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# Immune Indexes of Larks from Desert and Temperate Regions Show Weak Associations with Life History but Stronger Links to Environmental Variation in Microbial Abundance

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## ABSTRACT

Immune defense may vary as a result of trade-offs with other life-history traits or in parallel with variation in antigen levels in the environment. We studied lark species (Alaudidae) in the Arabian Desert and temperate Netherlands to test opposing predictions from these two hypotheses. Based on their slower pace of life, the trade-off hypothesis predicts relatively stronger immune defenses in desert larks compared with temperate larks. However, as predicted by the antigen exposure hypothesis, reduced microbial abundances in deserts should result in desert-living larks having relatively weaker immune defenses. We quantified host-independent and host-dependent microbial abundances of culturable microbes in ambient air and from the surfaces of birds. We measured components of immunity by quantifying concentrations of the acute-phase protein haptoglobin, natural antibody-mediated agglutination titers, complement-mediated lysis titers, and the microbicidal ability of whole blood. Desert-living larks were exposed to significantly

lower concentrations of airborne microbes than temperate larks, and densities of some bird-associated microbes were also lower in desert species. Haptoglobin concentrations and lysis titers were also significantly lower in desert-living larks, but other immune indexes did not differ. Thus, contrary to the trade-off hypothesis, we found little evidence that a slow pace of life predicted increased immunological investment. In contrast, and in support of the antigen exposure hypothesis, associations between microbial exposure and some immune indexes were apparent. Measures of antigen exposure, including assessment of host-independent and host-dependent microbial assemblages, can provide novel insights into the mechanisms underlying immunological variation.

## Introduction

Among the ideas to explain variation in immune defenses, the trade-off hypothesis predicts that consumption of limited resources by the immune system leads to trade-offs between immune function and other resource-demanding activities such as reproduction (Sheldon and Verhulst 1996; Ilmonen et al. 2000; Lochmiller and Deerenberg 2000; Norris and Evans 2000). Species with low reproductive rates and long life spans, referred to as a slow “pace of life,” should invest more in immunity than “fast-living” species with high reproductive rates and short life spans that favor current reproduction over self-maintenance activities such as immune defense (Roff 1992; Stearns 1992; Ricklefs and Wikelski 2002; Tieleman et al. 2005; Lee 2006). Alternatively, the antigen exposure hypothesis suggests that investment in immunity is shaped by exposure to antigens (Piersma 1997; Møller 1998; Blount et al. 2003; Matson 2006; Spottiswoode 2008). Environments with numerous antigens should select for robust immune systems that match the high antigenic challenge. However, since immune defense is resource demanding, exposure to fewer antigens should result in a reduction in immune investment, resulting in relatively weaker immune systems that nonetheless still match the infection risk (Lindström et al. 2004; Tschirren and Richner 2006).

Interestingly, opposing predictions about immune investment arise when applying the trade-off and antigen exposure hypotheses to birds from xeric and mesic environments (Horrocks et al. 2011). The pace of life is slower in birds living in deserts than in birds from the temperate zone, as exemplified

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by larks (Alaudidae), which in this context have especially well-characterized life histories, behaviors, and physiologies (Tieleman et al. 2004; Tieleman 2005; Williams and Tieleman 2005). Compared with their temperate counterparts, desert larks expend less on current reproduction and invest more in self-maintenance, laying smaller and fewer clutches per year, exhibiting reduced parental effort, and having higher predicted adult survival (Tieleman et al. 2004). Hence, according to the trade-off hypothesis, desert larks, with their slower pace of life, may invest more in immunity than temperate larks (Tieleman et al. 2005; Lee 2006). However, relative to mesic environments, deserts might pose a lower risk of infection by endo- and ectoparasites (Little and Earlé 1995; Moyer et al. 2002; but see Valera et al. 2003; Carrillo et al. 2007; Froeschke et al. 2010). The combined effects of low primary productivity, high temperatures, minimal precipitation, and high solar radiation likely limit the abundance and diversity of microbial assemblages in deserts (Tong and Lighthart 1997; Burrows et al. 2009; Tang 2009; Bachar et al. 2010). Thus, compared with temperate larks, desert-living larks might encounter fewer parasites and microbes, which, according to the antigen exposure hypothesis and in contrast to the trade-off hypothesis, may lead to lower investment in immune defense.

To assess the exposure of birds to microbes, we introduced a novel technique to quantify two abundance measures of broad, nonspecific microbial pressure: host-independent microbial concentrations present in ambient air and host-dependent densities of microbes on the surface of birds (Horrocks et al. 2012). Host-dependent measures, such as prevalence and load of specific parasites, can be affected by host immune defenses and may not reflect the magnitude of the host-independent antigenic challenge associated with a particular environment (Horrocks et al. 2011). Furthermore, in contrast to specific parasites, microbial pressures are faced by all animals, and the abundance of microbes can be measured either independently of an individual or in connection with particular individuals. Vectors are typically not required for transmission of microbes (Kulkarni and Heeb 2007), and variation in microbial pressure has physiological implications relevant for immune defense and host health (Ruiz-Rodríguez et al. 2009; Alcaide et al. 2010; Huttunen et al. 2010; Horrocks et al. 2012).

Indexes of immunity relevant for defense against microbes include the following: (i) concentrations of the acute-phase protein haptoglobin, which increase in response to inflammation and infection (Dobryszcka 1997; van de Crommenacker et al. 2010; Matson et al. 2012), binding heme to make iron unavailable for microbial growth (Gutteridge et al. 1987; Dobryszcka 1997; Quaye 2008) and promoting Th1 cellular immune responses that are particularly appropriate for countering microbial challenges (Arredouani et al. 2003); levels of (ii) natural antibodies and (iii) lytic enzymes, which work together to recognize, process, and eliminate microorganisms (Ochsenbein and Zinkernagel 2000); and (iv) the *in vitro* ability of whole blood to limit or kill microbes, a measure that integrates cellular and soluble mechanisms (Tieleman et al. 2005; Millet et al. 2007).

In this study, we disentangled the opposing predictions about immune investment generated by the trade-off and antigen exposure hypotheses. Studying birds with contrasting life histories in desert and temperate environments, we examined differences in concentrations of microbes in ambient air, in densities of microbes blown from the surface of birds, and in four immunological indexes related to defense against microbes. To minimize the potentially confounding effects of different behaviors, diets, or phylogenetic backgrounds, we focused our study on seven closely related lark species (Alaudidae; Tieleman et al. 2004).

## Material and Methods

### *Study Species and Study Locations*

We studied seven lark species during the breeding season. Arid-zone larks (hoopoe lark *Alaemon alaudipes*,  $n = 37$ ; Dunn's lark *Eremalauda dunnii*,  $n = 29$ ; bar-tailed desert lark *Ammomanes cincture*,  $n = 11$ ; black-crowned finchlark *Eremopterix nigriceps*,  $n = 27$ ; crested lark *Galerida cristata*,  $n = 13$ ) were captured in May and June 2006 and 2007 at two desert locations in Saudi Arabia. In 2006, all species were captured at Mahazat as-Sayd (henceforth Mahazat), a reserve in the Arabian Desert (22°15'N, 41°50'E). In 2007, the nomadic species, black-crowned finchlark and crested lark, were captured about 170 km away at the National Wildlife Research Center, Taif (21°15'N, 40°42'E). Mahazat is characterized by gravel plains, sparse vegetation, and annual mean rainfall of  $96 \pm 71$  mm ( $\pm$  SD). Spring conditions are hot and dry; mean air temperatures are around 30°C, and maximum temperatures often exceed 40°C (Tieleman and Williams 2002). Environmental conditions at Taif are less extreme than at Mahazat, although this area is still considered desert (Tieleman et al. 2003).

Temperate larks (woodlark *Lullula arborea*,  $n = 58$ ; skylark *Alauda arvensis*,  $n = 123$ ) were captured in the Aekingerzand, northern Netherlands (52°56'N, 06°18'E), between April and July 2006–2008, with some individuals sampled in multiple years. The Aekingerzand consists of heath and grazed meadowland, surrounded by agricultural fields and mixed woodland. Mean annual rainfall is  $853 \pm 160$  mm. Mean daily air temperature during the breeding season is 13.8°C (Koninkrijk Nederlands Meteorologisch Instituut).

Birds were captured using mist nets and clap traps. Sometimes we used playback calls or birdseed and mealworms to lure birds. Permission to work with wild birds in Saudi Arabia was obtained from the National Wildlife Research Centre. Procedures in the Netherlands were conducted under license from the Animal Experimentation Committee of the University of Groningen (DEC 5219, 5219A).

### *Air Sampling*

We used a portable impaction air sampler (Burkard, Rickmansworth, UK) that draws air at a constant rate through 1.0-

mm holes in a perforated plate and collects the particles passing through the holes onto an agar-filled petri dish below (Horrocks et al. 2012). Following incubation of the agar plate, we counted the number of colony-forming units (CFUs) to obtain an index of the concentration of culturable airborne microbial particles. Culture-based air-sampling devices continue to be used in medical and industrial settings (e.g., Dansby et al. 2008; Haas et al. 2010) because they provide an easily understandable measure of antigen exposure, despite issues relating to culturability (Rappé and Giovannoni 2003).

We used the air sampler to collect two types of data: concentrations of culturable microbes in ambient air and densities of culturable microbes blown from the surface of birds (Horrocks et al. 2012). In both cases, we used selective agars to culture three different microbial assemblages: (i) generalist aerobic bacteria (tryptic soy agar; Sigma-Aldrich, St. Louis, MO); (ii) gram-negative bacteria (MacConkey agar with crystal violet sodium, chloride, and 0.15% bile salts; Sigma-Aldrich); (iii) fungi (Sabouraud 4% glucose agar [Sigma-Aldrich], with 50 mg L<sup>-1</sup> Gentamicin antibiotic [Invitrogen, Breda, Netherlands]). Plates were incubated at 30°C and, because of different growth rates, numbers of CFUs were counted after 24 h (generalist bacteria), 72 h (fungi), and 96 h (gram-negative bacteria). We corrected all CFU counts by applying a “positive hole correction” for a 100-hole air sampler. This correction accounts for the possibility that multiple culturable particles passed through a single sampling hole but produced only a single countable particle (Andersen 1958).

#### Environmental Air Sampling

We sampled microbes in ambient air at microhabitats where birds foraged or rested (desert,  $n = 16$ ; temperate,  $n = 12$ ) over multiple days spanning periods of fieldwork (desert,  $n = 20$  d in 2007; temperate,  $n = 37$  d in 2008). Although we sampled during the same periods, environmental air samples do not correspond to specific bird-sampling events. We sampled throughout daylight hours to account for potential diurnal variation in microbial loads (Tong and Lighthart 1999). Sampling duration was optimized for both environments to avoid the situation where colonies were too numerous to count: air was sampled for 15–30 min per agar plate at the desert sites and for 5 min per agar plate at the temperate site. Sampling effort was standardized by multiplying the duration of sampling by the airflow rate (20 L min<sup>-1</sup>) and expressing the data as CFU m<sup>-3</sup> of air.

#### Sampling of Microbes on Birds

Sampling of microbes on birds took place in the desert in 2007 ( $n = 36$ ) and in the temperate zone in 2008 ( $n = 36$ ) during the breeding season. We isolated a bird in a sterilized plastic box (33 cm × 22 cm × 16 cm) with a fitted lid. The sterilized head of the air sampler was fitted through a hole in the side

of the box, and the air sampler was used to collect the microbes blown from the surface of the bird as the air sampler circulated the air in the box (Horrocks et al. 2012). To minimize cross contamination among birds and ourselves we collected microbes from birds immediately after capture, cleaned our hands with antibacterial hand wash before and after touching birds, and used single-use paper bird-handling bags. Birds were always sampled for 5 min per agar plate. The order of the three agar types varied randomly among birds, and duplicate plates were run for each agar type. We sampled species of varying body size, so we calculated body surface area (Walsberg and King 1978) and expressed counts as densities (CFU cm<sup>-2</sup> of body surface area). Because a standard sampling time was always used and because air was recirculated back into the box during sampling, these data are not based on air volume.

#### Blood Sampling and Immune Assays

After sampling microbial densities on birds, we bled (45–60 min after capture), weighed ( $\pm 0.1$  g), and measured (wing,  $\pm 0.1$  cm; tarsus,  $\pm 0.01$  cm) all birds. A further 226 birds (desert,  $n = 81$ ; temperate,  $n = 145$ ) were only bled (<10 min after capture), weighed, and measured. Up to 300  $\mu$ L of blood was collected from the brachial vein and stored on ice until processing later the same day. Samples were centrifuged to separate cellular and plasma fractions, which were frozen and stored at  $-20^\circ\text{C}$ . Birds were sexed by body measurements and by behavioral observations.

We measured haptoglobin concentrations (mg mL<sup>-1</sup>) with an assay that quantifies the heme-binding capacity of plasma (TP801; Tri-Delta Diagnostics, Morris Plains, NJ; Matson et al. 2012). We quantified natural antibody-mediated agglutination titers and complement-mediated lysis titers by incubating red blood cells from rabbits (B-0009D, Harlan, Bicester, UK) with serially diluted plasma samples from birds (Matson et al. 2005). We quantified the ability of whole blood to limit microbial growth using fresh blood samples, collected immediately upon capture from non-air-sampled birds only and transported to the laboratory for use within 30 min (Tieleman et al. 2005; Millet et al. 2007). We tested microbicidal ability against three organisms chosen to minimize the effects of exposure histories of birds: *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 10231), and *Staphylococcus aureus* (ATCC 6538; MicroBiologics, St. Cloud, MN). Blood, diluted with CO<sub>2</sub>-independent media (18045-054, Invitrogen, Breda, Netherlands), was incubated with bacteria under strain-specific conditions: *E. coli* was diluted 1 : 10 with blood-media mixture and incubated for 30 min; *C. albicans* and *S. aureus* were diluted 1 : 9 with blood-media mixture and incubated for 180 min. All other conditions were as described by Tieleman et al. (2005) and Matson et al. (2006). We calculated microbicidal ability as 1 – (number of CFUs on blood plates/number of CFUs on control plates). We interpreted higher values of all of these immune indexes as indicating greater immunological investment, regardless of whether the cause was greater risk (i.e., higher standing levels

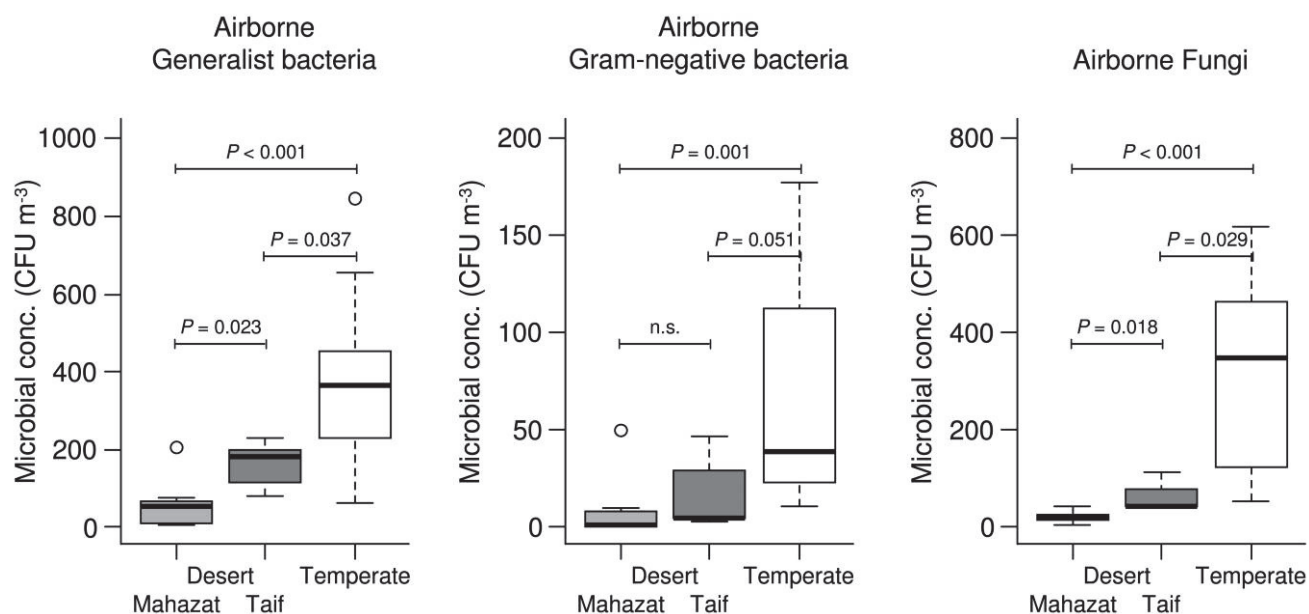


Figure 1. Concentrations of airborne microbes measured at two sites in the Arabian Desert and in the temperate Netherlands during spring. Data are presented as the interquartile range (IQR; boxes), median value (thick bars inside boxes), and minimum and maximum value, or 1.5 times the IQR (approximately 2 SD), whichever is smaller (whiskers). Data outside this range (“outliers”) are plotted individually as open circles.

of immunity) or greater experience (i.e., elevated levels due to exposure) of microbial abundance.

#### Statistical Analyses

We used Mann-Whitney  $U$ -tests and Fisher’s  $F$ -tests to examine environmental differences in mean values of airborne microbial concentrations. We used generalized and linear mixed models to examine environmental variation in bird-associated microbes and immune indexes. For each response variable we analyzed data from all years and a restricted data set containing only values collected from both environments in the same year(s). Results were qualitatively similar whichever data set was used; we report only results obtained using the larger data set. Full models contained the fixed effects of environment (desert or temperate), sex, year, and the interaction between environment and sex. We controlled for species differences and repeated measures by including species and individual nested within species as random effects. To examine species differences within and among environments, we refitted models by including species as a fixed effect and excluding the term environment and its interaction with sex. When the term species was significant, we used Tukey post hoc tests to identify which particular species were significantly different from each other. We simplified models using stepwise backward elimination based on log-likelihood ratio tests and  $P > 0.05$ . Residuals of models were checked for normality and homogeneity of vari-

ance by graphical examination. Analyses were performed using R, version 2.10.01 (R Development Core Team 2009).

We included time of day as a covariate in analyses of bird-associated microbes to account for potential temporal fluctuations in concentrations of airborne microbes (Tong and Lighthart 1999). We grouped bird-derived data for our two desert sites because immune indexes and bird-associated microbial densities do not differ between these sites during the breeding season (Horrocks et al. 2012). We also combined data on haptoglobin concentration and agglutination and lysis titers for individuals that were first sampled for microbes with the air sampler and those that were only bled and measured. There were no differences between these groups (haptoglobin:  $F_{1,62} = 3.39$ ,  $P = 0.07$ ; agglutination:  $F_{1,60} = 0.14$ ,  $P = 0.71$ ; lysis:  $F_{1,61} = 0.00$ ,  $P = 0.98$ ; Horrocks et al. 2012), despite differences in time of blood collection after capture (45–60 min vs. <10 min).

For the microbicidal assay, incubation with blood from some individuals led to the growth, rather than the death, of microbes and, consequently, negative assay values (*E. coli*, 21/90 individuals; *C. albicans*, 14/79; *S. aureus*, 65/83). We followed the approach of Buehler et al. (2008) and present the results of analyses based on assumptions of normality. Microbicidal ability can depend on the inoculation concentration, so we always included the mean control plate colony count as a covariate.

We examined correlations between total microbial density, which was the sum of all bacterial and fungal densities per bird,



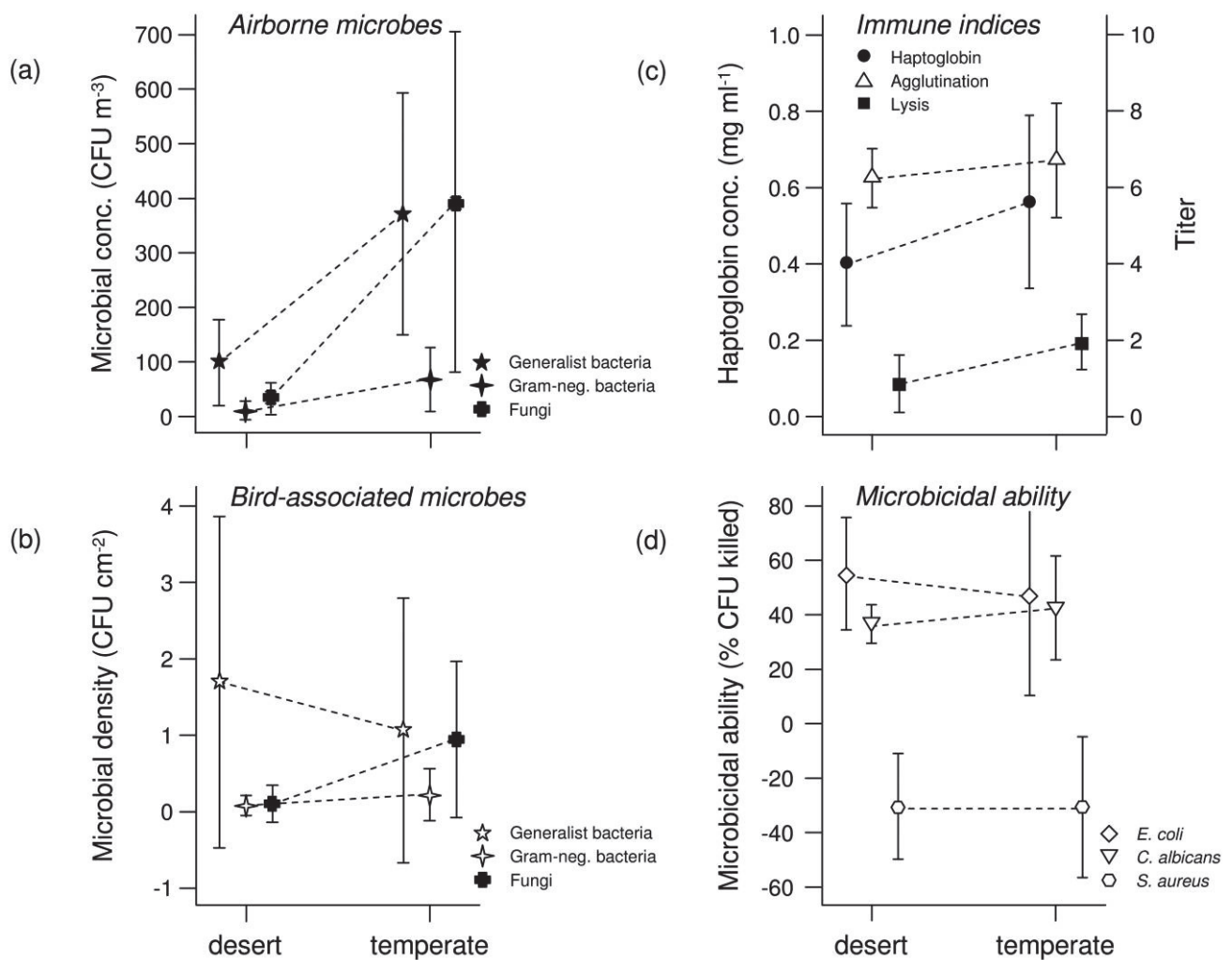


Figure 2. *a*, Concentrations of culturable airborne environmental microbes sampled from the Arabian Desert and the temperate Netherlands; *b*, densities of bird-associated culturable microbes; *c*, indexes of innate immunity (haptoglobin concentration, agglutination and lysis titers); and *d*, microbicidal ability (proportion of microbes killed) of blood sampled from larks living in these environments. Data are mean values  $\pm$  SD. In *b*–*d*, data are plotted after accounting for variation between years and species (fig. 4). Nonsignificant differences between environments are represented by open symbols, and significant differences between environments are represented by filled symbols.

and immune indexes (excluding microbicidal ability, since birds bled for measurement of microbicidal ability were not air sampled for microbial density). We calculated species means of total microbial density and individual deviations from these means to distinguish the contributions of within- and among-species variation (van de Pol and Wright 2009).

## Results

### Concentrations of Environmental Airborne Microbes

Mean airborne concentrations of all microbial groups were significantly lower in the desert than in the temperate environment (figs. 1, 2*a*). Within the desert, concentrations of generalist bacteria and fungi were significantly lower at Mahazat than at Taif (fig. 1). Gram-negative bacteria were the least numerous microbial group in both environments (figs. 1, 2*a*).

### Densities of Microbes Associated with Lark Species

Fungal densities were significantly lower on larks living in the desert compared with temperate larks ( $z = -3.74$ ,  $P < 0.001$ ; figs. 2*b*, 3), but densities of generalist bacteria ( $z = 0.97$ ,  $P = 0.33$ ) and gram-negative bacteria ( $z = -1.42$ ,  $P = 0.16$ ) associated with larks did not differ significantly between locations (figs. 2*b*, 3). Excluding three outliers that were greater than 2 SD from the mean did not change this result (fig. 3). Birds sampled later in the day carried higher densities of generalist bacteria ( $z = 2.11$ ,  $P = 0.035$ ) and fungi ( $z = 2.35$ ,  $P = 0.019$ ), but the size of this effect was small (an increase of approximately 1 CFU cm<sup>-2</sup> per 12 h in each case). Female larks shed more fungi than males ( $z = -2.01$ ,  $P = 0.045$ ), with a similar trend for generalist bacteria ( $z = -1.91$ ,  $P = 0.06$ ).

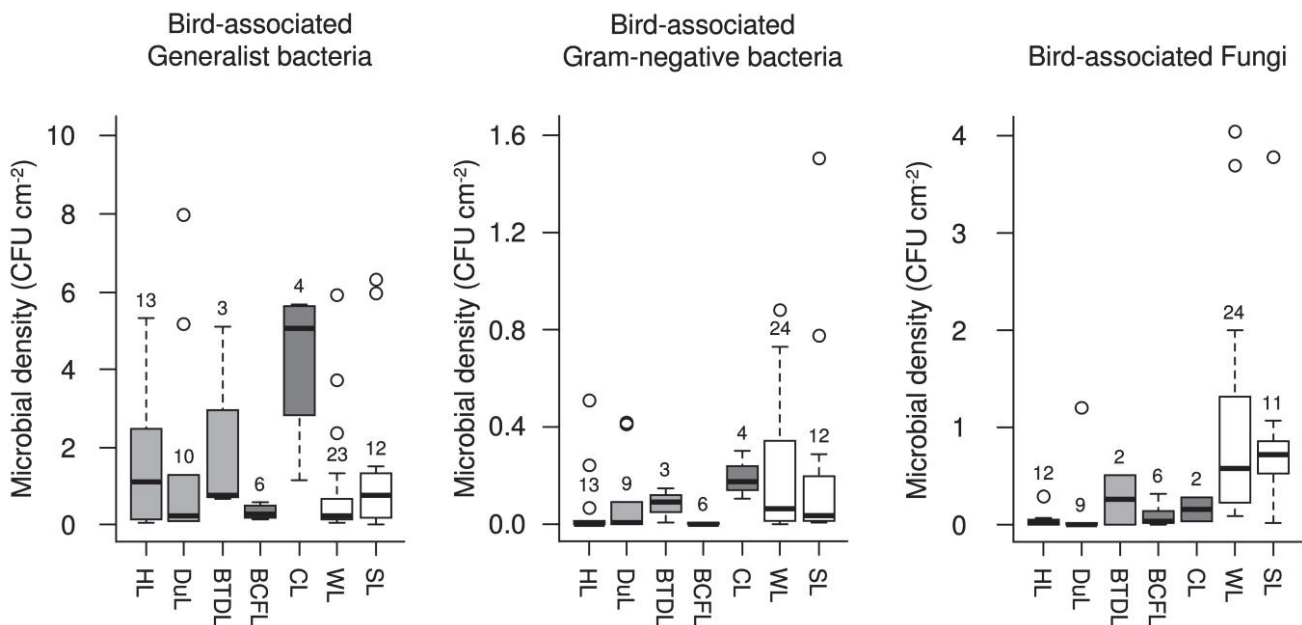


Figure 3. Densities of bird-associated microbes air sampled from seven lark species in the Arabian Desert ( $n = 5$  species) and the temperate Netherlands ( $n = 2$  species; white bars) in spring. Numbers above bars are sample sizes. HL = hoopoe lark; DuL = Dunn's lark; BTDL = bar-tailed desert lark; BCFL = black-crowned finch lark; CL = crested lark; WL = woodlark; SL = skylark. For an explanation of graph components, see legend for figure 1.

There were no sex differences for densities of gram-negative microbes ( $z = -0.25$ ,  $P = 0.80$ ).

Densities of bird-associated fungi differed significantly among species ( $F_{6,61} = 3.16$ ,  $P = 0.009$ ), but a post hoc test could not resolve the source of the significance (fig. 3). Densities of generalist bacteria ( $F_{6,64} = 1.99$ ,  $P = 0.08$ ) and gram-negative bacteria ( $F_{6,64} = 1.58$ ,  $P = 0.17$ ) associated with larks did not differ among species.

Repeatability of microbial abundance, using our air-sampling measurement method and based on the duplicate plates run per agar type for each bird, is shown in table 1 (Lessels and Boag 1987). Repeatability was significant for all three microbial groups, suggesting that measurement variability was greater among individuals than within individuals and indicating that our air-sampling technique provides a reliable and repeatable measurement of the microbial density associated with a bird. Note that we used averages of the duplicate plates per agar type as the estimate of microbial density for each individual.

#### Immune Indexes

Examining overall differences in immune indexes between environments, we found that haptoglobin concentrations ( $F_{1,5} = 8.45$ ,  $P = 0.034$ ) and lysis titers ( $F_{1,5} = 30.25$ ,  $P = 0.003$ ) were significantly lower in desert larks compared with lark species at the temperate site (table 2; figs. 2c, 4). Agglutination titers ( $F_{1,5} = 3.98$ ,  $P = 0.10$ ) and microbicidal ability against *Escherichia coli* ( $F_{1,5} = 0.03$ ,  $P = 0.87$ ), *Candida albicans* ( $F_{1,5} = 0.06$ ,  $P = 0.82$ ), and *Staphylococcus aureus*

( $F_{1,5} = 0.66$ ,  $P = 0.45$ ) showed no significant differences between environments (table 2; figs. 2b, 2c, 4).

When species were compared, while excluding environment as a factor, all immunological measures except microbicidal ability against *C. albicans* exhibited significant interspecific variation (table 3). Post hoc tests revealed that none of the desert lark species differed significantly from each other in any immune measure; however, the two temperate lark species differed significantly in microbicidal ability against *E. coli* (table 3).

#### Relationships between Immune Indexes and Total Microbial Load

Lysis titer was the only immune index that showed any relationship with total microbial load but only at the within-species level (see trend in table 4). Analyzing data for each environment separately revealed that the within-species relationship was strongly driven by desert birds ( $F_{1,27} = 5.61$ ,  $P = 0.025$ ) and was absent in temperate larks ( $F_{1,29} = 0.09$ ,  $P = 0.76$ ).

#### Discussion

Current ideas relating to physiological trade-offs and to antigen exposure can lead to contradictory predictions about immunological investment (Horrocks et al. 2011). We explored these opposing predictions simultaneously by contrasting the differences in immune defenses and host-independent and host-dependent microbial abundances in larks that are characterized by distinct life histories and that live in very different environ-

Table 1: Measurement repeatability ( $r$ ) and coefficient of variance (CV) of densities of bird-associated culturable microbes sampled from five desert and two temperate lark species in the Arabian Desert and the temperate Netherlands during spring

Microbial group and type of data	$r$	SE	$\chi^2$	$P$	Within-individual CV
Generalist bacteria:					
Raw	.60	.08	30.36	<.001	.52
Corrected	.62	.08	32.72	<.001	.58
Gram-negative bacteria:					
Raw	.45	.10	15.39	<.001	.85
Corrected	.40	.10	11.29	<.001	.85
Fungi:					
Raw	.55	.09	20.89	<.001	.67
Corrected	.25	.12	3.75	.053	.72

Note. Repeatability was calculated according to Lessels and Boag (1987; repeatability = among-individual variance/within-individual variance + among-individual variance). Statistical significance was calculated using a log-likelihood test, and standard errors (SE) for repeatability values were determined according to Becker (1984). Results are presented based on analyses using raw colony-forming unit counts and with corrected values obtained after applying a positive hole correction (see "Air Sampling"; Andersen 1958) and are based on the duplicate plates per agar type run for each individual.

ments. Larks living at a slow pace of life in an extremely xeric environment (Tieleman et al. 2004) exhibited similar or significantly lower levels of immune defenses than larks living at a faster pace of life in a mesic locale. Furthermore, slow-living desert larks encountered relatively less microbial abundance when compared with their fast-living counterparts in the temperate zone. The results of this study do not support an association between greater overall immunological investment and a slow pace of life, as predicted by the trade-off hypothesis, and are more consistent with predictions arising from the antigen exposure hypothesis, which links elevated exposure to antigens with stronger or more active immune systems (Horrocks et al. 2011).

#### *Life History and Immune Investment*

Our study provided no support for a link between physiological trade-offs and immunological investment, as predicted by the trade-off hypothesis. Larks in the desert, which optimize self-maintenance at the expense of current reproduction (Tieleman et al. 2004, 2008), did not display increased immune function compared with temperate larks, which optimize current reproduction over self-maintenance. The lack of correlation between immune indexes, particularly in vitro microbicidal abilities, and life-history parameters contrasts with other studies. For example, within the tropics, slow-living birds kill microbes better than faster-living species (Tieleman et al. 2005; Matson et al.

Table 2: Environmental variation in immune indexes measured in five desert and two temperate lark species in the Arabian Desert and the temperate Netherlands during spring

Immune index	Mean (intercept)	Difference	95% CI
Haptoglobin (mg mL <sup>-1</sup> )	<b>.37</b>	<b>.21</b>	<b>.02–.40</b>
Agglutination (titer)	5.58	1.03	–.30–2.36
Lysis (titer)	<b>.15</b>	<b>1.15</b>	<b>.61–1.67</b>
<i>Escherichia coli</i> (% killed)	.73	–.04	–.58–.51
<i>Candida albicans</i> (% killed)	.33	.03	–.28–.34
<i>Staphylococcus aureus</i> (% killed)	–.13	.13	–.27–.53

Note. Data are presented as mean values (intercept), estimate sizes (difference), and 95% confidence intervals around the difference (95% CI) for environmental variation in indexes of immunity measured in larks in the Arabian Desert ( $n = 5$  species) and temperate Netherlands ( $n = 2$  species) in spring. Desert larks were measured in 2006 and 2007, and temperate larks were measured in 2006–2008. Intercepts and differences are taken from final statistical models examining the role of environmental effects on each parameter. "Desert" is the reference category, and differences are given relative to this. Significant relationships are shown in bold.



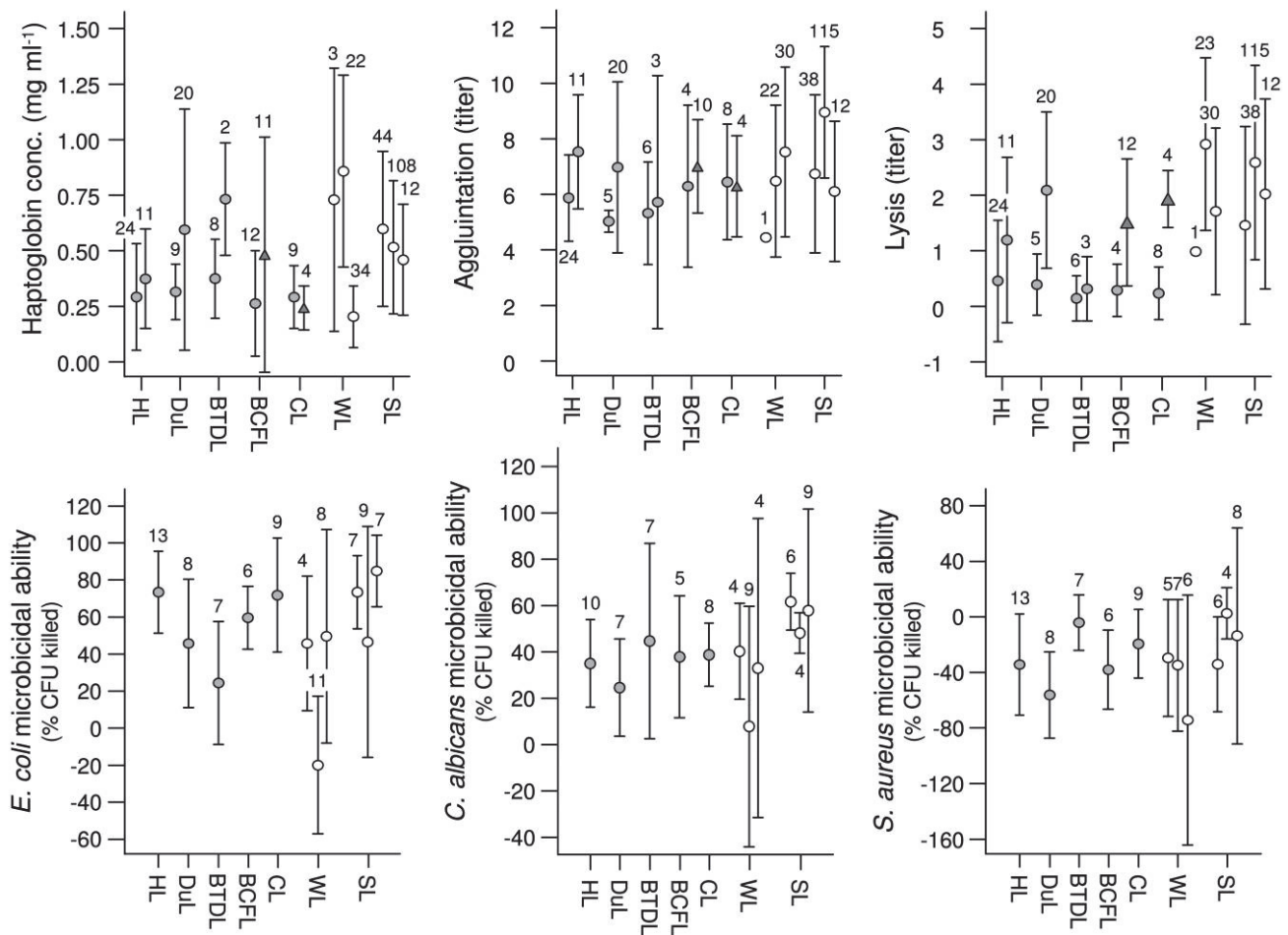


Figure 4. Haptoglobin concentration, agglutination and lysis titers, and microbicidal ability (proportion of microbes killed) of whole blood against *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus* measured in seven lark species in the Arabian Desert ( $n = 5$  species; gray symbols) and the temperate Netherlands ( $n = 2$  species; white symbols) during spring. Desert larks were measured in 2006 and 2007 in Mahazat as-Sayd (gray circles) and/or Taif (gray triangles). Temperate larks were measured in 2006–2008. Data are mean values  $\pm$  SD. Numbers above bars are sample sizes. HL = hoopoe lark; DuL = Dunn's lark; BTDL = bar-tailed desert lark; BCFL = black-crowned finchlark; CL = crested lark; WL = woodlark; SL = skylark.

2006). On the other hand, fast-living *Peromyscus* mice have greater microbicidal ability than moderate-living chipmunks or slow-living gray squirrels (Previtali et al. 2012), even though within the *Peromyscus* genus there is no relationship between reproductive life-history strategy and microbicidal ability (Martin et al. 2007). In another study, fast-living shoreline ecotypes of garter snakes *Thamnophis elegans* killed more microbes than their slow-living counterparts that live in montane meadows (Sparkman and Palacios 2009). With the exception of the garter snake study that involved populations living in separate environments, and where parasites were detected only in slow-living individuals (Sparkman and Palacios 2009), antigen exposure was not investigated in these studies, and they all focused on species living in shared environments. Hence, opposing predictions about how life history and antigen exposure might influence immunological investment could not be explored in these studies. Study systems that can generate such opposing

predictions are needed if the effects of life history and antigen exposure on immunity are to be successfully teased apart and if the generality of life-history trade-offs in shaping immunological investment is to be made clear.

Although we could not document a correlation between a slow pace of life and greater investment in the immune indexes that we measured, our results could provide a glimpse of how particular immune defense components may be associated with pace of life. We measured immunological components that are relevant for defense against general microbial exposure. However, other unmeasured branches of the immune system, such as antibody-mediated acquired immunity, may be selected for in association with the longer developmental times and higher survival rates that characterize a slow pace of life (Lee 2006; Lee et al. 2008; Sparkman and Palacios 2009). In this context, fast-living species, with shorter developmental times and increased reproductive rates, are hypothesized to rely more on

Table 3: General linear model statistics for the species variation in immune indexes measured in five desert and two temperate lark species in the Arabian Desert and the temperate Netherlands during spring

Immune measure	<i>F</i>	df	<i>P</i>	Species pairs
Haptoglobin (mg mL <sup>-1</sup> )	4.56	6, 263	<.001	WL-HL WL-BCFL WL-CL SL-HL
Agglutination (titer)	3.83	6, 245	.001	SL-DuL
Lysis (titer)	5.05	6, 247	<.001	WL-HL WL-BTDL SL-HL SL-BTDL SL-BCFL
<i>Escherichia coli</i> (% killed)	4.39	6, 80	<.001	HL-WL SL-WL SL-BTDL
<i>Candida albicans</i> (% killed)	2.05	6, 65	.07	SL-WL
<i>Staphylococcus aureus</i> (% killed)	2.19	6, 71	.05	SL-DuL

Note. The final column shows those species that differed significantly from each other (at the *P* = 0.05 level) in pairwise post hoc Tukey tests. HL = hoopoe lark; DuL = Dunn's lark; BTDL = bar-tailed desert lark; BCFL = black-crowned finch lark; CL = crested lark; WL = woodlark; SL = skylark. For each species pair, the species with the higher mean value for a particular immune parameter is always listed first.

faster-acting, innate immune defenses, despite the inflammatory and energetic costs that this entails (Lee 2006; Lee et al. 2008; Sparkman and Palacios 2009). Our results might be in agreement with the idea that fast-living species prioritize investment in inflammatory-mediated defenses more than slow-living species: we found that the acute-phase protein haptoglobin and complement-triggered cell lysis, both related to inflammatory responses, were greater in temperate larks. Nonetheless, without measures of specific antibody-mediated defenses, conclusions from this study about covariation among immunological components and how certain mixes of defenses may link to life histories must remain tentative.

#### Microbial Abundance and Immune Investment

The antigen exposure hypothesis predicted that immune indexes should be lower in desert larks that experienced reduced

microbial abundance. Ambient air in the desert contained lower concentrations of microbes than air from the temperate zone. In light of the significantly reduced levels of haptoglobin and lysis in desert larks, our results are therefore supportive of the antigen exposure hypothesis. The paucity of microbes in desert air likely relates to deserts being areas of low primary productivity (Field et al. 1998). This ecological characteristic, and related climatic conditions (low rainfall, high temperatures, and high solar radiation), makes deserts relatively inhospitable to microbial growth and could reduce microbial abundance (Tong and Lighthart 1997; Burrows et al. 2009; Tang 2009). In fact, soil microbial abundance decreases with increasing aridity (Bachar et al. 2010), and fungal abundance increases with increasing precipitation (Talley et al. 2002). Within the desert, microbial concentrations were higher at Taif, which receives more rain and experiences lower temperatures than does Ma-

Table 4: Relationships at the among-species and within-species level between total bird-associated microbial density and immune indexes measured in five desert and two temperate lark species in the Arabian Desert and the temperate Netherlands during spring

Immune measure	Among species				Within species			
	Estimate ± SE	<i>F</i>	df	<i>P</i>	Estimate ± SE	<i>F</i>	df	<i>P</i>
Haptoglobin (mg mL <sup>-1</sup> )	-.07 ± .06	1.22	1, 5	.32	.01 ± .02	.15	1, 59	.70
Agglutination (titer)	-.31 ± .40	.58	1, 5	.48	.11 ± .17	.40	1, 55	.53
Lysis (titer)	.11 ± .19	.34	1, 5	.59	.13 ± .08	3.21	1, 57	.08

Note. Within-species centering was used to partition the contribution of among- and within-species variation to any relationship.

hazat (Tieleman et al. 2003), further evidence that climatic conditions influence airborne microbial loads (Burrows et al. 2009).

Whereas differences in immune indexes between environments closely tracked differences in host-independent airborne microbial concentrations, they were largely unrelated to differences in bird-associated microbial densities. Only lysis showed a trend toward a positive correlation with total microbial load on birds, perhaps reflecting the importance of lytic mechanisms for eliminating antigens such as microbes and other foreign cells (Janeway et al. 2004). Intriguingly, this result contrasts with the covariation between bird-associated microbial densities and immune indexes observed when comparing between seasons in the desert (Horrocks et al. 2012). This might relate to the fact that, compared with concentrations of microbes in ambient air, the differences between environments in terms of densities of microbes sampled from birds were small. In fact, despite the pattern of relative abundances of the three microbial groups being the same in ambient air and when blown from birds (generalist bacteria > fungi > gram-negative bacteria in both environments), only fungi were significantly less common on desert larks compared with temperate larks.

Birds exposed to a greater abundance of airborne microbes might logically be expected to carry higher densities of microbes on their bodies. However, despite clear differences between environments in concentrations of airborne microbes, this was generally not the case. Our results therefore suggest that temperate larks are relatively good, or desert larks are relatively bad, at minimizing the abundance of microbes they carry. Higher airborne microbial abundances in the temperate zone may induce temperate larks to actively reduce the microbial load they harbor, perhaps via feather maintenance activities such as preening (Kulkarni and Heeb 2007). Conversely, constraints on activity budgets imposed by high temperatures (Tieleman and Williams 2002) might restrict preening in desert species, increasing the microbial abundances on their bodies. Other habitat-specific behaviors and the distribution of environmental microbes might also influence densities of bird-associated microbes (Saag et al. 2011). For example, desert larks use burrows and soil scrapes as thermal refuges (Williams et al. 1999) and often forage around the base of plants, where microbial abundance in desert soils is highest (Aanderud et al. 2008; Ben-David et al. 2011). Both these activities might increase exposure of desert larks to soil microbes (Shawkey et al. 2005). Hence, habitat-specific behaviors may cause desert-living larks to carry higher densities of microbes than expected based on airborne concentrations. Equally, physiological mechanisms might underlie this result. For example, differences in preen-wax properties between desert and temperate larks might also account for differences in the abundance of microbial assemblages associated with birds in these distinct environments (Shawkey et al. 2003; Haribal et al. 2009). Regardless of cause, the weak association between the abundances of microbes in the air and on birds highlights a new challenge for ecologists. That is, to properly understand how animals deal with the

antigenic challenges they may face, both host-independent and host-dependent measures of antigen exposure are required.

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### Literature Cited

- Aanderud Z.T., M.I. Shuldman, R.E. Drenovsky, and J.H. Richards. 2008. Shrub-interspace dynamics alter relationships between microbial community composition and belowground ecosystem characteristics. *Soil Biol Biochem* 40:2206–2216.
- Alcaide M., J.A. Lemus, G. Blanco, J.L. Tella, D. Serrano, J.J. Negro, A. Rodríguez, and M. García-Montijano. 2010. MHC diversity and differential exposure to pathogens in kestrels (*Aves: Falconidae*). *Mol Ecol* 19:691–705.
- Andersen A.A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. *J Bacteriol* 76:471–484.
- Arredouani M., P. Matthijs, E. Van Hoeyveld, A. Kasran, H. Baumann, J.L. Ceuppens, and E. Stevens. 2003. Haptoglobin directly affects T cells and suppresses T helper cell type 2 cytokine release. *Immunology* 108:144–151.
- Bachar A., A. Al-Ashhab, M. Soares, M. Sklarz, R. Angel, E. Ungar, and O. Gillor. 2010. Soil microbial abundance and diversity along a low precipitation gradient. *Microb Ecol* 60:453–461.
- Becker W.A. 1984. *A manual of quantitative genetics*. Academic Enterprises, Pullman, WA.
- Ben-David E.A., E. Zaady, Y. Sher, and A. Nejidat. 2011. Assessment of the spatial distribution of soil microbial communities in patchy arid and semi-arid landscapes of the Negev Desert using combined PLFA and DGGE analyses. *FEMS Microbiol Ecol* 76:492–503.
- Blount J.D., D.C. Houston, A.P. Møller, and J. Wright. 2003. Do individual branches of immune defence correlate? a comparative case study of scavenging and non-scavenging birds. *Oikos* 102:340–350.
- Buehler D.M., T. Piersma, K. Matson, and B.I. Tieleman. 2008.

- Seasonal redistribution of immune function in a migrant shorebird: annual-cycle effects override adjustments to thermal regime. *Am Nat* 172:783–796.
- Burrows S.M., W. Elbert, M.G. Lawrence, and U. Pöschl. 2009. Bacteria in the global atmosphere—part 1: review and synthesis of literature data for different ecosystems. *Atmos Chem Phys* 9:9263–9280.
- Carrillo C.M., F. Valera, A. Barbosa, and E. Moreno. 2007. Thriving in an arid environment: high prevalence of avian lice in low humidity conditions. *Ecoscience* 14:241–249.
- Dansby W., G. Purdue, J. Hunt, B. Arnoldo, D. Phillips, B. Moody, D. Kemp, L. Byrd, P. Walter, and J. Luby. 2008. Aerosolization of methicillin-resistant *Staphylococcus aureus* during an epidemic in a burn intensive care unit. *J Burn Care Res* 29:331–337.
- Dobryszczycka W. 1997. Biological functions of haptoglobin—new pieces to an old puzzle. *Eur J Clin Chem Clin Biochem* 35:647–654.
- Field C.B., M.J. Behrenfeld, J.T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* 281:237–240.
- Froeschke G., R. Harf, S. Sommer, and S. Matthee. 2010. Effects of precipitation on parasite burden along a natural climatic gradient in southern Africa—implications for possible shifts in infestation patterns due to global changes. *Oikos* 119:1029–1039.
- Gutteridge J.M.C. 1987. The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. *Biochim Biophys Acta Lipids Lipid Metab* 917:219–223.
- Haas D., M. Unteregger, J. Habib, H. Galler, E. Marth, and F. Reinthaler. 2010. Exposure to bioaerosol from sewage systems. *Water Air Soil Pollut* 207:49–56.
- Haribal M., A. Dhondt, and E. Rodriguez. 2009. Diversity in chemical compositions of preen gland secretions of tropical birds. *Biochem Syst Ecol* 37:80–90.
- Horrocks N.P.C., K.D. Matson, M. Shobrak, J.M. Tinbergen, and B.I. Tieleman. 2012. Seasonal patterns in immune indices reflect microbial loads on birds but not microbes in the wider environment. *Ecosphere* 3:art19.
- Horrocks N.P.C., K.D. Matson, and B.I. Tieleman. 2011. Pathogen pressure puts immune defense into perspective. *Integr Comp Biol* 51:563–576.
- Huttunen K., P. Kaarakainen, T. Meklin, A. Nevalainen, and M.-R. Hirvonen. 2010. Immunotoxicological properties of airborne particles at landfill, urban and rural sites and their relation to microbial concentrations. *J Environ Monit* 12:1368–1374.
- Ilmonen P., T. Taarna, and D. Hasselquist. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proc R Soc B* 267:665–670.
- Janeway C.A., P. Travers, M. Walport, and M.J. Shlomchik. 2004. *Immunobiology: the immune system in health and disease*. Garland, New York.
- Kulkarni S. and P. Heeb. 2007. Social and sexual behaviours aid transmission of bacteria in birds. *Behav Processes* 74:88–92.
- Lee K.A. 2006. Linking immune defenses and life history at the levels of the individual and the species. *Integr Comp Biol* 46:1000–1015.
- Lee K.A., M. Wikelski, W.D. Robinson, T.R. Robinson, and K.C. Klasing. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. *J Anim Ecol* 77:356–363.
- Lessels C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121.
- Lindström K., J. Foufopoulos, H. Pärn, and M. Wikelski. 2004. Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proc R Soc B* 271:1513–1519.
- Little R.M. and R.A. Earlé. 1995. Sandgrouse (Pterocleididae) and sociable weavers *Philetarius socius* lack avian haematzoa in semi-arid regions of South Africa. *J Arid Environ* 30:367–370.
- Lochmiller R.L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88:87–98.
- Martin L.B., II, Z.M. Weil, and R.J. Nelson. 2007. Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology* 88:2516–2528.
- Matson K.D. 2006. Are there differences in immune function between continental and insular birds? *Proc R Soc B* 273:2267–2274.
- Matson K.D., N.P.C. Horrocks, M.A. Versteegh, and B.I. Tieleman. 2012. Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response. *Comp Biochem Physiol A* 162:7–15.
- Matson K.D., R.E. Ricklefs, and K.C. Klasing. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev Comp Immunol* 29:275–286.
- Matson K.D., B.I. Tieleman, and K.C. Klasing. 2006. Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds. *Physiol Biochem Zool* 79:556–564.
- Millet S., J. Bennett, K.A. Lee, M. Hau, and K.C. Klasing. 2007. Quantifying and comparing constitutive immunity across avian species. *Dev Comp Immunol* 31:188–201.
- Møller A.P. 1998. Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos* 82:265–270.
- Moyer B.R., D.M. Drown, and D.H. Clayton. 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos* 97:223–228.
- Norris K. and M.R. Evans. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav Ecol* 11:19–26.
- Ochsenbein A.F. and R.M. Zinkernagel. 2000. Natural antibodies and complement link innate and acquired immunity. *Immunol Today* 21:624–630.
- Piersma T. 1997. Do global patterns of habitat use and migration strategies co-evolve with relative investments in im-



- munocompetence due to spatial variation in parasite pressure? *Oikos* 80:623–631.
- Previtali M.A., R.S. Ostfeld, F. Keesing, A.E. Jolles, R. Hanselmann, and L.B. Martin. 2012. Relationship between pace of life and immune responses in wild rodents. *Oikos*, doi: 10.1111/j.1600-0706.2012.020215.x.
- Quaye I.K. 2008. Haptoglobin, inflammation and disease. *Trans R Soc Trop Med Hyg* 102:735–742.
- Rappé M.S. and S.J. Giovannoni. 2003. The uncultured microbial majority. *Annu Rev Microbiol* 57:369–394.
- R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Ricklefs R.E. and M. Wikelski. 2002. The physiology/life-history nexus. *Trends Ecol Evol* 17:462–468.
- Roff D.A. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.
- Ruiz-Rodríguez M., J.J. Soler, F.S. Lucas, P. Heeb, M.J. Palacios, D. Martín-Gálvez, L. de Neve, T. Pérez-Contreras, J.G. Martínez, and M. Soler. 2009. Bacterial diversity at the cloaca relates to an immune response in magpie *Pica pica* and to body condition of great spotted cuckoo *Clamator glandarius* nestlings. *J Avian Biol* 40:42–48.
- Saag P., V. Tilgar, R. Mänd, P. Kilgas, and M. Mägi. 2011. Plumage bacterial assemblages in a breeding wild passerine: relationships with ecological factors and body condition. *Microb Ecol* 61:740–749.
- Shawkey M.D., K.L. Mills, C. Dale, and G.E. Hill. 2005. Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. *Microb Ecol* 50:40–47.
- Shawkey M.D., S.R. Pillai, and G.E. Hill. 2003. Chemical warfare? effects of uropygial oil on feather-degrading bacteria. *J Avian Biol* 34:345–349.
- Sheldon B.C. and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* 11:317–321.
- Sparkman A.M. and M.G. Palacios. 2009. A test of life-history theories of immune defence in two ecotypes of the garter snake, *Thamnophis elegans*. *J Anim Ecol* 78:1242–1248.
- Spottiswoode C. 2008. Cooperative breeding and immunity: a comparative study of PHA response in African birds. *Behav Ecol Sociobiol* 62:963–974.
- Stearns S.C. 1992. The evolution of life histories. Oxford University Press, Oxford.
- Talley S., P. Coley, and T. Kursar. 2002. The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecol* 2:7.
- Tang J.W. 2009. The effect of environmental parameters on the survival of airborne infectious agents. *J R Soc Interface* 6: S737–S746.
- Tieleman B.I. 2005. Physiological, behavioral and life history adaptations of larks along an aridity gradient: a review. Pp. 49–67 in G. Bota, J. Camprodon, S. Manosa, and M. Morales, eds. Ecology and conservation of steppe-land birds. Lynx, Barcelona.
- Tieleman B.I., T.H. Dijkstra, K.C. Klasing, G.H. Visser, G. Henk, and J.B. Williams. 2008. Effects of experimentally increased costs of activity during reproduction on parental investment and self-maintenance in tropical house wrens. *Behav Ecol* 19:949–959.
- Tieleman B.I. and J.B. Williams. 2002. Effects of food supplementation on behavioural decisions of hoopoe-larks in the Arabian Desert: balancing water, energy and thermoregulation. *Anim Behav* 63:519–529.
- Tieleman B.I., J.B. Williams, and P. Bloomer. 2003. Adaptation of metabolism and evaporative water loss along an aridity gradient. *Proc R Soc B* 270:207–214.
- Tieleman B.I., J.B. Williams, R.E. Ricklefs, and K.C. Klasing. 2005. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proc R Soc B* 272: 1715–1720.
- Tieleman B.I., J.B. Williams, and G.H. Visser. 2004. Energy and water budgets of larks in a life history perspective: parental effort varies with aridity. *Ecology* 85:1399–1410.
- Tong Y. and B. Lighthart. 1997. Solar radiation has a lethal effect on natural populations of culturable outdoor atmospheric bacteria. *Atmos Environ* 31:897–900.
- . 1999. Diurnal distribution of total and culturable atmospheric bacteria at a rural site. *Aerosol Sci Technol* 30: 246–254.
- Tschirren B. and H. Richner. 2006. Parasites shape the optimal investment in immunity. *Proc R Soc B* 273:1773–1777.
- Valera F., C.M. Carrillo, A. Barbosa, and E. Moreno. 2003. Low prevalence of haematozoa in Trumpeter finches *Bucanetes githagineus* from south-eastern Spain: additional support for a restricted distribution of blood parasites in arid lands. *J Arid Environ* 55:209–213.
- van de Crommenacker J., N.P.C. Horrocks, M.A. Versteegh, J. Komdeur, B.I. Tieleman, and K.D. Matson. 2010. Effects of immune supplementation and immune challenge on oxidative status and physiology in a model bird: implications for ecologists. *J Exp Biol* 213:3527–3535.
- van de Pol M. and J. Wright. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. *Anim Behav* 77:753–758.
- Walsberg G.E. and J.R. King. 1978. The relationship of the external surface area of birds to skin surface area and body mass. *J Exp Biol* 76:185–189.
- Williams J.B. and B.I. Tieleman 2005. Physiological adaptation in desert birds. *BioScience* 55:416–425.
- Williams J.B., B.I. Tieleman, and M. Shobrak. 1999. Lizard burrows provide thermal refugia for larks in the Arabian Desert. *Condor* 101:714–717.