

1 **Environmental proxies of antigen exposure explain variation in immune investment better**  
2 **than indices of pace-of-life**

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24

25 **ABSTRACT**

26 Investment in immune defences is predicted to co-vary with a variety of ecologically and  
27 evolutionarily relevant axes, with pace-of-life and environmental antigen exposure being  
28 two examples. These axes may themselves co-vary directly or inversely, and such  
29 relationships can lead to conflicting predictions regarding immune investment. If pace-of-life  
30 shapes immune investment then, following life history theory, slow-living, arid zone and  
31 tropical species should invest more in immunity than fast-living temperate species.  
32 Alternatively, if antigen exposure drives immune investment, then species in antigen-rich  
33 tropical and temperate environments are predicted to exhibit higher immune indices than  
34 species from antigen-poor arid locations. To test these contrasting predictions we  
35 investigated how variation in pace-of-life and antigen exposure influence immune  
36 investment in related lark species (*Alaudidae*) with differing life histories and predicted risks  
37 of exposure to environmental microbes and parasites. We used clutch size and total number  
38 of eggs laid year<sup>-1</sup> as indicators of pace-of-life, and aridity, and the climatic variables that  
39 influence aridity, as correlates of antigen abundance. We quantified immune investment by  
40 measuring four indices of innate immunity. Pace-of-life explained little of the variation in  
41 immune investment, and only one immune measure correlated significantly with pace-of-  
42 life, but not in the predicted direction. Conversely, aridity, our proxy for environmental  
43 antigen exposure, was predictive of immune investment, and larks in more mesic  
44 environments had higher immune indices than those living in arid, low-risk locations. Our  
45 study suggests that abiotic environmental variables with strong ties to environmental  
46 antigen exposure can be important correlates of immunological variation.

47

- 48 **Keywords:** Alaudidae, aridity, ecological immunology, lark, life history, pace-of-life
- 49 hypothesis.

## 50 INTRODUCTION

51 Explanations for variation in immune investment have often focused on the identification of  
52 evolutionary and ecological axes along which immune defences might co-vary. For example,  
53 ecological immunologists have exploited differences in pace-of-life to explain variation in  
54 immune defences. Limited resources and the costs associated with immunity suggest that  
55 immune investment must be counter-balanced against investment in other costly  
56 physiological processes such as growth and reproduction (Sheldon and Verhulst 1996;  
57 Ilmonen et al. 2000; Lochmiller and Deerenberg 2000; Norris and Evans 2000; Hegemann et  
58 al. 2013). Species at the fast end of the pace-of-life axis, with short lifespans and high  
59 reproductive rates, may allocate more of their limited resources to reproduction, and fewer  
60 to self maintenance activities such as immune defence. Conversely, 'slow-living' species that  
61 develop slowly, have low extrinsic mortality, and low reproductive rates, can prioritise self-  
62 maintenance activities and invest more heavily in immunity (Roff 1992; Stearns 1992;  
63 Ricklefs and Wikelski 2002).

64 Variation in exposure to environmental antigens represents another axis that might  
65 explain immune investment. Immune systems provide clear benefits in terms of protection  
66 against exogenous threats, including fitness-reducing micro- and macro-parasites. Immune  
67 investment might be greater when the risk of infection is higher (Tschirren and Richner  
68 2006; Horrocks et al. 2011a), which could be associated with environment, time, and other  
69 ecological factors (Piersma 1997; Møller 1998; Guernier et al. 2004, Hegemann et al. 2012,  
70 2013; Horrocks et al. 2012a, 2012b). For example, levels of environmental moisture shape  
71 endo- and ecto-parasitic communities, which show decreased prevalence, abundance and  
72 diversity in more arid environments (Little and Earlé 1995; Moyer et al. 2002; Valera et al.  
73 2003; Guernier et al 2004; Jex et al. 2007; Guerra et al. 2010; Froeschke et al. 2010; Pullan

74 and Brooker 2012). Combined with reduced moisture, the increased solar radiation and  
75 temperature extremes associated with arid environments also act to limit microbial  
76 assemblages (Tong and Lighthart 1997; Saranathan and Burt 2007; Burrows et al. 2009;  
77 Tang 2009; Bachar et al. 2010). If aridity is considered as a proxy for the level of antigenic  
78 exposure (Horrocks et al. 2014) then the requirement for immune investment should be  
79 greatest in cool, wet and humid environments where parasites and microbes are more likely  
80 to be encountered. This suggests a negative correlation between aridity and immune  
81 function.

82         Disentangling the relative contributions of pace-of-life and antigen exposure to  
83 immune variation is difficult because both axes may themselves co-vary (Horrocks et al.  
84 2011a). Where pace-of-life and antigen exposure co-vary positively, predictions about  
85 immune investment coincide, even if the causal factor responsible for immunological  
86 variation is not clear. For example, relative to temperate birds, those in the tropics might  
87 invest more in immunity due to their slower pace-of-life (Martin II et al. 2006; Wiersma et  
88 al. 2007), because of increased exposure to environmental antigens such as parasites  
89 (Møller 1998; Guernier et al. 2004), or perhaps as a result of both factors. Where pace-of-  
90 life and antigen exposure co-vary negatively, conflicting predictions can arise. For example,  
91 the slow pace-of-life of desert-living birds (Tieleman et al. 2004) predicts strong investment  
92 in immune defences, similar to birds in the tropics, even though deserts and the tropics may  
93 pose contrasting risks in terms of exposure to antigens (Horrocks et al. 2011a). Investigating  
94 the drivers and correlates of immune variation in diverse environments requires careful  
95 consideration of study system characteristics. If the goal is to separate the contributions of  
96 pace-of-life and antigen exposure, then these two factors must be as un-confounded as  
97 possible.

98           We investigated how immune investment is influenced by pace-of-life and antigen  
99 exposure - expressed as environmental aridity - by studying related species of songbirds  
100 (larks; Alaudidae) that inhabit environments differing in aridity. Larks are ideally suited to  
101 this study because of their ecological similarities in different environments (del Hoyo et al.  
102 2004), and because environmental moisture is already known to influence exposure to  
103 antigens in birds. Bacterial loads in nests, and infestation of nestlings by parasitic fly larvae  
104 correlate negatively with precipitation (Berger et al. 2003; Antoniazzi et al. 2011), while  
105 haematozoan infections and prevalence of lice and bacteria on feathers are reduced under  
106 more arid conditions (Little and Earlé 1995; Moyer et al. 2002; Valera et al. 2003;  
107 Saranathan and Burt 2007; Bush et al. 2009; Malenke et al. 2011; Horrocks et al. 2012b).  
108 Soil microbial abundance also correlates negatively with precipitation (Bachar et al. 2010;  
109 Drenovsky et al. 2010; Blankinship et al. 2011; Pasternak et al. 2013; Serna-Chavez et al.  
110 2013) and soil microbes contaminate birds and their nests (Shawkey et al. 2005; Ruiz de  
111 Castañeda et al. 2011; Potter et al. 2013).

112           We measured lark species living in hot, hyper-arid deserts and cooler, wetter, mesic  
113 locations, as well as those from cold desert and tropical locations. Cold desert larks have  
114 clutch sizes typical of a fast pace-of-life yet live in an environment predicted to pose a low  
115 risk of antigen exposure. Tropical larks display life-history traits consistent with a slow pace-  
116 of-life yet live in potentially high antigen exposure settings (Tables 1 and 2). For the  
117 remaining lark species in our study pace-of-life increases with decreasing aridity, a finding  
118 that is unaffected by phylogeny (Tieleman et al. 2003; 2004). This means that the  
119 environments most associated with species exhibiting a slow pace-of-life (which may select  
120 *for* immune investment) are the same environments that present the lowest exposure to  
121 environmental antigens (which may select *against* immune investment). These contrasts

122 make larks particularly suited for teasing apart the roles of life history and environmental  
123 antigen exposure in shaping investment in immune defences.

124 We assessed immune investment by measuring circulating levels of four non-specific  
125 immune indices that any environmental antigen that has breached defensive barriers such  
126 as the skin or mucosa might encounter (Janeway et al. 2004). Haptoglobin and  
127 ovotransferrin are acute phase proteins with immunomodulatory properties that counter  
128 microbial challenges and limit microbial growth by directly sequestering iron (Xie et al. 2002;  
129 Arredouani et al. 2003). Natural antibodies opsonize invading microorganisms to facilitate  
130 phagocytosis and activate the complement system, which leads to cell lysis (Ochsenbein and  
131 Zinkernagel 2000). We used clutch size and number of eggs laid year<sup>-1</sup> as indicators of pace-  
132 of-life (Saether 1998; Ricklefs 2000), and aridity, precipitation and mean ambient  
133 temperature as proxies for environmental antigen exposure. We predicted that if immune  
134 investment is driven by pace-of-life, then slow-living, arid zone and tropical larks should  
135 invest relatively more in immune defences than fast-living species from temperate and cold-  
136 arid environments. If antigen exposure is more important for determining investment in  
137 immune defences, then we predicted that immune indices should be lowest in lark  
138 populations from arid locations, and be higher in temperate and tropical larks living in  
139 environments with greater abundance of microbes and macro-parasites.

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141

## 142 **MATERIALS AND METHODS**

### 143 **Study populations, sampling, and indicators of pace-of-life**

144 We captured larks of 12 species in 11 climatically distinct locations during breeding and non-  
145 breeding periods from 2006 to 2009 (23 populations in total; Table 1). We collected <300µl

146 blood from the brachial vein of each bird and stored it on ice until processing by  
147 centrifugation to separate plasma and cellular fractions. The plasma was then frozen and  
148 stored at -20°C until it was used in immune assays. We gathered data on mean clutch size  
149 and number of eggs laid year<sup>-1</sup> (mean clutch size x mean number of clutches laid year<sup>-1</sup>;  
150 Table 2) directly from our own study populations and from Tieleman et al. (2004), Cramp  
151 (1988), del Hoyo et al. (2004) and Hegemann et al. (2012, 2013). All birds were sampled  
152 under licence from the relevant authorities.

153

#### 154 **Climatic variables and aridity index**

155 We obtained high-resolution (0.5 x 0.5 degree – approximately 55 x 55 km) gridded data on  
156 climatic variables for the period 1901-2009 from the Climatic Research Unit time series  
157 dataset (CRU TS 3.1; Harris et al. 2013). For each bird-sampling location we extracted mean  
158 annual values for precipitation (P, mm) and temperature (T, °C). We used these climatic  
159 variables to calculate de Martonne's aridity index  $A_M$  ( $P / T + 10$ ; de Martonne 1926).  $A_M$  and  
160 climatic variables for each lark population are shown in Table 1. Low values of  $A_M$  indicate  
161 arid conditions, whereas higher values are associated with increasingly mesic environments.

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#### 163 **Immune assays**

164 We determined haptoglobin concentrations (mg ml<sup>-1</sup>) using a functional assay that  
165 measures the haem-binding capacity of plasma (TP801; Tri-Delta Diagnostics, NJ, USA),  
166 following the 'manual method' instructions provided by the manufacturer and with  
167 incubation at 30°C for five minutes (Matson et al. 2012). We measured ovotransferrin  
168 concentrations (mg ml<sup>-1</sup>) according to Horrocks et al. (2011b). Three of the 23 populations  
169 were not measured due to blood volume limitations (Table 2). We quantified natural



170 antibody-mediated agglutination titres and complement-mediated lysis titres against rabbit  
171 red blood cells (B-0009D, Harlan, UK), according to the assay of Matson et al. (2005).

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### 173 **Statistical analyses**

174 We calculated mean values per population (i.e. per species per location) for each immune  
175 index and used these values to conduct regression analyses to investigate relationships  
176 between immune indices and life-history variables and aridity. Since our aridity index is a  
177 compound variable, we also ran separate models testing immune indices against each of the  
178 primary climatic variables precipitation and temperature. To account for potential non-  
179 independence among species due to common ancestry we used a phylogenetic generalised  
180 least squares (pgls) approach (Freckleton et al. 2002), simultaneously estimating maximum  
181 likelihood values of the parameter  $\lambda$  to test for phylogenetic signal in the model residuals  
182 (Pagel's lambda; Pagel 1999). A value of  $\lambda = 0$  indicates no phylogenetic signal, whereas  $\lambda = 1$   
183 suggests that trait evolution is consistent with a strong effect of phylogeny. We based our  
184 phylogeny on the phylogenetic tree of larks (Alström et al. 2013). For the seven species with  
185 multiple sampled populations we added branches to the tree for each population to create  
186 polytomies, with the branch length for each population within a species set to zero. We  
187 found no evidence for phylogenetic signal in any of our datasets:  $\lambda$  never differed  
188 significantly from zero and was always significantly different from one. Setting all branch  
189 lengths to one did not change this finding. Therefore, we present all results and figures  
190 based on simple linear regression models (ordinary least squares, ols; Freckleton 2009;  
191 Revell 2010), with species and geographically distinct populations treated as independent  
192 points. Because sample size varied among species and populations (Table 1), we weighted  
193 regression models by the square root of the number of individuals sampled in each

194 population (Sokal and Rohlf 1995). Since some species or populations were only sampled  
195 during one period (breeding or non-breeding; Table 1) we ran analyses using restricted  
196 datasets containing values per period, as well as with the entire dataset of all values. The  
197 results of these analyses were qualitatively similar and so we only present results based on  
198 the entire dataset. To disentangle the roles of pace-of-life and environmental antigen  
199 exposure in shaping immune investment, these factors should not be correlated. This was  
200 the case in our dataset: pace-of-life indicators and aridity were not significantly correlated  
201 (clutch size vs.  $A_M$ :  $P = 0.57$ ,  $r = -0.12$ ; number of eggs laid year<sup>-1</sup> vs.  $A_M$ :  $P = 0.16$ ,  $r = 0.31$ ).  
202 All statistical analyses were conducted using R 2.15.2 (R Core Team 2012).

203

204

## 205 **RESULTS**

206 Relationships between immune indices and pace-of-life parameters were non-significant  
207 and generally weak (Table 3; Fig. 1). The notable exception to this was the relationship  
208 between number of eggs laid year<sup>-1</sup> and agglutination titres (Table 3; Fig. 1f).

209 Lark populations consistently exhibited lower immune defences in more arid  
210 locations (Fig. 2). These negative correlations with aridity were significant for haptoglobin  
211 concentrations and lysis titres, and approached significance for agglutination titres (Table 3;  
212 Fig. 2). Haptoglobin concentrations and lysis titres were also positively and significantly  
213 correlated with mean annual precipitation (Table 3; Fig. 2). Ovotransferrin concentrations  
214 showed no relationship with aridity or mean annual precipitation (Figs 2d-e). Lysis titres  
215 correlated significantly and positively with mean annual temperature: larks from warmer  
216 locations had higher lysis titres (Table 3; Fig. 2i). All other immune indices correlated

217 negatively with mean annual temperature but these relationships were not significant  
218 (Table 3; Fig. 2).

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220

## 221 **DISCUSSION**

222 Contrary to the pace-of-life hypothesis, slow-living lark species did not invest more heavily  
223 in immune defences than their faster-living counterparts. Thus, our study adds to the list of  
224 publications that report no evidence for this hypothesis (Cutrera et al. 2010; Horrocks et al.  
225 2012a; Versteegh et al. 2012, Palacios et al. 2013), and contradicts those studies that  
226 provide at least some support for an association between pace-of-life and immune  
227 investment (Tieleman et al. 2005; Martin et al. 2006; Lee et al. 2008; Sparkman and Palacios  
228 2009; Previtalli et al. 2012). Taxonomic and methodological differences between studies,  
229 including measurement of different immune components and the use of different proxies  
230 for pace-of-life, may explain some of this variation. However, even within a single immune  
231 index and taxonomic group, interpretation of immune investment in relation to pace-of-life  
232 is not straightforward. The sole significant relationship in our study that might suggest a link  
233 between pace-of-life and immune investment was between agglutination titres and number  
234 of eggs laid year<sup>-1</sup>. However, this correlation was positive, and contrary to the pace-of-life  
235 hypothesis this suggests that lark species with a faster pace-of-life have higher agglutination  
236 ability. This finding contradicts earlier work showing greater agglutination ability in tropical  
237 species with a slower pace-of-life (Lee et al. 2008). It also conflicts with Versteegh et al.  
238 (2012), who found no relationship between agglutination titres and pace-of-life when  
239 studying stonechat subspecies in a common garden set-up. Natural antibody levels correlate  
240 positively with adaptive antibody responses (Parmentier et al. 2004) and these conflicting

241 patterns might arise because natural antibodies, although usually considered an innate  
242 immune defence, straddle the boundary between innate and adaptive immunity (Caroll and  
243 Prodeus 1998; Ochsenbein and Zinkernagel 2000). Studies that measure purely adaptive  
244 immune defences, such as induction of specific antibody responses, are needed to test this  
245 possibility. More generally, the equivocal nature of the evidence linking immune investment  
246 and pace-of-life suggests that further exploration of this topic is required.

247         In agreement with the antigen exposure hypothesis (Horrocks et al. 2011a),  
248 haptoglobin concentrations and lysis titres decreased with increasing aridity and were  
249 highest in populations of larks from less arid locations where exposure to abundant  
250 immunological challenges is expected to be higher (Little and Earlé 1995; Moyer et al. 2002;  
251 Valera et al. 2003; Guernier et al. 2004; Jex et al. 2007; Tang 2009; Bachar et al. 2010;  
252 Guerra et al. 2010; Froeschke et al. 2010). Agglutination titres showed a similar, but non-  
253 significant trend and only ovotransferrin concentrations showed no relationship with aridity.  
254 This might relate to the iron binding and transport function of ovotransferrin, which, aside  
255 from its anti-microbial and immunomodulatory properties, make ovotransferrin important  
256 for vascularisation and nerve and muscle growth in the developing embryo (Giansanti et al.  
257 2012).

258         In addition to links with aridity, we found significant positive associations between  
259 haptoglobin concentrations and lysis titres and precipitation, and between lysis titres and  
260 temperature. Previous authors have linked the risk of antigen exposure to climatic factors  
261 (Guernier et al. 2004; Gage et al. 2008; Guerra et al. 2010) and other abiotic environmental  
262 variables (e.g. salinity; Figuerola 1999; Piersma 1997; Mendes et al. 2005). Our study  
263 extends this approach by showing that immune indices that are related to infection risk also  
264 correlate with abiotic environmental variation. This demonstrates that abiotic measures can

265 serve as useful proxies for antigenic exposure when direct biotic measurements are  
266 unavailable. Even so, it is important to recognise that other factors might influence immune  
267 investment, and may also co-vary with abiotic environmental variation. For example,  
268 physiological differences between birds along our aridity gradient, such as variation in  
269 metabolic rate (Tieleman et al. 2003) might influence immune responses, independent of  
270 any effect of antigen exposure. Temperature-constrained activity budgets (Tieleman and  
271 Williams 2002) and the reduced productivity associated with arid environments (Del Grosso  
272 et al. 2008; Serna-Chavez et al. 2013) could both restrict food availability in arid-living larks,  
273 leading to a resource trade-off that negatively impacts immune function. However, food  
274 availability might also be expected to influence clutch size and number of clutches year<sup>-1</sup>  
275 (Tieleman et al. 2004; Lepage and Lloyd 2004), yet neither pace-of-life indicator correlated  
276 significantly with aridity. Earlier work showed that lark species in hyper-arid regions  
277 encountered lower microbial abundances than more mesic, temperate-living larks, and had  
278 correspondingly lower immune indices (Horrocks et al. 2012a). This supports our argument  
279 that aridity is a good proxy for risk of antigen exposure and consequently immune  
280 investment. Future work that takes a similar approach and focuses on direct measurement  
281 of relevant antigens will shed additional light on the associations we have identified  
282 between aridity, risk of antigen exposure, and immune defence. Combined with  
283 experimental studies, this work is also necessary to determine whether patterns of  
284 correlation between antigen exposure and immune responses reflect evolved responses to  
285 an antigen-rich environment or are simply a reflection of current exposure to antigens.

286 Comparative studies, both within and among species and environments, represent a  
287 powerful approach for disentangling the roles of pace-of-life and antigen exposure in  
288 shaping immune defences and for understanding immune defence variation in more general

289 ecological and evolutionary terms. By employing a study system in which pace-of-life  
290 variation is uncoupled from variation in antigen exposure, we demonstrated that  
291 investment in innate immune defences might be related more to the likelihood of  
292 encountering an immunological challenge than to pace-of-life. Including relevant measures  
293 of environmental antigens and associated exposure risk in ecoimmunology studies will  
294 provide exciting opportunities for advancing our understanding of immunological variation.

295

296

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530



531 **TABLES**

532 Table 1. Sample size (*n*), sampling period (breeding (B), non-breeding (NB), or sampled in both periods (both)), geographic origin and habitat  
 533 description, and climatic variables for 12 species of lark. The climatic variables are the aridity index  $A_M$  ( $P / T + 10$ ), and mean annual values for  
 534 precipitation (P) and temperature (T). A lower value of  $A_M$  indicates a more arid environment.

#	species	<i>n</i>	sampling	latitude	longitude	country	habitat	$A_M$	P (mm)	T (°C)
a	hoopoe lark <i>Alaemon alaudipes</i>	61	both	22° 20' N	41° 44' E	Saudi Arabia	hot desert	2.29	81.19	25.38
b		4	B	19° 53' N	16° 18' W	Mauritania	hot desert	2.01	69.33	24.55
c	bar-tailed desert lark <i>Ammomanes cincturus</i>	56	both	22° 20' N	41° 44' E	Saudi Arabia	hot desert	2.29	81.19	25.38
d	black-crowned finchlark <i>Eremopterix nigriceps</i>	14	B	21° 15' N	40° 42' E	Saudi Arabia	hot desert	6.43	200.20	21.12
e		19	both	22° 20' N	41° 44' E	Saudi Arabia	hot desert	2.29	81.19	25.38
f	crested lark <i>Galerida cristata</i>	4	B	21° 15' N	40° 42' E	Saudi Arabia	hot desert	6.43	200.20	21.12
g		18	both	22° 20' N	41° 44' E	Saudi Arabia	hot desert	2.29	81.19	25.38
h		2	NB	34° 22' N	62° 11' E	Afghanistan	cold desert	8.66	226.44	16.14
i	Dunn's lark <i>Eremalauda dunni</i>	35	both	22° 20' N	41° 44' E	Saudi Arabia	hot desert	2.29	81.19	25.38
j	short-toed lark <i>Calandrella brachydactyla</i>	2	NB	22° 16' N	41° 45' E	Saudi Arabia	hot desert	2.29	81.19	25.38
k	bimaculated lark <i>Melanocorypha bimaculata</i>	6	NB	36° 54' N	66° 53' E	Afghanistan	cold desert	7.96	214.88	16.98
l		14	NB	34° 54' N	67° 11' E	Afghanistan	cold desert	26.92	389.80	4.48
m		7	NB	34° 22' N	62° 11' E	Afghanistan	cold desert	8.66	226.44	16.14
n		6	B	36° 43' N	67° 06' E	Afghanistan	cold desert	9.50	243.17	15.61

535

536 Table 1 continued.

#	species	<i>n</i>	sampling	latitude	longitude	country	habitat	<i>A<sub>M</sub></i>	P (mm)	T (°C)
o	calandra lark <i>Melanocorypha calandra</i>	3	NB	36° 54' N	66° 53' E	Afghanistan	cold desert	7.96	214.88	16.98
p		6	NB	34° 54' N	67° 11' E	Afghanistan	cold desert	26.92	389.80	4.48
q		11	NB	34° 22' N	62° 11' E	Afghanistan	cold desert	8.66	226.44	16.14
r	red-capped lark <i>Calandrella cinerea</i>	5	B	0° 51' S	36° 25' E	Kenya	tropical	19.64	593.98	20.25
s		8	B	0° 34' S	36° 29' E	Kenya	tropical	33.05	839.22	15.39
t	rufous-naped lark <i>Mirafra africana</i>	4	B	0° 48' S	36° 32' E	Kenya	tropical	19.64	593.98	20.25
u		2	B	0° 34' S	36° 29' E	Kenya	tropical	33.05	839.22	15.39
v	skylark <i>Alauda arvensis</i>	144	both	52° 56' N	6° 18' E	Netherlands	temperate	40.50	777.01	9.19
w	woodlark <i>Lullula arborea</i>	60	both	52° 56' N	6° 18' E	Netherlands	temperate	40.50	777.01	9.19

537

538

539 Table 2. Mean clutch size and number of clutches per year, concentrations of haptoglobin and ovotransferrin, and agglutination and lysis titres  
 540 for 23 populations of 12 lark species. Values for life-history variables are from this study and from the literature (data source column).

#	species	clutch size	clutches year <sup>-1</sup>	haptoglobin (mg ml <sup>-1</sup> )	ovotransferrin (mg ml <sup>-1</sup> )	agglutination (titre)	lysis (titre)	data source*
a	hoopoe lark	2.99	1	0.28	7.47	5.85	0.86	2
b		2.88	1	0.25	7.41	4.50	1.63	3
c	bar-tailed desert lark	3.24	1	0.29	9.08	6.13	0.38	2
d	black-crowned finchlark	2.57	1	0.49	5.43	7.03	1.52	3, 4
e		2.00	1	0.27	9.11	5.83	0.58	3, 4
f	crested lark	4.15	2	0.25	15.28	6.31	1.94	2
g		4.15	2	0.25	5.18	6.24	0.53	2
h		4.75	2	0.07	11.20	5.25	0.00	2, 3
i	Dunn's lark	2.88	1	0.49	9.76	6.65	1.63	2
j	short-toed lark	3.50	2	0.41	9.18	11.00	1.00	2
k	bimaculated lark	3.96	1.5	0.19	-	5.17	2.08	3
l		3.96	1.5	0.33	14.53	4.90	2.63	3
m		3.96	1.5	0.11	12.72	4.21	0.21	3
n		3.96	1.5	0.17	-	7.63	4.88	3

541

542

543 Table 2 continued.

#	species	clutch size	clutches year <sup>-1</sup>	haptoglobin (mg ml <sup>-1</sup> )	ovotransferrin (mg ml <sup>-1</sup> )	agglutination (titre)	lysis (titre)	data source*
o	calandra lark	4.20	2	0.08	-	7.25	3.58	2
p		4.20	2	0.06	6.01	6.46	1.75	2
q		4.20	2	0.07	9.78	5.90	1.25	2
r	red-capped lark	1.83	2	0.15	9.10	4.50	0.13	1
s		1.89	2	0.57	7.25	5.17	2.42	1
t	rufous-naped lark	2.11	1	0.74	9.08	6.31	3.69	1, 4
u		2.00	1	0.19	10.20	5.63	3.63	1, 4
v	skylark	3.56	3.5	0.48	-	7.82	2.26	1, 5
w	woodlark	4.02	2.5	0.46	9.41	7.20	2.21	1

544 \* 1 Own data; 2 Tieleman et al. (2004); 3 Cramp (1988); 4 del Hoyo et al. (2004); 5 Hegemann et al. (2012, 2013).

545

546

547 Table 3. Results of linear models examining relationships between immune indices of 23  
 548 populations of 12 lark species in relation to pace-of-life proxies and to climatic proxies of  
 549 environmental antigen exposure. *P* values < 0.05 are shown in bold.

<b>response variable</b>	<b>explanatory variable</b>	<b>F</b>	<b><i>P</i></b>
haptoglobin (mg ml <sup>-1</sup> )	mean clutch size	F <sub>1,21</sub> = 2.86	0.11
	total eggs year <sup>-1</sup>	0.21	0.66
	aridity index A <sub>M</sub>	4.90	<b>0.038</b>
	mean annual precipitation (mm)	5.79	<b>0.025</b>
	mean annual temperature (°C)	0.31	0.59
ovotransferrin (mg ml <sup>-1</sup> )	mean clutch size	F <sub>1,17</sub> = 4.00	0.06
	total eggs year <sup>-1</sup>	1.74	0.21
	aridity index A <sub>M</sub>	0.53	0.72
	mean annual precipitation (mm)	0.00	0.95
	mean annual temperature (°C)	2.79	0.11
agglutination (titre)	mean clutch size	F <sub>1,21</sub> = 1.00	0.33
	total eggs year <sup>-1</sup>	8.34	<b>0.009</b>
	aridity index A <sub>M</sub>	3.14	0.09
	mean annual precipitation (mm)	2.31	0.14
	mean annual temperature (°C)	1.11	0.31
lysis (titre)	mean clutch size	F <sub>1,21</sub> = 0.23	0.64
	total eggs year <sup>-1</sup>	1.59	0.22
	aridity index A <sub>M</sub>	6.58	<b>0.018</b>
	mean annual precipitation (mm)	7.03	<b>0.015</b>
	mean annual temperature (°C)	8.32	<b>0.009</b>

550

551

552 **FIGURES**

553

554 Figure 1. Haptoglobin (a-b) and ovotransferrin (c-d) concentrations and agglutination (e-f)  
555 and lysis (g-h) titres as a function of mean clutch size and number of eggs year<sup>-1</sup> in 23  
556 populations of 12 lark species. Squares: hot desert larks; circles: cold desert larks;  
557 triangles: tropical larks; diamonds: temperate larks.

558

559 Figure 2. Haptoglobin (a-c) and ovotransferrin (d-f) concentrations and agglutination (g-i)  
560 and lysis (j-l) titres as a function of de Martonne's aridity index  $A_M$ , mean annual  
561 precipitation (mm) and mean annual temperature (°C) in 23 populations of 12 lark species  
562 measured along an environmental aridity gradient.  $A_M$  increases with decreasing aridity of  
563 the environment. Squares: hot desert larks; circles: cold desert larks; triangles: tropical  
564 larks; diamonds: temperate larks.

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566



