella* and others. We placed the horned lark as sister species to the Temminck's horned lark. We assumed that the short-toed lark was closely related to the Athi short-toed lark.

#### (c) *Phylogenetic constraint on BMR and TEWL?*

We found no significant phylogenetic autocorrelation in the data for mass-corrected TEWL or aridity, but did find a significant autocorrelation in the data for mass-corrected BMR (mass-corrected TEWL:  $p = 0.44$ ; aridity:  $p = 0.09$ ; mass-corrected BMR:  $p = 0.01$ ). Therefore, the positive association between TEWL and aridity was not influenced by phylogenetic constraint. The significant phylogenetic autocorrelation in the mass-corrected BMR data could be attributable to phylogenetic constraint or to ecological factors, indistinguishable alternatives (Westoby *et al.* 1995). Therefore, in addition to our conventional analysis, we tested whether the relationship between mass-corrected BMR and aridity could be confirmed using phylogenetic independent contrasts (Felsenstein 1985*b*). A stepwise multiple regression analysis with contrasts of log BMR as the dependent variable, and contrasts of log mass and of aridity as independent variables, showed significant effects of log mass and aridity on log BMR ( $r^2 = 0.88$ , d.f. = 9,  $F = 32.36$ ,  $p < 0.001$ ; log mass:  $t = 6.07$ ,  $p < 0.001$ ; arid-

ity:  $t = 4.83$ ,  $p = 0.001$ ). Hence, the result of the phylogenetic analysis was consistent with that of the conventional analysis: the BMR of larks decreases with increasing aridity.

#### 4. DISCUSSION

The BMR ( $n = 12$ ) and TEWL ( $n = 12$ ) of lark decreased along a gradient of increasing aridity, consistent with our proposal. These results support the conclusions of previous studies that found reduced BMR and TEWL in desert birds compared with species from mesic habitats (Dawson & Bennett 1973; Weathers 1979; Arad & Marder 1982; Williams 1996; Tieleman & Williams 2000), but the use of a continuous environmental classification in this study makes the argument more compelling. Although body mass alone explained 53% of the interspecific variation in BMR, adding aridity increased the explained variance to 91%. Similarly, body mass explained 72% of the variance in TEWL, while adding aridity increased the explained variance to 87%. A combination of low BMR and low TEWL could be favourable in birds from dry hot environments because it reduces food and water requirements and minimizes heat production.

The value of comparisons within a group of closely related species is illustrated by the deviations of the allometries for BMR and TEWL of larks from those of all

birds. The equation relating log body mass to log BMR in larks deviated from an allometry for all birds by  $-9\%$  for a 15 g lark and by  $+25\%$  for a 50 g lark (Tieleman & Williams 2000). The BMR of desert larks was close to allometric predictions, in contrast to the BMR of mesic larks, which far exceeded predictions. Predictions of TEWL based on the lark allometry were lower than those based on an allometry including all birds (Williams 1996) by  $-26\%$  for a 15 g lark and by  $-12\%$  for a 50 g lark. Although the TEWL of a lark from the desert was below allometric predictions, as expected, larks in general had a low TEWL. Hence, comparing the BMR or TEWL of a single lark species with the all-bird allometry would have led to erroneous conclusions about the adaptive significance of these traits.

The phylogeny of larks was characterized by long terminal branches and short internal branch lengths (figure 2). This, combined with the occurrence of larks in a wide array of habitats, may indicate that lark species rapidly radiated into different environments and lived for a significant part of their evolutionary history in diverse habitats. One might predict that phylogenetic constraints are small, natural selection has had ample time to eliminate suboptimal phenotypes and the current trait values are of adaptive significance in the current environment. The lack of phylogenetic effect in the mass-corrected TEWL data implies that phylogenetic relatedness is not a major evolutionary factor explaining interspecific differences in TEWL. The positive phylogenetic autocorrelation in the mass-corrected BMR data could indicate either a phylogenetic constraint or closely related species experiencing common ecological factors (Westoby *et al.* 1995). The correlation between BMR and aridity is not the result of phylogenetic constraint, because the results of the contrast analysis are consistent with those of the conventional statistics. Therefore, evolutionary forces other than phylogenetic constraint are likely to underlie the correlations between BMR, TEWL and environmental aridity.

Interspecific phenotype–environment correlations can indicate either genetic differences brought about by natural selection or phenotypically plastic responses to environmental conditions. Plastic responses include changes in adult phenotype depending on the environment (acclimation) and differences between phenotypes resulting from developmental conditions (ontogenetic plasticity). In a separate study, we found that the decrease in BMR and TEWL in larks along an aridity gradient cannot be attributed to acclimation to thermal environment, food availability or photoperiod (Tieleman *et al.* 2003).

In summary, decreasing levels of BMR and TEWL in larks correlate with increasing aridity. These physiological traits may have adaptive significance in the current environment, and natural selection is a likely process to explain our findings. Identifying evolutionary processes that cause correlative associations between traits remains difficult (Leroi 1994), but we have eliminated phylogenetic constraint and acclimation as likely alternative explanatory processes. The results of this study together with the decrease in BMR in mice along an environmental gradient of decreasing primary productivity (Mueller & Diamond 2001) suggest that the decrease in energy and water requirements with decreasing food and water availability is a general pattern found in birds and mammals.

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